Palaeoecology and population demographics of the extinct New Zealand moa (Aves: Dinornithiformes)

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Abstract

The cause(s) of the worldwide Late Pleistocene megafaunal extinctions and their effects on modern ecosystems has been debated by the scientific community since the widespread discovery of megafaunal fossils in the nineteenth century. New Zealand is a good case study because of its geographical isolation, resulting in the late colonisation of New Zealand by Polynesians in about 1280 AD. The subsequent extinction of New Zealand's megafauna, including the moa (Aves: Dinornithiformes), took place at a time of relative climatic stability, and is thought to be due to over-hunting and habitat destruction. The aim of this thesis, using moa as a case study, is to conduct a detailed examination of a megafaunal palaeoecosystem prior to the introduction of humans.

In this thesis, Chapter One reviews the literature concerning the causes and consequences of the Late Pleistocene megafaunal extinctions; the evolution of moa; New Zealand ecology; ancient DNA and its applications; and coalescent theory. The aims and structure of the thesis is then outlined.

To investigate the New Zealand palaeoecosystem using moa, it is first necessary to examine the quality of the recent moa fossil record. This is especially important because specimens used in temporal population demographic analyses need to be interpreted in the correct depositional context and timeframe. There have been a number of theories proposed to explain the deposition mechanisms of swamp deposits, or, more correctly, miring bone deposits, including large floods and stampeding during wildfires. Chapter Two discusses the taphonomy of three different New Zealand swamp deposits that were reconstructed using a novel ARC-GIS methodology specifically designed for this study. It concludes that the reconstructions are consistent with non-catastrophic periodic miring of individual moa.

To gain an insight into the faunal composition of North Canterbury, New Zealand in the Late Glacial period (10,000-14,000 years ago), and how moa responded to climate and habitat change, I led a team that re-excavated the Late Glacial Glencrieff miring bone deposit and reconstructed the palaeofauna. The analysis presented in Chapter Three shows the Glencrieff fauna was a characteristic glacial fauna, despite extensive climatic and habitat change during this period.

A new opportunity to examine moa palaeoecology has been presented by the discovery of moa coprolites. Previous reconstructions of moa diet have concluded that moa were predominantly browsers of trees and shrubs. There is considerable debate over the role of moa in the evolution of 'anti-browsing' growth characteristics found in many of the New Zealand flora. Chapter Four discusses the analysis of newly discovered coprolite deposits and subsequent reconstructions of moa diet. The results challenge historical perceptions of moa diet, showing there was considerable overlap in dietary preferences between different moa species, despite differences in skull and bill morphology, with the majority of plants eaten being less than 30 cm in height. In addition, plants with 'anti-browsing' growth characteristics were found in the coprolites, consistent with the moa anti-browsing hypothesis.

To further study moa ecology I led a genetic investigation of moa appearance using moa feathers. The current knowledge of moa plumage is limited because the majority of moa feathers are recovered as isolated specimens in caves and rockshelters, and cannot be related to specific species. In Chapter Five, ancient DNA (aDNA) is extracted from isolated sub-fossil feathers and used to identify species. Digital techniques used to reconstruct moa plumage indicate that four species of moa were characterised by either a plain brown slightly streaky plumage or a speckled plumage, with considerable overlap in plumage between species. The overlap may be due to convergent evolution of feather colour for camouflage against aerial predators as observed in many other New Zealand bird species, for example kiwi (*Apteryx* spp.), kakapo (*Strigops habroptilus*), kea (*Nestor notabilis*), takahe (*Porphyrio hochstetteri*) and weka (*Gallirallus australis*).

In Chapters Six and Seven I analyse how moa species have responded to changes in climate and habitat since the Last Glacial Maximum (LGM; 29-19 Kya in New Zealand). Ancient DNA and fossil records are used to examine the responses of the extinct crested moa (*Pachyornis australis*) and heavy-footed moa (*Pachyornis elephantopus*) to climate and habitat change. I show that crested moa tracked changes in its sub-alpine habitat since the LGM due to warming climate with little effect on population size. Concurrently, climate and habitat change promoted phylogeographic structuring and allometric size variation within heavy-footed moa. Importantly, while climate and habitat change had an effect on moa, it did not cause their ultimate extinction.

Chapter Eight further investigates the temporal population demographic methods used in Chapters Six and Seven. I discuss how sampling biases common to most aDNA datasets affect the robustness of the Bayesian Skyline Plot (BSP), a commonly used analytical method for inferring the past population demographic history of species or populations. The analyses indicate that sampling biases produced large variations in the BSP of Beringian Steppe Bison and *Pachyornis* moa, when the datasets were re-examined and re-sampled to simulate sampling biases. Importantly, this reveals the BSP may not accurately reflect the true demographic history of a species or population when analysing contemporary genetic data alone.

Finally, the thesis concludes with a discussion drawing together, and interpreting the outcomes and significance of the research and argues that the results of this research represent a significant addition to our present knowledge of the pre-human New Zealand megafaunal palaeoecosystem.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Gongora *et al.* 2008. Correction: Indo-European and Asian origins for Chilean and Pacific chickens revealed by mtDNA Correction. *Proceedings of the National Academy of Sciences of the USA* **105**: 14234.

Gongora *et al.* 2008. Reply to Storey *et al*.: More DNA and dating studies needed for ancient El-Arenal-1 chickens. *Proceedings of the National Academy of Sciences of the USA,* **105**, E100.

Storey *et al.* 2008. Pre-Columbian chickens, dates, isotopes, and mtDNA. *Proceedings of the National Academy of Science USA* **105**: E99.

Wood *et al.* 2008. A deposition mechanism for Holocene miring bone deposits, South Island, New Zealand. *Journal of Taphonomy* **6**: 1-20.

Wood *et al.* 2008. Coprolite deposits reveal the diet and ecology of the extinct New Zealand megaherbivore moa (Aves: Dinornithiformes). *Quaternary Science Reviews* **27**: 2593-2602.

Rawlence *et al.* 2009. DNA content and distribution in ancient feathers and potential to reconstruct the plumage of extinct avian taxa. *Proceedings of the Royal Society of London Biological Series* **276**: 3395-3402.

Slater *et al.* 2009. Evolutionary history of the Falklands wolf. *Current Biology* **19**: R937-38.

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This thesis is dedicated to my late father

Grant William Rawlence

(1946-1995)

No moa, no moa,
In old Ao-tea-roa.
Can't get 'em.
They've et 'em;
They've gone and there aint no moa!

A popular New Zealand song.

CHAPTER ONE
General Introduction

1. Overall aim of thesis

The overall aim of the thesis is to conduct a detailed examination of a megafaunal palaeoecosystem prior to the introduction of humans. New Zealand is an excellent case study because of its geographic isolation resulting in (i) a simplified ecosystem deficient in terrestrial mammals and (ii) the late colonisation by Polynesians about 1280 AD (Wilmshurst *et al.* 2008). This meant that the pre-human New Zealand ecosystem was fully functional 800 years ago in contrast to megafaunal ecosystems elsewhere, which were impacted by humans about 10 Kya. In addition there is overwhelming archaeological and palaeoenvironmental evidence suggesting that hunting and habitat destruction by early Maori resulted in the extinction of 41% of New Zealand's birds within 200 years (Anderson 1989a; Wilmshurst and Higham 2004; Tennyson and Martinson 2006) at a time of relative climatic stability (Burrows and Greenland 1979).

To examine the New Zealand palaeoecosystem, I will use the dominant herbivore, the extinct moa (Aves: Dinornithiformes) as a case study. Moa were large, wingless ratites that are endemic to New Zealand (Worthy and Holdaway 2002) with nine currently recognised species in six genera (Bunce *et al.* 2009). Moa were also the largest and most diverse terrestrial vertebrate herbivores in the pre-human ecosystem, and their extinction left an ecological vacuum that has not been filled by the introduction of exotic animals by Europeans (Wood *et al.* 2008; Forsyth *et al.* 2010; Kelly *et al.* 2010; Lee *et al.* 2010).

As a result of the late colonisation of New Zealand by Polynesians, there are extensive megafaunal fossil deposits dating as recently as 1000 years ago. The majority of these deposits are from swamps and caves. They contain moa bones (Worthy and Holdaway 1996), preserved gizzard contents (Burrows *et al*. 1981), coprolites (Horrocks *et al.* 2004), eggshell (Oskam *et al.* 2010), feathers (Hamilton 1894) and rare mummified remains (reviewed by Anderson 1989a; Worthy and Holdaway 2002). To critically assess the quality of information from the fossil record of New Zealand's palaeoecosystem, I aim to reconstruct the taphonomy and deposition mechanism of three swamp deposits, and the palaeofauna of the Late Glacial (10-14 Kya) Glencrieff swamp deposit. The data will help assess how representative fossil deposits are with respect to the underlying moa population, which will in turn influence the

interpretation of the population demographic history of moa inferred from genetic and palaeontological analyses.

There is considerable debate about the diet of moa and how they functioned within the New Zealand palaeoecosystem. Current knowledge of moa diet is based on a heavily biased fossil record of preserved moa gizzard contents which are restricted to only a few moa species and represent wetland and lowland forest habitats or vegetation within reach of mired birds (Greenwood and Atkinson 1977; Burrows *et al*. 1981; McGlone and Webb 1981; Worthy and Holdaway 2002; Wood 2007; Wood *et al.* 2008). Using extensive deposits of moa coprolites from southern New Zealand, I aim to reconstruct the diet of moa and how moa functioned within the palaeoecosystem. Another contentious area is the appearance and behaviour of moa (Vickers-Rich *et al.* 1985; Gill and Martinson 1991; Flannery and Schouten 2001; Worthy and Holdaway 2002; Tennyson and Martinson 2006). The fossil record contains numerous isolated moa feathers that have not been positively identified to species. I aim to develop a methodology to extract ancient DNA (aDNA) from sub-fossil feathers and reconstruct the plumage of moa. The plumage reconstructions will then be used to infer potential moa behaviour.

With knowledge about how moa functioned within the New Zealand palaeoecosystem and the biases associated with the interpretation of the fossil record, I will use moa remains to address questions concerning:

(i) The effect of major episodes of climate and habitat change on the phylogeography, population demography and allometric variation in body size of moa.
(ii) The ability of moa to respond to changes in habitat availability during major episodes of climate change by shifting their distributions accordingly.

New Zealand is unique because the effects of major episodes of climate and habitat change on a megafaunal ecosystem can be studied in isolation, in contrast with North and South America where human arrival overlaps with a period of major climate change.

Finally I will address how sampling biases in the fossil record affect inferences of population demographic history obtained from genetic datasets - using both the moa datasets generated in this thesis, and the Beringian Steppe Bison dataset of Shapiro *et al*. (2004).

2. Causes and consequences of the Late Pleistocene megafaunal extinctions

2.1 Megafauna

'Megafauna' is a collective term used to describe a group of animals that weigh over about 44 kg (Johnson 2002) or alternatively the largest animals in an ecosystem regardless of weight (Hansen and Galetti 2009). Animals classified as megafauna have a global distribution and include the enigmatic extinct terrestrial mammals - woolly mammoths (*Mammuthus primagenus*), mastodons (*Mammut* spp.) and woolly rhinos (*Coelodonta antiquitatis*); birds – Madagascan elephant birds (*Aepyornis* spp., *Mullerornis* spp.), dodo (*Raphus cucullatus*) and New Zealand moa (Aves: Dinornithiformes); and marine mammals - whales (ceatecea), southern elephant seals (*Mirounga leonina*) and the extinct Stellar's sea cow (*Hydrodamalis gigas*) from the Bering Sea.

2.2 Timing of the megafaunal extinctions

The Late Pleistocene and Holocene were characterised by the staggered extinction of megafaunal species worldwide. For example, megafaunal extinctions in North and South America occurred 12-9 and 12.9-10 Kya respectively (reviewed by Barnosky *et al.* 2004; Guthrie 2006), in contrast to extinctions in Australia 46-30 Kya (reviewed by Tuniz *et al.* 2009) and on islands in the Pacific 30-0.5 Kya (Holdaway and Jacomb 2000a, b; Steadman and Martin 2003; Tennyson and Martinson 2006). The variation in extinction timing corresponds with different major climatic and human migratory events. For example the extinctions in Australia occurred after humans arrived due to hunting and habitat modification (Brook *et al.* 2006; Prideaux *et al.* 2007), while the extinctions in Polynesia occurred just after Polynesians arrived in each island archipelago (Steadman and Martin 2003). It has been argued that the Late Pleistocene extinctions are not a true mass extinction event because they were staggered through time, rather than occurring at a single time point, and were highly size selective. This contrasts with the Cretaceous-Tertiary (or more correctly Palaeogene) extinction event 65 Mya, which resulted in the extinction of a large range of species including all of the dinosaurs, irrespective of size (Haynes 2000; Fiedel and Haynes 2004).

2.3 Causes of the megafaunal extinctions

Four main causes for the Late Pleistocene extinctions have been proposed, each with supporting and contrary evidence: human impact; climate change; disease; and extraterrestrial bolide impact. Human impact includes habitat destruction, over-hunting and the effect of commensal animals such as domesticated dogs (*Canis lupus familiaris*) and rats (*Rattus* spp.; Martin 1984; Anderson 1989a; Flannery 1994; Robert *et al.* 2001; Worthy and Holdaway

2002; Turney *et al.* 2008). Over-hunting has been hypothesised to be a continuum from fast (Martin 1984) to slow (Johnson 2006) blitzkrieg (i.e. anthropogenically driven megafaunal over-kill; Martin 1984). For example a fast blitzkrieg has been theorised to have occurred in New Zealand resulting in the extinction of over a third of bird species (Tennyson and Martinson 2006). In contrast, a slow blitzkrieg has been hypothesised to have occurred in Australia (Johnson 2006), because periodic hunting by Aborigines would have been enough to cause the extinction of megafauna with low reproductive rates, irrespective of size (Johnson 2002). Johnson (2006) theorised that the killing of one megafaunal individual per family per decade was capable of resulting in the loss of 14 megafaunal genera (Barnosky *et al.* 2004).

Climate change and associated habitat change have also been implicated in the extinctions and population bottlenecks experienced by several megafaunal taxa during the Late Pleistocene, which are recorded as dramatic declines in genetic diversity (Leonard *et al.* 2000; Barnes *et al.* 2002; Hofreiter *et al.* 2004; Shapiro *et al.* 2004; Dalen *et al.* 2005; Weinstock *et al.* 2009; Stiller *et al*. 2010). For example, based on temporal genetic data, Shapiro *et al.* (2004) showed that Beringian Steppe Bison experienced a dramatic population decline in the Late Pleistocene before, during and after the Last Glacial Maximum (LGM) 22-19 Kya (Yokoyama *et al.* 2000). In contrast, the mammoth is inferred to have had a comparatively stable population size during this time (Barnes *et al*. 2007; Debruyne *et al.* 2008). It is commonly argued that the survival of megafaunal species through the numerous glacial periods of the Pleistocene indicates that the extinctions or population declines during the last glacial period cannot be attributed to climate and habitat change (Barnosky *et al.* 2004).

In some geographical areas like North and South America, the megafaunal extinctions coincided with the Younger Dryas (a period of severe cooling climate 11-13 Kya) and human arrival, making it difficult to elucidate a single cause of the extinctions. Several studies have proposed multi-causal theories linking human arrival and climate change with megafaunal extinctions, with much of the current debate centred on the relative contributions of these two causes (Miller *et al.* 1999; Barnosky *et al.* 2004; Drummond *et al.* 2005; Johnson 2006). For example, Drummond *et al.* (2005) re-analysed the Shapiro *et al.* (2004) bison dataset and showed that bison underwent a population decline 10-12 Kya, coinciding with the arrival of humans in Beringia, the Younger Dryas and the hypothesised bolide impact (Firestone *et al.* 2007), in addition to the population decline prior to the LGM documented by Shapiro *et al.* (2004).

More unorthodox theories have also been proposed. The disease hypothesis was originally proposed by MacPhee (1999), and MacPhee and Marx (1999) and argues that the Late Pleistocene megafaunal extinctions were caused by a novel hyper-disease that was brought by immigrating human populations. This theory has been applied to New Zealand by Gemmell *et al.* (2004) to propose that the extinct moa were in decline before Polynesians colonised New Zealand due to increased mortality from introduced diseases from migratory bird species. This is illogical because there are no documented declines of taxa in the Late Quaternary fossil record outside of major episodes of climate and habitat change (Worthy and Holdaway 1994, 1995, 1996; Worthy 1997, 1998a, b; For further rebuttals of Gemmell *et al*. (2004) see *Section 3.3.7*). In addition, migratory bird species would have been arriving in New Zealand for millennia. If disease really did cause a decline in moa population size, why was it introduced just before Polynesians colonised New Zealand and not earlier? Another argument against the disease hypothesis is that the agent of disease (bacteria, virus or single cell parasite) has to be broad acting enough to affect multiple phylogenetically unrelated species. One modern candidate includes Phocine/Canine distemper virus (*Morbillivirus* spp.; Daszak *et al.* 2000). *Morbillivirus* spp. can jump the species barrier and has resulted in the local extinction of black-footed ferret (*Mustela nigripes*) and African wild dog (*Lycaon pictus*) populations. It is also a threat to European grey wolves (*Canis lupus*; reviewed by Daszak *et al.* 2000). There is limited evidence suggesting that disease contributed to the Late Pleistocene extinctions. However, there is evidence to suggest that global extinctions of specific taxa have been caused by disease. Wyatt *et al.* (2008) argued that the introduction of a trypanosome parasite by black rats (*Rattus rattus*) resulted in the extinction of endemic rats (*Rattus macleari*) on Christmas Island.

Recently, evidence of an extra-terrestrial bolide impact at 12.9 Kya over northern North America has been reported (Firestone *et al.* 2007; Kennett *et al.* 2008; Firestone 2009). Firestone *et al.* (2007) argued that the impact caused the Younger Dryas, the concomitant extinction of the North American megafauna and the rapid decline in the population size of humans (Clovis culture) in North American. However, several studies have challenged this theory. For example, Surovell *et al.* (2009) were unable to reproduce the results, while Buchanan *et al.* (2008) statistically analysed 1500 radiocarbon dates from archaeological sites in North America and found no evidence supporting a decline in the population size of humans in North America at the onset of the Younger Dryas. Interestingly, Fiedel (2009) showed that megafaunal species on the Caribbean and Arctic Islands survived until 4-5 Kya, 6.9-7.9 Kyr after the hypothesised bolide impact. In addition, the bolide impact hypothesis

only applies to the North American extinctions and cannot be used to explain other megafaunal extinctions in the Northern and Southern Hemisphere, as they are staggered through time before and after the hypothesised bolide impact.

2.4 Consequences of the megafaunal extinctions

Whatever the ultimate cause(s) of the megafaunal extinctions, they resulted in a significant loss of megafaunal diversity and had serious effects on modern ecosystems through the transformation of vegetation communities and loss of co-evolutionary relationships between plants and megafauna. As for the variation in extinction timing, the loss of generic diversity also varied with geographical location. In Africa and northern Eurasia, fewer megafaunal species became extinct during the Late Pleistocene (Africa: five genera become globally extinct and three genera locally extinct; Eurasia: five genera become globally extinct and four genera locally extinct; Barnosky *et al.* 2004) - potentially due to the longer presence of *Homo* spp. and adaptation of megafauna to humans (Martin 1984). In comparison, the loss of megafaunal and species diversity in the New World and the Pacific was considerably higher, theorised to be due to naivety of the megafauna. This is supported by robust evidence suggesting that when humans arrive on a virgin landmass, habitat destruction and faunal extinctions follow shortly after (Martin 1984). For example North and South America lost 33 and 50 genera respectively (combined global and local extinctions; Barnosky *et al.* 2004), while in the Pacific as many as 2000 species of birds have become extinct, although most of them were not megafauna (Steadman and Martin 2003).

It is hypothesised that the megafaunal extinctions caused major transformations to vegetation composition worldwide. Johnson (2009) hypothesised that there were three general changes: loss of open vegetation and habitat mosaics that were shaped by megafaunal species and their replacement with closed and uniform habitats; increased fire intensity; and declines and extinctions of plants that co-evolved with megafauna (see also *Section 3.3.5* and *3.3.6*). The mammoth steppe of northern Eurasia and Beringia has been used as an example of a megafaunal shaped environment, and the impact of the extinction of mammoths. During the Pleistocene glacial and interglacial periods, northern Eurasia and Beringia was characterised by a cool, dry, treeless grassland interspersed with wetter productive areas (van Geel *et al.* 2007, 2008; Kienast *et al.* 2008), supporting large numbers of woolly mammoths and *Equus* horses (Guthrie 2006; Johnson 2009). However, by 12.5 Kya, the mammoth steppe was changing towards a wetter habitat characterised by shrubland and tall forest (Anderson *et al.* 1991; Lozhkin *et al.* 1993; Bigelow and Powers 2001).

There is also evidence that fire intensity increased as a result of megafaunal extinctions. Johnson (2009) argued that Madagascar demonstrated this. In the dry southwest of Madagascar where the majority of megafaunal remains have been found, fire intensity was low before the megafaunal extinctions 2 Kya, but thereafter, plant biomass was higher and fire intensity subsequently increased (Burney 1993; Burney *et al.* 2003, 2004).

3. New Zealand and the megafaunal palaeoecosystem

In New Zealand, human arrival and major episodes of climate change did not overlap. The initial settlement by Polynesians is dated about 1280 AD (Wilmshurst *et al.* 2008). The faunal extinctions of large species such as moa, large flightless geese and ducks, and Haast's eagle (*Hapagornis moorei*) occurred within 200 years of settlement (Tennyson and Martinson 2006). This period was one of relative climatic stability compared with the early Holocene and last glacial period (Burrows and Greenland 1979). In addition, there is overwhelming palaeoenvironmental (Wilmshurst and Higham 2004) and archaeological (Anderson 1989a, b) evidence that over-hunting and habitat destruction by early Maori was responsible for the extinction of New Zealand's avian megafauna. Because of the late arrival of Polynesians in New Zealand and the extensive fossil record, New Zealand is one of only a handful of places where the pre-human megafaunal palaeoecosystem can be reconstructed and the effects of climate change on a megafaunal ecosystem can be examined in isolation. In contrast, it is known that human arrival and major episodes of climate change overlapped in many geographical areas, including North and South America (Barnosky *et al.* 2004). This is perhaps predictable because major episodes of climate change often facilitate human dispersal into new geographical areas. This overlap makes the elucidation of the causes of the Late Pleistocene extinctions in North and South America difficult. The research outlined in this section will concentrate on critically assessing the New Zealand fossil record and reconstructing the palaeoecosystem.

3.1 Geological and climatic history of New Zealand

The late colonisation of New Zealand by Polynesians is due to the geographical isolation of the New Zealand archipelago. The geological and climatic history of New Zealand is summarised in Table 1. The continental fragment that would eventually become New Zealand, called Zealandia, separated from eastern Gondwana (Antarctica and Australia) ca. 82 Mya (Campbell and Hutching 2007) with sea floor spreading in the Tasman Sea stopping ca. 60 Mya (Cooper and Millener 1993). Recent research by Rey and Muller (2010), based on palaeomagnetic studies of rocks on the floor of the Tasman Sea and mathematical computer

modelling, hypothesises that between 105-90 Mya, Zealandia (on the Pacific Plate) was still connected to eastern Gondwana. Subduction of the Pacific Plate beneath eastern Gondwana resulted in the formation of a mountain chain along the eastern margins of the super continent. However, between 90-82 Mya, the rate and depth of subduction decreased (Rey and Muller 2010). This resulted in (i) the connection between Zealandia and eastern Gondwana decreasing, causing the collapse of the eastern Gondwanan mountain chain; and (ii) increased buoyancy of the mantle, which stretched and pushed upwards towards the continental crust causing a rift system to develop (Rey and Muller 2010). The collapse of the mountain chain and formation of the rift system facilitated the separation of Zealandia from eastern Gondwana, which was complete by ca. 82 Mya (Cooper and Millener 1993; Rey and Muller 2010).

As the proto Tasman Sea widened and Zealandia moved into the Pacific, the continental crust thinned, causing Zealandia to sink (Campbell and Hutching 2007). By the Oligocene 34-22 Mya, the maximum level of marine transgression was reached. Recently debate has emerged concerning whether or not there was any emergent land present during this period. New geological evidence has challenged traditional depictions (Flemming 1979; Pocknall 1982) and has used the absence of terrestrial Oligocene-Early Miocene (25-22 Mya) rocks and the presence of extensive deposits of marine limestone of the same age to suggest that New Zealand was completely drowned. It would follow that the endemic flora and fauna subsequently dispersed to New Zealand (Pole 1994; Campbell and Landis 2001; Campbell and Hutching 2007; Campbell *et al.* 2008; Landis *et al.* 2008). However, the apparent absence of terrestrial Oligocene-Early Miocene rocks does not prove that there was no emergent land present during this time. It is possible that terrestrial rocks have been eroded in the 22 million years since the Oligocene drowning. Genetic and palaeontological evidence suggests there was emergent land during the Oligocene-Early Miocene, hypothesised to be about 18% of New Zealand's current land area (Flemming 1979; Stevens 1985; Cooper and Millener 1993; Cooper and Cooper 1995). Fossils from the early Miocene Saint Bathan's fauna suggest that a diverse assemblage survived the Oligocene drowning. Dated at 16-19 Mya, the Saint Bathan's fauna is characterised by a diverse range of avian, reptilian, mammalian and fish taxa with several Gondwanan groups represented including tuatara (*Sphenodon punctatus*) and leiopelmatid frogs (reviewed by Tennyson 2009). In addition, several non-Gondwanan avian taxa like hihi (*Notiomystis cincta*), wattlebirds (Callaeidae), ratites and parrots (*Nestor* spp., *Strigops habroptilus*) have molecular divergence dates prior to the Oligocene drowning (Driskell *et al.* 2007; Shepherd and Lambert 2007; Wright *et al.* 2008; Phillips *et al.* 2010).

Table 1 A summary of the geological and climatic history of New Zealand discussed in *Section 3.1*.

Time	Geological or climatic event	Consequences
105-90 Mya	Zealandia connected to eastern Gondwana.	-
90-82 Mya	Zealandia separates from Gondwana.	-
82 Mya	Separation of Zealandia complete.	Dispersal of taxa to New Zealand.
60 Mya	Seafloor spreading in Tasman sea stops.	Zealandia reaches current geographical position.
34-22 Mya	Oligocene drowning.	18% of New Zealand's current land area was emergent land. The remnant North and South Islands were not connected.
22-2 Mya	North Island progressively uplifted in a southwards direction.	Independent evolution of taxa cf. to South Island taxa.
16-19 Mya	Saint Bathan's Fauna.	Earliest dated moa fossils.
5-7 Mya	Southern Alps are uplifted along the Alpine fault.	Alpine habitats created.
2-1.5 Mya	North Island joins South Island for the first time in the past 30 Mya.	Faunal interchange.
450 Kya	Cook Straight forms.	North and South Island separated.
70-14 Kya	Otiran Glacial.	Sea level 120-130 metres lower. North and South Island unconnected. Tree line lowered by 800 metres. Sub-alpine grassland/shrubland mosaics at low altitudes.
29-19 Kya	Last Glacial Maximum.	Temperature 4-5ºC lower. Cool dry conditions.
19-14 Kya	Last Glacial.	Increased biological activity.
14-10 Kya	Late Glacial/Interglacial Transition.	Succession from grassland to shrubland (15-12 Kya) to forest (14-9 Kya). Sea level starts to rise.
13-11 Kya	New Zealand Glacial Reversal.	Severe climate oscillation.
10-0 Kya	Holocene/Aranuian Interglacial.	Warming temperature with periods of cooler temperature. Late Holocene climate comparatively stable.
6 Kya	Sea level stabilises.	-
ca. 1280 AD	Polynesians colonise New Zealand.	Megafaunal extinctions. Habitat destruction.

While it has been argued that numerous plant species like hebes (Scrophulariaceae), buttercups (Ranunculi) and daisies (*Celmisia* spp.; Breitwieser *et al.* 1999; Wagstaff *et al.* 2002; Winkworth *et al.* 2002; Gibbs 2006) dispersed to New Zealand after the Oligocene drowning, there is a fossil record of terrestrial plants spanning the entire Oligocene-Early Miocene (Gibbs 2006; Tennyson 2009). One exception to dispersal of plants after the Oligocene drowning may be kauri (*Agathis australis*; Stocker *et al.* 2002; Knapp *et al.* 2007; Lee *et al.* 2008). The genus *Agathis* shows a trans-Tasman distribution and has been interpreted as evidence that kauri has been present in New Zealand since before Zealandia separated from eastern Gondwana (Stocker *et al.* 2002). Recently, Knapp *et al.* (2007) reported divergence dates prior to the Oligocene drowning and could not reject the hypothesis that kauri survived the drowning event. This has recently been confirmed by the discovery of leaf fossils comparable to kauri (for an alternative explanation see Hill *et al.* 2008 and Biffen *et al.* 2010), in Late Oligocene-Early Miocene sediments in Southland, supporting the hypothesis that kauri was present in New Zealand throughout the Oligocene. However, Biffen *et al*. (2010) has recently challenged Knapp *et al.* (2007) conclusion on the basis that the fossil calibration points used by Knapp *et al.* (2007) resulted in an overestimation of the divergence date. Using alternative calibration points Biffen *et al*. (2010) calculated the divergence of kauri at 23 Mya, which does not reject the possibility of post Oligocene dispersal.

New geological evidence suggests that, following the Oligocene drowning, the remnant North Island, which was centred around modern day Northland, emerged in a progressively southwards direction, with the North and South Islands separated by the Manawatu Straight (Kamp and Furlong 2006; Bunce *et al.* 2009). The islands finally joined 1.5-2 Mya, for the first time in the past 30 Myr, only to be separated again by the formation of Cook Straight 450 Kya (Bunce *et al.* 2009). The Southern Alps were uplifted due to tectonic activity along the Alpine Fault ca. 5-7 Mya, coinciding with marked global cooling from the Miocene to the Pliocene (Campbell and Hutching 2007).

Climate change in New Zealand from the Otiran Glacial (70-14 Kya), the last glacial period of the Pleistocene (1.8 Mya-10 Kya), to the present, is well documented. Three major climatic intervals have been defined in New Zealand and include:

(i) Otiran Glacial (70-14 Kya)
(ii) Late Glacial (14-10 Kya)

(iii) Holocene (10-0 Kya)

The Otiran glacial encompasses the LGM (29-19 Kya; Newnham *et al.* 2007) and the Last Glacial (18-14 Kya; Williams *et al.* 2005). The LGM was characterized by mean annual temperatures 4-5ºC lower than today and cool, dry conditions (McGlone *et al*. 2010). In contrast, the Last Glacial was characterised by increased biological activity, including increased plant growth and precipitation (Williams *et al.* 2005). The Late Glacial (Worthy and Roscoe 2003), also known as the Interglacial Transition (Williams *et al.* 2005), was characterized by warming temperatures (14-13 Kya) and the New Zealand Glacial Reversal (13-11 Kya; Williams *et al.* 2005). This was a period of severe climate oscillation. The Holocene (or Aranuian) period (10 Kya-present) was characterised by warming temperatures interspersed with periods of cooler temperatures due to increased glacial activity (Williams *et al.* 2005). In the Late Holocene, the climate became comparatively more stable (Burrows and Greenland 1979).

The geomorphologic and vegetation changes that took place in New Zealand during the Otiran Glacial are well documented. During the LGM, sea levels were 120-130 metres lower. Despite this the North and South Islands remained unconnected (at least significantly) as shown through the lack of evidence of faunal interchange of endemic North and South Island avian species during this lowstand (Bunce *et al*. 2009). In previous glacial periods, there is geological evidence to suggest such a connection (Worthy and Holdaway 2002; Bunce *et al.* 2009). With colder temperatures, the tree line was lowered 800 metres (McGlone *et al.* 2010) and the Southern Alps were consistently covered in snow and ice (McKinnon 1997; Newnham *et al.* 1999). As a result, the high altitude sub-alpine grassland formed grassland/shrubland mosaics at low altitudes (Moar and Suggate 1979; Vandergoes *et al.* 2005; Burge and Schulmeister 2007) and the forest retreated to sheltered refugia and warmer northern refugia (Campbell and Hutching 2007). The sub-alpine grassland/shrubland mosaic was widespread at low altitudes until about 15-12 Kya (Moar and Suggate 1979; Vandergoes and Fitzimons 2003; Moar 2008), followed by a well-documented vegetation succession from grassland to shrubland (15-12 Kya) to forest (14-9 Kya), due to rapid climatic change during the Late Glacial and early Holocene. As a result of this rapid climate change, the sub-alpine grassland declined in extent and migrated up-slope from about 15 Kya until climate stabilised in the Late Holocene (Moar and Suggate 1979; Wardle 1991; Worthy and Holdaway 1995, 1996; Worthy 1997, 1998a, b; Moar 2008).

3.2 New Zealand fauna and its evolution

There has been considerable research into how the geological and climatic history of New Zealand affected the evolution of endemic and native fauna. When Zealandia separated from eastern Gondwana, it presumably carried a full complement of faunal taxa. Most species did not survive until the Late Holocene, with known major geological boundaries at the Cretaceous-Tertiary, mid-Oligocene and Late Miocene-Early Pliocene. Dinosaurs, pterosaurs, freshwater turtles (Wiffen 1991), crocodiles (Tennyson 2009) and a Mesozoic mammal lineage (Worthy *et al.* 2006) as well as several avian taxa (reviewed by Tennyson 2010) all became extinct before the Middle Pleistocene. New Zealand's fauna has been dominated by birds, reptiles and insects. Famous examples include kakapo, the world's largest and only nocturnal parrot; tuatara, the only extant representative of the sphenodontid order of reptiles (formally widespread in the Triassic) and giant weta (*Deinacrida* spp.), the flightless crickets described as behaving like invertebrate mice (Gibbs 2006).

This characteristic New Zealand fauna has either a Gondwanan vicariant or dispersal origin (reviewed by Tennyson 2009). Distinctive New Zealand taxa with a likely Gondwanan origin include leiopelmatid frogs (Roelants and Bossuyt 2005; Worthy *et al.* 2009), Acanthisittid wrens (Barker *et al.* 2002; Ericson *et al.* 2002), tuatara, geckos (Chambers *et al.* 2001; Hitchmough *et al.* 2009; Tennyson 2009) and the freshwater invertebrate community (Gibbs 2006). Taxa with a dispersal origin include most avian species (Tennyson 2009), mystacinid bats (Hand *et al.* 1998) and *Oligosoma* skinks (Hickson and Slack 1998; Hickson *et al.* 2000; Chapple *et al.* 2009). Most of the faunal taxa that arrived in New Zealand by dispersal came from Australia. However, there are examples of dispersal from New Caledonia (*Cyanoramphus* parakeets; Boon *et al.* 2000) and possibly South America (the extinct adzebill, *Aptornis* spp.; Houde 1997). However, the South American connection is not conclusive. Houde (1997) found adzebill was sister to the South American trumpeter (*Psophia* spp.) but bootstrap support was below 50%. Dispersals to New Zealand have been occurring since Zealandia separated from eastern Gondwana ca. 82 Mya, with recent examples including *Cyanoramphus* parakeets 400-600 Kya (Boon *et al.* 2000) and pukekos (*Porphyrio porphyrio*) after Polynesian colonisation of New Zealand in 1280 AD (Wilmshurst *et al.* 2008).

There are also many instances of island gigantism in the New Zealand fauna, hypothesised to be due to New Zealand's isolation and lack of mammalian predators. Gigantism is widespread across many taxonomic groups. Examples include giant collembola (springtail subfamily

Uchidanurinae), giant weta, kakapo and the megafauna. New Zealand's extinct megafauna include Haast's Eagle, adzebill, giant goose (*Cnemiornis* spp.) and the palaeognathus moa (Aves: Dinornithiformes). Haast's eagle, the top predator in the pre-human New Zealand ecosystem, was the largest eagle in the world with a wingspan up to 2.6 metres and weighing up to 15 kg. Recent aDNA research by Bunce *et al*. (2005) has shown that Haast's Eagle was most closely related to one of the smallest eagles, the Australian little eagle (*Aquila morphnoides*) that weighs only 815 grams. This study suggested that the ancestors of Haast's eagle arrived in New Zealand only 0.7-1.8 Mya and rapidly increased in size.

3.3 Moa

Moa, one of New Zealand's iconic megafaunal taxa, were large flightless birds endemic to New Zealand. They belonged to the palaeognath lineage (Hackett *et al.* 2008; Harshman *et al.* 2008; Phillips and Cooper 2008; Phillips *et al.* 2010), being birds with an archaic palate (Cooper *et al.* 2001). Moa (along with the flightless taxa listed below) were previously classified as 'ratites' (see *Section 3.3.1*) because they possessed a flat breastbone (Pycraft 1900). Other extinct palaeognath taxa include the flightless elephant birds from Madagascar (*Aepyornis* spp. *Mullerornis* spp.); several late Tertiary taxa from the Old and New Worlds; and a putative 'ratite' from the Eocene of Antarctica (Tambussi *et al.* 1994; Mayr 2009). The distribution of extant palaeognaths is currently restricted to the Southern Hemisphere, and includes the tinamou (Tinamidae) and rhea (*Rhea* spp.) from South America; ostrich (*Struthious camelus*) from Africa; emu (*Dromaius* spp.) and cassowary (*Casuarius* spp.) from Australia and New Guinea; and the kiwi (*Apteryx* spp.) from New Zealand.

3.3.1 How did the ancestors of moa get to New Zealand?

Ratites, including moa, have historically been presented as a classic example of Gondwanan vicariant biogeography (Cracraft 1974). Based on morphological evidence, moa and kiwi were thought to be sister taxa (Cracraft 1974; Anderson 1989a; Worthy 1989) that had been isolated on Zealandia when it separated from Gondwana. However, recent genetic studies have challenged this view and offer an insight into how the ancestors of moa arrived in New Zealand. Cooper *et al.* (1992) isolated 12S rDNA from four different moa species and concluded that kiwi and moa were not sister taxa as previously thought. Kiwi formed a recent (apical) Australasian clade with emu and cassowary, whereas moa were an old clade basal in the phylogeny and closely related to rhea. Cooper *et al.* (1992) concluded that the ancestors of moa were isolated on Zealandia when it separated from Gondwana and were an early divergence within ratites. However, Cooper *et al.* (1992) did not report any molecular

divergence dates to support this claim. This study further concluded that the position of kiwi with Australian ratites was inconsistent with a Gondwanan vicariant history. This suggests that the ancestors of kiwi either swam or island-hopped to New Zealand.

Cooper *et al.* (2001) extended this analysis by sequencing the complete mitochondrial (mtDNA) genomes of two species of moa, the extant ratites and two species of tinamou. They found that ratites were a monophyletic clade sister to the volant tinamous, with rhea basal followed by moa, ostrich and the Australasian clade. Fixing the divergence of moa from all other ratites at 82 Mya, when Zealandia separated from Gondwana, Cooper *et al.* (2001) calculated that all ratite taxa except kiwi had a Gondwanan vicariant history and diverged during the Late Cretaceous, followed by subsequent dispersal of kiwi to Zealandia 65-72 Mya by island-hopping. While it could be argued that ostrich required dispersal to get to Africa, because the divergence date of ostrich 73-78 Mya post-dated the separation of South America and Africa ca. 90 Mya, Cooper *et al.* (2001) argued that a vicariant history was still the most plausible explanation. Cooper *et al.* (2001) hypothesised that ancestral ostriches entered Indo-Madagascar via the Kerguelen Plateau, which linked Australia and Antarctica to Indo-Madagascar during the Late Cretaceous 80 Mya. Subsequent over-land dispersal into Eurasia, hypothesised to be the location of ostrich evolution (Sauer 1972; Olson 1985), was followed by the colonisation of Africa (Cooper *et al.* 2001). Independent research by Haddrath and Baker (2001) mirrored that of Cooper *et al.* (2001) and found that ratites were monophyletic and sister to tinamous, with moa basal, compared to Cooper *et al.* (2001) where rhea were basal. Haddrath and Baker (2001) concluded that ratites were consistent with a Gondwanan vicariant history except ostrich and kiwi, which required dispersal. Molecular clock estimates put the divergence time of moa at 78.9 Mya and kiwi at 62 Mya.

With the advent of genomic analyses and increases in analytical methodology, more detailed analyses of how the ancestors of moa got to New Zealand have been undertaken. Hackett *et al.* (2008) sequenced 32 Kb of nuclear DNA from 19 independent loci from 169 avian species including the extant ratites. This study showed that the volant tinamous clustered within the flightless ratites. Such evidence suggested that ratites are not monophyletic as was previously thought (Sibley and Ahlquist 1990; Cooper *et al.* 1992; 2001; Elzanowski 1995; Lee *et al.* 1997; Haddrath and Baker 2001; Livezey and Zusi 2007). This is interesting because some of the phylogenetic analyses conducted by Haddrath and Baker (2001) placed tinamous inside of ratites.

Harshman *et al.* (2008) sequenced 20 independent nuclear loci from all extant ratites, and representatives of Tinamidae, Neoavian and crocodilian out-groups. They showed that ratites were paraphyletic. Harshman *et al*. (2008) concluded that there have been at least three losses of flight in 'ratite' evolution and that 'ratites' do not have a Gondwanan vicariant history. This study found that ostriches were basal, followed by tinamous, rhea and the Australasian clade, with losses of flight in ostriches, rheas and the Australasian clade. Harshman *et al.* (2008) also argued that long branch attraction, base composition bias, gene trees versus species trees and sequence alignment errors were not the cause of ratite paraphyly. However, unlike previous studies, Harshman *et al.* (2008) did not include the extinct moa or elephant birds, meaning that the sister group of tinamous was unclear, and did not report any molecular divergence dates.

Phillips *et al.* (2010) extended the Harshman *et al.* (2008) study by analysing the complete mtDNA genomes of ratites, including moa, two new kiwi mtDNA genomes and more outgroup taxa. Phillips *et al.* (2010) phylogeny matched Harshman *et al.* (2008) and showed that moa were the sister taxa of tinamous, suggesting at least four losses of flight in ratites, including moa. Because tinamous grouped with moa, the separation of Zealandia from eastern Gondwana at 82 Mya for the divergence between moa and other ratites could not be used, thus Phillips *et al*. (2010) used a new suite of fossil calibration points to calculate the divergence times within ratites. In contrast to Cooper *et al.* (2001) the divergence dates are associated with the Cretaceous-Tertiary extinction event and the break-up of southern Gondwana (South America, Antarctica, Zealandia and Australia) with moa and tinamou diverging 60 Mya (95% HPD 38.3-81.6 Mya). The conclusions of Phillips *et al.* (2010) are further supported by the analysis of nuclear RAG-1 data (Phillips and Cooper 2008).

Both Harshman *et al.* (2008) and Phillips *et al.* (2010) offer insight into why previous studies like Cooper *et al.* (1992, 1997, 2001) and Haddrath and Baker (2001) found that ratites were monophyletic. Harshman *et al.* (2008) and Phillips *et al.* (2010) partitioned sequence data into first, second and third codon positions, with the third codon position R-Y coded to reduce signal saturation. They also partitioned RNA sequences into stems and loops. Importantly, Harshman *et al*. (2008) and Phillips *et al*. (2010) did not enforce ratite monophyly, but allowed rate heterogeneity among variable sites and partitions, and calculated molecular divergence dates using the more biologically realistic relaxed molecular clock models (Drummond *et al*. 2006). The problems of previous analyses were further compounded by the high rate of molecular evolution in tinamous relative to other ratites. In the absence of other

taxa with high rates of molecular evolution, tinamous clustered basal in the phylogeny, which reinforced ratite monophyly.

The Phillips *et al.* (2010) study suggests that the common ancestor of moa and tinamous arrived in Zealandia from South America or Antarctica about 60 Mya, after Zealandia had separated from eastern Gondwana. It is therefore highly probable that the volant common ancestor of moa and tinamous flew to Zealandia. However, it is also possible that moa had a vicariant origin as the upper 95% HPD for the divergence of moa and tinamous is 81.6 Mya, which is comparable to the separation of Zealandia from eastern Gondwana at 82 Mya. The earliest known fossils attributable to moa are dated at 16-19 Mya (Tennyson *et al.* 2008), but this disparity between the genetic and palaeontological dates is probably due to the paucity of terrestrial Tertiary fossil localities in New Zealand. It is also possible that moa are more closely related to extinct putative Antarctic 'ratites' (Tambussi *et al.* 1994; Mayr 2009) than South American tinamous. Tinamous, Antarctic 'ratites' and moa could potentially form a clade, with tinamous sister to Antarctic ratites and moa.

3.3.2 Evolutionary radiation of moa

There has also been considerable research into the evolutionary radiation of moa. Since the first description of moa (Owen 1840), their taxonomy has been in constant review with 64 species in 20 genera previously described in the literature (reviewed by Worthy & Holdaway 2002). Currently just nine species are recognised in six genera, based on a combination of molecular, morphological, palaeoecological and phylogeographic data (Figure 1; Cooper 1997; Cooper *et al.* 1992, 2001; Worthy & Holdaway 2002; Bunce *et al*. 2003, 2009; Huynen *et al*. 2003; Baker *et al*. 2005; Lambert *et al*. 2005; Worthy *et al.* 2005; OSNZ Checklist Committee 2010). However, the taxonomy of some moa taxa is still unresolved, with small sample sizes and a lack of robust morphometric analyses fuelling debate into whether genetic distance measures underpin meaningful taxonomic structure or population genetic structuring (Baker *et al.* 2005; Lambert *et al.* 2005; Baker 2007; Bunce *et al.* 2009). For example, Lambert *et al.* (2005) and Bunce *et al.* (2009) have shown that some clades of upland moa (*Megalapteryx didinus*) differ by as much as 4-5% sequence divergence. In the absence of morphological differences between individuals from these clades, questions were asked about whether genetic distance measures represented deep phylogeographic structuring or cryptic species (Bunce *et al.* 2009). The diversity of moa species is greater than other ratite clades except tinamous, promoting the question how did this diversity evolve and what were the divergence times of the various different moa taxa?

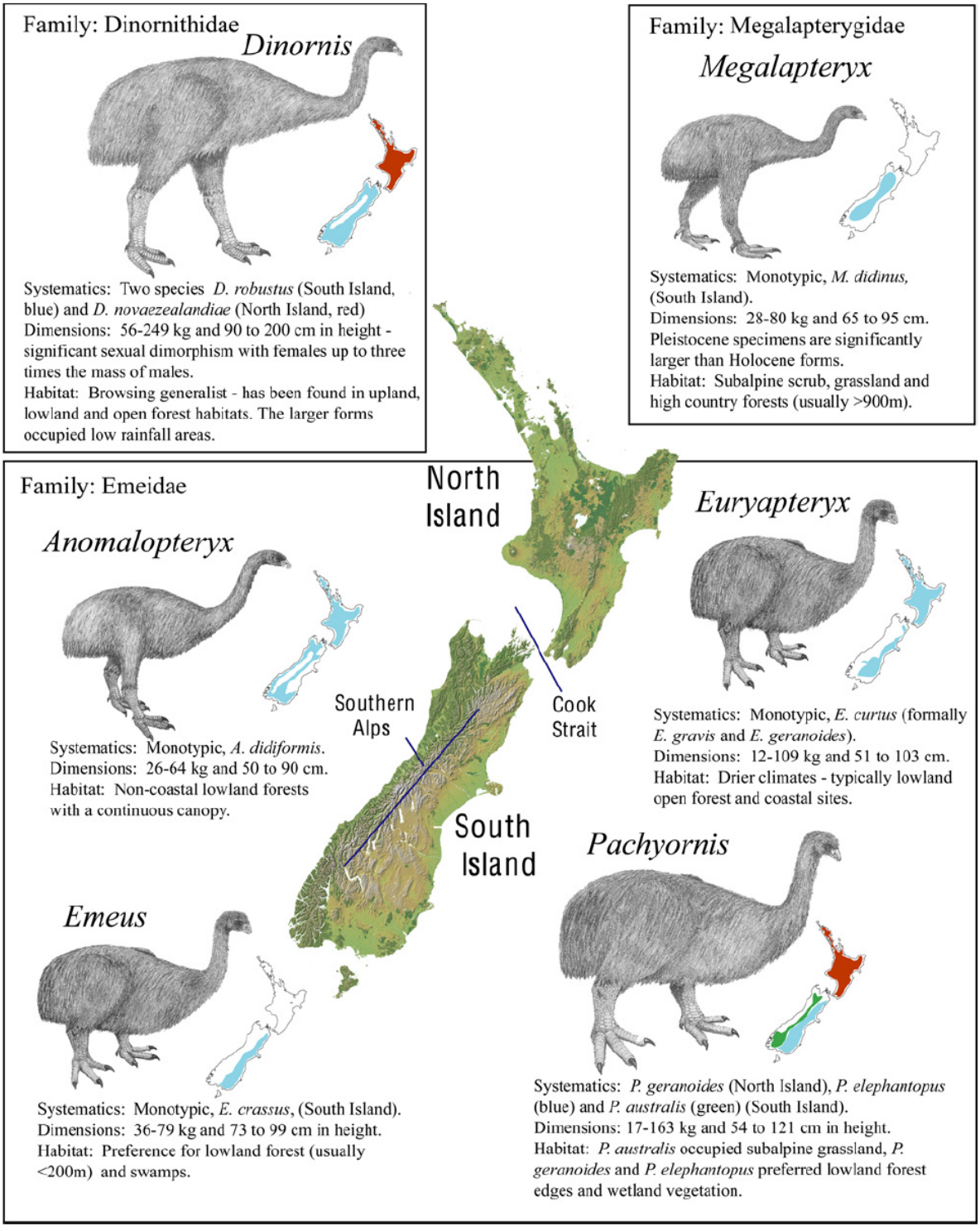

Figure 1 Taxonomy, geographical distribution and habitat of the extinct New Zealand moa (Aves: Dinornithiformes). Reproduced from Bunce *et al*. (2009). The colours on the maps of New Zealand refer to the geographical distribution of each moa species. For *Dinornis*, red refers to *D. novaezelandiae*, while blue refers to *D. robustus*. For *Pachyornis*, red refers to *P. geranoides*, blue refers to *P. elephantopus*, and green refers to *P. australis*.

The first genetic investigations into the evolutionary radiation of moa in New Zealand were conducted by Cooper *et al.* (1992), Cooper and Cooper (1995) and Cooper (1997). Historically, moa were separated into two families, based on shared morphological characteristics: Dinornithidae (containing the genus *Dinornis*) and Emeidae (containing the genera *Pachyornis, Euryapteryx, Emeus, Anomalopteryx* and *Megalapteryx*). Cooper *et al.* (1992), Cooper and Cooper (1995) and Cooper (1997) showed that *Megalapteryx* was basal to all other moa genera, followed by Dinornithidae then Emeidae. The basal position of *Megalapteryx* was in contrast to morphological analyses, which suggested that *Megalapteryx* was closely related to *Anomalopteryx* (reviewed by Worthy and Holdaway 2002). Recent morphological analyses have confirmed the branching order of *Megalapteryx*, Dinornithidae and Emeidae (Worthy and Holdaway 2002; Bourdon *et al.* 2009).

Subsequent studies focused on the genus *Dinornis*, which occurred on both the North and South Islands. *Dinornis* was separated into three species on the basis of size, irrespective of the island of origin (Worthy and Holdaway 2002): *D. giganteus*, *D. novaezealandiae* and *D. struthoides*. Bunce *et al.* (2003) and Huynen *et al.* (2003) extracted aDNA from specimens covering the taxonomic and geographic range of *Dinornis* and showed that the genus comprised reciprocally monophyletic North (*D. novaezealandiae*) and South (*D. robustus*) Island clades. Molecular dating indicated that the two clades diverged during the Middle Pleistocene (Bunce *et al*. 2003). In addition, Huynen *et al.* (2003) showed that Mappin's moa (*Pachyornis geranoides*) from the North Island had well defined western and eastern clades, thus generating the debate over whether genetic distance measures underpin meaningful taxonomic structure or population genetic structuring. Baker *et al*. (2005) argued that based on genetic distance, the western and eastern clades of Mappin's moa were separate lineages, while Baker (2007) advocated full species status for these lineages.

With the development of DNA barcoding (Hebert *et al.* 2004), Lambert *et al.* (2005) investigated whether DNA barcoding could be applied to extinct taxa. DNA barcoding uses a short piece of the mitochondrial Cytochrome Oxidase I (COI) gene to identify specimens to known reference species based on a pre-defined threshold percentage sequence divergence. Using the COI barcode and mtDNA control region sequences, Lambert *et al.* (2005) confirmed the results of Cooper *et al*. (1992), Cooper and Cooper (1995) and Cooper (1997). However, the Lambert *et al.* (2005) study was based on small sample sizes and did not report any molecular divergence dates to assess phylogeographic hypotheses.

Baker *et al*. (2005) with a larger dataset (sample size and genetic loci) than Lambert *et al.* (2005) conducted a phylogenetic analysis of moa and found 14 monophyletic lineages, with nine corresponding to currently recognised species. While Baker *et al.* (2005) did not state that the five new lineages were new species, Baker (2007) later advocated full species status. However, both Baker *et al.* (2005) and Baker (2007) did not consider that palaeoecology, geography and habitat preferences influence species limits, in addition to genetic distance (Bunce *et al.* 2009). In addition, Baker *et al.'s* (2005) and Baker's (2007) taxonomic conclusions contain significant taxonomic and nomenclatural errors. For example, Baker *et al.* (2005) genetically typed two upland moa (*M. didinus*) specimens from northwest Nelson and Otago that differed by 4.47% sequence divergence. Baker *et al.* (2005) concluded that the specimens represented two lineages of upland moa and questioned the previous synonymy of *M. didinus* and *M. benhami* by Worthy (1988), which was based on the absence of morphological differences between these two species. *Megalapteryx benhami* was a large glacial morph of *M. didinus* and was found only in northwest Nelson/West Coast, while the type specimen of *M. didinus* was found in Otago (Worthy 1988). Baker *et al.* (2005) assigned the name *M. benhami* to the Otago specimen and *M. didinus* to the northwest Nelson specimen, contrary to the fossil record and the rules of taxonomic nomenclature (Jeffrey 1989).

To determine the divergence times of moa within New Zealand, Baker *et al.* (2005) constructed a phylogeny of ratites and tinamous based on the Cooper *et al.* (2001) and Haddrath and Baker (2001) datasets, with each gene partitioned and assigned a unique model of evolution. In addition, Baker *et al.* (2005) fixed the basal divergence of moa from all other ratites at 82 Mya, when Zealandia separated from eastern Gondwana, and enforced ratite monophyly. Using this dataset, Baker *et al.* (2005) calculated the date of the most recent common ancestor of moa in their dataset at 18.5 Mya, after the height of the Oligocene drowning 22 Mya (Flemming 1979; Stevens 1985; Cooper and Millener 1993; Cooper and Cooper 1995). This study concluded that moa went through a significant population bottleneck as a result of the Oligocene drowning, followed by a cycle of lineage splitting 4-10 Mya, coinciding with the formation of the Alpine fault and uplift of the Southern Alps in the South Island. The diversity of moa species and phylogeographic structuring within some species was hypothesised to be the result of the isolation of moa in the North and South Islands by the formation of Cook Straight 5 Mya, contraction and expansion into and out of 'refugia' during the Pleistocene, and migration between the North and South Islands during glacial periods. In light of Hackett *et al.* (2008), Harshman *et al.* (2008) and Phillips *et al.*

(2010), fixing the divergence date of moa from all other ratites using the separation of Zealandia from eastern Gondwana and enforcing ratite monophyly is invalid given the common ancestor of moa and tinamou was volant and diverged about 60 Mya.

Bunce *et al.* (2009) re-analysed the Baker *et al.* (2005) dataset and included more specimens and new geological data. In light of the findings of Hackett *et al.* (2008) and Harshman *et al.* (2008) that ratites are paraphyletic, Bunce *et al.* (2009) did not use the 82 Myr date for the separation of Zealandia from eastern Gondwana. Instead this study used new analytical methods and included new secure avian fossil calibration points. One such calibration point was the split between rhea and cassowary/kiwi/tinamou/moa at 56-83 Mya. The split was based on the minimum age of *Diogenornis*, an extinct ratite genus from South America, at 56 Mya, and the absence of members of this clade from Northern Hemisphere and South American fossil deposits prior to 83 Mya (Bunce *et al.* 2009 Supplementary Information). By R-Y coding the third codon position in protein coding sequences (as opposed to standard coding as exercised by Baker *et al.* 2005) and including new fossil calibration points, Bunce *et al.* (2009) calculated that the most recent common ancestor of moa within their dataset was 5.8 Mya, compared with 18.5 Mya calculated by Baker *et al.* (2005). Bunce *et al.* (2009) further argued that moa were present on the remnant South Island during the Oligocene drowning. This study further argued that any moa present on the remnant North Island, centred on modern day Northland, did not contribute to subsequent moa diversity because no divergence dates pre-dated the Oligocene drowning ca. 22 Mya. This was supported by three facts: the time to most recent common ancestor (tMRCA) of moa post-dated the Oligocene drowning; the earliest fossil moa are dated to 16-19 Mya in the Saint Bathan's fauna on the South Island; and most North Island moa taxa have a derived position in the phylogeny. Further support is provided by the apparent diversification of moa genera over the past 6 Myr. New habitats were formed with the uplift of the Southern Alps and dispersal into North Island habitats about 1.5-2 Mya, when the North Island joined the South Island for the first time in 30 Myr. Bunce *et al.* (2009) ultimately concluded that subsequent isolation in the North Island and South Island during Pleistocene glacial cycles and the formation of Cook Straight 450 Kya (Bunce *et al.* 2009; in contrast to 5 Mya reported by Baker *et al.* 2005), along with volcanism, habitat change and geographic barriers resulted in differing patterns of phylogeographic structuring within moa. An example of this is the little bush moa's (*Anomalopteryx didiformis*) clearly defined North and South Island clades, with no inter-clade morphological divergence (Baker *et al*. 2005; Bunce *et al*. 2009). This reflects a recent allopatric split by Cook Straight.

Assuming Bunce *et al.'s* (2009) hypothesis is correct, the following questions are pertinent: Did moa diversify between their colonisation of New Zealand ca. 60 Mya (Phillips *et al.* 2010) and the Oligocene drowning 22 Mya; did the Oligocene drowning cause the widespread extinction of moa taxa; and was there a single moa lineage prior to 6 Mya when genetic data suggests moa diversified? Fossil eggshell from the Saint Bathan's fauna suggests at least two moa lineages were living in the South Island as recently as 3 Myr after the Oligocene drowning (Tennyson *et al.* 2008). Therefore it is possible that at least two moa lineages survived the Oligocene drowning.

3.3.3 Reverse sexual dimorphism in moa

Research into the radiation of moa within New Zealand has been complicated by unrecognised sexual dimorphism, and geographical or temporal variation in body size within species. During the nineteenth and early twentieth centuries, scientists had limited knowledge of species boundaries in moa. For example, the genus *Pachyornis* in the North Island was split into *P. septentrionalis* and *P. mappini* on the basis of size. With the development of univariate and multivariate statistical analyses of morphological characters, aDNA analysis and radiocarbon dating of bone, an appreciation of allometric size variation (including sexual dimorphism) has been gained. Worthy (1987; see also Worthy and Holdaway 2002) has shown that within discrete paleontological deposits, some moa species had unimodel or bimodal distributions in the length of leg bones (femur, tibiotarsus, tarsometatarsus). Unimodel distributions have been used to suggest the sexes were the same size; while bi-model distributions have been used to suggest that the sexes were different sizes. Comparison with other extant ratites suggested that the large bones belonged to females (Worthy and Holdaway 2002).

As noted in *Section 3.3.2*, phylogenetic analysis of previously recognised species of *Dinornis* (*D. giganteus*, *D. novaezealandiae* and *D. struthoides*; separated solely on the basis of size) showed that this genus consisted of reciprocally monophyletic North and South Island species (*D. novaezelandiae* and *D. robustus* respectively; Bunce *et al.* 2003; Huynen *et al.* 2003). To investigate why there was a large variation in body size within the North and South Island species and whether sexual dimorphism could explain this variation, genetic sexing of *Dinornis* bones was conducted (Bunce *et al.* 2003; Huynen *et al.* 2003). The genetic analysis showed that the larger individuals (previously classified as *D. giganteus* and *D. novaezealandiae*) were female, while smaller individuals (previously *D. struthoides*) were male. This confirmed that some species of moa exhibited extreme reverse sexual size

dimorphism. Huynen *et al.* (2003) extended the analysis further to additional moa species pairs that had previously been separated on size: *P. septentrionalis* and *P. mappini*, *Euryapteryx curtus* and *E. exilis,* and *Emeus huttonii* and *E. crassus*, and again found that the larger 'species' were female and the smaller 'species' male.

3.3.4 Allometric size variation in moa

Statistical analysis (univariate and multivariate), aDNA analyses and radiocarbon dating have also shown that moa exhibited allometric size (mass and height) variation in response to changing climate. For example, the genus *Megalapteryx* historically contained three species: the large *M. benhami*, the medium-sized *M. didinus* and the small *M. hectori*. Morphological analysis (Worthy 1988) showed that although there were allometric (size) differences between these three species, the shape of the bones were the same. Subsequent radiocarbon dating has shown that bones from *M. benhami* were dated to the Late Glacial and Otiran Glacial while bones from *M. didinus* and *M. hectori* were from the Holocene. Additionally, genetic analysis (Bunce *et al.* 2009) indicates that these three taxa are spread throughout the *Megalapteryx* clade, confirming Worthy (1988) conclusion they belonged to the same species, namely upland moa (*M. didinus*). Examples of the pattern of large glacial morphs and small interglacial morphs in moa have also been found in stout-legged/coastal moa (*E. curtus*, formerly *E. gravis* and *E. curtus*) and Mappin's moa. The pattern of temporal size variation shows that some species of moa followed Bergman's Rule (Bergmann 1847), which states that as warm blooded animals move down a temperature gradient (temporal, altitude or latitude) the size of the animals gets larger.

3.3.5 Moa-plant co-evolution

Another area of considerable research and scientific debate is the co-evolution of moa and plants during the ancestral moa radiation. New Zealand has a large number of phylogenetically diverse plant species exhibiting a range of distinctive plant growth characteristics. These include divarication (plants with branch angles of greater than 90º, interlacing branches, reduced number of leaves on outer branches and tough stems), heteroblasty (plants with different juvenile and adult growth habits, with many species of divaricating plants also exhibiting heteroblasty), mimicry, toxins, spines, unpalatibility, photosynthetic stems, fibrous leaves, stinging hairs or low nutrient status (Worthy and Holdaway 2002). These growth characteristics have puzzled scientists as to their evolutionary cause. For example, juvenile lancewoods (*Pseudopanax crassifolius*) have long, tough, spiky, brown, unpalatable leaves, while the adult plants (above 3 metres in height, which has been

hypothesised as the height giant moa could reach) have bushy, green palatable foliage (Fadzly *et al*. 2009). Historically, there have been two main theories explaining these distinctive growth characteristics.

The moa-browsing hypothesis was proposed by Greenwood and Atkinson (1977). It states that these growth characteristics evolved in response to moa browsing. This study used divarication and heteroblasty as the main evidence supporting moa browsing hypothesis. This hypothesis also has support from subsequent studies (Batcheler 1989; Atkinson and Greenwood 1989; Worthy and Holdaway 2002; Bond *et al.* 2004; Burns and Dawson 2006, 2009; Bond and Silander 2007; Fadzly *et al.* 2009; Lee & Gould 2009; Lee *et al.* 2009). For example, closely related or sister taxa of plants with "anti-browsing" characteristics lack these growth characteristics on islands without moa e.g. Chatham Islands 800 km east of New Zealand. The spear grass genus *Aciphylla* in New Zealand has spines and spiky leaves, while those on the Chatham Islands have no spines and soft leaves (Worthy and Holdaway 2002). Similarly, islands with large avian herbivores (like Madagascar where several species of elephant birds existed until their extinction about 2 Kya; Burney 1993; Burney *et al.* 2003, 2004) have flora with similar anti-browsing growth characteristics (Bond and Silander 2007; Burns and Dawson 2006).

An alternative theory, proposed by Cockayne (1912) and Rattenbury (1962), argued that the cold and windy climatic conditions of the Pleistocene caused the evolution of these growth characteristics. This theory was proposed again by McGlone and Webb (1981) and other authors (Day 1998; Howell *et al*. 2002) in response to Greenwood and Atkinson (1977). McGlone and Webb (1981) argued that the presence of divaricating flora in moa gizzards (Burrows *et al.* 1981) strongly suggested that divarication did not stop moa browsing and therefore could not be responsible for its evolution. However, Atkinson and Greenwood (1980, 1989) and Lowry (1980) challenged this argument by stating that these growth characteristics reduced moa browsing, giving these plants an evolutionary advantage over plants without anti-browsing growth strategies. Recently, Oligo-Miocene fossils of woody shrubs with growth characteristics similar to modern divaricating plants have been found (Campbell *et al.* 2000). This suggests such growth characteristics have been a feature of the New Zealand flora for millions of years and contradicts the climate hypothesis.

Some authors argue that the evolution of these growth forms was probably the result of a combination of moa browsing and climate (Worthy and Holdaway 2002; Gibbs 2006). It is

not known how many of plants with these growth characteristics moa ate, as preserved gizzard contents are biased towards forest edge/wetland habitats or plants within reach of mired moa, and contain mostly twigs (Burrows *et al.* 1981; Wood 2007; Wood *et al.* 2008).

3.3.6 Moa diet

One way to determine whether moa ate plants with 'anti-browsing' growth characteristics is to reconstruct moa diet. Historically, moa were thought to be omnivores like kiwi (Haast 1872) or browsers of grassland like sheep and cows, only eating fruit and leaves when seasonally available (Buick 1931; Oliver 1949, 1955). However, these theories were not based on any dietary evidence. The first evidence for moa diet was published by Hamilton (1892) who found preserved moa gizzard contents comprising seeds, twigs and branches. Allan *et al.* (1941), Burrows *et al.* (1981) and Wood (2007) have also recovered seeds and twigs but not grass from moa gizzards, suggesting that moa were browsers of trees and shrubs in forested habitats. Skull and bill anatomy and structure also suggested that the various moa species had different dietary and feeding preferences (Worthy and Holdaway 2002). For example, the pointed bill and large muscle attachments of the heavy-footed moa skull suggest a more robust diet than stout-legged moa, which had a broad bill and small muscle attachments (Worthy and Holdaway 2002).

Radiocarbon dating of moa bones and palynological (pollen) analysis of sediment cores from numerous fossil deposits have made it possible to estimate the habitat preferences of the different moa species, and narrow down the possible plant species in moa diet. Worthy and Holdaway (2002) defined three habitat zones that moa occupied: upland zone, lowland wet forest zone and lowland dry climate zone. The alpine zone (above the tree line) is characterised by herblands and tussock grasslands. The dominant species in the upland zone was upland moa, with crested (*Pachyornis australis*) and South Island giant moa common. In comparison, lowland wet forest zones were characterised by tall closed canopy forest and the dominance of Mappin's moa and little bush moa in the North Island, and little bush moa in the South Island. The lowland dry climate zone was characterised by a mosaic of shrubland, open canopy forest and grassland. In the North Island this zone was dominated by coastal/stout-legged moa (*E. curtus*), with North Island giant and Mappin's moa common. In the eastern South Island, the common moa species were eastern (*Emeus crassus*), stout-legged, heavy-footed (*Pachyornis elephantopus*) and South Island giant moa. Within the eastern South Island heavy-footed moa preferred open tussock grassland/shrubland mosaics while the other species preferred more open forest habitats.

$\delta^{13}C$ and $\delta^{15}N$ isotopic signatures from moa bones have also provided insights into moa diet (Worthy and Holdaway 2002) and can be used as proxy indicators of climate and habitat change (Stevens *et al.* 2008). For example, $\delta^{13}C$ has been used as a proxy for the amount of forest cover (Worthy and Holdaway 2002). Trends towards more negative $\delta^{13}C$ values over time indicate a change from open shrubland/grassland habitat to forest (Worthy and Holdaway 2002). Similarly $\delta^{15}N$ has been used previously as a proxy for temperature and precipitation, with increasing $\delta^{15}N$ isotope values indicative of increasing temperature and precipitation (Amundson *et al.* 2003). In addition, increasing $\delta^{15}N$ isotope values are also indicative of trophic level (e.g. herbivore, insectivore, omnivore, and carnivore) because protein in tissue becomes more enriched with ^{15}N the higher up the food chain (Minagawa and Wada 1984). For example, when the $\delta^{15}N$ isotope signal of the enigmatic adzebill (*Aptornis* spp.), an extinct giant rail from New Zealand, was compared with the extinct herbivorous Finsch's duck (*Cheonetta finschi*), moa, and the extinct insectivorous owlet-nightjar (*Aegotheles novaezelandiae*), the adzebill samples had higher $\delta^{15}N$ signals than owlet-nightjar (Worthy and Holdaway 2002). Worthy and Holdaway (2002) interpreted this result to mean that adzebill were carnivores because they ate animals higher up the food chain than insects. However, caution needs to be exercised when using $\delta^{13}C$ and $\delta^{15}N$ isotopes to infer diet. Comparison of the isotope signal of sub-fossil kakapo (a strict herbivore) from Central Otago (Wood 2006) to adzebill shows similar $\delta^{15}N$ isotope signatures in the two species (Wood unpublished data). This suggests regional and temporal factors are involved in determining isotope levels, for example changes in temperature and precipitation.

Despite the palaeodietary data for moa from gizzard contents, unbiased information is not available. Gizzard contents are biased towards wetland and lowland forest habitats or vegetation within reach of mired birds (Wood 2007; Wood *et al.* 2008). The gizzard contents are also heavily biased towards South Island giant moa, with only a few examples from eastern and heavy-footed moa (Worthy and Holdaway 2002; Wood 2007), and none from North Island species. Evidence about moa diet from coprolites is also sparse. Horrocks *et al.* (2004) analysed coprolites from Takahe Valley rockshelter in Fiordland, upon the assumption they were from upland moa. Bones of upland moa have been found in several caves and rockshelters in the area (Worthy 1998a; Worthy and Holdaway 2002). There is also the possibility that the coprolites were from another sympatric species little bush moa. However, Horrocks *et al.* (2004) did not genetically identify the defecator species and combined multiple coprolites into the one sample for dietary analysis and radiocarbon dating, potentially combining coprolites from multiple species and time periods.

3.3.7 Moa plumage

Moa plumage is another area where information is quite limited. The only definitive evidence on moa plumage comes from feathers preserved *in situ* on a mummified leg of an upland moa (Hamilton 1894) showing that this species was feathered to its toes, an adaptation to cold, snowy habitat (Worthy and Holdaway 2002). The absence of feather bases on mummified tarsometatarsi of other moa show they were not feathered to the toes. The feathers on the mummified upland moa leg had dark bases and white tips, suggesting speckled plumage. The other eight mummified moa specimens (reviewed by Anderson 1989a; Worthy and Holdaway 2002), where feathers are present, have only the bases of feathers preserved, thus giving no indication of plumage. Isolated feathers from multiple fossil localities comparable to known moa feathers have also been described since the mid 19th century, with multiple slight variations in colour including white, plain brown and white tipped (reviewed by Oliver 1949). It is not known whether other moa species had speckled plumage, what colour the other species of moa were, and whether there was more than one plumage type per species or individual. Because most moa feathers have not been identified to species, subsequent reconstructions of moa have had to assume that upland moa were speckled and all other moa were plain brown (Anderson 1989a; Worthy 1989; Vickers Rich *et al.* 1985; Vickers-Rich *et al.* 1995; Gill and Martinson 1991; Flannery and Schouten 2001; Murray and Vickers Rich 2003; Tennyson and Martinson 2006).

3.3.8 Moa population demographics

Despite the wealth of research conducted on moa diet and plumage, very little research has been conducted into how moa populations responded to major episodes of climate and habitat change. New Zealand has a rich fossil record of avifauna spanning the last 50 Kyr (Worthy 1999; Worthy and Holdaway 2002; Tennyson and Martinson 2006). From temporal and geographical comparisons of fossil deposits throughout New Zealand, it is evident that no vertebrate species known from the fossil record in the past 50 Kyr went extinct before Polynesians arrived in New Zealand (Worthy and Holdaway 2002). However, it is evident that the Otiran Glacial (70-10 Kya) significantly altered the distribution and relative abundance of moa compared to the current interglacial. During the Otiran Glacial and Late Glacial, wetter western regions of the South Island were dominated by heavy-footed moa and crested moa. During the Holocene, there was a localised faunal replacement by South Island giant moa and little bush moa. In contrast, drier eastern regions of the South Island during the Otiran Glacial and Late Glacial were characterised by a moa fauna dominated by heavy-footed and eastern moa, with South Island giant moa comparatively rare (Worthy 1993a,

1997; Worthy & Holdaway 1995, 1996; Worthy & Grant-Mackie 2003). In the Holocene, the relative fossil abundance of taxa changed with eastern moa and South Island giant moa dominating, and heavy-footed moa comparatively rare (Worthy & Holdaway 1996).

While palaeontological evidence suggests that the population size of moa has changed over time, there is limited genetic evidence to support this assertion. Gemmell *et al.* (2004) hypothesised that moa were already in decline before Polynesians arrived in New Zealand, due to volcanism and increased mortality from introduced avian diseases. Using aDNA sequences from Bunce *et al.* (2003) and Huynen *et al.* (2003), Gemmell *et al.* (2004) estimated the time to most recent common ancestor of *Dinornis* at 1-6 Kya. This study then estimated the effective population size of *Dinornis* at 300,000-1.4 million. This estimate is actually the effective population size of females because mtDNA is passed through the female line. Gemmell *et al.* (2004) assumed that all other moa species had a similar effective population size to *Dinornis* and converted the overall effective population size of moa to a census population size of 3-12 million.

To convert effective population size to census population size, Gemmell *et al.* (2004) used a three-step conversion process. First, they converted the effective population size of females to the total effective population size using the sex ratio of *Dinornis* of 1:1.4 (Bunce *et al.* 2003). However, recent research by Allentoft *et al.* (2010) has shown that the sex ratio of moa is heavily biased towards females, so the Bunce *et al*. (2003) sex ratio is probably an under-estimate. Allentoft *et al.* (2010) used genetic sexing of moa bones from Holocene North Canterbury swamp deposits and calculated the sex ratio at >2 females: 1 male, suggesting increased mortality of males compared to females during juvenile and sub-adult growth stages, creating an excess of females. There may be some taphonomic biases associated with this, with larger females more likely to get mired. However, the sex ratio estimate includes species with low levels of sexual dimorphism. Second, Gemmell *et al*. (2004) later converted the total effective population size to the number of breeding adults by assuming the ratio of the number of breeding adults to total effective population size is two. This ratio also assumed that *Dinornis* had a constant population size. Thirdly, Gemmell *et al.* (2004) converted the number of breeding adults to census population size using an estimate of the proportion of adult *Dinornis* to juveniles from Pyramid Valley moa swamp in North Canterbury (Worthy and Holdaway 2002) of 84% adults and 16% juveniles. This ratio is also heavily biased because of the over-representation of females in the Pyramid Valley deposit (Allentoft *et al.* 2010).

Gemmell *et al*. (2004) compared their estimate of moa population size (3-12 million) to an ecological estimate of 158,000 moa at the time of Polynesian arrival (Holdaway & Jacomb 2000a) and concluded that moa were in decline before Polynesians colonised New Zealand. However, the comparison of genetic and ecological estimates of population size is not valid for several reasons. Firstly, the Gemmell *et al.* (2004) estimate is based on a small sample size and temporal range (Forsyth *et al.* 2009) with only 57 specimens from the Late Holocene. Secondly, Gemmell *et al.* (2004) assumed a mutation rate of 2.08% per million years (Quinn 1992) and 9.6% per million years (Lambert *et al.* 2002), calculated from avian and Adelie penguin datasets respectively, rather than calculating a moa mutation rate. Thirdly, Gemmell *et al.* (2004) assumed the population size of each species of moa was the same as *Dinornis*. However, relative fossil abundance data shows that the frequency of moa species differed within and between habitats (Worthy and Holdaway 1994, 1995, 1996; Worthy 1997, 1998a, b), suggesting that the nine currently recognised moa species did not have the same population size. Finally, estimating moa population size is complicated by differences between how genetic and ecological estimates of population size are calculated. Genetic estimates use the amount of genetic variation within a population to calculate population size, while ecological estimates are based on the carrying capacity of a habitat based on the behaviour of living species (e.g. home range size). Anderson (1989a) estimated the number of moa during the Holocene at 70,500, while Holdaway and Jacomb (2000a) estimated 158,000, suggesting a moa density of 0.3-0.6 individuals km^{-2} (Forsyth *et al.* 2009).

Based on the available palaeontological and genetic data, there are several areas in our knowledge of moa population demography that require attention. Despite the abundant palaeontological data, it is not known how the effective population size of moa changed in response to major episodes of climate and habitat change; what effect contracting and expanding habitat had on the phylogeography of moa; and whether there was a loss of genetic diversity with major climatic events and declining habitat. Studies on Holarctic megafaunal species, like bison (Shapiro *et al.* 2004; Drummond *et al.* 2005), brown bears (Barnes *et al.* 2001; Valdiosera *et al.* 2008), Arctic fox (Dalen *et al.* 2007) and cave bears (Stiller *et al.* 2010) suggest that several taxa underwent dramatic population bottlenecks during the Late Pleistocene due to replacement events, climate change or an inability to respond to changes in habitat availability.

Importantly, it is not known how representative each fossil deposit (the palaeofaunal composition of a deposit and/or how it changes over time within a deposit) is with respect to

the underlying moa population, which will in turn affect the interpretation of the population demographic history of moa inferred from genetic and palaeontological analyses. To answer this it is important to know how each fossil deposit formed with respect to:

(i) The time frame of deposition.
(ii) Whether moa have been trapped *in situ* or reworked into the fossil deposit.
(iii) Whether moa remains are articulated or disassociated.
(iv) Whether the fossil locality is stratified or time averaged.
(v) The geological and geographical setting of the fossil locality.

Historically, there has been considerable debate into how some fossil deposits have formed, with catastrophic scenarios used to explain the taphonomy of these deposits including dying in the swamp from drinking poisoned water (Booth 1875), stampeding during wildfires (Booth 1875; Buick 1931) or large floods transporting the remains of moa into mires (Howorth 1887; Tregear 1893; Hutton 1900).

3.4 Arrival of Polynesians in New Zealand

3.4.1 When did Polynesians arrive in New Zealand?

Previous research outlined in *Section 3.3* has concentrated on critically assessing the New Zealand fossil record and reconstructing the palaeoecosystem. Intimately linked with this research is determining when Polynesians colonised New Zealand and the effect human arrival had on the palaeoecosystem. The date of Polynesian colonisation of New Zealand has been debated among the scientific community since Europeans discovered New Zealand in 1642. Historically there have been three main theories to explain when Polynesians colonised New Zealand. The "short chronology" theory postulates that Polynesians colonised New Zealand in the 13th century AD in line with archaeological, palaeoenvironmental, radiocarbon and genetic evidence (Anderson 1991; Murry-McIntosh *et al.* 1998; Higham *et al.* 1999; McGlone and Wilmshurst 1999). The "middle chronology" theory postulates that a small number of Polynesians arrived around 800 AD and were archaeologically invisible (Green 1975; Davidson 1984) until the earliest dated sites about 1280 AD (Higham *et al.* 1999; McGlone and Wilmshurst 1999). The "long chronology" theory postulates that Polynesians colonised New Zealand between 0-500 AD (Sutton 1987), although there is no archaeological evidence to support this. The "long chronology" theory gained support with the publication of a series of radiocarbon dates of kiore/Pacific rat (*Rattus exulans*) bones dated to about 200 BC (Holdaway 1996, 1999). Holdaway (1996, 1999) argued that Polynesians arrived in New

Zealand about 200 BC with kiore, and were archaeologically invisible until the earliest dated archaeological sites at about 1280 AD. Holdaway (1996, 1999) also hypothesised that kiore caused a wave of extinctions of small vertebrate taxa weighing less than 150 grams (Holdaway 1996, 1999, Worthy and Holdaway 2002) despite no vertebrate species going extinct in the fossil record before Polynesian colonisation in 1280 AD (Wilmshurst *et al*. 2008).

Subsequent research (Anderson 1996, 2000b, 2002; Smith and Anderson 1998; Holdaway *et al.* 2002; Anderson and Higham 2004; Higham *et al.* 2005) failed to obtain radiocarbon dates of kiore bones similar to the dates published by Holdaway (1996, 1999). Further dating of rat-gnawed seeds preserved in New Zealand sediments has also shown that no rat-gnawed seeds are older than the 13th century AD (Wilmshurst and Higham 2004; Wilmshurst *et al.* 2008). In addition, Wilmshurst *et al.* (2008) dated kiore bones from some of the same sites originally sampled by Holdaway (1996, 1999) and got dates no earlier than the 13th century AD. Stable isotopes further suggested a terrestrial signal and not one depleted in ^{14}C (Wilmshurst *et al.* 2008), which had been suggested as a reason for the old dates of Holdaway (1996, 1999; Beavan and Sparks 1998; Beavan-Athfield 2006). An old date would have been obtained if kiore had been eating fish or shellfish from Maori middens, a source of old marine ^{14}C. The research of Wilmshurst and Higham (2004) and Wilmshurst *et al.* (2008) strongly suggests the date of colonisation of New Zealand by Polynesians was about 1280 AD and not 200 BC, supporting the short chronology.

The cause of the old dates published by Holdaway (1996, 1999) has been suggested to be due to either the failure of pre-treatment procedures to remove old ^{14}C from kiore bone collagen extracts or intra laboratory contamination of collagen extracts with old ^{14}C (Anderson 1996, 2000b, 2002; McGlone and Wilmshurst 1999; Higham *et al.* 2005). Thirty-three kiore bones were radiocarbon dated to before the 13th century in 1995-96 at the Rafter Radiocarbon Accelerator Unit, Lower Hutt, New Zealand (Holdaway 1996, 1999). However, 30 additional kiore bones, dated at the same laboratory in 1997-98, were all younger than 1280 AD (Anderson 1996, 2000b, 2002; Smith and Anderson 1998; Holdaway *et al.* 2002; Anderson and Higham 2004; Higham *et al.* 2005). Additional kiore bones (30 in total) sampled by Wilmshurst *et al*. (2008) from nine of the same fossil deposits and stratigraphic layers as Holdaway (1996, 1999) were radiocarbon dated at the Oxford Radiocarbon Accelerator Unit, Oxford, UK, using ultrafiltration of collagen extracts (Bronk Ramsey *et al.* 2004a, b; Jacobi *et al.* 2006). Ultrafiltration removes high and low molecular weight contaminants that can

contain old ^{14}C. The radiocarbon dates of Wilmshurst *et al.* (2008) overlapped with the radiocarbon dates from the Rafter Radiocarbon Accelerator Unit during 1997-1998 and not the samples processed during 1995-1996.

3.4.2 Consequences of Polynesian colonisation.

Given the recent colonisation of New Zealand by Polynesians in 1280 AD (Wilmshurst *et al.* 2008), the consequences for the pre-human ecosystem were dire. There is overwhelming palaeoenvironmental (Wilmshurst and Higham 2004) and archaeological (Anderson 1989a) evidence suggesting that over-hunting and widespread habitat destruction by early Maori, and predation by introduced kiore (reviewed by Tennyson and Martinson 2006) resulted in the extinction of 41% of New Zealand's bird species within 200 years of settlement, including the megafaunal moa. New Zealand is dotted with early Maori moa butchering sites that support Martin's (1984) blitzkrieg model of megafaunal extinction. Holdaway and Jacomb (2000a) suggested that in some areas of New Zealand moa were locally extinct within 50-100 years of settlement, with some late survivors clinging on in less accessible areas (Anderson 2000a, Holdaway and Jacomb 2000b). The situation is similar in New Zealand marine mammal, fish, insect and floral populations. There were widespread local extinctions in these populations soon after Polynesian colonisation due to over-hunting by early Maori (marine mammal and fish populations; Smith 1989) or widespread predation by kiore (invertebrate and floral populations; Brook 2000; Wilmshurst and Higham 2004; Tennyson and Matinson 2006; Wilmshurst *et al.* 2008). Finally, habitat destruction was very severe with 40% of New Zealand's forest cover burnt within 200 years of Polynesian settlement to facilitate hunting and easier travel, transforming the landscape into open tussock grasslands prone to increased fire intensity.

4. Ancient DNA

Ancient DNA provides a new means to re-construct the pre-human palaeoecosystem of New Zealand, which would be the first detailed view of a megafaunal ecosystem anywhere in the world. Furthermore, the temporal proximity and detailed fossil record permit analysis of the effects of climate change and Polynesian colonisation on this ecosystem. Areas of key interest include palaeoecological reconstructions of moa diet, behaviour and appearance, and moa population demographics through time.

4.1 Brief history of aDNA

Ancient DNA is defined as highly degraded, fragmented and chemically modified DNA extracted from historical, archaeological or sub-fossil specimens. The field of aDNA started in the early 1980s with the cloning of DNA from Egyptian mummies (Paabo 1985), and the extinct quagga (*Equus quagga quagga*; Higuchi *et al.* 1984). The development of the Polymerase Chain Reaction (PCR; Mullis & Faloona 1987) in the late 1980s allowed the *in vitro* amplification of surviving aDNA molecules, resulting in several studies on aDNA from taxa including the thylacine (*Thylacinus cynocephalus*; Thomas *et al.* 1989), ancient humans (Paabo *et al.* 1988; Hagelberg & Clegg 1991), moa (Cooper *et al.* 1992) and plants (Brown *et al.* 1994) ranging in age from 100-8000 years. Claims of ancient DNA from geologically older specimens were also reported, including 17-20 Myr plant fossils (Golenberg *et al.* 1990, 1991; Soltis *et al.* 1992), 30-130 Myr insects and plants preserved in amber (Cano *et al.* 1992a,b; Poinar *et al.* 1993; de Salle *et al.* 1992; Cano *et al.* 1993) and 80 Myr dinosaur bones (Woodward *et al.* 1996), though these are widely discredited and have been shown to be un-reproducible and/or the result of contamination (Allard *et al.* 1995; Hedges & Schweitzer 1995; Zischler *et al.* 1995; Austin *et al.* 1997, 1998; Guitierrez *et al.* 1998).

As the aDNA field matured over the next 20 years, research has shown that aDNA can be recovered from various types of material up to 800 Kyr under ideal conditions (Willerslev and Cooper 2005; Willerslev *et al*. 2007). Apparently reproducible aDNA has been retrieved from sediments and ice cores up to 800 Kyr, showing that before the glaciation of Greenland, the area was characterised by temperate taxa rather than Arctic taxa (Willerslev *et al.* 2007). Specimens suitable for aDNA analysis include animal tissue (bone, teeth, muscle, hair and feathers), coprolites, sediment, plants and insects (Hagelberg & Clegg 1991; Cooper *et al.* 1992; Lindahl 1993a, b, 1995; Brown *et al.* 1994; Willerslev *et al.* 2003; Gilbert *et al.* 2004; Reiss 2006; Gilbert *et al.* 2007; Haak *et al.* 2008; Thomsen *et al*. 2009).

Since 2001, the field of aDNA research has focussed on attempts to sequence whole mtDNA genomes and population demographic analyses (discussed in *Section 4.3.3*). Cooper *et al.* (2001) published the first mtDNA genomes of an extinct species, the South Island giant moa and eastern moa, shortly followed by the little bush moa and another eastern moa individual by Haddrath and Baker (2001; excluding the control region). Amplification and sequencing of whole mtDNA genomes became more achievable with the advent of multiplex PCR, which allowed the amplification of multiple non-overlapping fragments in two PCR reactions (Krause *et al.* 2006; Gilbert *et al.* 2007).

Recently, the development of next generation sequencing technologies (e.g. 454 sequencing; Margulies *et al.* 2005) has facilitated a rapid technological advance in aDNA research. Sequencing of multiple whole mtDNA (Gilbert *et al.* 2007) and nuclear genomes is now possible. For example, Poinar *et al.* (2006) sequenced 28 million base pairs of nuclear DNA from the woolly mammoth, while Miller *et al.* (2008) announced the completion of the draft mammoth nuclear genome. In 2010, Green *et al.* (2010) published the draft nuclear genome of the Neandertal (*Homo neanderthalensis*), showing that one to four percent of Europeans and Asians, but not Africans, appear to share some very limited ancestry with Neandertals.

4.2 Caveats with ancient DNA research

Unlike modern genetic studies there are several problems within aDNA research that must be addressed if published results are to be seen as robust after peer review and subsequent scientific scrutiny. The first caveat is that aDNA is heavily fragmented. After death, the DNA within cells is broken down by endogenous nucleases, bacterial decomposition, hydrolysis and oxidation (Paabo 1989; Lindahl 1993a,b; Brotherton *et al.* 2007). As a consequence, the amplification size of aDNA is typically limited to below 500 bp (Paabo 1989), except in exceptional preservation circumstances (e.g. permafrost). The post-mortem fragmentation processes appear more pronounced on low copy number DNA molecules like nuclear DNA, compared to high copy number DNA molecules like mtDNA, because the probability of multi-copy DNA surviving the processes of DNA degradation are higher than low copy DNA molecules (Hofreiter 2000). As such, most aDNA studies are restricted to multi-copy DNA unless exceptional preservation circumstances exist (Poinar *et al.* 2006; Phillips & Cooper 2008; Miller *et al.* 2008). In addition, cold conditions are preferable for aDNA preservation as they slow down the decomposition process, while hot, wet conditions speed up decomposition processes until DNA is too degraded for genetic analysis (Hoss *et al.* 1996; Paabo *et al.* 2004) or there is no DNA left in the specimen. This is because cold or permafrost conditions slow down the post-mortem bacterial and chemical degradation of DNA (Hoss *et al.* 1996).

A second caveat is that aDNA is also heavily chemically degraded. Aside from fragmentation, which simply reduces template size and eventually prevents PCR amplification, there are three common types of DNA damage. These are mis-coding lesions (MSL), abasic sites and cross-linking. The only significant form of MSL occurs when hydrolytic damage causes a deamination reaction, resulting in the loss of an amino group from the nucleotide cytosine. This results in a cytosine to uracil (U; an analogue of thymine, T) transition that causes incorrect nucleotides to be incorporated into the PCR product by DNA polymerases

(Friedberg *et al.* 1995; Paabo *et al.* 2004). This modification is known as "Type II DNA Damage" and is seen as C to T or guanine (G)-adenine (A) transitions in DNA sequence alignments, depending on the DNA strand sequenced (Hansen *et al.* 2001; Hofreitier *et al.* 2001; Gilbert *et al.* 2003; Briggs *et al.* 2007; Brotherton *et al.* 2007). Until recently, scientists hypothesised there was also Type I DNA Damage MSL. This was seen to occur when a deamination reaction resulted in a transition from A to hypoxanthine (HX). Because HX is an analogue of G, it would be seen as A-G or T-C transitions in DNA sequence alignments (Hansen *et al.* 2001; Hofreitier *et al.* 2001; Gilbert *et al.* 2003). However, Briggs *et al.* (2007) and Brotherton *et al.* (2007) have argued that there is no authentic evidence for Type I DNA Damage MSL and such artefacts result from PCR's ability to generate non-endogenous sequence artefacts (Hansen *et al*. 2001; Stiller *et al.* 2006; Gilbert *et al.* 2007; Brotherton *et al.* 2007).

Another common form of aDNA damage is abasic sites. Abasic sites are generated when the glycosidic bonds between nucleotide bases and the sugar phosphate backbone of DNA are broken. This results in the release of free nucleotides and an abasic site (i.e. no nucleotide attached to the sugar phosphate backbone), which prevents most DNA polymerases from extending the PCR product (Lindhall and Nyberg 1972; Lindhall and Karlstro 1973; Schaaper *et al.* 1983, Paabo *et al.* 2004). Abasic sites will typically also undergo further chemical rearrangement, promoting strand breakage (Shapiro 1981; Friedberg *et al*. 1995; Paabo *et al.* 2004). The third common form of aDNA damage is cross-linking (Hofreiter *et al.* 2000). Crosslinking occurs when condensation reactions between sugars in the sugar phosphate backbone of DNA react with primary amine groups (from DNA or proteins), resulting in Maillard products that inhibit PCR amplification. Poinar *et al.* (1998) advocated the use of PTB to cleave Maillard products and release the DNA for PCR. Poinar *et al*. (1998) assumed Maillard products were present in coprolites because they had detected the sugar components (not Maillard products themselves) using gas chromatography mass spectrometry and aDNA could be amplified after the use of PTB. Subsequent studies have argued that PTB is not necessary to extract aDNA from samples where crosslinking may be an issue (Kemp *et al.* 2006; Rohland and Hofreiter 2007). Kemp *et al.* (2006) showed that the addition of PTB during aDNA extraction did not result in the amplification of aDNA from coprolites. However, repeat silica extractions of PTB treated aDNA extracts removed inhibitors allowing PCR amplification, questioning the use of PTB in aDNA extractions from coprolites. In a more robust study, Rohland and Hofreiter (2007) compared different aDNA extraction methods using quantitative PCR. This study showed that the addition of PTB did not increase

the yield of aDNA. Rohland and Hofreiter (2007) argued that Maillard products were either less common than previously thought or cross linking between sugars and the primary amine groups in DNA cannot be broken with the addition of PTB.

The third important point is that aDNA research is prone to contamination by modern DNA. This is due to the sensitivity and power of PCR, and the preference of DNA polymerases for modern DNA over fragmented and chemically modified aDNA. Two key controls to detect contamination include negative extraction (DNA extraction is conducted using the extraction regents only) and PCR (PCR is conducted using the PCR reagents only) controls. The reported claims of aDNA from fossils millions of years old have been shown to result from modern contamination. For example, Zischler *et al.* (1995) showed that the Woodward *et al.* (1994) 'dinosaur' DNA sequence was a human mtDNA nuclear copy (numt). Numts occur when portions of the mitochondrial genome are copied into the nuclear genome. Because the nuclear genome on average has a slower mutation rate than the mitochondrial genome, the evolutionary rate of numts will be slower than mtDNA. As a result numts will tend to fall basal to authentic mtDNA sequences in phylogenetic analyses. This explains how a human numt clustered with reptilian mtDNA sequences (Zichler *et al*. 1995) and was mistaken for dinosaur DNA by Woodward *et al.* (1994). Similarly, DNA sequences from insects preserved in amber were shown to be unreproducible (Austin *et al.* 1997, 1998) while Guitierrez *et al.* (1998) argued these sequences might be contamination. Contamination issues are still a major problem for current aDNA research despite rigorous criteria for authenticity being established as early as 1989 (Paabo 1989; see below). For example, Green *et al.* (2006) published one million base pairs of Neandertal nuclear DNA, 78% of which was subsequently suggested to be contamination from modern human DNA (Wall & Kim 2007) although this was later revised down to 11-40% (Green *et al.* 2009). Wall and Kim (2007) cleverly re-analysed the highly divergent results of Noonan *et al.* (2006) and Green *et al.* (2006), which were from the same Neandertal specimen. For each dataset, Wall and Kim (2007) estimated the modern human-Neandertal divergence time, modern European-Neandertal divergence time and the genetic contribution of Neandertals to modern Europeans. Wall and Kim (2007) showed that while there was no significant difference between the two datasets in the timing of the human-Neandertal divergence, there were considerable differences for European-Neandertal divergence times (35 Kya for Green *et al.* and 325 Kya for Noonan *et al*.). Also, the genetic contribution of Neandertals to modern Europeans differed (0% for Noonan *et al.* and 94% for Green *et al*.). Wall and Kim (2007) also showed that when the Green *et al.* (2006) dataset was divided into short (< 100 bp) and long (>100 bp) sequences, the short fragments had a similar

divergence time and genetic contribution to Noonan *et al.* (2006), while the long fragments had similar values to Green *et al.* (2006). This strongly suggests that the Green *et al.* (2006) dataset was contaminated with modern human DNA. This was later confirmed when the long fragments were compared to the HapMap modern human single nucleotide polymorphism (SNP) database, which showed that the long fragments had human specific SNPs (Wall and Kim 2007).

In light of the high level of contamination in aDNA studies during the 1990s, and the worrying trend of further contaminated studies like Green *et al.* (2006), criteria of authenticity are important if published results are to be seen as robust after peer review and subsequent scientific scrutiny. In response to this trend, Cooper and Poinar (2000) published a summary of criteria of authenticity (several other criteria have been published including: Paabo 1989; Lindahl 1993a, b; Handt *et al.* 1994; Hofreiter 2000; Hofreiter *et al.* 2001). Criteria of authenticity are methodological and analytical steps that should be taken to minimise the risk that published aDNA sequences are the result of contamination by exogenous DNA or numts. However, even if the criteria of authenticity list below are followed, sequences could still easily result from contamination. For example, replication in independent laboratories is a useless criterion if the sample has been contaminated before being handed to the geneticist. The criteria, summarised by Cooper and Poinar (2000; excluding biochemical preservation, quantitation and associated remains) are as follows:

(i) To minimise the risk, and increase the detection of modern DNA contamination in ancient samples, aDNA extraction and PCR set up must be conducted in a geographically isolated aDNA laboratory.

(ii) Multiple negative extraction and PCR controls must be included to detect contamination.

(iii) PCR success rates/copy number should be inversely related to product size. For example, there should be more small fragments and less large fragments in aDNA extracts. In addition, reproducible mtDNA sequences should be obtained if single copy nuclear DNA is amplified.

(iv) Results should be repeatable within labs and by independent labs from the same specimen. If sequences cannot be replicated, it suggests that the results might be the product of contaminating modern DNA.

(v) Sequences should make phylogenetic sense (e.g. moa DNA sequences should cluster with other moa). Sequences that do not make phylogenetic sense could be due to contamination by modern DNA, Type II DNA Damage MSL, or the amplification of numts, which has been documented in moa (Cooper *et al.* 2001) and many other species.

(vi) To detect DNA damage and to verify sequences, cloning or sequencing each DNA strand (5`-3` or 3`-5`) from independent PCR reactions should be carried out (Krings *et al.* 1997; Hofreiter *et al.* 2001; Krause *et al.* 2006).

4.3 Applications of aDNA.

Despite the large number of caveats with aDNA research, there is a huge range of scientific applications from the investigation of human evolution (e.g. Green *et al.* 2010) to the domestication of animals (e.g. Gongora *et al.* 2008a; see reviews Wayne *et al.* 1999; Hofreiter 2000; Hofreiter *et al.* 2001; Paabo *et al.* 2004; Willerslev and Cooper 2005). In this thesis, I will concentrate on the application of aDNA to palaeoecological reconstructions of diet and phenotype, and phylogeography and population demographics.

4.3.1 Diet reconstructions

The first palaeodietary study utilising aDNA and palaeobotanical techniques was published in 1998. Poinar *et al.* (1998) extracted aDNA from the coprolites of the Shasta ground sloth (*Nothrotheriops shastensis*) dated to 19 Kya, which had been excavated from a cave in Nevada and amplified, cloned and sequenced a small fragment of the chloroplast ribulose-biphosphate carboxylase (*rbcl*) gene. Comparing the sequences against *rbcl* sequences in Genbank that had been positively identified to species, Poinar *et al.* (1998) were able to show that the Shasta ground sloth diet consisted of six families and two orders of plants characteristic of floral zones currently 800 metres higher in altitude. In contrast, macroscopic examination of floral components produced only a partially overlapping dataset compared to the *rbcl* dataset. This is potentially due to the different coprolite sub-samples used for aDNA and plant macrofossil analysis, or more likely a bias in the preservation of dietary components. For example seeds have a higher probability of preservation than fruit or leaves (Wood 2007). However, there were several limitations in the Poinar *et al.* (1998) study. The analysis of diet was based on an extremely small sample size, with only one fecal bolus analysed. In addition, the *rbcl* fragment used to determine plant species could only identify chloroplast sequences to the family and order level. Subsequent re-analysis of the Poinar *et al.* (1998) dataset by Hofreiter *et al.* (2000) identified nine families and orders of plants by

comparing *rbcl* sequences against sequences from Genbank and reference herbarium specimens from Nevada. Similarly small sample size and the limitations of the *rbcl* locus to identify floral species below the family/order level have also been associated with the subsequent palaeodietary studies discussed below.

The Poinar *et al.* (1998) study was extended by Hofreiter *et al.* (2000) who analysed Shasta ground sloth coprolites from Nevada spanning 11-28 Kya. Hofreiter *et al.* (2000) showed that the Shasta ground sloth diet consisted of 13 orders and families of plants comprising trees, herbs and shrubs. Hofreiter *et al.* (2000) was the first study to examine temporal changes in diet and palaeovegetation and used the plant taxa present in the coprolites as an indicator of environmental conditions and showed that the climate in Nevada was drier 11 Kya, compared to 12-28 Kya.

Palaeodietary studies have also been conducted on hunter-gather populations of ancient humans. Poinar *et al*. (2001) analysed the diet of Native Americans in Texas from three coprolites dated to 2165 +/- 60 to 2370 +/- 60 years BP using molecular and macroscopic techniques. The analysis showed that the diet of Native Americans in the area consisted of pronghorn antelope, bighorn sheep, cottontail rabbit, rodents, fish and eight different orders and families of plants. Poinar *et al.* (2001) argued that this was a more diverse and nutritious diet than agricultural populations.

Studies of palaeodiet were subsequently conducted on coprolites from temperate geographical regions such as South America. Kuch *et al.* (2002) analysed the diet of rodent middens dated to 11.7 Kya from the Atacama Desert, while Hofreiter *et al.* (2003) analysed the diet of the Cuchillo Cura ground sloth showing that the diet consisted of herbs, grass and shrubs common in the area today. Hofreiter *et al.* (2001) hypothesised that changes in the diet of the Cuchillo Cura ground sloth over time could not account for their local extinction as the habitat was similar prehistorically and at present. While not studying palaeodiet per see, Willerslev *et al.* (2003) analyzed cave sediment from southern New Zealand dated to 3-0.6 Kya, which they suggested may have consisted of large volumes of disaggregated moa coprolites. Willerslev *et al.* (2003) identified two species of extinct moa and 29 plants to the order and family level in the sediments, but could not identify the potential DNA contribution from moa coprolites. A similar study by Haile *et al*. (2007) identified three species of moa, an anatid (duck) species and 14 families and orders of plants from multiple stratigraphic layers from two rockshelters in Hawke's Bay, North Island, New Zealand.

The latest study to investigate the palaeodiet of an extinct species used a multidisciplinary approach to analyse the dung found in the intestinal track of a mammoth preserved in the Siberian permafrost dated at 18.5 Kya (van Geel *et al.* 2008). Using microscopic, chemical and molecular techniques, van Geel *et al.* (2008) were able to reconstruct the diet, season of death and the palaeoenvironment. Ancient DNA, and plant macro (e.g. leaf fragments, twigs) and micro (e.g. pollen) remains indicated that the mammoth ate grasses and sedges as well as willow twigs, herbs and mosses, characteristic of the mammoth steppe flora, while chemical analysis also indicated that the mammoth ate dung to supplement its diet.

Finally, Miller *et al.* (1999, 2005) conducted an important palaeodietary study, utilising $\delta^{13}C$ isotope signatures, into the diet of the extant emu (*Dromaius* spp.) and the extinct *Genyornis newtonii*. *G. newtonii* is a dromornithid bird from the Late Pleistocene of Australia that has been hypothesised to be a browser of trees and shrubs based on skull and bill morphology (Murray and Megirian 1998). In addition, radiocarbon dating of preserved *G. newtonii* eggshell across three different climate regimes in Australia has shown that *G. newtonii* became extinct ca. 50 +/- 5 Kya (Miller *et al.* 1999). To determine the diet of emu and *G. newtonii*, and whether changes in habitat could explain the extinction of *G. newtonii*, Miller *et al*. (1999, 2005) reconstructed the diet from $\delta^{13}C$ isotope signatures from preserved eggshell spanning the past 140 Kyr. The analysis showed that prior to 50 Kya emu had a broad dietary range consisting of C_3 (trees and shrubs) and C_4 (grass) plants (C_3 and C_4 refer to the photosynthetic pathway used by plants). In contrast, after 45 Kya the diet of emu was restricted to C_3 plants. Compared to the broad dietary range of emu prior to 50 Kya, the diet of *G. newtonii* was much more restricted (only 40% of the variation in the $\delta^{13}C$ signatures of emu) suggesting a browsing diet. Miller *et al.* (1999, 2005) argued that aboriginal fire-stick farming resulted in a succession from a shrubland/grassland mosaic to an environment dominated by grassland. While the generalist emu could change dietary preferences, the specialized *G. newtonii* could not and became extinct.

4.3.2 Phenotype reconstructions

Ancient DNA has also been used to reconstruct the external appearance of extinct species. The first studies reconstructed the skin and hair colour of mammoths, Neandertals and horses. Rompler *et al.* (2006) sequenced the melanocortin-1 receptor (MC1R) gene from mammoth remains to show that mammoths had dark and light colour morphs, while Lalueza-Fox *et al.* (2007) suggested that Neandertals also had differing degrees of skin and hair pigmentation. Incorporating an additional five colour genes, Ludwig *et al.* (2009) examined coat colour

variation prior to and at the beginning of horse domestication by typing Late Pleistocene and Holocene horse remains from Sibera and Europe. They found no variation in Late Pleistocene horses, while variation increased rapidly from about 5 Kyr BP, after the earliest evidence of horse domestication 5.5 Kyr BP. Ludwig *et al.* (2009) argued that selective breeding accounted for coat colour variation and that it was likely that horse domestication predated the increase in coat colour variation.

The reconstruction of external appearance has taken a technological jump with the sequencing of 79% of the nuclear genome of a male palaeo-eskimo dated at 4 Kya from preserved hair found in Greenland (Rasmussen *et al.* 2010). Focusing on specific SNPs linked to phenotypic characteristics, Rasmussen *et al.* (2010) reconstructed the external phenotype of the male palaeo-eskimo and showed that he had brown eyes, light skin colour, thick dark hair and shovel-graded front teeth. These phenotypic characteristics are not surprising given the Asian ancestry of palaeo Eskimo populations. Previous studies conducting phenotypic reconstructions have inferred the external phenotype from DNA sequence variants rather than directly linking preserved hair or skin samples of known colour to an extinct species of unknown external appearance, despite the abundance of mammoth hair (Gilbert *et al.* 2007). The exception is Rasmussen *et al.* (2010) where the phenotype (thick dark brown hair) matched the inferred phenotype from genetic data.

4.3.3 Phylogeography and population demographics

Another area that aDNA research has focused on is the phylogeography of extinct and extant megafauna and the effects of the LGM and other periods of major climatic change on population demographics. Species that have been studied include brown bears (Barnes *et al.* 2002), cave bears (Stiller *et al.* 2010), bison (Shapiro *et al.* 2004; Drummond *et al.* 2005), mammoths (Barnes *et al.* 2007; Debruyne *et al.* 2008), hyenas (Rohland *et al*. 2005) and cave lions (Burger *et al.* 2004).

These studies have argued that climate change has had a dramatic effect on phylogeography. Barnes *et al.* (2002) showed that the strict reciprocally monophyletic phylogeographic pattern of modern brown bears in North America was only established during the Holocene. Instead, the Pleistocene was characterised by local extinctions and re-invasions, due to the presence/absence of the North American ice sheets and competition from other species (see below). In contrast Hofreiter *et al.* (2004) investigated the phylogeography of cave bears, brown bears, cave hyenas and Neandertals in Europe prior to the LGM. They found no

evidence for phylogeographic structuring prior to the LGM, in contrast to many extant European species which have marked phylogeographic structuring (reviewed by Hewitt 1996; Taberlet *et al.* 1998). Hofreiter *et al.* (2004) hypothesised that present day phylogeographic structure was due to the LGM and resulted from population bottlenecks associated with glacial refugia.

Ancient DNA studies have also shown that there have been major bottlenecks during the last glacial period prior to the decline or extinction of megafaunal populations. Shapiro *et al.* (2004) and Drummond *et al.* (2005) showed that Beringian Steppe Bison went through a major population bottleneck in the lead up the LGM. Declines in genetic diversity can also result from other factors like competition between species. Barnes *et al.* (2002) failed to find any brown bear (*Ursus arctos*) fossils in North America from 35-21 Kya and hypothesised that this local extinction, the resultant decline in genetic diversity and re-invasion 21 Kya was due to competition from the short faced bear (*Arctodus simus*), whose fossil remains are concentrated from 35-21 Kya. However, Matheus *et al*. (2004) has found a brown bear skull fragment dating to approximately 25-27 Kya at Edmonton, Alberta, Canada, on the southern side of the Laurentide and Cordilleran ice sheets. In contrast, Kuhn *et al.* (2010) examined the phylogeography of modern and Holocene caribou (*Rangifer tarandus caribou*) from the Southern Yukon, Canada and showed that there was a partial replacement event approximately 1 Kya. They hypothesised that this was due to volcanic eruptions in the area that deposited the White River Tephra and/or the Medieval Warm period, that both occurred about 1 Kya, although there is no evidence directly linking these events to the partial replacement of caribou in the area.

The majority of the phylogeographic studies utilizing aDNA have been conducted on Holarctic species, with very few studies on terrestrial Southern Hemisphere megafauna. Weinstock *et al.* (2009) used short mtDNA sequences and two specimens to suggest that haplotypes of two Late Pleistocene vicunas were not present in modern vicuna populations, arguing that there was a loss of genetic diversity co-incident with the vicuna population decline from the Pleistocene to Holocene. However, the small sample size of this study did not allow investigation of the role of climate change in the phylogeography and extinction of Southern Hemisphere megafauna and therefore it is not known whether climate change affected the phylogeography of Southern Hemisphere megafauna; and whether there were declines in genetic diversity with major climatic events, declining habitat or prior to extinction. I will use moa from the Late Pleistocene of New Zealand to examine these issues.

5. The Coalescent

5.1 Coalescent theory

The effective population size of taxa through time can be reconstructed using coalescent theory, which is an extension of classical population genetics theory developed by numerous authors in the 1980s (Kingman 1982; Hudson 1983a, b; Tajima 1983). Coalescent theory incorporates (i) the random coalescent history of lineages, (ii) mutations that create new lineages and (iii) recombination. This allows the coalescent process to construct and analyse samples of random genealogies (Rosenberg and Nordborg 2002) and is a powerful tool to estimate population demographic parameters such as mutation rates, time to most recent common ancestor and effective population size (Drummond *et al.* 2007).

The principle of coalescent theory is that if a single unstructured population is thought of as a group of randomly mating individuals, the result will be the random coalescence of lineages through time. Eventually all lineages will coalesce into a common ancestor. Using this principle, the main mathematical model of coalescent theory is that the average time to coalescence is a product of population size, making the coalescent rate dependant on the number of lineages and population size, with the more lineages, the faster the coalescence rate (Rosenberg and Nordborg 2002). However, there are several caveats with the use of coalescent theory, most importantly when the assumptions of neutral evolution, population continuity and an unstructured population are broken, namely by selection or phylogeographic structuring. Selection results in the biased reproduction of some lineages, so lineages do not randomly coalesce (Gillespie 2000; Kreitman 2000). Phylogeographic structuring can also mimic selection because lineages can only coalesce within a given phylogeographic unit and the apparent time to the most recent common ancestor of the whole population increases (Nordbord 2001).

5.2 Practical realisations

The practical applications of coalescent theory for aDNA research are through the software programmes BEAST (Drummond and Rambaut 2007) and BayeSSC (Excoffier *et al.* 2000; Anderson *et al.* 2006). BEAST utilizes the coalescent process and the Metropolis Hastings Markov Chain Monte Carlo (MCMC) algorithm to analyse a DNA sequence alignment and calculate population demographic parameters including mutation rate, effective population size, and time to most recent common ancestor (Drummond and Rambaut 2007; Drummond *et al.* 2007). BEAST also implements the Bayesian Skyline Plot (BSP; Drummond *et al.* 2005), which can be used to estimate a posterior distribution of effective population size

(Theta, θ) change at each coalescent interval from a sample of random genealogies without specifying a parametric population demographic model i.e. exponential or logistic growth (Drummond *et al.* 2005). The BSP has been used to investigate the demographic histories of extinct and extant megafauna including Beringian Steppe Bison (Drummond *et al.* 2005), woolly mammoths (Barnes *et al.* 2007; Debruyne *et al.* 2008); musk ox (Campos *et al.* 2010), southern elephant seals (Bruyn *et al.* 2008) and bowhead whales (Ho *et al.* 2008). These studies have compared the population size changes inferred from the BSP with known human arrival and major climatic events to elucidate the causes of the Late Pleistocene megafaunal extinctions and the dramatic population bottlenecks that megafauna experienced during the Late Pleistocene and Holocene. Utilising the BSP, Debruyne *et al.* (2008) showed that woolly mammoths had a constant population size through time, compared to Beringian Steppe Bison, which underwent a severe population decline leading up to the LGM (Drummond *et al.* 2005). The BSP has also been used to analyse serially sampled viral datasets including Hepatitis C (Drummond *et al.* 2005), HIV (Lemey *et al.* 2006) and Influenza A (Rambaut *et al.* 2008) to examine the population demographics of these pathogens. Drummond *et al.* (2005) showed that Hepatitis C underwent a rapid increase in effective population size between 1920 and 1950, probably due to viral contamination of injectable anti-schistosomiasis treatment.

Despite the utility of the BSP in reconstructing the demographic history of taxa, there are some problems associated with the BSP methodology and inferences of population demographic history from the BSP. The coalescent states that the amount of genetic variation is proportional to the population size, theta (Beebee and Rowe 2004). However, the common Type Two DNA Damage MSL in aDNA (seen as C to T and A to G transitions) can artificially increase the amount of genetic variation in a dataset. Axelsson *et al.* (2008) claimed that the BSP could not accurately estimate effective population size in aDNA datasets because it did not take into account Type Two DNA Damage MSL. This study showed that when all transitions were removed from the Shapiro *et al.* (2004) bison aDNA dataset, the signal for population expansion and decline reported by Drummond *et al.* (2005) disappeared and a constant population size was reconstructed. However, the study made no attempt to distinguish between Type Two DNA Damage MSL and real C-T and A-G transitions. Furthermore, Rambaut *et al.* (2008) re-analysed the bison dataset incorporating models that take into account Type Two DNA Damage MSL and accurately reconstructed the Drummond *et al.* (2005) bison BSP. However, DNA damage models are still somewhat over simplistic and need further improvement (Heled and Drummond 2008).

One of the major assumptions of the BSP is panmixia of individuals within a single population coalescent (Drummond *et al.* 2005) which restricts BSP analyses to a single unstructured population. When this assumption is met, theta approximates the effective population size. Violation of this assumption will always result in elevations of theta, which will not accurately approximate effective population size and therefore changes to the shape of the BSP. However, sampling biases inherent in some aDNA datasets can also violate this assumption. Sampling biases include the following categories:

(i) Temporal biases: Samples are not evenly distributed through time but are clustered in discrete time periods.

(ii) Geographic biases: Samples are not evenly distributed across the species range (in total or during a given time period).

(iii) Phylogenetic biases:
   a. Samples are derived from more than one phylogeographic unit or population.
   b. Different clades are sampled at different times. This is akin to replacement events that have been observed in brown bear populations (Barnes *et al*. 2002).
   c. Only one clade is sampled.
   d. Unrecognised phylogeographic structure is present in the dataset.

(iv) Biomolecular preservation biases: DNA preservation is not uniform across all samples, so some samples are represented by more or less sequence data than others or DNA is not present at all.

Only the effects of temporal and phylogenetic biases have been investigated, albeit in a limited fashion. These analyses suggest that the BSP is not affected by temporal sampling biases, at least in viral datasets (Rambaut *et al.* 2008), but is affected by phylogenetic biases, namely phylogeographic structuring. Different phylogeographic units or populations within a species can have different demographic signals (Shapiro *et al.* 2004; Grazziotin *et al.* 2006; Crandall *et al.* 2008; Debruyne *et al*. 2008; Campos *et al.* 2010). For example, Bruyn *et al.* (2009) analysed southern elephant seal populations in Antarctica (Victoria Land) and Macquarie Island ultilising the BSP, which was characterised by constant population size. However, when Bruyn *et al.* (2009) combined these two populations the BSP was characterised by an increase in population size from 15-1 Kya, followed by a marked decline 1-0 Kya. To date, the effect of sampling biases on the BSP has not been thoroughly investigated.

Contrasting the BSP, is another commonly used palaeodemographic tool, the programme BayeSSC (Excoffier *et al.* 2000; Anderson *et al.* 2006). BayeSSC uses the coalescent to simulate modern and ancient sequence data and subsequent population genetic statistics for user-specified population histories. The simulated and observed values are then statistically compared and hypotheses evaluated to determine the most probable population demographic history given the observed data. In addition, unlike the BSP, where the prior model of population size change is constant or linear, in BayeSSC the simulated and observed data is fitted to a prior model of demographic history.

BayeSSC has two major advantages over the BSP. The first is that sequences can be placed in time-bins defined by climatic periods or palaeontological/archaeological stratigraphic layers. Time-binning also removes the need for every sequence to be radiocarbon dated like BSP analyses saving considerable consumables costs. The latest version of the BSP allows age priors to be placed on undated sequences (Korsten *et al.* 2009). However, this can only be done for a small number of samples where strict upper and lower bounds on the age of the specimen are known i.e. layer-dated samples. The second advantage of BayeSSC over the BSP is that BayeSSC can analyse datasets that are phylogeographically structured. In BayeSSC multiple populations can be modeled, that can diverge and coalesce. In BSP analyses the assumption of a single unstructured population must be met otherwise theta will not approximate effective population size.

BayeSSC has been used to reconstruct the demographic history of a variety of extant and extinct taxa including hominids (*Homo* spp.), rodents and brown bears (Hadly *et al*. 2004; Belle *et al.* 2006; Chan *et al.* 2006; Belle *et al.* 2008; Valdiosera *et al.* 2008; Ramakrishnan and Hadly 2009). Belle *et al.* (2006) showed that ancient Etruscans are unlikely to be the ancestors of modern Tuscans, while Malmstrom *et al.* (2009) showed that Neolithic hunter gatherers from Scandinavia were more closely related to contemporary Baltic populations than modern Scandinavians. Both Belle *et al.* (2006) and Malmstrom *et al*. (2009) concluded that population replacements have occurred from prehistoric to modern times. However, both these studies suffered from small sample sizes (Rowley-Conway 2009), which may influence the interpretation of the results. Further research will determine whether the hypotheses of Belle *et al.* (2006) and Malmstrom *et al.* (2009) are valid.

6. Specific aims of thesis

6.1 Taphonomic deposition mechanisms of Holocene moa swamp deposits

The quality of the palaeontological information from the fossil record must be critically assessed before questions about the effects of major episodes of climate and habitat change on moa population demographics can be addressed. The aim of Chapter Two is to determine the deposition mechanism of Holocene miring bone deposits (i.e. moa swamp deposits) in New Zealand. Three swamp deposits will be excavated and the data used to reconstruct GIS based 3D representations of the taphonomy of these fossil deposits. This research will help determine the deposition mechanisms of each fossil locality including:

(i) The time span of deposition.
(ii) Whether moa have been trapped *in situ* or reworked into the fossil deposit
(iii) Whether moa skeletons are articulated or disassociated.
(iv) Whether the fossil deposit is stratified or time averaged.
(v) The geological and geographical setting of the fossil deposit.

As outlined in *Section 3.3.8*, there has been considerable debate into how miring bone deposits have formed with a range of catastrophic scenarios proposed (Booth 1875; Howorth 1887; Tregear 1893; Buick 1931). New GIS software applications provide the opportunity to reconstruct the taphonomy of fossil deposits, and critically assess each deposition scenario in light of the available data. In addition, this research will provide insight into how representative each fossil deposit is (the palaeofaunal composition of a deposit and/or how it changes over time within a deposit) with respect to the underlying moa population, which will in turn affect the interpretation of the population demographic history of moa inferred from genetic and palaeontological analyses. Specimens also need to be interpreted in the correct deposition context and timeframe. For example, each fossil deposit within a cave system must be treated as a discrete deposit with a different taphonomy. In Honeycomb Hill on the South Islands' northwest coast, 'The Cemetery' deposit contains the associated skeletons of moa that became trapped in the cave and can be individually radiocarbon dated. In contrast, 'The Graveyard' deposit contains disassociated moa bones that were washed into the cave, meaning each bone needs to be radiocarbon dated at considerable cost. However, unlike other fossil localities with disassociated remains, 'The Graveyard' has clear stratigraphic layers with tight upper and lower bounds (Worthy 1993b), so undated bones from each layer can be assigned an accurate estimate of the stratigraphic age of the layer.

6.2 Palaeofaunal reconstruction of the Glencrieff moa swamp deposit

An accurate reconstruction of the palaeofauna of Glencrieff is possible due to the reconstruction of the taphonomy of the Glencrieff moa swamp deposit in Chapter Two. The aim of Chapter Three is to use palaeontological techniques to reconstruct the palaeofauna of the Late Glacial Glencrieff moa swamp deposit, dating to 10-12 Kya (Worthy and Holdaway 1996). The palaeoecosytem of Late Glacial (10-14 Kya) North Canterbury was experiencing rapid climatic and vegetation change from the Otiran Glacial to the Holocene (Worthy and Holdaway 1995, 1996; Worthy and Roscoe 2003; Moar 2008). Precipitation had increased compared with the preceding Otiran Glacial (70-14 Kya). In addition, the New Zealand Glacial Reversal (11-13 Kya) resulted in significant temperature oscillations (Williams *et al.* 2005). The habitat was also changing from an open tussock grassland/shrubland mosaic to tall shrubland (Worthy and Holdaway 1995, 1996; Moar 2008). The Glencrieff palaeofaunal reconstruction will be compared and contrasted with other Otiran Glacial (70-14 Kya), Late Glacial (14-10 Kya) and Holocene (10-0 Kya) sites from North Canterbury and the South Island (Worthy and Holdaway 1994, 1995, 1996; Worthy 1997, 1998a, b) to determine:

(i) How characteristic the Glencrieff palaeofauna is of the Late Glacial given the rapid climatic and vegetation change during this time period.
(ii) How the North Canterbury palaeoecosytem changed through time in response to climate and habitat change.

The results will provide a dataset of moa relative fossil abundance and faunal composition that can be used to:

(i) Build models of moa population demography that can be simulated in BayeSSC and compared against the empirical genetic results to determine which model of population demography best fits the empirical data.
(ii) Compared to changes in moa population demography inferred from the BSP.

6.3 Reconstructing the diet of moa

The fossil record can also be used reconstruct the diet of moa and determine how moa functioned in the pre-human ecosystem of New Zealand. Recently discovered moa coprolite deposits (Wood *et al*. 2008) have provided a unique opportunity to address this issue because very little is known about the diet of moa. Therefore the aim of Chapter Four is to reconstruct the dietary preferences of moa using palaeobotantical data from moa coprolites. In addition,

an aDNA extraction protocol for coprolites will be developed to extract moa DNA and genetically identify each coprolite to the species level.

This research will increase our knowledge of moa diet in four main areas:

(i) How accurate are previous reconstructions of moa diet based on preserved gizzard contents? As outlined in *Section 3.3.6* reconstructions of moa diet from preserved gizzard contents are potentially biased towards lowland and wetland forest habitats or vegetation within reach of mired birds (Burrows *et al.* 1981; Wood 2007), and are also heavily biased towards South Island giant moa (Worthy and Holdaway 2002; Wood 2007). Analysis of coprolites from rockshelter deposits in sub-alpine and semi-arid areas of southern New Zealand will provide new dietary data from a diverse range of habitats, potentially leading to a less biased view of moa diet.

(ii) The evolutionary role of moa in the moa anti-browsing versus climate debate into the origins of characteristic plant growth strategies like divarication and heteroblasty. While finding the remains of plants with 'anti-browsing' growth strategies in moa coprolites does not mean that moa were the evolutionary cause of these growth characteristics, it would be consistent with the moa anti-browsing hypothesis.

(iii) The plasticity of moa diet. It is important to know whether moa were generalists or specialists, as this would affect how moa populations responded to episodes of major climatic and habitat change. Research on mammals, reptiles and birds has shown that specialists are at greater risk of extinction than generalists (McKinney 1997; Foufopoulos and Ives 1999; Owen and Bennett 2000; Harcourt *et al.* 2002). For example, as outlined in *Section 4.3.1*, the habitat specialist *G. newtonii* became extinct 50 +/- 5 Kya during a period of major habitat change hypothesised to have resulted from anthropogenic burning of the landscape. In contrast, the emu, which is a generalist, did not go extinct (Miller *et al.* 1999, 2005; Roberts *et al.* 2001).

(iv) The validity of the Pleistocene re-wilding hypothesis (Martin 2005; Donlan *et al.* 2005). This hypothesis advocates the use of species closely related to extinct megafauna to re-wild habitats in an effort to conserve modern ecosystems. It has already been suggested that deer (Batchelor 1989) and ostriches (Nicholls 2006) may be surrogates for moa based on feeding ecology, though this has been refuted by recent studies (Bond *et al.* 2004; Wood *et al.* 2008;

Forsyth *et al.* 2009; Lee *et al.* 2009). Determining the diet of moa and comparing this to the diet of introduced ungulates like deer and goat, will help determine whether exotic ungulates can be used as surrogates for the extinct moa and also provides further data to address the validity of Pleistocene re-wilding.

6.4 Reconstructing the plumage of moa

Ancient DNA and palaeontological techniques also provide an opportunity to investigate what information about moa plumage can be extracted from preserved moa feathers in the fossil record and to use this plumage information to examine potential moa behaviour. The aim of Chapter Five is to (i) determine whether aDNA can be extracted and amplified from sub-fossil feathers, and (ii) reconstruct the plumage of moa.

To address (i) whether aDNA can be extracted and amplified from sub-fossil feathers, I will use techniques pioneered for the extraction of aDNA from hair, an analogous structure to feathers (Gilbert *et al.* 2007). Ancient DNA analysis of feathers has been restricted to the analysis of historical museum specimens (Payne and Sorenson 2002; Sefc *et al.* 2003; Horvath *et al.* 2005). The survival of DNA in sub-fossil feathers has not been demonstrated, despite the abundance of moa feathers in museum collections. In addition, molecular methodologies have assumed DNA is present only in the base of the feather (or more correctly, the calamus). Because the calamus is frequently missing in sub-fossil feathers, due to breakage during or after deposition, it is important to determine if DNA can be isolated from the distal portions of feathers (that is, the rachis and barbs).

The reconstruction of moa plumage will involve computer software to (i) determine whether the colour of sub-fossil feathers has faded since deposition, which will affect the accuracy of plumage reconstructions and (ii) to reconstruct the plumage of moa. Comparison of the DNA based reconstructions against previously published hypotheses of moa plumage (Gill & Martinson 1991; Vickers-Rich *et al.* 1995; Flannery & Schouten 2001; Tennyson & Martinson 2006) will help determine the accuracy of these hypotheses. With the exceptions of upland moa, previous reconstructions have not been based on feathers positively identified to species.

6.5 Responses of moa to climate change in pre-human New Zealand

With knowledge about how moa functioned within the pre-human ecosystem of New Zealand and the biases associated in the interpretation of the New Zealand fossil record (Chapters Two

to Five), the aim of Chapters Six and Seven is to use the fossil record to investigate the effects of major episodes of climate and associated habitat change on the phylogeography and demographic history of two different moa species, the crested (*Pachyornis australis*) and heavy-footed moa (*Pachyornis elephantopus*). I will also investigate how climate change has affected the allometric variation in body size of heavy-footed moa.

Crested moa were restricted to northwest Nelson/West Coast on the South Island in sub-alpine grassland/shrubland (Worthy 1989). Originally described by Oliver (1955) and validated by Worthy (1989), the phylogenetic status of crested moa is somewhat still un-resolved as it is not known whether the phylogenetic position of the crested moa clade is within or outside the heavy-footed moa clade (Bunce *et al*. 2009). Conservatively I accept crested moa as a distinct species based on discrete morphological characteristics (Oliver 1955; Worthy 1989) in accordance with Bunce *et al.* (2009). The palaeontological data suggests that crested moa were more common during the Otiran Glacial (70-14 Kya) and Late Glacial (14-10 Kya) than the Holocene (10-0 Kya), with complete faunal replacement of crested and heavy-footed moa by little bush moa and South Island giant moa in lowland areas from the Pleistocene to the Holocene (Worthy 1993b, 1994, 1997, 1999; Worthy and Holdaway 1994; Worthy and Roscoe 2003). It is also not known when crested moa became extinct as no genetically verified Holocene remains have been radiocarbon dated and no archaeological remains of crested moa have been found (Worthy and Holdaway 2002; Tennyson and Martinson 2006).

In contrast, heavy-footed moa were restricted to the eastern South Island (Worthy and Holdaway 2002), with rare individuals in northwest Nelson/West Coast during the Late Glacial and Otiran Glacial (Worthy 1993b, 1994, 1997, 1999; Worthy and Holdaway 1994). Originally described by Owen (1856) as *Dinornis elephantopus*, recent aDNA analyses have suggested that heavy-footed moa were split into at least two phylogeographic units (Lambert *et al*. 2005; Baker *et al.* 2005; Bunce *et al*. 2009). However, it is not known whether this represents phylogeographic structuring or species diversity (Bunce *et al.* 2009). Conservatively, I accept heavy-footed moa as a single species with phylogeographic structuring in accordance with Bunce *et al.* (2009). The palaeontological data suggests that heavy-footed moa underwent a decline in relative fossil abundance from the Otiran Glacial to the Holocene in most areas of the South Island coincident with fragmentation of open tussock grassland/shrubland mosaics and the succession of grassland to shrubland to forest (Worthy

and Holdaway 1995, 1996; Worthy 1997, 1998a, b; Worthy and Grant Mackie 2003; Moar 2008).

There are three important reasons for investigating how climate and habitat change in pre-human New Zealand affected moa population demographics. First, the population demographic history of moa is not known. Second, palaeontological data argues for a large impact from climate change on the distribution and relative fossil abundance of moa species. For example it has been shown that in North Canterbury during the Otiran Glacial (70-14 Kya) and Late Glacial (14-10 Kya) heavy-footed, eastern and stout-legged moa dominated, while during the Holocene eastern and South Island giant moa dominated, with heavy-footed and stout-legged rare components of the palaeofauna (Worthy and Holdaway 1995, 1996). Finally, studies of Holarctic species suggest climate change caused large declines in the genetic diversity of megafauna through population bottlenecks during the Late Pleistocene (Barnes *et al.* 2002; Burger *et al.* 2004; Shapiro *et al.* 2004; Drummond *et al.* 2005; Rohland *et al.* 2005; Dalen *et al.* 2007; Hofreiter and Stewart 2008).

This research will help answer the following questions:

(i) Did climate change, in the absence of Polynesians, have a significant impact on the phylogeography and population demographics of moa?

(ii) Are changes in the phylogeography and population demographics of moa reflected in changes in the relative fossil abundance of moa through time, and vice versa (Chapter Three)? For example, replacement events in brown bears (Barnes *et al.* 2002) and population declines in the lead up to the LGM and Late Glacial in bison (Shapiro *et al.* 2004; Drummond *et al.* 2005) are not reflected in the fossil record of these species. In contrast bison numbers increased during the Late Glacial (Guthrie 2006).

(iii) Did climate change contribute to the ultimate extinction of moa or predispose them to over-hunting and habitat destruction by early Maori? A related question is whether moa populations were stable when Polynesians arrived in New Zealand in about 1280 AD (Wilmshurst *et al.* 2008). Gemmell *et al*. (2004) argued that moa were in decline before Polynesians arrived in New Zealand, however, as outlined in *Sections 2.3* and *3.3.7*, there are many assumptions and scientific criticisms of Gemmell *et al.* (2004) analysis.

(iv) Did moa respond to changes in habitat availability by changing their distributions accordingly? Eldredge (1989) proposed that taxa can 'recognise' familiar habitats and track them accordingly. The alternative hypothesis is that taxa are not able to track their habitat (or aren't able to track it fast enough) and either shift their niche or go locally extinct (Bennett *et al.* 1991). Whether moa are generalists or specialists (Chapter Four) will also impact the ability of moa to respond to changes in habitat availability.

6.6 Robustness of the BSP to biased sampling

The aim of Chapter Eight is to assess whether the palaeodemographic history of taxa inferred from the BSP is affected by sampling biases in the fossil record inherent in most aDNA datasets. To date, no in-depth investigation has been conducted into how temporal, geographic, phylogenetic and biomolecular preservation biases (as outlined in *Section 5.2*) affect the BSP.

Using three datasets: a simulated non-biased dataset, a Beringian Steppe Bison dataset with minimal known biases (Shapiro *et al.* 2004; Drummond *et al.* 2005) and the moa datasets generated in Chapters Six and Seven with numerous sampling biases, I aim to examine how these sampling biases affect the BSP by deliberately introducing biases into the datasets through the exclusion of specific sequences relating to time periods, clades, geography and sequence length. For example, to simulate a temporal bias, Late Glacial (10-14 Kya) specimens could be excluded from the analysis to examine the impact on the BSP. This research aims to answer the following questions:

(i) How does the BSP perform in non-ideal situations such as biased sampling?
(ii) How accurate are previous inferences of palaeodemographic history from the BSP and are the interpretations or conclusions drawn from BSP valid?
(iii) What are the limitations of the BSP? For example, are geographical, temporal, phylogenetic and biomolecular preservation biases likely to affect datasets with a strong demographic signal compared to datasets with a weak signal?
(iv) How should scientists construct time-structured aDNA datasets for BSP analysis so sampling biases are kept to a minimum and the assumptions of the BSP are not violated.

There are significant implications of this research for the reconstruction of the palaeodemographic history of moa and other species elsewhere in the world. Determining the effects of sampling biases on inferences of moa population demography will influence what

can be concluded from the BSP about the effects of climate and habitat change on moa population demographics.

7. Thesis structure and appendices

The thesis is presented as a series of seven papers that are published or prepared for journal submission. As such, they are presented in journal format, preceded by a title page and statement of authorship. The thesis concludes with a discussion drawing together and interpreting the outcomes and significance of the research. The appendices include three papers and an unpublished manual, based on research during my PhD tenure.

Appendix One: *BEAST v1.4 user manual*

Appendix One is an unpublished user manual for the software programme BEAST (Drummond and Rambaut 2007), available for download from http://beast.bio.ed.ac.uk. It was written as part of an Australian Research Council Environmental Futures Network funded visit to work with Dr Alexei Drummond at the University of Auckland. I brought together all the disparate information on BEAST and contributed to the writing of this manual.

Appendix Two: *Did Polynesians introduce chickens to South America?*

Appendix Two is a paper published in *Proceedings of the National Academy of Sciences USA* (Gongora *et al.* 2008a). It argues there is no evidence for the introduction of chickens to South America by Polynesians, contrary to a previous analysis of radiocarbon and aDNA data (Storey *et al.* 2008a). I performed some of the genetic data analysis and contributed to the writing of this paper. Subsequent to the publication of Gongora *et al.* (2008a), Storey *et al.* (2008b) published a reply stating that while there was no direct evidence for Polynesians introducing chickens to South America, this was still the most parsimonious hypothesis. Storey *et al.* (2008b) also reported pre-Columbian radiocarbon dates for an additional two chicken specimens. In reply to Storey *et al.* (2008b), we published a reply (Gongora *et al.* 2008b), arguing for more aDNA research and radiocarbon dating to examine the origins of chickens in South America. The rationale behind this argument was that the archaeological chicken from South America and several from Polynesia belonged to a clade that is found worldwide, providing no support for the South American sequence representing a genetic signal for Polynesian expansion. In contrast, unique sequences from Easter Island, a likely stepping-stone for colonisation of South America, belonged to an Indonesian clade that has not been found in South America. Additional radiocarbon dates from more specimens in

independent laboratories are also needed to ensure the South American dates are reliable (see Wilmshurst *et al.* 2008).

Appendix Three: *Evolutionary history of the extinct Falklands wolf*

Appendix Three is a paper published in *Current Biology* (Slater *et al*. 2009). It argues that the Falklands wolf (*Dusicyon australis*) is most closely related to the South American maned wolf (*Chrysocyon brachyurus*), and colonised the Falkland Islands about 330,000 years ago. I collected a specimen of the Falklands wolf from Otago Museum, Dunedin while on fieldwork in New Zealand and extracted DNA from this specimen.

8. References

Allan RS, Percival E, Duff RS, Falla RA 1941. Preliminary report on excavations at Pyramid Valley Swamp, Waikari, North Canterbury. Records of the Canterbury Museum 4: 325-353.

Allard MW, Yound D, Huynen Y 1995. 'Detecting dinosaur DNA'. Science 268: 1192.

Allentoft ME, Bunce M, Scofield RP, Hale ML, Holdaway RN 2010. Highly skewed sex ratios and biased fossil deposition of moa: ancient DNA provides new insights on New Zealand's extinct megafauna. Quaternary Science Reviews 29: 753-762.

Amundson R, Austin AT, Schuur EAG, Yoo K, Matzek V, Kendall C, Uebersax A, Brenner D, Baisden WT 2003. Global patterns of the isotopic composition of soil and plant nitrogen. Global Biogeochemical Cycles 17: 1031-1040

Anderson, A 1989a. Prodigious birds. Cambridge, UK: Cambridge University Press.

Anderson A 1989b. Mechanisms of overkill in the extinction of New Zealand moas. Journal of Archaeological Science 16: 137-151.

Anderson A 1991. The chronology of colonization in New Zealand. Antiquity 65: 767-795.

Anderson A 1996. Was *Rattus exulans* in New Zealand 2000 years ago? AMS radiocarbon ages from Shag River Mount. Archaeology of Oceania 31: 178-184.

Anderson A 2000a. Less is moa. Science 289: 1472-1474.

Anderson A 2000b. Differential reliability of 14C AMS ages of *Rattus exulans* bone gelatin in South Pacific prehistory. Journal of the Royal Society of New Zealand 30: 243-261.

Anderson A 2002. Faunal collapse, landscape change and settlement history of Remote Oceania. World Archaeology 33: 375-390.

Anderson AJ, Higham TFG 2004. The age of rat introduction in New Zealand. Further evidence from Earthquakes #1, North Otago. New Zealand Journal of Archaeology 24: 135-147.

Anderson CNK, Ramakrishnan U, Chan YL, Hadly EA 2006. Serial SimCoal: A population genetics model for data from multiple populations and points in time. Bioinformatics 21: 1733-1734

Anderson PM, Bartlein PJ, Brubaker LB, Gajewski K, Ritchie JC 1991. Vegetation-pollen-climate relationships for arcto-boreal region of North America and Greenland. Journal of Biogeography 18: 565-582.

Atkinson IAE, Greenwood RM 1980. Divaricating plants and moa browsing: A reply. New Zealand Journal of Ecology 3: 165-167.

Atkinson IAE, Greenwood RM 1989. Relationships between moas and plants. New Zealand Journal of Ecology 12 (supplement): 67–96.

Austin JJ, Ross AJ, Smith AB, Fortey RA, Thomas RH 1997. Problems of reproducibility – does geologically ancient DNA survive in amber preserved insects? Proceedings of the Royal Society of London Series B 264: 467-74

Austin JJ, Smith AB, Fortey RA, Thomas RH 1998. Ancient DNA from amber inclusions: A review of the evidence. Ancient Biomolecules 2: 167-176.

Axelsson E, Willerslev E, Gilbert MTP, Nielsen R 2008. The effect of ancient DNA damage of inferences of demographic histories. Molecular Biology and Evolution 2: 2181-2187.

Baker AJ 2007. Molecular advances in the study of geographic variation and speciation in birds. Ornithological Monographs 63:18–29.

Baker AJ, Huynen LJ, Haddrath O, Miller CD, Lambert DM 2005. Reconstructing the tempo and mode of evolution in an extinct clade of birds with ancient DNA: The giant moas of New Zealand. Proceedings of the National Academy of Sciences USA 102: 8257–8262.

Barker FK, Barrowclough GF, Groth JG 2002. A phylogenetic hypothesis for passerine birds: taxonomic and biogeographic implications of an analysis of nuclear DNA sequence data. Proceedings of the Royal Society of London Series B 269: 295-308.

Barnes I, Matheus P, Shapiro B, Jensen D, Cooper A 2002. Dynamics of Pleistocene population extinctions in Beringian brown bears. Science 295: 2267-2270.

Barnes IB, Shapiro B, Lister A, Kuznetsova T, Sher A, Guthrie D, Thomas MG 2007. Genetic structure and extinction of the woolly mammoth, *Mammuthus primigenius*. Current Biology 17: 1072-1075.

Barnosky AD, Koch PL, Feranec RS, Wing SL, Shabel AB 2004. Assessing the causes of Late Pleistocene extinctions on the continents. Science 306: 70-75.

Batcheler CL 1989. Moa browsing and vegetation formations, with particular reference to deciduous and poisonous plants. New Zealand Journal of Ecology 12 (supplement): 57–65.

Beavan-Athfield N 2006. Comment on "Diet derived variations in radiocarbon and stable isotopes: A case study from Shag River mount, New Zealand. Radiocarbon 48: 117-121.

Beavan NR, Sparks RJ 1998. Factors influencing 14C ages of the Pacific Rat *Rattus exulans*. Radiocarbon 40: 601-603.

Beebee T, Rowe G 2004. An introduction to molecular ecology. Oxford, UK. Oxford University Press.

Belle EMS, Benazzo A, Ghirotto S, Colonna V, Barbujani G 2009. Comparing models of the genealogical relationships among Neandertal, Cro-Magnoid and modern Europeans by serial coalescent simulations. Heredity 102: 218-225.

Belle EMS, Ramakrishnan U, Mountain SL, Barbujani 2006. Serial coalescent simulations suggest a weak genealogical relationship between Etruscans and modern Tuscans. Proceedings of the National Academy of Sciences USA 103: 8012-8017.

Bennett KD, Tzedakis PC, Willis KJ 1991. Quaternary refugia of north European trees. Journal of Biogeography 18: 103-115.

Bergman C 1847. Uber die verhaltnisse der warmeokonomie der thiere su ihrer grosse. Gottinger Studien Pt. 1: 595-708.

Biffen E, Hill RS, Lowe AJ 2010. Did kauri (*Agathis*: Araucariaceae) really survive the Oligocene drowning of New Zealand? Systematic Biology DOI:10.1093/sysbio/syq030.

Bigelow NH and Powers WR 2001. Climate, vegetation, and archaeology 14,000-9,000 cal yr B.P. in Central Alaska. Arctic Anthropology 38: 171-195.

Boon WM, Kearvell JC, Daugherty CH, Chambers GK 2001. Molecular systematics and conservation of kakariki (*Cyanoramphus* species). Department of Conservation New Zealand. Science for Conservation 176.

Booth BS 1875. Description of the Moa Swamp at Hamilton. Transactions and Proceedings of the Royal Society of New Zealand 7: 123-138.

Bond WJ, Lee WG, Craine JM 2004. Plant structural defenses against browsing birds: A legacy of New Zealand's extinct moas. Oikos 104: 500–508.

Bond WJ, Silander WJ 2007. Springs and wire plants: Anachronistic defenses against Madagascar's extinct elephant birds. Proceedings of the Royal Society of London Series B 274: 1985–1992.

Bourdon E, de Ricqles A, Cubo J 2009. A new Transantarctic relationship: morphological evidence for a Rheidae-Dromaiidae-Casuariidae clade (Aves, Palaeognathae, Ratitae). Zoological Journal of the Linnaean Society 156: 641-663.

Breitwieser I, Glenny DS, Thorne A, Wagstaff SJ 1999. Phylogenetic relationships in Australasian Gnaphalieae (Compositae) inferred from ITS sequences. New Zealand Journal of Botany 37: 399-412.

Briggs AW, Stenzel U, Johnson PLF, Green RE, Kelso J, Prufer K, Meyer M, Krause J, Ronan MT, Lachmann M, Paabo S 2007. Patterns of damage in genomic DNA sequences from a Neandertal. Proceedings of the National Academy of Sciences USA 104: 14616-14621.

Bronk Ramsey C, Higham TFG, Bowles A, Hedges R 2004a. Improvement to the pretreatment of bone at Oxford. Radiocarbon 46: 155-163.

Bronk Ramsey C, Higham TFG, Leach P 2004b. Towards high precision AMS: process and limitations. Radiocarbon 46: 7-24.

Brook BW, Burney DA, Flannery TF, Gagan MK, Gillespie R, Johnson CN, Kershaw P, Magee JW, Martin PS, Miller GH, Peiser B, Roberts RG 2007. Would the Australian megafauna have become extinct if humans had never colonized the continent? Comments on "A review of the evidence for a human role in the extinction of Australian megafauna and an alternative explanation" by S. Wroe and J. Field. Quaternary Science Reviews 26: 560-564.

Brook FJ 2000. Prehistoric predation of the land snail *Placostylus ambagiosus* Suter (Stylommatophora: Bulimulidae), and evidence for the timing of establishment of rats in northernmost New Zealand. Journal of the Royal Society of New Zealand 30: 227-241.

Brotherton P, Endicott P, Sanchez JJ, Beaumont M, Barnett R, Austin J, Cooper A 2007. Novel high-resolution characterization of ancient DNA reveals C > U-type base modification events as the sole cause of post mortem miscoding lesions. Nucleic Acids Research 35: 5717-5728.

Brown TA, Allaby RG, Brown KA, O'Donoghue K, Sallares R 1994. DNA in wheat seeds from European archaeological sites. Cellular and Molecular Life Sciences 50: 571-5.

Bruyn M, Hall BL, Chauke LF, Baroni C, Koch PL, Hoelzel AR 2009. Rapid response of a marine mammal species to Holocene climate and habitat change. PLoS Genetics 5: e1000554.

Buchanan B, Collard M, Edinborough K 2008. Paleoindian demography and the extraterrestrial impact hypothesis. Proceedings of the National Academy of Sciences USA 105: 11651-11654.

Buick TL 1931. The mystery of the moa. New Plymouth, New Zealand: Thomas Avery & Sons Limited.

Bunce M, Szulkin M, Lerner HRL, Barnes I, Shapiro B, Cooper A, Holdaway RN 2005. Ancient DNA provides new insights into the evolutionary history of New Zealand's extinct giant eagle. PLoS Biology 3: e9.

Bunce M, Worthy TH, Ford T, Hoppitt W, Willerslev E, Drummond A, Cooper A 2003. Extreme reversed sexual size dimorphism in the extinct New Zealand moa *Dinornis*. Nature 425: 172–175.

Bunce M, Worthy TH, Phillips MJ, Holdaway RN, Willerslev E, Haile J, Shapiro B, Scofield RP, Drummond A, Kamp PJJ, Cooper A 2009. The evolutionary history of the extinct ratite moa and New Zealand Neogene paleogeography. Proceedings of the National Academy of Science USA 106: 20646-20651.

Burge PI, Schulmeister J 2007. An MIS 5a to MIS 4 (or early MIS 3) environmental and climatic reconstruction from the northwest South Island, New Zealand, using beetle fossils. Journal of Quaternary Science 22: 501-516.

Burger J, Rosendahl W, Loreille OP, Hemmer H, Eriksson T, Gotherstrom A, Hiller J, Collins MJ, Wess T, Alt KW 2004. Molecular phylogeny of the extinct cave lion *Panthera leo spelaea*. Molecular Phylogenetics and Evolution 30: 841-849.

Burney DA 1993. Holocene environmental changes in arid southwestern Madagascar. Quaternary Research 40: 98-106.

Burney DA, Robinson GS, Pigott Burney L 2003. *Sporormiella* and the late Holocene extinctions in Madagascar. Proceedings of the National Academy of Science USA 100: 10800-10805.

Burney DA, Burney PJ, Godfrey LR, Jungers WL, Goodman SM, Wright HT, Jull AJT 2004. A chronology for late prehistoric Madagascar. Journal of Human Evolution 47: 25-63.

Burns KC, Dawson JW 2006. A morphological comparison of leaf heteroblasty between New Caledonia and New Zealand. New Zealand Journal of Botany 44: 387–396.

Burrows CJ, Greenland DE 1979. An analysis of the evidence for climatic change in the last thousand years: Evidence from diverse natural phenomena and from instrumental records. Journal of the Royal Society of New Zealand 9: 321-373.

Burrows CJ, McCulloch B, Trotter MM 1981. The diet of moas based on gizzard contents samples from Pyramid Valley, North Canterbury, and Scaife's Lagoon, Lake Wanaka, Otago. Records of the Canterbury Museum 9: 309–336.

Cano RJ, Poinar H, Poinar G 1992a. Isolation and partial characterisation of DNA from the bee *Proplebeia dominicana* (Apidae: Hymenoptera) in 25-40 million year old amber. Medical Science Research 20: 249-51.

Cano RJ, Poinar HN, Roubik DW, Poinar GO 1992b. Enzymatic amplification and nucleotide sequencing of portions of the 18s rRNA gene of the bee *Proplebeia dominicana* (Apidae: Hymenoptera) isolated from 25-40 million year old Dominican amber. Medical Science Research 20: 619-22.

Cano RJ, Poinar HN, Pieniazek NJ, Acra A, Poinar Jr GO 1993. Amplification and sequencing of NDA from a 120-135 million year old weevil. Nature 363: 536-8.

Campbell HJ, Adams CJ, Mortimer N 2008. Exploring the Australian geological heritage of Zealandia and New Zealand. Proceedings of the Royal Society of Victoria 120: 38-50.

Campbell H, Hutching G 2007. In search of ancient New Zealand. New Zealand: Penguin Group.

Campbell H, Landis C 2001. New Zealand awash. New Zealand Geographic 51: 6-7.

Campbell JD, Lee DE, Lee WG 2000. A woody shrub from the Miocene Nevis Oil Shale, Otago, New Zealand – a possible fossil divaricate? Journal of the Royal Society of New Zealand 30: 147-153.

Chambers GK, Boon WM, Buckley TR, Hitchmough RA 2001. Using molecular methods to understand the Gondwanan affinities of the New Zealand biota: Three case studies. Australian Journal of Botany 49: 377-387.

Chan YL, Anderson CNK, Hadly EA 2006. Bayesian estimation of the timing and severity of a population bottleneck from ancient DNA. PLoS Genetics 2: e59.

Chapple DG, Ritchie PA, Daugherty CH 2009. Origin, diversification, and systematics of the New Zealand skink fauna (Reptilia: Scincidae). Molecular Phylogenetics and Evolution 52: 470-487.

Checklist Committee OSNZ 2010. Checklist of the birds of New Zealand, Norfolk and Macquarie Islands, and the Ross Dependency, Antarctica (4th ed.) Wellington, New Zealand: Ornithological Society of New Zealand and Te Papa Press.

Cockayne L 1912. Observation concerning evolution derived from ecologic studies in New Zealand. Transactions and Proceedings of the New Zealand Institute 44: 1-50.

Cooper A 1997. Ancient DNA and avian systematics: From Jurassic Park to modern island extinctions. In: Mindell D (Ed.) Avian Molecular Evolution and Molecular Systematics, pp. 345-373. New York, USA: Academic Press.

Cooper A, Cooper RA 1995. The Oligocene bottleneck and New Zealand biota: genetic record of a past environmental crisis. Proceedings of the Royal Society of London Series B 261: 293-302.

Cooper A, Lalueza-Fox C, Anderson A, Rambaut A, Austin J, Ward R 2001. Complete mitochondrial genome sequences of two extinct moas clarify ratite evolution. Nature 409: 704–707.

Cooper A, Mourer-Chauvire C, Chambers GK, von Haeseler A, Wilson AC, Paabo S 1992. Independent origins of New Zealand moas and kiwis. Proceedings of the National Academy of Science USA 89: 8741–8744.

Cooper A, Poinar HN 2000. Ancient DNA: Do it right or not at all. Science 289: 1139.

Cooper RA, Millener PR 1993. The New Zealand biota: historical background and new research. Trends in Ecology and Evolution 8: 429-433.

Cracraft J 1974. Phylogeny and evolution of the ratite birds. Ibis 116: 494-521.

Crandall ED, Frey MA, Grosberg RK, Barber PH 2008. Contrasting demographic histories and phylogeographical patterns in two Indo-Pacific gastropods. Molecular Ecology 17: 611-626.

Dalen L, Fuglei E, Hersteinsson P, Kapel CMO, Roth JD, Samelius G, Tannerfeldt M, Angerbjorn 2005. Population history and genetic structure of a circumpolar species: The Arctic fox. Biological Journal of the Linnaean Society 84: 79-89.

Dalen L, Nystrom V, Valdiosera C, Germonpre M, Sablin M, Turner E, Angerbjorn A, Arsuaga SL, Gotherstrom A 2007. Ancient DNA reveals lack of postglacial habitat tracking in the Arctic fox. Proceedings of the National Academy of Science USA 104: 6726-6729.

Daszak P, Cunningham AA, Hyatt AD 2000. Emerging infectious diseases of wildlife – threats to biodiversity and human health. Science 287: 443-449.

Davidson J 1984. The prehistory of New Zealand. Auckland, New Zealand: Longman Paul.

Day JS 1998. Light conditions and the evolution of heteroblasty (and the divaricate form) in New Zealand. New Zealand Journal of Ecology 22: 43–54.

Debruyne R, Chu G, King CE, Bos K, Kuch M, Schwarz C, Szpak P, Grocke DR, Matheus P, Zazula G, Guthrie D, Froese D, Buigues B, Marliave C, Flemming C, Poinar D, Fisher D, Southon J, Tikhonov AN, MacPhee RDE, Poinar HN 2008. Out of America: Ancient DNA evidence for a New World origin of Late Quaternary woolly mammoths. Current Biology 18: 1320-1326.

DeSalle R, Gatesy J, Wheller W, Grimaldi 1992. DNA sequences from a fossil termite in Oligo-Miocene amber and their phylogenetic implications. Science 257: 1933-6.

Donlan J, Greene HW, Berger J, Bock CE, Bock JH, Burney DA, Estes JA, Foreman D, Martin DS, Roemer GW, Smith FA, Soule ME 2005. Rewilding North America. Nature 436: 913–914.

Driskell A, Christidis L, Gill BJ, Boles WE, Barker FK, Longmore NW 2007. A new endemic family of New Zealand passerine birds: adding heat to a biodiversity hotspot. Australian Journal of Zoology 55: 73-78.

Drummond AJ, Ho SYW, Phillips MJ, Rambaut A 2006. Relaxed phylogenetics and dating with confidence. PLoS Biology 4: e88.

Drummond AJ, Ho SYW, Rawlence N, Rambaut A 2007. A Rough Guide to BEAST 1.4. Unpublished user manual. http://beast.bio.ed.ac.uk.

Drummond AJ, Rambaut A 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7: 214.

Drummond AJ, Rambaut A, Shapiro B, Pybus OG 2005. Bayesian coalescent inference of past population dynamics from molecular sequences. Molecular Biology and Evolution 22: 1185-1192.

Eldredge N 1989. Macroevolutionary dynamics: species, niches and adaptive peaks. MacGraw Hill, New York, USA.

Elzanowski A 1995. Cretaceous birds and avian phylogeny. Cour Forsch Inst Senck 181: 37-53.

Ericson PGP, Christidis L, Cooper A, Irestedt M, Jackson J, Johansson US, Norman JA 2002. A Gondwanan origin of passerine birds supported by DNA sequences of the endemic New Zealand wrens. Proceedings of the Royal Society of London Series B 269: 235-241.

Excoffier L, Novembre J, Schneider S 2000. SIMCOAL: a general coalescent program for simulation of molecular data in interconnected populations with arbitrary demography. Journal of Heredity 91: 506-509.

Fadzly N, Jack C, Schaefer HM, Burns KC 2009. Ontogenetic colour changes in an insular tree species: signalling to extinct browsing birds? New Phytologist 184: 495-501.

Fiedel SJ 2009. What caused the megafaunal extinctions? The case against bolide impact. Journal of Cosmology 2: 286-288.

Fiedel S, Haynes G 2004. A premature burial: comments on Grayson and Meltzer's "Requiem for overkill". Journal of Archaeological Science 31: 131-131.

Firestone R 2009. The case for the Younger Dryas extraterrestrial impact event: mammoth, megafauna and Clovis extinction. Journal of Cosmology 2: 256-285.

Firestone RB, West A, Kennett JP, Becker L, Bunch TE, Revay ZS, Schultz PH, Belgya T, Kennett DJ, Erlandson JM, Dickenson OJ, Goodyear AC, Harris RS, Howard GA, Kloosterman JB, Lechler P, Mayewski PA, Montgomery J, Poreda R, Darrah T, Que Hee SS, Smith AR, Stich A, Topping W, Wittke JH, Wolback WS 2007. Evidence for an extraterrestrial impact 12,900 years ago that contributed to the megafaunal extinctions and the Younger Dryas cooling. Proceedings of the National Academy of Science USA 104: 16016-16021.

Flannery T 1994. The Future Eaters. Australia: Reed Publishing.

Flannery T, Schouten P 2001. A gap in nature: Discovering the world's extinct animals. Australia: The Text Publishing Company.

Flemming CA 1979. The geological history of New Zealand and its life. Auckland, New Zealand: Auckland University Press and Oxford University Press.

Forsyth DM, Wilmshurst SM, Allen RB, Coomer DA 2010. Impacts of introduced deer and extinct moa on New Zealand ecosystems. New Zealand Journal of Ecology 34: 48-65.

Foufopoulos J, Ives AR 1999. Reptile extinctions on land-bridge islands: Life-history attributes and vulnerability to extinction. American Naturalist 153: 1-25.

Friedberg EC, Walker GC, Siede W 1995. DNA repair and mutagenesis. Washington DC, USA: ASM Press.

van Geel B, Aptroot A, Baittinger C, Birks HH, Bull ID, Cross HB, Evershed RP, Gravendeel B, Kompanje EJO, Kuperus P, Mol D, Nierop KGJ, Pals JP, Tikhonov AN, Reenen G, Tienderen PH 2008. The ecological implications of a Yukutian mammoth's last meal. Quaternary Research 69: 361-376.

van Geel B, Zazula GD, Schwegerc CE 2007. Spores of coprophilous fungi from under the Dawson tephra (25,300 14C years BP), Yukon Territory, northwestern Canada. Palaeogeography, Palaeoclimatology, Palaeoecology 252: 481-485.

Gemmell NJ, Schwartz MK, Robertson BC. 2004. Moa were many. Proceedings of the Royal Society of London Series B 271 (Supplement 6): S430-432.

Gibbs G 2006. Ghosts of Gondwana. Nelson, New Zealand: Craig Potton Publishing.

Gilbert MTP, Hansen AJ, Willerslev E, Rudbeck L, Barnes I, Lynnerup N, Cooper A 2003. Characterisation of genetic miscoding lesions caused by postmortem damage. American Journal of Human Genetics 72: 48-61.

Gilbert MTP, Cuccui J, White W, Lynnerup N, Titball RW, Cooper A, Prentice MB 2004. Absence of *Yersinia pestis*-specific DNA in human teeth from five European excavations of putative plague victims. Microbiology 150: 341-354.

Gilbert MTP, Tomsho LP, Rendulic S, Packard M, Drautz DI, Sher A, Tikhonov A, Dalén L, Kuznetsova T, Kosintsev P, Campos PF, Higham T, Collins MJ, Wilson AS, Shidlovskiy F, Buigues B, Ericson PGP, Germonpré M, Götherström A, Iacumin P, Nikolaev V, Nowak-Kemp M, Willerslev E, Knight JR, Irzyk GP, Perbost CS, Fredrikson KM, Harkins TT, Sheridan S, Miller W, Schuster SC 2007. Whole-genome shotgun sequencing of mitochondria from ancient hair shafts. Science 317: 1927–1930.

Gill B, Martinson P 1991. New Zealand's extinct birds. New Zealand: Random Century.

Gillespie JH 2000. Genetic drift in an infinite population: the pseudo hitchhiking model. Genetics 155: 909-919.

Golenberg EM 1991. Amplification and analysis of Miocene plant fossil DNA, Philosophical Transactions of the Royal Society of London Series B 333: 419-27.

Golenberg EM, Giannassi DE, Clegg MT, Smiley CJ, Durbin M, Henderson D, Zurawski G 1990. Chloroplast DNA sequence from a Miocene Magnolia species. Nature 344: 656-8

Gongora J, Rawlence NJ, Mobegi VA, Jianlin H, Alcalde JA, Matus JT, Hanotte O, Moran C, Austin JJ, Ulm S, Larson G, Cooper A 2008a. Indo-European and Asian origins for modern Chilean and Pacific archaeological chickens revealed by mtDNA. Proceedings of the National Academy of Sciences USA, 105 (30), 10308-10313

Gongora J, Rawlence NJ, Mobegi VA, Jianlin H, Alcalde JA, Matus JT, Hanotte O, Moran C, Austin JJ, Ulm S, Larson G, Cooper A 2008b. Reply to Storey et al.: More DNA and dating studies needed for ancient El-Arenal-1 chickens. Proceedings of the National Academy of Science 105: E48

Grazziotin FG, Monzel M, Echeverrigaray S, Bonatto SL 2006. Phylogeography of the *Bothrops jararaca* complex (Serpentes: Viperidae): past fragmentation and island colonisation in the Brazilian Atlantic Forest. Molecular Ecology 15: 3969-3982.

Green RC 1975. In: Kuschel G (Ed) Biogeography and ecology of New Zealand. Junk, The Hague, The Netherlands, pp 591-641.

Green RE, Briggs AW, Krause J, Prufer K, Burbano HA, Siebauer M, Lachmann M, Paabo S 2009. The Neandertal genome and ancient DNA authenticity. EMBO Journal 28: 2494-2502.

Green RE, Krause J, Briggs AW, Maricic T, Stenzel U, Kircher M, Patterson K, Li H, Zhai W, Fritz MHY, Hansen NF, Durand EY, Malaspinas AS, Jensen JD, Marques-Bonet T, Alkan C, Prüfer K, Meyer M, Burbano HA, Good JM, Schultz R, Aximu-Petri A, Butthof A, Höber B, Höffner B, Siegemund M, Weihmann A, Nusbaum C, Lander ES, Russ C, Novod N, Affourtit J, Egholm M, Verna C, Rudan P, Brajkovic D, Kucan E, Guic I, Doronichev VB, Golovanova LV, Lalueza-Fox C, Rasilla M, Fortea J, Rosas A, Schmitz RW, Johnson PLF, Eichler EE, Falush D, Birney E, Mullikin JC, Slatkin M, Nielsen R, Kelso J, Lachmann M, Reich D, Pääbo P 2010. A draft sequence of the Neandertal genome. Nature 328: 710-722.

Green RE, Krause J, Ptak SE, Briggs AW, Ronan MT, Simons JF, Du L, Egholm H, Rothberg JM, Paunovic M, Paabo S 2006. Analysis of one million base pairs of Neanderthal DNA. Nature 444: 330-336.

Greenwood RM, Atkinson IAE 1977. Evolution of divaricating plants in New Zealand in relation to moa browsing. New Zealand Ecological Society Proceedings 24: 21-23.

Guitierrez G, Marin A 1998. The most ancient DNA recovered from an amber-preserved specimen may not be as ancient as it seems. Molecular Biology and Evolution 15: 926-929.

Haak W, Brandt G, Jong HN, Meyer C, Ganslmeier R, Heyd V, Hawkesworth C, Pike AWG, Meller H, Alt KW 2008. Ancient DNA, Strontium isotopes, and osteological analysis shed light on social and kinship organization of the Later Stone Age. Proceedings of the National Academy of Science USA 105: 18226-18231.

Haast J 1872. Moas and moa hunters. Address to the Philosophical Institute of Canterbury. Transactions and Proceedings of the New Zealand Institute 4: 66-90.

Hackett SJ, Kimball RT, Reddy S, Bowie RCK, Braun EL, Braun MJ, Chojnowksi JL, Cox WA, Han KL, Harshman J, Huddleston CJ, Marks BD, Miglia KJ, Moore WS, Sheldon FH, Steadman DW, Witt CC, Yuri T 2008. A phylogenomic study of birds reveals their evolutionary history. Science 320: 1763-1768.

Haddrath O, Baker AJ 2001. Complete mitochondrial DNA genome sequences of extinct birds: Ratite phylogenetics and the vicariance biogeography hypothesis. Proceedings of the Royal Society of London Series B 268:939–945.

Hadly EA, Ramakrishnan U, Chan YL, Tuinen M, O'Keefe K, Spaeth PA, Conroy CJ 2004. Genetic response to climatic change: insights from ancient DNA and phylochronology. PLoS Biology 2: e290.

Hagelberg E, Clegg JB 1992. Isolation and characterisation of DNA from archaeological bone. Proceedings of the Royal Society of London Series B 244: 45-50.

Haile J, Holdaway R, Oliver K, Bunce M, Gilbert MTP, Nielsen R, Munch K, Ho SYW, Shapiro B, Willerslev E 2007. Ancient DNA chronology within sediment deposits: Are palaeobiological reconstructions possible and is DNA leaching a factor? Molecular Biology and Evolution 24: 982–989.

Hamilton A 1892. Notes on moa gizzard stones. Transactions and Proceedings of the New Zealand Institute 24: 172-175.

Hamilton A 1894. On the feathers of a small species of Moa (*Megalapteryx*) found in a cave at the head of the Waikaia River, with a notice of a moa-hunters camping place on the Old Man Range. Transactions and Proceedings of the New Zealand Institute 27: 232–238.

Hand SJ, Murray P, Megirian D, Archer M, Godthelp H 1998. Mystacinid bats (Microchiroptera) from the Australian Tertiary. Journal of Paleontology 72: 538-545.

Handt O, Krings M, Ward RH, Paabo S 1994. The retrieval of ancient human DNA sequences. American Journal of Human Genetics 59: 376-386.

Hansen DM, Galetti M 2009. The forgotten megafauna. Science 324: 42-43.

Hansen J 2001. Macroscopic plant remains from Mediterranean Caves and Rockshelters: Avenues of interpretation. Geoarchaeology 16: 401–432.

Harcourt AH, Copeton SA, Parks SA 2002. Rarity, specialisation and extinction risk in primates. Journal of Biogeography 29: 445-456.

Harshman J, Braun EL, Braun MJ, Huddleston CJ, Bowie RCK, Chojnowski JL, Hackett SJ, Han KL, Kimball RT, Marks BD, Miglia KJ, Moore WS, Reddy S, Sheldon FH, Steadman DW, Steppan SJ, Witt CC, Yuri T 2008. Phylogenomic evidence for multiple losses of flight in ratite birds. Proceedings of the National Academy of Science USA 105:13462–13467.

Haynes G (Ed) 2000. American megafaunal extinctions at the end of the Pleistocene. Springer, USA.

Hebert PDN, Stoeckle MY, Zemlak TS, Francis C 2004. Identification of birds through DNA barcodes. PLoS Biology 10: 1657-1663.

Hedges SB & Schweitzer MH 1995. Detecting dinosaur DNA. Science 268: 1191-2.

Heled J, Drummond AJ 2008. Bayesian inference of population size history from multiple loci. BMC Evolutionary Biology 8: 289.

Hewitt G 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. Biological Journal of the Linnean Society 58: 247-276.

Hickson R, Slack K 1998. Phylogeny recapitulates geography – New Zealand's skinks in the Oligocene. In: Cooper RA, Jones CM (Eds). Geology and Genes. Wellington, Geological Society of New Zealand Miscellaneous Publication No. 97. Pp. 39-41.

Hickson RE, Slack KE, Lockhart P 2000. Phylogeny recapitulates geography, or why New Zealand has so many species of skinks. Biological Journal of the Linnean Society 70: 415-433.

Higham TFG, Anderson A, Jacomb C 1999. Dating the first New Zealanders: The chronology of Wairau Bar. Antiquity 73: 420-427.

Higham TFG, Anderson A, Bronk Ramsey C, Tompkins C 2005. Diet-derived variations in radiocarbon and stable isotopes: A case study from Shag River mouth, New Zealand. Radiocarbon 47: 367-375.

Higuchi RG, Bowman B, Freiberger M, Ryder O, Wilson 1984. DNA sequences from the quagga, an extinct member of the horse family. Nature 312: 282-4

Hill RS, Lewis T, Carpenter RJ, Whang SS 2008. *Agathis* (Araucariaceae) macrofossils from Cainozoic sediments in south-eastern Australia. Australian Systematic Botany 21: 162-177.

Hitchmough R, Nielsen S, Bauer A, Jackman T 2009. New Zealand's diplodactyline geckos – a speciose endemic radiation with probable vicariant, Gondwanic origins, In: Trewick S, Hillier N, Cooper R (Eds). Geology and Genes IV. Wellington, Geological Society of New Zealand Miscellaneous Publication No. 126, p 27.

Ho SYW, Saarma U, Barnett R, Haile J, Shapiro B 2008. The effect of inappropriate calibration: Three case studies in molecular ecology. PLoS One 3: e1615.

Hofreiter M 2000. Ancient DNA. Nature Reviews Genetics 2: 353-359.

Hofreiter M, Betancourt JL, Sbriller AP, Markgraf V, McDonald 2001. Phylogeny, diet and habitat of an extinct ground sloth from Cuchillo Cura, Neuquen Provance, southwest Argentina. Quaternary Research 59: 364-378.

Hofreiter M, Capelli C, Krings M, Waits L, Conard N, Munzel S, Rabeder G, Nagel D, Paunovic M, Jambresi G, Meyer S, Weiss G, Paabo S 2002. Ancient DNA analyses reveal high mitochondrial sequence diversity and parallel morphological evolution of Late Pleistocene cave bears. Molecular Biology and Evolution 19: 1244-1250.

Hofreiter M, Kuch M, McDonald G, Martin P, Paabo S 2003. Nuclear gene sequences from a Late Pleistocene sloth coprolite. Quaternary Research 59: 364–378.

Hofreiter M, Poinar HN, Spaulding WG, Bauer K, Martin PS, Possnert G, Paabo S 2000. A molecular analysis of ground sloth diet through the last glaciation. Molecular Ecology 9: 1975–1984.

Hofreiter M, Serre D, Rohland N, Rabeder G, Nagel D, Conard N, Munsel S, Paabo S 2004. Lack of phylogeography in European mammals before the last glaciation. Proceedings of the National Academy of Science USA 101: 12963-12968.

Hofreiter M, Stewart J 2009 Ecological change, range fluctuations and population dynamics during the Pleistocene. Current Biology 19: R584-594.

Holdaway RN 1996. The arrival of rats in New Zealand. Nature 384: 225-226.

Holdaway RN 1999. A spatio-temporal model for the invasion of the New Zealand archipelago by the Pacific rat *Rattus exulans*. Journal of the Royal Society of New Zealand 29: 91-105.

Holdaway RN, Jacomb C 2000a. Rapid extinction of the moas (Aves: Dinornithiformes): model, test and implications. Science 287: 2250-2254.

Holdaway RN, Jacomb C 2000b. Less is moa: Response. Science 289: 1472-1474.

Horrocks M, D'Costa D, Wallace R, Gardner R, Kondo R 2004. Plant remains in coprolites: diet of a sub-alpine moa (Dinornithiformes) from southern. New Zealand Emu 104: 149–156.

Horvath MB, Martinez-Cruz B, Negro JJ, Kalmar L, Godoy JA 2005. An overlooked DNA source for non-invasive genetic analysis in birds. Journal of Avian Biology 36: 84-88.

Hoss M, Jaruga P, Zastawny TH, Dizdarouglu M, Paabo S 1996. DNA damage and DNA sequence retrieval from ancient tissues. Nucleic Acids Research 24: 1304-1307.

Houde P, Cooper A, Leslie E, Shand AE, Montano GA 1997. Phylogeny and evolution of 12S rRNA in gruiformes (Aves). In: Mindell DP (Ed), Avian Molecular Evolution and Systematics, pp 117-154. San Diego, USA: Academic Press.

Howell CJ, Kelly D, Turnbull MH 2002. Moa ghosts exorcised? New Zealand's divaricate shrubs avoid photoinhibition. Functional Ecology 16: 232-240.

Howorth HH 1887. The Mammoth and the Flood: An Attempt to Confront the Theory of Uniformity with the Facts of Recent Geology. London, UK: Low, Marston, Searle, & Rivington.

Hudson RR 1983a. Testing the constant-rate neutral allele model with protein sequence data. Evolution 37: 203-217.

Hudson RR 1983b. Properties of a neutral allele model with intragenic recombination. Theoretical Population Biology 23: 183-201.

Hutton FW 1900. The Geological History of New Zealand. Transactions and Proceedings of the New Zealand Institute 32: 159-183.

Huynen L, Millar CD, Scofield RP, Lambert DM 2003. Nuclear DNA sequences detect species limits in ancient moa. Nature 425: 175–178.

Jacobi RM, Higham TFG, Bronk Ramsey CB 2006. AMS radiocarbon dating of Middle and Upper Palaeolithic bone in the British Isles: Improved reliability using ultrafiltration. Journal of Quaternary Science 21: 557-573.

Jeffrey CS 1989. Biological Nomenclature. Edward Arnold, London.

Johnson CN 2002. Determinants of loss of mammal species during the Late Quaternary 'megafauna' extinctions: Life history and ecology, but not body size. Proceedings of the Royal Society of London Series B 269: 2221-2227

Johnson CN 2006. Australia's mammal extinctions. CSIRO Publishing, Australia.

Johnson CN 2009. Ecological consequences of the Late Quaternary extinctions of megafauna. Proceedings of the Royal Society of London Biological Series 276: 2509-2519.

Kamp PJJ, Furlong KP 2006. Neogene plate tectonic reconstructions and geodynamics of North Island sedimentary basins. New Zealand Petroleum Conference Proceedings, p 16.

Kelly D, Ladley JJ, Robertson AW, Anderson SH, Wotton DM, Wiser SK 2010. Mutualisms with the wreckage of an avifauna: The status of bird pollination and fruit-dispersal in New Zealand. New Zealand Journal of Ecology 34: 66-85.

Kemp BM, Monroe C, Smith DG 2006. Repeat silica extraction: a simple technique for the removal of PCR inhibitors from DNA extracts. Journal of Archaeological Science 33: 1680-1689.

Kennett DJ, Kennett JP, West GJ, Erlandson JM, Johnson JR, Hendy IL, West A, Culleton BJ, Jones TL, Stafford Jr TW 2008. Wildfire and abrupt ecosystem disruption on California's Northern Channel Islands at the Allerod-Younger Dryas boundary (13.0-12.9 Ka). Quaternary Science Reviews 27: 2528-2543

Kienast F, Tarasov P, Schirrmeister L, Grosse G, Andreev AA 2008. Continental climate in the east Siberian Arctic during the last interglacial: implications from palaeobotanical records. Global Planetary Change 60: 535-562.

Kingman JFC 1982. On the genealogy of large populations. Journal of Applied Probability 19A: 27-43.

Knapp M, Mudaliar R, Havell D, Wagstaff SJ, Lockhart PJ 2007. The drowning of New Zealand and the problem of *Agathis*. Systematic Biology 56: 862-870.

Korsten M, Ho SYW, Davidson J, Pahn D, Wulla E, Roht M, Tumanov IL, Kojola I, Andersone-Lilley Z, Ozolins J, Pilot M, Mertzanis Y, Giannakopoulos A, Vorobiev AA, Markov NI, Saveljev A, Yapunova EA, Abramov AV, Mannil P, Valdmann H, Pazetnov SV, Pazetnov VS, Rokov AM, Saarma U 2009. Sudden expansion of a single

brown bear maternal lineage across northern continental Eurasia after the last ice age: A general demographic model for mammals? Molecular Ecology 18: 1963-1979.

Krause J, Dear PH, Pollack JL, Slatkin M, Spriggs H, Barnes I, Lister AM, Ebersberger I, Paabo S, Hofreiter M 2006. Multiplex amplification of the mammoth mitochondrial genome and the evolution of Elephantidae. Nature 439: 724-27.

Kreitman M 2000. Methods to detect selection in populations with applications to the human. Annual Review in Genomics and Human Genetics 1: 539-559.

Krings M, Stone A, Schmitz R, Krainitzki H, Stoneking M, Paabo S 1997. Neandertal DNA sequences and the origin of modern humans. Cell 90: 19-30.

Kuch M, Rohland N, Betancourt JL, Latorre C, Steppan C, Poinar HN 2002. Molecular analysis of a 11,700-year-old rodent midden from the Atacama Desert, Chile. Molecular Ecology 11: 913-924.

Kuhn TS, McFarlane KA, Groves P, Mooers AO, Shapiro B. 2010. Modern and ancient DNA reveal recent partial replacement of caribou in the southwest Yukon. Mol. Ecol. 19: 1312-1323.

Lambert DM, Baker A, Huynen L, Haddrath O, Herbert PDN, Miller CD 2005. Is a large-scale DNA-based inventory of ancient life possible? Journal of Hereditary 96: 279–284.

Lambert DM, Ritchie PA, Miller CD, Holland B, Drummond A, Baroni C 2002. Rates of evolution in ancient DNA from Adelie penguins. Science 295: 2270-2273.

Landis CA, Campbell HJ, Begg JG, Mildenhall DC, Paterson AM, Trewick SA 2008. The Waipounamu Erosion Surface: Questioning the antiquity of the New Zealand land surface and terrestrial fauna and flora. Geological Magazine 145: 173-197.

Lee DE, Bannister JM, Lindqvist JK 2007. Late Oligocene-Early Miocene leaf macrofossils confirm a long history of *Agathis* in New Zealand. New Zealand Journal of Botany 45: 565-578.

Lee K, Feinstein J, Cracraft J 1997. The Phylogeny of Ratite Birds: Resolving Conflicts between Molecular and Morphological Data Sets. In: Mindell DP (Ed). Avian Molecular Evolution and Systematics. Academic Press, San Diego, USA. Pp 173-211.

Lee WG, Gould K 2009. Three birds with one stone: Moas, heteroblasty and the New Zealand flora. New Phytologist 184: 282-284.

Lee WG, Wood JR, Rogers GM 2010. Legacy of avian-dominated plant-herbivore systems in New Zealand. New Zealand Journal of Zoology 34: 28-47.

Lemey P, Rambaut A, Pybus OG 2006. HIV evolutionary dynamics within and among hosts. AIDS Reviews 8: 124-40.

Leonard JA, Wayne RA, Cooper A 2000. Population genetics of ice age brown bears. Proceedings of the National Academy of Sciences USA 97: 1651-1654.

Lindahl T 1993a. Instability and decay of the primary structure of DNA. Nature 362: 709-15

Lindahl T 1993b. Recovery of antidiluvian DNA. Nature 365: 700

Lindahl T 1995. Recognition and processing of damaged DNA. Journal of Cell Science 49: 73-7

Lindhall T, Karlstro O 1973. Heat-induced depyrimidination of deoxyribonucleic acid in neutral solution. Biochemistry 12: 5151-54.

Lindhall T, Nyberg B 1972. Rate of depurination of native deoxyribonucleic acid. Biochemistry 11:3610.

Livezey BC, Zusi RL 2007. Higher-order phylogeny of modern birds (Theropoda, Aves: Neornithes) based on comparative anatomy. II. Analysis and discussion. Zoological Journal of the Linnean Society 149: 1-95.

Lowry JB 1980. Evolution of divaricating plants in New Zealand in relation to moa browsing. New Zealand Journal of Ecology 3: 165.

Lozhkin AV, Anderson PM, Eisner WR, Ravako LG, Hopkins DM, Brubaker LB, Colinvaux PA, Miller MC 1993. Late Quaternary lacustrine pollen records from southwestern Beringia. Quaternary Research 39: 314-324.

Ludwig A, Pruvost M, Reissmann M, Benecke N, Brockmann GA, Castanos P, Cieslak M, Lippold S, Llorente L, Malaspinas AS, Slatkin M, Hofreiter M 2009. Coat colour variation at the beginning of horse domestication. Science 324: 485.

McGlone MS, Newnham RM, Moar NT 2010. The vegetation cover of New Zealand during the Last Glacial Maximum: do pollen records under-represent woody vegetation? In: Haberle SG, Stevenson J, Prebble M (Eds). Altered Ecologies: Fire, Climate and Human Influence on Terrestrial Landscapes. Terra Australis 32. ANU E-Press, Australian National University, Canberra, Australia.

McGlone MS, Webb CJ 1981. Selective forces influencing the evolution of divaricating plants. New Zealand Journal of Ecology 4: 20-28.

McGlone MS, Wilmshurst JM 1999. Dating initial Maori environmental impact in New Zealand. Quaternary International 59: 5-16.

McKinney ML 1997. Extinction vulnerability and selectivity: Combining ecological and palaeontological views. Annual Review of Ecology and Systematics 28: 495-516.

McKinnon M 1997. New Zealand Historical Atlas. Auckland, New Zealand: Bateman.

MacPhee RDE 1999. Extinctions in Near Time: Causes, Contexts and Consequences. New York, USA: Kluwer Academic/Plenum.

MacPhee RDE, Marx PA 1999. The 40,000 year plague: Humans, hyperdisease, and first contact extinctions. In S. Goodman and GB Patterson (eds.), Natural Change and Human Impact in Madagascar, pp. 169-217. Washington DC, USA: Smithsonian Institution Press

Malmstrom H, Gilbert MTP, Thomas MG, Brandstrom M, Stora J, Molnar P, Anderson PK, Bendixen C, Holmlund G, Gotherstrom A, Willerslev E 2009. Ancient DNA reveals lack of continuity between Neolithic hunter gatherers and contemporary Scandinavians. Current Biology 19: 1758-1762.

Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen YJ, Chen Z, Dewell SB, Du L, Fierro JM, Gomes XV, Goodwin BC, He W, Helgesen S, Ho CH, Irzyk GP, Jando SC, Alenquer MLI, Jarvie JP, Jirage KB, Kim JB, Knight JR, Lanza JR, Leamon JH, Lefkowitz SM, Lei M, Li J, Lohman KL, Lu H, Makhijani VB, McDade KE, McKenna MP, Myers EW, Nickerson E, Nobile JR, Plant R, Puc BP, Ronan MT, Roth GT, Sarkis GJ, Simons JF, Simpson JW, Srinivasan M, Tartaro KR, Tomasz A, Vogt KA, Volkmer GA, Wang SH, Wang Y, Weiner MP, Yu P, Begley RF, Rothberg JM 2005. Genome sequencing in open microfabricated high density picoliter reactors. Nature 437: 376-380.

Martin PS 1984. Prehistoric overkill: The global model. In: Martin PS, Klein RG (Eds.), Quaternary Extinctions: A prehistoric revolution, pp. 354-403. Tuscon USA: Arizona University Press.

Martin PS 2005. Twilight of the mammoths: Ice age extinctions and the rewilding of America. University of California Press, Berkeley, USA.

Matheus P, Burns J, Weinstock J, Hofreiter M 2004. Pleistocene brown bears in the mid-continent of North America. Science 306: 1150.

Mayr G 2009. Palaeogene fossil birds. USA: Springer.

Miller GH, Fogel ML, Magee JW, Gagan MK, Clarke SJ, Johnson BJ 2005. Ecosystem collapse in Pleistocene Australia and a human role in megafaunal extinction. Science 309: 287-290.

Miller GH, Magee JW, Johnson BJ, Fogel ML, Spooner NA, McCulloch MT, Ayliffe LK 1999. Pleistocene extinction of *Genyornis newtoni*: Human impact on Australian megafauna. Science 283: 205-208.

Miller M, Drautz DI, Ratan A, Pusey B, Qi J, Lesk AM, Tomsho LP, Packard MD, Zhao F, Sher A, Tikhonov A, Raney B, Patterson N, Lindblad-Toh K, Lander ES, Knight JR, Irzyk GP, Fredrikson KM, Harkins TT, Sheridan S, Pringle T, Schuster SC 2008. Sequencing the nuclear genome of the extinct woolly mammoth. Nature 456: 387-390.

Minagawa M, Wada E 1984. Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}N$ and animal age. Geochemica et Cosmochimica Acta 48: 1135-1140.

Moar NT 2008. Late Quaternary Vegetation. In: Winterbourn M, Knox G, Burrows C, Marsden I (Eds.) The Natural History of Canterbury. Christchurch, New Zealand: Canterbury University Press.

Moar NT, Suggate RP 1979. Contributions to the Quaternary history of New Zealand flora 8: Interglacial and glacial vegetation in the Westport District South Island. New Zealand Journal of Botany 17: 361-87.

Mullis KB, Faloona FA 1987. Specific synthesis of DNA in vitro via a polymerase catalysed chain reaction. Methods in Enzymology 155: 335-50.

Murray PF, Megirian D 1998. The skull of dromornithid birds: anatomical evidence for their relationship to Anseriformes. Records of the South Australian Museum 31: 51-97.

Murray PF, Vickers-Rich P 2003. Magnificent mihirungs: The colossal flightless birds of the Australian dreamtime. USA: Indiana University Press.

Murry-McIntosh RP, Scrimshaw BJ, Hatfield PJ, Penny D 1998. Testing migration patterns and estimating founding population size in Polynesia by using human mtDNA sequences. Proceedings of the National Academy of Science USA 95: 9047-9052.

Newnham RM, Lowe DJ, Williams PW 1999. Quaternary environmental change in New Zealand: a review. Progress in Physical Geography 23: 567-610.

Newnham RM, Vandergoes MJ, Hendy CH, Lowe DJ, Preusser F 2007. A terrestrial palynological record for the last two glacial cycles from southwestern New Zealand. Quaternary Science Reviews 26: 517-535.

Nicholls H 2006. Restoring Nature's Backbone. PloS Biology 4: e202.

Noonan JP, Coop G, Kudaravalli S, Smith D, Krause J, Alessi J, Chen F, Platt D, Paabo S, Pritchard JK, Rubin EW 2006. Sequencing and analysis of Neanderthal genomic DNA. Science 314: 1113-1118.

Nordborg M 2001. In: Balding DJ, Bishop MJ, Cannings C (Eds). Handbook of statistical genetics, pp 213-238. Chichester, UK: John Wiley and Sons.

Oliver WRB 1949. The moas of New Zealand and Australia. Wellington, New Zealand: Dominion Museum.

Oliver WRB 1955. New Zealand birds (2nd edn). Wellington, New Zealand: Reed.

Olson SL. The fossil record of birds. In: Farner DS, King JR, Parkes KC (Eds). Avian Biology Vol. VIII. pp 79-238. Orlando, USA: Academic Press.

Oskam CL, Haile J, McLay E, Rigby P, Allentoft ME, Olsen ME, Bengtsson C, Miller GH, Schwenninger JL, Jacomb C, Walter R, Baynes A, Dortch J, Parker-Pearson M, Gilbert MTP, Holdaway RN, Willerslev E, Bunce M 2010. Fossil avian eggshell preserves ancient DNA. Proceedings of the Royal Society of London Series B 277: 1991-2000.

Owen R 1840. On the bone of an unknown struthious bird from New Zealand, meeting of November 12, 1839. Proceedings of the Zoological Society London for 1839, Part VII, no lxxxiii: 169-171.

Owen R 1856. On *Dinornis* (Part VII): Containing a description of the bones of the leg and foot of the *Dinornis elephantopus*, Owen. Proceedings of the Zoological Society of London 1856: 54-61.

Owens IPF, Bennett PM 2000. Ecological basis of extinction in birds: habitat loss versus human persecution and introduced predators. Proceedings of the National Academy of Science USA 97: 12144-12148.

Paabo S 1985. Molecular cloning of ancient Egyptian mummy DNA. Nature 314: 644-5

Paabo S 1989. Ancient DNA: Extraction, characterization, molecular cloning, and enzymatic amplification. Proceedings of the National Academy of Science USA 86: 1939–1943.

Paabo S, Gifford JA, Wilson AC 1988. Mitochondrial DNA sequences from a 7000 year old brain. Nucleac Acids Research 16: 9775-83.

Paabo S, Poinar H, Serre D, Jaenicke-Despres V, Hebler J, Rohland N, Kuch M, Krause J, Vigilant L, Hofreiter M 2004. Genetic analyses from ancient DNA. Annual Review of Genetics 38: 645-679.

Payne RB, Sorenson MD 2002. Museum collections as sources of genetic data. Bonner Zoologische Beitrage 51: 97-104.

Phillips MJ, Cooper A 2008. Independent evolution of flightless palaeognaths followed the extinction of the dinosaurs. Society for Avian Paleontology and Evolution Scientific Program and Abstracts, Australian Museum.

Phillips MJ, Gibb GC, Crimp EA, Penny D 2010. Tinamous and moa flock together: Mitochondrial genome sequence analysis reveals independent losses of flight among ratites. Systematic Biology 59: 90-107.

Pocknall DT 1982. Palynology of late Oligocene Pomahaka Estuarine Bed sediments, Waikoikoi, Southland, New Zealand. New Zealand Journal of Botany 20: 263-287.

Poinar HN, Cano RJ, Poinar Jr GO 1993. DNA from an extinct plant. Nature 363: 667.

Poinar HN, Hofreiter M, Spaulding WG, Martin PS, Stankiewicz BA, Bland H, Evershed RP, Possnert G, Paabo S 1998. Molecular coproscopy: Dung and diet of the extinct ground sloth *Nothrotheriops shastensis*. Science 218: 402–406.

Poinar HN, Kuch M, Sobolik KD KD, Barnes I, Stankiewicz AB, Kuder T, Spaulding WG, Bryant VM, Cooper A, Paabo S 2001. A molecular analysis of dietary diversity for three archaic Native Americans. Proceedings of the National Academy of Sciences USA 98: 4317-4322.

Poinar HN, Schwarz C, Qi J, Shapiro B, MacPhee RDE, Buigues B, Tikhonov A, Huson DH, Tomsho LP, Auch A, Rampp M, Miller W, Schuster SC 2006. Metagenomics to palaeogenomics: Large-scale sequencing of mammoth DNA. Science 311: 392-394.

Pole M 1994. The New Zealand flora – entirely long-distance dispersal? Journal of Biogeography 21: 624-635.

Prideaux GJ, Long JA, Ayliffe LK, Hellstrom JC, Pillans B, Boles WE, Hutchinson MN, Roberts RG, Cupper ML, Arnold LJ, Devine PD, Warburton NM 2007. An arid-adapted middle Pleistocene vertebrate fauna from south-central Australia. Nature 445: 422-425.

Pycraft WP 1900. On the morphology and phylogeny of the palaeognathae (Ratitae and Crypturi) and Neognathae (Carinatae). The Transactions of the Zoological Society of London 15: 149-290.

Quinn TW 1992. The genetic legacy of Mother Goose: Phylogeographic patterns of lesser snow goose *Chen caerulescens caerukscens* maternal lineages. Molecular Ecology 1: 105-117.

Ramakrishnan U, Hadly EA 2009. Using phylochronology to reveal cryptic population histories: Review and synthesis of 29 ancient DNA studies. Molecular Ecology 18: 1310-1330.

Rambaut A, Ho SYW, Drummond AJ, Shapiro B 2009. Accommodating the effect of ancient DNA damage of inferences of demographic histories. Molecular Biology and Evolution 26: 245-248.

Rambaut A, Pybus OG, Nelson MI, Viboud C, Taubenberger JK, Holmes EC 2008. The genomic and epidemiological dynamics of human influenza A virus. Nature 453: 615-619.

Rasmussen M, Li Y, Lindgreen S, Pedersen JS, Albrechtsen A, Moltke I, Metspalu M, Metspalu E, Kivisild T, Gupta R, Bertalan M, Nielsen K, Gilbert MTP, Wang Y, Raghavan M, Campos PF, Kamp HM, Wilson AS, Gledhill A, Tridico S, Bunce M, Lorenzen ED, Binladen J, Guo X, Zhao J, Zhang X, Zhang H, Li Z, Chen M, Orlando L, Kristiansen K, Bak M, Tommerup N, Bendixen C, Pierre TL, Grønnow B, Meldgaard M, Andreasen C, Fedorova SA, Osipova LP, Higham TFG, Ramsey CB, Hansen TO, Nielsen FC, Crawford MH, Brunak S, Sicheritz-Ponten T, Villems R, Nielsen R, Krogh A, Wang J, Willerslev E 2010. Ancient human genome sequence of an extinct Palaeo-

Eskimo. Nature 463: 757-762.
Rattenbury JA 1962. Cyclic hybridisation as a survival mechanism in the New Zealand forest flora. Evolution 16: 348–363.
Reiss RA 2006. Ancient DNA from ice age insects: Proceed with caution. Quaternary Science Reviews 25: 1877-1893.
Rey PF, Muller RD 2010. Fragmentation of active continental plate margins owing to the buoyancy of the mantle wedge. Nature Geoscience 3: 257-261.
Roberts RG, Flannery TF, Ayliffe LK, Yoshida H, Olley JM, Prideaux GJ, Laslett GM, Baynes A, Smith MA, Jones R, Smith BL 2001. New ages for the last Australian megafauna: Continent-wide extinction about 46,000 years ago. Science 292: 1888-92.
Roelants K, Bossuyt F 2005. Archaeobatrachian paraphyly and Pangaean diversification of crown-group frogs. Systematic Biology 54: 111-126.
Rohland N, Hofreiter M 2007. Comparison and optimization of ancient DNA extraction. Biotechniques 42: 343-352.
Rohland N, Pollack JL, Nagel D, Beauval C, Airvaux J, Paabo S, Hofreiter M 2005. The population history of extant and extinct hyenas. Molecular Biology and Evolution 22: 2435-2443.
Rompler H, Rohland N, Lalueza-Fox C, Willerslev E, Kuznetsova T, Rabeder G, Bertranpetit J, Schoneberg T, Hofreiter M 2006. Nuclear gene indicates coat-colour polymorphism in mammoths. Science 313: 62.
Rosenberg NA, Nordborg M 2002. Genealogical trees, coalescent theory and the analysis of genetic polymorphisms. Nature Reviews Genetics 3: 390-390.
Rowley-Conway 2009. Human prehistory: Hunting for the earliest farmers. Current Biology 19: R948-949.
Sauer EGF 1972. Ratite eggshells and phylogenetic questions. Bonner Zoologische Beiträge 23: 3-48.
Schaaper RM, Kunkel TA, Loeb LA 1983. Infidelity of DNA-synthesis associated with bypass of apurinic sites. Proceedings of the National Academy of Science USA 80: 487-91.
Sefc KM, Payne RB, Sorenson MD 2003. Microsatellite amplification from museum feather samples: Effects of fragment size and template concentration on genotyping errors. Auk 120: 982-989.
Shapiro B, Drummond AJ, Rambaut A, Wilson MC, Matheus PE, Sher AV, Pybus OG, Gilbert MTP, Barnes I, Binladen J, Willerslev E, Hansen AJ, Baryshnikov GF, Burns JA, Davydov S, Driver JC, Froese DG, Harington CR, Keddie G, Kosintsev P, Kunz ML, Martin LD, Stephenson RO, Storer J, Tedford R, Zimov S, Cooper A 2004. Rise and fall of the Beringian Steppe Bison. Science 306: 1561-1565.
Shapiro R 1981. Damage to DNA caused by hydrolysis. In: Seenberg E, Kleppe K (Eds). Chromosome Damage and Repai, pp 3-12. New York, USA: Plenum.
Shepherd LD, Lambert DM 2007. The relationships and origins of the New Zealand wattlebirds (Passeriformes, Callaeatidae) from DNA sequence analysis. Molecular Phylogenetics and Evolution 43: 480-492.
Sibley GC, Ahlquist JA 1990. Phylogeny and classification of birds. Yale University Press, New Haven, USA.
Slater GJ, Thalmann O, Leonard JA, Schweizer RM, Koepfli KP, Pollinger JP, Rawlence NJ, Austin JJ, Cooper A, Wayne RK 2009. Evolutionary history of the Falklands wolf. Current Biology 19: R937-38.
Smith IWG 1989. Saying so doesn't make it so. In: Sutton DG (Ed). New Zealand Archaeological Association Monograph 17, pp 76-108. Dunedin, New Zealand: New Zealand Archaeological Association.
Smith IWG, Anderson AJ 1998. Radiocarbon dates from archaeological rat bones: the Pleasant River case. Archaeology in Oceania 33: 88-91.

Soltis S, Soltis DE, Smiley CJ 1992. An rbcL sequence from a Miocene Taxodium (bald cypress). Proceedings of the National Academy of Science USA 89: 449-51.

Steadman DW, Martin PS 2003. The late Quaternary extinction and future resurrection of birds on Pacific islands. Earth Science Reviews 61: 133-147.

Stevens GR 1985. Lands in Collision. New Zealand Department of Scientific and Industrial Research.

Stevens RE, Jacobi R, Street M, Germonpre M, Conard NJ, Munzel SC, Hedges REM 2008. Nitrogen isotope analysis of reindeer (*Rangifer tarandus*), 45,000 BP to 9,000 BP: Palaeoenvironmental reconstructions. Palaeogeography, Palaeoclimatology, Palaeoecology 262: 32-45.

Stiller M, Green RE, Ronan M, Simons JF, Du L, He W, Egholm M, Rothberg JM, Keates SG, Ovodov ND, Antipina EE, Baryshnikov GF, Kuzmin YV, Vasilevski AA, Wuenschell GE, Termini T, Hofreiter M, Jaenicke-Després V, Pääbo S 2006. Patterns of nucleotide misincorporations during enzymatic amplification and direct large-scale sequencing of ancient DNA. Proceedings of the National Academy of Science USA 103: 13578-13584.

Stockler K, Daniel IL, Lockhart PJ 2002. New Zealand kauri (*Agathus australis* (D. Don) Lindhl., Araucariaceae) survives Oligocene drowning. Systematic Biology 51: 827-832.

Storey AA, Ramirez JM, Quiroz D, Burley DV, Addison DJ, Walter R, Anderson AJ, Hunt TL, Athens JS, Huynen L, Matisoo-Smith EA 2008a. Radiocarbon and DNA evidence for a pre-Columbian introduction of Polynesian chickens to Chile. Proceedings of the National Academy of Science USA 104: 10335-10339.

Storey A, Quiroz D, Ramirez JM, Beavan-Athfield N, Addison DJ, Walter R, Hunt T, Athens JT, Huynen L, Matisoo-Smith EA 2008b. Pre-Columbian chickens, dates, isotopes, and mtDNA. Proceedings of the National Academy of Science USA 105: E99.

Surovell TA, Holliday VT, Gingerich JAM, Ketron C, Haynes CV, Hilman I, Wagner DP, Johnson E, Claeys P 2009. An independent evaluation of the Younger Dryas extraterrestrial impact hypothesis. Proceedings of the National Academy of Science USA 106: 18155-19158.

Sutton DG 1987. A paradigmatic shift in Polynesian prehistory: Implications for New Zealand. New Zealand Journal of Archaeology 9: 135-155.

Taberlet P, Fumagalli L, Wust-Saucy AG, Cosson JF 1998. Comparative phylogeography and postglacial colonization routes in Europe. Molecular Ecology 7: 453-464.

Tajima F 1983. Evolutionary relationship of DNA sequences in finite populations. Genetics 105: 437-460.

Tambussi CP, Noriega J, Gazdzicki A, Tatur A, Reguero MA, Vizcaino SF 1994. The first occurrence of ratite birds in the Paleocene of Antarctica. In SM Zalewki (Ed.), XXI Polar Symposium, pp. 45-58. Warsaw: Polish Academy of Sciences.

Tennyson AJ 2010. The origin and history of New Zealand's terrestrial vertebrates. New Zealand Journal of Ecology 34: 6-27.

Tennyson A, Martinson P 2006. Extinct birds of New Zealand. Wellington, New Zealand: Te Papa Press.

Tennyson AJD, Worthy TH, Scofield RP, Jones C, Hand SJ 2008. Fossil evidence for moa in the early Miocene and its implications for New Zealand's 'Oligocene drowning'. Society of Avian Palaeontology and Evolution, Sydney, NSW, Australia, 18-23 August 2008 Scientific Programme and Abstracts.

Thomas RH, Schaffner W, Wilson AC, Paabo S 1989. DNA phylogeny of the extinct marsupial wolf. Nature 340: 465-67.

Thomsen PF, Elias S, Gilbert MTP, Haile J, Munch K, Kuzmina S, Froese DG, Sher A, Holdaway RN, Willerslev 2009. Non-destructive sampling of ancient insect DNA. PLoS One 4: e5048.

Tregear E 1893. The extinction of the moa. Transactions and Proceedings of the New Zealand Institute 25: 413-426.

Turney CSM, Flannery TF, Roberts RG, Reid C, Fifield LK, Higham TFG, Jacobs Z, Kemp N, Colhoun EA, Kalin RM, Ogle N 2008. Late-surviving megafauna in Tasmania, Australia, implicate human involvement in their extinction. Proceedings of the National Academy of Science USA 105: 12150-12153.

Tuniz C, Gillespie R, Jones C 2009. The bone readers: Atoms, genes and the politics of Australia's deep past. Australia: Allen and Unwin.

Valdiosera CE, Garcia-Garitagoitia JL, Carcia N, Doadrio I, Thomas MG, Hanni C, Arsuaga JL, Barnes I, Hofreiter M, Orlando L, Gotherstrom A 2008. Surprising migration and population size dynamics in ancient Iberian brown bears (*Ursus arctos*). Proceedings of the National Academy of Science USA 105: 5123-5128.

Vandergoes MJ, Fitzimons SJ 2003. The Last Glacial-Interglacial Transition (LGIT) in south Westland, New Zealand: palaeoecological insight into mid-latitude Southern Hemisphere climate change. Quaternary Science Reviews 22: 1461-1476.

Vandergoes MJ, Newnham RM, Hendy CH, Lovell TV, Preusser F, Fitzsimons SJ, Hogg, A, Kasper HU, Schluchter C 2005. Regional isolation forcing of Late Quaternary climate change in the Southern Hemisphere. Nature 436: 242-245.

Vickers-Rich P, van Tets GF, Knight F 1985. Kadimakara: Extinct vertebrates of Australia. USA: Princeton University Press.

Vickers-Rich P, Trusler P, Rowley MJ, Cooper A, Chambers GK, Bock WJ, Millener PR, Worthy TH, Yaldwyn JC 1995. Morphology, myology, collagen and DNA of a mummified moa, *Megalapteryx didinus* (Aves: Dinornithiformes) from New Zealand. Tuhinga Records of the Museum of New Zealand Te Papa Tongarewa 4: 1–26.

Wagstaff SJ, Bayly MJ, Garnock-Jones PJ, Albach DC 2002. Classification, origin, and diversification of the New Zealand hebes (Scrophulariaceae). Annuals of the Missouri Botanical Gardens 89: 38-63.

Wall JD, Kim SK 2007. Inconsistencies in Neanderthal genomic DNA sequences. PLoS Genetics 3: e175.

Wardle P 1991. Vegetation of New Zealand. Cambridge, UK: Cambridge University Press.

Wayne RK, Leonard JA, Cooper A 1999. Full of sound and fury: History of ancient DNA. Annual Review of Ecology and Systematics 30: 457-477.

Wiffen J 1991. Valley of the Dragons: The story of New Zealand's dinosaur woman. New Zealand: Reed Publishers.

Weinstock J, Shapiro B, Preito A, Marin JC, Gonzalez BA, Gilbert MTP, Willerslev E 2009. The Late Pleistocene distribution of vicunas (*Vicugna vicugna*) and the 'extinction' of the gracile llama ('*Lama gracilis*'): New molecular data. Quaternary Science Reviews 28: 1269-1373.

Willerslev E, Cooper A 2005. Ancient DNA. Proceedings of the Royal Society of London Series B 272: 3-16.

Willerslev E, Cappellini E, Boomsma W, Nielsen R, Hebsgaard MB, Brand TB, Hofreiter M, Bunce M, Poinar HN, Dahl-Jensen D, Johnsen S, Steffensen JP, Bennike O, Schwenninger JL, Nathan R, Armitage S, Hoog CJ, Alfimov V, Christl M, Beer J, Muscheler R, Barker J, Sharp M, Penkman KEH, Haile J, Taberlet P, Gilbert MTP, Casoli A, Campani E, Collins MJ 2007. Ancient biomolecules from deep ice cores reveal a forested southern Greenland. Science 317: 111-114.

Willerslev E, Hansen AJ, Binladen J, Brand TB, Gilbert MTP, Shapiro B, Bunce M, Wiuf C, Gilichinsky DA, Cooper A 2003. Diverse plant and animal genetic records from Holocene and Pleistocene sediments. Science 300: 791-795.

Williams PW, King DNT, Zhao JX, Collerson KD 2005. Late Pleistocene to Holocene composite speleotherm ^{18}O and ^{13}C chronologies from South Island, New Zealand – did a global Younger Dryas really exist? Earth and Planetary Science Letters 230: 301-317.

Wilmshurst JM, Higham TFG 2004. Using rat gnawed seeds to independently date the arrival of Pacific rates and humans to New Zealand. The Holocene 14: 801-806.

Wilmshurst JM, Anderson AJ, Higham TFG, Worthy TH 2008. Dating the late prehistoric dispersal of Polynesians to New Zealand using the commensal Pacific rat. Proceedings of the National Academy of Science USA 105, 7676–7680.

Winkworth RC, Wagstaff SJ, Glenny D, Lockhart PJ 2002. Plant dispersal N.E.W.S from New Zealand. Trends in Ecology and Evolution 17: 514-520.

Wright TF, Schirtzinger EE, Matsumoto T, Eberhard JR, Graves GR, Sanchez JJ, Capelli S, Muller H, Scharpegge J, Chambers GK, Fleischer RC 2008. A multilocus molecular phylogeny of the parrots (Psittaciformes): Support for a Gondwanan Origin during the Cretaceous. Molecular Biology and Evolution 25: 2141-2156.

Wood JR 2006. Subfossil kakapo (*Strigops habroptilus*) remains from near Gibraltar Rock, Cromwell Gorge, Central Otago, New Zealand. Notornis 53: 191-193.

Wood JR 2007. Moa gizzard content analyses: further information on the diets of *Dinornis robustus* and *Emeus crassus*, and the first evidence for the diet of *Pachyornis elephantopus* (Aves: Dinornithiformes). Records of the Canterbury Museum 21: 27–39.

Wood JR, Rawlence NJ, Rogers GM, Austin JJ, Worthy TH, Cooper A 2008. Coprolite deposits reveal the diet and ecology of the extinct New Zealand megaherbivore moa (Aves, Dinornithiformes). Quaternary Science Reviews: 2593–2602.

Woodward SR, Weyand NJ, Bunnell M 1994. DNA sequence from Cretaceous Period bone fragments. Science 266: 1229-32.

Worthy TH 1987. Sexual dimorphism and temporal variation in the North Island moa species *Euryapteryx curtus* (Owen) and *Pachyornis mappini* Archey. National Museum of New Zealand Records 3: 59-70.

Worthy TH 1988. A re-examination of the moa genus *Megalapteryx*. Notornis 35: 99-108.

Worthy TH 1989. Mummified moa remains from Mt Owen, northwest Nelson. Notornis 36: 36–38.

Worthy TH 1993a. A review of fossil bird bones from loess deposits in eastern South Island, New Zealand. Records of the Canterbury Museum 10: 95-106.

Worthy TH 1993b. Fossils of Honeycomb Hill. Wellington, New Zealand: Museum of New Zealand Te Papa Tongawera.

Worthy TH 1994. Late Quaternary changes in the moa fauna on the West Coast of the South Island, New Zealand. Records of the South Australian Museum 27: 125-134.

Worthy TH 1997. Quaternary fossil fauna of South Canterbury, South Island, New Zealand. Journal of the Royal Society of New Zealand 27: 67-162.

Worthy TH 1998a. Quaternary fossil faunas of Otago, South Island, New Zealand. Journal of the Royal Society of New Zealand 28: 421–521.

Worthy TH 1998b. The Quaternary fossil avifauna of Southland, South Island, New Zealand. Journal of the Royal Society of New Zealand 28: 537-589.

Worthy TH 1999. Changes induced in the New Zealand avifauna by climate during the last glacial-interglacial period. Proceedings of the 4th International Meeting of the Society of Avian Paleontology and Evolution (Washington, D.C., June 1996). Smithsonian Contributions to Palaeobiology 89: 111-123.

Worthy TH, Bunce M, Cooper A, Scofield P 2005. *Dinornis* – an insular oddity, a taxonomic conundrum reviewed. Proceedings of the International Symposium ‘‘Insular Vertebrate Evolution: The Palaeontological Approach’’., eds Alcover JA, Bover P (Monografies de la Societat d’Histo` ria Natural de les Balears.), Vol 12, pp 377–390.

Worthy TH, Grant-Mackie JA 2003. Late-Pleistocene avifaunas from Cape Wanbrow, Otago, South Island, New Zealand. Journal of the Royal Society of New Zealand 33: 427-485.

Worthy TH, Hand SJ, Lee MSY, Hutchinson M, Tennyson AJD, Scofield RP, Marshall B, Worthy JP, Nguyen JMT, Boles WE, Archer M 2009. New Zealand’s St Bathans Fauna: an update on its composition and relationships. In: Trewick S, Hillier N, Cooper R

(Eds). Geology and Genes IV. Wellington, New Zealand. Geological Society of New Zealand Miscellaneous Publication No. 126. Pp 40-43.

Worthy TH, Holdaway RN 1994. Quaternary fossil faunas from caves in Takaka valley and on Takaka Hill, northwest Nelson, South Island, New Zealand. Journal of the Royal Society of New Zealand 24: 297-391.

Worthy TH, Holdaway RN 1995. Quaternary fossil faunas from caves on Mt Cookson, North Canterbury, New Zealand. Journal of the Royal Society of New Zealand 25: 333-370.

Worthy TH, Holdaway RN 1996. Quaternary fossil faunas, overlapping taphonomies, and palaeofaunal reconstruction in North Canterbury, South Island, New Zealand. Journal of the Royal Society of New Zealand 26: 275-361.

Worthy TH, Holdaway RN 2002. The Lost World of the Moa. Canterbury University Press, Christchurch.

Worthy TH, Roscoe D 2003. Takaka Fossil Cave – a stratified Late Glacial to Late Holocene deposit from Takaka Hill, New Zealand. Tihinga 14: 41-60.

Worthy TH, Tennyson AJD, Archer M, Musser AM, Hand AJ, Jones C, Douglas BJ, McNamara JA, Beck RMD 2006. Miocene mammal reveals a Mesozoic ghost lineage on insular New Zealand, southwest Pacific. Proceedings of the National Academy of Science USA 103: 19419-19423.

Wyatt KB, Campos PF, Gilbert MTP, Kolokotronis SO, Hynes WH, DeSalle R, Ball SJ, Daszak P, MacPhee RDE, Greenwood AD 2008. Historical mammal extinction on Christmas Island (Indian Ocean) correlates with introduced infectious disease. PLoS One 3: e3602.

Yokoyama Y, Lambeck K, Deckker PD, Johnston P, Fifield LK 2000. Timing of the Last Glacial Maximum from observed sea level minima. Nature 406: 713-716.

Zischler H, Hoss M, Handt O, Haeseler A, Kuyl AC, Goudsmith J 1995. Detecting dinosaur DNA. Science 268: 1192-93.

CHAPTER TWO

A deposition mechanism for Holocene miring bone deposits, South Island, New Zealand

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I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed Date 8/July/2009

Trevor H. Worthy

Excavated Bell Hill moa swamp, provided Bell Hill data, interpreted data and evaluated manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed. Date 13 July 2009

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Excavated Glencrieff moa swamp, provided Glencreiff data, interpreted data and evaluated manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

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Created the ArcView GIS script and assisted in creating 3D reconstructions in ArcView.

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A Deposition Mechanism for Holocene Miring Bone Deposits, South Island, New Zealand

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Localised deposits of Late Pleistocene and Holocene bird bones occur in wetlands throughout New Zealand. These are characterised by dense accumulations of mostly disarticulated bones, with assemblages dominated by large, flightless bird taxa; in particular the extinct ratite moa (Aves: Dinornithiformes). A wide range of deposition mechanisms were historically proposed for these sites, including large floods and stampedes during wildfires. We outline a simple method for analysing the orientation and spatial distribution of bones within these deposits using GIS software, and apply this method to the interpretation of three such deposits from South Island, New Zealand. The results are consistent with non-catastrophic, periodic miring of individual moa. Long bones within these sites were preferentially orientated and subhorizontally inclined, indicating post-deposition disarticulation and movement of the bones within the sediment by sediment liquefaction and raking from the legs of mired birds, with a possible influence from water flow. Small, light skeletal elements were significantly under represented in the deposits. This may be due to post-mortem scavenging or weathering of vertebra and crania, 'pumping' to the surface of light, buoyant elements during liquefaction events, or crushing of these elements by subsequently mired birds.

Keywords: BONE, DEPOSITION, GIS, HOLOCENE, MIRING, MOA, NEW ZEALAND, WETLAND

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Introduction

New Zealand has one of the richest late Quaternary avifaunal records in the world (Worthy & Holdaway, 2002). The four types of deposit from which the greatest accumulations of the bones have been recovered are roosting sites of predatory birds (e.g. Worthy, 1997; Worthy & Zhao, 2006), pitfalls in caves and loess swallow-holes (e.g. Fraser, 1873; Archey, 1941; Clark *et al*., 1996; Worthy & Holdaway, 1994; Worthy, 1998a), coastal sand dunes (e.g. Worthy, 1998b; Worthy, 1998c) and mirings (e.g. Hamilton, 1889; Forbes, 1892; Duff, 1941; Holdaway & Worthy, 1996). Bone deposits in the latter are dominated by large flightless taxa, especially the extinct ratite moa (Aves: Dinornithiformes) and flightless geese (*Cnemiornis calcitrans; C. gracilis*) (Worthy & Holdaway, 1997). Similar late Quaternary miring deposits of megafaunal bones have been recorded elsewhere, e.g. Australia (Gillespie *et al*., 1978); North America (Graham & Graham, 1990; Spencer *et al.,* 2003); Europe (Furrer, 2007). Despite the relatively widespread occurrence of miring bone deposits in New Zealand (Fig. 1), no synthesis describing the range of stratigraphic and taphonomic variation seen at these sites has previously been published.

New Zealand miring bone deposits sometimes contain bones from hundreds of individual moa (Fig. 2), and such deposits were extensively mined to supplement local and international museum collections during the mid-late 19th Century (Worthy & Holdaway, 2002). Sediments associated with these bone deposits are varied, ranging from lime-rich sands and gyttja (Gregg, 1972) to dark, organic-rich mud and mossy peat (Worthy & Holdaway, 1996). However, the distribution of miring bone deposits appears closely linked to geology. Most occur within regions of calcite-rich schist or calcareous Tertiary marine sediments, where groundwater pH is sufficiently buffered to favour bone preservation. Holocene miring bone deposits are not known from areas of New Zealand with widespread acidic igneous rocks, such as Stewart Island (Worthy, 1998c) (Fig. 1).

Important early discovered bone deposits include Glenmark, Hamiltons, Te Aute, Enfield, and Kapua (Worthy & Holdaway, 2002). However, few data relating to sediment stratigraphy, or the position of bones within the sites were recorded. Also, before the advent of radiocarbon dating there was no way to accurately assess the relative age of individuals within a deposit. Therefore, interpretation of such deposits proved problematic for early scientists. Lyell (1850: 700) presented a deposition mechanism for mired quadrupeds in European peats: *"There are two ways in which animals become occasionally buried in the peat of marshy grounds; they either sink down into the semifluid mud, underlying a turfy surface, upon which they have rashly ventured, or, at other times, a bog 'bursts', and animals may be involved in the peaty alluvium"*. Despite this simple observation-based explanation, a range of catastrophic scenarios were subsequently suggested as deposition mechanisms for New Zealand miring bone deposits. Booth (1875) suggested they may contain the bones of birds that had died from drinking poisonous spring waters. Flooding was widely cited as a deposition mechanism for the bone deposits (e.g. Tregear, 1893; Hutton, 1900). Howorth (1887) noted: *"...in the caves, on the mud-flats near the sea-shore, and on the turbary deposits, both of the North and South islands, large collections of bones are found mixed together in utter confusion, as though a number of struthious*

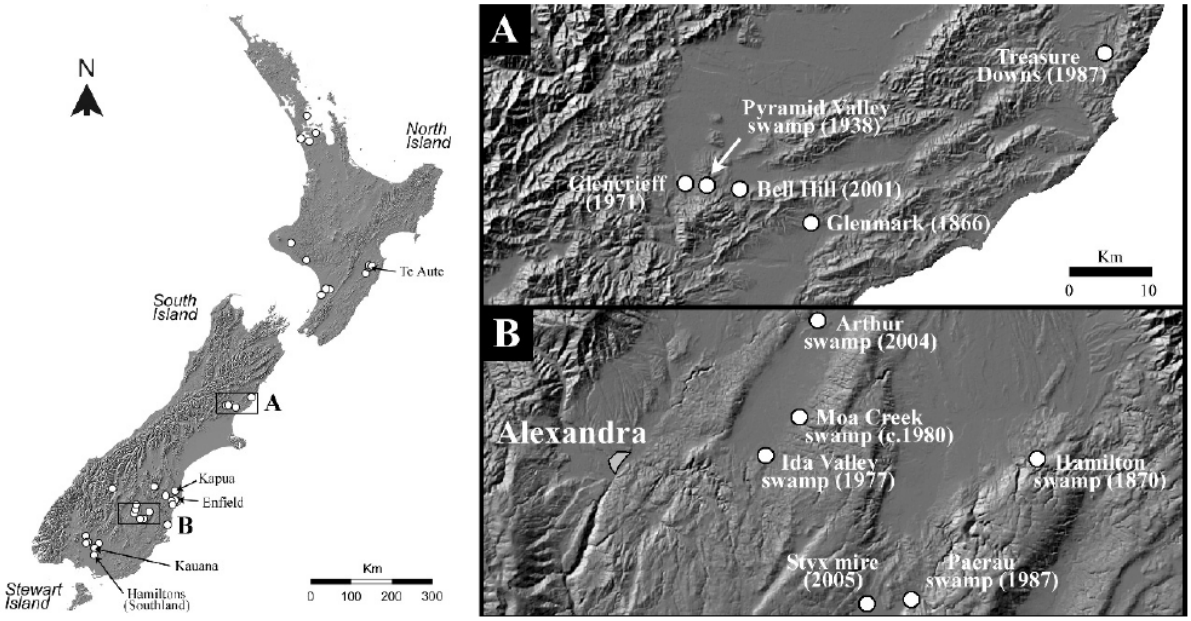

Figure 1. Location of major Late Pleistocene and Holocene miring bone deposits in New Zealand. A, major miring bone deposits in the Waikari area, North Canterbury, with years of first excavations, showing location of Glencrieff and Bell Hill sites; B, major miring bone deposits in the Central Otago region, with years of first excavations, showing location of Styx.

birds of different genera and species, overtaken and driven together by a common peril, had perished in one general catastrophe".

The 'panic as a result of wildfire' hypothesis was widely held until the early 20th Century, despite Booth's (1875: 130) dismissal of this hypothesis: "*in such a case, for them to stop, gather in a small waterhole, and allow them-selves to be thus smothered, would manifest an amount of stupidity that none of the brute creation has been known to possess.*" Buick (1931: 234) described the scenario as he imagined it: "*Impelled by intense fear of flames and blinding smoke driven by a north wind, the birds would congregate in large flocks when, if after travelling many miles of rough country, safety and satiation of a parching thirst seemed to lie in an open swamp where their implacable pursuer might not reach them, the leaders would plunge into the morass, only to be trampled to death by the oncoming mass of avian terror. Before the avalanche of frightened feather had ceased, the swamp would be full, and, if some escaped, there were still sufficient heaped high upon a suffocating pile to make a holocaust of such dimensions as to cause appreciable mortality in the ranks of the Moas, and to bewilder, with its mystery and enormity, the enquiring mind of modern man.*"

However, some authors did not believe the bones were instantaneously deposited, but that they had accumulated over a long period of time. Booth (1875) observed that bones near the surface of Hamiltons were in better condition, and therefore probably younger, than those at depth. He suggested that birds had been

Figure 2. Disarticulated and densely intermingled moa leg bones, Glencrieff, January 2007. This taphonomic feature is typical of many New Zealand miring bone deposits.

drawn to such sites during severe winter frosts, when the temperature of the spring water was warmer than that of the surrounding ground. Pyke (1890) suggested that the birds may have become mired while searching for food. The idea that wetland margins were part of the natural habitat of some moa species, and that individual birds were trapped when venturing too far into these sites, is supported by radiocarbon dating, which shows that bones in these deposits have accumulated over hundreds or thousands of years (Worthy & Holdaway, 2002). This is now the most widely accepted interpretation of these bone deposits.

The first spatial measurements of bones within a New Zealand miring bone deposit were made at Hamiltons, Otago (Fig. 1), in 1870 (Booth, 1875), in an attempt to test whether the deposit had formed by birds becoming mired, or if the bones had been washed in. If the former was the case, it was hypothesised that orientation of bones within the deposit should represent the standing position of a bird (i.e. proximal higher than distal). The position of 71 femora and 38 foot bones were recorded. No pattern was obvious, so Booth (1875) dismissed the miring hypothesis. Excavations of the bone deposit at Pyramid Valley, North Canterbury (Fig. 1), from 1939 to 1973, yielded over 183 individual moa skeletons, and bones of 46 bird species, three of which were previously undescribed (Worthy & Holdaway, 2002). This site has become one of the most studied late Quaternary fossil deposits in New Zealand, and was the first miring bone deposit where detailed records of stratigraphy were made (e.g. Duff, 1941; Moar, 1970; Gregg, 1972). An excavation at Glencrieff, North Canterbury (Fig. 1) in 1993 was the first time detailed measurements of bone depths, orientations, and inclinations were made in a miring bone deposit (Worthy & Holdaway, 1996). Some minor taphonomic data were also collected during the excavations at Hamiltons, Southland (1974) and Kauana (1994) (Worthy, 1998d). Since then, measurements of the three dimensional position of individual bones have been recorded during three excavations of miring bone deposits. In this paper we analyse these spatial data with particular focus on moa bones. We compare the results with those obtained by Worthy & Holdaway (1996) and interpret aspects of the deposition mechanisms acting in New Zealand miring bone deposits.

Site terminology

In the past, New Zealand miring bone deposits have commonly been referred to as either 'moa swamps' (e.g. Booth, 1875; Falla, 1941) or 'mires' (e.g. Burrows, 1989). Although both have been used by palaeontologists to describe the same deposits, ecologists apply specific definitions to these terms. We have referred to these deposits collectively as miring bone deposits. Mire is an ecological term broadly synonymous with peatland (Johnson & Gerbeaux, 2004), and the term 'miring' has often been used to describe the trapping of animals in such sites (e.g. Burrows, 1989; Graham & Graham, 1990; Worthy & Holdaway, 1996). Following the definitions of Johnson & Gerbeaux (2004), major New Zealand miring bone deposits occur in at least two wetland classes. The sites at Styx and Paerau (Fig. 1) are examples of 'fens'; oligotrophic to mesotrophic wetland where the water table is close to the peat surface and there is a significant geotrophic, or spring, water input. The Pyramid Valley site (Fig. 1) is an example of a 'swamp';

which are characterised by having a significant input of ground water runoff, and often have water tables higher than the ground surface. Bone deposits may also potentially occur in hillside 'seepages' e.g. The Deans, upper Waipara River, North Canterbury (Worthy & Holdaway, 1996). No New Zealand bone deposits are known from 'bogs'; acidic, ombrotrophic wetlands. Differentiation of bone deposits using this classification system will provide more information on the typical stratigraphy and deposition mechanisms that might be present in these different wetland types.

Study sites

Glencrieff

The Glencrieff site is located approximately 6.5 km southwest of Hawarden, North Canterbury (42° 58' 07.45" S, 172° 34' 01.84" E) (Fig. 1). A full account of the history, stratigraphy, and extent of the bone deposit was given by Worthy & Holdaway (1996). Eaves's private collection from this site has subsequently been 'lost' overseas to a private collection in the United Kingdom. A pit measuring 5 m × 2 m, with a 0.5 m extension for a 2 m length of the pit's northern face, was excavated in January 2007, following location of bones by probing with a metal rod. The pit is equivalent to squares I0, I1, I2, I3, J0, J1, J2, J3, and half of G1 and G2, where rows I and J extend southwest from row G on the grid system mapped by Worthy & Holdaway (1996) (Fig. 3).

Styx

The Styx site is located on the western side of the Styx Basin, Central Otago (45° 25' 14.49" S, 169° 51' 03.47" E) (Fig. 1). The site consists of a circular fen within a linear wet depression running parallel to the base of a terrace. An L-shaped pit measuring 2 m × 1.5 m was excavated in the centre of the fen in April 2005, following the discovery of bones there by probing with a metal rod.

Bell Hill

The Bell Hill deposit is located approximately 6 km southwest of Hawarden, North Canterbury (42° 58' 11.91" S, 172° 39' 59.26" E) (Fig. 1). Bones were accidentally discovered by a mechanical digger during excavation of a

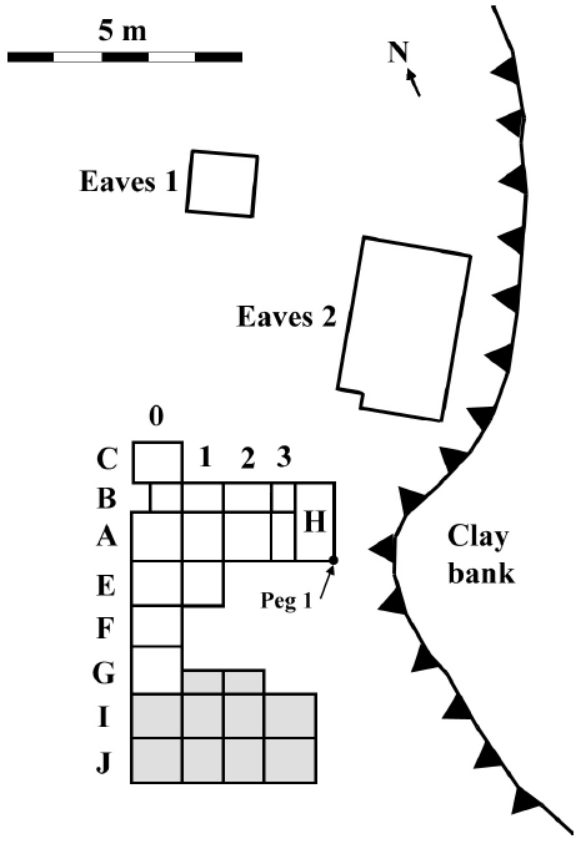

Figure 3. Sketch plan of Glencrieff site (based on Worthy & Holdaway, 1996), showing previously excavated squares, and location of the 2007 excavation (grey squares).

gully. Undisturbed deposit below the excavated surface, and along the edge of the gully, was surveyed on a grid of 1 m squares. This paper describes data associated with bones excavated from squares D12, E11, E12, F11, F12, G11, G12, and H12 in 2001.

Methods

Field data collection

Glencrieff and Styx

X and Y axes were assigned to each excavation pit, and the bearing of each axis from the point of origin was recorded (hereafter referred to as β and χ for X and Y axes respectively). Prior to the removal of individual long bones from the excavation pit, the following spatial measurements were recorded: X, Y and Z coordinates to the proximal tip of the bone (recorded as distance in mm along each axis from point of origin); the horizontal direction of the bone (α), measured as a bearing from the proximal to distal end of the bone; and the inclination of the bone (θ), measured as the angle between the bone and a horizontal plane intersecting its proximal end. Where $Z_{proximal} > Z_{distal}$, the inclination of the bone was recorded as a negative value. Following removal of the bone from the pit, its length (δ) was also recorded. These data are now recorded with specimens catalogued at Canterbury Museum (Glencrieff, uncatalogued) and Otago Museum (Styx, catalogue OM Av10809).

Bell Hill

X, Y and Z coordinates were obtained for the point midway between the proximal and distal ends of each bone (point M). Bearings (α) and inclinations (θ) of bones were recorded towards the proximal ends of bones. The co-ordinates are recorded with each specimen catalogued in Museum of New Zealand (catalogue range MNZ S.39931-S.40739).

Data formatting and GIS analysis

Cartesian coordinates for the distal end of each bone at Glencrieff and Styx were obtained using the following set of equations:

$$X_{distal} = X_{proximal} + (\sqrt{((\delta)^2 - (\delta \times \cos(90-\theta))^2)} \times \cos(\alpha - \beta))$$

$$Y_{distal} = Y_{proximal} + (\sqrt{((\delta)^2 - (\delta \times \cos(90-\theta))^2)} \times \cos(\alpha - \chi))$$

$$Z_{distal} = Z_{proximal} + (\delta \times \cos(90-\theta))$$

Lengths of individual bones from Bell Hill were not recorded, so approximate average lengths of each element for each species were used as δ. Cartesian coordinates for the proximal and distal ends of bones from Bell Hill, based on mean lengths, were obtained using the following set of equations, where τ is the opposite bearing to α ($\tau = \alpha + 180$, if $\alpha < 180$; $\tau = \alpha - 180$, if $\alpha > 180$):

$$X_{proximal} = X_M + (\sqrt{((0.5\delta)^2 - (0.5\delta \times \cos(90-\theta))^2)} \times \cos(\alpha - \beta))$$

$$Y_{proximal} = Y_M + (\sqrt{((0.5\delta)^2 - (0.5\delta \times \cos(90-\theta))^2)} \times \cos(\alpha - \chi))$$

$$Z_{proximal} = Z_M + (0.5\delta \times \cos(90-\theta))$$

$$X_{distal} = X_M + (\sqrt{((0.5\delta)^2 - (0.5\delta \times \cos(90+\theta))^2)} \times \cos(\tau - \beta))$$

$$Y_{distal} = Y_M + (\sqrt{((0.5\delta)^2 - (0.5\delta \times \cos(90+\theta))^2)} \times \cos(\tau - \chi))$$

$$Z_{distal} = Z_M + (0.5\delta \times \cos(90+\theta))$$

Data representing the three dimensional position of long bones from individual bird species within each mire site were saved as a comma separated value (.csv) file with 7 values on each line representing a single bone: element type, $X_{proximal}$, $Y_{proximal}$, $Z_{proximal}$, X_{distal}, Y_{distal} and Z_{distal}. The file was run in a vbscript conversion program (Appendix 1) in ArcScene 9.2 (ArcGIS 9.0) to produce a z-enabled shape-file with lines representing the bones. A .csv file, in the same format, containing data for lines marking the edges of the excavated pit was also run in the conversion program.

Results

Stratigraphy

Sediment stratigraphy was relatively consistent between sites. At Glencrieff and Styx the stratigraphy consisted of basal gravels acting as an aquifer, overlain by a layer of grey-blue clay and bone-bearing peat (Fig. 4). The thickness of these layers varied within individual sites. At Bell Hill the stratigraphy had been disturbed prior to examination, although it was noted that the bone-bearing peat layer was resting upon a Tertiary green sand unit. At the edge of the peat was a bank of weathered colluvium derived from the calcareous marl that stratigraphically overlies the green sand, and forms a hill above the site.

Bone orientation and spatial distribution

Plotting the relative position of long bones (femora, tibiotarsi, tarsometatarsi) in ArcScene allowed the spatial distribution and orientation of bones within each deposit to be viewed (Fig. 5). Different colours were used to assess spatial patterns in the distribution of moa species, age categories and element types within each deposit (Fig. 5), although no obvious patterns were identified.

The orientation of bird long bones in all three miring deposits was non-random. Most bones had subhorizontal inclination (Fig. 6). Long bones within all three individual deposits showed a preferential orientation (Fig. 6), although the direction and strength of this orientation varied between sites. The preferential orientation of long bones at Bell Hill was north-northeast/south-southwest; at Glencrieff was north/south; and at Styx was west/east.

Long bones were not equally distributed throughout the excavated pits, but formed concentrations in certain areas (Fig. 5). At Glencrieff, bones were concentrated close to the clay slope at the edge of the wetland. Bones were also dense close to the clay slope on the edge of the Bell Hill deposit. However, due to disturbance from a mechanical excavator, the density of bones further out from the slope could not be ascertained. At Styx, bones were concentrated in the deepest peat at the centre of the circular fen. Excavations at the edge of the fen showed that no bones occurred in peat presently < 50cm thick.

The depths of proximal ends of long bones were normally distributed at all three sites (Fig. 7). At Glencrieff and Styx these were found most commonly at <1m depth below the ground surface, but extended deeper at Bell Hill, where the upper part of the deposit had been removed by a mechanical digger.

The density of long bones also varied between excavated pits, ranging from 49 m^{-3} at Bell Hill, to 28.9 m^{-3} at Styx, and 6.5 m^{-3} at Glencrieff.

Few radiocarbon dates were available for bones from the excavation pits examined

in this paper. However, radiocarbon dates from Bell Hill suggest that some age stratification of bones exists within these deposits (Fig. 8). Two articulated feet of *Dinornis robustus* were found on the top of the peat, the proximal ends of the tarsometatarsi, and possibly the rest of the skeleton, having been lost due to recent erosion of the peat surface. One of these bones had an age of 914 ± 75 14C years BP (NZA-13749) (799 ± 124 cal. years BP; SHCal04 calibration curve). A bone lying on the greensand base was older, at 2107 ± 65 14C years BP (NZA-13750) (2083 ± 217 cal. years BP; SHCal04 calibration curve).

Element bias

Analysis of moa skeletal elements recovered from Bell Hill shows different elements had different probabilities of preservation and recovery. This is not an excavation bias as all sediments from these excavation squares were sieved through a 6 mm mesh. The largest and heaviest leg bones (tibiotarsi, femora and tarsometatarsi) were the most commonly recovered elements, whereas smaller, lighter elements (e.g. phalanges, vertebrae) were less common (Fig. 9). The same pattern was also found at Styx and Glencrieff.

Discussion

Site location and stratigraphy

The sediment stratigraphy of the mires can be used to interpret aspects of their development and deposition mechanism. It

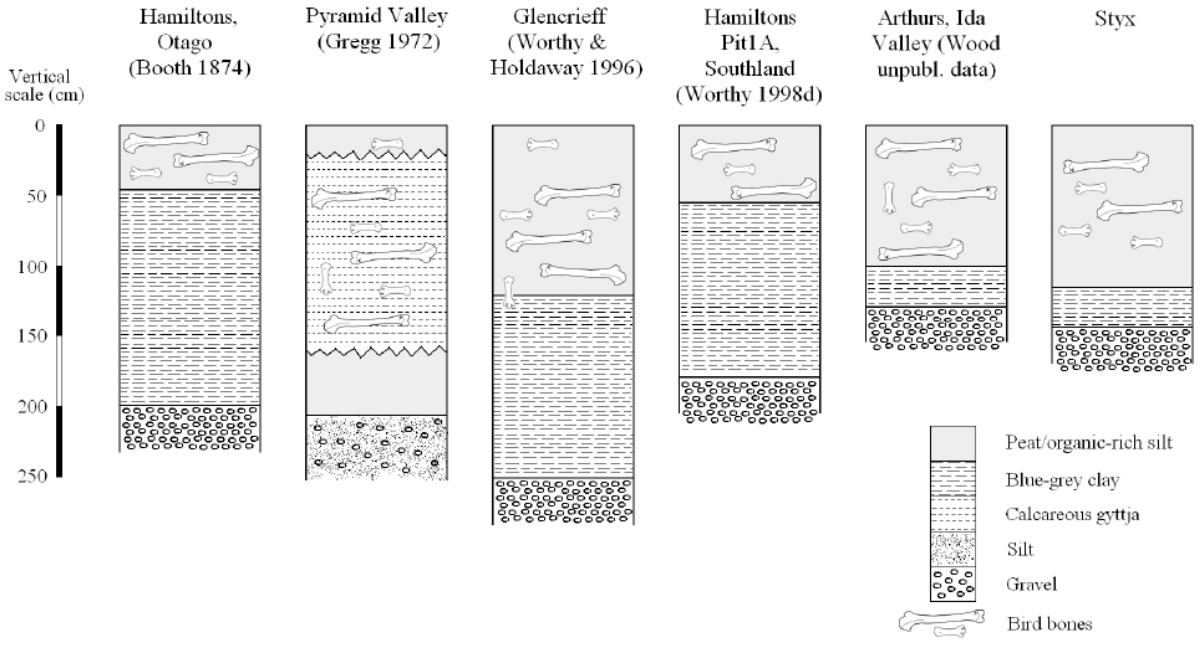

Figure 4. Stratigraphy of six Late Pleistocene and Holocene miring bone deposits from South Island, New Zealand. Sediment layer thicknesses shown for Hamiltons, Otago, are means of the range of values given by Booth (1875).

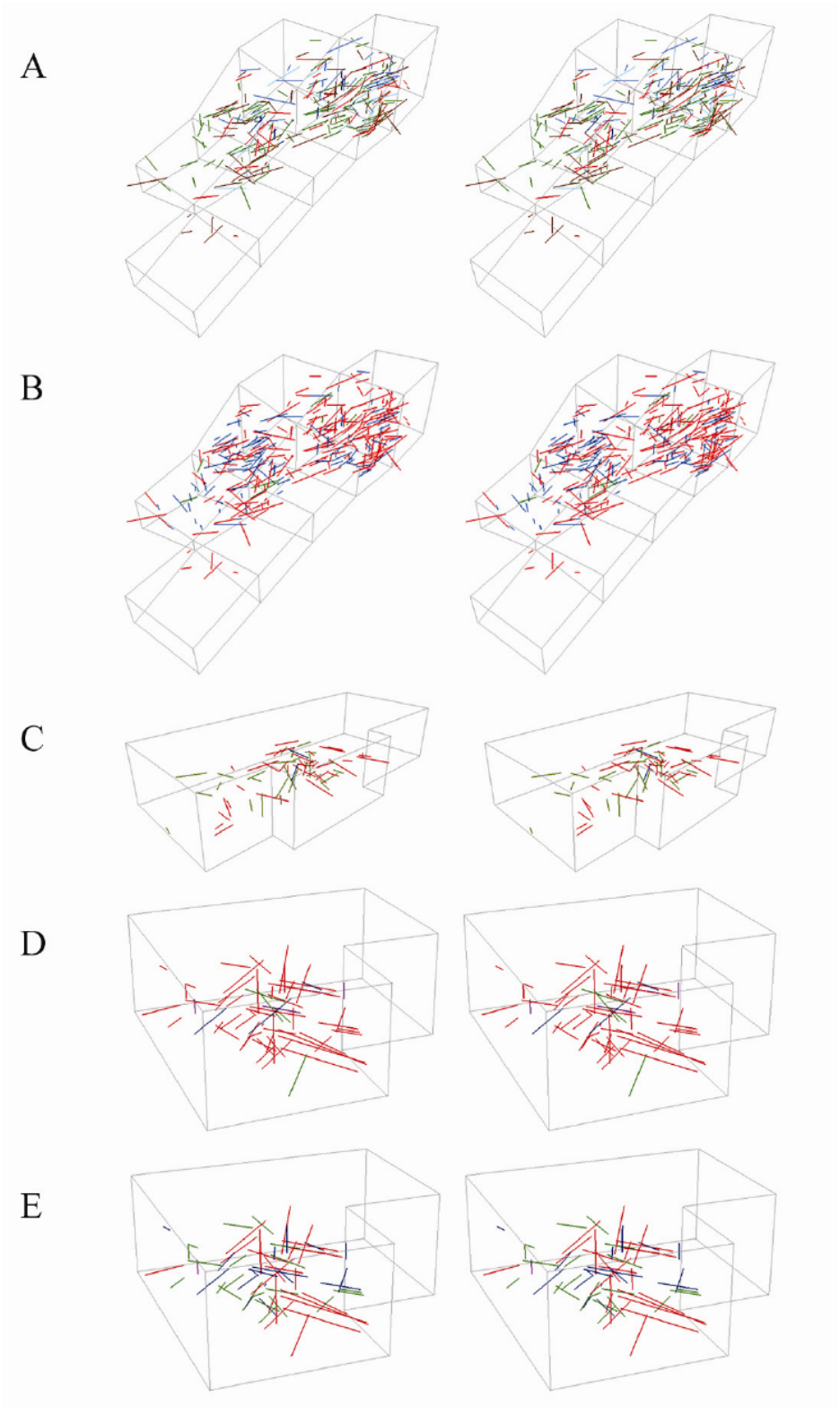

Figure 5. Stereo-pair representations of miring bone deposits: A and B, Bell Hill, North Canterbury; C, Glencrieff, North Canterbury; D and E, Styx, Central Otago.

A, C and D show position of long bones from different species: red, *Pachyornis elephantopus*; brown, *Euryapteryx gravis*; green, *Emeus crassus*; blue, *Dinornis robustus* (shown in A as: light blue, female; medium blue, male; dark blue, sex unknown).

B shows position of long bones from different aged individuals: blue, juvenile; green, subadult; red, adult.

E shows position of different avian elements: blue, tarsometatarsi; red, tibiotarsi; green, femora; purple, radii (*Cnemiornis calcitrans*).

Figure 6. Bone orientation data from Bell Hill, Glencrieff, and Styx excavations: A, orthographic plan views of miring bone deposits showing position of bird long bones; B, horizontal bearing of bird long bones (distal ends), and; C, inclination of bone, measured as the angle formed between bird long bones and horizontal planes through their proximal ends. Stereoplot figures were plotted using the GEOrient v9.x program developed by Dr. Rod Holcombe. m.r.d. = mean resultant direction.

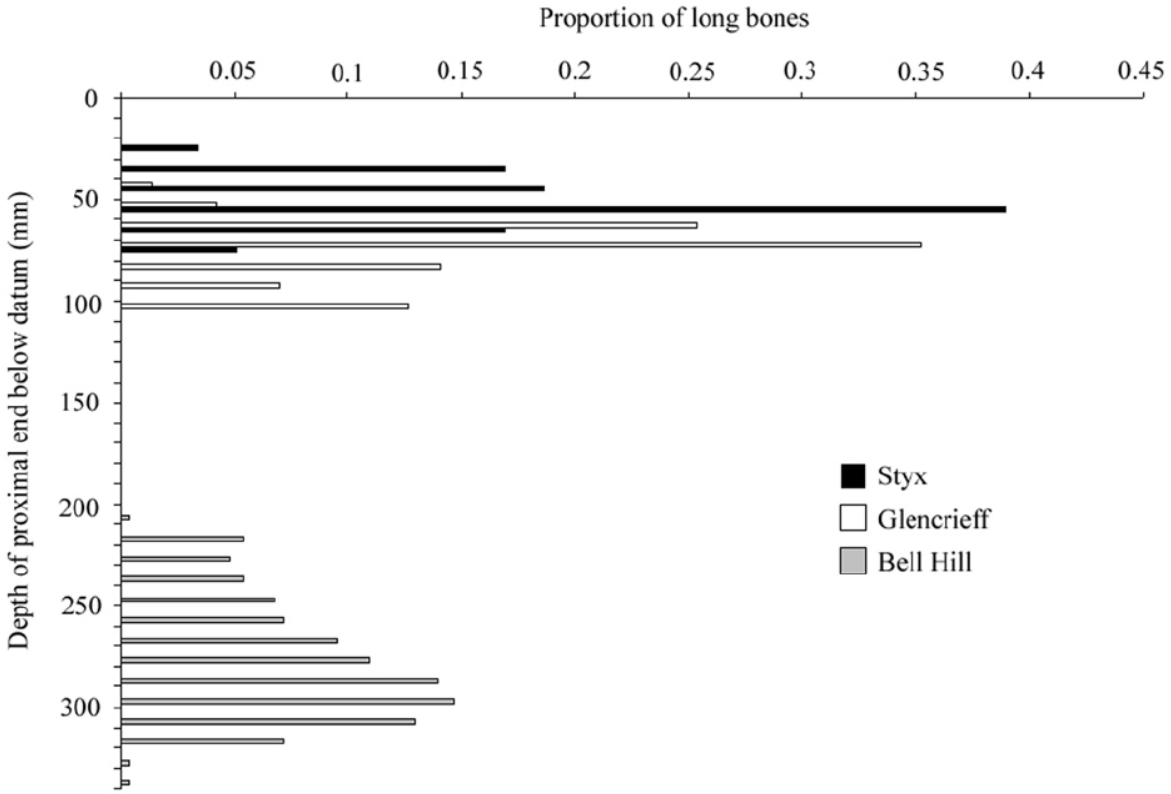

Figure 7. Distribution of proximal ends of bones with respect to depth below datum (present ground surface).

was noted that most of these sites have an artesian inflow. At Glencrieff and Styx, this appears to be from pressurised water in the underlying gravels being forced through fractures in the confining clay layers. At Bell Hill, incision of a shallow valley has intersected the contact between a porous greensand and confining marl, and it is from this contact that the spring flows. There are also mound springs such as Makirikiri (Worthy, 1989) where water from surrounding hills rises on a terrace and forms a mound with pooled water in the spring mouth. Bones can be very deep in such sites e.g. >5m in Makirikiri and Kauana (Worthy 1998d). The stratigraphy of the swamp site in Pyramid Valley reported by Gregg (1972) (Fig. 4) differed to that of these sites. It suggests that birds became mired when walking into, or possibly breaking through a thin crust on top of, a calcareous gyttja deposited on the bed of a shallow lake (Gregg, 1972).

The results indicate that fluid sediments would have had to accumulate to depths of at least 50 cm before they began trapping moa. In the early history of some sites, mired moa may have been able to walk about on a hard clay or sand base. However, growth of the peat in these sites would have meant subsequently mired moa were effectively 'swimming' in the sediment.

Spatial distribution

Taphonomic data collected from all three sites were very similar to those reported from the 1993/94 excavations at Glencrieff by Worthy & Holdaway (1996), and measurements made at Hamiltons and Kauana in Southland (Worthy, 1998d). Similar characteristics between all sites include the subhorizontal alignment of long bones, normal distribution of bone frequency with respect to depth, and under representation of small, light skeletal elements. Preferential orientation of long bones to a particular compass bearing was recorded at Glencrieff, Bell Hill and Styx.

We suggest two possible influences on the orientation of bones: the struggling action of mired birds, and the local groundwater flow direction. At Bell Hill and Glencrieff, long bones were preferentially orientated parallel to the boundary between the peat and adjacent clay banks/bank. The orientation of bones excavated at Glencrieff in 1993 (Worthy & Holdaway, 1996) differed slightly to those excavated in 2007. However, these were still subparallel to the edge of the adjacent terrace, which deflects to the northeast near the location of the 1993 excavation (Worthy & Holdaway, 1996). The direction of groundwater flow in these sites is also parallel to the terrace edge. Groundwater may have some influence on the alignment of bones in these sites when the sediment was liquefied, but the rate of flow is likely to be too low to make a significant contribution to bone alignment. Worthy & Holdaway (1996) concluded that the section of deposit they excavated was a 'channel' for outflow of the spring. The section of the deposit examined in this paper, and also the Eaves excavations, could be interpreted as extensions of this, revealing a sinuous channel subparallel to the eastern bank. Greater water flow along this more liquid route, towards a northern outlet, and bounded by marginal peat on the bank and a floating peat morass in the centre may be sufficient to explain the alignment of bones. However, another

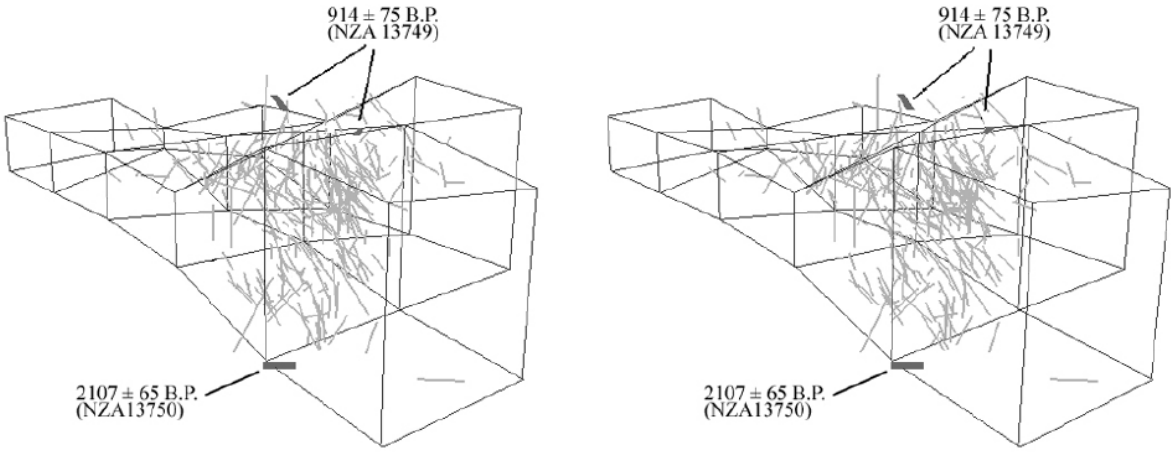

Figure 8. Stereo-pair representation of moa long bones in the Bell Hill excavation pits, showing position and age of radiocarbon dated specimens.

possible explanation exists in the probable actions of moa that were mired in these sites. Moa that ventured off the stable clay banks into these wetlands would have broken through a thin peaty crust and found themselves mired in semi-liquid sediment. These birds are likely to have tried to escape by scrabbling up onto the clay banks. The raking action of the moa legs on the clay bank as they struggled to escape would have resulted in the concentration and preferential alignment of bones parallel to the bank.

Long bones in Styx were preferentially orientated perpendicular to the adjacent terrace. However, the layout of Styx mire is different to that of Glencrieff and Bell Hill, and the orientation was not as significant as that seen at Bell Hill. The Styx bone deposit is within a spring fen, 25-30 m

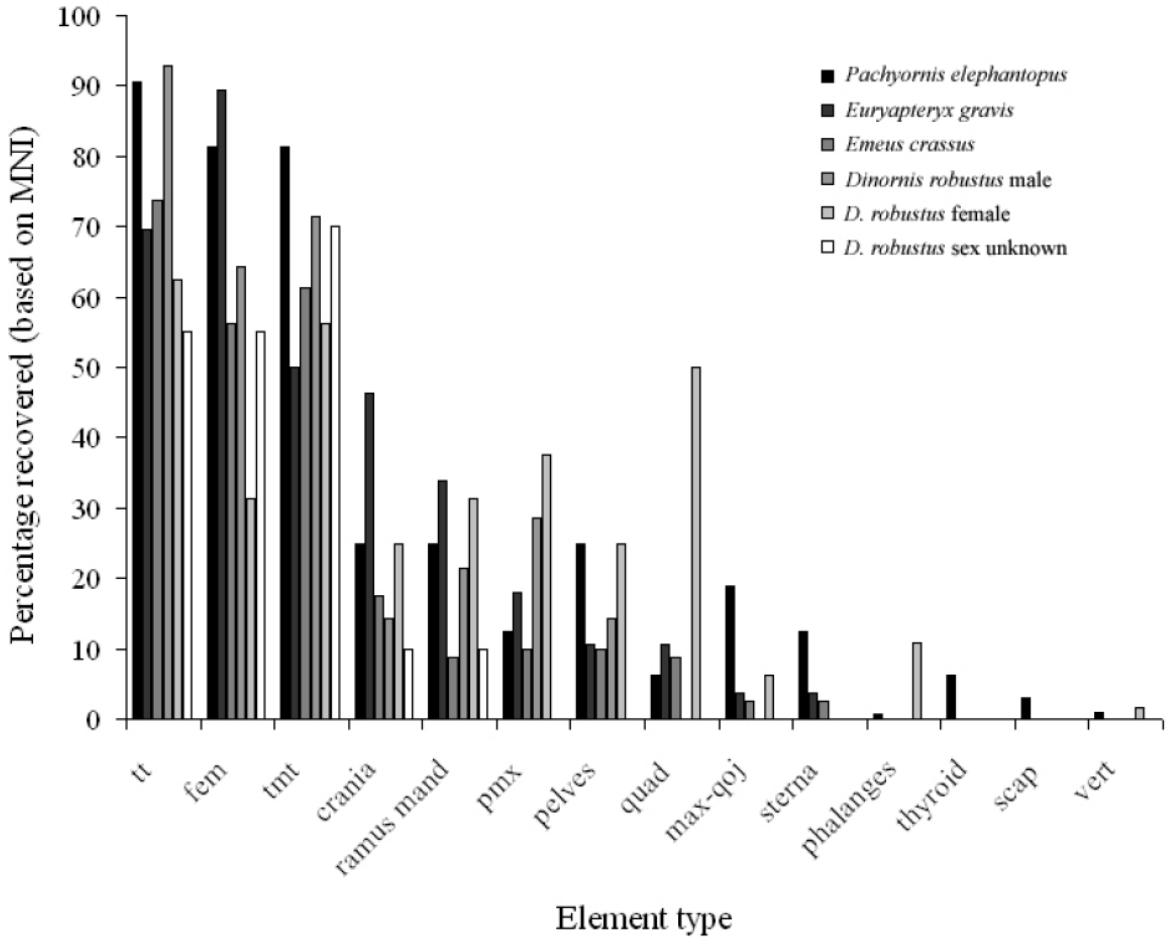

Figure 9. Percentage of preserved/recovered moa skeletal elements from Bell Hill, based on expected number of specimens from minimum number of individuals (MNI). tt, tibiotarsi; fem, femora; tmt, tarsometatarsi; ramus mand, ramus mandibulae; pma, premaxillae; quad, quadrates; max-qoj, quadratojugal; scap, scapulocoracoid; vert, vertebrae.

from the base of the adjacent terrace. Between the spring and the terrace, and running parallel to the base of the terrace, is a natural depression containing a small stream. The natural flow of water from the spring bog would be towards this depression, thus perpendicular to the terrace edge. Whether the bone orientation at Styx was real, and due to the effect of water flow, or is an artefact of having too few measurements, cannot be determined. However, some deposits may show no bone orientation, as was noted at Hamiltons, Southland (Worthy, 1998d). At both Styx and Hamiltons, Southland, there is no obvious one direction that moa would have tried to escape from the miring, which may account for possible random orientation of bones at these sites.

The subhorizontal alignment of most long bones within the miring deposits reflects the natural buoyancy position of bones in a fluid or semi-fluid state. Miring bone deposits are typically wet, yet most appear insufficiently fluid to allow easy migration of bones. Therefore, much of the orientation of bones may have taken place during discrete liquefaction events, such as the struggling of trapped birds.

Element bias

Under-representation and poor preservation of small, fragile skeletal elements was also noted in the moa assemblage from Kauana, Southland (Worthy, 1998d), and has been reported for assemblages of large mammals from the Rancho La Brea tar seeps (Spencer *et al.,* 2003). Spencer *et al.* (2003) proposed that this taphonomic bias at Rancho La Brea was due to removal of certain skeletal elements from the site by large scavengers, however this is not as applicable to the New Zealand situation. We suggest three possible explanations for the bias in preservation and recovery of different moa skeletal elements (Fig. 9). First, when the bird walked into the wetland and became mired, the legs would have been submerged in anaerobic sediment, and therefore had increased chance of preservation. Following the death of the bird, any parts remaining on the surface (cervical vertebrae, cranial elements) would have been prone to scavenging by Haast's eagle (*Harpagornis moorei*), Eyles' harrier (*Circus eylesi*) and kea (*Nestor notabilis*) (Archey, 1941; Worthy & Holdaway, 1996), transport away from the site by surface water flow, and weathering (Eyles, 1955). This latter process was particularly evident at Styx, where only the lower halves of most pelves were preserved. Second, liquefaction of sediment by struggling birds would have acted to 'pump out' the smaller, lighter bones, lifting them to the surface through buoyancy, where they would have had less chance of preservation, and where outlet flows can remove them from the site (e.g. at Bell Hill, and likely at Glencrieff). This probably explains the under representation of phalanges, which should have been preserved due to submergence in the sediment. Third, fragile elements in the bone deposit, such as sterna, would have had a greater chance of being crushed by struggling birds than the more robust leg bones. We do not believe that dissolution contributed to the loss of small elements in the mirings examined, as there was little evidence for this process on the surfaces of larger bones.

Deposition

In New Zealand's late Quaternary bone deposits it is possible to discriminate the individual events that have contributed to the accumulations, and through radiocarbon

determinations show that deposition usually occurred over hundreds or a few thousand years. If, however, such deposits underwent slight geological compression and were viewed from a period distant in time there would be the temptation to invoke catastrophic events to explain the deposits (e.g. Rogers, 1990; Henrici & Fiorillo, 1993). Our data show that the presence of disarticulation definitely allows for the interpretation of considerable accumulation time for a bone deposit, but even mass deposits of articulated specimens do not need imply catastrophic events. In Pyramid valley, most moa specimens are articulated yet are likely to have been deposited over a range of 2000-4000 years (Holdaway & Worthy, 1997). So, if the deposit was slightly compressed and seen in some millions of years time, effectively instantaneous burial could be erroneously invoked. There is no evidence for catastrophic events being causal factors in any miring bone deposit in New Zealand.

GIS as a spatial distribution tool

Our method works as a simple way of viewing relative positions of bones from an excavation using widely available GIS software. Bones can be plotted as lines or tubes, and rotated on a computer monitor to be viewed from different angles. Functions of the program can also be used to view the excavation pit and bones as three dimensional stereo-pair images or red-blue anaglyphs. These provide a more detailed overview of the site, and the orientation of bones within the deposit, than is available in the field. Similar methods have previously been used in other studies (e.g. Jennings & Hasiotis, 2006) and have a wide range of applications in paleontology and archaeology. Further improvements of the method may include accurate representation of the bones by three-dimensional scans.

Conclusions

All three deposits examined in this study seem to have formed in a similar way; with the periodic miring of individual birds, subsequent disarticulation and settling of bones within semi-liquid sediment, and preferential orientation either through struggling of mired birds or water flow. Further excavations are required to reveal whether subhorizontal inclination and preferential alignment of long bones are characteristic taphonomies for New Zealand miring bone deposits. The methods presented in this paper provide a simple framework for the type of data that should be collected during future excavations, both scientific and salvage-based, of New Zealand miring bone deposits. Data on more sites will help interpret the full range of deposition mechanisms and post-depositional processes operating within these sites.

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References

Archey, G. (1941). The moa. *Bulletin of the Auckland Institute and Museum,* 1: 1-245.

Booth, B.S. (1875). Description of the Moa Swamp at Hamilton. *Transactions and Proceedings of the Royal Society of New Zealand*, 7: 123-138.

Buick, T.L. (1931). *The mystery of the moa*. Thomas Avery & Sons Limited, New Plymouth.

Burrows, C.J. (1989). Moa browsing: evidence from the Pyramid Valley mire. *New Zealand Journal of Ecology*, 12 (supplement): 51-56.

Clark, G.R., Petchey, P., McGlone, M.S. & Bristow, P. (1996). Faunal and floral remains from Earnscleugh Cave, Central Otago, New Zealand. *Journal of The Royal Society of New Zealand*, 26: 363-380.

Duff, R.S. (1941). Notes on the excavations at Pyramid Valley, Waikari. *Records of the Canterbury Museum*, 4: 330-338.

Eyles, J.R. (1955). Field notes on the excavations. *Records of the Canterbury Museum*, 6: 257-260.

Falla, R.A. (1941). Preliminary report on excavations at Pyramid Valley Swamp, Waikari, North Canterbury: the avian remains. *Records of the Canterbury Museum,* 4: 339-353.

Forbes, H.O. (1892). On a recent discovery of the remains of extinct birds in New Zealand. *Nature*, 45: 416-418.

Fraser, T. (1873). A description of the Earnsclough (*sic.*) moa cave. *Transactions and Proceedings of the New Zealand Institute,* 5: 102-105.

Furrer, H., Graf, H.R. & Mäder, A. (2007). The mammoth site of Niederweningen, Switzerland. *Quaternary International*, 164-165: 85-97.

Gillespie, R., Horton, D.R., Ladd, P., Macumber, P.G., Rich, T.H., Thorne, R. & Wright, R.V.S. (1978). Lancefield Swamp and the Extinction of the Australian Megafauna. *Science*, 200: 1044-1048.

Graham, M.A. & Graham, R.W. (1990). Holocene Records of *Martes pennanti* and *Martes americana* in Whiteside County, Northwestern Illinois. *American Midland Naturalist*, 124: 81-92.

Gregg, D.R. (1972). Holocene stratigraphy and Moas at Pyramid Valley, North Canterbury, New Zealand. *Records of the Canterbury Museum*, 9: 151-158.

Hamilton, A. (1889). Notes on a deposit of moa-bones in the Te Aute Swamp, Hawke's Bay. *Transactions and Proceedings of the New Zealand Institute*, 21: 311-318.

Harris, W.F. (1955). Progress report on pollen statistics from Pyramid Valley swamp. *Records of the Canterbury Museum*, 6: 279-290.

Henrici, A.C. & Fiorillo, A.R. (1993). Catastrophic death assemblage of *Chelomophrynus bayi* (Anura, Rhinophrynidae) from the middle Eocene Wagon Bed Formation of Central Wyoming. *Journal of Paleontology*, 67: 1016-1026.

Holdaway, R.N. & Worthy, T.H. (1997). A reappraisal of the late Quaternary fossil vertebrates of Pyramid Valley Swamp, North Canterbury, New Zealand. *New Zealand Journal of Zoology*, 24: 69-121.

Howorth, H.H. (1887). *The Mammoth and the Flood: An Attempt to Confront the Theory of Uniformity with the Facts of Recent Geology*. S. Low, Marston, Searle, & Rivington, London.

Hutton, F.W. (1900). The Geological History of New Zealand. *Transactions and Proceedings of the New Zealand Institute*, 32: 159-183.

Jennings, D.S. & Hasiotis, S.T. (2006). Taphonomic analysis of a dinosaur feeding site using geographic information systems (GIS), Morrison Formation, southern Bighorn Basin, Wyoming, USA. *Palaios,* 21: 480-492.

Johnson, P. & Gerbeaux, P. (2004). *Wetland types in New Zealand*. Department of Conservation, Wellington.

Lyell, C. (1850). *Principles of Geology*. John Murray, London.

Moar, N.T. (1970). A new pollen diagram from Pyramid Valley Swamp. *Records of the Canterbury Museum*, 8: 455-461.

Pyke, V. (1890). *The moa (Dinornis) and the probable cause of its extinction*. Evening Post Office, Wellington.

Rogers, R.R. (1990). Taphonomy of three dinosaur bone beds in the upper Cretaceous Two Medicine Formation of Northwestern Montana: Evidence for drought-related mortality. *Palaios,* 5: 394-413.

Spencer, L.M., Van Valkenburgh, B. & Harris, J.M. (2003). Taphonomic analysis of large mammals recovered from the Pleistocene Rancho La Brea tar seeps. *Paleobiology*, 29: 561-575.

Tregear, E. (1893). The Extinction of the Moa. *Transactions and Proceedings of the New Zealand Institute*, 25: 413-426.

Worthy, T.H. (1989). An analysis of moa bones (Aves: Dinornithiformes) from three lowland North Island swamp sites: Makirikiri, Riverlands and Takapau Road. *Journal of The Royal Society of New Zealand*, 19: 419-432.

Worthy, T.H. (1997). Quaternary fossil fauna of South Canterbury, South Island, New Zealand. *Journal of The Royal Society of New Zealand,* 27: 67-162.

Worthy, T.H. (1998a). Quaternary fossil faunas of Otago, South Island, New Zealand. *Journal of The Royal Society of New Zealand*, 28: 421-521.

Worthy, T.H. (1998b). Fossils indicate *Pelecanoides georgicus* had large colonies at Mason Bay, Stewart Island, New Zealand. *Notornis*, 45: 229-246.

Worthy, T.H. (1998c). Fossil avifaunas from Old Neck and Native Island, Stewart Island – Polynesian middens or natural sites? *Records of the Canterbury Museum*, 12: 49-82.

Worthy, T.H. (1998d). The Quaternary fossil avifauna of Southland, South Island, New Zealand. *Journal of The Royal Society of New Zealand*, 28: 537-589.

Worthy, T.H. & Holdaway, R.N. (1994). Quaternary fossil faunas from caves in Takaka Valley and on Takaka Hill, northwest Nelson, Sout Island, New Zealand. *Journal of The Royal Society of New Zealand*, 24: 297-391.

Worthy, T.H. & Holdaway, R.N. (1996). Quaternary fossil faunas, overlapping taphonomies, and palaeofaunal reconstruction in North Canterbury, South Island, New Zealand. *Journal of The Royal Society of New Zealand,* 26: 275-361.

Worthy, T.H. & Holdaway, R.N. (1997). A reappraisal of the late Quaternary fossil vertebrates of Pyramid Valley Swamp, North Canterbury, New Zealand. *New Zealand Journal of Zoology*, 24: 69-121.

Worthy, T.H. & Holdaway, R.N. (2002). *The lost world of the Moa*. Canterbury University Press, Christchurch.

Worthy, T.H. & Zhao, J.X. (2006). A late Pleistocene predator-accumulated avifauna from Kids Cave, West Coast, South Island, New Zealand. *Alcheringa Special Issue*, 1: 389-408.

Appendix 1

```
MoaBones.vbs
Purpose: Loops through a csv file to spatially
enable 3D bone xyz observation
SM Jones
5 April 2007

'Create the Geoprocessor object
set gp = wscript.createobject
("esriGeoprocessing.GPDispatch.1")

'Display the file name
gp.addmessage ("")
gp.addmessage ("**********************")
gp.addmessage ("*  Plot Moa Bones*")
gp.addmessage ("**********************")
gp.addmessage ("*SM Jones*")
gp.addmessage ("*April 2007*")
gp.addmessage ("**********************")
gp.addmessage ("")

'Get the parameters
strFN = gp.getparameterastext(0)
strWorkspace = gp.getparameterastext(1)
strFC = gp.getparameterastext(2)
strHR = gp.getparameterastext(3)

'Set the output file name
if instr(strFC,".shp") < 1 then
  strFC = strFC & ".shp"
end if

'Set up the geodatabase parameters
gp.workspace = strWorkspace

'Check for the existance of the feature class
if gp.exists(strWorkspace & "\" & strFC) then
  gp.addmessage "Deleting " & strWorkspace &
"\" & strFC
  gp.delete_management strWorkspace & "\" &
strFC
end if

'Create the new feature class
gp.addmessage "Creating " & strWorkspace &
"\" & strFC & " ..."
gp.createfeatureclass_management strWorkspace,
strFC, "polyline", "", "disabled", "enabled"
gp.addmessage "Add the MoaID field ..."
gp.addfield_management strWorkspace & "\" &
strFC, "MoaID", "text","#","#","10"
gp.addfield_management strWorkspace & "\" &
strFC, "frX", "short"
gp.addfield_management strWorkspace & "\" &
strFC, "frY", "short"
gp.addfield_management strWorkspace & "\" &
strFC, "frZ", "short"
gp.addfield_management strWorkspace & "\" &
strFC, "toX", "short"
gp.addfield_management strWorkspace & "\" &
strFC, "toY", "short"
gp.addfield_management strWorkspace & "\" &
strFC, "toZ", "short"

'Create the cursor object
set rows = gp.insertcursor (strWorkspace & "\" & strFC)

'Create the filesystem objects to read from
set fn = createobject
("Scripting.FileSystemObject")
set f = fn.getfile(strFN)
set lidar = f.openastextstream (1, 0)

'Read the header rows in the file
intHR = cint(strHR)
if intHR > 0 then
  for n = 1 to intHR
    rec = lidar.readline()
  next
end if

'Read the first line
rec = lidar.readline()

gp.addmessage ("")
gp.addmessage
("************************************")

'Make the array
set ptarray = gp.createobject("array")

'Loop through the records
do while not lidar.atendofstream
```

```
  'Split the comma separated file
  strList = split(rec,",")

  'Only work with records where there is a before
and end point
  if strList(1) <> "" and strList(4) <> ""  then
   'Create a new row template from rows
   set row = rows.newrow()
   'Set the attrbutes for each obervation
   set pt = gp.createobject ("point")
   pt.x = strList(1)
   pt.y = strList(2)
   pt.z = strList(3)
   ptarray.add pt
   set pt = nothing
   set pt = gp.createobject ("point")
   pt.x = strList(4)
   pt.y = strList(5)
   pt.z = strList(6)
   ptarray.add pt
   set row = rows.newrow()
   row.shape = ptarray
   row.MoaID = strList(0)
   row.frX = strList(1)
   row.frY = strList(2)
   row.frZ = strList(3)
   row.toX = strList(4)
   row.toY = strList(5)
   row.toZ = strList(6)

   'Insert row into new feature class
   rows.insertrow(row)

   'Print success message
   gp.addmessage strList(0) & " created
successfully"
   'Empty the array
   ptarray.removeall
  end if

 'next record
 rec = lidar.readline()

loop

'Close the gigantic file
lidar.close()

gp.addmessage ("")
gp.addmessage
("**************************************")
gp.addmessage ("*All completed*")
gp.addmessage
("**************************************")
gp.addmessage ("*SM Jones*")
gp.addmessage ("*February 2007*")
gp.addmessage
("**************************************")
gp.addmessage ("")

'Refresh the workspace
gp.refreshcatalog(strWorkspace)

'Uninitialize
set row = nothing
set rows = nothing
set pt = nothing
set ptarray = nothing
set fn = nothing
set f = nothing
set lidar = nothing
set gp = nothing
```

CHAPTER THREE

New palaeontological data from the excavation of the Late Glacial Glencrieff miring bone deposit, North Canterbury, South Island, New Zealand

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Prepared for submission to Journal of the Royal Society of New Zealand.

STATEMENT OF AUTHORSHIP

Chapter Three

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Prepared for submission to: *Journal of the Royal Society of New Zealand*

Nicolas. J. Rawlence

Excavated Glencrieff moa swamp, bone identification to species, bone collagen preparation and radiocarbon dating, data analysis and interpretation, wrote paper and acted as corresponding author.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed ..... Date 09/7/09

Jamie R. Wood

Excavated Glencrieff moa swamp, bone identification to species, gizzard contents analysis, plant macrofossil analysis and evaluated manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed ..... Date 9/7/09

Paul Scofield

Excavated Glencrieff moa swamp, bone identification to species, accession of bones into Canterbury Museum collections and evaluated manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed ..... Date 20/8/09.

Chapter Three

New palaeontological data from the excavation of the Late Glacial Glencrieff miring bone deposit, North Canterbury, South Island, New Zealand

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ABSTRACT

The avifauna from the Glencrieff swamp deposit in North Canterbury, South Island, New Zealand is described. Radiocarbon ages of moa bones bracket miring at the site to between 10,000-12,000 (uncalibrated) years BP. Heavy footed moa (*Pachyornis elephantopus*) and eastern moa (*Emeus crassus*) dominated the moa assemblage at the site, while South Island giant moa (*Dinornis robustus*) and stout-legged moa (*Euryapteryx curtus*, formerly *E. gravis*) were rare. The total assemblage from the site consists of at least 1896 bones from 18 species of birds, of which nine are extinct and a further three locally extinct. In addition, we report on the discovery of the oldest known moa gizzard contents and comment on significant changes in site conditions that are threatening the continued preservation of this significant fossil deposit.

KEYWORDS avifauna, gizzard contents, Glencrieff, moa, palaeontology, New Zealand

INTRODUCTION

The Glencrieff swamp, a miring bone deposit, offers an important view of the avifauna in Late Glacial North Canterbury (Fig. 1). Extensive excavation of bones from the site has occurred over the past 40 years. In 1971 a partial skeleton of the South Island giant moa (*Dinornis robustus*) was excavated by Canterbury Museum staff and the Eaves family, the previous owners of Glencrieff (Worthy & Holdaway 1996). Further excavations in 1991 by the Eaves family resulted

in 356 moa bones being found, which were retained as a private collection that was illegally exported overseas and is now missing (Worthy & Holdaway 1996). In 1993-94 Worthy and Holdaway (1996) conducted three further excavations, mapping the site and the extent of the bone deposits. In order to obtain new palaeontological and taphonomic data for analysing the deposition mechanism at the Glencrieff deposit, an additional sub-deposit, previously identified by Worthy and Holdaway (1996) was excavated in 2007 (Wood *et al.* 2008b). This paper describes the results of the 2007 excavation and synthesises the avifauna of Glencrieff. Additionally, it documents the oldest known moa gizzard contents and comments upon recent changes in site preservation conditions since the excavations by Worthy and Holdaway (1996).

INSTITUTIONAL ABREVIATIONS

New Zealand museums: MNZ, Museum of New Zealand Te Papa Tongarewa, Wellington (ex National Museum of New Zealand); CM, Canterbury Museum, Christchurch.

SITE DISCRIPTION

Glencrieff is located on the Wilson's (previously Eaves') farm near Hawarden, North Canterbury (42° 58' 07.45" S, 172° 34' 01.84''E), South Island, New Zealand (Fig. 1). Worthy and Holdaway (1996) and Wood *et al.* (2008b) presented an in-depth account of the stratigraphy, taphonomy and extent of the bone deposit.

METHODS

Excavation

The bone deposit identified by Worthy and Holdaway (1996) to the south of their 1993-94 excavation was re-located by probing the ground with a metal rod. This deposit was excavated in a pit measuring 5 m x 2 m, with an extension of 0.5 m x 2 m along the pit's northern face, adjoining the 1993-94 excavation. The pit is equivalent to squares I0 - I3, J0 - J3 and half of G1 and G2 (Worthy & Holdaway 1996; Wood *et al.* 2008b). The exact positions of all crania, sterna, pelves, femora, tibiotarsi, fibula, tarsometatarsi and preserved gizzard contents were measured, with respect to the X and Y axes of the excavation pit, along with their depth, orientation and inclination. These measurements were used by Wood *et al.* (2008b) to create a three dimensional reconstruction of the deposit.

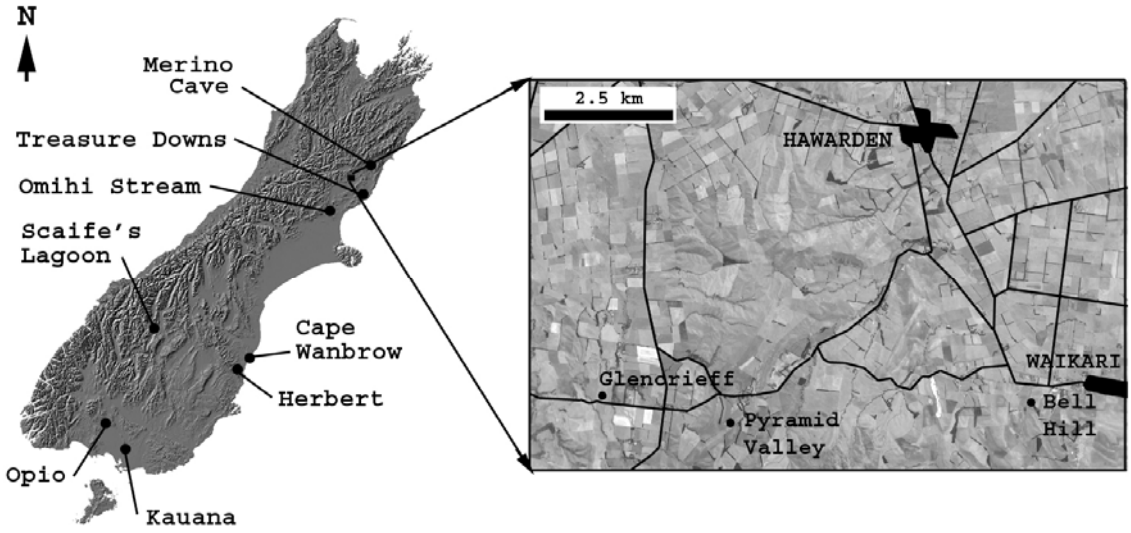

Fig. 1 Location of fossil deposits discussed in this paper, and location of the Late Glacial Glencrieff miring bone deposit in North Canterbury, South Island, New Zealand (insert).

Excavated remains were identified and accessioned into the Canterbury Museum collections. Gizzard contents are stored frozen at Landcare Research, Lincoln.

Identification

Bones were identified to species level through comparison to reference material held in Canterbury Museum and by application of the identification key published by Worthy and Holdaway (2002; Appendix Two). Minimum numbers of individuals (MNI) were determined for each taxon from the most frequent skeletal element (maximum of left or right side only). Where possible, preserved gizzard contents were associated with moa species by articulated moa ribs and sterna.

Nomenclature

We follow the taxonomy of the New Zealand Bird Checklist Committee OSNZ (2010) for extinct and extant New Zealand birds. The geological periods referred to here are Holocene, the present interglacial period, beginning at 10,000 years BP; Late Glacial, a period of rapid climatic and vegetational change, from 14,000-10,000 years BP; and the Otiran Glaciation, the last New Zealand glacial period, from approximately 70,000-14,000 years BP, encompassing the Last Glacial Maximum, 29,000 – 19,000 years BP (Worthy & Roscoe 2003; Williams *et al.* 2005; Newnham *et al.* 2007).

Radiocarbon dating

To augment the three published radiocarbon dates available for moa bones from Glencrieff (Worthy & Holdaway 1996), five heavy-footed moa (*Pachyornis elephantopus*) bones originating from the excavation by Worthy and Holdaway (1996) were radiocarbon dated using three different modifications of the Oxford Radiocarbon Accelerator Unit protocols for collagen extraction (see below; Bronk Ramsey *et al.* 2004a, b; Jocobi *et. al.* 2006). This was conducted at the University of Wollongong (UW) and the Australian National University (ANU). The stratigraphic position of radiocarbon dated bones within the Glencrieff deposit is shown in Table 3 and Figure 3. We did not radiocarbon date any bones from the Canterbury Museum, Eaves or 2007 excavations. Relative fossil abundance data from these deposits and the Worthy and Holdaway (1996) excavation suggests that Glencrieff had a palaeofaunal composition characteristic of the Late Glacial-Otiran Glacial (Worthy and Holdaway 1996; this study). In addition, analysis of *Sporormiella* spores (a dung fungi used as an indicator of vertebrate

herbivore biomass; Burney *et al.* 2003; Gill *et al.* 2009), from the Moar (2008) Glencrieff sediment core and preserved moa gizzard contents indicates that avian herbivore (i.e. moa) biomass at Glencrieff was high during the Late Glacial (Wood, unpublished data). However, the biomass was relatively low during the Holocene (Wood, unpublished data). This strongly suggests that miring and bone deposition at Glencrieff occurred during the Late Glacial (10,000-14,000 years BP) only.

Each radiocarbon date was assigned a specific ANU laboratory number. For dates with laboratory numbers ANU1605-1610, collagen preparation, combustion and graphitisation was conducted by Rawlence and Chris Turney (UW) using the following method. Bone samples weighing 0.5-1 g were cleaned, powdered and demineralised overnight in 0.5 M hydrochloric acid (HCl), then treated with 0.1 M sodium hydroxide (NaOH) for 30 minutes to remove humic acids and make an impure collagen extract. The collagen extract was further treated with 0.5 M HCl for 15 minutes to remove atmospheric carbon dioxide (CO_2) absorbed by the NaOH. A soluble gelatine fraction was extracted by treatment with water (pH 3 by the addition of HCl) for 20 hours at 75°C, leaving a residue of mainly humics. The gelatin fraction was filtered through 8µm Eezi ultrafilters by centrifugal dialysis to remove large contaminating molecules greater than 102 kD. The gelatine solution was subsequently filtered through 30 kD molecular weight cut off Vivaspin ultrafilters (15 ml capacity) by centrifugal dialysis to remove small molecular weight contaminants. The pure collagen fraction was freeze-dried and the percentage collagen calculated (defined as the amount of pure collagen, in grams, divided by the weight of the initial bone sample). Approximately 0.5-1 mg of freeze-dried collagen was loaded into glass combustion tubes, along with silver wire (to remove sulphur contaminants such as hydrogen sulphide and sulphur dioxide) and copper oxide (to provide oxygen (O_2) in the combustion reaction). Before combustion, O_2 and CO_2 were evacuated from the combustion tubes, and the tubes were heat sealed. Collagen samples were combusted at greater than 400°C overnight. Combusted samples were purified and graphitised on a closed system continuous line. Graphitised samples were run on the ANU single stage accelerator mass spectrometer (SSAMS) by Stewart Fallon and Keith Fifield (ANU), with the appropriate blanks, carbon blanks and known age samples.

The following modifications were made to the methodology for dates with laboratory numbers ANU4079-4937 and ANU7612-7625: The 8µm Eezi filter centrifugal dialysis step was not included as this is not necessary to retrieve pure collagen (Fiona Peachy pers comm. 2008); and

silver powder was used instead of silver wire in the combustion step, providing a greater surface area to remove contaminants. Collagen preparation was performed by Rawlence, while combustion, graphitisation and radiocarbon dating were performed with Richard Gillespie, Stewart Fallon and Keith Fifield (ANU). It was subsequently discovered that the iron catalyst used in the graphitisation reaction for dates ANU4079-4937 contributed some modern ^{14}C to the sample (pMC 0.24-0.38 +/- 0.03-0.04), despite standard cleaning procedures (reduction by hydrogen at 400ºC). Subsequently, for dates ANU7612-7625, a new modern ^{14}C free iron catalyst was used (pMC weighted average 0.080 +/- 0.007). Combustion, graphitisation and radiocarbon dating were conducted by Stewart Fallon and Keith Fifield (ANU) using purified collagen prepared for the dates ANU4079-4937.

Dates are reported as radiocarbon ages, based on Libby $T^{1/2}$ = 5568 years, uncorrected for calendar variation, in years before present, where present is 1950. Radiocarbon ages were calibrated using the programme OxCal4.0 (available at www.c14.arch.ox.ac.uk) and the IntCal04 calibration curve (Reimer *et al.* 2004). Radiocarbon dates were not calibrated using the ShCal04 calibration curve (McCormac *et al.* 2004) because the curve does not extend beyond 10,000 radiocarbon years BP. Calibrated dates are reported as 95% confidence calibrated ages (+/- 2 s.d) in years BP. To correct radiocarbon dates, sub-samples of purified collagen were used to determine the %N, %C and the C: N ratio at UW by colleagues.

Gizzard contents analysis

Moa gizzard contents were analysed following the methodology of Wood (2007). Gizzard contents were wet-sorted using a dissecting microscope at 10x magnification. Identifiable plant macro remains were counted and representative examples of seeds, leaves and twigs were stored in 70% ethanol. Seeds were identified using Webb and Simpson (2001). Wood was identified following the methodology of Meylan and Butterfield (1978). Wood samples were soaked in water and transverse sections were cut with razor blades. All measurements were made to the nearest 0.1 mm using vernier callipers.

RESULTS

Avifauna

A total of 1896 identified bones (excluding vertebrae, ribs, pubes, ischia and phalanges from the 2007 excavation) from 18 species of birds have been excavated from Glencrieff since excavations began in 1971.

Moa fauna

At least 16 individual moa comprising four species were represented in the 2007 excavation (Table 1). These include: *P. elephantopus* (MNI = 8, including one subadult); eastern moa, *Emeus crassus* (MNI = 6); *D. robustus* (MNI = 1); and stout-legged moa, *Euryapteryx curtus* (formerly *E. gravis*, MNI = 1). The *E. curtus* specimen represents the first of this species discovered at Glencrieff. Several of the moa pelves had indentations attributed to either Haast eagle (*Harpagornis moorei*) claws or kea (*Nestor notabilis*) beak marks. At least 51 individual moa are represented in the total fauna from Glencrieff. Specimens from the 2007, Worthy and Holdaway (1996), Eaves and Canterbury Museum excavations are summarised in Table 1 and Appendix A.

As was noted by Worthy and Holdway (1996), most of the bones excavated in 2007 were disarticulated. However, we found several incidences of associated and articulated remains. Examples included the pelvis + LR femora + LR tibiotarsi + LR fibulae + LR tarsometatarsi of a *P. elephantopus* and the vertebrae + pelvis + sternal ribs + gizzard contents of an *E. crassus* (Fig. 2). Despite the prevalence of disarticulation, we could assign most leg bones (femora, tibiotarsi and tarsometatarsi) to individual left and right pairs on the basis of comparing size and shape.

The majority of the known bone deposits within Glencrieff have now been excavated, with probing by a metal rod revealing no fossil bones to the south of the 2007 excavation. The area to the north of the Worthy and Holdaway (1996) excavation was not probed as the only bones known to remain in situ have previously been reported (Worthy and Holdaway 1996) between squares B2- B3-H and the Eaves deposit.

Table 1 A summary of moa specimens excavated from Glencrieff in all known excavations. For MNZ S and CM Av numbers see Appendix A. In species lists, x/y = number of bones/MNI. Bones are listed as femora (fem), tibiotarsi (tt), tarsometatarsi (tmt) and sometimes identified as left (L), right (R) or partial (pt) elements.

Species	**2007 excavation**	**Total for all collections***
Heavy-footed moa (*Pachyornis elephantopus*)	3 pelves, 4 sterna, 6L 4R fem, 6L 7R (1L 1R subad) tt, 4L 8R tmt, 1 complete skull, **43/8**	13 pelves, 14 sterna, 22L 20R fem, 21L 18R tt (1L 1R subad), 11L 8R fib, 19L 19 tmt, 1 complete skull, 3 crania[#], 8 mandibles, 229 vertebrae, 192 ribs/pubes/ischia, 213 phalanges, **830/22**
Eastern moa (*Emeus crassus*)	4 pelves, 1 sterna, 3L 4R fem, 4L 6R tt, 4L 5R tmt, **31/6**	17 pelves, 10 sterna, 14L 15R fem, 19L 21R tt, 7L 10R fib, 15L 15R tmt, 11 crania[†], 4 mandibles, 227 vertebrae, 282 ribs/pubes/ischia, 187 phalanges, **873/21**
South Island giant moa (*Dinornis robustus*)	1R fem, 1R tt, **2/1**	2 pelves, 2 sterna, 2L 3R fem, 4L 4R tt, 3L 1R fib, 3L 3R tmt, 10 vertebrae, 13 phalanges, 1 sesamoid, 67 bones from one discrete skeleton[‡], **118/5**
Stout-legged moa (*Euryapteryx curtus*)	1L fem, 1 L tmt, **2/1**	1L fem, 1L tmt, **2/1**
Emeid juv. moa chick	n/a	**13/3**
Unident. Moa	Vertebrae	Vertebrae
Unident. Moa	Ribs	Ribs
Unident. Moa	8L 10R fib, **18/10**	8L 10R fib, **18/10**
Unident. Moa	Phalanges	Phalanges

* Includes the 2007, 1993-94 Worthy and Holdaway, 1991 Eaves and 1971 Canterbury Museum excavations (Worthy & Holdaway, 1996).

[#] Includes 22 cranial bones.

[†] Includes 19 cranial bones.

[‡] This discrete skeleton (CM Av253373) was excavated in 1971 by Canterbury Museum staff from an unknown location within Glencrieff. It is presumably not from the deposits excavated in 1991, 1993-94 or 2007 because disarticulated bones rather than discrete skeletons were excavated (Worthy & Holdaway 1996).

Fig. 2 Articulated pelvis, sternal ribs, vertebrae and gizzard contents of an eastern moa (*Emeus crassus*), Glencreiff, 2007. Lens cap diameter is 58 mm.

Neongath avifauna

Seven neongath (or neoavian) bird species were discovered during the 2007 excavation (Table 2) and have been summarized with previous finds of neoavian bird species in Appendix A. A notable missing taxon from the 2007 excavation was Haast's eagle, which was found during the Worthy and Holdaway (1996) excavation. Fourteen species of neoavian birds are represented in the total fauna from Glencrieff (see Appendix A).

Table 2 List of neongath (or neoavian) bird specimens excavated from Glencrieff, 2007. In species lists, x/y = number of bones/MNI. For CM Av numbers see Appendix A. Bones are listed as carpometacarpus (cmc), femora (fem), tibiotarsi (tt), tarsometatarsi (tmt) and sometimes partial (pt) elements.

Species	**Element, X/Y**
Brown teal (*Anas chlorotis*)	1 radius, 1/1
Kea (*Nestor notabilis*)	2 pt maxilla, 2/1
Grey Warbler (*Gerygone igata*)	1 tmt, 1/1
Weka (*Gallirallus australis*)	1 corocoid, 1/1
Kereru (*Hemiphaga novaeseelandiae*)	1 cmc, 1/1
Hodgen's Rail (*Gallinula hodgenorum*)	1 coracoid, 1 fem, 2 tt, 4/1
Rail sp. (*Rallidae*)	1 vertebrae, 1/1

Radiocarbon ages

Twelve radiocarbon ages were obtained from five *P. elephantopus* bones (Table 3). Radiocarbon ages from bones excavated by Worthy and Holdaway (1996) were between 11,390 +/- 130 (ANU1610) and 9,070 +/- 80 (ANU4937) years BP, and are within the range of previously published dates (10,470 +/- 130 to 11,898 +/- 82; Worthy & Holdaway 1996), except ANU4937. ANU4937 was dated using the contaminated iron catalyst (pMC 0.24-0.38 +/- 0.03-0.04). Given subsequent re-dating of this specimen (MNZ S 32670.8; Table 3) at 10,680 +/- 70 (ANU7625) with the modern ^{14}C free iron catalyst (pMC weighted average 0.080 +/- 0.007) we interpret ANU4937 as the result of contamination and disregard this date. To determine whether there was stratigraphy in the Worthy and Holdaway (1996) excavation, the radiocarbon ages were plotted against the excavation depth of the dated specimens (Fig. 3). The results indicate there is no stratigraphic relationship between excavation depth and radiocarbon age from the Worthy and Holdaway (1996) excavation (Fig. 3).

Table 3 Determining the age range of moa bones excavated from Glencrieff by Worthy and Holdaway (1996).

Species	**Museum No.**	**Excavation square and depth**	**Prep. Code^**	**Lab. No.**	**^{14}C age, years BP (1 s.d.)**	**Calibrated age (2 s.d.), years BP#**	**% C**	**% N**	**δ^{13}C**	**C:N ratio**
E. crassus	MNZ S32688	Sq A1, -70 cm	ABA	NZA4018	10, 480 +/- 120*	12,386-12,624	n/a	n/a	-24.72	n/a
P. elephantopus	n/a	Peg 1, -85 cm	ABA	NZA4162	11, 898 +/- 82*	13,706-13,824	n/a	n/a	-21.48	n/a
P. elephantopus	MNZ S 32670.9	Sq A1, -95 cm	UF1	ANU1607	11,230 +/- 210	12,832-13,534	42.8	14.6	-23.6	2.81
			UF2	ANU4923	10,750 +/- 80	12,819-12,944				
			UF3	ANU7612	10,510 +/- 80	12,154-12,770				
			Combined calibrated age, years BP			**12,154-13,534**				
P. elephantopus	MNZ S 32670.8	Sq A2, -100 cm	UF3	ANU7625	10,680 +/- 70	12,408-12,851	39.6	13.9	-23.5	2.86
			UF2	ANU4937	9,070 +/- 80	9,928-10,492 (9,891-10,407)				
			Combined calibrated age, years BP			**9,928-12,851**				
E. crassus	MNZ S 32690	Sq A1, -105 cm	ABA	NZA4079	10, 470 +/- 130*	12,382-12,620	n/a	n/a	-23.5	n/a
P. elephantopus	MNZ S 32670.3	Sq A1, -105cm	UF1	ANU1606	11,490 +/- 80	13,196-13,537	43.1	14.9	-23.3	2.76
			UF2	ANU4925	11,180 +/- 70	12,938-13,215				
			UF3	ANU7614	10,980 +/- 70	12,842-13,064				
			Combined calibrated age, years BP			**12,842-13,537**				
P. elephantopus	MNZ S 32670.7	Sq B2/B3, -110 cm	UF1	ANU1610	11, 390 +/- 130	13,001-13,547	30.3	10.5	-23.0	2.85
P. elephantopus	MNZ S 32670.2	Sq B2, -120 cm	UF2	ANU4924	10,760 +/- 70	12,678-12,881	40.2	12.9	-23.8	3.04
			UF3	ANU7613	10,610 +/- 80	12,395-12,817				
			UF1	ANU1605	10,580 +/- 90	12,239-12,824				
			Combined calibrated age, years BP			**12,239-12,881**				

^ ABA: Acid Base Acid collagen preparation, followed by Worthy and Holdaway (1996); UF1: Ultrafiltration with Eezi and Vivaspin filters, combustion with silver wire; UF2: Ultrafiltration with Vivaspin filters, combustion with silver powder, contaminated iron catalyst; UF3: Ultrafiltration with Vivaspin filters, combustion with silver powder, modern ^{14}C free iron catalyst.

* Dates presented by Worthy and Holdaway (1996).

Radiocarbon dates were calibrated using the programme OxCal4.0 (available at www.c14.arch.ox.ac.uk) and the IntCal04 (Reimer *et al.* 2004) or ShCal04 (in parentheses; McCormac *et al.* 2004) calibration curves.

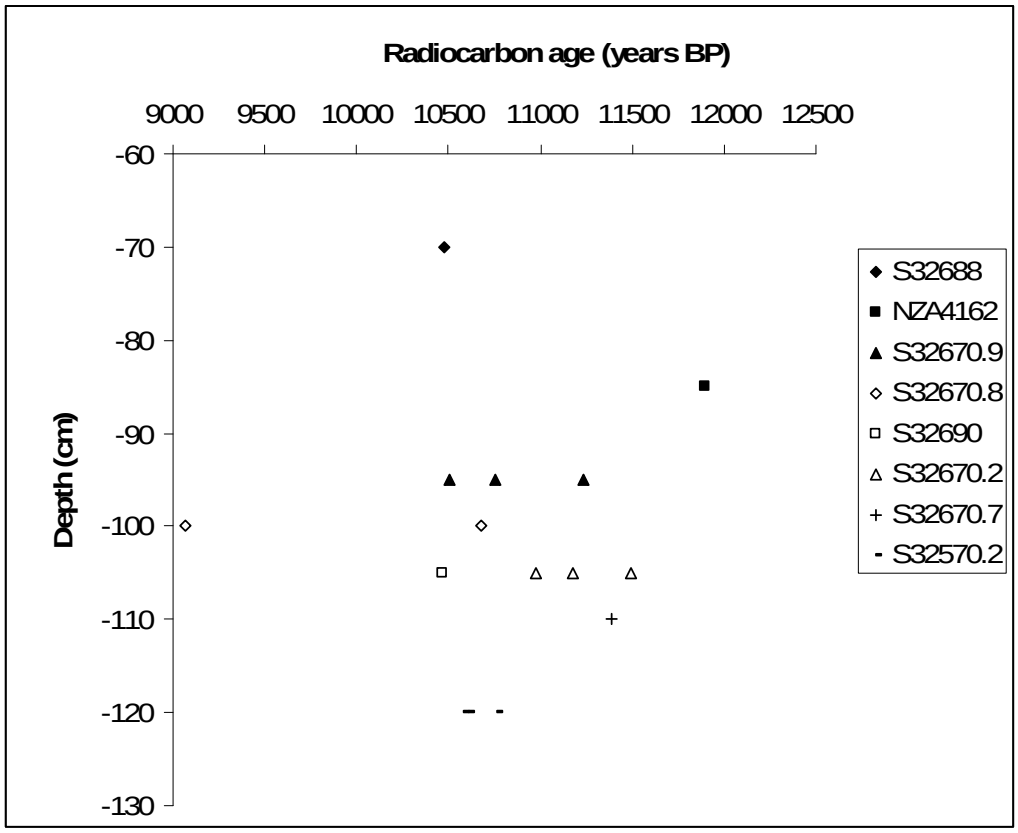

Fig. 3 Relationship of radiocarbon age to excavation depth for dated moa bones at Glencrieff. Numbers refer to museum accession numbers (Table 3).

Moa gizzard contents

The preserved contents of 16 individual moa gizzards were recovered during the 2007 excavation and represent the first gizzards identified and recovered from Glencrieff (Fig. 4). Unlike moa gizzard contents from the Late Holocene Pyramid Valley (Burrows *et al.* 1981) and Treasure Downs (Wood 2007) calcareous mires, at Glencrieff and Scaifes Lagoon (Burrows *et al.* 1981) the boundary between the gizzard contents and peat matrix is indistinct, making the distinguishing of gizzard contents somewhat difficult.

Gizzard contents were characterized by dense accumulations of gizzard stones, and were, in some instances, associated with articulated moa ribs and sterna, thus allowing identification of the moa species. The gizzard contents are associated with one *P. elephantopus,* one *E. crassus* and 14 unidentified moa. Associated bone radiocarbon dates (Table 3) show that they are the oldest moa gizzard contents found in New Zealand, and include only the third example of gizzard content of *P. elephantopus* (Wood 2007).

No large seeds or twigs were noted in the gizzard content samples from Glencrieff, which is surprising as such material is considered characteristic of both gizzard contents (Burrows *et al.* 1981; Wood 2007) and coprolites (Horrocks *et al.* 2004; Wood *et al.* 2008a) from other sites. Small seeds, representing wetland taxa (*Limosella australis*, *Juncus* spp., *cf. Juncus* spp., *Carex* spp., *Ranunculus* spp., *Colobanthus* spp., Chenopodiaceae) were found within the gizzard contents, but may have originated from the surrounding matrix rather than the gizzard because of the difficulty in distinguishing between the peat matrix and gizzard contents. Matted tussock grass leaves, *cf.* Poaceae, were recovered from the inside of two sterna, one identified as *P. elephantopus* (Fig. 4), suggesting that tussock grass formed part of the diet of *P. elephantopus*.

During the 2007 excavation a small (approximately 150 mm diameter), discrete deposit of concentrated plant material was collected from amongst the moa bones but was not associated with moa gizzard stones. The sample included at least 74 twigs (identified as *Nothofagus* spp. *cf. menziesii*, silver beech), with lengths ranging from 9 - 43 mm and were normally distributed (mean = 20.4 mm, s.d. = 8.1 mm). Similar distributions of twig lengths have been reported associated with both moa gizzard contents (Wood 2007) and nesting material (Wood 2008). The sample also included at least 18 leaf fragments of an unidentified monocot.

Fig. 4 In situ heavy-footed moa (*Pachyornis elephantopus*) gizzard resting between the rib cage and sternum. Between the sternum and gizzard was a matted layer of grass material.

Recent changes in site preservation conditions

Some significant changes in site preservation conditions have occurred at Glencrieff in the 14 years between the 1993-94 excavation by Worthy and Holdaway (1996) and 2007. In 1993, a blue clay layer between the overlying peat and the basement gravels was liquid and under pressure (Worthy pers comm. 2008). In the subsequent 14 years, there has been significant drying and desiccation of the site. The water table has dropped and the blue-clay layer has dried out. In addition, there were numerous large desiccation cracks that extended from the soil surface to water table. In places, the blocks of peat created by these cracks had formed hard concretions around the bones.

Throughout the peat layer, we also found abundant roots from poplar trees (*Poplus* spp.) lining the west edge of the site. The roots appeared to be preferentially growing on the surfaces of the moa leg bones and throughout the more porous pelves. Bones higher in the stratigraphy were not as well preserved as those at lower levels due to the combination of poplar roots and low water table. Extensive erosion of the bone surface, especially of less dense elements like pelves, was evident for bones nearer the surface of the 2007 deposit. It is therefore possible that the dropping of the water table, and the desiccation and deterioration of Glencrieff is due to the poplars.

DISCUSSION

Period of deposition

The radiocarbon ages for Glencrieff (Table 3) indicate that the fossil deposit is about 10,000-12,000 years BP, placing it during the Late Glacial. The age range suggests that Glencrieff started trapping moa approximately 12,000 years BP and stopped about 10,000 years BP. It is probable that the peat is older than 12,000 years BP because it is likely that the peat had to be at least 50 cm deep to mire moa (Wood *et al.* 2008b). There is no correlation between the radiocarbon dates and stratigraphic position (Table 1, Fig. 3), supporting the conclusions of Wood *et al.* (2008b) that periodic peat liquefaction events and raking of fossil bones by the legs of mired moa caused disarticulation and intermingling of bones. If the radiocarbon dates encompass approximately the entire time period of miring at Glencrieff, this would give a minimum average miring rate of one moa per 28 years.

Taphonomy

The horizontal to sub-horizontal alignment of moa bones in the Worthy and Holdaway (1996) and 2007 deposits (Wood *et al.* 2008b), and the discrete nature of the 2007 and Eaves deposits, suggest that there were at least three different "soft spots" in the Glencrieff wetland where birds became mired. We interpret the site of the 2007 excavation as the spring upwelling or an extension of the spring outflow channel that flowed along the eastern edge of the mire (Wood *et al.* 2008b).

All the recovered moa bones derived from individuals trapped in the mire. In comparison, many of the small bird bones that were found (Table 2) are likely to be from individuals that died around the mire, with subsequent incorporation into the fossil deposit. The orientation of the bones in the deposit is hypothesised to have resulted from rare peat liquefaction events when moa became mired in the swamp and broke through the peat cap, with the raking action of moa legs resulting in the concentration and alignment of bones sub parallel to the eastern bank (Wood *et al.* 2008b).

These taphonomic observations suggest that an artesian spring developed on the terrace at Glencrieff during the Late Glacial (Worthy and Holdaway 1996; Wood *et al.* 2008b). This facilitated the formation of floating peat deposits. Cracks or rifts in the peat would have resulted in the miring of moa, until the peat became too thick to mire moa in the Early Holocene. Rare liquefaction events may also have resulted in bones migrating through the overlying peat into the underlying liquid blue clay and being deposited on the basement gravels.

Avifauna and ecological implications

Of the 18 species of birds excavated from Glencrieff, nine are extinct, while a further three are locally extinct (Table 4; Wilson 2004; Robertson *et al.* 2007). Of the remaining five species, kea and kakariki (*Cyanoramphus* spp.) are absent from the lowlands, while kereru (*Hemiphaga novaeseelandiae*) is restricted to Banks Peninsula and lowland forest remnants (Robertson *et al.* 2007). This reflects a significant decline in avifaunal diversity since the arrival of Polynesians (1280AD; Wilmshurst *et al.* 2008) and later Europeans (Tennyson & Martinson 2006) in New Zealand.

Table 4 Status of avian taxa excavated from Glencrieff. In species lists, X = extinct.

Species	Extinct	Locally extinct
South Island giant moa (*Dinornis robustus*)	X	
Heavy footed moa (*Pachyornis elephantopus*)	X	
Eastern moa (*Emeus crassus*)	X	
Stout-legged moa (*Euryapteryx curtus*)	X	
Kiwi (*Apteryx* spp.)		X
New Zealand Quail (*Coturnix novaezelandiae*)	X	
Kakariki (*Cyanoramphus* spp.)		restricted distribution
New Zealand snipe (*Coenocorypha iredalei*)	X	
Laughing Owl (*Sceloglaux albifacies*)	X	
New Zealand pipit (*Anthus novaeseelandiae*)		
Haast's Eagle (*Harpagornis moorei*)	X	
Brown teal (*Anas chlorotis*)		X
Kea (*Nestor notabilis*)		restricted distribution
Grey Warbler (*Gerygone igata*)		
Weka (*Gallirallus australis*)		X
Kereru (*Hemiphaga novaeseelandiae*)		restricted distribution
Hodgen's Rail (*Gallinula hodgenorum*)	X	
Rail sp. (*Rallidae*)	n/a	n/a

Pachyornis elephantopus and *E. crassus* dominate the Late Glacial moa assemblage of Glencrieff, with rare *D. robustus* and *E. curtus*. When compared to assemblages from other Otiran Glacial-Early Holocene sites in the eastern South Island, it is evident that Glencrieff represents a characteristic fauna of this time period. This is despite extensive climatic and vegetation change during the period of deposition at Glencrieff (Worthy 1993a; Worthy & Holdaway 1995, 1996; Moar 2008). Other Otiran Glacial-Early Holocene sites in the eastern South Island, including Omihi Stream (Worthy & Holdaway 1996), Cape Wanbrow (Worthy & Grant-Mackie 2003), Merino Cave (Worthy & Holdaway 1995), Herbert (Oliver 1949), and Albury Park (Worthy 1997a), have similar faunal assemblages to Glencrieff. They are characterised by the dominance of *P. elephantopus*, with either *E. crassus* or *E. curtus* also dominant (depending on the elevation and aspect of the fossil locality), and *D. robustus* comparatively rare. This is in contrast to Otiran Glacial-Late Glacial sites in Southland, including Opio (pers. obs.) and Kauana (Worthy 1998b) where *E. curtus* is the dominant moa, with fewer *E. crassus*, *P. elephantopus* and *D. robustus.*

By the Late Holocene the composition of the avifauna in North Canterbury had altered. Warming temperatures and the establishment of tall podocarp forest by the Early Holocene and mixed southern beech forest (*Nothofagus* spp.) and podocarp forest by the Middle to Late Holocene resulted in significant shrinking of the open tussock grassland/shrubland mosaic habitat favored by *P. elephantopus* (Moar 2008). Late Holocene fossil deposits in North Canterbury, including Pyramid Valley (Holdaway & Worthy 1997), Bell Hill (Worthy, unpublished data), Glenmark (Worthy & Holdaway 1996) and Treasure Downs (McCulloch, in Mourer-Chauvire 1987; Worthy pers comm. 2009), are dominated by *E. crassus*, and either *E. curtus* or *D. robustus* (depending on elevation and aspect of the fossil locality), with *P. elephantopus* comparatively rare. Other Late Holocene sites in the eastern South Island generally follow this trend, including several sites in South Canterbury (Worthy 1997a) and North Otago (Worthy 1998a). However, Late Holocene sites in Central Otago were dominated by *P. elephantopus*, with co-dominant *E. curtus* or *E. crassus* (Worthy 1998a), reflecting the increased abundance of the open grassland/shrubland mosaic in Central Otago favoured by *P. elephantopus* (Wood & Walker 2008).

Worthy and Holdaway (1996) found the absence of *E. curtus* from Glencrieff intriguing because the species was present in low numbers at other Otiran-Late Glacial sites in Canterbury (Worthy 1993a; Worthy & Holdaway 1996). They hypothesised that *E. curtus* had a preference for hill country where soils were drier, with *E. crassus* replacing *E. curtus* at lower altitudes. This is evident when the relative proportion of moa species in the Late Holocene Pyramid Valley and Bell Hill fossil deposits are compared. Pyramid Valley, located in a basin, has as assemblage dominated by *E. crassus* and *D. robustus*, with *E. curtus* and *P. elephantopus* present but rarer (Holdaway & Worthy 1997). Bell Hill, located on a hillside, has significantly more *E. curtus* than Pyramid Valley, and *D. robustus* is rare (unpublished data, Worthy pers comm. 2009). The single *E. curtus* from Glencrieff is therefore interpreted as a rare individual on the margin of its preferred habitat. This may also apply to rare *E. curtus* individuals from Otiran Glacial-Late Glacial deposits in the wetter northwest Nelson/West Coast regions of the South Island (Worthy 1993b, 1994, 1997b; Worthy & Holdaway 1994).

Conclusions

The fossil avifaunal assemblage at Glencrieff documents the presence of 18 species of birds during the Late Glacial, nine of which are extinct with a further three locally extinct (Table 4).

Comparisons with other Otiran Glacial-Late Holocene fossil deposits in the eastern South Island (Oliver 1949; Worthy & Holdaway 1995, 1996; Holdaway & Worthy 1997; Worthy 1997a, 1998a; Worthy & Grant-Mackie 2003) indicate that the Glencrieff avifaunal assemblage represents a characteristic Otiran Glacial-Early Holocene fauna, despite extensive climatic and vegetation change during this time period. The 2007 excavations have also documented the oldest moa gizzard contents yet found (Fig. 4), and the first occurrence of *E. curtus* at Glencrieff. The seasonal wetting and drying of the deposit and the extraction of water by the poplars surrounding Glencrieff will continue to degrade the remaining bones. It is interesting to note that before significant drying of the deposit after the 1993-94 excavations by Worthy and Holdaway (1996), bones may have migrated through the peat in rare liquefaction events into the underlying liquid blue clay and deposited on the basement gravels. Given the diversity of avian taxa present at Glencrieff, it would be interesting to know what taxa are preserved on these basement gravels as this will help gain a fuller understanding of the palaeofauna in North Canterbury during the Late Glacial. Future radiocarbon dating of moa bones from the 2007 and 1971 Canterbury Museum excavations (Worthy & Holdaway 1996) will determine whether bone deposition within each deposit at Glencrieff occurred during the same time period. Loss of the Eaves collection overseas is unfortunate as radiocarbon dating of this deposit would be important to gain a complete understanding of the taphonomy and deposition at this significant fossil locality.

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REFERENCES

Bronk Ramsey C, Higham TFG, Bowles A, Hedges R 2004a. Improvements to the pretreatment of bone at Oxford. Radiocarbon 46: 155-163.

Bronk Ramsey C, Higham TFG, Leach P 2004b. Towards high precision AMS: progress and limitations. Radiocarbon 46: 7-24.

Burney DA, Robinson GS, Pigott Burney L 2003. *Sporormiella* and the late Holocene extinctions in Madagascar. Proceedings of the National Academy of Science USA 100: 10800-10805.

Burrows CJ, McCulloch B, Trotter MM 1981. The diet of moas based on gizzard contents from Pyramid Valley, North Canterbury, and Scaife's Lagoon, Lake Wanaka, Otago. Records of the Canterbury Museum 9: 309-336.

Checklist Committee OSNZ 2010. Checklist of the birds of New Zealand, Norfolk and Macquarie Islands, and the Ross Dependency, Antarctica (4th ed.) Wellington, Ornithological Society of New Zealand and Te Papa Press.

Gill JL, Williams JW, Jackson ST, Linninger KB, Robinson GS 2009. Pleistocene megafaunal collapse, novel plant communities, and enhanced fire regimes in North America. Science 326: 1098-1100.

Holdaway RN, Worthy TH 1997. A reappraisal of the late Quaternary fossil vertebrates of Pyramid Valley Swamp, North Canterbury, New Zealand. New Zealand Journal of Zoology 24: 69-121.

Horrocks M, D'Costa D, Wallace R, Gardner R, Kondo R 2004. Plant remains in coprolites: diet of a subalpine moa (Dinornithiformes) from southern New Zealand. Emu 104: 149–156.

Jacobi RM, Higham TFG, Bronk Ramsey CB 2006. AMS radiocarbon dating of Middle and Upper Palaeolithic bone in the British Isles: improved reliability using ultrafiltration. Journal of Quaternary Science 21: 557-573.

McCormac FG, Hogg AG, Blackwell PG, Buck CE, Higham TFG, Reimer PJ 2004. ShCal04 Southern Hemisphere calibration, 0-11.0 Cal Kyr BP. Radiocarbon 46: 1087-1092.

Meylan BA, Butterfield BG 1978. The structure of New Zealand woods. Department of Scientific and Industrial Research, Wellington, New Zealand.

Moar NT 2008. Late Quaternary Vegetation. In: Winterbourn M, Knox G, Burrows C, Marsden I (eds.) 2008. The Natural History of Canterbury. Christchurch, Canterbury University Press.

Mourer-Chauvire C (ed.) 1987. Letter of information of the Society of Avian Paleontology and Evolution 1. http://www2.nrm.se/ve/birds/sape/sapenews1html.en.

Newnham RM, Vandergoes MJ, Hendy CH, Lowe DJ, Preusser F 2007. A terrestrial palynological record for the last two glacial cycles from southwestern New Zealand. Quaternary Science Reviews 26: 517-535.

Oliver WRB 1949. The moas of New Zealand and Australia. Wellington, Dominion Museum.

Reimer PJ, Baillie MGL, Bard E, Bayliss A, Beck JW, Bertrand CJH, Blackwell PG, Buck CE, Burr GS, Cutler KB, Damon PE, Edwards RL, Fairbanks RG, Friedrich M, Guilderson TP, Hogg AG, Hughen KA, Kromer B, McCormac G, Manning S, Bronk Ramsey C, Reimer RW, Remmele S, Southon JR, Stuiver M, Talamo S, Taylor FW, van der Plicht J, Weyhenmeyer CE 2004. INTCAL04 terrestrial radiocarbon age calibration, 0-26 Cal Kya BP. Radiocarbon 46: 1029-1058.

Robertson CJR, Hyvonen P, Fraser MJ, Pickard CR 2007. Atlas of bird distribution in New Zealand 1999-2004. Ornithological Society of New Zealand.

Tennyson A, Martinson P 2006. Extinct birds of New Zealand. Te Papa Press, Wellington, New Zealand.

Webb CJ, Simpson MJA 2001. Seeds of New Zealand: gymnosperms and dicotyledons. Manuka Press, Christchurch, New Zealand.

Williams PW, King DNT, Zhao JX, Collerson KD 2005. Late Pleistocene to Holocene composite speleotherm ^{18}O and ^{13}C chronologies from South Island, New Zealand – did a global Younger Dryas really exist? Earth and Planetary Science Letters 230: 301-317.

Wilmshurst JM, Anderson AJ, Higham TFG, Worthy TH 2008. Dating the late prehistoric dispersal of Polynesians to New Zealand using the commensal Pacific rat. Proceedings of the National Academy of Sciences USA 105: 7676-7680.

Wilson KJ 2004. Flight of the Huia: Ecology and conservation of New Zealand's frogs, reptiles, birds and mammals. Canterbury University Press, Christchurch, New Zealand.

Wood JR 2007. Moa gizzard content analysis: further information on the diet of *Dinornis robustus* and *Emeus crassus*, and the first evidence for the diet of *Pachyornis elephantopus* (Aves: Dinornithiformes). Records of the Canterbury Museum 21: 27-39.

Wood JR 2008. Moa (Aves: Dinornithiformes) nesting material from rockshelters in the semi-arid interior of South Island, New Zealand. Journal of the Royal Society of New Zealand 38: 115-129.

Wood JR, Walker S 2008. Macrofossil evidence for pre-settlement vegetation of Central Otago's basin floors and gorges. New Zealand Journal of Botany 46: 239-255.

Wood JR, Rawlence NJ, Rogers GM, Austin JJ, Worthy TH, Cooper A 2008a. Coprolite deposits reveal the diet and ecology of the extinct New Zealand megaherbivore moa (Aves: Dinornithiformes). Quaternary Science Reviews 27: 2593-2602.

Wood JR, Worthy TH, Rawlence NJ, Jones SM, Read SE 2008b. A deposition mechanism for Holocene miring bone deposits, South Island, New Zealand. Journal of Taphonomy 6: 1-20.

Worthy TH 1993a. A review of fossil bird bones from loess deposits in eastern South Island, New Zealand. Records of the Canterbury Museum 10: 95-106.

Worthy TH 1993b. Fossils of Honeycomb Hill. Wellington, Museum of New Zealand Te Papa Tongawera.

Worthy TH 1994. Late Quaternary changes in the moa fauna on the West Coast of the South Island, New Zealand. Records of the South Australian Museum 27: 125-134.

Worthy TH 1997a. Quaternary fossil fauna of South Canterbury, South Island, New Zealand. Journal of the Royal Society of New Zealand 27: 67-162.

Worthy TH 1997b. Fossil deposits in the Hodge Creek Cave System, on the northern foothills of Mt Arthur, Nelson, South Island, New Zealand. Notornis 44: 111-124.

Worthy TH 1998a. Quaternary fossil faunas of Otago, South Island, New Zealand. Journal of the Royal Society of New Zealand 28: 421-521.

Worthy TH 1998b. The Quaternary fossil avifauna of Southland, South Island, New Zealand. Journal of the Royal Society of New Zealand 28: 537-589.

Worthy TH, Grant-Mackie JA 2003. Late-Pleistocene avifaunas from Cape Wanbrow, Otago, South Island, New Zealand. Journal of the Royal Society of New Zealand 33: 427-485.

Worthy TH, Holdaway RN 1994. Quaternary fossil faunas from caves in Takaka valley and on Takaka Hill, northwest Nelson, South Island, New Zealand. Journal of the Royal Society of New Zealand 24: 297-391.

Worthy TH, Holdaway RN 1995. Quaternary fossil faunas from caves on Mt Cookson, North Canterbury, New Zealand. Journal of the Royal Society of New Zealand 25: 333-370.

Worthy TH, Holdaway RN 1996. Quaternary fossil faunas, overlapping taphonomies, and palaeofaunal reconstruction in North Canterbury, South Island, New Zealand. Journal of the Royal Society of New Zealand 26: 275-361.

Worthy TH, Holdaway RN 2002. The lost world of the moa: Prehistoric life of New Zealand. Christchurch, Canterbury University Press.

Worthy TH, Roscoe D 2003. Takaka Fossil Cave – a stratified Late Glacial to Late Holocene deposit from Takaka Hill, New Zealand. Tihinga 14: 41-60.

APPENDIX A Vertebrate Fossils from Glencrieff miring bone deposit.

SITE NAME	**Glencrieff Moa swamp**
GRID REFERENCE	M33 747042
REPOSITORY OF MATERIAL	CM, MNZ, K. Eaves numbered E1-E216
COLLECTER	R. Scarlett – 1/4/71, K. Eaves – 1992, THW, RNH – Feb/March 1993, 24 March, 1994, NJR, JRW, PS – Feb 2007
TAPHONOMY	Swamp miring
FOSSIL RECORD NUMBER	M33/f25

Species	**Catalogue no.**	**x/y**
Dinornis robustus	CM Av253373	67/1
Dinornis robustus	Eaves colln; MNZ S32684-5, CM Av unregistered	51/4
Pachyornis elephantopus	Eaves colln; MNZ S32668-73, 32675 32679, 32681, 32698, 32700 (2 juv), CM Av unregistered	830/22
Emeus crassus	Eaves colln; MNZ S32674, 32680, 32682, 32686, 32696-7, 32699 (1 juv, 4 subad), CM Av unregistered	873/20
Euryapteryx curtus	CM Av unregistered	2/1
Emeid moa chick	Eaves colln, MNZ S32683, 32691	13/3
Unident. moa vertebrae	CM Av unregistered	Lots
Unident. moa ribs, pubes, ischia	CM Av unregistered	Lots
Unident. moa fibulae	CM Av unregistered	18/10
Unident. moa phalanges	CM Av unregistered	Lots
Apteryx spp.	S33490	1/1
Coturnix novaezelandiae	S33476-483	13/2
Cyanoramphus spp.	S33486	1/1
Coenocorypha iredalei	S33484-5	2/1
Sceloglaux albifacies	S33491-2	4/1
Anthus novaeseelandiae	S33489	1/1
Harpagornis moorei	S28377-8	2/2
Anas chlorotis	S33487-8, CM Av unregistered	6/1
Nestor notabilis	S28376, CM Av unregistered	3/1
Gerygone igata	CM Av unregistered	1/1
Gallirullus australis	CM Av unregistered	1/1
Hemiphaga novaezeelandiae	CM Av unregistered	1/1
Gallinula hodgenorum	CM Av unregistered	4/1
Rallidae sp.	CM Av unregistered	1/1

CHAPTER FOUR

Coprolite deposits reveal the diet and ecology of the extinct New Zealand megaherbivore moa (Aves: Dinornithiformes)

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Quaternary Science Reviews (2009) **27**, 2593-2602.

Jamie R. Wood

Excavated coprolites, designed experiment, performed plant macrofossil analysis, interpreted data, wrote manuscript and acted as corresponding author.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed Date..8/July/2009

Nicolas. J. Rawlence

Designed experiment, performed DNA and phylogenetic analysis, interpreted data and wrote manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed Date..8/7/09

Geoffrey M. Rogers

Contributed discussion on moa and plant ecology, and evaluated manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed Date..9/7/09

Jeremy J. Austin

Performed DNA analysis, supervised development of work and evaluated manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed Date..8/7/09.

Trevor. H. Worthy

Excavated coprolites, designed experiment, interpreted data and evaluated manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed .Date..13 July 09

Alan Cooper

Designed experiment, supervised development of work, evaluated manuscript and provided funding.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed Date..9/7/09

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ABSTRACT

The discovery in New Zealand of Late Holocene deposits of coprolites from extinct avian megaherbivores has provided a unique opportunity to gain a detailed insight into the ecology of these birds across ecologically diverse habitats. Macrofossil analysis of 116 coprolites of the giant ratite moa (Aves, Dinornithiformes) reveals a diverse diet of herbs and low shrubs in both semi-arid and high rainfall ecological zones, overturning previous models of moa as dominantly browsers of trees and shrubs. Ancient DNA analysis identified coprolites from four moa species (South Island giant moa, *Dinornis robustus*; upland moa, *Megalapteryx didinus*; heavy-footed moa, *Pachyornis elephantopus* and stout-legged moa, *Euryapteryx gravis*), revealing a larger dietary variation between habitat types than between species. The new data confirm that moa fed on a variety of endemic plant taxa with unusual growth forms previously suggested to have co-evolved with moa. Lastly, the feeding ecologies of moa are shown to be widely different to introduced mammalian herbivores.

1. Introduction

The ecological consequences of the extinction of the Pleistocene megafauna (MacPhee, 1999; Owen-Smith, 1987) remain a contentious topic, along with the potential co-evolutionary role of megafauna in the origin of plant growth and reproduction traits (Atkinson and Greenwood, 1989; Bond and Silander, 2007; Bond et al., 2004; Burns and Dawson, 2006). Both issues have major implications for interpreting and conserving modern ecosystems, and the potential for their re-wilding (Donlan et al., 2005). However, a lack of detailed dietary evidence for nearly all extinct megaherbivore species has prevented detailed testing of these issues, or even of general palaeoecological reconstructions that might aid re-wilding attempts to reconstruct pre-human ecosystems.

New Zealand provides a unique opportunity to analyse a recently extinct megafaunal ecosystem in detail, and to test the accuracy of current approaches to palaeoecological reconstruction. The terrestrial fauna was free of mammals (except for three species of bat) (Worthy and Holdaway, 2002), and dominated by 10 species of avian megaherbivore, the ratite moa (Aves, Dinornithiformes). There has been much debate about the palaeoecology of the different moa taxa, and their potential role in the evolution of a range of distinctive plant growth characteristics found across a taxonomically diverse range of the New Zealand flora (Atkinson and Greenwood, 1989). These unusual forms include divarication, mimicry, and heteroblasty amongst others. In contrast, hypotheses of moa-plant co-evolution have been described as "ghost stories" (McGlone and Clarkson, 1993), with at least two of the most notable growth forms (divarication and heteroblasty) also suggested to be responses to cold, arid and windy climatic conditions during the Late Pleistocene (Day, 1998; Howell et al., 2002; Rattenbury, 1962). However, it also has been noted that these forms are well represented on other islands where birds were dominant herbivores (Bond and Silander, 2007; Burns and Dawson, 2006). Because of their large, robust bodies, moa were originally interpreted as grassland grazers (as reviewed by Worthy, 1991), but analyses of several gizzard contents excavated from swamps has led to the current view that at least some genera (*Dinornis*, *Emeus*, *Euryapteryx*, *Pachyornis*) were browsers of trees and shrubs along forest margins (Burrows et al., 1981; Wood, 2007). However, the gizzards are biased towards diets in wetland and lowland forest habitats, or potentially vegetation within reach of a mired bird (Wood, 2007), and other direct evidence of diet is sparse.

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Coprolites have previously been used in palaeodietary studies of several extinct megaherbivores, but generally only for relatively low-resolution macrofossil analyses (e.g. Davis et al., 1984; Horrocks et al., 2004; James and Burney, 1997; Mead et al., 1986). Ancient DNA (aDNA) analysis provides the opportunity to identify both the defecating species and the dietary components, but the few studies of megafaunal coprolites have featured limited numbers of samples and have generally only been able to resolve floral components to order or family level (Hofreiter et al., 2000, 2003; Poinar et al., 1998). The only two palaeodietary studies of extinct avian herbivore coprolites (Horrocks et al., 2004; James and Burney, 1997) were limited by small sample sizes, and did not use aDNA so could only infer the identity of the defecating species. In New Zealand, Late Holocene coprolites of the extant kakapo (*Strigops habroptilus*), a large flightless parrot, have also been studied (Horrocks et al., 2008).

Moa coprolites could potentially provide dietary information across a broad range of habitats for the ten currently recognised moa species, but have only been rarely reported. We excavated rockshelter and cave sites from across southern New Zealand and recovered almost 2000 avian coprolites across a broad geographic area. From a subset of these we used plant macrofossils and aDNA to analyse the dietary specialisations of three sympatric moa species in two distinct ecological zones, in order to complement current moa palaeoecological reconstructions and investigate the issue of moa-plant co-evolution.

2. Regional setting

The newly identified coprolite deposits are located in the Otago region, South Island, New Zealand (Fig. 1). These occur in two broad ecological zones. First, Daley's Flat is situated in the Dart River Valley, a glacially-carved valley in West Otago, that receives >1.5 m mean annual rainfall (Tait et al., 2001). The coprolites were collected from dry shelters beneath large boulders (within a ca 60 ha rockfall deposit) in closed canopy silver beech (*Nothofagus menziesii*) forest, although grassland and patchy scrub occur in places on the valley floor. Despite a lack of palaeovegetation data from West Otago, other evidence suggests the vegetation is relatively unmodified compared to the pre-settlement situation. Pollen records from similar montane valley sites along central South Island (Burrows and Russell, 1990; McGlone et al., 1997, 2004; Moar, 1971, 1973) indicate a non-synchronous but repeated pattern of post-glacial shrubland establishment (14,000–10,000 years BP), followed by spread of tall podocarp forest (13,600–7500 years BP) and later expansion of *Nothofagus* dominated forest (>7500–2000 years BP). Present forest patterns in the mid- to upper-valley (around Daley's Flat) (Mark, 1977) suggest little or no post-settlement loss of forest, which is characteristic of central and eastern regions of South Island (e.g. McGlone, 2001; McGlone and Moar, 1998), although some reduction of palatable plant species in the forest understorey is likely due to introduced mammalian herbivores. In contrast, Central Otago is non-glaciated and has a semi-arid climate. Coprolites were excavated from four rockshelters (Sawers', Kawarau, Roxburgh Gorge B and Roxburgh Gorge C) (Fig. 1), located in river gorges that receive <500 mm mean annual rainfall (Tait et al., 2001). The present vegetation of Central Otago is highly modified due to anthropogenic burning, widespread land clearing for pastoralism in the 19th Century, and a suite of introduced grasses and weeds. Pre-settlement vegetation in the Central Otago lowlands was characterised by low shrubland and diverse herbs (McGlone and Moar, 1998; Wood and Walker, 2008).

3. Materials and methods

3.1. DNA extraction, PCR and sequencing

DNA extractions were carried out on a sub-sample of 43 of the coprolites on which macrofossil analysis was performed (22 from Dart River Valley and 21 from Central Otago), in the physically isolated, dedicated ancient DNA facility at ACAD (Australian Centre for Ancient DNA, University of Adelaide) following standard ancient DNA procedures (Cooper and Poinar, 2000). Coprolite pieces were ground to a fine powder using a Mikrodismembrater (Braun) and tungsten ball bearings. The powder was hydrated for 24 h in 10 mL of 10 mM Tris–HCl, pH 8.0. DNA was extracted from up to 0.6 g of hydrated powder using the MoBio Power Soil Kit following the

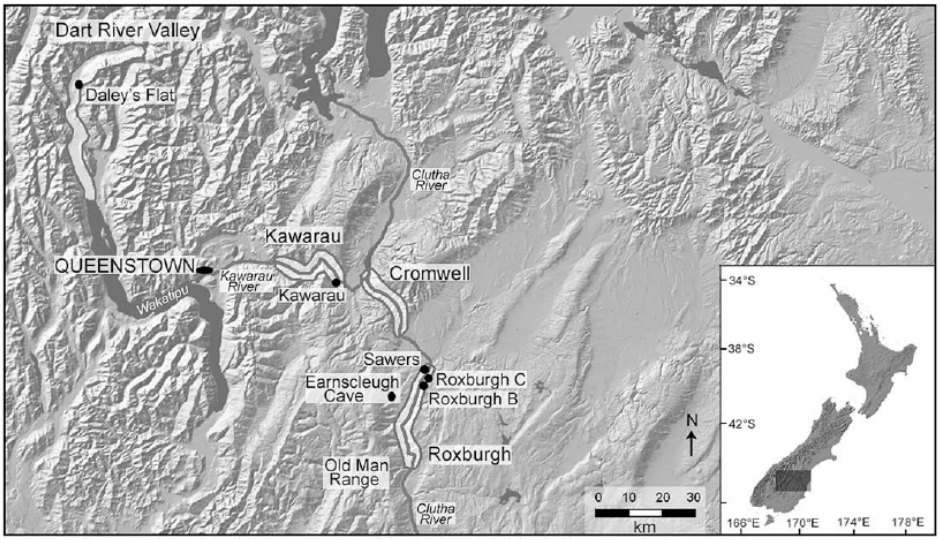

Fig. 1. The Otago region, South Island, New Zealand, showing the location of moa coprolite deposits studied, and the three Central Otago river gorges (Kawarau, Cromwell and Roxburgh).

manufacturer's instructions. Multiple negative extraction controls were included with each batch of extractions. Repeat extractions of a subset of the coprolites were performed to confirm that sequence results and specific status of the defecator was authentic. The defecating moa species were identified using mitochondrial DNA control region sequences (see Supplementary data), following PCR amplification of fragments of 200 bp, 48 bp, 31 bp or 11 bp in length (excluding primers) from each coprolite DNA extract. Moa nomenclature used here is based on that of Worthy and Holdaway (2002), incorporating subsequent revisions (Bunce et al., 2003; Huynen et al., 2003; Worthy, 2005).

PCR reactions were conducted in 25 μL volumes containing 2 mg/mL RSA (Sigma), 1× PCR buffer (Platinum, Invitrogen), 2 mM $MgSO_4$, 200 μM dNTPs, 1 μM each primer, 1 unit Platinum Taq HiFi (Invitrogen), 1–2 μL DNA. PCR reactions were set up in the ancient DNA facility at ACAD. Multiple negative PCR controls were included with each batch of PCR amplifications. PCR conditions were as follows: 94 °C 3 min; 55 cycles of 94 °C 30 s, 55 °C 30 s and 68 °C 45 s; followed by 68 °C 10 min, then holding at 15 °C. The PCR amplification reaction and all downstream procedures were carried out in the ACAD post-PCR lab on the main campus of the University of Adelaide.

PCR products were visualised on 4% 1× TBE agarose gel. PCR products >100 bp (including primers) were purified using the AMPURE magnetic bead system (Agencourt) following the manufacturers instructions. Those <100 bp (including primers) were purified using EXOSAP (4 units Exo1, 0.6 units SAP) by incubation at 37 °C for 30 min and 80 °C 15 min.

PCR products were sequenced in both directions using the M13USP, M13RSP or PCR primers (see Supplementary data) using Big Dye Terminator technology (BigDye v3.1) and separated on an ABI 3130XC capillary sequencer. Sequences obtained from the coprolites have been deposited in the GenBank database (accession numbers FJ214598–FJ214618), and are provided in the supplementary information.

3.2. Macrofossil analysis

Plant taxa browsed by moa were determined from macrofossils preserved within coprolites. A sample of 116 coprolites (81 from Dart River Valley and 35 from Central Otago), identified as likely belonging to moa due to their large size, were selected for macrofossil analysis. Approximately 10–20% of each coprolite was removed for DNA analysis. The remaining portion was softened in a standard household detergent solution for 2–3 weeks and then disaggregated by gentle mashing. Sediment from each disaggregated coprolite was examined in a Petri dish under a dissecting microscope at 10–40× magnification. All seeds, leaf cuticle fragments and invertebrate remains were picked from the dish with fine forceps and stored in glass vials of 70% ethanol. Seeds were identified using reference images (Webb and Simpson, 2001), and by comparison with specimens in the Otago Regional Herbarium, New Zealand. Where greater detail was required for identification, seeds were examined using a Cambridge scanning electron microscope. Leaf cuticles were mounted in glycerol on glass slides, and identified by comparison with the Allan Herbarium cuticle photograph reference collection, Lincoln, New Zealand. Remaining coprolite sediment residues and associated plant macrofossils are held at Otago Museum, Dunedin, New Zealand (OM Av10656–10767).

3.3. ^{14}C age determination

AMS radiocarbon dates were obtained for coprolites or contemporaneous organic sediment from three rockshelters. Samples underwent standard ABA pre-treatment and graphitisation at Waikato Radiocarbon Dating Laboratory, and were analysed at Rafter Radiocarbon Laboratory. Radiocarbon dates were calibrated using the Southern Hemisphere calibration curve (McCormac et al., 2004).

3.4. Statistical analyses

Differences in coprolite seed assemblages between moa species in Dart River Valley, and between Central Otago and Dart River Valley, were assessed using the general linear model function of the statistical program Minitab v.15. Seed count data (see Supplementary information) were log_{10}-transformed prior to analysis and residuals were plotted to ensure normality. Potential moa preferences for different plant types (differences between percentage of seeds in coprolites and bulk rockshelter sediments) were assessed by two-sample *t*-test.

4. Results

4.1. DNA identification

Of the 43 coprolites analysed for moa DNA, 24 were positively identified as moa (Fig. 2). Extractions of the remaining 19 either failed to amplify, or amplified DNA fragments of the incorrect length. It was noted that the majority of coprolites that gave amplifiable moa DNA had relatively hard exteriors, and this, along with constant aridity of the deposition site, may be important factors for DNA preservation in coprolites. A single clean sequence

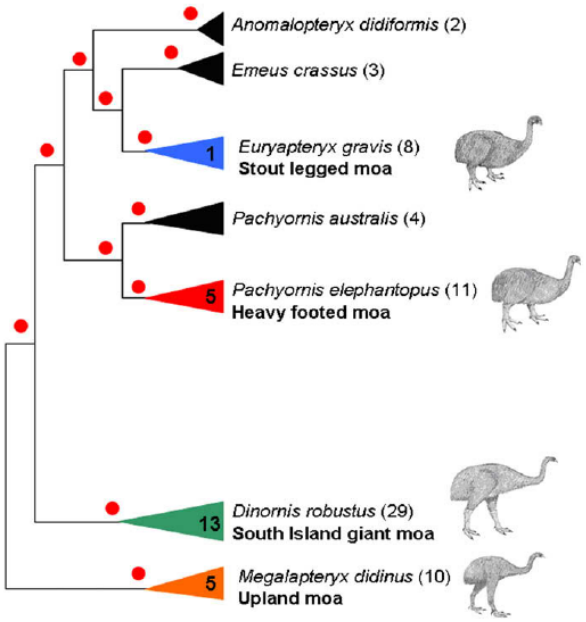

Fig. 2. Maximum parsimony strict consensus phylogeny (ten random sequence addition replications, 500 bootstrap replicates) of reference moa (2914 bp of concatenated CR, 12S, COIII, ATP6, Cyt b, ND3, ND4, ND5, tRNA Lys or 379 bp CR data depending on specimen) constructed using PAUP*4.0b10. All major branches and clades in the phylogeny below have bootstrap values greater than 90% (represented by red dots above the relevant branches/clades. See Supplementary figure 2 for phylogeny). To determine specific defecator status, partial control region sequences from moa coprolites were placed onto this phylogeny using a full heuristic maximum parsimony search with the bootstrap phylogeny as a backbone constraint. Highlighted clades contain sequences from moa coprolites, with the number in the clade representing the number of coprolite sequences obtained. Numbers in brackets next to the species designation for each clade represent the number of sequences within a given clade.

(i.e. not multiple sequences, indicative of more than one PCR product the same size) was obtained from each coprolite sample, and sequences from repeat extractions and PCRs were identical to the initial results. Comparison of the sequences against a comprehensive database of moa control region sequences (77 published and 300 unpublished sequences, encompassing the entire geographic range of each species in the South Island) was sufficient to identify the moa species which had deposited each coprolite. Because only a single sequence was obtained from each sample, and the samples were taken from the coprolite interior, the results are assumed to be from the depositing individual, rather than the result of contamination within the rockshelter sediments.

DNA sequences identified coprolites from four moa species: *Dinornis robustus* (South Island giant moa, $n = 13$), *Megalapteryx didinus* (upland moa, $n = 5$), *Pachyornis elephantopus* (heavy-footed moa, $n = 5$), and *Euryapteryx gravis* (stout-legged moa, $n = 1$). The single *E. gravis* coprolite provided only an 11 bp sequence that is identical in both *E. gravis* and little bush moa (*Anomalopteryx didiformis*), but was identified as the former species because bones of *A. didiformis* are not known from Central Otago (Worthy, 1998; Wood, 2008a). This tentative identification is not important in the interpretation of the results. The other 92 coprolites could be attributed to moa on the basis of size and morphological similarity with the identified specimens, although as no inter-specific variation in coprolite morphology was detectable (Fig. 3) these could not be assigned to species. The species identifications obtained for coprolites by aDNA analysis fit those expected from known Holocene moa distributions, based on analysis of fossil bone deposits. Coprolites of *D. robustus*, *P. elephantopus* and *M. didinus* were identified from Dart River Valley, and bones of all three species were found beneath rockshelters in the immediate area. Coprolites of *P. elephantopus*, *M. didinus* and *E. gravis* were identified from Central Otago, and were all common in the region during the Holocene (Worthy, 1998).

4.2. Macrofossils

Coprolites were mostly comprised of amorphous organic material interpreted as digested leaf, fruit or woody tissues. Identifiable plant macrofossils (Table 1) were dominated by seeds (recognised in 58.6% of coprolites examined), although intact leaves, leaf margins, thorns and cuticle fragments were also present. Few wood fragments were recovered, and none were large enough to identify by microstructural analysis. Frequent intact seeds permitted high resolution (typically to species) analysis of the taxonomic diversity and composition of plant taxa eaten. Remains of at least 18 plant taxa were identified in coprolites from Dart River Valley, and at least 19 in the coprolites from Central Otago, with 7 taxa common between both regions (Table 1). Invertebrate remains were also present in the coprolites, but were not frequent enough to dismiss their accidental ingestion with vegetation. Identified remains included fragments of beetle, oribatid mites, and post-depositional fly exuviae.

4.3. Coprolite ages

All calibrated radiocarbon ages of coprolite horizons in Central Otago rockshelters were < 3500 years BP (Table 2) and the similar stratigraphy observed in undated sites suggests contemporaneity (Wood and Walker, 2008). The exact age of the coprolites from Dart River Valley is not known, although they must post-date the retreat

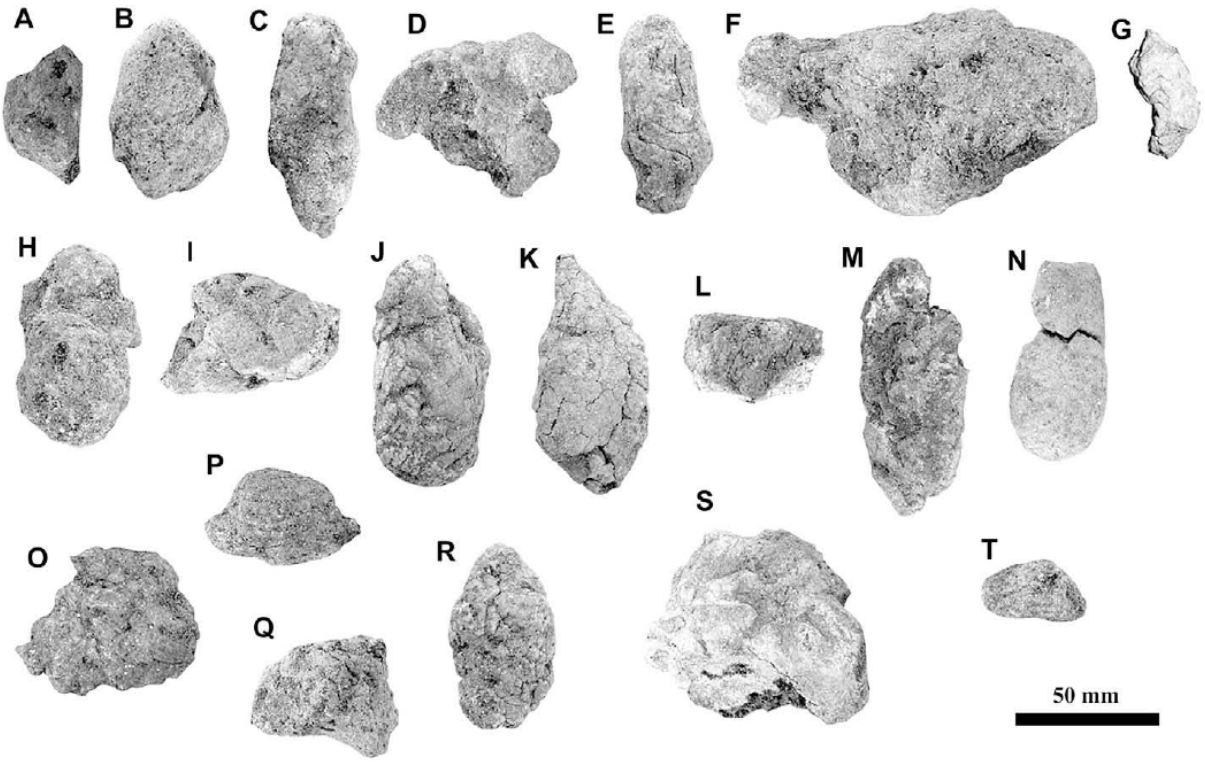

Fig. 3. Morphology of coprolites from three moa species identified from a DNA analysis: A–C, *Pachyornis elephantopus*; D–H, *Megalapteryx didinus*; I–T, *Dinornis robustus*. All coprolites shown are from Dart River Valley, except G (from Old Man Range, Central Otago).

Table 1
Summary of macroremains identified from moa coprolites

Plant taxa	Dart River Valley				Central Otago			
	Diro	*Pael*	*Medi*	Unid.	*Pael*	*Medi*	*Eugr*	Unid.
	($n = 13$)	($n = 3$)	($n = 4$)	($n = 61$)	($n = 2$)	($n = 1$)	($n = 1$)	($n = 31$)
Trees and tall shrubs								
Carmichaelia sp.	–	–	–	0.02 *s*	–	–	–	–
Coprosma spp.	0.46 *s*	0.67 *s*	0.25 *s*	0.20 *s* 0.07 *l*	0.5 *l*	–	–	0.03 *s*
Coprosma or *Olearia*	–	–	–	–	4 *l*	–	1 *l*	0.10 *l*
Hebe sp.	–	–	–	–	–	–	8 *s*	–
Melicytus sp.	–	–	–	–	0.5 *s*	–	–	0.03 *s*
Nothofagus menziesii	0.08 *l*	–	–	0.02 *l*	–	–	–	–
Olearia sp.	–	–	–	–	–	–	1 *s*	–
Gymnosperm	–	0.33 *s*	–	0.05 *s* 0.02 *l*	–	–	–	–
	0.46 *s* **0.08 *l***	**1 *s***	**0.25 *s***	**0.27 *s*** **0.11 *l***	**0.5 *s*** **4.5 *l***	–	**9 *s*** **1 *l***	**0.06 *s*** **0.10 *l***
Lianes								
Muehlenbeckia axillaris	0.31 *s*	–	0.25 *s*	0.18 *s*	1.5 *s*	–	–	0.55 *s*
Rubus sp.	–	–	–	–	–	–	–	0.6 *s* 0.32*t*
	0.31 *s*	–	**0.25 *s***	**0.18 *s***	**1.5 *s***	–	–	**1.15 *s*** **0.32*t***
Subshrubs and dicot herbs								
Ceratocephala pungens	–	–	–	–	0.5 *s*	–	–	0.48 *s*
Colobanthus sp.	–	–	–	–	–	–	–	0.06 *s*
Coprosma petriei	5.77 *s*	6 *s*	11.75 *s*	1.34 *s*	–	–	–	–
Coriaria plumosa	5.23 *s*	–	1.5 *s*	0.23 *s*	–	–	–	–
Einadia triandra	–	–	–	–	3 *s*	–	–	0.48 *s*
Chenopodiaceae cf. *E. allanii*	–	–	–	–	–	–	–	1.68 *s*
Gaultheria crassa	1 *s*	–	0.5 *s*	1.82 *s*	–	–	–	–
Gonocarpus aggregatus	0.38 *s*	0.67 *s*	0.5 *s*	0.11 *s*	–	–	–	–
Lagenifera pumila	0.08 *s*	1.33 *s*	0.25 *s*	0.13 *s*	–	–	–	–
Leucopogon fraseri	1.69 *s* 3.85 *l*	4.33 *s* 11 *l*	10.5 *s* 5.25 *l*	1.85 *s* 2.16 *l*	–	–	–	0.06 *l*
Myosotis pygmaea s.l.	–	–	–	–	–	–	–	0.03 *s*
Myosurus minimus novae-zelandiae	–	–	–	–	–	–	–	0.58 *s*
Oxalis exilis	–	–	–	–	–	–	–	0.71 *s*
Pratia angulata	0.08 *s*	–	0.25 *s*	0.15 *s*	–	–	–	–
Ranunculus gracilipes	0.46 *s*	77 *s*	16.5 *s*	1.7 *s*	–	–	–	–
Ranunculus sp.	1.23 *s*	3 *s*	1 *s*	0.13 *s*	–	1 *s*	–	–
Urtica incisa	0.15 *s*	1.33 *s*	0.5 *s*	0.7 *s*	–	–	–	–
Wahlenbergia pygmaea	0.23 *s*	–	0.25 *s*	0.15 *s*	–	–	–	–
Unidentified	–	–	–	–	–	*c*	–	*c*
	16.3 *s* **3.85 *l***	**93.66 *s*** **11 *l***	**43.5 *s*** **5.25 *l***	**8.31 *s*** **2.16 *l***	**3.5 *s***	**1 *s***	–	**4.02 *s*** **0.06 *l***
Monocot herbs								
Carex sp.	0.38 *s*	7.67 *s*	1 *s*	0.25 *s*	–	–	–	0.58 *s*
Juncus sp.	–	0.33 *s*	–	0.02 *s*	–	–	–	1.35 *s*
Poaceae	–	–	–	–	1.5 *s*	–	–	0.06 *s*
Unidentified	–	–	–	–	–	*c*	–	*c*
	0.38 *s*	**8.0 *s***	**1 *s***	**0.27 *s***	**1.5 *s***	–	–	**1.99 *s***
Invertebrate fragments	0.15	1.67	–	1.10	2	3	–	0.61

Data are mean number per coprolite; bold numbers are totals. Moa species are: *Diro*, *Dinornis robustus*; *Pael*, *Pachyornis elephantopus*; *Medi*, *Megalapteryx didinus*; *Eugr*, *Euryapteryx gravis*; Unid., unidentified. Macroremains are: *s*, seeds; *l*, leaves; *t*, thorns, *c*, leaf cuticle.

Table 2
Radiocarbon dates from sites associated with moa coprolites examined in this study

Lab. no.	Site	Material dated	$\delta^{13}C$ (‰)	$\delta^{14}C$	% modern	Radiocarbon age, years BP	Calibrated age (2 s.d.), years BP
Wk-16348	Kawarau Gorge rockshelter	Coprolite (27 cm depth)	-29.1 ± 0.2	-122.9 ± 3.3	88.1 ± 0.4	1017 ± 34	954–795
Wk-16382	Roxburgh Gorge rockshelter B	Plant material from coprolite layer	-26.0 ± 0.2	-306.8 ± 8.6	69.5 ± 0.9	2928 ± 100	3319–2778
Wk-19082	Sawers' rockshelter	Plant material from coprolite layer	-27.8 ± 0.2	-205.7 ± 3.0	79.5 ± 0.3	1843 ± 35	1823–1605
NZA-4615	Earnscleugh Cave	*Euryapteryx gravis* bone	−24.37	?	?	2176 ± 76	2322–1930
NZA-4596	Earnscleugh Cave	*Sceloglaux albifacies* bone	−19.78	?	?	1552 ± 68	1528–1295

Ages are calibrated using Southern Hemisphere Calibration Curve (McCormac et al., 2004). Dates for Kawarau Gorge, Roxburgh Gorge and Sawers' rockshelters from Wood and Walker (2008), and Earnscleugh Cave from Clark et al. (1996).

of the Dart Glacier beyond Daley's Flat, and therefore are probably also of mid to late Holocene age.

4.4. Moa diet and habitat

The coprolite contents show that all the moa taxa consumed a diverse range of plants (at least 30 taxa) with a strong dominance of herbs and subshrubs (<1 m tall) (Table 1, Fig. 4). Overall, different moa species within the same habitat type appeared to eat a very similar range of plant species (Table 1). The relative abundances of different plant types (i.e. trees and tall shrubs, lianes, subshrubs and dicot herbs, and monocot herbs) also did not vary greatly between species; there was no significant variation seen in the types of plants eaten by three sympatric moa species in the Dart River Valley ($F = 1.93$, $P = 0.088$) (Fig. 4), where the number of identified coprolites was sufficient to allow such a comparison. The smaller number of identified coprolites ($n = 4$, of three species) from Central Otago prevented a similar comparison between species. Surprisingly, variation between the seed assemblages of coprolites from Dart River Valley and Central Otago ($F = 4.17$, $P = 0.006$) exceeded the interspecific variation observed in the Dart River Valley alone (Fig. 4), and is presumed to reflect a strong habitat influence. Plant species identified in coprolites from Central Otago are typical of the shrubland with herb-rich understoreys that existed throughout the Central Otago river gorges during the late Holocene (Wood and Walker, 2008). Plant species identified in coprolites from the Dart River Valley are characteristic of moraine and rockfall habitats, and valley floor grasslands, of West Otago (Mark, 1977).

Seed assemblages in bulk sediment from coprolite-bearing horizons in three Central Otago rockshelters (Wood and Walker, 2008) provide a proxy for palaeovegetation communities, and therefore a unique opportunity to assess plant taxa preferences of moa. The data appear to suggest that not only did subshrubs and herbs form a larger part of the diet of moa in Central Otago, but that they were preferentially selected for by moa (Fig. 5). However, these potential preferences were not statistically significant (for the difference between the two preference extremes; monocot herbs and trees/tall shrubs, $t = -2.58$, $P = 0.123$).

5. Discussion

5.1. Moa coprolite deposits

That moa utilised rockshelters and caves, whether for shelter, roosting, or nesting, is supported by numerous moa remains (bones, eggshell, feathers, DNA) obtained from such sites throughout New Zealand (e.g. Ambrose, 1970; Haile et al., 2007; Hamilton, 1894; Hartree, 1999; White, 1877; Wood, 2008b). Large coprolites, putatively identified as being from moa, have previously been reported from caves and rockshelters in southern New Zealand (Duff, 1952; Hamilton, 1894; Ritchie, 1982; Trotter, 1970; White, 1877). For the first time, our study has confirmed that some coprolites found in such sites are attributable to moa. Evidence from Central Otago suggests that some coprolites may be associated with nesting sites (Wood, 2008b).

The range of size and morphology seen in coprolites recovered suggests that other extinct birds may also be represented in the deposits, including South Island goose (*Cnemiornis calcitrans*) and Finsch's duck (*Chenonetta finschi*).

Desiccation of organic matter, such as coprolites, is dependent on a consistently arid microclimate in the site of deposition, preventing decay by fungi and invertebrates (Hansen, 2001). Whereas published radiocarbon ages of fossil remains from Central Otago sites that are not strictly dependant on aridity for preservation (e.g. swamps, loess, colluvium) have a relatively even temporal distribution throughout the Late Pleistocene and Holocene, radiocarbon ages of remains from dry caves and rockshelters (including the coprolite deposits reported on here) are clustered in the late Holocene (Wood 2008). This reflects the most intense period of ENSO (El Niño Southern Oscillation) cycling within the last 150,000 years (Johnson, 2006), which led to the mid-Holocene aridification of the Central Otago region (McGlone and Moar, 1998). This increased aridity may have directly influenced coprolite

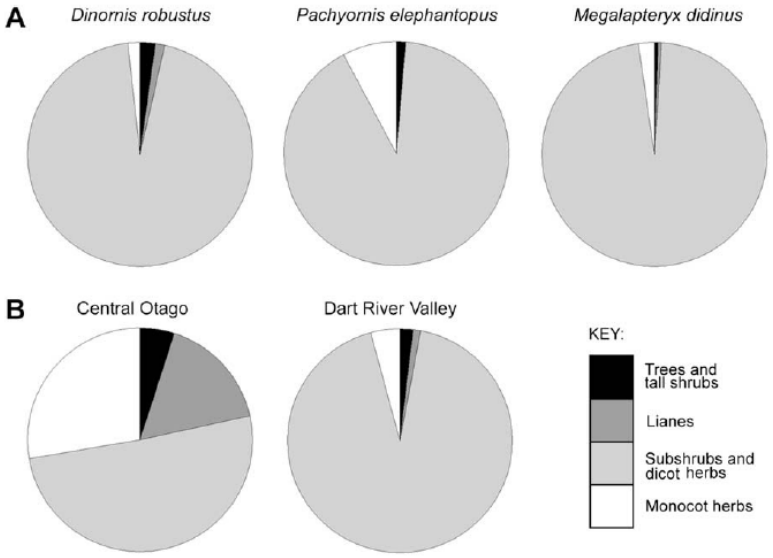

Fig. 4. Diet variation in moa represented by percentage of seeds in coprolites. (A) Interspecific variation between three sympatric moa species (*Dinornis robustus*, $n = 13$; *Pachyornis elephantopus*, $n = 3$; *Megalapteryx didinus*, $n = 4$) in the Dart River Valley. (B) Geographic- or habitat-related variation between total samples from Central Otago ($n = 35$) and Dart River Valley ($n = 81$).

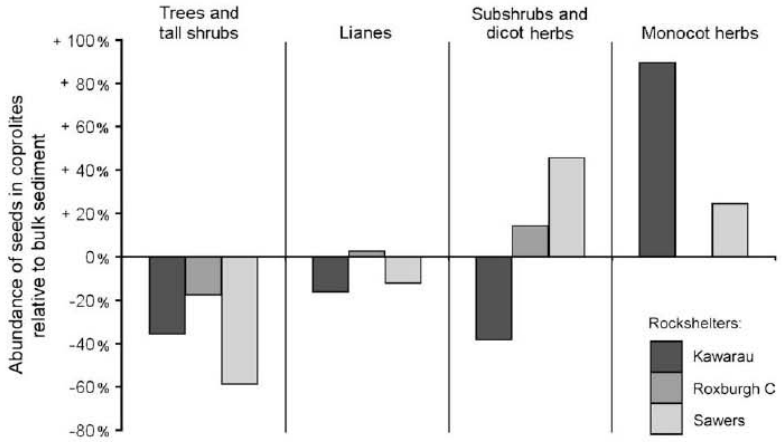

Fig. 5. Selection of plant taxa by moa in Central Otago, represented by comparison of seed assemblages from moa coprolites and bulk-sediment from coprolite-bearing horizons (proxy of local palaeovegetation composition) (Wood and Walker, 2008). Preferential selection by moa is suggested by positive values.

preservation, and therefore, continuous stratigraphic records of desiccated coprolites extending beyond ca 4000 years BP may be rare.

5.2. Moa diet and habitat

We suggest that some of the variation in plant macrofossil content between individual coprolites is possibly due to seasonal diet variation, with fruit/seed being widely available during summer months but less common during winter. A similar seasonal pattern was present in data from a study of Hawaiian moa-nalo coprolites (James and Burney, 1997), where pollen/spore counts were high ($n > 200$) in 33% of coprolites examined (suggesting deposition of these during spring/summer), but were low ($n < 40$) in 67% (suggesting deposition during winter).

While faecal seed analysis is often used to determine diets of primarily frugivorous birds, such as cassowary (*Casuarius* spp.) (e.g. Bradford et al., 2008; Wright, 2005), the correlation with diet of more general herbivores is less well known. A potential bias in our interpretation of moa diet is that amorphous organic material in the coprolites was not able to be identified, and may include plant species not represented by seeds. However, we believe seeds in coprolites are a good indicator of the plant taxa eaten by moa, as they are likely to be ingested whether a bird is feeding on fruit, or incidentally in association with browsing of foliage, twigs or whole plants. In support of this, studies of relatively undigested plant remains from moa gizzards reveal the majority of plant species identified were represented by seeds (87% in Burrows et al., 1981; 72% in Wood, 2007). Although seeds are indicative of plant taxa consumed, they do not necessarily reveal details of the diet such as the parts of plants that are being preferentially eaten. For example, our data indicate that three moa species (*M. didinus, D. robustus*, and *P. elephantopus*) were feeding on a very similar range of plants in the Dart River Valley (Fig. 2). However, we cannot resolve whether one of these species was, for example, preferentially feeding on fruit, and another on twigs and leaves. Some variation of this sort might be expected from the morphological variation in moa bills (Atkinson and Greenwood, 1989; Worthy and Holdaway, 2002). We also compare seed assemblages from coprolites and bulk rockshelter sediment to provide an estimate of plant types preferred by moa in Central Otago (Fig. 5). The underlying assumption is that most seeds within the bulk sediment were deposited by falling from vegetation growing around each site. A potential problem is that disaggregated coprolites may contribute to the bulk sediment, and therefore the seed assemblage within the sediment is not an independent measure of local vegetation. However, any contribution of seeds from coprolites into the bulk sediment is likely to reduce the amount of variation between the two assemblages, and therefore provide an underestimate of any moa preferences.

While the similar dietary preferences between different moa taxa are surprising, there is also a remarkable similarity between moa and several still extant native avian herbivores. For example, the diet of kakapo (*Strigops habroptilus*), an endangered large flightless parrot, includes many of the plants found in moa gizzards and coprolites, including highly fibrous *Olearia* twigs and *Phormium* leaves (Atkinson and Merton, 2006; Horrocks et al., 2008). It is possible that for generalist herbivores the preferential selection of nitrogen-rich food, especially juvenile plants, may result in significant dietary overlap, with plant taxa eaten being differentiated by plant availability and accessibility rather than quality. This is likely to be particularly evident on islands with depauperate floras, but may have also been a factor in continental ecosystems with megafauna.

Seeds provide a good indication of the habitat that the moa were utilising although it is possible that some bias may exist in seeds that pass through into the droppings intact. Larger and denser seeds (e.g. *Prumnopitys*, *Elaeocarpus*) may be retained in the gizzard longer than small seeds (Wilson, 1989), and therefore may be proportionally under-represented in coprolites. However, we do not see this as biasing habitat interpretations, as moa gizzard contents from North Canterbury (Burrows et al., 1981), and coprolites from Dart River Valley, both contained abundant seeds of *Coprosma* spp., yet those in the gizzards were of forest species whereas those in the coprolites were of a non-forest, prostrate species.

Two of the moa species identified from Dart River Valley (*P. elephantopus* and *M. didinus*) are characteristic of open shrubland and low vegetation (Worthy and Holdaway, 2002). The third, *Dinornis robustus*, occurred in these habitats but also in heavily forested regions (Worthy and Holdaway, 2002). Despite the bone assemblage being representative of shrubland or grassland habitat, the lack of forest plant remains in the coprolites was unexpected, as they were excavated from sites currently beneath a closed forest canopy. Three possible explanations exist. First, following the Holocene retreat of glaciers from the valley, forest would likely have had to reinvade from the south (near Lake Wakatipu), and therefore may not have been widespread in the upper valley when

the coprolites were deposited. Second, the coprolites may represent a period soon after the rockfall occurred, and prior to regeneration of forest. Third, moa may have roosted and nested in the rockshelters within the forest, but moved out of the forest to preferentially feed on plants in open habitats. Such movement may have also been altitudinal; however this is unlikely as we did not identify any exclusively subalpine plant taxa in the coprolites.

Remarkably, for such large birds (up to 250 kg and 2 m tall at back), more than half of the identified plant taxa are <30 cm in height. This pattern is in direct contrast to the dietary inferences from the gizzard contents, and current concepts of moa, in particular *Dinornis*, as being dominantly a tree and shrub browser (Burrows et al., 1981; Wood, 2007). One explanation for this discrepancy may involve the extreme sexual dimorphism in *Dinornis*, where females are up to 280% the weight and 150% the height of males (Bunce et al., 2003), which might suggest strong sexual variation in food selection. Examined gizzard contents from *Dinornis* have almost exclusively been from females (Wood, 2007), and therefore the *Dinornis* coprolites from Dart River may represent the varying diet of males. Another alternative is that *Dinornis*, which had a wide distribution across varying habitat types (Worthy and Holdaway, 2002), also had a broad dietary range, and therefore its feeding tier varied with the available vegetation.

The coprolite results, synthesised with previous dietary data, provide an important new assessment of the diet of three moa genera. Our coprolite data reveal that *D. robustus* and *P. elephantopus* were substantial grazers; however, gizzard content analyses (Burrows et al., 1981; Wood, 2007) have indicated that they also browsed trees and tall shrubs along forest margins. Our results suggest that *M. didinus* was predominantly a herb grazer (Fig. 4, Table 1). Some twigs were identified in putative *M. didinus* coprolites by Horrocks et al. (2004), but aDNA identification of these coprolites was not carried out. Therefore, we do not include these in the synthesis on the possibility that they may be from another moa species that existed in the area (e.g. *A. didiformis*, *D. robustus*). We also identified a single coprolite from *E. gravis*, and although few plant remains were identified in this specimen, our data complement gizzard content analyses that suggest this species fed on leaves and fruit of trees and shrubs (Gregg, 1972).

These feeding differences are reflected in morphology, where *Dinornis* and *Pachyornis* have comparatively robust bills, large temporal fossae and large gizzard stone sets (Wood, 2007; Worthy and Holdaway, 2002), all of which are consistent with diets that feature relatively high proportions of fibrous leaves and twigs. Intriguingly, dietary variation detected between the genera was relatively minor compared to the amount of variation observed between the different ecological zones (Fig. 4). This raises interesting questions about how these megafaunal species partitioned niches in the many areas of apparent sympatry throughout New Zealand, and seems to support the suggestion by Atkinson and Greenwood (1989) that niche partitioning in moa may not have been important until food was scarce, such as during droughts. Further to this, the habitat component of niche partitioning may have been more pronounced during Pleistocene glacial periods when shrubland and grassland habitats would have been widespread (McGlone et al., 1993) and forest was restricted to topographically-sheltered sites. Graviportal and short-statured moa genera (*Pachyornis*, *Euryapteryx*, *Emeus*, *Megalapteryx*) that specialised in utilising such habitats (Worthy and Holdaway, 2002) would have been common, with partitioning of *Dinornis* to more forested habitats. A warming climate led to the ascendancy of forest (McGlone et al., 1993) and, across much of lowland New Zealand, shrubland and grassland habitats were restricted to small pockets edaphic conditions, cold air drainage, or interspersed within a forest matrix and maintained by disturbances such as fire and herbivory (Rogers et al., 2005). Accordingly, *P. elephantopus*, *M. didinus* and *D. robustus* exhibit significant habitat overlap during the Holocene.

5.3. Co-evolution and ecological surrogacy

Coprolites and gizzards are now known to contain plant taxa representing at least eight plant growth forms hypothesised to be responses to moa-browsing (Atkinson and Greenwood, 1989;

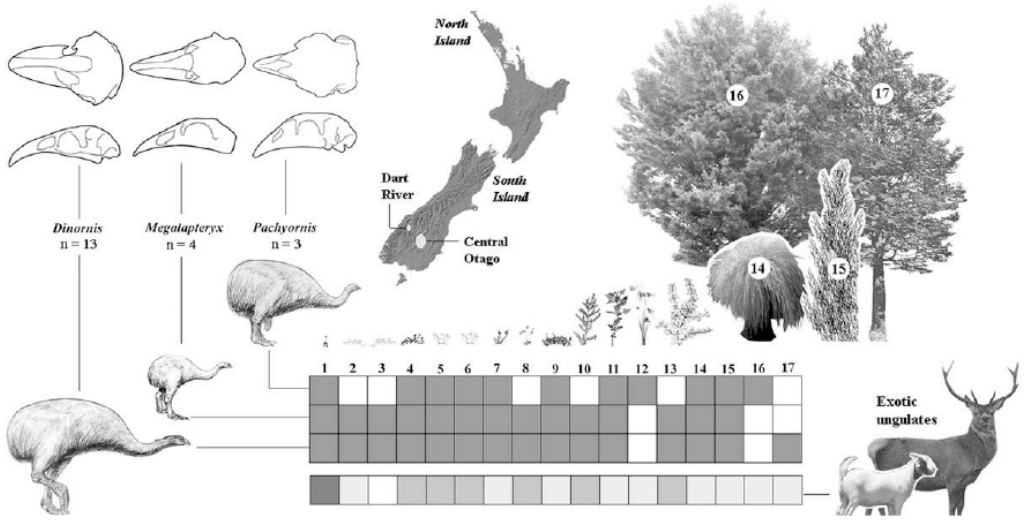

Fig. 6. Similarity in plant taxa eaten by moa species at Dart River and difference with those eaten by exotic ungulates. Plant taxa 1–17 are those identified by macrofossils in moa coprolites, Dart River: 1, *Lagenifera pumila*; 2, *Pratia angulata*; 3, *Muehlenbeckia axillaris*; 4, *Gonocarpus aggregatus*; 5, *Ranunculus* sp.; 6, *R. gracilipes*; 7, *Leucopogon fraseri*; 8, *Wahlenbergia pygmaea*; 9, *Coprosma petriei*; 10, *Coriaria plumosa*; 11, *Urtica incisa*; 12, *Juncus* sp.; 13, *Gaultheria crassa*; 14, *Carex* sp.; 15, *Coprosma* spp.; 16, Gymnosperm; 17, *Nothofagus menziesii*. All plants are shown to scale with moa except 16,17 which are shown approximately 1/5 maximum height. Ungulate diet data (Forsyth et al., 2006) are presented as: dark grey, plant taxa preferred by ungulates; medium grey, plants recorded as being eaten by ungulates; light grey, plant taxa avoided by ungulates; white, no published records of ungulates browsing the plant taxa.

Batcheler, 1989; Greenwood and Atkinson, 1977), including divarication, toxicity, photosynthetic stems, prostrate-filiramulate habit, fibrous leaves, stinging hairs and low nutrient status, confirming that plants with these characteristics were indeed part of moa diet, and supporting the co-evolution hypotheses.

In Central Otago, several of the plant species detected in the coprolites are now rare, due to anthropogenically increased fire frequency and pastoralism. New Zealand has four tiny (ca 20 mm diameter) dryland 'spring annual' herbs, including *Ceratocephala pungens* and *Myosurus minimus novae-zelandiae* (Ranunculaceae), both of which are currently endangered and lack dispersers (Rogers et al., 2002, 2007). However, distinctive seeds of these two taxa were unexpectedly common in coprolites from some Central Otago sites, suggesting potential ecological relationships existed between moa and these plants. Although seed dispersal mutualisms are rarely specific to single animal taxa (Herrera, 2002), moa are likely to have played a major role in dispersing the seeds of a range of plants which relied on avian herbivores for dispersal. If so, then reduced avian herbivory is a potentially novel threat-syndrome for New Zealand's herbaceous flora. Furthermore, the presence of spring annuals in the coprolites, as well as a range of other taxa (e.g. *Einadia*, Poaceae) supports the "foliage is the fruit" hypothesis (Janzen, 1984), which posits that herbs with dry indehiscent seeds closely associated with nutritious foliage are adapted for dispersal by large herbivores.

Importantly, the coprolite data strongly refute the idea that modern populations of introduced ungulates (deer and goats) might act as browsing surrogates for moa (Caughley, 1983), as the coprolites show moa ate a high proportion of plant taxa that are avoided by ungulates (Fig. 6), including several that are potentially toxic to mammals (e.g. *Coriaria*, Ranunculaceae) (see Supplementary data). Conversely, the data reveal that in some non-forest habitat types, the feeding ecologies of moa were similar to extant ratites that utilise open habitats (emu, *Dromaius novaehollandiae*; ostrich, *Struthio camelus*; Rhea, *Rhea* spp.). The diets of these birds are comprised mostly of leaves, flowers, fruits and seeds of grasses, dicot herbs and low shrubs (Marchant and Higgins, 1990; Noble, 1991). However, moa gizzard content studies (Burrows et al., 1981; Wood, 2007) and cafeteria experiments (Bond et al., 2004) suggest that, in more forested habitats, some moa species were able to exploit woody twigs to an extent unparalleled by extant ratites. Such data question the suggested suitability of ostriches (*Struthio camelus*) to act as ecological "analogues" of moa (Nicholls, 2006), by filling a similar evolutionary niche. While a similar ecological function may be fulfilled in rare non-forest situations, ostrich, emu and rhea would clearly not be proxies for moa in all indigenous habitat types. Neither would the forest-dwelling cassowary, whose diet consists largely of fallen fruits (Bradford et al., 2008; Wright, 2005). Overall, the new dietary information raises the issue of how many apparently closely related proxy species for Pleistocene rewilding (Donlan et al., 2005) would actually be valid.

6. Conclusions

Our preliminary study, utilising recently discovered late Holocene coprolite deposits, has begun to shed light on the diet and ecology of moa. Importantly, we have shown that (1) some moa species, previously thought of as tree and shrub browsers, in fact had widely varied diets that included grazing on tiny herbs in non-forest habitats; (2) several plant taxa eaten by moa exhibit adaptations that were previously hypothesised to have co-evolved with these birds; and (3) the feeding ecology of moa does not reflect that of introduced ungulates, currently the dominant megaherbivores in New Zealand terrestrial ecosystems. The discovery of the existence of a geographically and ecologically widespread record of megaherbivore coprolites in New Zealand now provides a unique opportunity to reconstruct the first detailed ecological picture of extinct megafauna, encompassing different habitats, seasonal variation, and species and sexual differences. Future study of this resource, using a range of aDNA, macro- and micropalaeontological techniques, promises to rapidly expand our knowledge of New Zealand's lost avian herbivores.

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Supplemental material

Supplementary information for this manuscript can be downloaded at doi: 10.1016/j.quascirev.2008.09.019.

References

Ambrose, W., 1970. Archaeology and rock drawings from the Waitaki Gorge. Records of the Canterbury Museum 8, 383–437.
Atkinson, I.A.E., Greenwood, R.M., 1989. Relationships between moas and plants. New Zealand Journal of Ecology 12 (supplement), 67–96.
Atkinson, I.A.E., Merton, D.V., 2006. Habitat and diet of kakapo (*Strigops habroptilus*) in the Esperance Valley, Fiordland, New Zealand. Notornis 53, 37–54.
Batcheler, C.L., 1989. Moa browsing and vegetation formations, with particular reference to deciduous and poisonous plants. New Zealand Journal of Ecology 12 (supplement), 57–65.
Bond, W.J., Silander, W.J., 2007. Springs and wire plants: anachronistic defences against Madagascar's extinct elephant birds. Proceedings of the Royal Society B 274, 1985–1992.
Bond, W.J., Lee, W.G., Craine, J.M., 2004. Plant structural defences against browsing birds: a legacy of New Zealand's extinct moas. Oikos 104, 500–508.
Bradford, M.G., Dennis, A.J., Westcott, D.A., 2008. Diet and dietary preferences of the southern cassowary (*Casuarius casuarius*) in North Queensland, Australia. Biotropica 40, 338–343.
Bunce, M., Worthy, T.H., Ford, T., Hoppitt, W., Willerslev, E., Drummond, A., Cooper, A., 2003. Extreme reversed sexual size dimorphism in the extinct New Zealand moa *Dinornis*. Nature 425, 172–175.
Burns, K.C., Dawson, J.W., 2006. A morphological comparison of leaf heteroblasty between New Caledonia and New Zealand. New Zealand Journal of Botany 44, 387–396.
Burrows, C.J., Russell, J.B., 1990. Aranuian vegetation history of the Arrowsmith Range, Canterbury I. Pollen diagrams, plant macrofossils, and buried soils from Prospect Hill. New Zealand Journal of Botany 28, 323–345.
Burrows, C.J., McCulloch, B., Trotter, M.M., 1981. The diet of moas based on gizzard contents samples from Pyramid Valley, North Canterbury, and Scaife's Lagoon, Lake Wanaka, Otago. Records of the Canterbury Museum 9, 309–336.
Caughley, G.J., 1983. The Deer Wars. The Story of Deer in New Zealand. Heinemann, Auckland.
Clark, G.R., Petchey, P., McGlone, M.S., Bristow, P., 1996. Faunal and floral remains from Earnscleugh Cave, Central Otago, New Zealand. Journal of The Royal Society of New Zealand 26, 363–380.
Cooper, A., Poinar, H.N., 2000. Ancient DNA: do it right or not at all. Science 289, 1139.
Davis, O.K., Agenbroad, L.D., Martin, P.S., 1984. The Pleistocene dung blanket of Bechan Cave, Utah. In: Genoways, H.H., Dawson, M.R. (Eds.), Contributions in Quaternary Vertebrate Paleontology. Carnegie Museum of Natural History Special Publication, pp. 267–282.
Day, J.S., 1998. Light conditions and the evolution of heteroblasty (and the divaricate form) in New Zealand. New Zealand Journal of Ecology 22, 43–54.
Donlan, J., Greene, H.W., Berger, J., Bock, C.E., Bock, J.H., Burney, D.A., Estes, J.A., Foreman, D., Martin, D.S., Roemer, G.W., Smith, F.A., Soulé, M.E., 2005. Re-wilding North America. Nature 436, 913–914.
Duff, R.S., 1952. Recent Maori occupation of Notornis Valley, Te Anau. Journal of the Polynesian Society 61, 90–119.
Forsyth, D.M., Coomes, D.A., Nugent, G., Hall, G.M.J., 2006. Diet and diet preferences of introduced ungulates (Order: Artiodactyla) in New Zealand. New Zealand Journal of Zoology 29, 323–343.
Greenwood, R.M., Atkinson, I.A.E., 1977. Evolution of divaricating plants in New Zealand in relation to moa browsing. Proceedings of the New Zealand Ecological Society 24, 21–29.
Gregg, D.R., 1972. Holocene stratigraphy and moas at Pyramid Valley, North Canterbury, New Zealand. Records of the Canterbury Museum 9, 151–158.

Haile, J., Holdaway, R., Oliver, K., Bunce, M., Gilbert, M.T.P., Nielsen, R., Munch, K., Ho, S.Y.W., Shapiro, B., Willerslev, E., 2007. Ancient DNA chronology within sediment deposits: Are paleobiological reconstructions possible and is DNA leaching a factor? Molecular Biology and Evolution 24, 982–989.

Hamilton, A., 1894. On the feathers of a small species of Moa (*Megalapteryx*) found in a cave at the head of the Waikaia River, with a notice of a moa-hunters camping place on the Old Man Range. Transactions and Proceedings of the New Zealand Institute 27, 232–238.

Hansen, J., 2001. Macroscopic plant remains from Mediterranean Caves and Rockshelters: Avenues of interpretation. Geoarchaeology 16, 401–432.

Hartree, W.H., 1999. A preliminary report on the nesting habits of moas in the East Coast of the North Island. Notornis 46, 457–460.

Herrera, C.M., 2002. Seed dispersal by vertebrates. In: Herrera, C.M., Pellmyr, O. (Eds.), Plant–Animal Interactions: An Evolutionary Approach. Blackwell, Oxford, pp. 185–208.

Hofreiter, M., Poinar, H.N., Spaulding, W.G., Bauer, K., Martin, P.S., Possnert, G., Pääbo, S., 2000. A molecular analysis of ground sloth diet through the last glaciation. Molecular Ecology 9, 1975–1984.

Hofreiter, M., Kuch, M., McDonald, G., Martin, P., Pääbo, S., 2003. Nuclear gene sequences from a Late Pleistocene sloth coprolite. Quaternary Research 59, 364–378.

Horrocks, M., D'Costa, D., Wallace, R., Gardner, R., Kondo, R., 2004. Plant remains in coprolites: diet of a subalpine moa (Dinornithiformes) from southern. New Zealand Emu 104, 149–156.

Horrocks, M., Salter, J., Braggins, J., Nichol, S., Moorhouse, R., Elliot, G., 2008. Plant microfossil analysis of coprolites of the critically endangered kakapo (*Strigops habroptilus*) parrot from New Zealand. Review of Palaeobotany and Palynology 149, 229–245.

Howell, C.J., Kelly, D., Turnbull, M.H., 2002. Moa ghosts exorcised? New Zealand's divaricate shrubs avoid photoinhibition. Functional Ecology 16, 232–240.

Huynen, L., Millar, C.D., Scofield, R.P., Lambert, D.M., 2003. Nuclear DNA sequences detect species limits in ancient moa. Nature 425, 175–178.

James, H.F., Burney, D.A., 1997. The diet and ecology of Hawaii's extinct flightless waterfowl: evidence from coprolites. Biological Journal of the Linnean Society 62, 279–297.

Janzen, D.H., 1984. Dispersal of small seeds by big herbivores: foliage is the fruit. The American Naturalist 123, 338–353.

Johnson, C., 2006. Australia's mammal extinctions: a 50 000 year history. Cambridge University Press, Melbourne, 278 pp.

MacPhee, R.D.E., 1999. Extinctions in Near Time: Causes, Contexts and Consequences. Kluwer Academic/Plenum, New York.

McCormac, F.G., Hogg, A.G., Blackwell, P.G., Buck, C.E., Higham, T.F.G., Reimer, P.J., 2004. SHCal04 Southern Hemisphere calibration, 0–11.0 cal kyr BP. Radiocarbon 46, 1087–1092.

McGlone, M.S., 2001. The origin of the indigenous grasslands of southeastern South Island in relation to pre-human woody ecosystems. New Zealand Journal of Ecology 25, 1–15.

McGlone, M.S., Clarkson, B.D., 1993. Ghost stories: moa, plant defences and evolution in New Zealand. Tuatara 32, 1–21.

McGlone, M.S., Moar, N.T., 1998. Dryland Holocene vegetation history of Central Otago and Mackenzie Basin, South Island, New Zealand. New Zealand Journal of Botany 36, 91–111.

McGlone, M.S., Salinger, M.J., Moar, N.T., 1993. Paleovegetation studies of New Zealand's climate since the last glacial maximum. In: Wright, H.E., Kutzbach, J.E. (Eds.), Global Climates Since the Last Glacial Maximum. University of Minnesota Press, USA, pp. 294–316.

McGlone, M.S., Moar, N.T., Meurk, C.D., 1997. Growth and vegetation history of alpine mires on the Old Man Range, Central Otago, New Zealand. Arctic and Alpine Research 29, 32–44.

McGlone, M.S., Turney, C.S.M., Wilmshurst, J.M., 2004. Late-glacial and Holocene vegetation and climatic history of the Cass Basin, central South Island, New Zealand. Quaternary Research 62, 267–279.

Marchant, S., Higgins(co-ordinators), P.J., 1990. Handbook of Australia, New Zealand and Antarctic Birds. In: Ratites to Ducks, Vol. 1. Oxford University Press, Melbourne.

Mark, A.F., 1977. Vegetation of Mt. Apriring National Park, New Zealand. National Parks Authority, Wellington.

Mead, J.I., Agenbroad, L.D., Davis, O.K., Martin, P.S., 1986. Dung of *Mammuthus* in the arid Southwest, North America. Quaternary Research 25, 121–127.

Moar, N.T., 1971. Contributions to the Quaternary history of the New Zealand flora: 6. Aranuian pollen diagrams from Canterbury, Nelson, and North Westland, South Island. New Zealand Journal of Botany 9, 80–145.

Moar, N.T., 1973. Contributions to the Quaternary history of the New Zealand flora: 7. Two Aranuian pollen diagrams from central South Island. New Zealand Journal of Botany 11, 291–304.

Nicholls, H., 2006. Restoring Nature's Backbone. PloS Biology 4, e202.

Noble, J.C., 1991. On ratites and their interactions with plants. Revista Chilena de Historia Natural 64, 85–118.

Owen-Smith, N., 1987. Pleistocene extinctions: the pivotal role of megaherbivores. Paleobiology 13, 351–362.

Poinar, H.N., Hofreiter, M., Spaulding, W.G., Martin, P.S., Stankiewicz, B.A., Bland, H., Evershed, R.P., Possnert, G., Pääbo, S., 1998. Molecular coproscopy: Dung and diet of the extinct ground sloth *Nothrotheriops shastensis*. Science 218, 402–406.

Rattenbury, J.A., 1962. Cyclic hybridisation as a survival mechanism in the New Zealand forest flora. Evolution 16, 348–363.

Ritchie, N.A., 1982. The prehistoric role of the Cromwell Gorge, New Zealand. New Zealand Journal of Archaeology 4, 21–43.

Rogers, G.M., Walker, S., Tubbs, M., Henderson, J., 2002. Ecology and conservation status of three "spring annual" herbs in dryland ecosystems of New Zealand. New Zealand Journal of Botany 40, 649–669.

Rogers, G.M., Walker, S., Lee, W.G., 2005. The role of disturbance in dryland New Zealand: past and present. In: Science for Conservation, 258. Department of Conservation, Wellington.

Rogers, G.M., McOverton, J., Price, R., 2007. Land use effects on "spring annual" herbs in rare non-forest ecosystems of New Zealand. New Zealand Journal of Botany 45, 317–327.

Tait, A.B., Basher, R., Thompson, C., Burgess, S., McKenzie, R., Mullan, B., Porteous, A., Salinger, J., Shankar, U., Wratt, D., 2001. The Climate of Otago: Patterns of Variation and Change. Otago Regional Council, Dunedin.

Trotter, M.M., 1970. Archaeological investigations in the Aviemore area, South Island. Records of the Canterbury Museum 8, 439–453.

Webb, C.J., Simpson, M.A., 2001. Seeds of New Zealand Gymnosperms and Dicotyledons. Manaaki Whenua, Lincoln.

White, T., 1877. Notes on Moa Caves, etc., in the Wakitipu (sic.) District. Transactions and Proceedings of the New Zealand Institute 9, 97–102.

Wilson, M.F., 1989. Gut retention times of experimental pseudoseeds by emu. Biotropica 21, 210–213.

Wood, J.R., 2007. Moa gizzard content analyses: further information on the diets of *Dinornis robustus* and *Emeus crassus*, and the first evidence for the diet of *Pachyornis elephantopus* (Aves: Dinornithiformes). Records of the Canterbury Museum 21, 27–39.

Wood, J.R., 2008a. Pre-settlement Paleoecology of Central Otago's Semi-arid Lowlands. Unpublished PhD thesis, University of Otago, Dunedin. 440 pp.

Wood, J.R., 2008b. Moa (Aves: Dinornithiformes) nesting material from the semi-arid interior of South Island, New Zealand. Journal of The Royal Society of New Zealand 38, 115–129.

Wood, J.R., Walker, S., 2008. Macrofossil evidence for pre-settlement vegetation of Central Otago's basin floors and gorges. New Zealand Journal of Botany 45, 239–255.

Worthy, T.H., 1991. An overview of the taxonomy, fossil history, biology and extinction of moas. Acta XX Congressus Internationalis Ornithologici, 555–562.

Worthy, T.H., 1998. Quaternary fossil faunas of Otago, South Island, New Zealand. Journal of The Royal Society of New Zealand 28, 421–521.

Worthy, T.H., 2005. Rediscovery of the types of *Dinornis curtus* Owen and *Palapteryx geranoides* Owen, with a new synonomy (Aves: Dinornithiformes). Tuhinga: Records of the Museum of New Zealand Te Papa Tongarewa 16, 33–43.

Worthy, T.H., Holdaway, R.N., 2002. The Lost World of the Moa. Canterbury University Press, Christchurch.

Wright, D.D., 2005. Diet, keystone resources and altitudinal movement of dwarf cassowaries in relation to fruiting phenology in a Papua New Guinea rain forest. In: Dew, J.L., Boubli, J.P. (Eds.), Tropical Fruits and Frugivores: The Search for Strong Interactors. Springer, The Netherlands, pp. 205–236.

Coprolite deposits reveal the diet and ecology of the extinct New Zealand megaherbivore moa (Aves, Dinornithiformes)

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Supplementary Information: Ancient DNA

Moa primers

The amplified regions were selected because they can discriminate between all ten species of moa currently recognised (except the 11 bp control region fragment that is identical for *Euryapteryx gravis* and *Anomalopteryx didiformis*, but these species can be distinguished on the basis of mutually exclusive geographic distributions). The PCR primers for these regions are as follows:

200 bp fragment (242 bp including primers):
Moa CR262F 5`- GCGAAGACTGACTAGAAGC -3`
Moa CR441R 5 CGCATACCGGGTCTGTTTATGC -3`

48 bp fragment (90 bp including primers):
Moa CR262F
Moa CR329R 5`-ATACGAGAAATAGGGATTAAGC-3`

31 bp fragment (67 bp including primers):
Moa CR262F
Moa CR294R 5`-GCGAGATTTGAACAGTACG-3`

11 bp fragment (47 bp including primers):
Moa CR204F 5`-AGATTTATARCTCGGACA-3`
Moa CR294R

To improve sequencing results from short PCR fragments, additional sequences were added to primers to form tags. The PCR primers Moa CR262F, Moa CR204F were tagged with the M13USP primer (5`-TGTAAAACGACGGCCAGT-3`), while the primers Moa CR329R and Moa CR294R were tagged with the M13RSP primer (5`-CAGGAAACAGCTATGACCAT-3`). Preliminary experiments indicated that tagging of standard PCR primers with M13 primers greatly improved DNA sequence quality.

Sequence verification

Multiple PCR negative controls were included with each batch of PCR amplifications. Repeat PCR amplifications of DNA extracts were performed to check sequence results. DNA sequences were checked for sequencing ambiguities using Sequencer and aligned using Clustal W with representative published moa mitochondrial control region sequences available on GenBank (Bunce et al., 2003; Huynen et al., 2003; Baker et al., 2005) and unpublished sequences. Only sequences from South Island moa specimens were included in the analysis. These included South Island giant moa, *Dinornis robustus* (20 published and 23 unpublished), heavy footed moa, *Pachyornis elephantopus* (17 published and 154 unpublished), crested moa, *Pachyornis australis* (4 published and 55 unpublished), stout legged moa, *Euryapteryx gravis* (16 published and 21 unpublished), eastern moa, *Emeus crassus* (15 published and 9 unpublished), upland moa, *Megalapteryx didinus* (4 published and 30 unpublished) and little bush moa, *Anomalopteryx didiformis* (3 published and 14 unpublished). The defecating species and number of haplotypes (and thus the minimum number of individuals) was determined through comparison of the coprolite and known moa sequences, and by phylogenetic analysis of the coprolite sequences within a matrix of representative moa sequences by maximum parsimony with 10 sequence addition replications supported by 1000 bootstrap analyses using MEGA 4.0 (Kumar et al., 2004).

Table 1. Coprolite samples that were positively identified as moa, and their haplotype/minimum number of individual affinities (number of different mitochondrial control region lineages within a site). Haplotypes have been determined for the 48 bp of moa mitochondrial DNA control region sequence (excluding primers) amplified with the primers Moa CR262F-Moa CR329R (D=*Dinornis robustus*, P=*Pachyornis elephantopus*, E=*Euryapteryx gravis*, M=*Megalapteryx didinus*, 1-6=different haplotypes). Control region sequences from the smaller fragments (indicated by *) either cluster with one/range of haplotypes or can only be identified to specific level. N/A: sequences less than 30 base pairs in length (not accepted by Genbank). Sequences for these individuals can be obtained from Supplementary Figure 1.

ACAD #	Otago Museum #	GenBank accession #	Region	Deposit	Sp. ID (DNA)	Haplotype/ Individual	DNA sequence length (not including primers)
A2062a	OMAv10720	FJ214609	West Otago	Dart	*Dinornis robustus*	D1	48
A2063a	OMAv10667	FJ214607	West Otago	Dart	*Dinornis robustus*	D1	48
A2064a	OMAv10670	FJ214612	West Otago	Dart	*Dinornis robustus*	D6	31
A2065a	OMAv10716	FJ214610	West Otago	Dart	*Dinornis robustus*	D6	48
A2066a	OMAv10707	FJ214613	West Otago	Dart	*Dinornis robustus*	D3	200
A2069a	OMAv10750	FJ214603	Central Otago	Roxburgh Gorge B	*Pachyornis elephantopus*	P1	31
A2070a	OMAv10715	FJ214618	West Otago	Dart	*Dinornis robustus*	D4	48
A2071a	OMAv10709	FJ214606	West Otago	Dart	*Pachyornis elephantopus*	P2	47
A2072a	OMAv10674	FJ214614	West Otago	Dart	*Dinornis robustus*	D3	47
A2074a	OMAv10740	FJ214605	Central Otago	Kawarau rockshelter	*Pachyornis elephantopus*	P3	48
A2079a	OMAv10436	FJ214598	Central Otago	Old Man Range	*Megalapteryx didinus*	M1	48
A2082a	OMAv10681	FJ214599	West Otago	Dart	*Megalapteryx didinus*	M2*	31
A2092a	OMAv10436	N/A	Central Otago	Earnscleugh	*Euryapteryx*	E1	11

				Cave	*gravis*		
A2101a	OMAv10690	N/A	West Otago	Dart	*Pachyornis elephantopus*	P2/P4*	11
A2102a	OMAv10686	FJ214615	West Otago	Dart	*Dinornis robustus*	D2/D3*	30
A2103a	OMAv10732	FJ214616	West Otago	Dart	*Dinornis robustus*	D2	47
A2105a	OMAv10677	FJ214600	West Otago	Dart	*Megalapteryx didinus*	M2	48
A2106a	OMAv10719	FJ214601	West Otago	Dart	*Megalapteryx didinus*	M2	48
A2107a	OMAv10718	FJ214602	West Otago	Dart	*Megalapteryx didinus*	M2	48
A2108a	OMAv10711	FJ214604	West Otago	Dart	*Pachyornis elephantopus*	P4	48
A2109a	OMAv10675	FJ214617	West Otago	Dart	*Dinornis robustus*	D5	44
A2110a	OMAv10717	FJ214611	West Otago	Dart	*Dinornis robustus*	D6	48
A2111a	OMAv10684	FJ214608	West Otago	Dart	*Dinornis robustus*	D1	48
A2112a	OMAv10684	N/A	West Otago	Dart	*Dinornis robustus*	D6*	11

Figure 1. DNA sequence alignment containing the single sequence we obtained from each of the coprolites positively identified as being defecated by moa. The primers for the regions amplified are shown in relation to sequences obtained above the alignment except for the Moa CR441R primer. The three fragments amplified are also shown (Moa CR262F-Moa CR329R, Moa CR262F- Moa CR294R and Moa CR204F-Moa CR294R). The haplotype/minimum number of individual affinities are shown to the left of the alignment.

```
  MoaCR262F GCGAAGACTGACTAGAAGC>                                                <GCTTAATCCCTATTTCTCGTAT MoaCR329R
                        MoaCR204F AGATTTATARCT CGGACA>          <CGTACTGTTCAAATCTCGC MoaCR294R

Diro A2062A Dart River          ATAGATTTATAGCT-CGGACATAACCTYAACCCGTACCGTTCAAATCTC D1
Diro A2063A Dart River          .............-................................... D1
Diro A2111A Dart River          .............-................................... D1
Diro A2103A Dart River          .............-...........T..................... D2
Diro A2102A Dart River          .............-...........T....???????????????? D2/D3*
Diro A2066A Dart River          .............-...........T.........T........... D3^
Diro A2072A Dart River          .............-...........T.........T........... D3
Diro A2070A Dart River          ............T...........T.........T........... D4
Diro A2109A Dart River          .............-........G...T.........T..?.....??? D5
Diro A2065A Dart River          .............-.........TCT.........T........... D6
Diro A2110A Dart River          .............-.........TCT.........T........... D6
Diro A2112A Dart River          ????????????????-??????....TCT....???????????????? D6*#
Diro A2064A Dart River          .............-.........TCT....???????????????? D6*
Eugr A2092A Earnscleugh         ????????????????-??????..CA..CT...???????????????? E1#
Pael A2069A Roxburgh Gorge B    .............-...........CTT...???????????????? P1
Pael A2071A Dart River          ?............T..A.......TCTT........T........... P2
Pael A2074A Kawarau Gorge A     .............-.........T.CTT........T........... P3
Pael A2108A Dart River          .............-..A.......TCTT........T........... P4
Pael A2101A Dart River          ????????????????-??????....TC?T...???????????????? P2/P4*#
Medi A2079A Old Man Range       ......C....A.C-.......GC...CT.T......T........T.T M1
Medi A2105A Dart River          ......C....A.C-T......GC...CT.T......T........T.T M2
Medi A2106A Dart River          ......C....A.C-T......GC...CT.T......T........T.T M2
Medi A2107A Dart River          ......C....A.C-T......GC...CT.T......T........T.T M2
Medi A2082A Dart River          ......C....A.C-T......GC...CT.T.???????????????? M2*
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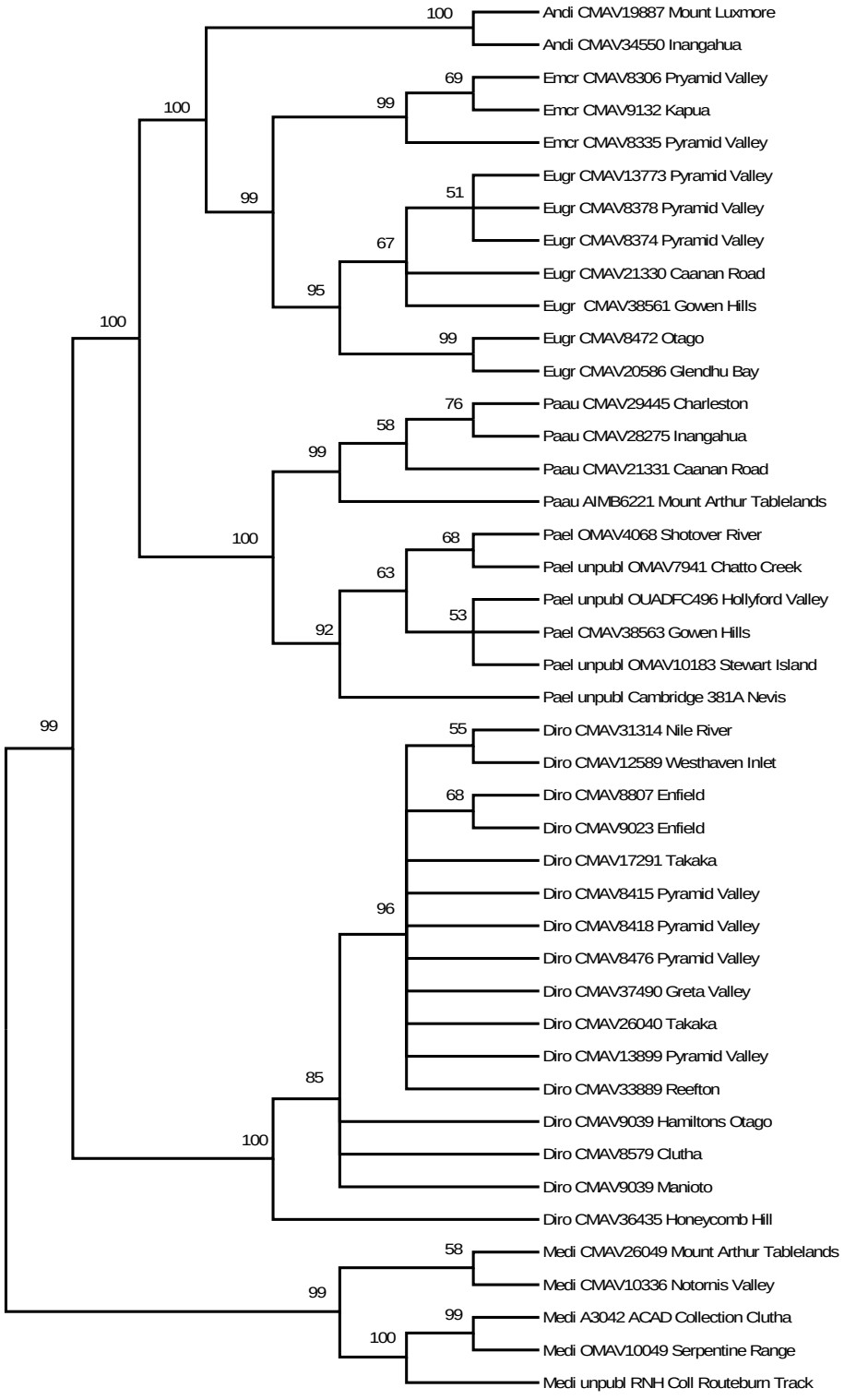
* DNA sequences provisionally assigned to haplotypes (based on 48 bp of full length sequence)
^ Diro A2066A Dart River amplified for the fragment Moa CR262F-Moa CR441R, of which only the 48 bp corresponding to Moa CR262F-Moa CR329R is shown in this figure. The complete sequence is available on Genbank (FJ214613).
# Sequences less than 30 base pairs in length (not accepted by Genbank).

Figure 2. Maximum parsimony strict consensus phylogeny (ten random sequence addition replications, 500 bootstrap replicates) of reference moa (2914 bp of concatenated CR, 12S, COIII, ATP6, Cyt b, ND3, ND4, ND5, tRNA Lys or 379 bp CR data depending on specimen) constructed using PAUP*4.0b10.



References

Baker, A.J., Huynen, L.J., Haddrath, O., Millar, C.D., Lambert, D.M. 2005. Reconstructing the tempo and mode of evolution in an extinct clade of birds with ancient DNA: The giant moas of New Zealand. Proceedings of the National Academy of Sciences 102, 8257-8262.

Bunce, M., Worthy, T.H., Ford, T., Hoppitt, W., Willerslev, E., Drummond, A., Cooper, A. 2003. Extreme reversed sexual size dimorphism in the extinct New Zealand moa *Dinornis*. Nature 425, 172-175.

Huynen, L., Millar, C.D., Scofield, R.P., Lambert, D.M. 2003. Nuclear DNA sequences detect species limits in ancient moa. Nature 425, 175-178.

Kumar, S., Tamura, K., Nei, M. 2004. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Briefings in Bioinformatics 5, 150-163.

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Supplementary Information: Plant macrofossil

Table 1

Plant and invertebrate macrofossils in identified moa coprolites. Eugr, *Euryapteryx gravis*; Medi, *Megalapteryx didinus*; Pael, *Pachyornis elephantopus*; Dino, *Dinornis robustus*. O, Old Man Range cave; RB, Roxburgh Gorge rockshelter B; K, Kawarau Gorge rockshelter; E, Earnscleugh Cave.

	DART RIVER VALLEY																				CENTRAL OTAGO			
																					O	K	RB	E
	Medi A2082a	Medi A2105a	Medi A2106a	Medi A2107a	Pael A2071a	Pael A2108a	Pael A2101a	Drob A2062a	Drob A2063a	Drob A2064a	Drob A2065a	Drob A2066a	Drob A2070a	Drob A2072a	Drob A2102a	Drob A2103a	Drob A2109a	Drob A2110a	Drob A2111a	Drob A2112a	*Medi A2079a*	Pael A2074a	Pael A2069a	Eugr A2092a
Trees and shrubs																								
Coprosma sp.			1*s*		1*s*		1*s*				6*s*											1*l*		
Coprosma or *Olearia*																							8*l*	1*l*
Hebe sp.																								8*s*
Melicytus sp.																							1*s*	
Olearia sp.																								1*s*
Gymnosperm						1*s*																		
Nothofagus menziesii								1*l*																
Lianes																								
Muehlenbeckia axillaris			1*s*							1*s*	2*s*					1*s*							3*s*	
Dicot herbs																								
Ceratocephala pungens																							1*s*	
Coprosma petriei		3*s*	43*s*	1*s*	14*s*		4*s*			28*s*	43*s*					4*s*								
Coriaria plumosa		2*s*	4*s*						1*s*	2*s*						61*s*		4*s*						
Einadia triandra																						1*s*	5*s*	
Gaultheria crassa				2*s*												13*s*								
Gonocarpus aggregatus		2*s*			1*s*	1*s*			1*s*	2*s*	2*s*													
Lagenifera pumila			1*s*			4*s*			1*s*															
Leucopogon fraseri		4*s*	38*s*	21*l*	10*s*, 31*l*	2*s*, 2*l*	1*s*		3*s*	19*s*, 44*l*								6*l*						
Myosurus minimus novae-zelandiae																								
Pratia angulata				1*s*							1*s*													
Ranunculus gracilipes		53*s*	13*s*		225*s*	6*s*			5*s*		1*s*													
Ranunculus sp.			4*s*		1*s*	8*s*				13*s*	2*s*					1*s*					1*s*			
Urtica incisa			2*s*		1*s*	3*s*				2*s*														
Wahlenbergia pygmaea			1*s*						1*s*		2*s*													
Monocot herbs																								
Carex sp.		2*s*	2*s*		20*s*	3*s*			3*s*	2*s*														

Juncus sp.		1*s*						
Poaceae							3*s*	
Unidentified dicot						*c*		
Unidentified monocot						∞*c*		
Invertebrate frag.		14				3	2	2
Acari: Cryptostigmata								1
Exuviae	11	3	2		2			

Table 2

Summary of plant and invertebrate remains in unidentified moa coprolites from Central Otago sites. S = seeds, l = leaves; c = leaf cuticle. Samples OMAv10761, 10763, 10765 (Sawers'), 10739, 10741-46 (Kawarau Gorge), 10748, 10751, OU unregistered (Roxburgh Gorge B) and 10754 (Roxburgh Gorge C) had no identifiable macroremains and are not shown on this table.

	Sawers' rockshelter					**Kawarau Gorge**		**Roxburgh Gorge B**					**Roxburgh Gorge C**				
	OMAv10760	OMAv10762	OMAv10764	OMAv10766	OMAv10767	OMAv10738	OMAv10747	OMAv10749	OMAv10752	OMAv10753	OU31901-6a	OU31901-6b	OMAv10755	OMAv10756	OMAv10757	OMAv10758	OMAv10759
Trees and shrubs																	
Coprosma sp.												1*s*					
Coprosma or *Olearia* sp.	1*l*								2*l*								
Melicytus sp.															1*s*		
Lianes																	
Muehlenbeckia axillaris		3*s*	1*s*	6*s*	4*s*					1*s*				1*s*		1*s*	
Rubus sp.								2*s*				10*t*					
Dicot herbs																	
Ceratocephala pungens			3*s*		5*s*				1*s*				5*s*	1*s*			
Colobanthus sp.							2*s*										
Einadia triandra	1*s*	1*s*									1*s*		2*s*	5*s*		5*s*	
Chenopodiaceae cf. *E. allanii*	1*s*	5*s*		46*s*													
Leucopogon sp. cf. *fraseri*	1*l*																1*l*
Myosurus minimus novae-zelandiae													17*s*				1*s*
Colobanthus sp.								2*s*		1*s*			1*s*	1*s*			
Oxalis exilis								1*s*		2*s*			18*s*			1*s*	
Myosotis pygmaea s.l.														1*s*			
Monocot herbs																	
Juncus sp.		5*s*	3*s*	16*s*	1*s*	7*s*			1*s*	1*s*	2*s*	4*s*				1*s*	1*s*
Carex sp.				1*s*	1*s*	16*s*											
Poaceae					2*s*												
Bryophytes																	
Unidentified fragments				12													
Invertebrate frag.			3	5	4	2								2	1	2	
Acari: Cryptostigmata	1										1			3			

Table 3

Summary of plant and invertebrate remains in unidentified moa coprolites from Dart River, OMAv10656-10696. S = seeds, l = leaves; c = leaf cuticle. Samples OMAv10660, 10662-64, 10669, 10672, 10678, 10683, 10688-89, and 10691-92 had no identifiable macroremains and are not shown on this table.

	OMAv10656	OMAv10657	OMAv10658	OMAv10659	OMAv10661	OMAv10665	OMAv10666	OMAv10668	OMAv10671	OMAv10673	OMAv10676	OMAv10679	OMAv10680	OMAv10682	OMAv10685	OMAv10687	OMAv10693	OMAv10694	OMAv10695	OMAv10696
Trees and shrubs																				
Carmichaelia sp.																				
Coprosma sp.			1*s*											3*s*						1*s*, 1*l*
Gymnosperm	1*s*																			1*l*
Nothofagus menziesii																				
Lianes																				
Muehlenbeckia axillaris				1*s*					1*s*		1*s*			6*s*						1*s*
Subshrubs and dicot herbs																				
Coprosma petriei		1*s*				8*s*		1*s*	6*s*		14*s*	1*s*		3*s*				7*s*	2*s*	1*s*
Coriaria plumosa	1*s*									4*s*					1*s*					1*s*
Gaultheria crassa									8*s*		57*s*			8*s*						8*s*
Gonocarpus aggregatus	1*s*													1*s*	1*s*					
Leucopogon fraseri			3*l*	1*l*	2*l*	13*s*, 1*l*	1*s*		9*l*	6*l*	17*s*, 2*l*	1*s*, 2*l*	7*s*, 1*l*	10*s*, 33*l*	3*s*		1*s*	2*s*		1*s*, 3*l*
Lagenifera pumila	4*s*										1*s*				1*s*					
Pratia angulata									1*s*		7*s*									
Ranunculus gracilipes	55*s*					7*s*					2*s*		4*s*	3*s*	5*s*			1*s*		
Ranunculus sp.	2*s*																			1*s*
Urtica incisa					2*s*				5*s*					3*s*			18*s*			2*s*
Wahlenbergia pygmaea									3*s*		1*s*			1*s*	1*s*					
Monocot herbs																				
Juncus sp.											1*s*									
Carex sp.	2*s*		3*s*			2*s*									3*s*					
Invertebrate frag.																2			1	
Exuviae	14					6				2		1		3		2			1	
Acari: Cryptostigmata												1								

Table 4

Summary of plant and invertebrate remains in unidentified moa coprolites from Dart River, OMAv10697-10736. S = seeds, l = leaves; c = leaf cuticle. Samples OMAv10698-703, 10713, 10721, and 10725 had no identifiable macroremains and are not shown on this table.

	OMAv10697	OMAv10704	OMAv10705	OMAv10706	OMAv10708	OMAv10710	OMAv10712	OMAv10714	OMAv10722	OMAv10723	OMAv10724	OMAv10726	OMAv10728	OMAv10729	OMAv10730	OMAv10731	OMAv10733	OMAv10734	OMAv10735	OMAv10736
Trees and shrubs																				
Carmichaelia sp.			1*s*																	
Coprosma sp.			1*l*	1*s*		5*s*, 2*l*											1*s*			
Gymnosperm			1*s*						1*s*											
Nothofagus menziesii																1*l*				
Lianes																				
Muehlenbeckia axillaris														1*s*						
Subshrubs and dicot herbs																				
Coprosma petriei	1*s*		4*s*	2*s*	3*s*				1*s*			1*s*		24*s*				1*s*	1*s*	
Coriaria plumosa	1*s*			1*s*		2*s*	1*s*						1*s*	1*s*						
Gaultheria crassa				6*s*		19*s*	1*s*		1*s*										3*s*	
Gonocarpus aggregatus	2*s*						1*s*									1*s*				
Leucopogon fraseri	2*s*, 5*l*	1*l*	9*s*, 1*l*	4*s*, 42*l*		11*l*	25*s*, 2*l*	1*l*	1*s*	2*l*				15*s*	1*l*	1*s*	3*l*			
Lagenifera pumila	1*s*						1*s*													
Pratia angulata			1*s*																	
Ranunculus gracilipes	1*s*		12*s*	1*s*	3*s*		7*s*		1*s*		1*s*			2*s*						
Ranunculus sp.		1*s*			1*s*									2*s*				1*s*		
Urtica incisa	4*s*					1*s*							1*s*	2*s*			1*s*		1*s*	3*s*
Wahlenbergia pygmaea					2*s*													1*s*		
Monocot herbs																				
Juncus sp.																				
Carex sp.					2*s*		1*s*							1*s*				1*s*		
Invertebrate frag.			1						2											
Exuviae							25					4								
Acari: Cryptostigmata																				

Table 5

Plant taxa recorded in moa coprolites and gizzard content (Falla, 1941; Gregg, 1972; Burrows et al., 1981; Horrocks et al., 2004; Wood, 2007; this study) compared with ungulate diet data (Forsyth et al., 2006).

Macrofossils recorded from moa coprolites or gizzard content	**Recorded being eaten by ungulates in NZ?**	**Ungulate diet preference in NZ: - (avoided); 0 (not selected); + (preferred)**
Bryophyta	Yes	-
Calystegia sepium	No	
Carex sp.	Yes	-
Carpodetus serratus	Yes	-, 0
Ceratocephala pungens	No	
Cladium sp.	No	
Clematis sp.	Yes	0, +
Colobanthus sp.	Yes	
Coprosma sp. (small-leaved)	Yes	-
Coprosma sp. (large-leaved)	Yes	+
Cordyline australis	Yes	+
Coriaria plumosa	Yes	
Corokia cotoneaster	Yes	0
Cyathodes empetrifolia	No	- (*C. juniperina*)
Dacrycarpus dacrydioides	Yes	-
Einadia allanii	No	
E. triandra	No	
Elaeocarpus hookerianus	Yes	0, +
Eleocharis cf. *acuta*	No	
Gaimardia sp.	No	
Gaultheria crassa	Yes	
Gonocarpus sp.	Yes	
Gymnosperm	Yes	- (both *Podocarpus* and *Prumnopitys* sp.)
Hebe cf. *pimelioides*	Yes	
Juncus sp.	Yes	-
Lagenifera sp.	Yes	+
Leptospermum scoparium	Yes	-
Leucopogon fraseri	Yes	- (*L. fasciculatus*)
Lophomyrtus obcordata	Yes	-
Malvaceae	Yes	
Melicope simplex	Yes	-
Melicytus sp.	Yes	
Muehlenbeckia australis	Yes	-
M. axillaris	No	
M. complexa	Yes	
Myoporum laetum	Yes	-
Myosotis pygmaea	Yes	
Myosurus minimus	No	
Myrsine divaricata	Yes	-, 0
Nertera sp.	Yes	- and +

Nothofagus cliffortioides	Yes	-
Nothofagus menziesii \	Yes	-
Olearia sp.	Yes	- and +
Oxalis exilis	Yes	-
Pennantia corymbosa	Yes	- and +
Phormium tenax	Yes	- and +
Phyllocladus alpinus	Yes	- (*P. trichomanoides*)
Pimelea sp.	Yes	
Pittosporum sp.	Yes	0, +
Plagianthus regius	Yes	
Podocarpus hallii	Yes	-, 0
Podocarpus totara	Yes	-
Polystichum vestitum	Yes	- and +
Pratia angulata	Yes	-
Prumnopitys taxifolia	Yes	-
Pseudopanax ferox	No	
Ranunculus gracilipes	Yes	
Ranunculus sp.	Yes	
Rubus sp.	Yes	-, 0
Scirpus sp.	No	
Tetrapathaea tetrandra	No	
Teucridium parviflorum	No	
Urtica incisa	Yes	-
Wahlenbergia pygmaea	Yes	

References

Burrows, C.J., McCulloch, B., Trotter, M.M. 1981. The diet of moas based on gizzard contents samples from Pyramid Valley, North Canterbury, and Scaife's Lagoon, Lake Wanaka, Otago. Records of the Canterbury Museum 9, 309-336.

Falla, R. 1941. The avian remains. Preliminary report on excavations at Pyramid Valley Swamp, Waikari, North Canterbury. Records of the Canterbury Museum 4, 325-353.

Forsyth, D.M., Coomes, D.A., Nugent, G., Hall, G.M.J. 2006. Diet and diet preferences of introduced ungulates (Order: Artiodactyla) in New Zealand. New Zealand Journal of Zoology 29, 323-343.

Gregg, D.R. 1972. Holocene stratigraphy and moas at Pyramid Valley, North Canterbury, New Zealand. Records of the Canterbury Museum 9, 151-158.

Horrocks, M., D'Costa, D., Wallace, R., Gardner, R., Kondo, R. 2004. Plant remains in coprolites: diet of a subalpine moa (Dinornithiformes) from southern New Zealand. Emu 104, 149-156.

Wood, J.R. 2007. Moa gizzard content analyses: further information on the diets of *Dinornis robustus* and *Emeus crassus*, and the first evidence for the diet of *Pachyornis elephantopus* (Aves: Dinornithiformes). Records of the Canterbury Museum 21, 27-39.

CHAPTER FIVE

DNA content and distribution in ancient feathers and potential to reconstruct the plumage of extinct avian taxa

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STATEMENT OF AUTHORSHIP

Chapter Five

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Proceedings of the Royal Society of London Biological Series (2009), 276: 3395-3402.

Nicolas. J. Rawlence

Designed experiment, collected samples, performed DNA and phylogenetic analysis, interpreted data and wrote paper.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed ..... Date..... 8/7/09

Jamie R. Wood

Designed experiment, excavated rockshelters, performed colour fading analysis, reconstructed moa plumage, interpreted data and evaluated manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed ..... Date..... 8 July 2009

Kyle N. Armstrong

Performed DNA analysis and evaluated manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed ..... Date..... 9/7/09.

Alan Cooper

Designed experiment, supervised development of research, evaluated manuscript and provided funding.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed ..... Date..... 9/7/09

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Feathers are known to contain amplifiable DNA at their base (calamus) and have provided an important genetic source from museum specimens. However, feathers in subfossil deposits generally only preserve the upper shaft and feather 'vane' which are thought to be unsuitable for DNA analysis. We analyse subfossil moa feathers from Holocene New Zealand rockshelter sites and demonstrate that both ancient DNA and plumage information can be recovered from their upper portion, allowing species identification and a means to reconstruct the appearance of extinct taxa. These ancient DNA sequences indicate that the distal portions of feathers are an untapped resource for studies of museum, palaeontological and modern specimens. We investigate the potential to reconstruct the plumage of pre-historically extinct avian taxa using subfossil remains, rather than assuming morphological uniformity with closely related extant taxa. To test the notion of colour persistence in subfossil feathers, we perform digital comparisons of feathers of the red-crowned parakeet (*Cyanoramphus novaezelandiae novaezelandiae*) excavated from the same horizons as the moa feathers, with modern samples. The results suggest that the coloration of the moa feathers is authentic, and computer software is used to perform plumage reconstructions of moa based on subfossil remains.

Keywords: ancient DNA; feathers; moa; phenotype; plumage reconstruction

1. INTRODUCTION

The arrival of humans and their associated mammalian species in New Zealand at approximately AD 1280 (Wilmshurst *et al.* 2008) resulted in the extinction of 41 per cent of New Zealand's breeding bird species (Tennyson & Martinson 2006). These species are relatively well known osteologically because of New Zealand's rich late Quaternary avifaunal fossil record (Worthy & Holdaway 2002). While most are known only from their bones, a few partially mummified remains have also been found (Anderson 1989; Worthy 1989; Vickers-Rich *et al.* 1995), while isolated feathers have been recovered from a range of late Holocene rockshelter sediments (Wood 2008; Wood *et al.* 2008). The majority of subfossil feathers found in New Zealand have been attributed to the extinct palaeognathus (ratite) moa (Aves: Dinornithiformes), although none has been confirmed genetically. If DNA could be recovered routinely from subfossil feathers, it would create many opportunities for genetic studies of extinct taxa and populations, as well as providing an important insight into the appearance of extinct species. However, the survival of DNA in subfossil feathers has not yet been demonstrated, and there is little evidence about the quality or location of DNA in different parts of feathers.

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DNA has been extracted previously from the base, or calamus, of feathers from modern and historical museum specimens (Payne & Sorenson 2002; Sefc *et al.* 2003; Horváth *et al.* 2005). Extraction protocols have generally ignored the distal components of the feather, such as the rachis and barbs (including barbules and barbicels) (figure 1), which comprises the bulk of the feather structure, because it has been thought that there is no amplifiable DNA in these structures. The distribution of mitochondrial DNA (mtDNA) throughout paralogous structures like hair (Gilbert *et al.* 2007) and reptilian scales (Fetzner 1999; Feldman & Spicer 2002) raises the possibility that mtDNA might also be present in all parts of the feather structure. Because the calamus is commonly absent from subfossil feathers (owing to breakage before, or during, deposition), the recovery of DNA from distal feather components would have major implications for historical and ancient DNA research.

Ancient DNA can be a powerful tool when reconstructing the phenotype of extinct and extant species. It has been used previously to reconstruct mammoth and horse coat colour (Rompler *et al.* 2006; Ludwig *et al.* 2009) and to suggest that Neanderthals had differing degrees of skin and hair pigmentation (Lalueza-Fox *et al.* 2007). However, these studies used ancient DNA to identify colour genes, including the melanocortin-1 receptor (*MCR1*) gene, rather than directly linking hair samples of known colour to an extinct species of unknown external appearance. Recently, it has been

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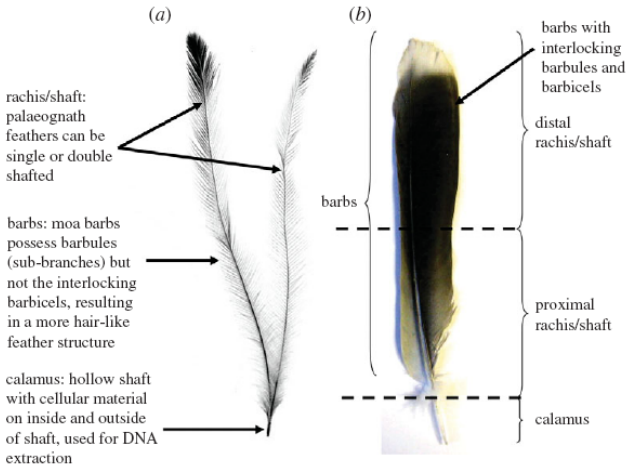

Figure 1. (*a*) Morphological structure of a palaeognathus feather, here shown by emu (*Dromaius novaehollandiae*). The main shaft (rachis) supports side branches (barbs) and together makes up the distal component of feathers. In vaned feathers, barbs are held together by small sub-branches termed barbules, which in turn are held together by interlocking hooks or barbicels. (*b*) A schematic representation of the distal feather components sampled in this study using a neo-avian feather to illustrate the role of barbules and barbicels in forming a vaned structure characteristic of flighted birds.

demonstrated that even fossilized feathers from the Cretaceous and Eocene (Vinther *et al.* 2008) can be preserved as carbonaceous traces of melanosomes (pigment containing organelles). When compared with modern taxa, such fossils provide information on the colour pattern of the original feather (Vinther *et al.* 2008). However, reconstructions of specific taxa using this methodology are only possible if the fossilized feathers can be identified positively to species. When there is no fossil feather information, reconstruction attempts have relied on the phenotype of closely related extant taxa (e.g. Vickers-Rich *et al.* 1985; Gill & Martinson 1991; Flannery & Schouten 2001; Murray & Vickers-Rich 2003; Tennyson & Martinson 2006). In the rare cases in which subfossil feathers have been preserved, it has generally been assumed that the colours reflect accurately their original appearance (White 1885; Hamilton 1894). However, historical museum and subfossil feathers are prone to fading from exposure to sunlight or other factors (Oliver 1955; G. Pohland 2007, unpublished data), and it is conceivable that feathers excavated from cave sediment may have also altered. One approach to investigating this issue is to use standardized Munsell colour chips (Villafuerte & Negro 1998) to compare subfossil and modern feathers from the same species and to quantify colour fading for a given site and horizon. The assumption is then made that other feathers in the deposit have been protected similarly from fading and alteration.

To determine whether amplifiable ancient DNA can be extracted from subfossil feathers, we performed trials on nine moa feathers collected from rockshelter sediments in the semi-arid region of Central Otago, South Island, New Zealand. The giant, graviportal moa were the dominant terrestrial herbivores in New Zealand's pre-human terrestrial ecosystems and a striking example of an avian radiation into different niches and habitats. Moa have been the focus of considerable palaeontological (Worthy & Holdaway 2002), palaeoecological (Wood *et al.* 2008) and evolutionary research (Cooper *et al.* 1992; Bunce *et al.* 2003; Huynen *et al.* 2003; Baker *et al.* 2005; Lambert *et al.* 2005). However, relatively little is known about their external phenotype or interspecific plumage variation. Most mummified moa specimens, where skin is present, have just the bases of feathers preserved (e.g. Hutton & Coughtrey 1875; Forrest 1987). As a consequence, it has been difficult to assign isolated moa feathers to species. Ancient DNA analysis offers the potential to link these feathers to known fossil taxa and provides insight into their plumage and appearance. To better understand where DNA is distributed in different parts of a feather, we also tested whether amplifiable DNA was detectable in the rachis and barbs of 10 moa feathers from one of the sites, as well as three modern emu feathers (figure 1). Lastly, to determine whether moa feathers could be used to reconstruct plumage characteristics, we quantified digitally the amount of colour fading by comparing subfossil feathers from another species recovered from the same deposits with living relatives.

2. MATERIAL AND METHODS

(a) *Materials*

We analysed a total of 19 moa feathers excavated from Sawers', Roxburgh Gorge B and Roxburgh Gorge C rockshelters, in Central Otago, South Island, New Zealand, and held in the collections of the Otago Museum, Alexandra Museum and the Australian Centre for Ancient DNA (ACAD) (table S1, electronic supplementary material). The excavated feathers were characteristic of palaeongaths and specimens identified previously as belonging to moa (figure 1; Worthy & Holdaway 2002). The calamus (5 mm) was removed from a set of nine moa feathers (table S1, electronic supplementary material) for genetic analysis, and then each feather was photographed with a Nikon digital camera for digital reconstruction of original colour and plumage.

To investigate the location of DNA in feathers, the rachis and barbs from a further 10 moa feathers from Sawers' rockshelter were examined in a later series of extractions. The two

sets of samples were collected at different times from different excavations (table S1, electronic supplementary material). Naturally shed modern emu (*Dromaius novaehollandiae*) feathers were also used as a positive control (table S1, electronic supplementary material).

(b) *Molecular methods*

To determine whether DNA could be extracted from subfossil feathers, the calamus from the first nine moa feathers was cut in half longitudinally and further diced with a sterile scalpel blade to help facilitate enzymatic digestion. Ancient DNA was isolated using the Qiagen DNeasy Blood and Tissue Kit following the manufacturer's instructions. Multiple negative extraction and amplification controls were included. All DNA extractions and the setup of PCR reactions were performed in the physically remote, isolated and dedicated ancient DNA facility using appropriate logistical and methodological procedures (Cooper & Poinar 2000).

PCR amplifications targeted 31, 180 or 205 bp of the moa mtDNA control region (excluding primers) using the primer pairs 262F/294R (31 bp), 262F/419R (180 bp) and 185F/294R (205 bp; from Cooper *et al.* 2001; Bunce *et al.* 2003; Wood *et al.* 2008) (table S2, electronic supplementary material). Unsuccessful PCR amplifications were subsequently repeated with the following primer pair: 204F/294R (11 bp excluding primers) (Wood *et al.* 2008; table S2, electronic supplementary material) to determine whether smaller DNA fragments were present. To improve the sequencing results from the short PCR products, the 262F and 294R primers were tagged with M13USP and M13RSP primers, respectively, as described by Wood *et al.* (2008). PCR reactions were conducted in 25 µl volumes containing a final concentration of 2 mg ml^{-1} rabbit serum albumen (Sigma), 1× PCR buffer (Invitrogen), 2 mM $MgSO_4$, 200 µM each dNTP, 1 µM each primer, 1 U Platinum Taq DNA Polymerase High Fidelity (Invitrogen) and 1–2 µl template DNA. PCR conditions were as follows: 94°C for 3 min, 55 cycles of 94°C for 30 s, 55°C for 30 s and 68°C for 45 s, with a final extension of 68°C for 10 min. PCR amplification reactions and all downstream post-PCR procedures were carried out in a modern molecular biology laboratory at the University of Adelaide.

To test whether amplifiable DNA was present in the distal portion of both modern and subfossil feathers, an additional 10 moa and 3 modern emu feathers were divided into sections for separate DNA extractions. The entire distal portion (rachis and barbs) of the moa feathers was examined, while for emus, separate DNA extractions were performed on subsamples comprising: the calamus; the distal; and proximal halves of the rachis (only); and all of the barbs (figure 1). Each subsample was minced with a scalpel blade and soaked in 1 : 10 bleach solution for 30 min to remove potential contamination from exterior surfaces, and then rinsed three times with Millipore ultrapure water. DNA was isolated using the Qiagen DNeasy Blood and Tissue Kit following the manufacturer's instructions, with the addition of 20 µl of dithiothreitol to the ATL lysis buffer to aid digestion of keratin and left overnight at 55°C on a rotary mixer to completely dissolve the samples. PCR amplifications targeting 31, 180 or 205 bp of the moa mtDNA control region (excluding primers) were performed as described earlier. For emu feathers, a 108 bp fragment of the mtDNA 12S ribosomal RNA gene was amplified following the PCR method outlined earlier using the primer pair 12SE/12SH2 reported in Cooper *et al.* (2001) (table S2, electronic supplementary material).

PCR products were visualized on a 2–3.5% 1× TBE agarose gel. If primer dimers were present after PCR amplification, PCR products were purified using the AMPure magnetic bead system (Agencourt), otherwise PCR products were purified using 4 U Exo1 and 0.6 U SAP (Fermentas) by incubation at 37°C for 30 min and 80°C for 15 min. Both template strands were sequenced for each mtDNA fragment, using independent PCR reactions for each strand in ancient samples. All sequences were determined using Big Dye Terminator v. 3.1 chemistry and an ABI 3130XL capillary sequencer. All sequences have been deposited in GenBank (GQ253938–GQ253945) or table S3 (electronic supplementary material) if less than 50 bp in length.

(c) *Data analysis*

All sequences were imported into SEQUENCHER (Genecodes) and consensus sequences assembled. Sequences derived from emu feathers were identified using GenBank BLAST searches. mtDNA sequences obtained from moa feathers were aligned to a database of moa control region sequences (77 published from Cooper *et al.* 2001; Bunce *et al.* 2003; Huynen *et al.* 2003, 2008; Baker *et al.* 2005 and 300 sequences encompassing the entire geographical range of each moa species in New Zealand; unpublished data) using the CLUSTALW algorithm implemented in MEGA 4.0 (Kumar *et al.* 2004). Moa species were identified through similarity to known moa sequences in the database, with matches of 98–100%. To verify the initial identifications, a reference dataset of previously published long (2914 bp) moa sequences (Baker *et al.* 2005) was used to construct a robust maximum parsimony strict consensus bootstrap phylogeny (10 random sequence addition replications and 500 bootstrap replicates) with PAUP*4.0b10 (Swofford 2000). The reference moa dataset comprised concatenated *CR, 12S, COIII, Cyt-b, ND3, ND4, ND5* and *tRNA Lys* sequences from 25 specimens and an additional 379 bp *CR* data from 29 specimens lacking the full 2914 bp. The moa feather sequences were identified to species using the bootstrap phylogeny as a backbone constraint by placing the partial control region sequences onto the tree using a full heuristic maximum parsimony search.

(d) *Preservation of colour in subfossil feathers*

The degree of colour fading in moa feathers was tested using subfossil feathers of the extant red-crowned parakeet (*Cyanoramphus novaezelandiae novaezelandiae*) excavated from moa feather-bearing sediment horizons within Roxburgh Gorge C rockshelter. These were compared with similar-sized feathers collected recently from captive birds following the digital methodology of Villafuerte & Negro (1998). Feathers were photographed on a white background using the auto colour setting on a Nikon digital camera, at a standard distance (300 mm) and focal length (35 mm), with fluorescent lighting positioned 300 mm above the feathers. Three Munsell colour chips (10YR7/4, 10YR6/6 and 10YR5/4) were placed beside all the photographed feathers. Raw images (NEF format) were opened in Adobe PHOTOSHOP 7.0 using the Camera Raw 3.7 plug-in. The magic wand tool in PHOTOSHOP was used to select each colour chip, and mean red, green and blue (RGB) values were obtained for each. These were plotted against theoretical RGB values for each chip obtained using Munsell CONVERSION v. 7.0.1, to calculate a linear regression for each primary colour. This

was then used to shift colour values in each photograph, to permit comparison between images. Feather colours were measured by selecting a bright portion of the mid-barb towards the distal end of the feather.

(c) *Reconstruction of moa plumage*

Standardized digital photographs of moa feathers were opened in Adobe Photoshop 7.0, and the polygonal lasso tool was used to select around the edge of each feather. The clipped feathers were copied as a new layer onto a blank canvas, and the layer was duplicated multiple times to create copies of each feather. Each new layer was then moved so that neighbouring feathers were slightly overlapping, in order to reconstruct the plumage.

3. RESULTS

(a) *Ancient DNA from the calamus of subfossil feathers*

We successfully amplified and sequenced 31–361 bp of the mtDNA control region from the calamus samples of seven moa feathers (electronic supplementary material, tables S1 and S3). A single unambiguous sequence was obtained from each of the seven feathers. Extractions from the remaining two moa feathers failed to amplify, despite attempts using multiple primer combinations.

The percentage uncorrected sequence divergences for pairwise comparisons of moa taxa for the 31, 180 and 205 bp control region sequences ranged approximately between 3–35%, 3–14% and 5–21%, respectively, demonstrating that there is enough genetic variation within each fragment length to distinguish the various moa species. All seven feather sequences were between 98 and 100 per cent identical to a reference moa sequence, and four moa species were identified: upland moa (*Megalapteryx didinus*, $n = 4$), South Island giant moa (*Dinornis robustus*, $n = 1$), stout-legged moa (*Euryapteryx gravis*, $n = 1$) and heavy-footed moa (*Pachyornis elephantopus*, $n = 1$). The species identifications from the moa feathers matched the known Holocene distributions of these taxa based on the analysis of fossil bone (Worthy 1998) and coprolite (Wood *et al.* 2008) deposits.

(b) *DNA from the rachis and barbs of feathers*

It was also possible to amplify and sequence mtDNA from the combined rachis and barbs of moa feathers (excluding the calamus) (electronic supplementary material, tables S1 and S3). We amplified and sequenced 31–180 bp of mtDNA control region from four rachis/barb samples of ten moa feathers from Sawers' rockshelter. All four moa sequences were 98–100% identical to a reference moa sequence. Three moa species were identified: South Island giant moa ($n = 1$), heavy-footed moa ($n = 1$) and stout-legged moa ($n = 2$). For the emu feathers, mtDNA was found to be amplifiable from both the rachis and isolated barbs, respectively. For all three emu feathers, a 108 bp fragment of 12S was amplified and sequenced from each of the four sections (figure 1, electronic supplementary material, table S1). A single unambiguous sequence was obtained from all 12 PCR products, which matched 100 per cent to emu sequences on GenBank (e.g. AF338711.1). There were no PCR products in the extraction and PCR negatives. It is important to note that the precise location of the mtDNA within the feather structure (e.g. within the keratin cells of the rachis and barbs) was not precisely determined. There is also the possibility that despite the bleach treatment, exogenous DNA on the feather might contribute a significant part of the amplifiable DNA. A similar possibility exists for analogous studies of ancient DNA from mammoth (Gilbert *et al.* 2007) and thylacine hair (Miller *et al.* 2009), and historical and modern DNA from reptile (Fetzner 1999; Feldman & Spicer 2002) and fish scales (Yue & Orban 2001).

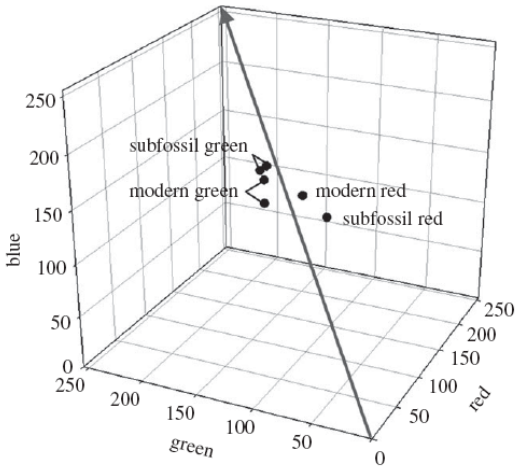

Figure 2. Quantification of the amount of colour fading in subfossil red facial and green contour feathers of red-crowned parakeet (*C. novaezelandiae novaezelandiae*) from the Late Holocene Roxburgh Gorge rockshelter B with modern red-crowned parakeet feathers in RGB colour space. The graph indicates that the amount of colour fading in subfossil parakeet feathers is minimal. The colours of moa feathers from the same deposits are also likely to be relatively unmodified.

(c) *Preservation of colour in subfossil feathers*

The digital colour comparison illustrated that the colour of subfossil red-crowned parakeet feathers reflected accurately that of the modern feathers (figure 2) indicating that any fading of the subfossil feathers was minor. For green contour feathers, the variation between modern specimens was greater than that observed between some modern and subfossil specimens. We assumed that the colours of moa feathers from the same deposits are also likely to be relatively unmodified, and therefore provide accurate data for plumage reconstructions.

(d) *Reconstruction of moa plumage*

Two different morphological and colour types were apparent among the moa feathers (figure 3). The first, present in South Island giant moa, stout-legged moa, upland moa and some heavy-footed moa consisted of slender, medium to long, single or double shafted feathers. These feathers were tan to light brown at the base, grading into dark brown to black at the tip (figure 3*a*). They were suggestive of a relatively plain, but slightly streaky plumage (figure 4*d*) similar to brown kiwi (*Apteryx australis*) which has similarly patterned feathers

Figure 3. Characteristic morphology and colour of moa feathers identified from ancient DNA sequences. (*a*) Feathers identified as upland moa (*M. didinus*), South Island giant moa (*D. robustus*), stout-legged moa (*E. gravis*) and heavy-footed moa (*P. elephantopus*) exhibited overlapping morphology and colour. The three best examples are shown. From left to right: upland moa (OM Av10793.1), upland moa (OM Av10791.1), South Island giant moa (OM Av10793.2). (*b*) White-tipped feather (A 06.49.18) identified as heavy-footed moa. Scale bar, 10 mm.

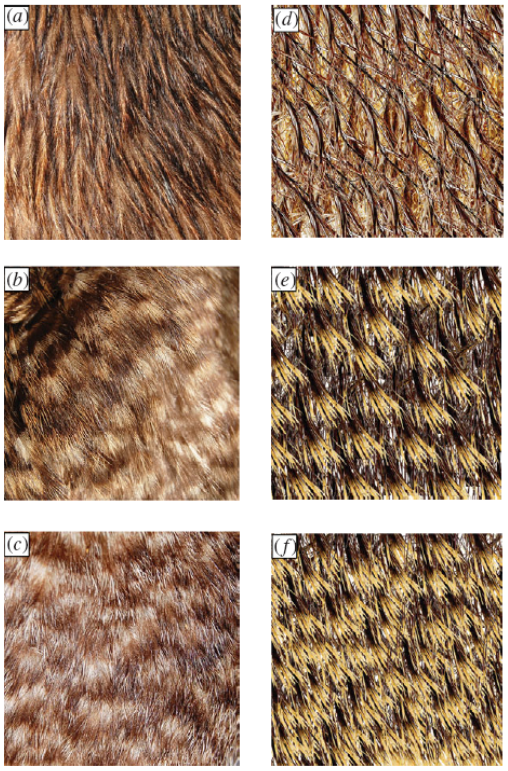

Figure 4. (*a*) Plumage of brown kiwi (*A. australis*); (*b*) plumage of great-spotted kiwi (*A. haastii*); (*c*) plumage of little-spotted kiwi (*A. owenii*); (*d*) reconstruction of upland moa (*M. didinus*), South Island giant moa (*D. robustus*), stout-legged moa (*E. gravis*) and heavy-footed moa (*P. elephantopus*) plumage based on a dark feather; (*e* and *f*) reconstruction of upland moa and heavy footed moa plumage based on white-tipped feathers, (*e*) densely and (*f*) sparsely spaced.

(figure 4*a*). The second feather type was present in heavy-footed moa and was a short feather that was dark brown to black for the basal two-thirds and white at the tip (figure 3*b*). *In situ*, these feathers would create a speckled plumage pattern (figure 4*e*,*f*). Similar-patterned light-tipped feathers are seen in great-spotted kiwi (*Apteryx haastii*) and little-spotted kiwi, *Apteryx owenii* (figure 4*b*,*c*).

4. DISCUSSION

The discovery that mtDNA can be amplified from isolated subfossil feathers has allowed the identification of four species of New Zealand moa and provided some important information about the plumage characteristics of this extinct group. Previous studies of historical museum feather specimens (Payne & Sorenson 2002; Sefc *et al.* 2003; Horváth *et al.* 2005) have been able to recover genetic information, but we have demonstrated that with a suitably stringent approach (appropriate protocols, targeted primer design for short fragments and low-contamination facilities), it is possible to obtain taxonomically informative sequences both from subfossil feathers and notably from parts of feathers not considered previously to be of use for genetic analysis. There appears to be great potential for genetic studies of birds, both extinct and extant, using feathers where the calamus is not preserved or present, such as from subfossil sites or discarded material in nests. The ability to use the distal portions of feathers also has considerable implications for genetic research on museum specimens, as destructive sampling could be minimized and soft tissue samples such as toe pads could be left intact.

In order to use subfossil feathers to reconstruct the external appearance of extinct avian taxa, we must be confident that the amount of colour fading is minimal. The results reported here show that the colour of subfossil feathers has not faded significantly in the relatively cool, dry conditions of some New Zealand rockshelters. This is potentially aided by the protection from UV exposure in sediments. The genetic identification of four species of moa using isolated feathers provides unique insights into the appearance of these species. Perhaps the most striking is that some heavy-footed moa appear to have had a speckled appearance (figures 3*b* and 4*e*,*f*). This pattern (figure 3*b*) has been shown previously to be characteristic only for upland moa based on feathers attached *in situ* to the mummified remains of this species (Hamilton 1894). The results reported here suggest that speckled patterning may have been present in both the heavy-footed and upland moa. In contrast to the situation above, the other feather type

analysed in this study was similar in upland moa, South Island giant moa, stout-legged moa and some heavy-footed moa, indicating a plain or slightly streaky appearance (figures 3*a* and 4*d*). These feathers could not be separated on either morphological or colour characteristics. This also suggests that upland moa and heavy-footed moa had feathers of differing morphology and colour on different parts of the body or that they might have varied between sexes. This variation among species and plumage patterns would not have been revealed without genetic data, and the analysis of further samples are likely to lead to improved reconstructions. It may also be possible to reconstruct more accurate representations of moa plumage by comparing moa feathers with other extant palaeognathus taxa with differing feather morphology and colour such as the emu (patterned) and cassowary (*Casuarius* spp.) (non-descript). In addition, because of the similarity of moa and kiwi plumage (figure 4), comparisons with known kiwi colour morphs (Morris & Smith 1988) can be made.

It is likely that the convergent colouring of some moa feathers has been driven by selection on plumage to avoid predation by aerial predators such as Haasts' eagle (*Harpagornis moorei*). This concept is supported by the drab camouflage plumage of several other endemic avian New Zealand taxa with similar terrestrial habits (e.g. species of kiwi and kakapo, *Strigops habroptilus*). It is also probable that moa plumage differed between open and closed-canopy habitat types, sexes and ages—with more disruptive plumage patterns present in the open habitats. The overlapping feather morphology and colour between the identified moa species also raises the possibility that assortative mating in moa was controlled more by call recognition (especially given suggestions of species differences in vocal tract morphology; Worthy & Holdaway 2002), habitat or other unidentified plumage characteristics.

In addition to DNA from subfossil bone (Baker *et al.* 2005), coprolites (Poinar *et al.* 1998; Hofreiter *et al.* 2000), hair (Gilbert *et al.* 2007) and sediment (Willerslev *et al.* 2003), ancient DNA from subfossil feathers offer insights into the presence and absence of species on temporal scales. For example, the taxonomic information from moa coprolites (Wood *et al.* 2008) and subfossil feathers from the same horizons in Roxburgh Gorge B and Sawers' rockshelters indicate that multiple species of moa are present, raising the possibility of competition between moa for prime rockshelter and nesting sites. Interestingly, the success rate for extraction of amplifiable ancient DNA from subfossil moa feathers is much higher than coprolites excavated from the same horizons (4 of 21 coprolites versus 11 of 19 feathers from Central Otago rockshelters had amplifiable ancient DNA; Wood *et al.* 2008). Furthermore, the size range of amplifiable mtDNA fragments from subfossil feathers (31–205 bp from the calamus, 31–180 bp from distal portions) is comparable to hair (60–130 bp; Gilbert *et al.* 2007) and coprolites (11–273 bp; Poinar *et al.* 1998; Hofreiter *et al.* 2000; Wood *et al.* 2008), depending on the age and preservation conditions of samples.

Our findings suggest that preserved subfossil feathers from extinct avian taxa in sites around the world are a major potential resource for multi-disciplinary research. Semi-arid sites in Antarctica (Emslie & Patterson 2007), South America (Paabo 1989; Poinar *et al.* 1998; Hofreiter *et al.* 2000; authors' personal observation, 2008), North America (Borson *et al.* 1998; Gilbert *et al.* 2008), Siberia (Stone 2002; Gilbert *et al.* 2007), Europe (Loreille *et al.* 2001) and New Zealand (Worthy & Holdaway 2002; Wood *et al.* 2008) have preserved bone, skin, fur, hair, coprolites and feathers. In New Zealand, Late Holocene feathers attributed to the extinct Finsch's duck (*Chenonetta finschi*) have been found in a range of rockshelter deposits. The potential also exists that key enigmatic extinct New Zealand taxa such as the adzebill (*Aptornis* spp.) and giant goose (*Cnemiornis* spp.) may also have preserved feathers in subfossil deposits. Until now, subfossil feathers from these palaeontological and archaeological contexts (Borson *et al.* 1998; Emslie & Patterson 2007) have not been analysed for ancient DNA and this represents an important new area of research.

Subfossil and museum feathers represent a valuable untapped resource of genetic information. In addition, the presence of DNA in the rachis and barbs of moa and modern emu feathers suggests that multiple parts of a feather may contain DNA, providing a method for genetic analysis requiring minimal destruction of valuable specimens. The combination of ancient DNA approaches with the use of appropriate tests for colour fading such as that illustrated here promises to improve our reconstructions of extinct avian taxa.

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REFERENCES

Anderson, A. 1989 *Prodigious birds*. Cambridge, UK: Cambridge University Press.

Baker, A. J., Huynen, L. J., Haddrath, O., Miller, C. D. & Lambert, D. M. 2005 Reconstructing the tempo and mode of evolution in an extinct clade of birds with ancient DNA: the giant moas of New Zealand. *Proc. Natl Acad. Sci. USA* **102**, 8257–8262. (doi:10.1073/pnas.0409435102)

Borson, N., Berdan, F., Stark, E., States, J. & Wettstein, P. J. 1998 Origin of an Anasazi scarlet macaw feather artifact. *Am. Antiquity* **63**, 131–142. (doi:10.2307/2694780)

Bunce, M., Worthy, T. H., Ford, T., Hoppitt, W., Willerslev, E., Drummond, A. & Cooper, A. 2003 Extreme reversed sexual size dimorphism in the extinct New Zealand moa *Dinornis*. *Nature* **425**, 172–175. (doi:10.1038/nature01871)

Cooper, A. & Poinar, H. N. 2000 Ancient DNA: do it right or not at all. *Science* **289**, 1139. (doi:10.1126/science.289.5482.1139b)

Cooper, A., Mourer-Chauvire, C., Chambers, G. K., von Haeseler, A., Wilson, A. C. & Paabo, S. 1992 Independent origins of New Zealand moas and kiwis. *Proc. Natl Acad. Sci. USA* **89**, 8741–8744. (doi:10.1073/pnas.89.18.8741)

Cooper, A., Fox-Lalueza, C., Anderson, A., Rambaut, A., Austin, J. & Ward, R. 2001 Complete mitochondrial genome sequences of two extinct moas clarify ratite evolution. *Nature* **409**, 704–707. (doi:10.1038/35055536)

Emslie, S. D. & Patterson, W. 2007 Abrupt recent shift in delta13C and delta15N values in Adelie penguin eggshell in Antarctica. *Proc. Natl Acad. Sci. USA* **104**, 11 666–11 669. (doi:10.1073/pnas.0608477104)

Feldman, C. R. & Spicer, G. S. 2002 Mitochondrial variation in sharp-tailed snakes (*Contia tenuis*): evidence of a cryptic species. *J. Herpetol.* **36**, 648–655.

Fetzner, J. W. 1999 Extracting high quality DNA from shed reptile skin: a simplified method. *Biotechniques* **26**, 1052–1054.

Flannery, T. & Schouten, P. 2001 *A gap in nature: discovering the world's extinct animals*. Australia: The Text Publishing Company.

Forrest, R. M. 1987 A partially mummified skeleton of *Anomalopteryx didiformis* from Southland. *J. R. Soc. N. Z.* **17**, 399–408.

Gilbert, M. T. P. *et al.* 2007 Whole-genome shotgun sequencing of mitochondria from ancient hair shafts. *Science* **317**, 1927–1930. (doi:10.1126/science.1146971)

Gilbert, M. T. P. *et al.* 2008 DNA from pre-clovis coprolites in Oregon, North America. *Science* **320**, 786–789. (doi:10.1126/science.1154116)

Gill, B. & Martinson, P. 1991 *New Zealand's extinct birds*. New Zealand: Random Century.

Hamilton, A. 1894 On the feathers of a small species of moa (*Megalapteryx*) found in a cave at the head of the Waikaia River, with a notice of a moa-hunters camping place on the Old Man Range. *Trans. Proc. N. Z. Inst.* **27**, 232–238.

Hofreiter, M., Poinar, H. N., Spaulding, W. G., Bauer, K., Martin, P. S., Possnert, G. & Paabo, S. 2000 A molecular analysis of ground sloth diet through the last glaciation. *Mol. Ecol.* **9**, 1975–1984. (doi:10.1046/j.1365-294X.2000.01106.x)

Horváth, M. B., Martínez-Cruz, B., Negro, J. J., Kalmár, L. & Godoy, J. A. 2005 An overlooked DNA source for non-invasive genetic analysis in birds. *J. Avian Biol.* **36**, 84–88. (doi:10.1111/j.0908-8857.2005.03370.x)

Hutton, F. W. & Coughtrey, M. 1875 Description of some moa remains from the Knobby Ranges. With anatomical notes. *Trans. Proc. N. Z. Inst.* 7, 266–273.

Huynen, L., Miller, C. D., Scofield, R. P. & Lambert, D. M. 2003 Nuclear DNA sequences detect species limits in ancient moa. *Nature* **425**, 175–178. (doi:10.1038/nature01838)

Huynen, L., Lissone, I., Sawyer, S. & Lambert, D. 2008 Genetic identification of moa remains recovered from Tiniroto, Gisborne. *J. R. Soc. N. Z.* **38**, 231–235.

Kumar, S., Tamura, K. & Nei, M. 2004 MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief. Bioinform.* **5**, 150–163. (doi:10.1093/bib/5.2.150)

Lalueza-Fox, C. *et al.* 2007 A melanocortin 1 receptor allele suggests varying pigmentation among Neanderthals. *Science* **318**, 1453–1455. (doi:10.1126/science.1147417)

Lambert, D. M., Baker, A., Huynen, L., Haddrath, O., Herbert, P. D. N. & Miller, C. D. 2005 Is a large-scale DNA-based inventory of ancient life possible? *J. Hered.* **96**, 279–284. (doi:10.1093/jhered/esi035)

Loreille, O., Roumat, E., Verneau, O., Bouchet, F. & Hänni, C. 2001 Ancient DNA from *Ascaris*: extraction amplification and sequences from eggs collected in coprolites. *Int. J. Parasitol.* **31**, 1101–1106. (doi:10.1016/S0020-7519(01)00214-4)

Ludwig, A. *et al.* 2009 Coat colour variation at the beginning of horse domestication. *Science* **324**, 485. (doi:10.1126/science.1172750)

Miller, W. *et al.* 2009 The mitochondrial genome sequence of the Tasmanian tiger (*Thylacinus cynocephalus*). *Genome Res.* **19**, 213–220. (doi:10.1101/gr.082628.108)

Morris, R. & Smith, H. 1988 *Wild south: saving New Zealand's endangered birds*. TVNZ in Association with Century Hutchinson: New Zealand.

Murray, P. & Vickers-Rich, P. 2003 *Magnificent mihirungs: the colossal flightless birds of the Australian dreamtime*. USA: Indiana University Press.

Oliver, W. 1955 *New Zealand birds*, 2nd edn. Wellington, New Zealand: Reed.

Paabo, S. 1989 Ancient DNA: extraction, characterization, molecular cloning, and enzymatic amplification. *Proc. Natl Acad. Sci. USA* **86**, 1939–1943. (doi:10.1073/pnas.86.6.1939)

Payne, R. B. & Sorenson, M. D. 2002 Museum collections as sources of genetic data. *Bonner Zoologische Beitrage* **51**, 97–104.

Poinar, H. N., Hofreiter, M., Spaulding, W. G., Martin, P. S., Stankiewicz, B. A., Bland, H., Evershed, R. P., Possnert, G. & Paabo, S. 1998 Molecular coproscopy: dung and diet of the extinct ground sloth *Nothrotheriops shastensis*. *Science* **218**, 402–406. (doi:10.1126/science.281.5375.402)

Rompler, H., Rohland, N., Lalueza-Fox, C., Willerslev, E., Kuznetsova, T., Rabeder, G., Bertranpetit, J., Schoneberg, T. & Hofreiter, M. 2006 Nuclear gene indicates coat-colour polymorphism in mammoths. *Science* **313**, 62. (doi:10.1126/science.1128994)

Sefc, K. M., Payne, R. B. & Sorenson, M. D. 2003 Microsatellite amplification from museum feather samples: effects of fragment size and template concentration on genotyping errors. *Auk* **120**, 982–989. (doi:10.1642/0004-8038(2003)120[0982:MAFMFS]2.0.CO;2)

Stone, R. 2002 *Mammoth: the resurrection of an ice age giant*. New York, NY: Basic Books.

Swofford, D. L. 2000 PAUP*. *Phylogenetic analysis using parsimony (*and other methods)*. Version 4. Sunderland, MA: Sinauer Associates.

Tennyson, A. & Martinson, P. 2006 *Extinct birds of New Zealand*. Wellington, New Zealand: Te Papa Press.

Vickers-Rich, P., van Tets, G. F. & Knight, F. 1985 *Kadimakara: extinct vertebrates of Australia*. USA: Princeton University Press.

Vickers-Rich, P., Trusler, P., Rowley, M. J., Cooper, A., Chambers, G. K., Bock, W. J., Millener, P. R., Worthy, T. H. & Yaldwyn, J. C. 1995 Morphology, myology, collagen and DNA of a mummified moa, *Megalapteryx didinus* (Aves: Dinornithiformes) from New Zealand. *Tuhinga Rec. Mus. N. Z. Te Papa Tongarewa* 4, 1–26.

Villafuerte, R. & Negro, J. J. 1998 Digital imaging for colour measurement in ecological research. *Ecol. Lett.* **1**, 151–154. (doi:10.1046/j.1461-0248.1998.00034.x)

Vinther, J., Briggs, D. E. G., Prum, R. O. & Saranathan, V. 2008 The colour of fossil feathers. *Biol. Lett.* **4**, 522–525. (doi:10.1098/rsbl.2008.0302)

White, T. 1885 Remarks of the feathers of two species of moa. *Trans. Proc. N. Z. Inst.* **18**, 83–84.

Willerslev, E., Hanson, A. J., Binladen, J., Brand, T. B., Gilbert, M. T. P., Shapiro, D., Bunce, M., Wuif, C., Gilichinsky, D. A. & Cooper, A. 2003 Diverse plant and animal genetic records from Holocene and Pleistocene sediments. *Science* **300**, 791–795. (doi:10.1126/science.1084114)

Wilmshurst, J. M., Anderson, A. J., Higham, T. F. G. & Worthy, T. H. 2008 Dating the late prehistoric dispersal

of Polynesians to New Zealand using the commensal Pacific rat. *Proc. Natl Acad. Sci. USA* **105**, 7676–7680. (doi:10.1073/pnas.0801507105)

Wood, J. 2008 Moa (Aves: Dinornithiformes) nesting material from rockshelters in the semi-arid interior of South Island, New Zealand. *J. R. Soc. N. Z.* **38**, 115–129.

Wood, J. R., Rawlence, N. J., Rogers, G. M., Austin, J. J., Worthy, T. H. & Cooper, A. 2008 Coprolite deposits reveal the diet and ecology of the extinct New Zealand megaherbivore moa (Aves, Dinornithiformes). *Quat. Sci. Rev.* **27**, 2593–2602. (doi:10.1016/j.quascirev.2008.09.019)

Worthy, T. H. 1989 Mummified moa remains from Mt Owen, northwest Nelson. *Notornis* **36**, 36–38.

Worthy, T. H. 1998 Quaternary fossil faunas of Otago, South Island, New Zealand. *J. R. Soc. N. Z.* **28**, 421–521.

Worthy, T. H. & Holdaway, R. N. 2002 *The lost world of the moa.* Christchurch, New Zealand: Canterbury University Press.

Yue, G. H. & Orban, L. 2001 Rapid isolation of DNA from fresh and preserved fish scales for polymerase chain reaction. *Mar. Biotechnol.* **3**, 199–204. (doi:10.1007/s10126-001-0010-9)

DNA content and distribution in ancient feathers and potential to reconstruct the plumage of extinct avian taxa

Supplementary Information

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Sequences have been deposited in Genbank (accession numbers GQ253937-GQ253945) or Table S3 if less than 50 bp in length.

Table S1 Moa and emu feather specimens included in this study. Sequence refers to the partial mtDNA control region fragment amplified using primers reported in Cooper *et al.* (2001); Bunce *et al.* (2003) and Wood *et al.* (2008) (Table S1).

Genus	Species	Museum No.[1]	Rockshelter	Sequence	Sub-sample	Genbank No.[2]
Megalapteryx	*didinus*	OM Av10793.1	Roxburgh Gorge B	CR262-294	Calamus	Table S3
Megalapteryx	*didinus*	OM Av10791.1	Roxburgh Gorge C	CR262-419		GQ253937
Megalapteryx	*didinus*	A 06.49.20.1	Sawers	CR262-419		GQ253938
Megalapteryx	*didinus*	A 06.49.20.2	Sawers	CR262-419		GQ253939
Dinornis	*robustus*	OM Av10793.2	Roxburgh Gorge B	CR262-419		GQ253940
Euryapteryx	*gravis*	A 06.49.20.3	Sawers	CR185-419		GQ253941
Pachyornis	*elephantopus*	A 06.49.18	Sawers	CR262-294		Table S3
?	?	OM Av10797	Cainmuir Gully B	n/a		n/a
?	?	OM Av10791.2	Roxburgh Gorge C	n/a		n/a
?	?	ACAD7148	Sawers	n/a	Rachis, barbs	n/a
Dinornis	*robustus*	ACAD7149	Sawers	CR262-294		Table S3
Pachyornis	*elephantopus*	ACAD7150	Sawers	CR262-294		Table S3
Euryapteryx	*gravis*	ACAD7151	Sawers	CR262-294		Table S3
?	?	ACAD7152	Sawers	n/a		n/a
?	?	ACAD7153	Sawers	n/a		n/a
?	?	ACAD7154	Sawers	n/a		n/a
Euryapteryx	*gravis*	ACAD7155	Sawers	CR262-419		GQ253942
?	?	ACAD7158	Sawers	n/a		n/a
?	?	ACAD7159	Sawers	n/a		n/a
Dromaius	*novaehollandiae*	ACAD4885-89[3]	Adelaide	12SE-12SH2	Calamus, proximal rachis, distal rachis, barbs	GQ253943
Dromaius	*novaehollandiae*	ACAD4891-95[3]	Adelaide	12SE-12SH2		GQ253944
Dromaius	*novaehollandiae*	ACAD4897-4901[3]	Adelaide	12SE-12SH2		GQ253945

[1] OM: Otago Museum; A: Alexandra Museum; ACAD: Australian Centre for Ancient DNA.

[2] Sequences less than 50 bp can be found in Table S3 (not accepted by Genbank).

[3] ACAD number refers to DNA extracts from each feather.

Table S2 Primer sequences used in this study.

Primer	Sequences (5`-3`)	Reference
CR185F	GTACATTCCCTGCATTGGCTC	Cooper *et al.* 2001; Bunce *et al.* 2003
CR204F	AGATTTATARCTCGGACA	Wood *et al.* 2008
CR262F	GCGAAGACTGACTAGAAGC	Cooper *et al.* 2001; Bunce *et al.* 2003
CR294R	GCGAGATTTGAACAGTACG	Cooper *et al.* 2001; Bunce *et al.* 2003
CR419R	GGGTTGCTGATTTCTCGTGA	Cooper *et al.* 2001; Bunce *et al.* 2003
12SE	CCCACCTAGAGGAGCCTGTTC	Cooper *et al.* 2001
12SH2	CCTTGACCTGTCTTGTTAGC	Cooper *et al.* 2001

Table S3 Mitochondrial DNA control region sequences less than 50 bp in length from sub-fossil moa feathers.

Museum number	Sequence
OM Av10793.1	CTATAACCCGGACATGCCCTTTACC
A 06.49.18	TTATAGCTCGAACATAACTCTTACC
ACAD7149	ATAGATTTATAGCTCGGACATAATCTTAACC
ACAD7150	ATAGATTTATAGCTCGAACATAACTYTTACC
ACAD7151	ATAGATTTATAGCTCGGACATACACTCTACC

Supplementary References

Bunce, M., Worthy, T.H., Ford, T., Hoppitt, W., Willerslev, E., Drummond, A. & Cooper, A. 2003 Extreme reversed sexual size dimorphism in the extinct New Zealand moa *Dinornis*. *Nature* **425**, 172-175.

Cooper, A., Fox-Lalueza, C., Anderson, A., Rambaut, A., Austin, J. & Ward, R. 2001 Complete mitochondrial genome sequences of two extinct moas clarify ratite evolution. *Nature* **409**, 704-707.

Wood, J.R., Rawlence, N.J., Rogers, G.M., Austin, J.J., Worthy, T.H. & Cooper, A. 2008 Coprolite deposits reveal the diet and ecology of the extinct New Zealand megaherbivore moa (Aves, Dinornithiformes). *Quat. Sci. Rev.* **27**, 2593-2602.

CHAPTER SIX

The extinct crested moa (*Pachyornis australis*) tracked its habitat with warming climate since the last glacial maximum

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Chapter Six

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Nicolas. J. Rawlence

Designed experiment, collected samples, conducted fieldwork, performed DNA and demographic analysis, performed collagen preparation and radiocarbon dating, interpreted data and wrote paper.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed Date.....20/04/10

Jessica Metcalf

Performed collagen preparation and radiocarbon dating, helped with demographic analyses and evaluated manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed Date.....27/4/18

Jamie R. Wood

Helped with fieldwork and interpretation of results, evaluated manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed Date.....20 April 2010.....

Jeremy J. Austin

Helped with fieldwork, supervised development of research, evaluated manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed Date.....22/4/10.....

Alan Cooper

Designed experiment, supervised development of research, evaluated manuscript and provided funding.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed .....Date... 22/4/0...

Chapter Six

The extinct crested moa (*Pachyornis australis*) tracked its habitat with warming climate since the Last Glacial Maximum.

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Abstract

The response of taxa to changing habitat availability by altering their distributions accordingly has been called habitat tracking. In contrast, taxa that are not able to track their habitat either shift their niche or go locally extinct. New Zealand offers a unique opportunity to test hypotheses about species' ability to track habitat in a temperate setting because of a well characterised palaeoclimatic and vegetation history, and an extensive fossil record spanning the last 50,000 years. We investigated whether the extinct crested moa (*Pachyornis australis*) tracked the migrating and declining sub-alpine shrubland/grassland habitat since the Last Glacial Maximum using ancient DNA, radiocarbon dating and stable dietary isotope analyses. The combined analyses show that crested moa tracked the sub-alpine habitat with minimal declines in population size. The results suggest that failure of populations to track habitat with warming climate may not be a general phenomenon and that other explanations need to be sought for the different responses of populations to changes in habitat availability.

Introduction

Understanding how species respond to climate change is central to understanding historical processes that have shaped intra-specific phylogeography, and to improving predictions of the impacts of future climate change. One theory, which became known as the habitat tracking hypothesis, was first proposed by Eldredge (1989). The theory states that taxa can 'recognise' familiar habitats and track them accordingly, perceiving little to no change in habitat. Eldredge (1989) supported his theory with evidence of changes in the distribution of the Late Cenozoic

beetle fauna of Europe (Coope 1979). For example, Coope (1979) showed that the beetle fauna found in the United Kingdom 44 Kya is now restricted to boreal and montane regions in Arctic Russia. The habitat tracking hypothesis has also been proposed as an explanation for punctuated equilibria in the fossil record, where morphological evolution occurs in rare, rapid, localised events (Gould and Eldredge 1977).

The alternative to the habitat tracking hypothesis is that taxa are not able to track their habitat (or are not able to track it rapidly enough) and either shift their niche or go locally extinct. Support for this hypothesis has come from the analysis of Quaternary pollen records in Europe (Bennett *et al.* 1991). The data showed that forest tree species that extended their range into northern Europe from southern refugia, during warming climatic conditions, went locally extinct in northern Europe, rather than contracting their range south, during cooling climatic conditions.

It has been shown that the ranges of many Arctic or cold adapted taxa expanded during glacial periods and contracted during interglacial periods, with the opposite occurring in temperate or warm adapted taxa (Stewart and Lister 2001; Dalen *et al.* 2005, 2007; Provan and Bennett 2008). However, Dalen *et al*. (2007) hypothesised that the failure of cold adapted taxa to track declining habitat with warming climate might be a general phenomenon, with declines in preferred habitat resulting in local extinctions. This differs from Eldredge (1989) hypothesis, which assumes taxa can continually 'recognise' familiar habitats, rather than going locally extinct when habitats decline (Bennett *et al*. 1991; Dalen *et al*. 2007). Dalen *et al.* (2007) showed that Scandinavia was re-colonised by Arctic foxes from Beringia rather than mid-latitude Europe, with mid-latitude European populations going extinct at the end of the Pleistocene. The results suggested that if a highly mobile species like the Arctic fox failed to track changes in habitat availability, less mobile species were even less likely to do so. However, it has not been conclusively shown that Arctic foxes failed to respond to changes in habitat availability because Dalen *et al.* (2007) did not conduct stable dietary isotope analyses of bones to reconstruct diet or incorporate palynological data from fossil localities to reconstruct habitat. In addition, the failure of taxa to respond to changes in habitat availability by shifting their distributions accordingly is potentially complicated by a series of complex secondary interactions including geographical barriers (Hewitt *et al.* 1999), interspecies competition (Bennett *et al.* 1991), predator-prey relationships, behavioral constraints (Dalen *et al.* 2007) or over-hunting by humans (Nogues-Bravo *et al.*

2008). This questions the Dalen *et al.* (2007) hypothesis that failure of taxa to track changes in habitat availability may be a general phenomenon.

New Zealand offers a unique opportunity to test hypotheses about the ability of taxa to track changes in habitat availability in a temperate climate. There is already evidence for habitat tracking in temperate populations. The integration of fossil data into datasets of the modern distributions of animals has shown that the geographical range of multiple taxa in North America (Graham *et al.* 1996) and Eurasia (Lister and Stuart 2008) shifted in a gleasonian manner from the Last Glacial Maximum (LGM) to the Late Holocene. For example, Stuart *et al.* (2004) showed that during cold climatic conditions, the temperate adapted giant deer (*Megaloceros giganteus*) was expatriated from Europe and replaced by the cold adapted woolly mammoth (*Mammuthus primagenius*) as forest was replaced by tundra, with the opposite occurring during warm climatic conditions. Unlike Europe and North America, New Zealand does not have many of the complex secondary interactions that can affect the ability of taxa to track changes in habitat availability. Prior to the settlement of New Zealand by Polynesians in 1280 AD (Wilmshurst *et al*. 2008), there was only one dominant herbivore, the moa, and no mammalian predators, minimising secondary interactions from other vertebrate groups.

Climate change in New Zealand through the Otiran Glacial (70-14 Kya), Late Glacial (14-10 Kya) and Holocene (10-0 Kya) is well documented. The Otiran Glacial encompasses the LGM (29-19 Kya; Newnham *et al.* 2007) and the Last Glacial (18-14 Kya; Williams *et al.* 2005). During the LGM mean annual temperatures were 4-5ºC lower than today and cool, dry conditions were prevalent (McGlone *et al*. 2010). The Last Glacial was characterised by increased biological activity, like increased plant growth, and precipitation (Hellstrom *et al.* 1998; Williams *et al.* 2005). The Late Glacial (14-10 Kya) followed the Otiran Glacial (Worthy and Roscoe 2003), and is sometimes referred to as the Interglacial Transition (Williams *et al.* 2005). The Late Glacial was characterised by warming temperatures (14-13 Kya) and the New Zealand Glacial Reversal (13-11 Kya; Williams *et al.* 2005), a period of severe climate oscillation. The Holocene period (10 Kya-present) was characterised by warming temperatures interspersed with periods of cooler temperatures due to increased glacial activity (Williams *et al.* 2005) but comparatively stable climate during the Late Holocene (Burrows and Greenland 1979).

The geomorphological and vegetation changes in New Zealand through the Otiran Glacial, Late Glacial and Holocene are also well documented. During the LGM sea levels were 120-130 metres lower with the North and South Islands unconnected (at least significantly), compared to previous glacial periods, as shown through the lack of evidence of faunal interchange of endemic North and South Island avian species during the LGM (Worthy and Holdaway 2002; Bunce *et al.* 2009). With colder temperatures, the tree line was lowered by 800 metres (McGlone *et al.* 2010) and the Southern Alps were covered in permanent snow and ice (McKinnon 1997; Newnham *et al.* 1999; Figure 1). This was followed by the expansion of high altitude sub-alpine grassland forming a grassland/shrubland mosaic at low altitudes (Moar and Suggate 1973, 1979; Vandergoes *et al.* 2005; Burge and Schulmeister 2007; Figure 1) and the retreat of forest to sheltered southern and warmer northern refugia (Campbell and Hutching 2007). The sub-alpine grassland/shrubland mosaic was widespread at low altitudes until about 15-12 Kya depending on the area (Moar and Suggate 1973, 1979; Vandergoes and Fitzsimons 2003), followed by a well-documented succession of grassland to shrubland (15-12 Kya) to forest (14-10 Kya), due to rapid climatic change during the Late Glacial (14-10 Kya). Subsequently, the sub-alpine habitat declined in extent and migrated up-slope from about 15 Kya until climate stabilised in the Late Holocene (Moar and Suggate 1973, 1979; Wardle 1991; Worthy and Holdaway 1995, 1996; Figure 1).

We wanted to study the impacts of these climatic, geomorphological and vegetational changes on the endemic New Zealand fauna. We focused on the extinct crested moa (*Pachyornis australis*), which was one of nine species of extinct palaeognathus moa (Aves: Dinornithiformes) endemic to New Zealand (Oliver 1949; Worthy 1989a; Bunce *et al.* 2009; Checklist Committee OSNZ 2010). The location of crested moa remains in areas of high altitude sub-alpine grassland/shrubland in northwest Nelson/West Coast during the Pleistocene and Holocene (Worthy 1989b; Worthy and Holdaway 2002; Tennyson and Martinson 2006; Figure 1, 2), suggests crested moa inhabited the relatively narrow sub-alpine altitudinal zone that migrated up-slope since the LGM (Moar and Suggate 1973, 1979; Wardle 1991; Worthy and Holdaway 1995, 1996). Crested moa were more common in glacial periods when the sub-alpine habitat was widespread at lower altitudes (Figure 1).

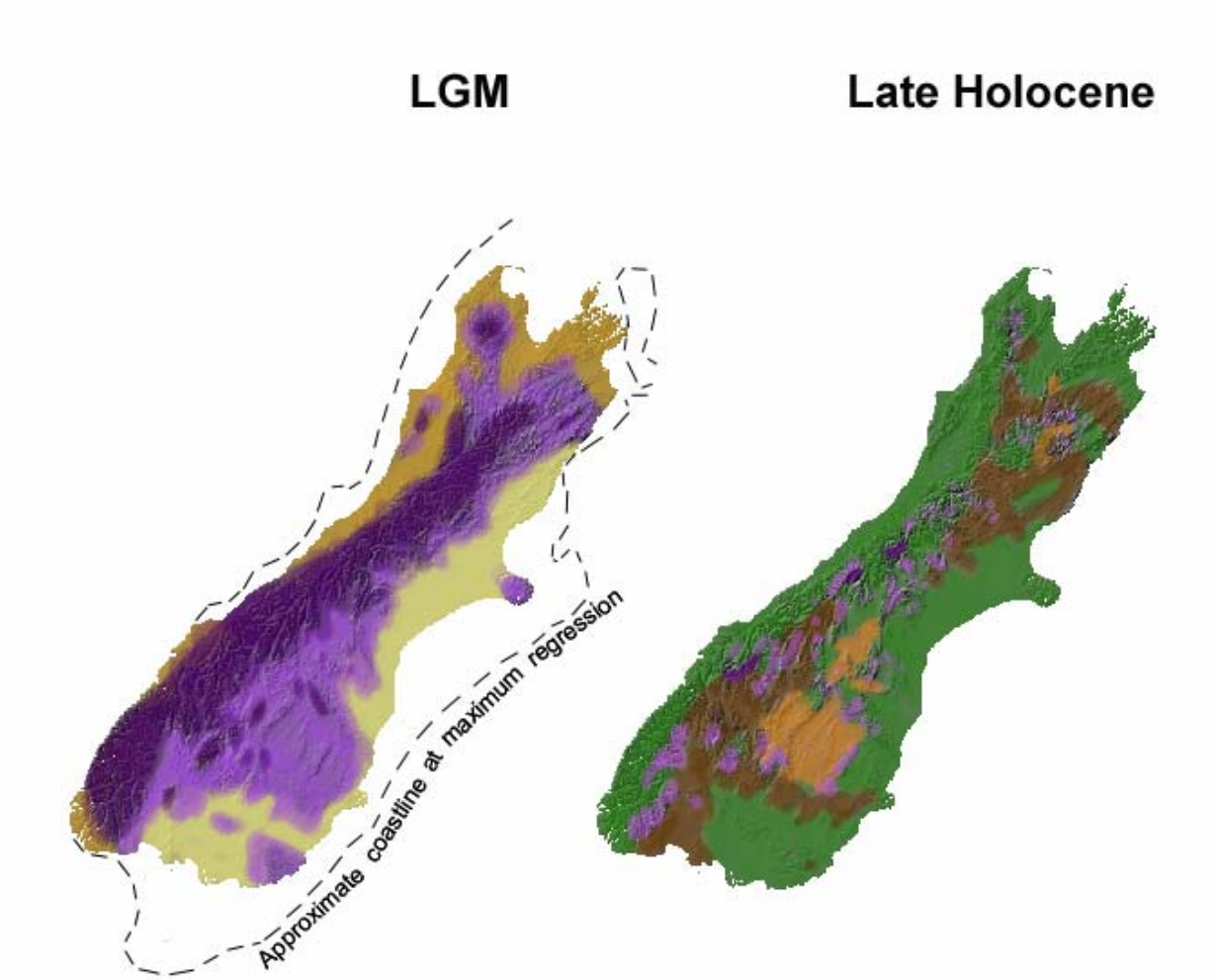

Figure 1 Major habitat types on the South Island, New Zealand, during the Last Glacial Maximum (29-19 Kya) and the Late Holocene, based on Barrell *et al.* (2005). Purple, permanent ice; Lilac, subalpine; brown, beech forest; green, podocarp-broadleaf forest; orange, shrublands/grasslands; yellow, open grasslands with marginal woody vegetation.

Due to the comparative rarity of crested moa remains compared to other moa species (Tennyson and Martinson 2006) only a small number of bones have been radiocarbon dated (Worthy 1993; Bunce *et al.* 2009), therefore it has not been possible to determine whether crested moa survived the pronounced climate and habitat change during the Late Glacial and Early Holocene. The youngest confirmed remains have been dated to 10,165 +/- 50 years BP (Bunce *et al.* 2009). Late Holocene remains attributed to crested moa have been found in Otago (Worthy 1998a) and Southland (Worthy 199b) but these have yet to be verified by DNA analysis and as such do not constitute solid evidence for crested moa survival.

There are three potential hypotheses in general relating to the ability of crested moa to track changes in the distribution of the sub-alpine habitat with warming climate since the LGM:

i) Crested moa tracked the sub-alpine habitat. There would be no change in the temporal phylogeographic pattern with younger DNA sequences identical to or recently derived from older sequences.
ii) Crested moa became extinct. There would be declines in haplotype diversity towards extinction.
iii) Crested moa went locally extinct and the study area was subsequently re-colonised from adjacent areas. This would lead to a marked change in the temporal phylogeographic pattern with younger sequences different to older sequences.

We aim to address the following questions:

i) Did crested moa track the migrating and declining sub-alpine shrubland/grassland habitat after the LGM?
ii) Did crested moa decline in population size with declining habitat?
iii) Did crested moa go extinct at the end of the Pleistocene with climate and habitat change or did it survive until the Late Holocene?

To address (i), the ability of crested moa to track the sub-alpine habitat will be assessed by temporal phylogeographic analysis, radiocarbon dating, and $\delta^{13}C$ and $\delta^{15}N$ isotope analysis of bones.

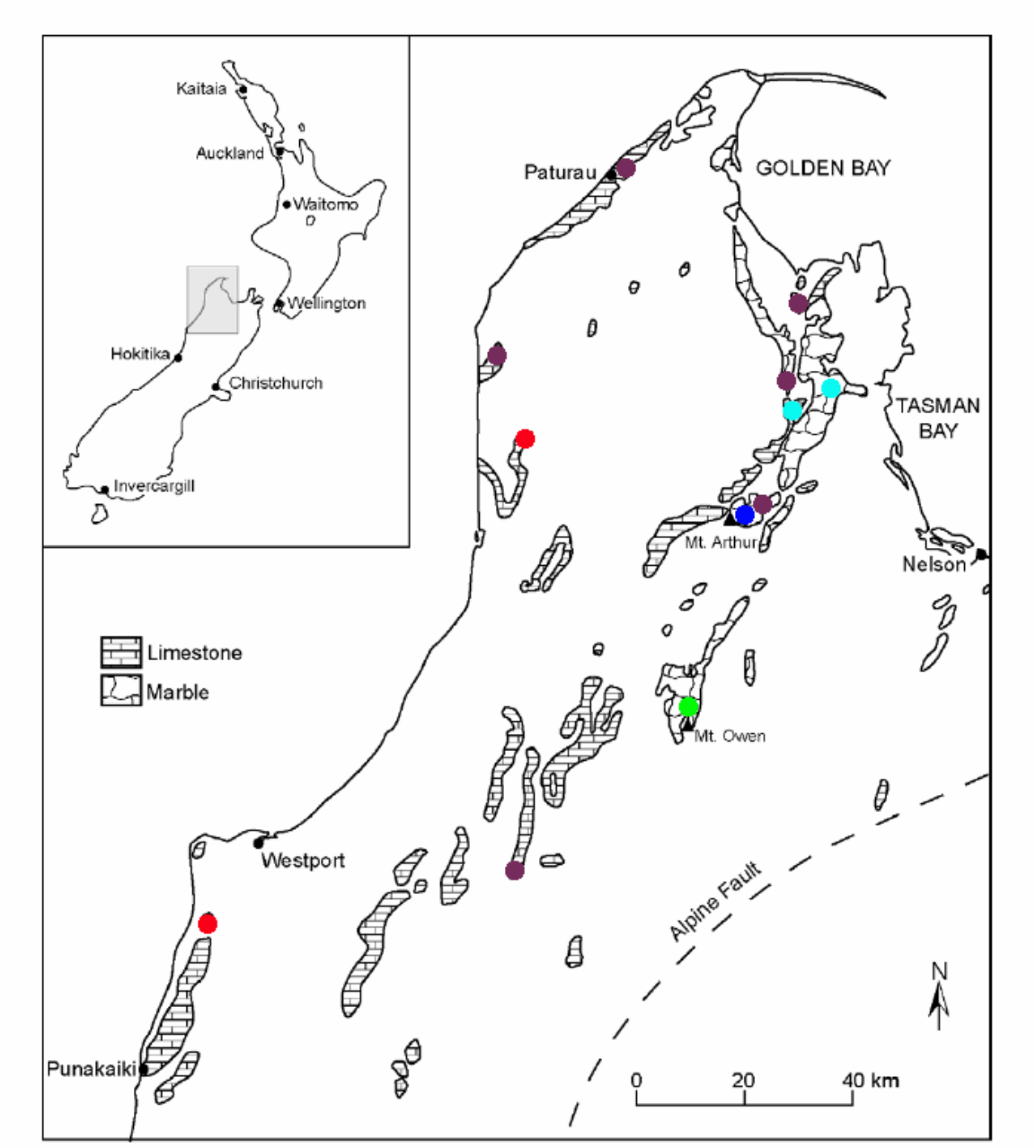

Figure 2 Distribution of fossil localities containing crested moa (*Pachyornis australis*) overlaid on the distribution of kaarst areas in northwest Nelson/West Coast (modified from Williams *et al.* 2005). Each coloured point represents a cave system(s) containing multiple discrete fossil deposits. Green: Mount Owen Kaarst; Blue: Mount Arthur Kaarst; Sky Blue: Takaka Hill/Cobb Dam Kaarst; Red: West Coast Kaarst; Purple: Fossil localities where a broad stratigraphic date could not be assigned or where specimens potentially belonged to multiple haplotypes because of missing sequence data.

Radiocarbon dating of bones will place the genetic sequences and isotope signatures in a temporal context that can be compared against known climatic and palaeovegetation records. $\delta^{13}C$ and $\delta^{15}N$ signatures from crested moa will be used as indirect indicators of climate and habitat change (Stevens *et al.* 2008) to determine what environment crested moa was living in since the LGM. $\delta^{13}C$ has been used previously as a proxy for the amount of forest cover (Worthy and Holdaway 2002), with trends towards more negative $\delta^{13}C$ values indicating a change from open shrubland/grassland to forest (Worthy and Holdaway 2002). $\delta^{15}N$ has been used previously as a proxy for temperature and precipitation, with increasing $\delta^{15}N$ values indicative of increasing temperature and precipitation (Amundson *et al.* 2003). To address (ii), changes in crested moa population size will be analysed with the Bayesian Skyline Plot (BSP; Drummond *et al.* 2005) and BayeSSC, a coalescent-based modelling programme that incorporates ancient DNA (aDNA) data (Excoffier *et al.* 2000; Anderson *et al.* 2006). These analytical methods have been used previously to examine the palaeodemographic history of species including bison (Drummond *et al.* 2005), mammoths (Barnes *et al.* 2007; Debruyne *et al.* 2008), musk ox (Campos *et al.* 2010) and brown bears (Valdiosera *et al.* 2008). Finally, the issue of the timing of the extinction of crested moa will be addressed using radiocarbon dating of crested moa remains.

Materials and Methods

DNA extraction, amplification and sequencing

Whole genomic DNA was extracted from 39 crested moa bones (Figure 2, Table S1). Strict aDNA procedures were followed (Cooper and Poinar 2000) to minimise contamination of samples by exogenous DNA, with multiple negative extraction and amplification controls, and independent replication to detect contamination. All DNA extractions and PCR reactions were set up and performed in a geographically isolated, dedicated aDNA laboratory at the Australian Centre for Ancient DNA (ACAD). PCR amplification reactions and all downstream procedures were carried out in the ACAD post-PCR lab on the main campus of the University of Adelaide.

Approximately 0.5-1 g bone powder/shavings of dense cortical bone was removed from specimens using a cordless drill at low speed with an 8 mm wood drill bit. To avoid sampling the same individual twice, only common elements (e.g. femora) of the left or right orientation were sampled from each deposit. Samples were powdered in sterilised stainless steel containers using a Braun Mikrodismembrator bead mill. Approximately 200-600 mg of bone powder was decalcified overnight in 0.5 M EDTA (pH 8.0) and digested in digestion buffer (10 mM Tris-HCl

pH 8.0; 1% sodium dodecyl sulphate; 10 mM NaCl; 10 mg/mL dithiothreitol; 0.5 mg/mL proteinase K). The digestion buffer was incubated overnight at 55°C on a rotary mixer. The digestion buffer was added to an equal volume of Tris-saturated phenol, rotated for 10 minutes and centrifuged at 6000 rpm for 5 minutes at room temperature. The aqueous phase was then removed. The phenol extraction was repeated twice and then repeated with an equal volume of chloroform. The aqueous phase was concentrated using microcon ultra-4 filters (Millipore, molecular weight cut off 50 kD) and centrifugal dialysis to approximately 100-200 uL.

Two overlapping fragments (~190 bp and ~185 bp excluding primers) of the mitochondrial DNA (mtDNA) control region hyper-variable region one (HVRI) and one fragment (~150 bp excluding primers) of hyper-variable region two (HVRII) were amplified using the primer pairs 185F/294R, 262F/419R and 1070F/1231R (Cooper *et al.* 2001; Bunce *et al.* 2009). To confirm species assignment based on morphology and to obtain further distributional data, unsuccessful PCR amplifications were subsequently repeated with the primer pair 262F/329R (Cooper *et al*. 2001; Wood *et al*. 2008), which amplified a 49 bp product that can distinguish between all nine species of moa currently recognised. PCR reactions were conducted in 25 uL volumes containing 2 mg/mL rabbit serum albumen (RSA; Sigma), 1 x PCR buffer (Platinum, Invitrogen), 2 mM $MgSO_4$, 200uM dNTPs, 1 uM each primer, 1 unit Platinum Taq Hifi (Invitrogen) and 1-2 uL DNA. PCR conditions were as follows: 94°C for three minutes, 55 cycles of 94°C for 30 seconds, 55°C for 30 seconds and 68°C for 45 seconds, with a final extension of 68°C for 10 minutes. Negative extraction and PCR controls were included with each PCR reaction. Positive PCR controls consisted of moa DNA extracts from well-preserved bones excavated from the Late Holocene Bell Hill swamp, North Canterbury, New Zealand.

PCR products were visualised on a 2% 1 x TBE agarose gel. If primer dimers were present, PCR products were purified using the AMPURE magnetic bead system (Agencourt) following the manufactures instructions, otherwise PCR products were purified using EXOSAP (4 units Exo1, 0.6 unit SAP; Fermentas) by incubation at 37°C for 30 min and 80°C 15 min. The forward and reverse sequence for each mtDNA control region fragment was sequenced from independent PCR products with the primers listed above using Big Dye Terminator technology (BigDye v3.1) and an ABI 3130XL capillary sequencer.

Altitudinal data

Altitudinal data for each fossil locality was sourced from unpublished excavation records (Trevor Worthy pers comm. 2009). Altitude in metres above sea level (m asl) was measured using a standard handheld global positioning system device.

Collagen extraction and radiocarbon dating

Only six of the crested moa specimens used for genetic analysis had been previously radiocarbon dated (Worthy 1993; Bunce *et al.* 2009; Table S2). To augment these dates, we extracted collagen from, and radiocarbon dated, a further 13 specimens (Table S2) using the facilities at the Oxford Radiocarbon Accelerator Unit (ORAU), the University of Wollongong (UW) and the Australian National University (ANU). Collagen extraction and radiocarbon dating at ORAU was conducted following ORAU protocols (see Bronk Ramsey *et al.* 2004a, b; Jacobi *et al.* 2006). To correct radiocarbon dates, $\delta^{13}C$, $\delta^{15}N$ and the C:N ratio were determined at ORAU as part of the radiocarbon dating procedure (Table S2). Collagen extraction and radiocarbon dating at UW/ANU was conducted following the methodology in Chapter Three (MNZ S25867-68; Table S2).

The size of the dataset was increased by calculating mean stratigraphic radiocarbon ages for fossil deposits using this dataset and published resources (see Worthy and Holdaway 2002), and assigning mean stratigraphic ages to undated specimens from each locality (Table S1).

Sequence alignments

Individual sequences were imported into the program Sequencer (GeneCodes) and contiguous sequences (contigs) constructed for each individual. Partial mtDNA control region sequences obtained from each individual were aligned with previously published crested moa sequences (Baker *et al.* 2005; Bunce *et al.* 2009) using the Clustal W algorithm implemented in MEGA 4.0 (Kumar *et al*. 2004). For BSP analysis, an alignment of sequences with finite and stratigraphic radiocarbon ages was constructed using MEGA. For TCS (Clement *et al.* 2002), Arlequin v3.1 and BayeSSC analyses, sequences were imported into MacClade v4.0 to determine the number of haplotypes present. To check the accuracy of MacClade when some contigs contained missing sequence data, constant sites were excluded in PAUP4.0b* (Swofford 1998) and the number of haplotypes was determined by eye and crosschecked against results from McClade. Incomplete sequences that potentially belonged to multiple haplotypes were excluded from further analysis.

Undated sequences that could not be reliably assigned a stratigraphic age were also excluded from further analysis.

Temporal phylogeographic analysis

Temporal phylogeographic analyses (also referred to as three dimensional phylogeographic analyses by Prost *et al.* 2010) assess changes in:

(i) The level of phylogeographic structuring through time.
(ii) The proportion of shared haplotypes over pre-defined time periods.
(iii) Changes in the distribution of haplotypes within a phylogeographic network.

For crested moa the following time periods are defined based on intervals of pronounced climate and habitat change (Moar and Suggate 1973, 1979; Vandergoes *et al.* 2005; Burge and Schulmeister 2007):

(i) Otiran Glacial (70-14 Kya) encompassing the LGM (29-19 Kya) and Last Glacial (18-14 Kya).
(ii) Late Glacial (14-10 Kya) encompassing the New Zealand Glacial Reversal (13-11 Kya).
(iii) Holocene (10-0 Kya).

The level of temporal phylogeographic structuring within crested moa was examined by constructing a statistical parsimony network using the programme TCS. For each time period, haplotypes were colour coded based on their presence or absence and geographical location. The colour coded networks for each time period were overlaid onto each other in chronological order, with haplotypes shared between time periods connected by dotted lines (Figure 4).

BSP analysis

The BSP of the crested moa population was constructed using BEAST v1.4.8 (Drummond & Rambaut 2007) with a group size of 10 (number of coalescent intervals in the analysis to reduce noise from outliers in the dataset) and the GTR+I+G model of nucleotide substitution. The analysis was run for 50 million generations, sampling demographic parameters every 1000 generations to ensure enough genealogies are sampled. The first 10% of runs were discarded as

burn-in, so that only the portions of the data that are in equilibrium were analysed. Three independent runs were pooled to generate the BSP. Results were visualised using Tracer v1.8 (http://beast.bio.ed.ac.uk/Tracer) to construct the BSP. To assess the effects of sequence error, due to post-mortem DNA damage, on the BSP, the analysis was repeated using BEAST v1.5.2 with the following age dependant sequence error models: transitions only and all substitutions (Rambaut *et al.* 2009).

Population genetic statistics

BayeSSC analysis compares empirical population genetic summary statistics against simulated values to determine the model of population demography that best fits the empirical data. To calculate summary statistics, crested moa sequences were divided into the following time bins based on intervals of pronounced climate and habitat change (Moar and Suggate 1973, 1979; Vandergoes *et al.* 2005; Burge and Schulmeister 2007): Otiran Glacial (70-14 Kya), Late Glacial (14-10 Kya) and Holocene (10-0 Kya). As recommended by Ramakrishnan and Hadly (2009) for population genetic analysis of a single population, the programme Arlequin 3.0 was used to calculate the number of polymorphic sites and the average pairwise difference between sequences within each time period (Table S3). Because of the amount of missing sequence data within the crested moa dataset, all nucleotide positions in DNA alignments were used by Arlequin to calculate summary statistics.

BayeSSC analysis

Because BayeSSC analysis uses generation time rather than years to model population demography, radiocarbon ages were calibrated (+/- 2 s.d.) using the programme OxCal4.0 (http://c14.arch.ox.ac.uk/oxcal) and the IntCal04 calibration curve (Reimer *et al.* 2004). Assuming a generation time for moa of ten years, the mean calibrated age for each dated sequence was converted into generation time. Ten years was chosen as a conservative estimate because the generation time of Emeid moa (that includes crested moa) of four to nine years (Turvey *et al.* 2005) may be an underestimate (Bourdon *et al.* 2009). Turvey *et al.* (2005) calculated the generation time of moa using lines of arrested growth (LAGs) that are laid down in bone and can be counted like tree rings to determine the age when moa reach osteological maturity. However, recent research by Bourdon *et al.* (2009) has shown that unlike tree rings, LAGs can be re-absorbed in ratites, meaning inferences of generation time may be inaccurate.

Due to the rarity of Holocene crested moa remains, estimates of Late Holocene population size cannot be inferred. To estimate the effective population size of crested moa during the Late Holocene, a uniform prior was placed on population size. Because mutation rates in BayeSSC are in mutations per base pair per generation, the evolutionary rate from the BSP analysis of 1.79% per million years (95% HPD 0.43-3.47%) was converted to 0.00089 mutations per base pair per generation using the above parameters. The evolutionary rate of 1.79% per million years was calculated using radiocarbon and stratigraphic dated crested moa sequences under a BSP coalescent prior following the methodology above for BSP analysis. The moa control region mutation rate of 8.7% per million years (95% HPD 2.34-20.4%; Bunce *et al.* 2009) was not used in the BayeSSC analysis because the rate was calculated using DNA sequences from all three highly divergent moa families (Megalapterygidae, Dinornithidae and Emediae) and is probably an overestimate of the crested moa mutation rate due to the lack of phylogeographic structuring within crested moa (Figure 4). The transition/transversion ratio of 0.217 was calculated using the programme MEGA from complete HVRI and II sequences with radiocarbon and stratigraphic ages, as the calculation cannot incorporate specimens with missing sequence data.

Model One: Constant population size through time

This model corresponds to a single population that remained constant through time (Figure 3) and was used to determine whether there is enough power in the crested moa dataset to reject constant population size. There were three time bins (stat groups) within this model based on intervals of pronounced climate and habitat change (Moar and Suggate 1973, 1979; Vandergoes *et al.* 2005; Burge and Schulmeister 2007): Otiran Glacial (70-14 Kya), Late Glacial (14-10 Kya) and Holocene (10-0 Kya). For each subsequent model the same time-bins were used.

Model Two: Decreasing population size from the Late Glacial to the Holocene.

This model corresponds to a single population that declined from the Late Glacial to the Holocene by a magnitude of 1.5 (Figure 3). The decline was based on the palaeodemographic history of crested moa inferred from the BSP (Figure 6). There was also a well documented succession of shrubland to forest 14-10 Kya, when the sub-alpine habitat migrated up-slope and declined in extent (Moar and Suggate 1973, 1979; Wardle 1991; Worthy and Holdaway 1995, 1996).

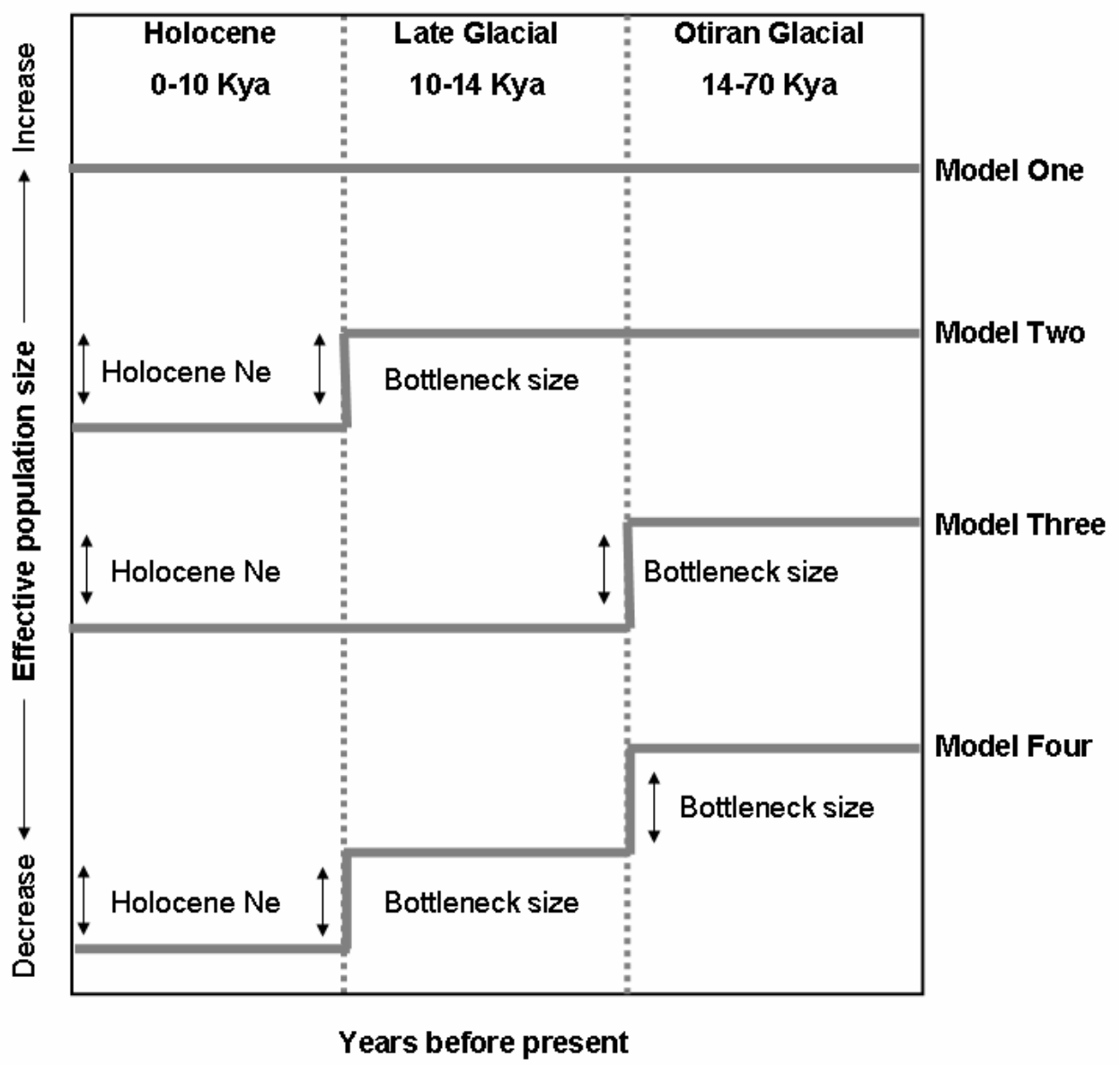

Figure 3 Models of crested moa (*Pachyornis australis*) population demography simulated in BayeSSC. For each model the Holocene effective population size (Nef) and bottleneck size was estimated. The population bottleneck size represents an undefined decline in population size.

Model Three: Decreasing population size from Otiran Glacial to the Late Glacial.
This model, based on the palaeodemographic history of crested moa inferred from the BSP analysis (Figure 6), corresponds to a single population that declined from the Otiran Glacial to the Late Glacial by a magnitude of two (Figure 3). There was also a vegetational succession from grassland to shrubland 15-12 Kya (Moar and Suggate 1973, 1979; Wardle 1991; Worthy and Holdaway 1995, 1996).

Model Four: Decreasing population size from the Otiran Glacial to the Late Glacial and from the Late Glacial to the Holocene.
This model, based on the inferred palaeodemographic history of crested moa from the BSP (Figure 6), corresponds to a single population that declined from the Otiran Glacial to the Late Glacial by a magnitude of two and from the Late Glacial to the Holocene by a magnitude of 1.5 (Figure 3).

The four models (Figure 3) were run for 50,000 generations to simulate population genetic summary statistics from genealogies produced under the coalescent process, which could be compared against empirical data to determine the demographic model that best fits the empirical data. Simulated and observed statistics were compared in the R statistics programme using the akima, lattice and lockfit analysis packages, and the R analysis script (www.standford.edu/group/hadlylab/ssc/index.html) to determine posterior distributions on priors using Approximate Bayesian Computation (Beaumont *et al.* 2002). Only simulations with a Euclidean distance (a measure of fit between simulated and observed values for each simulation) within a 0.1 tolerance interval (delta value) were analysed. Simulations with low delta values are more likely to represent the true demographic history than simulations with high delta values. For each prior the mode (the value for population size with the highest posterior probability) was chosen and the analysis repeated with the fixed mode instead of a prior distribution. The demographic model that best fitted the empirical data was determined by comparing model outcomes using the Aikaike Information Criterion (AIC). The AIC is a penalised likelihood value that penalises against the number of priors incorporated into the model (Burnham and Anderson 2002). A final analysis was run for 50,000 generations with a uniform prior on the size of the population bottleneck (population size after the bottleneck was one to ten times smaller than prior to the bottleneck) to determine the most likely population size decline.

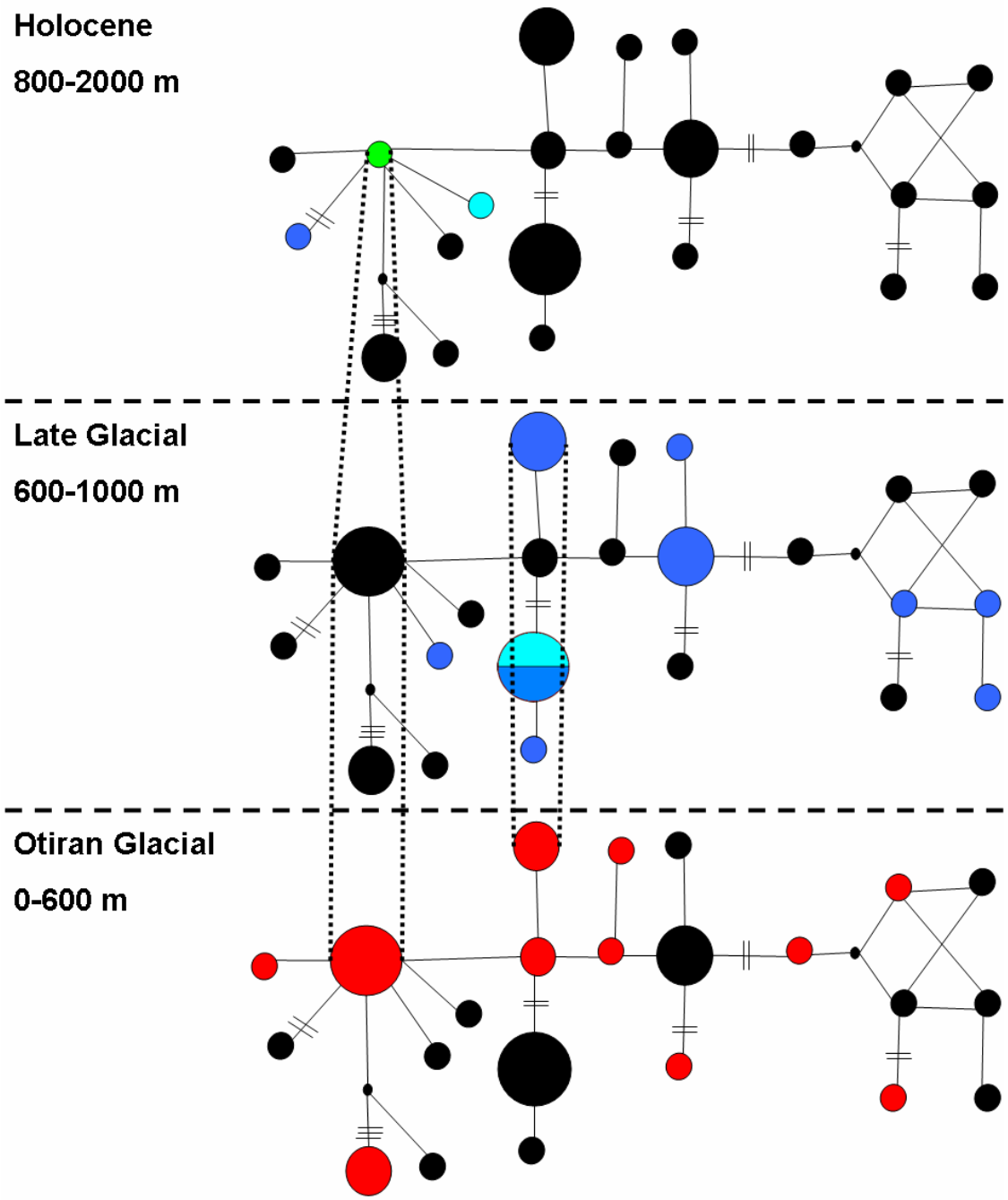

Figure 4 Temporal phylogeographic network of crested moa (*Pachyornis australis*). Each circle represents a distinct haplotype; the size of the circle corresponds to the number of sequences within each haplotype. Each line represents one mutation unless separated by dashes, where each dash represents one mutation. Small black circles represent inferred mutations. Sub-networks are connected via shared haplotypes by dotted lines. Green: Mount Owen Kaarst; Blue: Mount Arthur Kaarst; Sky Blue: Takaka Hill/Cobb Dam Kaarst; Red: West Coast Kaarst.

Results

Sequence authentication

To provide support for the authenticity of the aDNA sequences, evaluate template damage and to check sequence accuracy we replicated ca. 15% of the crested moa dataset by re-amplifying and re-sequencing six individuals for each HVRI and II fragment at ACAD. Of the six individuals (A2555-2556, 2587, 3744, 3748 and 3769; Table S1) replicated at ACAD (total of 498 bp for each individual and 2998 bp in total), no sequencing errors were observed when replicated sequences were compared with the original sequences. In addition, five specimens (GU139063-67; Table S1) previously extracted and sequenced at the Henry Welcome Ancient Biomolecules Centre in Oxford (Bunce *et al*. 2009) were independently replicated in this study. The independently replicated sequences were identical to the original sequences published by Bunce *et al.* (2009). In addition, the recovery of identical sequences despite the diversity within crested moa provides additional support for the authenticity of the aDNA sequences.

Temporal phylogeographic analysis

The temporal phylogeographic analysis suggests there was no phylogeographic structuring within crested moa as a whole and also when radiocarbon age (Holocene, Late Glacial or Otiran Glacial) and altitude are taken into account (Figure 4). Within each time period, haplotypes are spread throughout the network. Population continuity through time is supported by haplotype sharing between the Otiran Glacial, Late Glacial and Holocene.

Radiocarbon dating, $\delta^{15}N$ and $\delta^{13}C$ analysis

Radiocarbon dating revealed three discrete fossil deposits that contained crested moa remains dated to the Late Holocene (Table S2). The deposits include Cheops Cave on Mount Arthur, Magnesite Quarry at Cobb Dam, and Blowhole Cave on Mount Owen (Figure 2). The youngest remains, from Blowhole Cave, are 564 +/- 26 years BP (641-527 calendar years BP). This equates to 1309-1423 AD, after Polynesians settled in New Zealand in approximately 1280 AD (Wilmshurst *et al.* 2008).

When the radiocarbon dates from Table S2 are combined with previously published dates (see Worthy and Holdaway 2002; Bunce *et al.* 2009), there is a strong trend (R^2 0.5305) towards increasing altitude of specimens with decreasing radiocarbon age (Figure 5a).

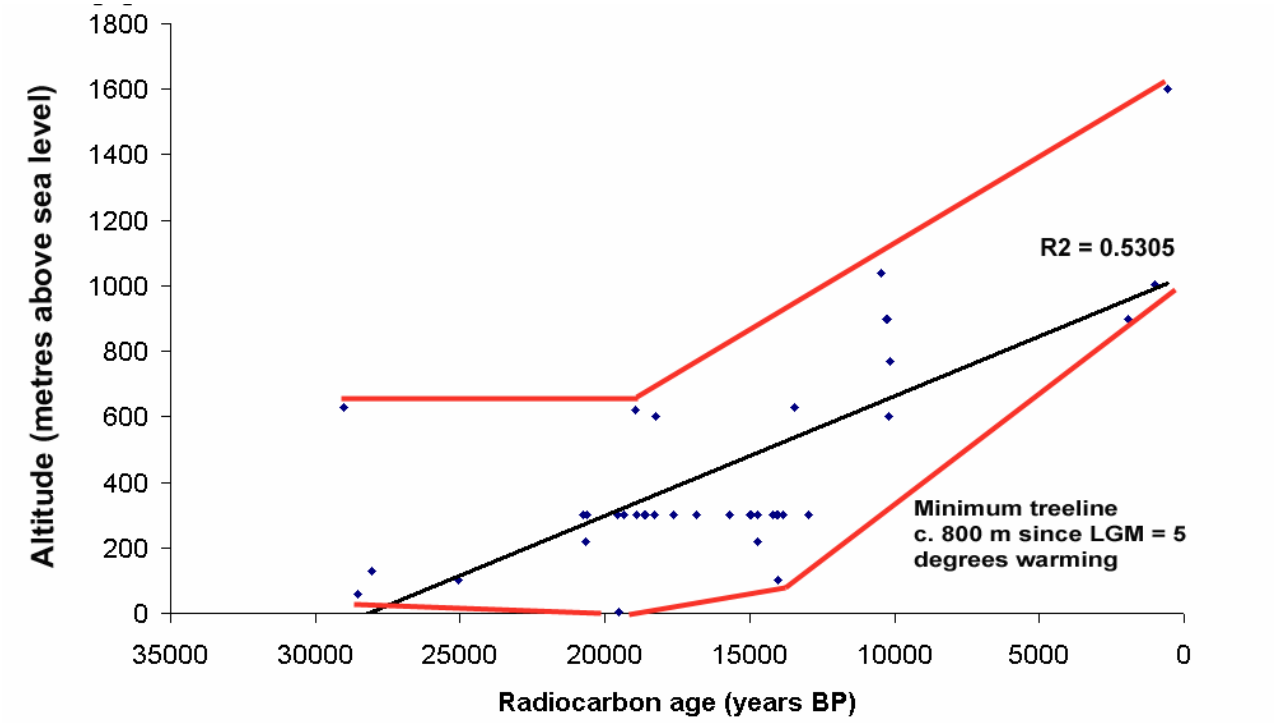

Figure 5 A: Positive relationship (R^2: 0.5305) of radiocarbon age of crested moa (*Pachyornis australis*) fossils with increasing altitude and migration of the sub-alpine zone up-slope since the LGM. The graph indicates that Holocene (0-10 Kya) specimens were found at higher altitudes than Late Glacial (10-14 Kya) specimens. In turn Late Glacial specimens are found at higher altitudes than Otiran Glacial (70-14 Kya) specimens. Red lines represent the upper and lower extent of the sub-alpine zone derived from McGlone et al. (2010).

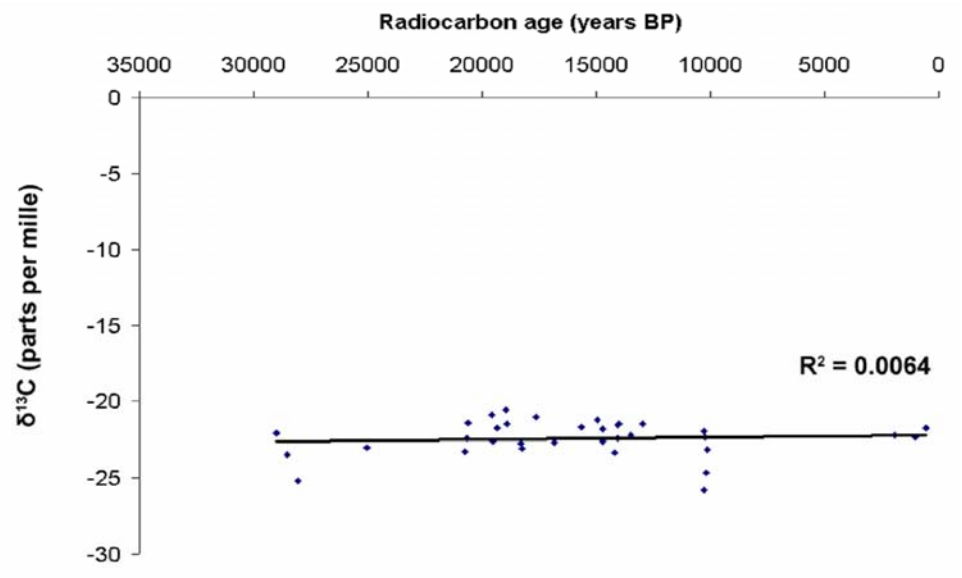

Figure 5B: Relationship of $\delta^{13}C$ signature with radiocarbon age in crested moa (*Pachyornis australis*). The analysis indicates that there is no relationship (R^2: 0.0064).

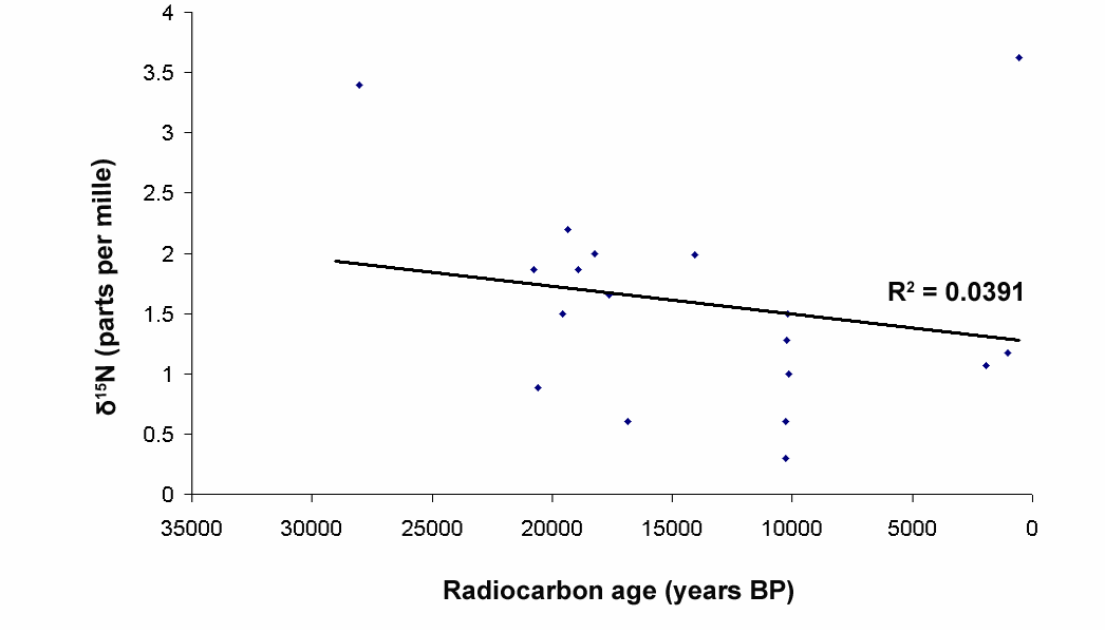

Figure 5C: Relationship of $\delta^{15}N$ signature with radiocarbon age in crested moa (*Pachyornis australis*). The analysis indicates that there is no relationship (R^2: 0.0391).

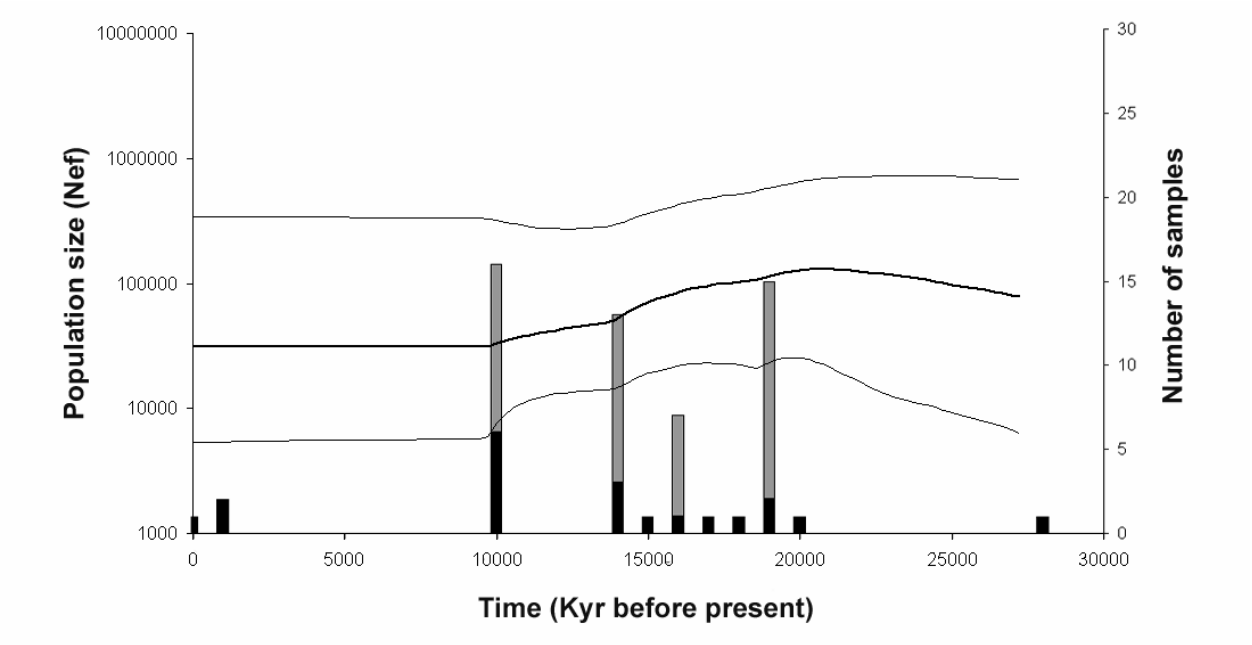

Figure 6 Bayesian skyline plot (BSP) of crested moa (*Pachyornis australis*). The black line represents the median BSP, while the grey lines represent the 95% credible intervals. Each column represents the number of radiocarbon dated sequences: black, number of finite radiocarbon dates; grey, number of undated sequences with mean stratigraphic ages assigned from well dated fossil deposits.

Specimens from the LGM (29-29 Kya) are widely distributed below 600 m asl, while Last Glacial (18-14 Kya) specimens show the same altitudinal range but are concentrated around 300 m asl. Late Glacial (14-10 Kya) specimens are restricted to 600-1000 m asl, while Holocene (10-0 Kya) specimens are restricted to 800-2000 m asl. There is no trend in the $\delta^{13}C$ (Figure 5b) and $\delta^{15}N$ (Figure 5c) signatures relative to radiocarbon age ($\delta^{13}C$ R^2 0.0064, $\delta^{15}N$ R^2 0.0391).

Population demographic analysis

The palaeodemographic history inferred from the BSP suggests that crested moa increased in population size from 28-20 Kya, followed by a slow decline from 20-10 Kya and then held a relatively constant population size until the Late Holocene (Figure 6). However, due to the wide credible intervals a constant population through time size cannot be rejected. The analysis of "transitions only" and "all substitutions" to assess the effects of sequence error on the BSP (Rambaut *et al.* 2009), did not alter the results (data not shown).

Table 1 Statistical performance of crested moa (*Pachyornis australis*) palaeodemographic models in BayeSSC analysis. The time-bins analysed are as follows: OG: Otiran Glacial (70-14 Kya); LG: Late Glacial (14-10 Kya); H: Holocene (10-0 Kya). ↓ represents a decline, - represents constant population size. Grey shaded models are not statistically significant from each another.

Model	Nef (mode)	Decline factor (mode)	AIC
4: OG ↓ LG ↓ H (3 priors)	700	1.25, 1.35	68.19
2: OG-LG ↓ H (2 priors)	1000	1.3	65.63
3: OG ↓ LG-H (2 priors)	1250	1.3	62.50
1: OG-LG-H constant (1 prior)	2250	N/A	57.56
2: OG-LG ↓ H (1 prior)	1550	1.5	56.54
3: OG ↓ LG-H (1 prior)	1380	2	56.19
4: OG ↓ LG ↓ H (1 prior)	800	2, 1.5	55.85

BayeSSC analysis suggests the most likely model of population demography is a decline in population size from the Otiran Glacial to the Late Glacial by a factor of two and from the Late Glacial to the Holocene by a factor of 1.5 (Table 1). However, the BayeSSC results are not statistically significant (Burnham and Anderson 2002; Ramakrishnan *et al.* 2009), because all four models (Figure 3), including constant population size, are within two AIC values of each

other (Table 1). This means that constant population size, or population declines from the Otiran Glacial to the Late Glacial, Late Glacial to the Holocene or both are equally likely.

Discussion

Did crested moa go extinct at the end of the Pleistocene due to climate and habitat change?

The radiocarbon dating carried out in this study (Table S2) independently supports the hypothesis that crested moa did not go extinct at the end of the Pleistocene with declining habitat but survived until the Late Holocene and the arrival of Polynesians in New Zealand about 1280 AD (Wilmshurst *et al.* 2008). Dating of further crested moa remains found in high altitude sub-alpine shrubland and grassland above 800 m asl will no doubt result in more verified Holocene remains (Worthy 1989b; authors' pers. obs. 2010).

Did crested moa track their migrating and declining habitat due to warming climate?

The temporal phylogeographic analyses (Figure 4) and radiocarbon dates (Figure 5a) suggest that crested moa showed a distribution that followed altitudinal changes through time, rather than phylogeographic changes. In addition, the lack of phylogeographic structuring, and the shared haplotypes between the Otiran Glacial (70-14 Kya), Late Glacial (14-10 Kya) and Holocene (10-0 Kya) suggests that the crested moa population was relatively continuous through time.

The radiocarbon dating analysis (Figure 5a) suggests that during the LGM (29-19 Kya) and Last Glacial (18-14 Kya) crested moa were restricted to approximately 0-600 m asl, which was up to the edge of permanent snow and ice (Worthy 1997). McGlone *et al.* (2010) have argued that a fall of 5ºC equates to a lowering of the vegetation zones in the South Island by 800 m. The resulting treeline during the LGM is comparable to the lower altitudinal limit of the crested moa distribution during the LGM and Last Glacial. In contrast, during the Late Glacial, crested moa were restricted to between approximately 600-1000 m asl. Habitat below 600 m asl was changing to shrubland and forest (Moar and Suggate 1973, 1979; Worthy 1993, 1994), which is likely to have potentially excluded crested moa. Areas above 1000 m asl still retained permanent snow and ice cover (Worthy 1997). By the Late Holocene, crested moa were restricted to approximately 800-2000 m asl, with forest widespread below 1200 m asl, potentially restricting crested moa to areas of open grassland/shrubland above this altitude (Worthy 1997). Below 1200 asl, there is complete faunal turnover in fossil assemblages from the Late Glacial to the Holocene in the study area, with crested moa and heavy-footed moa (*Pachyornis elephantopus*) replaced by the

cosmopolitan South Island giant moa (*Dinornis robustus*) and forest adapted little-bush moa (*Anomalopteryx didiformis*; Worthy 1993, 1994).

The analysis of radiocarbon dates (Figure 5a) suggests that crested moa appear to have tracked their sub-alpine habitat as it migrated up-slope after the LGM (Moar and Suggate 1973, 1979; Wardle 1991; Worthy and Holdaway 1995, 1996). The lack of an obvious trend in the $\delta^{13}C$ and $\delta^{15}N$ signatures (Figure 5b, c) supports this hypothesis and suggests that crested moa have inhabited an altitudinal zone with similar habitat and environmental conditions since the LGM. This is in contrast to $\delta^{13}C$ data from upland moa (*Megalapteryx didinus*). Worthy and Holdaway (2002) published $\delta^{13}C$ signatures from upland moa bones excavated from deposits at Honeycomb Hill (300 m asl, N=five) and Te Ana Titi (100 m asl, N=four) within the study area, ranging in age from 19-4 Kya. The $\delta^{13}C$ signatures show a significant decline about 12 Kya from -20.5‰ to -30.1‰. While the sample size is small, the trend towards more negative $\delta^{13}C$ values is seen in two different fossil deposits, arguing against a local phenomenon. The negative trend probably reflects (i) a change in dietary preferences, a form of adaptation to environmental change, given that geographical variation in moa diet is greater than interspecific variation in diet (Wood *et al.* 2008), and (ii) the return of forest to areas below 1200 m asl from 14-10 Kya (Moar and Suggate 1973, 1979; Wardle 1991; Worthy and Holdaway 1995, 1996).

Did crested moa decline in population size due to migrating and declining habitat?

Since crested moa appear to have tracked changes in the availability of habitat since the LGM, interesting questions arise as to the effects on population demography. The palaeodemographic history inferred from the BSP (Figure 6) suggests crested moa increased in population size from 28-20 Kya which corresponds to the expanding sub-alpine habitat during the LGM, but decreased in population size from 20-10 Kya, despite the sub-alpine habitat being widespread at low altitudes until 15-12 Kya (Moar and Suggate 1973, 1979; Vandergoes and Fitzimons 2003). The BSP analysis also suggests that crested moa had a constant population size during the Holocene despite further contractions of sub-alpine habitat during the early Holocene (Figure 1, 6). However, due to the wide credible intervals in the BSP analysis, a constant population size from 28-0 Kya cannot be rejected and therefore the results should be treated with caution. It is important to note however, that while the results of the BayeSSC analysis was not statistically significant (Table 1; Burnham and Anderson 2002; Ramakrishnan *et al.* 2009), the BayeSSC

analysis supports the palaeodemographic history of crested moa inferred from the BSP, suggesting that there is a signal for population decline from the Otiran Glacial to the Holocene.

The small loss of genetic diversity in crested moa, reflected in the inferred palaeodemographic history (Figure 6, Table 1), supports Hewitt (1999) assertion that slower contractions and expansions in mountainous areas result in larger amounts of retained genetic diversity. This is in contrast to rapid expansions out of refugia that produce large areas of low genetic diversity. The BSP and BayeSSC results also refute the hypothesis of Gemmell *et al.* (2004) that moa populations were in decline during the Late Holocene prior to Polynesian colonisation due to widespread volcanism and increased mortality from introduced avian diseases. In crested moa, there is no evidence for declining populations during the Late Holocene.

The results from the palaeodemographic analyses suggest climate and habitat change did cause a small decline in population size from the Otiran Glacial to the Holocene but did not cause the ultimate extinction of crested moa. Crested moa appear to have had a constant population size throughout the Holocene, including when Polynesians colonised New Zealand in about 1280 AD (Wilmshurst *et al.* 2008) at a time of relative climate stability (Burrows and Greenland 1979). Though no verifiable crested moa remains have been found in early Maori middens (Tennyson and Martinson 2006) it is highly probable that crested moa were hunted to extinction as the youngest natural crested moa remains postdate Polynesian colonisation. In addition, there is overwhelming palaeoenvironmental (Wilmshurst and Higham 2004, Wilmshurst *et al.* 2008) and archaeological (Anderson 1989) evidence indicating that human overkill and habitat destruction by early Maori was responsible for the extinction of the moa. Given the results and conclusions of this study and similar patterns in other moa species (based on fossil distributions through time; see Worthy and Holdaway 2002) it appears probable that (i) moa did not fluctuate greatly in population size in relation to climate and habitat change; and (ii) moa populations were stable when Polynesians colonised New Zealand.

The habitat-tracking hypothesis revisited

The results and conclusions from this study suggest that crested moa were a cold adapted species, because the range of crested moa declined during the present interglacial (compared to warm adapted species where ranges expand). This means that comparisons between crested moa and other cold adapted or Arctic species can be made. First, the apparent failure of some Arctic and

cold adapted species to track declining habitat with warming climate may not be a general phenomenon as advocated by Dalen *et al*. (2007). Crested moa did not go extinct from declines in habitat and warming temperatures, in contrast to some highly mobile Arctic fox populations (Dalen *et al.* 2007).

Second, the ability of taxa to track changes in habitat availability by shifting their distribution accordingly may be complicated by complex secondary interactions like interspecific competition, geographic barriers, predator-prey relationships or behavioral constraints. For example, Bennett *et al.* (1991), investigated how European tree species responded to climate change during the Late Quaternary, and proposed "*that interspecific competition in southern European forests during warm stages may be at least as important as climatic extremes in cold stages for Quaternary tree extinctions*". This concept can be extended to animal taxa where interspecies competition for dietary resources could affect the ability of taxa to respond to changing environmental conditions and habitat availability. Numerous studies have shown that in North America about 12.5 Kya, there was a significant vegetation succession from grassland characteristic of the mammoth steppe to unpalatable shrubland and tundra (Anderson *et al.* 1991; Lozhkin *et al.* 1993; Bigelow and Powers 2001; Guthrie 2006; van Geel *et al.* 2007, 2008; Kienast *et al.* 2008). This resulted in a change in the dominant herbivore assemblage from mammoths and *Equus* horses, to bison (McDonald 1981; Guthrie 2006). The dietary preferences of mammoths, *Equus* horses and bison overlapped significantly (Akersten *et al*. 1998; Coltrain *et al*. 2004; Feranec *et al.* 2009) but there were major differences in how these species digested food and behaved (Guthrie 2006; Scott 2009). Mammoths and *Equus* horses use a caecum, or hindgut fermentation, allowing them to digest large amounts of low quality food with little nutritional gain. In comparison, bison are ruminants and use foregut fermentation, allowing them to obtain the maximum nutritional value from low quality food (Guthrie 2006; Scott 2009). This lead Scott (2009) to hypothesise that the extinction of mammoths and *Equus* horses was due to interspecies competition with bison for dietary resources. Bison are highly aggressive and territorial (Scott 2009) and may have prevented mammoths and *Equus* horses from responding to changes in habitat availability.

Geographical barriers can also influence the ability of populations to track changes in habitat, regardless of the populations' mobility. Hewitt (1999) reviewed the literature concerning the post glacial colonisation of Europe by taxa after the last glacial period. The study showed that in about

72% of cases, the Alps were a significant geographic barrier that blocked the expansion of taxa from Italian refugia into Europe, resulting in northern Europe being re-colonised from refugia in Western and Eastern Europe.

Finally, over-hunting by humans, if present, could also potentially affect the ability of populations to respond to changes in habitat availability. Nogues-Bravo *et al.* (2008) used radiocarbon, palaeodistribution and palaeoclimatic data to model the woolly mammoth climate envelope at 42, 30 and 21 Kya and projected the climate envelope onto 126 and 6 Kya time slices. This study showed that the modeled geographical range of woolly mammoth contracted by 89% between 42 and 6 Kya, probably causing a decline in population size that made mammoths vulnerable to over-hunting. Nogues-Bravo *et al.* (2008) hypothesised that in the absence of humans, mammoth populations may have survived in areas of suitable habitat in Eurasia.

The results and conclusions presented in this study, on the ability of crested moa to respond to changes in habitat availability, suggests that the habitat tracking hypothesis (Eldredge 1989) may be more applicable to situations where complex secondary interactions are minimised or absent. In addition, the presence of complex secondary interactions may be at least as important as the failure of taxa to track changes in habitat availability in explaining the population declines and extinctions of that characterized the Late Pleistocene ecosystem.

Acknowledgments

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References

Akersten WA, Foppe TM, Jefferson GT 1998. New source of dietary data for extinct herbivores. Quaternary Research 30: 92-97.

Amundson R, Austin AT, Schuur EAG, Yoo K, Matzek V, Kendall C, Uebersax A, Brenner D, Baisden WT 2003. Global patterns of the isotopic composition of soil and plant nitrogen. Global Biogeochemical Cycles 17: 1031-1040.

Anderson A 1989. Prodigious birds. Cambridge University Press, Cambridge, UK.

Anderson CNK, Ramakrishnan U, Chan YL, Hadly EA 2006. Serial SimCoal: A population genetics model for data from multiple populations and points in time. Bioinformatics 21: 1733-1734.

Anderson PM, Bartlein PJ, Brubaker LB, Gajewski K, Ritchie JC 1991. Vegetation-pollen-climate relationships for arcto-boreal region of North America and Greenland. Journal of Biogeography 18: 565-582.

Baker AJ, Huynen LJ, Haddrath O, Miller CD, Lambert DM 2005. Reconstructing the tempo and mode of evolution in an extinct clade of birds with ancient DNA: The giant moas of New Zealand. Proceedings of the National Academy of Science USA 102: 8257-8262.

Barnes IB, Shapiro B, Lister A, Kuznetsova T, Sher A, Guthrie D, Thomas MG 2007. Genetic structure and extinction of the woolly mammoth, *Mammuthus primigenius*. Current Biology 17: 1072-1075.

Barrell DJA, Alloway BV, Shulmeister J, Newnham RM (Eds) 2005. Towards a climate event stratigraphy for New Zealand over the past 30,000 years. Institute of Geological and Nuclear Sciences science report 2005/07, 12p. and poster.

Beaumont M, Zhang W, Balding D 2002. Approximate Bayesian computation in population genetics. Genetics 162: 2025.

Bennett KD, Tzedakis PC, Willis KJ 1991. Quaternary refugia of north European trees. Journal of Biogeography 18: 103-115.

Bigelow NH and Powers WR 2001. Climate, vegetation, and archaeology 14,000-9,000 cal yr B.P. in Central Alaska. Arctic Anthropology 38: 171-195.

Bourdon E, Castanet J, Ricqles A, Scofield P, Tennyson A, Lamrous H, Cubo J 2010. Bone growth marks reveal protracted growth in New Zealand kiwi (Aves, Apterygidae). Biology Letters 5: 639-642.

Bronk Ramsey C, Higham TFG, Bowles A, Hedges R 2004a. Improvements to the pretreatment of bone at Oxford. Radiocarbon 46: 155-163.

Bronk Ramsey C, Higham TFG, Leach P 2004b. Towards high precision AMS: progress and limitations. Radiocarbon 46: 7-24.

Bunce M, Worthy TH, Phillips MJ, Holdaway RN, Willerslev E, Haile J, Shapiro B, Scofield RP, Drummond A, Kaamp PJJ, Cooper A 2009. The evolutionary history of the extinct ratite moa and New Zealand Neogene paleogeography. Proceedings of the National Academy of Science USA 106: 20646-20651.

Burge PI, Schulmeister J 2007. An MIS 5a to MIS 4 (or early MIS 3) environmental and climatic reconstruction from the northwest South Island, New Zealand, using beetle fossils. Journal of Quaternary Science 22: 501-516.

Burnham KP, Anderson DR 2002. Model selection and inference: a practical information – theoretic approach (2nd ed). Springer-Verlag, New York.

Burrows CJ, Greenland DE 1979. An analysis of the evidence for climate change in New Zealand in the last thousand years: evidence from diverse natural phenomena and from instrumental records. Journal of the Royal Society of New Zealand 9: 321-373.

Campbell H, Hutching G 2007. In search of ancient New Zealand. Penguin Group, New Zealand.

Campos PF, Willerslev E, Sher A, Orlando L, Axelsson E, Tikhonov A, Aaris-Sorensen K, Greenwood AD, Kahlke RD, Kosintsev P, Krakhmalnaya T, Kuznetsova T, Lemey P, MacPhee R, Norris CA, Shepherd K, Suchard MA, Zazula GD, Shapiro B, Gilbert MTP 2010. Ancient DNA analyses exclude humans as the driving force behind late Pleistocene musk ox (*Ovibos moschatus*) population dynamics. Proceedings of the National Academy of Science USA 107: 5675-5680.

Checklist Committee OSNZ 2010. Checklist of the birds of New Zealand, Norfolk and Macquarie Islands, and the Ross Dependency, Antarctica (4th ed.) Wellington, Ornithological Society of New Zealand and Te Papa Press.

Clement M, Posada D, Crandall KA 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9: 1657-1659.

Coltrain JB, Harris JM, Cerling TE, Ehleringer JR, Dearing MD, Ward J, Allen J 2004. Rancho La Brea stable isotope biogeochemistry and its implications for the palaeoecology of late Pleistocene, coastal southern California. Palaeogeography, Palaeoclimatology, Palaeoecology 205: 199-219.

Coope GR 1979. Late Cenozoic Coleoptera: Evolution, Biogeography, and Ecology. Annual Review of Ecology and Systematics 10: 247-267.

Cooper A, Fox-Lalueza C, Anderson A, Rambaut A, Austin J, Ward R 2001. Complete mitochondrial genome sequences of two extinct moas clarify ratite evolution. Nature 409: 704–707.

Cooper A, Poinar HN 2000. Ancient DNA: do it right or not at all. Science 289: 1139.

Dalen L, Fuglei E, Hersteinsson P, Kapel CMO, Roth JD, Samelius G, Tannerfeldt M, Angerbjorn 2005. Population history and genetic structure of a circumpolar species: The arctic fox. Biological Journal of the Linnaean Society 84: 79-89.

Dalen L, Nystrom V, Valdiosera C, Germonpre M, Sablin M, Turner E, Angerbjorn A, Arsuaga SL, Gotherstrom A 2007. Ancient DNA reveals lack of postglacial habitat tracking in the arctic fox. Proceedings of the National Academy of Science USA 104: 6726-6729.

Debruyne R, Chu G, King CE, Bos K, Kuch M, Schwarz C, Szpak P, Grocke DR, Matheus P, Zazula G, Guthrie D, Froese D, Buigues B, Marliave C, Flemming C, Poinar D, Fisher D, Southon J, Tikhonov AN, MacPhee RDE, Poinar HN 2008. Out of America: Ancient DNA evidence for a New World origin of Late Quaternary woolly mammoths. Current Biology 18: 1320-1326.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7: 214.

Drummond AJ, Rambaut A, Shapiro B, Pybus OG 2005. Bayesian coalescent inference of past population dynamics from molecular sequences. Molecular Biology and Evolution 22: 1185-1192.

Dynesius M, Jansson R 2000. Evolutionary consequences of changes in species' geographical distributions driven by Milankovitch climate oscillations. Proceedings of the National Academy of Science USA 97: 9115-9120.

Eldredge N 1989. Macroevolutionary Dynamics: Species, Niches and Adaptive Peaks. MacGraw Hill, New York, USA.

Eldredge N 1995. Reinventing Darwin: the great evolutionary debate. Wiley Interscience, New York.

Excoffier L, Novembre J, Schneider S 2000. SIMCOAL: a general coalescent program for simulation of molecular data in interconnected populations with arbitrary demography. Journal of Heredity 91: 506-509.

Feranec RS, Hadly EA, Paytan 2009. Stable isotopes reveal seasonal competition for resources between late Pleistocene bison (*Bison*) and horse (*Equus*) from Rancho La Brea, Southern California. Palaeogeography, Palaeoclimatology, Palaeoecology 271: 153-160.

van Geel B, Aptroot A, Baittinger C, Birks HH, Bull ID, Cross HB, Evershed RP, Gravendeel B, Kompanje EJO, Kuperus P, Mol D, Nierop KGJ, Pals JP, Tikhonov AN, Reenen G, Tienderen PH 2008. The ecological implications of a Yukutian mammoth's last meal. Quaternary Research 69: 361-376.

van Geel B, Zazula GD, Schwegerc CE 2007. Spores of coprophilous fungi from under the Dawson tephra (25,300 14C years BP), Yukon Territory, northwestern Canada. Palaeogeography, Palaeoclimatology, Palaeoecology 252: 481-485.

Gemmell NJ, Schwartz MK, Robertson BC 2004. Moa were many. Proceedings of the Royal Society of London Series B 271: S430-432.

Gould SJ, Eldredge N 1977. Punctuated equilibria: the tempo and mode of evolution reconsidered. Palaeobiology 3: 115-151.

Graham RW, Lundelies EL, Graham MA, Schroeder EK, Toomey RS, Anderson E, Barnosky AD, Burns JA, Churcher CS, Grayson DK, Guthrie D, Harrington CR, Jefferson GT, Martin LD, McDonald G, Morlan RE, Semken HA, Webb D, Werdelin L, Wilson MC 1996. Spatial response of mammals to late Quaternary environmental fluctuations. Science 272: 1601-06.

Guthrie RD 2006. New carbon dates link climatic change with human colonisation and Pleistocene extinctions. Nature 441: 207-209.

Hellstrom J, McCulloch M, Stone J 1998. A detailed 31,000-year record of climate and vegetation change, from the isotope geochemistry of two New Zealand speleothems. Quaternary Research 50: 167-178.

Hewitt GM 1999. Post-glacial re-colonisation of European biota. Biological Journal of the Linnaean Society 68: 87-112.

Huntley B, Birks JHB 1983. An Atlas of Past and Present Pollen Maps for Europe: 0-13,000 Years Ago. Cambridge University Press, Cambridge, UK.

Jacobi RM, Higham TFG, Bronk Ramsey CB 2006. AMS radiocarbon dating of Middle and Upper Palaeolithic bone in the British Isles: Improved reliability using ultrafiltration. Journal of Quaternary Science 21: 557-573.

Kienast F, Tarasov P, Schirrmeister L, Grosse G, Andreev AA 2008. Continental climate in the east Siberian Arctic during the last interglacial: implications from palaeobotanical records. Global Planetary Change 60: 535-562.

Kumar S, Tamura K, Nei M 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Briefings in Bioinformatics 5: 150-163.

Lister AM, Stuart AJ 2008. The impact of climate change on large mammal distribution and extinction: Evidence from the last glacial/interglacial transition. C. R. Geoscience 340: 615-620.

Lozhkin AV, Anderson PM, Eisner WR, Ravako LG, Hopkins DM, Brubaker LB, Colinvaux PA, Miller MC 1993. Late Quaternary lacustrine pollen records from southwestern Beringia. Quaternary Research 39: 314-324.

McDonald JN 1981. North American bison, their classification and evolution. University of California Press, Berkeley, USA.

McGlone MS, Newnham RM, Moar NT 2010. The vegetation cover of New Zealand during the Last Glacial Maximum: Do pollen records under-represent woody vegetation? In: Haberle SG, Stevenson J, Prebble M (Eds). Altered Ecologies: Fire, Climate and Human Influence on Terrestrial Landscapes. Terra Australis 32. ANU E-Press, Australian National University, Canberra, Australia.

McKinnon M 1997. New Zealand Historical Atlas. Bateman, Auckland, New Zealand.

Moar NT, Suggate RP 1973. Pollen analysis of Late Otiran and Aranuian sediments at Blue Spur Road (S51), North Westland. New Zealand Journal of Geology and Geophysics 16: 333-344.

Moar NT, Suggate RP 1979. Contributions to the Quaternary history of New Zealand flora 8: Interglacial and glacial vegetation in the Westport District South Island. New Zealand Journal of Botany 17: 361-87.

Newnham RM, Lowe DJ, Williams PW 1999. Quaternary environmental change in New Zealand: A review. Progress in Physical Geography 23: 567-610.

Newnham RM, Vandergoes MJ, Hendy CH, Lowe DJ, Preusser F 2007. A terrestrial palynological record for the last two glacial cycles from southwestern New Zealand. Quaternary Science Reviews 26: 517-535.

Nogues-Bravo D, Rodriguez J, Hortal J, Batra P, Araujo MB 2008. Climate change, humans, and the extinction of the woolly mammoth. PLoS Biology 6: e79.

Oliver WRB 1949. The moas of New Zealand and Australia. Dominion Museum, Wellington, New Zealand.

Pounds JA, Fogden MPL, Campbell JH 1999. Biological responses to climate change on a tropical mountain. Nature 398: 611-615.

Prost S, Smirnov N, Fedorov VB, Sommer RS, Stiller M, Nagel D, Knapp M, Hofreiter M 2010. Influence of climate warming on Arctic mammals? New insights from ancient DNA studies of the Collared Lemming *Dicrostonyx torquatus*. PLoS One 5: e10447.

Provan J, Bennett KD 2008. Phylogeographic insights into cryptic glacial refugia. Trends in Ecology and Evolution 23: 564-571.

Ramakrishnan U, Hadly EA 2009. Using phylochronology to reveal population histories: Review and synthesis of 29 ancient DNA studies. Molecular Ecology 18: 1310-1330.

Rambaut A, Ho SYW, Drummond AJ, Shapiro B 2009. Accommodating the effect of ancient DNA damage of inferences of demographic histories. Molecular Biology and Evolution 26: 245-248.

Reimer PJ, Baillie MGL, Bard E, Bayliss A, Beck JW, Bertrand CJH, Blackwell PG, Buck CE, Burr GS, Cutler KB, Damon PE, Edwards RL, Fairbanks RG, Friedrich M, Guilderson TP, Hogg AG, Hughen KA, Kromer B, McCormac G, Manning S, Bronk Ramsey C, Reimer RW, Remmele S, Southon JR, Stuiver M, Talamo S, Taylor FW, van der Plicht J, Weyhenmeyer CE 2004. INTCAL04 terrestrial radiocarbon age calibration, 0-26 Cal Kya BP. Radiocarbon 46: 1029-1058.

Scott E. 2009. Extinctions, scenarios, and assumptions: Changes in latest Pleistocene large herbivore abundance and distribution in western North America. Quat. Int. 217: 225-239.

Stevens RE, Jacobi R, Street M, Germonpre M, Conard NJ, Munzel SC, Hedges REM 2008. Nitrogen isotope analysis of reindeer (*Rangifer tarandus*), 45,000 BP to 9,000 BP: Palaeoenvironmental reconstructions. Palaeogeography, Palaeoclimatology, Palaeoecology 262: 32-45.

Stewart JR, Dalen L 2005. Is the glacial refugium concept relevant for northern species? A comment on Pruett and Winker. Climate Change 86: 19-22.

Stewart JR, Lister AM 2001. Cryptic northern refugia and the origins of the modern biota. Trends in Ecology and Evolution 16: 608-613.

Still CJ, Foster PN, Schneider SH 1999. Simulating the effects of climate change on tropical montane cloud forests. Nature 398: 608-610.

Stuart AJ, Kosintsev PA, Higham TFG, Lister AM 2004. Pleistocene to Holocene extinction dynamics in giant deer and woolly mammoth. Nature 431: 684-689.

Swofford DL 1998. PAUP*: Phylogenetic analysis using parsimony (*and other methods). Version 4.0b2. Sinauer Associates, Sunderland, Massachuesetts, USA.

Taberlet P, Fumagalli L, Wust-Saucy AG, Cosson JF 1998. Comparative phylogeography and postglacial colonization routes in Europe. Molecular Ecology 7: 453-464.

Tennyson A, Martinson P 2006. Extinct birds of New Zealand. Te Papa Press, Wellington, New Zealand.

Thomas CD, Cameron A, Green RE, Bakkenes M, Beaumont LJ, Collingham TC, Erasmus BFN, Siqueira MF, Grainger A, Hannah L, Hughes L, Huntley B, Jaarsveld AS, Midgley GF, Miles L, Ortega-Huerta MA, Perterson AT, Phillips OL, Williams SE 2004. Extinction risk from climate change. Nature 427: 145-148.

Turvey ST, Green OR, Holdaway RN 2005. Cortical growth marks reveal extended juvenile development in New Zealand moa. Nature 435: 940-943.

Valdiosera CE, Garcia-Garitagoitia JL, Carcia N, Doadrio I, Thomas MG, Hanni C, Arsuaga JL, Barnes I, Hofreiter M, Orlando L, Gotherstrom A 2008. Surprising migration and population size dynamics in ancient Iberian brown bears (*Ursus arctos*). Proceedings of the National Academy of Science USA 105: 5123-5128.

Vandergoes MJ, Fitzimons SJ 2003. The Last Glacial-Interglacial Transition (LGIT) in south Westland, New Zealand: Palaeoecological insight into mid-latitude Southern Hemisphere climate change. Quaternary Science Reviews 22: 1461-1476.

Vandergoes MJ, Newnham RM, Hendy CH, Lovell TV, Preusser F, Fitzsimons SJ, Hogg, A, Kasper HU, Schluchter C 2005. Regional isolation forcing of Late Quaternary climate change in the Southern Hemisphere. Nature 436: 242-245.

Wardle P 1991. Vegetation of New Zealand. Cambridge University Press, Cambridge, UK.

Williams D, Dunkerley D, Deckter P, Kershaw P, Chappell J 1998. Quaternary Environments. Hodder Arnold.

Williams PW, King DNT, Zhao JX, Collerson KD 2005. Late Pleistocene to Holocene composite speleotherm ^{18}O and ^{13}C chronologies from South Island, New Zealand – did a global Younger Dryas really exist? Earth and Planetary Science Letters 230: 301-317.

Wilmshurst JM and Higham TFG 2004. Using rat gnawed seeds to independently date the arrival of Pacific rates and humans to New Zealand. The Holocene 14: 801-806.

Wilmshurst JM, Anderson AJ, Higham TFG, Worthy TH 2008. Dating the late prehistoric dispersal of Polynesians to New Zealand using the commensal Pacific rat. Proceedings of the National Academy of Science USA 105: 7676-7680.

Wood JR, Rawlence NJ, Rogers GM, Austin JJ, Worthy TH, Cooper A 2008. Coprolite deposits reveal the diet and ecology of the extinct New Zealand megaherbivore moa (Aves: Dinornithiformes). Quaternary Science Reviews 27: 2593-2602.

Worthy TH 1989a. Validation of *Pachyornis australis* Oliver (Aves: Dinornithiformes), a medium sized moa from the South Island New Zealand. New Zealand Journal of Geology and Geophysics 32: 255-266.

Worthy TH 1989b. Moa of the sub-alpine zone. Notornis 36: 191-196.

Worthy TH 1993. Fossils of Honeycomb Hill. Museum of New Zealand Te Papa Tongawera, Wellington, New Zealand.

Worthy TH 1994. Late Quaternary changes in the moa fauna on the West Coast of the South Island, New Zealand. Records of the South Australian Museum 27: 125-134.

Worthy TH 1997. Fossil deposits in the Hodge Creek Cave System, on the northern foothills of Mt Arthur, Nelson, South Island, New Zealand. Notornis 44: 111-124.

Worthy TH 1998a. Quaternary fossil faunas of Otago, South Island, New Zealand. Journal of the Royal Society of New Zealand 28: 421-521.

Worthy TH 1998b. The Quaternary fossil avifauna of Southland, South Island, New Zealand. Journal of the Royal Society of New Zealand 28: 537-589.

Worthy TH, Holdaway RN 1994. Quaternary fossil faunas from caves in Takaka valley and on Takaka Hill, northwest Nelson, South Island, New Zealand. Journal of the Royal Society of New Zealand 24: 297-391.

Worthy TH, Holdaway RN 1995. Quaternary fossil faunas from caves on Mt Cookson, North Canterbury, New Zealand. Journal of the Royal Society of New Zealand 25: 333-370.

Worthy TH, Holdaway RN 1996. Quaternary fossil faunas, overlapping taphonomies, and palaeofaunal reconstruction in North Canterbury, South Island, New Zealand. Journal of the Royal Society of New Zealand 26: 275-361.

Worthy TH, Holdaway RN 2002. The lost world of the moa: Prehistoric life of New Zealand. Canterbury University Press, Christchurch, New Zealand.

Worthy TH, Roscoe D 2003. Takaka Fossil Cave – a stratified Late Glacial to Late Holocene deposit from Takaka Hill, New Zealand. Tihinga 14: 41-60.

The extinct crested moa (*Pachyornis australis*) tracked its habitat with warming climate since the Last Glacial Maximum.

Supplementary Information

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Table S1 Information regarding crested moa (*Pachyornis australis*) samples used in this study. For the ^{14}C date category, 'radiocarbon' means the specimen has a finite radiocarbon date, 'stratigraphic' means the specimen is undated but has a mean stratigraphic date assigned from well dated fossil deposits. Grey bars indicate specimens that could potentially belong to multiple haplotypes or could not be placed into a discrete time bin and were excluded from the TCS, Arlequin and BayeSSC analysis.

Sample	Moa species (DNA)	Sample location	Museum number*	AMS ^{14}C date	AMS ^{14}C date category
GU139066	*P. australis*	Charleston	CM Av29445	14045 +/- 65	radiocarbon
DQ055487	*P. australis*	Charleston	CM Av29445	-	-
GU139064	*P. australis*	Gradungula Passage	In situ	18925 +/- 80	radiocarbon
A3781	*P. australis*	Gradungula Passage	NRS348, in situ	19575 +/- 80	radiocarbon
A3783	*P. australis*	Gradungula Passage	NRS350, in situ	20760 +/- 90	radiocarbon
A3784	*P. australis*	Gradungula Passage	NRS351, in situ	19753	stratigraphic
A3785	*P. australis*	Gradungula Passage	NRS352, in situ	19753	stratigraphic
A3726	*P. australis*	Moa Cave Extension	MNZ S25863.2	17645 +/- 60	radiocarbon
A3727	*P. australis*	Moa Cave Extension	MNZ S25863.1	19335 +/- 70	radiocarbon
A3722	*P. australis*	Graveyard	MNZ S23797	19007	stratigraphic
A3723	*P. australis*	Graveyard	MNZ S23820.1	19007	stratigraphic
A3724	*P. australis*	Graveyard	MNZ S23820.2	19007	stratigraphic
A3725	*P. australis*	Graveyard	MNZ S23822	19007	stratigraphic
A3735	*P. australis*	Graveyard	MNZ S23031.1	19007	stratigraphic
A3744	*P. australis*	Graveyard	MNZ S23810.1	19007	stratigraphic
A3745	*P. australis*	Graveyard	MNZ S23031.3	19007	stratigraphic
A3748	*P. australis*	Graveyard	MNZ S23810.2	19007	stratigraphic
A3790	*P. australis*	Graveyard	NRS357, in situ	19007	stratigraphic
A3793	*P. australis*	Graveyard	NRS360, in situ	19007	stratigraphic
A3794	*P. australis*	Graveyard	NRS361, in situ	19007	stratigraphic
A3795	*P. australis*	Graveyard	NRS362, in situ	19007	stratigraphic
A3796	*P. australis*	Graveyard	NRS363, in situ	19007	stratigraphic
A3742	*P. australis*	Wren Wrecker	MNZ S25655	16860 +/- 75	radiocarbon
A3798	*P. australis*	Moa Cave	NRS365, in situ	16248	stratigraphic
A3799	*P. australis*	Moa Cave	NRS366, in situ	16248	stratigraphic

A3802	*P. australis*	Moa Cave	NRS369, in situ	16248	stratigraphic
A3803	*P. australis*	Moa Cave	NRS370, in situ	16248	stratigraphic
A3805	*P. australis*	Moa Cave	NRS372, in situ	16248	stratigraphic
A3806	*P. australis*	Moa Cave	NRS373, in situ	16248	stratigraphic
A2555	*P. australis*	Cemetery	MNZ S25864	15000 +/- 200	radiocarbon
A2556	*P. australis*	Cemetery	MNZ S25868	14730 +/- 170	radiocarbon
A2557	*P. australis*	Cemetery	MNZ S25867	14950 +/- 150	radiocarbon
A3718	*P. australis*	Cemetery	MNZ S25869	14407	stratigraphic
A3738	*P. australis*	Cemetery	MNZ S25871	14407	stratigraphic
A3772	*P. australis*	Cemetery	NRS339, in situ	14407	stratigraphic
A3773	*P. australis*	Cemetery	NRS340, in situ	14407	stratigraphic
A3774	*P. australis*	Cemetery	NRS341, in situ	14407	stratigraphic
A3775	*P. australis*	Cemetery	NRS342, in situ	14407	stratigraphic
A3776	*P. australis*	Cemetery	NRS343, in situ	14407	stratigraphic
A3777	*P. australis*	Cemetery	NRS344, in situ	14407	stratigraphic
A3778	*P. australis*	Cemetery	NRS345, in situ	14407	stratigraphic
A3779	*P. australis*	Cemetery	NRS346, in situ	14407	stratigraphic
A3810	*P. australis*	Megamania	CM Av un-registered	-	-
DQ055487	*P. australis*	Inangahua	CM Av28275	-	-
A3739	*P. australis*	Irvines Tomo	MNZ S27881	28520 +/- 20	radiocarbon
A3740	*P. australis*	Commentary Cave	MNZ S35298.1	28050 +/- 300	radiocarbon
A3713	*P. australis*	Takaka Hill	WCM WO90.47	10210 +/- 45	radiocarbon
AY299914	*P. australis*	Takaka	CM Av21331	-	-
GU139065	*P. australis*	Bone Cave	CM Av21331	10165 +/- 50	radiocarbon
A2594	*P. australis*	Magnesite Quarry	NM un-registered	1021 +/- 26	radiocarbon
GU139067	*P. australis*	Moa Trap Cave	MNZ S33754	10450 +/- 45	radiocarbon
A3757	*P. australis*	Moa Arch	NRS324, in situ	10280 +/- 45	radiocarbon
A3758	*P. australis*	Moa Arch	NRS325, in situ	10260	stratigraphic
A3759	*P. australis*	Moa Arch	NRS326, in situ	10260	stratigraphic
A3760	*P. australis*	Moa Arch	NRS327, in situ	10260	stratigraphic
A3761	*P. australis*	Moa Arch	NRS328, in situ	10265 +/- 45	radiocarbon
A3762	*P. australis*	Moa Arch	NRS329, in situ	10260	stratigraphic
A3763	*P. australis*	Moa Arch	NRS330, in situ	10260	stratigraphic
A3764	*P. australis*	Moa Arch	NRS331, in situ	10260	stratigraphic

A3765	*P. australis*	Moa Arch	NRS332, in situ	10260	stratigraphic
A3766	*P. australis*	Moa Arch	NRS333, in situ	10235 +/- 45	radiocarbon
A3768	*P. australis*	Moa Arch	NRS335, in situ	10260	stratigraphic
A3769	*P. australis*	Moa Arch	NRS336, in situ	10260	stratigraphic
A3770	*P. australis*	Moa Arch	NRS337, in situ	10260	stratigraphic
GU139063	*P. australis*	Cheops	MNZ S41344	1928 +/- 27	radiocarbon
DQ023685	*P. australis*	Mount Arthur Tablelands	AIM B6221	-	-
A3736	*P. australis*	Bulmer Cave	MNZ S23569	564 +/- 26	radiocarbon
A2598	*P. australis*	Nelson District	NM un-registered	-	-

* NM: Nelson Museum; MNZ: Museum of New Zealand Te Papa Tongawera; NRS: Nic Rawlence sample, left *in situ* with permanent tag attached to the bone with sampling details; CM: Canterbury Museum; WCM: Waitomo Caves Museum.

Table S2 Radiocarbon dates and isotope data generated in this study. Shaded grey rows are previously published radiocarbon dates.

Sample	Moa species (DNA)	Sample location	Museum no.	Lab no.	^{14}C date	C:N ratio	δ^{15} N	δ^{13}C
GU139066	*P. australis*	Charleston	CM Av29445	OxA-12431	14045 +/- 65*	3.322	1.985	-22.415
GU139064	*P. australis*	Gradungula Passage	In situ	OxA-12435	18925 +/- 80*	3.38	1.863	-21.471
A3781	*P. australis*	Gradungula Passage	NRS348, in situ	OxA-20284	19575 +/- 80	3.31984	1.50	-20.92
A3783	*P. australis*	Gradungula Passage	NRS350, in situ	OxA-20285	20760 +/- 90	3.28455	0.88	-21.44
A3726	*P. australis*	Moa Cave Extension	S25863.2	OxA-20366	17645 +/- 60	3.3703	1.65	-21.03
A3727	*P. australis*	Moa Cave Extension	MNZ S25863.1	OxA-20367	19335 +/- 70	3.21983	2.20	-21.73
A3742	*P. australis*	Wren Wrecker	MNZ S25655	OxA-20286	16860 +/- 75	3.38665	0.60	-22.66
A2555	*P. australis*	Cemetery	MNZ S25864	NZA-7646	15000 +/- 200#	n/a	n/a	n/a
A2556	*P. australis*	Cemetery	MNZ S25868	ANU-1611	14730 +/- 170	n/a	n/a	-21.8
A2557	*P. australis*	Cemetery	MNZ S25867	ANU-1612	14950 +/- 150	n/a	n/a	-21.2
A3739	*P. australis*	Irvines Tomo	MNZ S27881	NZA-3049	28520 +/- 20^	n/a	n/a	-23.5
A3740	*P. australis*	Commentary Cave	MNZ S35298.1	OxA-20294	28050 +/- 300	3.30909	3.40	-25.19
A2597	*P. australis*	Takaka Hill	NM unregistered	OxA-20290	18235 +/- 80	3.38795	2.00	-23.09
A3713	*P. australis*	Takaka Hill	WCM WO90.47	OxA-20291	10210 +/- 45	3.33134	-1.50	-24.69
GU139065	*P. australis*	Bone Cave	CM Av21331	OxA-12430	10165 +/- 50*	3.346	1.001	-23.174
A2594	*P. australis*	Magnecite Quarry	NM unregistered	OxA-20289	1021 +/- 26	3.23975	1.17	-22.23
GU139067	*P. australis*	Moa Trap Cave	MNZ S33754	OxA-12669	10450 +/- 45*	3.1	-0.8	-22.5
A3757	*P. australis*	Moa Arch	NRS324, in situ	OxA-20295	10280 +/- 45	3.27772	0.60	-25.75
A3761	*P. australis*	Moa Arch	NRS328, in situ	OxA-20296	10265 +/- 45	3.23137	0.30	-21.97
A3766	*P. australis*	Moa Arch	NRS333, in situ	OxA-20297	10235 +/- 45	3.32588	1.28	-22.32
AC923	*P. australis*	Cheops	MNZ S41344	OxA-20288	1928 +/- 27	3.25158	1.07	-22.24
A3736	*P. australis*	Bulmer Cave	MNZ S23569	OxA-20287	564 +/- 26	3.27223	3.62	-21.73

* Radiocarbon dates published in Bunce et al. (2009)

Radiocarbon date published in Worthy (1993)

^ Radiocarbon date published by Worthy and Holdaway (1994).

Table S3 Summary population genetic statistics for crested moa (*Pachyornis australis*) calculated in Arlequin.

	Sequences	**Length (bp)**	**Haplotypes**	**Polymorphic sites**	**Pairwise difference**
Holocene	3	497	3	3	2.00
Late Glacial	14	497	6	12	2.69
Otiran Glacial	26	497	12	16	3.60

CHAPTER SEVEN

The effect of Late Quaternary climate change on heavy-footed moa (*Pachyornis elephantopus*)

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STATEMENT OF AUTHORSHIP

Chapter Seven

The effect of Late Quaternary climate change on the extinct heavy-footed moa (*Pachyornis elephantopus*)

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Nicolas. J. Rawlence

Designed experiment, collected samples, conducted fieldwork, collected morphological data, performed DNA and demographic analysis, performed collagen preparation and radiocarbon dating, performed morphological analyses, interpreted data and wrote paper.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed ..... Date... 20/04/10

Jessica Metcalf

Performed collagen preparation and radiocarbon dating, helped with demographic analyses and evaluated manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed ..... Date... 26/4/10

Jamie R. Wood

Helped with fieldwork, collecting of morphological data and interpretation of results, evaluated manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed ..... Date.....20 April 2010.....

Jeremy J. Austin

Helped with fieldwork, supervised development of research, evaluated manuscript.

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Signed Date.....22/4/10.

Alan Cooper

Designed experiment, supervised development of research, evaluated manuscript and provided funding.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed Date.....22/4/10

Chapter Seven

The effect of Late Quaternary climate change on heavy-footed moa (*Pachyornis elephantopus*).

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Abstract

Studies of Holarctic fauna have argued that contraction into and expansion out of glacial refugia has had a significant effect on their phylogeography and population demography. Morphological studies have also argued that climate change has had a dramatic effect on body size variation. New Zealand offers a unique opportunity to investigate the effects of climate change on a megafaunal species in the absence of humans because of the late colonisation of New Zealand by Polynesians in about 1280 AD at a time of relative climatic stability. We investigated how climate change affected the phylogeography, population demography and allometric variation in body size of the extinct heavy-footed moa (*Pachyornis elephantopus*). Phylogeographic analysis showed that heavy-footed moa was structured into two well supported clades, northern and southern heavy-footed moa, that diverged during the Late Pleistocene, while demographic analyses showed that heavy-footed moa had a relatively constant population size through time. Morphological analysis indicates that both northern and southern heavy-footed moa exhibited allometric size variation in accordance with Bergman's Rule. The results suggest that while climate change may have affected moa phylogeography and body size, it did not cause the ultimate extinction of moa.

Introduction

One of the key debates surrounding the effects of anthropogenic climate change is the effect of global warming on the population demographics and distribution of species (Pounds *et al*. 1999; Overpeck *et al.* 2002; Benton and Twitchett 2003; Parmesan and Yohe 2003; Root *et al.* 2003;

Thomas *et al.* 2004). Global warming has already been implicated in the extinction of the golden toad (*Bufo periglenes*) from Costa Rica (Pounds *et al.* 1999) with predictions that many habitats will contract and fragment, resulting in 15-37% of species committed to extinction by 2050 including mammals, reptiles, birds, invertebrates and insects (Thomas *et al.* 2004). However, there are many uncertainties involved in predictions of the effects of anthropogenic climate change and understanding how past climate change has affected species is central to estimating the impacts of and responses to future anthropogenic climate change (Thuiller 2003, 2004). Like anthropogenic climate change, the consequences of past climate change have been debated among scientists, especially the effects of habitat contraction and expansion on the phylogeography, population demographics and extinction of species (Hewitt 2000; Lessa *et al.* 2003).

The contraction/expansion hypothesis (Hewitt 2000) is a key concept in interpreting the responses of species to past climate change. Hewitt (2000) proposed that in times of unfavourable climate, taxa retreated to refugia as their habitat contracted and fragmented. When climate became favourable, taxa dispersed into areas of expanding habitat. The consequences of this movement would have large effects on the phylogeography of taxa. Contraction into refugia created vicariant populations that diverged in allopatry, while rapid expansion phases might maintain phylogeographic structure but result in large areas of low genetic diversity (Graham *et al.* 1996; Ibrahim *et al.* 1996; Taberlet *et al.* 1998; Hewitt 2000; Lessa *et al.* 2003). For example, Cooper *et al.* (1995) showed that the common meadow grasshopper in Europe has five main phylogeographic regions each of which correspond to different refugia. In support of this, Hofreiter *et al.* (2004) showed that there was no phylogeographic structure in several European species prior to the Last Glacial Maximum (LGM), arguing that isolation in refugia during the LGM promoted phylogeographic structuring (Hewitt 2000).

Ancient DNA (aDNA) studies have also shown that climate change has strongly affected the genetic diversity and population demographics of taxa. For example, Shapiro *et al.* (2004) and Drummond *et al.* (2005) inferred that Beringian Steppe Bison underwent a significant decline in effective population size in the lead up to the LGM. Several other aDNA studies have shown declines in genetic diversity coincident with major climatic transitions in hyenas (Rohland *et al.* 2005), lions (Burger *et al.* 2004), Arctic fox (Dalen *et al.* 2007) and musk ox (Campos *et al.* 2010). A loss of genetic diversity can also result from population replacement events. Barnes *et*

al. (2002) showed that the current phylogeographic pattern of brown bears in North America was established during the Holocene and not the Pleistocene. Instead, the Pleistocene was characterised by local extinctions, population replacements and re-invasions, due the presence and absence of the North American Laurentide and Cordilleran ice sheets and competition from the extinct giant short faced bear (*Arctodus simus*).

Climate change has also been linked with allometric variation in body size (mass and/or height) in numerous avian and mammalian taxa (Yom-Tov 2001; Millien 2004; Yom-Tov and Yom-Tov 2004; Millien *et al.* 2006; Yom-Tov *et al.* 2006). For example, some species of the extinct New Zealand moa (Worthy and Holdaway 2002) and the North American bushy-tailed wood rat (*Neotoma cinerea*; Smith *et al.* 1995) were larger during the last glacial period compared to the present interglacial. While changes in body size are not fully understood, it is thought to be an adaptive response to changing climate and habitat. Two main patterns in allometric variation in body size have been described: Bergman's Rule and Allan's Rule. Bergman's Rule (Bergman 1847) states that as warm blood animals move down a temperature gradient (temporal, latitude or altitude) the mean size of animals gets larger as an adaptive response to regulating temperature. Allan's Rule (Allan 1877) builds upon Bergman' Rule and states that as the mass of animals increase, the length of limbs decrease. Recently, Teplitsky *et al.* (2008) has argued that declines in mean body size in accordance with Bergman's Rule were a phenotypic response to environmental stresses rather than warming temperature. This seems counterintuitive because changes in temperature can be an environmental stressor. Teplitsky *et al.* (2008) showed that the mean body size of red-billed gulls in a population from New Zealand declined from 1958-2004 with increasing temperature and decreasing survivability, with no change in breeding values. Breeding values (a statistic that incorporates an individuals age, hatching year, and environmental, maternal and genetic effects), were used as a proxy for genetic change in the population resulting from inbreeding, genetic drift or selection (Teplitsky *et al.* 2008). However, the breeding values had low statistical power, so a genetic response could not be ruled out (Teplitsky *et al*. 2008).

The mechanisms behind allometric variation in body size are not well understood. It is also not well understood whether allometric responses to climate change track demographic responses? For example, Hadly *et al.* (2004) showed that over the past 3 Kyr the northern pocket gopher (*Thomomys talpoides*) in North America underwent a population decline with warming climate in

conjunction with a significant decline in body size but did not explicitly link the two responses (Hadly 1997; Hadly *et al.* 1998). Guthrie (2003) has also shown that caballoid horses belonging to the *Equus ferus* species complex underwent a rapid decline in body size before their extinction 12.5 Kya, hypothesizing that the decline was due to climatic and vegetation shifts, leading to new environmental conditions and increased interspecies competition for dietary resources with bison (*Bison* spp.).

New Zealand is an ideal location to investigate how climate change has affected the phylogeography, demography and allometric variation in the body size of taxa. New Zealand has a well documented fossil record spanning the past 50 Kyr that documents changes in the distribution and relative fossil abundance of taxa but does not document the extinction of any species in New Zealand until after Polynesian colonisation about 1280 AD (Wilmshurst *et al.* 2008). The extinctions that followed also occurred at a time of relative climatic stability (Burrows and Greenland 1979). The late colonisation of New Zealand by Polynesians and the extensive fossil record allows the effects of climate change on taxa to be investigated in the absence of human impact like habitat destruction and over-hunting. The separation of climatic and anthropogenic impacts on taxa is not possible for Northern Hemisphere locations like North America where human arrival and major episodes of climate change coincide. For example Drummond *et al*. (2005) showed that bison underwent a second population decline about 12 Kya, coincident with marked climate change, human arrival and a hypothesised extra terrestrial bolide impact (Firestone *et al*. 2007).

We focused on the extinct heavy-footed moa (*Pachyornis elephantopus*). The fossil record indicates that heavy-footed moa underwent changes in relative fossil abundance and distribution in response to changing climate. During the Holocene (0-10 Kya), Late Glacial (10-14 Kya) and Otiran Glacial (14-70 Kya) heavy-footed moa were mainly restricted to the eastern South Island within areas of open tussock grassland/shrubland mosaics (Worthy 1993a, 1997, 1998a, b; Worthy and Holdaway 1995, 1996; Worthy and Grant Mackie 2003; Moar 2008; Figure 1). Rare individuals have also been found in northwest Nelson/West Coast during the Late Glacial and Otiran Glacial (Figure 1; Worthy 1993b, 1994, 1999; Worthy and Holdaway 1994).

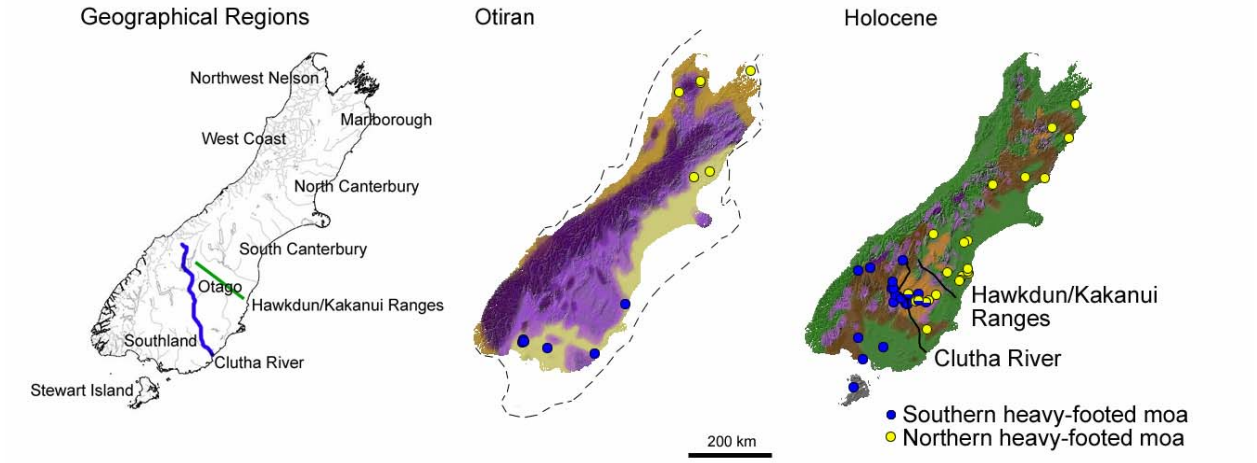

Figure 1 Geographical regions mentioned in the text and distribution of fossil localities containing heavy-footed moa (*Pachyornis elephantopus*) overlaid on the distribution of major habitat types on the South Island, New Zealand, during the Last Glacial Maximum (Otiran) and Late Holocene, based on Barrell *et al.* (2005). Purple, permanent ice; Lilac, sub-alpine; brown, beech forest; green, podocarp-broadleaf forest; orange, shrublands/grasslands; yellow, open grasslands with marginal woody vegetation. Due to the resolution of vegetation types, Holocene fossil localities within areas of podocarp-broadleaf vegetation are actually found in areas of open tussock grassland/shrubland favoured by heavy-footed moa. In the Otiran palaeovegetation reconstruction, the dotted line represents the approximate coastline at maximum regression.

In addition, there is a well documented vegetation succession from widespread open tussock grassland/shrubland, to tall shrubland, to forest (Figure 1), resulting in the fragmentation and contraction of the grassland/shrubland mosaic from the Otiran Glacial to the Holocene (Moar and Suggate 1973, 1979; Wardle 1991; Worthy and Holdaway 1995, 1996; Worthy 1997, 1998a, b; Vandergoes and Fitzimons 2003; Moar 2008). This is mirrored by the decline in relative fossil abundance of heavy-footed moa and changes in their distribution from the Otiran Glacial to the Holocene in most areas of the South Island (Worthy and Holdaway 1995, 1996; Worthy 1997, 1998a, b). Finally, as discussed above, Worthy and Holdaway (2002) have shown that some moa species exhibited allometric variation in body size through time, hypothesized to be in response to temperature changes.

We aim to address the following questions:

i) How did climate change affect the phylogeography and dispersal patterns of heavy-footed moa?
ii) How did climate change affect the population demographic history of heavy-footed moa in the absence of humans?
iii) How did climate change affect body size variation in heavy-footed moa?
iv) Do changes in body size track demographic changes in population size?

How climate change affected the phylogeography and dispersal patterns of heavy-footed moa will be assessed by phylogeographic analysis and radiocarbon dating. The programme BEAST (Drummond and Rambaut 2007) was used to reconstruct the genealogy of heavy-footed moa in a Bayesian framework. Radiocarbon dating of bones and dating of major divergences within heavy-footed moa will place the phylogeny in a temporal context that can be compared against known climatic and palaeovegetation records. Previous research has suggested that heavy-footed moa may be separated into two well supported clades (Lambert *et al.* 2005; Baker *et al.* 2005; Bunce *et al.* 2009). However, it is currently not known whether the apparent phylogeographic structuring within heavy-footed moa is an artefact of small sample size (Goldstein & Desalle 2003; Munoz-Fuentes *et al.* 2005; Austin *et al*. 2009) or a real phenomenon. Lambert *et al.* (2005) and Baker *et al.* (2005) analysed only 12 heavy-footed moa specimens, while Bunce *et al*. (2009) analysed a further nine specimens. How climate change affected the population demographic history of heavy-footed moa will be analysed with the Bayesian Skyline Plot (BSP;

Drummond *et al.* 2005) and BayeSSC (Excoffier *et al.* 2000; Anderson *et al.* 2006). These analytical methods have been used previously to examine the palaeodemographic histories of species including musk ox (Campos *et al.* 2010), brown bears (Valdiosera *et al.* 2008), bison (Drummond *et al.* 2005) and mammoths (Barnes *et al.* 2007; Debruyne *et al.* 2008). How climate change affected allometric variation in the body size of heavy-footed moa will be assessed by comparing femoral mid shaft width measurements, an indicator of mass and height (Worthy and Holdaway 2002), from well-dated fossil deposits throughout the Late Quaternary. Finally, to determine whether changes in body size track demographic changes in population size the patterns of allometric size variation will be compared to the palaeodemographic history of heavy-footed moa inferred from the BSP.

Materials and Methods

Samples

One hundred and forty-five heavy-footed moa bones encompassing the geographical and temporal range of the species (Figure 1) were sampled from New Zealand museum collections (Table S1). Approximately 0.5-1 g bone powder/shavings of dense cortical bone was removed from specimens using a cordless drill at low speed with an 8 mm wood drill bit. To avoid sampling the same individual twice, only common elements (e.g. tibiotarsi) of the left or right orientation were sampled from each deposit. Morphological measurements were taken from every southern heavy-footed moa individual sampled for aDNA analysis compared to 18 northern heavy-footed moa individuals.

DNA extraction, amplification and sequencing

Whole genomic DNA extraction, PCR amplification of the mitochondrial DNA (mtDNA) control region hyper variable region one (HVRI) and two (HVRII) fragments, and DNA sequencing was conducted following the methodology in Chapter Six.

Collagen extraction and radiocarbon dating

Only three of the heavy-footed moa specimens in this study had been previously radiocarbon dated (Bunce *et al.* 2009; Table S2). To facilitate phylogeographic and palaeodemographic analyses, we extracted collagen from, and radiocarbon dated, a further 42 specimens (Table S2), using the facilities at the Oxford Radiocarbon Accelerator Unit (ORAU), University of Colorado (CU), University of California Urvine (UCI), University of Wollongong (UW) and the Australian

National University (ANU). Collagen extraction and radiocarbon dating at ORAU was conducted following the ORAU protocols (see Bronk Ramsey *et al.* 2004a, b; Jocobi *et. al.* 2006); at CU/UCI following Orlando *et al.* (2009); and at UW/ANU following the methodology in Chapter Three. The size of the dataset was increased by calculating mean stratigraphic radiocarbon ages for fossil deposits using this dataset and published resources (see Worthy and Holdaway 2002) and assigning stratigraphic ages to undated specimens from each locality (Table S1).

Sequence alignments

Contiguous sequences and sequence alignments for Bayesian Skyline Plot (BSP; Drummond *et al.* 2005), Arlequin and BayeSSC (Excoffier *et al.* 2000; Anderson *et al.* 2006) analyses were constructed following the methodology in Chapter Six. Alignments for phylogeographic analysis using BEAST (Drummond and Rambaut 2007) were constructed following the methodology for Arlequin and BayeSSC analyses in Chapter Six.

Phylogeographic analysis

To examine the level of phylogeographic structuring within heavy-footed moa a maximum clade credibility phylogeny was constructed using BEAST v1.4.8, with the GTR + I + G model of nucleotide substitution and a constant population size coalescent tree prior. A maximum clade credibility phylogeny is the phylogeny from the posterior distribution with the maximum sum of posterior probabilities. A constant population prior was chosen because the BSP analysis (Figure 6) failed to reject constant population size. Little bush moa (*Anomalopteryx didiformis*) was used as an out-group to root the phylogeny because *Anomaloptyeryx* is the sister taxon of *Pachyornis* (Bunce *et al.* 2009). Crested moa (*Pachyornis australis*) and Mappin's moa (*Pachyornis geranoides*) were not used as out-groups because the branching order within the *Pachyornis* clade is currently unresolved (Baker *et al.* 2005; Lambert *et al.* 2005; Bunce *et al*. 2009).

To date the major divergences within heavy-footed moa, the substitution rate was fixed at 8.7% per million years (Bunce *et al.* 2009). This substitution rate was calculated by Bunce *et al.* (2009) from radiocarbon and stratigraphic-dated sequences from the moa genera *Megalapteryx*, *Dinornis* and *Pachyornis* and is a conservative estimate of the moa substitution rate. A varying substitution rate was not chosen because the phylogeographic analysis was restricted to a single species and it is unlikely that the substitution rate would vary across branches in the phylogenetic tree. A varying substitution rate would also increase the variance on the divergence time estimates.

Therefore the short-term rate of 8.7% per million years is justified. A heavy-footed moa or *Pachyornis* substitution rate was not used because (i) the branching order of *Pachyornis* is unresolved and (ii) the out-group belongs to a different genus to *Pachyornis*.

Two independent runs were combined; each consisting of 50 million generations, sampling parameters every 10,000 generations to ensure enough genealogies were sampled. The first 10% of runs were discarded as burn-in. Results were visualised in Tracer v1.8 (http://beast.bio.ed.ac.uk/Tracer). The maximum clade credibility phylogeny was constructed in TreeAnnotator and viewed in FigTree.

Bayesian skyline analysis

The population demographic history of heavy-footed moa was investigated using the BSP. The phylogeographic analysis indicated that the heavy-footed moa phylogeny was split into two highly supported but geographically restricted clades: a northern and southern clade (Figure 3). Phylogeographic structuring within heavy-footed moa violates the key BSP assumption of a single unstructured population (Drummond *et al*. 2005). To meet this assumption, the heavy-footed moa dataset was divided into northern and southern subsets. The BSPs for northern and southern heavy-footed moa were constructed following the methodology in Chapter Six. The effects of sequence error, due to post-mortem DNA damage, on the BSP (Rambaut *et al.* 2008), was analysed following the methodology in Chapter Six.

The phylogeographic analysis also indicated that southern heavy-footed moa were phylogeographically structured into highly supported clades north and south of the Clutha River (Figure 1; Figure 5). To investigate if there were different geographic signals contributing to the demographic history of southern heavy-footed moa inferred from the BSP, the dataset was subdivided into sequences north versus south of the Clutha River and the BSP constructed for each geographical region following the methodology in Chapter Six. In addition, because Southland (Figure 1) and Otago are characterised by a different habitats (Worthy 1998a, b; Figure 1), the population demographics of southern heavy-footed moa in Southland was also investigated.

The northern heavy-footed moa dataset was further subdivided into sequences from Marlborough/North Canterbury and South Canterbury/Otago (Figure 1) and the BSP constructed

for each geographical region following the methodology in Chapter Six. This was based on the palaeovegetation reconstruction of the South Island during the Holocene by Barrell *et al.* (2005) that suggested that northern heavy-footed moa were potentially subdivided into two populations, with a forest barrier between them. Heavy-footed moa remains have been found in areas of open tussock grassland/shrubland during the Pleistocene and Holocene. Though not supported by the phylogeographic analysis (Figure 3) the forest barrier may have been a geographical barrier to dispersal or a filter, meaning that different geographic signals may be contributing to the palaeodemographic history of northern heavy-footed moa inferred from the BSP.

Population genetic statistics

BayeSSC analysis compares empirical population genetic summary statistics against simulated values to determine the model of population demography that best fits the empirical data. To calculate summary statistics, northern heavy-footed moa sequences from Marlborough/North Canterbury (and North Canterbury only) were divided into the following time bins based on intervals of pronounced climate and habitat change (Moar and Suggate 1973, 1979; Vandergoes *et al.* 2005; Burge and Schulmeister 2007; Moar 2008): Otiran Glacial (70-14 Kya), Late Glacial (14-10 Kya) and Holocene (10-0 Kya). Population genetic and BayeSSC analyses were restricted to Marlborough and North Canterbury because of the paucity of Late Glacial and Otiran Glacial northern heavy-footed moa specimens in South Canterbury/Otago.

In southern heavy-footed moa there is a paucity of Late Glacial specimens, therefore sequences were divided into Pleistocene (> 10 Kya) and Holocene (10-0 Kya) time bins. Based on the phylogeographic analyses (Figure 5) and habitat differences between Southland and Otago (Worthy 1998a, b; Figure 1), sequences from south of the Clutha River or Southland were also analysed.

The number of polymorphic sites and the average pairwise difference between sequences, for each time bin and geographical region (Table S3), was calculated using the programme Arlequin 3.0, following the methodology in Chapter Six. Polymorphic sites and pairwise differences are recommended by Ramakrishnan and Hadly (2009) for intra population comparisons using BayeSSC.

BayeSSC analysis

BayesSSC analysis was conducted following the methodology in Chapter Six with the following modifications. The evolutionary rate from the BSP analysis of 3.9817% per million years (95% HPD 0.12% - 7.45%) and 2.5474% per million years (95% HPD 0.68% - 4.76%) for northern and southern heavy-footed moa respectively was converted to 0.001983 and 0.0001287 mutations per base pair per generation using the above parameters. The evolutionary rates were calculated in BEAST using radiocarbon and stratigraphic dated northern and southern heavy-footed moa sequences under a BSP coalescent prior following the methodology above for BSP analysis. The transition/transversion ratio of 0.43529 and 0.4938 for northern and southern heavy-footed moa respectively was calculated in MEGA 4.0 from only complete sequences with radiocarbon and stratigraphic ages, as the calculation cannot incorporate specimens with missing sequences data.

Model One: Constant population size.

This model corresponds to a single population that remained constant through time (Figure 2) and will be used to determine whether there is enough power in the northern and southern heavy-footed moa datasets to reject constant population size. In northern heavy-footed moa there are three time bins (stat groups) within this model based on intervals of pronounced climate and habitat change (Moar and Suggate 1973, 1979; Vandergoes *et al.* 2005; Burge and Schulmeister 2007; Moar 2008): Otiran Glacial (70-14 Kya), Late Glacial (14-10 Kya) and Holocene (10-0 Kya). In southern heavy-footed moa there are two time bins within this model: Pleistocene (70-10 Kya) and Holocene (10-0 Kya).

Model Two: Increasing population size from the Pleistocene to Holocene

This model corresponds to a single southern heavy-footed moa population (or south of the Clutha) that increased in size from the Otiran Glacial to the Holocene (Figure 2). This model is based on an increase in the relative fossil abundance of southern heavy-footed moa from the Pleistocene to the Late Holocene (Worthy 1998a, b; Otago Museum unpublished data of relative fossil abundance data from Owaka, Southland). For specific modelled increases in population size, see Table 2.

Model Three: Decreasing population size from the Late Glacial to the Holocene

This model corresponds to a single population that declined from the Late Glacial to the Holocene (Figure 2). The decline in population size were based on four different parameters: the

relative fossil abundance of heavy-footed moa (Worthy and Holdaway 1995, 1996; Worthy 1997, 1998a, b); the palaeodemographic history of heavy-footed moa inferred from the BSP (Figure 6); the amount of open tussock grassland/shrubland mosaic, calculated from Barrell *et al.* (2005) palaeovegetation reconstruction of the South Island during the LGM and Holocene; and the well documented succession of tall shrubland/grassland to podocarp forest during the early Holocene (Moar 2008; Chapter Three). For specific modelled declines in population size, see Table 2.

Model Four: Decreasing population size from the Otiran Glacial to the Late Glacial and from the Late Glacial to the Holocene.

This model corresponds to a single northern heavy-footed moa population that declined from the Otiran Glacial to the Late Glacial and from the Late Glacial to the Holocene (Figure 2). The declines in population size were based on two different parameters: the relative fossil abundance of heavy-footed moa (Worthy and Holdaway 1995, 1996; Worthy 1997, 1998a, b) and the palaeodemographic history inferred from the northern heavy-footed moa BSP (Figure 6a). For specific modelled declines in population size, see Table 2.

Morphological analysis

To determine the effects of climate change on the allometric variation in body size of heavy-footed moa, we assembled an extensive dataset of femoral mid shaft width measurements for heavy-footed moa (N = 96) and combined this data with published measurements (N = 81; Worthy and Holdaway 2002), encompassing a total of 12 discrete fossil deposits (Table S4). Mid-shaft width is an indicator of mean body size (both mass and height; Figure S6) and has been used previously to examine allometric size change through time in moa (Worthy and Holdaway 2002). For each deposit the mean femoral mid shaft width and standard deviation (when available) were calculated (Table S4). To assess temporal body size variation, the mean femoral shaft width measurements and standard deviations were plotted against the mean stratigraphic age for each fossil deposit. To control for possible geographical variation in body size, only northern heavy-footed moa from Canterbury were analysed. Because southern heavy-footed moa are from a single geographical region (i.e. southern New Zealand) all individuals were analysed.

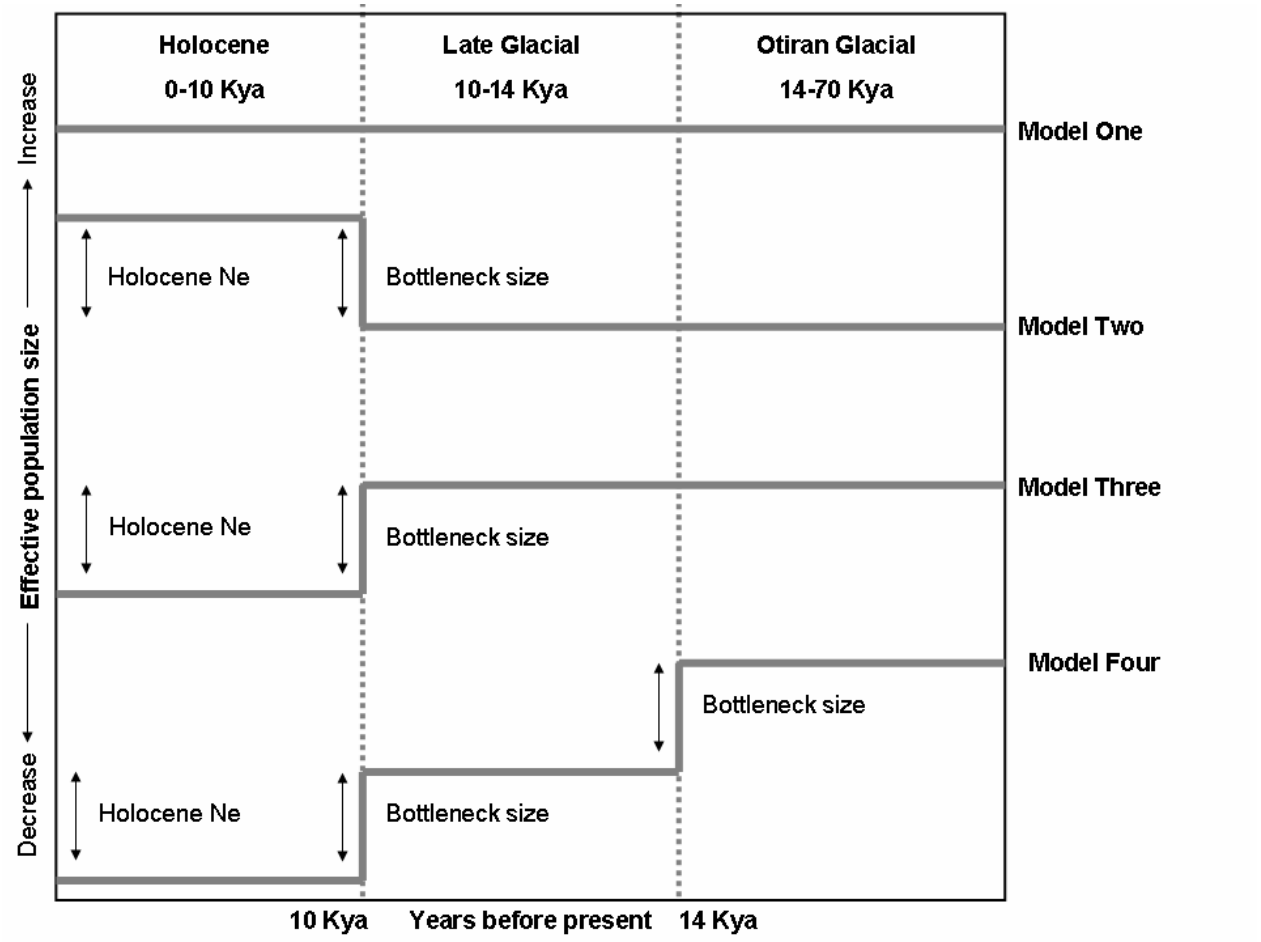

Figure 2 Models of heavy-footed moa (*Pachyornis elephantopus*) population demography simulated in BayeSSC. For each model the Holocene effective population size (Nef) and bottleneck size were estimated. The population bottleneck size represents an undefined decline in population size.

Results

Sequence authentication

To provide support for the authenticity of the aDNA sequences we replicated nine percent of the heavy-footed moa dataset by re-amplifying and re-sequencing 13 individuals for each HVRI and II fragment at ACAD (total of 498 bp for each individual and 6474 bp in total). Of the 13 individuals (A2047, 2631, 2634, 2643-44, 2666, 2696, 2700-01, 2760, 3719, 3756, 3872, 3886; Table S1), no sequencing errors were observed when replicated sequences were compared with the original sequences. In addition, eight specimens (GU139068, GU139070, GU139072-77; Table S1) previously extracted and sequenced at the Henry Welcome Ancient Biomolecules Centre in Oxford (Bunce *et al.* 2009) were independently replicated in this study. The independently replicated sequences were identical to the original sequences published by Bunce *et al.* (2009). In addition, the recovery of identical sequences despite the diversity within heavy-footed moa provides additional support for the authenticity of the aDNA sequences.

Phylogeographic structuring

The phylogeographic analysis shows that there were two well supported clades within heavy-footed moa: a northern and southern clade (Figure 1 and 3). The northern clade (Figure 4) was restricted to the eastern South Island from the northern tip to the Clutha River in the south. Otiran and Late Glacial sequences are restricted to North Canterbury and rare individuals from northwest Nelson/West Coast, with all other sequences dating to the Early to Late Holocene (Figure S1). There is no support for the separation of Marlborough/North Canterbury and South Canterbury/Otago into separate sub-clades (Figure 4). The northern clade contained 27 radiocarbon dated sequences ranging in age from 32,230-663 years BP (Table S2).

The southern clade (Figure 5) was distributed from Otago, north of the Hawkdun/Kakanui Ranges to Stewart Island, with northern and southern heavy-footed moa sympatric in Otago (Figure 1). The southern clade contained 26 radiocarbon-dated sequences ranging in age from 50,200-654 years BP (Table S2). Within the southern clade there are two well-supported sub-clades (Figure 5). The first sub-clade contained sequences that are present north of the Clutha River with two closely related sequences found in individuals south of the Clutha River. All of the sequences are Late Holocene in age (Figure S2). The second sub-clade contained sequences found south of the Clutha River, with three representative sequences found in individuals north of the Clutha River. The sequences range from Otiran Glacial to Holocene in age (Figure S2).

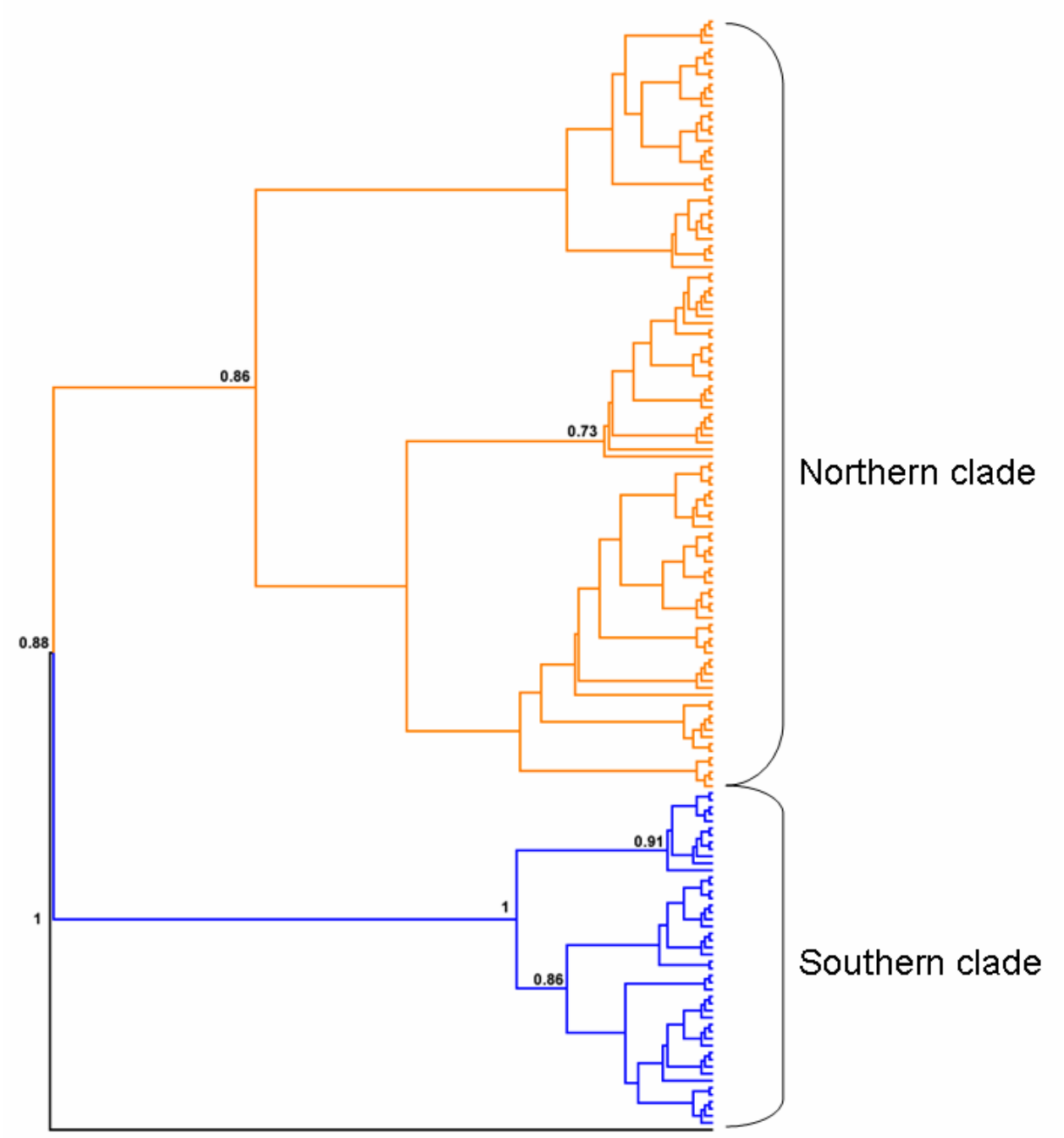

Figure 3 Maximum clade credibility phylogram of heavy-footed moa (*Pachyornis elephantopus*), with branch lengths proportional to the number of substitutions. Heavy-footed moa are structured into well supported clades, a northern and southern clade. Only posterior probabilities greater than 0.5 are shown on the phylogram.

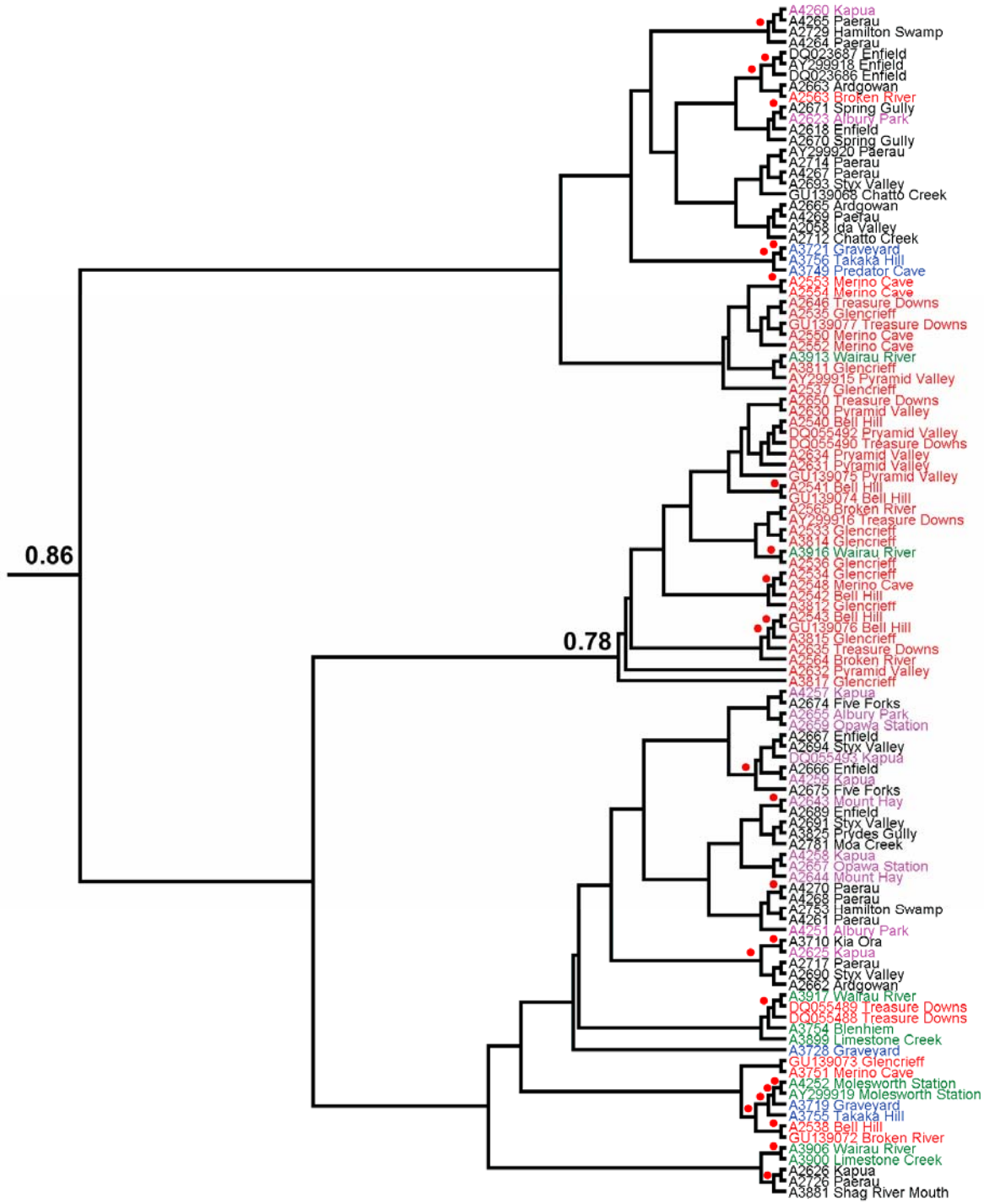

Figure 4 Maximum clade credibility phylogram of northern heavy-footed moa (*Pachyornis elephantopus*), with branch lengths proportional to the number of substitutions. Posterior probability support (> 0.5) is indicated at each node in black (or by red dots). Blue: Northwest Nelson/West Coast; Green: Marlborough; Red: North Canterbury; Fuchsia: South Canterbury; and Black: Otago (North Otago/Central Otago).

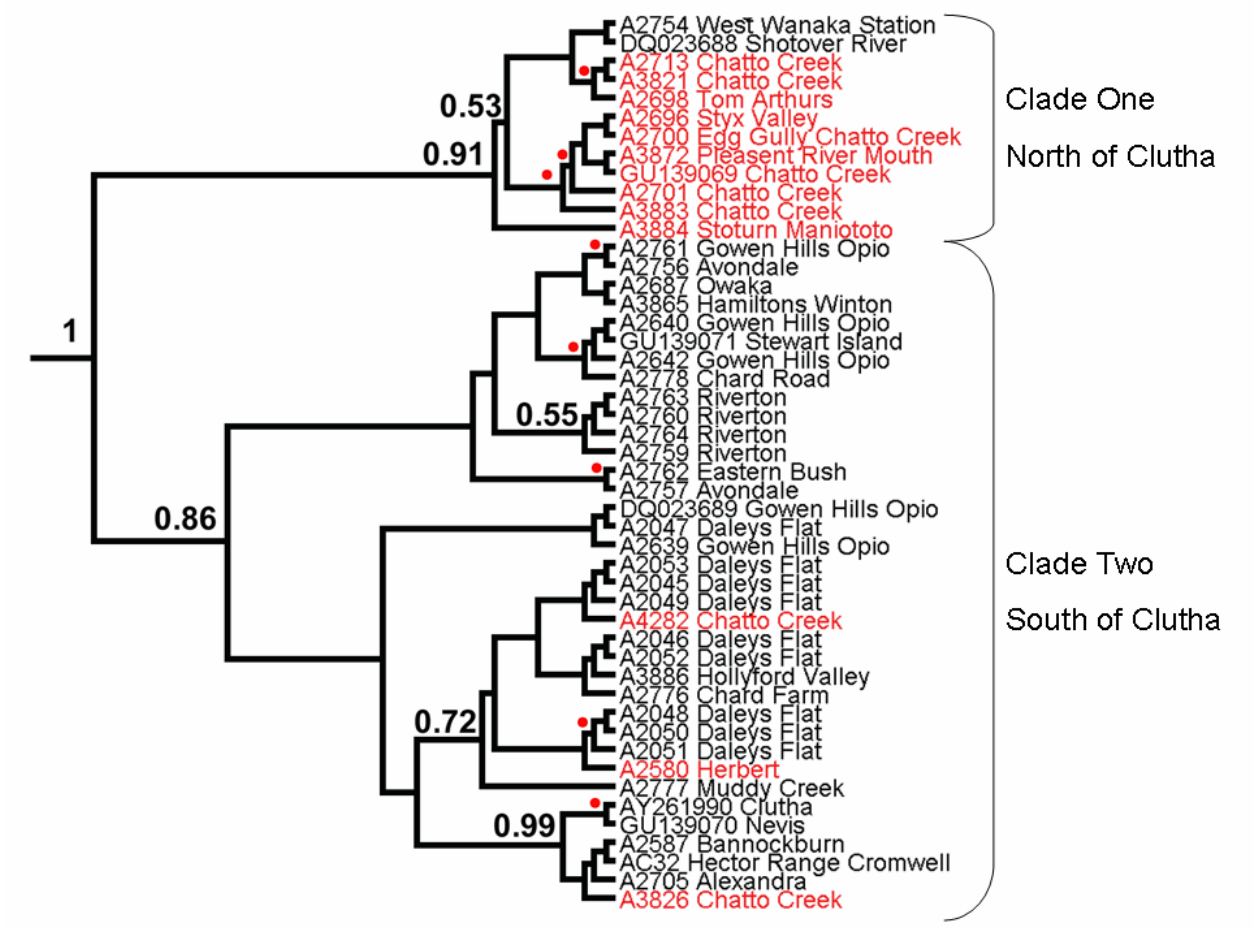

Figure 5 Maximum clade credibility phylogram of southern heavy-footed moa (*Pachyornis elephantopus*), with branch lengths proportional to the number of substitutions. Posterior probability support (> 0.5) is indicated at each node in black (or by red dots). There are two well supported clades within southern heavy-footed moa, restricted to north and south of the Clutha River. Red: north of the Clutha; Black: south of the Clutha.

The time dependency of molecular rates means that calculating divergences times within heavy-footed moa is difficult (Ho *et al.* 2005; Ho and Larson 2006). Within this limitation, the mean divergence times of the most recent common ancestor (tMRCA) of the well-supported major clades in the heavy-footed moa phylogeny are within the Late Pleistocene (Table 1). Heavy-footed moa are estimated to have diverged into the northern and southern clades about 500,700 years ago (95% HPD 283,900 – 758,300 Kya), while southern-heavy footed moa split into clades north and south of the Clutha River about 125,900 years ago (95% HPD 67,953 – 197,100 Kya; Table 1).

Table 1 Estimated dates for heavy-footed moa divergence events. Divergence events were calculated by fixing the substitution rate at 8.7% million years (Bunce *et al.* 2009). Mya, millions of years; Kya, thousands of years; CI, confidence interval; MRCA, most recent common ancestor.

	Date, Mya (Kya)	
MRCA/Taxon Split	**Mean**	**95% CI**
Heavy-footed moa	0.5007 (500,700)	0.2839-0.7583 (283,900-758,300)
Northern heavy-footed moa	0.29 (290,000)	0.1226-0.6562 (122,600-656,200)
Southern heavy-footed moa	0.1259 (125,900)	0.067953-0.1971 (67, 953-197,100)
North of the Clutha River	0.06785 (67,850)	0.019043-0.131 (19,043-131,000)
South of the Clutha River	0.091689 (91,689)	0.046684-0.1478 (46,684-147,800)

Demographic analysis

The palaeodemographic history of northern heavy-footed moa inferred from the BSP, suggested that the northern heavy-footed moa population increased in size from 40-22 Kya, declined from 22-9 Kya, increased from 9-4 Kya and declined from 4 Kya to the hypothesised extinction of moa in the 14th century AD (Tennyson and Martinson 2006; Figure 6a). However, due to the wide credible intervals a constant population size through time cannot be rejected for northern heavy-footed moa and subsequent BSPs presented in this study. The analysis of "transitions only" to assess the effects of sequence error, due to post mortem DNA damage, on the northern heavy-footed moa BSP (Rambaut *et al.* 2008) did alter the BSP (Figure S3), suggesting that there is some evidence for post mortem DNA damage in this dataset. However a constant population size could not be rejected so this result should be treated with caution.

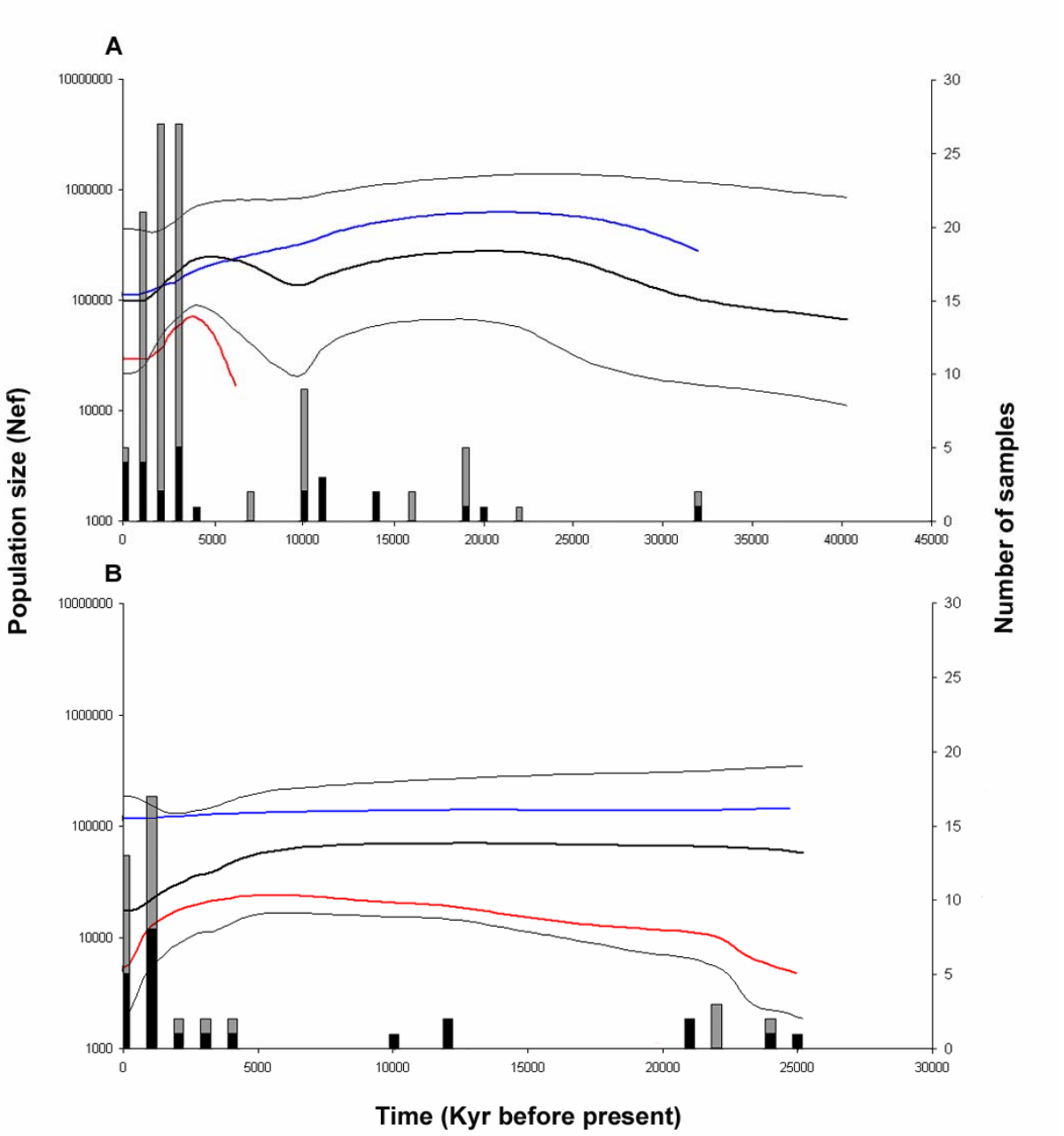

Figure 6 A: Bayesian skyline plot (BSP) of northern heavy-footed moa and B: southern heavy-footed moa. The black line represents the median BSP, while the grey lines represent the 95% credible intervals. Each column represents the number of radiocarbon dated sequences for the analysis of the complete dataset: black, number of finite radiocarbon dates; grey, number of undated sequences with mean stratigraphic ages assigned. In A: the blue line represents Marlborough/North Canterbury, while the red line represents South Canterbury/Otago. In B: the blue line represents Southland, while the red line represents the south of the Clutha River.

Table 2 Statistical performance of heavy-footed moa (*Pachyornis elephantopus*) palaeodemographic models in the BayeSSC analysis. The time-bins analysed are as follows: PL: Pleistocene (70-10 Kya); OG: Otiran Glacial (70-14 Kya); LG: Late Glacial (14-10 Kya); H: Holocene (10-0 Kya). ↓ represents a decline, - represents constant population size. Grey shaded rows represent models that are not statistically significant from one another.

Model	Nef (mode)	Decline factor (mode)	AIC
Northern heavy-footed moa			
North Canterbury			
OG ↓ LG ↓ H BSP (1 prior)	500	3.33, 2	64.09
OG ↓ LG ↓ H fossil (1 prior)	550	1.57, 4.44	62.60
OG-LG ↓ H fossil (1 prior)	600	4.44	61.16
OG-LG-H constant (1 prior)	1,600	N/A	59.45
Marlborough/North Canterbury			
OG ↓ LG ↓ H fossil (1 prior)	500	1.57, 4.44	65.54
OG ↓ LG ↓ H BSP (1 prior)	520	3.33, 2	65.34
OG-LG ↓ H fossil (1 prior)	700	4.44	58.84
OG-LG-H constant (1 prior)	2,000	N/A	58.75
Southern heavy-footed moa			
Complete dataset			
PL ↓ H BSP (1 prior)	17,500	2	40.49
PL ↓ H habitat (1 prior)	10,000	3.83	39.39
PL ↓ H fossil (1 prior)	16,000	1.82	38.71
PL ↑ H (1 prior)	42,000	2 (0.55)	39.25
PL-H constant (1 prior)	30,000	N/A	37.08
Southland			
PL ↓ H habitat (1 prior)	6,000	3.83	41.57
PL ↓ H fossil (1 prior)	8,000	2.49	39.41
PL ↑ H (1 prior)	50,000	0.4	39.00
PL-H constant (1 prior)	20,000	N/A	38.61
South of Clutha River			
PL ↓ H fossil (2 priors)	15,000	1.25	41.93
PL ↓ H BSP (1 prior)	14,500	2	40.08
PL ↓ H habitat (1 prior)	7,500	3.83	39.44
PL ↑ H (1 prior)	40,000	0.4	39.25
PL-H constant (1 prior)	24,000	N/A	39.13
PL ↓ H fossil (1 prior)	15,000	1.82	38.91

Two different demographic signals were reconstructed when the northern heavy-footed moa dataset was divided into sequences from Marlborough/North Canterbury and South Canterbury/Otago (Figure 6a; S4). The Marlborough/North Canterbury population increased in size from about 32-20 Kya, followed by a slow decline from 20 Kya to the extinction of moa in the 14th century AD (Tennyson and Martinson 2006). In contrast, the BayeSSC analysis suggests the most likely model of population demography is a constant population size through time (Table 2). However, the BayeSSC results are not statistically significant (Burnham and Anderson 2002; Ramakrishnan and Hadly 2009), because Model Three (Declining population size from the Late Glacial to the Holocene based on the decline in relative fossil abundance data; Figure 2, Table 2) is within two AIC values of Model One (constant population size; Table 2). The palaeodemographic history inferred from the BSP of the South Canterbury/Otago population is similar to the Late Holocene demography of northern heavy-footed moa, and is characterised by an increase in population size from about 7-4 Kya, followed by a decline from 4 Kya to the extinction of moa (Figure 6a, S4b).

In contrast to northern heavy-footed moa, the BSP analysis suggested that southern heavy-footed moa had a constant population size from 25-5 Kya, followed by a population decline from 5 Kya (Figure 6b). The analysis of "transitions only" and "all substitutions" did not alter the BSP (data not shown). For southern heavy-footed moa, BayeSSC analysis suggested the most likely model of population demography was a constant population size (Table 2). When only sequences from Southland and south of the Clutha River were analysed, the BSP inferred a constant population size for Southland, while inferring for south of the Clutha an increase in population size from 25-5 Kya, followed by a decline from 5 Kya (Figure 6b, S5). BayeSSC analysis suggested that the most likely model of population demography for heavy-footed moa from Southland and south of the Clutha was a constant population size. However, the BayeSSC results are not statistically significant. For Southland, an increase or decrease in population size from the Pleistocene to Holocene is as equally likely as constant population size through time. For south of the Clutha, no model of population demography could be rejected (Table 2).

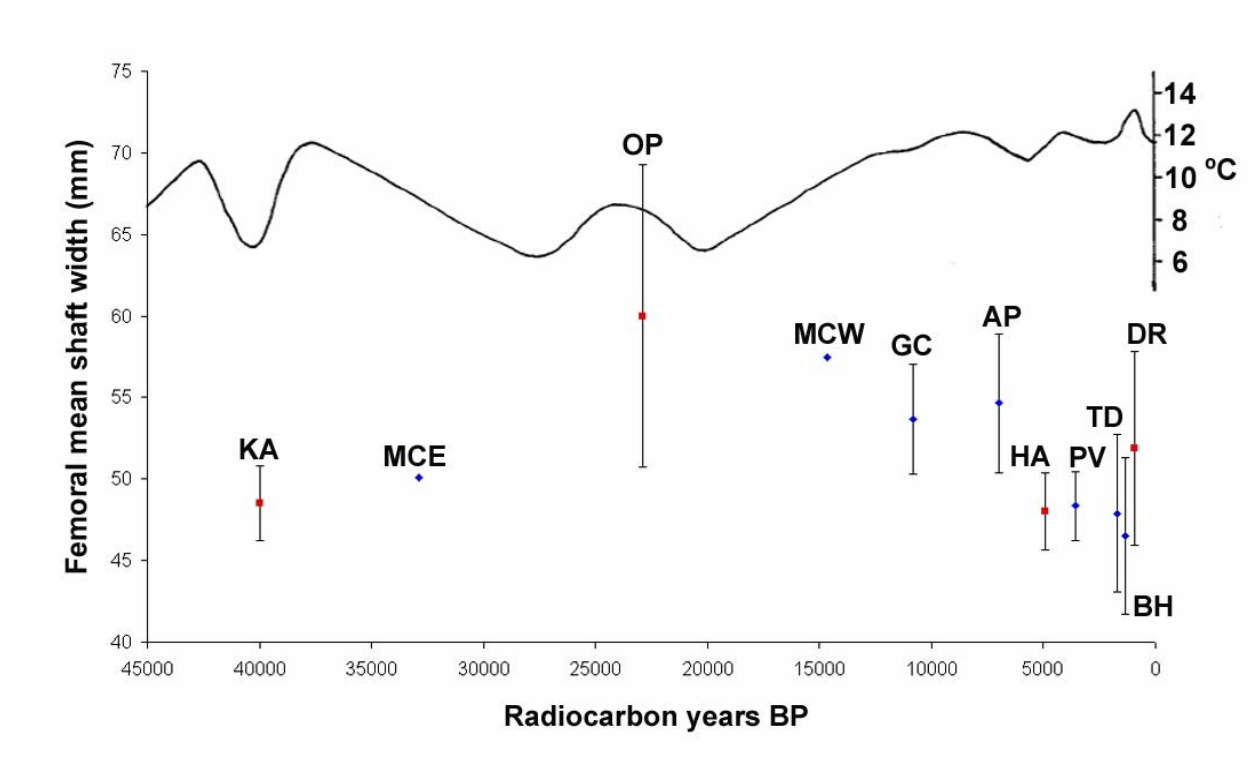

Figure 7 Temporal size variation in heavy-footed moa (*Pachyornis elephantopus*) femora from dated fossil deposits in relation to temperature changes illustrating Bergman's Rule. Each data point represents the mean femoral shaft width of each deposit with standard deviations (where available). Blue = northern heavy-footed moa, red = southern heavy-footed moa, KA = Kauana, MCE = Merino Cave East, OP = Opio, MCW = Merino Cave West, GC = Glencrieff, AP = Albury Park, HA = Hamilton's, Winton, PV = Pyramid Valley, TD = Treasure Downs, BH = Bell Hill, DR = Dart River. The mean annual temperature curve was determined from $\delta^{18}O$ values of speleothems (after Hendy 1969). Note the *x* axis (radiocarbon years BP) is in the reverse order relative to Figures 6 and 8.

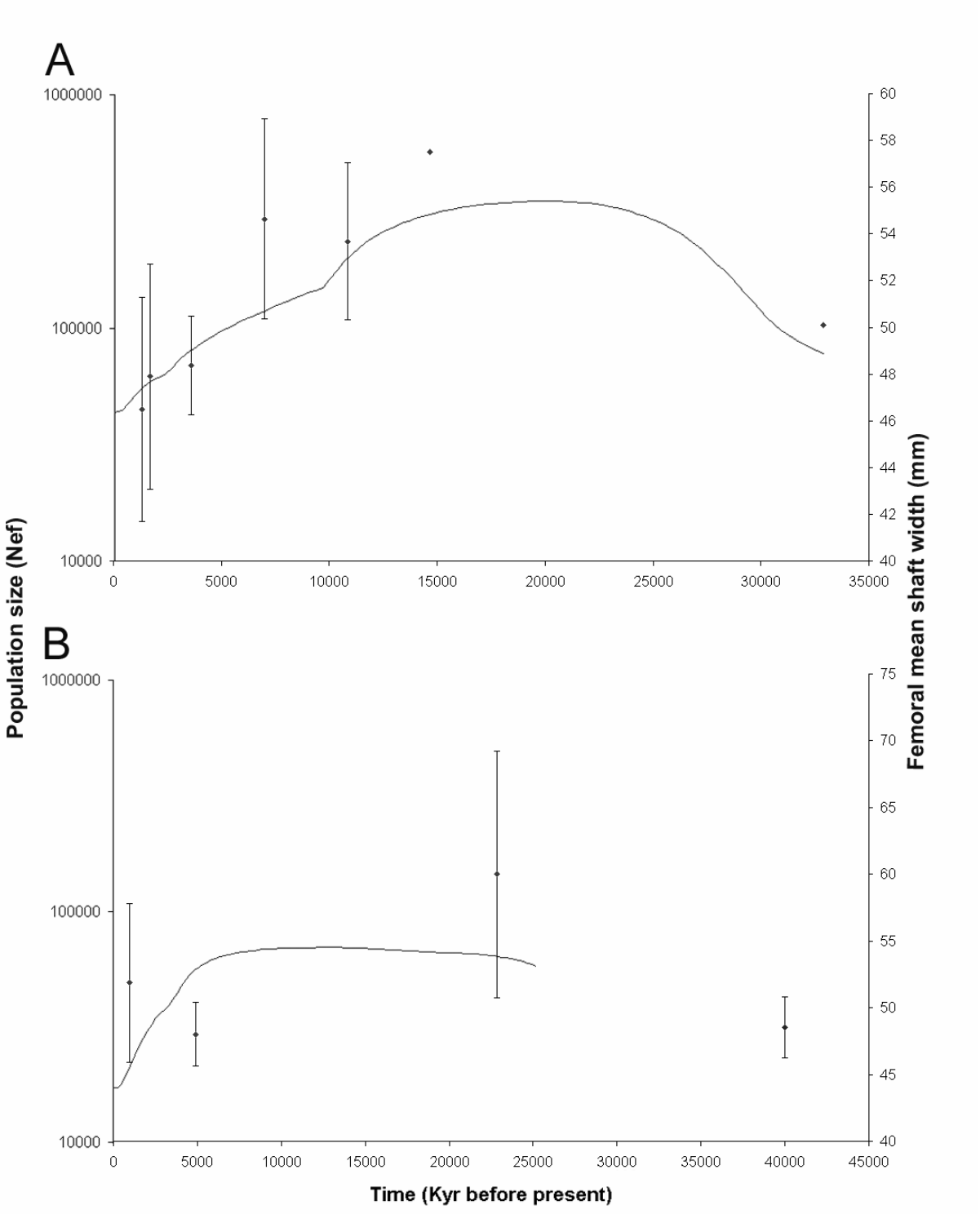

Figure 8 Allometric and demographic responses to climate change in heavy-footed moa (*Pachyornis elephantopus*). A: northern heavy-footed moa; B: southern heavy-footed moa. Note the "Time" and "Population size" scales are the reverse of Figure 6.

Allometric variation in body size

The morphological analysis revealed that heavy-footed moa exhibited allometric variation in body size through time (Figure 7; Table S4). Both northern (from North Canterbury to control for possible geographical variation in body size) and southern heavy-footed moa increased in mean body size (mass and height; Figure S6) up to the LGM (29-19 Kya), and decreased afterwards, with southern heavy-footed moa generally larger than northern heavy-footed moa. In the Holocene there was an altitudinal component to allometric variation in body size, with southern heavy-footed at higher altitudes larger than those at lower altitudes. Southern heavy-footed moa specimens from the Dart River in the sub-alpine zone are larger than those from Hamilton's Swamp near sea level (Figure 7; Table S4). When the trend in body size variation in northern heavy-footed moa is compared to the BSP for the North Canterbury/Marlborough population, it is evident that increases and decreases in effective population size are matched by changes in mean body size (Figure 8a). This was also seen in southern heavy-footed moa, where the decline in effective population size from the Pleistocene to Holocene is matched by a decline in the mean body size of individuals (Figure 8b). However, we cannot comment on the match between changes in population size and allometry in southern heavy-footed moa during the Pleistocene, because the BSP does not extend back to 40 Kya.

Discussion

We have shown that heavy-footed moa comprised two highly supported distinct phylogeographic units, a northern and southern clade, with additional highly supported phylogeographic structuring within the southern clade. Population demographic analyses suggested that heavy-footed moa were characterised by population expansions, declines or constant population size throughout the Late Quaternary, though constant population size could not be rejected for the BSP analyses in this study. Morphological analysis indicated that both northern and southern heavy-footed moa exhibited allometric variation in body size through time.

Climate change may have affected heavy-footed moa phylogeography.

The results from this study suggest that heavy-footed moa were structured into two well supported northern and southern phylogeographic clades, corroborating the findings of Baker *et al.* (2005), Lambert *et al.* (2005) and Bunce *et al.* (2009). It was not apparent from previous studies whether this was an artefact of small sample size or represented phylogeographic structuring. Our new data suggests that the phylogeographic structuring was a real phenomenon,

with northern and southern heavy-footed moa diverging about 500,700 years ago (95% HPD 283,900-758,300 years ago; Table 1).

The mean divergence dates for the major splits within the heavy-footed moa phylogeny (Figure 3, 4 and 5) are within the Late Pleistocene, which was characterised by regular glacial and interglacial cycles. This suggests that the phylogeographic structuring within heavy-footed moa may be the result of contraction and expansion into and out of areas of suitable habitat, in this case open tussock grassland/shrubland mosaics (Worthy and Holdaway 1995, 1996). During glacial periods, the alpine zone was depressed by approximately 800 metres and sea level lowered by about 120 metres (McGlone *et al.* 2010). This resulted in Central Otago being covered in permanent snow and ice (Figure 1; Darby *et al.* 2004). We hypothesise that this excluded heavy-footed moa from Central Otago and subdivided heavy-footed moa into two 'refugia' in Southland and the eastern South Island. During interglacials, the alpine zone lifted, resulting in the expansion of suitable habitat into Central Otago (Figure 1; Darby *et al.* 2004). We hypothesise that this was followed by the expansion of northern and southern heavy-footed moa into Central Otago. Radiocarbon dating of fossil localities in Central Otago indicates that none are older than 5 Kya (Worthy 1998a). However, this may be a taphonomic artefact. Further excavations and radiocarbon dating of fossil localities in Central Otago may result in the discovery of Late Glacial or Otiran Glacial sub-fossil deposits. Whereas the alpine zone may have acted as a barrier during the glacial periods, the Clutha River (Figure 1) potentially acted as a barrier or filter during interglacial periods, with repeated glacial-interglacial cycles reinforcing the phylogeographic structure seen in heavy-footed moa. The Clutha River system divides the eastern and southern South Island into two geographical areas boarded by the Pacific Ocean to the east and the Southern Alps to the west. Compared to the braided rivers (which are made up of small shallow channels separated by temporary gravel islands) of the eastern South Island, the Clutha River is a deep, fast flowing river that potentially acted as a barrier or filter to dispersal. In support of this, there is no phylogeographic structuring in moa across the larger region of braided rivers in the South Island (Baker *et al*. 2005; Lambert *et al.* 2005; Bunce *et al*. 2009). In addition, before the Clutha River was dammed for hydroelectric power, there was a shallow point on the Clutha River at its junction with the Manuherikia River that may have facilitated the rare dispersal of moa (Darby *et al.* 2004). Interestingly, no heavy-footed moa specimens belonging to the northern clade were found south of the Clutha River. None of the 21 specimens from Central and West Otago, immediately south of the Clutha River, belonged to the northern clade.

However, 12/36 specimens from Central Otago, immediately north of the Clutha River, belonged to the southern clade. This is probably a taphonomic artefact rather than different dispersal abilities of northern and southern heavy-footed moa, as there is evidence for dispersal of southern heavy-footed moa across the Clutha River in both directions (see below). Exploration for new sub-fossil deposits and further excavations of existing sub-fossil deposits may result in northern heavy-footed moa being found south of the Clutha River.

When northern and southern heavy-footed moa are examined in detail, there are different patterns of phylogeographic structuring within each clade. The results from the phylogeographic analysis suggest that within northern heavy-footed moa there is little phylogeographic structuring. This suggests that the vegetation succession from the Otiran Glacial to the Late Holocene of grassland to shrubland to forest, that resulted in the fragmentation of heavy-footed moa habitat within a forest matrix, and the formation of a potential Holocene forest ecological barrier (Worthy 1997; Worthy and Holdaway 1996; Barrell *et al.* 2005), did not result in any phylogeographic structure. Palynological analysis of pollen cores from throughout Canterbury indicates that widespread shrubland had changed to forest by about 9 Kya in North and Mid Canterbury, with the succession occurring about 6 Kya in South Canterbury (Knox 1969; Moar 1971, 2008; McGlone 1988). The succession of grassland to shrubland to forest is common throughout the eastern South Island, occurring later in drier regions like South Canterbury (Knox 1969; Moar 1971, 2008; McGlone 1988). It may be that while heavy-footed moa could have been separated into two populations during the Holocene, the separation was not long enough to form reciprocally monophyletic clades (Figure 4). Further exploration for sub-fossil deposits between North and South Canterbury would help resolve whether heavy-footed moa were absent from this area during the Holocene. However, it cannot be fully assessed whether there was phylogeographic structuring during glacial periods because bones preserved in Otiran and Late Glacial loess deposits in Canterbury and Otago (Worthy 1993a) did not have sufficient aDNA preservation to permit genetic analysis. Ancient DNA could not be extracted from the loess specimens tested (Rawlence unpublished data). This resulted in all Late Glacial and Otiran Glacial northern heavy-footed moa specimens being restricted to North Canterbury and northwest Nelson/West Coast.

The results also indicate that there is no phylogeographic structuring between northern heavy-footed moa from northwest Nelson/West Coast, dating to the Late Glacial and Otiran Glacial, and the eastern South Island. Heavy-footed moa have not been found in Holocene deposits in

northwest Nelson/West Coast. The Holocene vegetation of this area below 1200 metres above sea level (asl) is characterised by forest, with only the cosmopolitan South Island giant moa (*Dinornis robustus*) and forest adapted little bush moa (*Anomalopteryx didiformis*) present. Areas above 1200 m asl are characterised by sub-alpine shrubland and grassland, with upland moa (*Megalapteryx didinus*) and crested moa (*Pachyornis australis*) common (Worthy 1993b, 1994, 1997; Worthy and Holdaway 1994). This suggests that during the Late and Otiran Glacial either multiple dispersals, or a single dispersal of a small number of individuals retaining ancestral genetic polymorphism, occurred from the eastern South Island into northwest Nelson/West Coast (Worthy 1993b, 1994, 1997; Worthy and Holdaway 1994).

In contrast to northern heavy-footed moa, the phylogeographic analysis suggested that southern heavy-footed moa were divided into two well-supported sub-clades, north and south of the Clutha River (Figure 1 and 5), which diverged about 125,900 years ago (95% HPD 67,953-197,100 years ago). The phylogeographic analysis also suggested there was some migration between the two clades (Figure 5). In clade one, "north of the Clutha River", two closely related sequences (A2754 West Wanaka Station and DQ023688 Shotover River) from specimens found south of the Clutha River may represent a single dispersal south of the Clutha River. In contrast, in clade two, "south of the Clutha River", three sequences (A4282 Chatto Creek, A2580 Herbert and A3826 Chatto Creek), from specimens found north of the Clutha River, may represent at least three dispersals north of the Clutha River. Like heavy-footed moa as a whole, the phylogeographic pattern in southern heavy-footed moa may represent contraction and expansion into and out of Central Otago. We hypothesise that during the onset of interglacial periods southern heavy-footed moa expanded their range northwards from Southland, across the Clutha River into Otago. The onset of glacial conditions and the depression of the alpine zone may have resulted in Central Otago acting as a geographical barrier, potentially confining some southern heavy-footed moa to Otago. The return of interglacial conditions may have allowed southern heavy-footed to extend their range back into Central Otago and south of the Clutha River. However, this cannot be fully resolved because of the paucity of southern heavy-footed moa specimens north of the Clutha River, especially Late Glacial and Otiran Glacial specimens. Comparing the two heavy-footed moa clades, it appears that the Pleistocene glacial/interglacial cycles may have had a large effect on southern heavy-footed moa phylogeography but little effect on northern-heavy-footed moa phylogeography.

Climate change had an effect on heavy-footed moa population size.

Population demographic studies on European and North American taxa (Burger *et al.* 2004; Shapiro *et al.* 2004; Drummond *et al.* 2005; Rohland *et al.* 2005; Campos *et al.* 2010; Stiller *et al.* 2010) have shown that levels of genetic diversity and effective population size changed through time in response to climate change. In light of the differences in ecology, population and range size, habitat and abiotic factors between heavy-footed moa and North American megafaunal taxa, some comparisons between inferred palaeodemographic histories in response to Late Quaternary climate change can be made. The results from the demographic analyses suggest that heavy-footed moa did not respond strongly to climate change like bison, which went through a significant decline in population size prior to, during and after the LGM (Shapiro *et al.* 2004; Drummond *et al.* 2005). Instead, heavy-footed moa appear to be similar to mammoths, where constant population size through time could not be rejected (Barnes *et al.* 2007; Debruyne *et al.* 2008). The failure to reject constant population size through time in heavy-footed moa and mammoths is in direct contrast to the fossil record. The fossil record suggests that heavy-footed moa underwent a decline in relative fossil abundance from the Otiran Glacial to the Holocene (Worthy and Holdaway 1995, 1996; Worthy 1997, 1998a, b). In mammoths, the fossil record also suggests that mammoths underwent a decline in relative fossil abundance about 12.5 Kya (Guthrie 2006).

The BSP analysis suggests that the inferred palaeodemographic history of northern and southern heavy-footed moa is a composite of different geographic signals. For example, northern heavy-footed moa populations in Marlborough and North Canterbury were characterised by an inferred decline in population size and relative fossil abundance (Worthy and Holdaway 1994, 1995) from the Otiran Glacial through to the Late Holocene in concert with the established vegetation succession of grassland to shrubland to forest in Marlborough and North Canterbury (Worthy and Holdaway 1995, 1996; Moar 2008). However, the BayeSSC analysis failed to reject all four palaeodemographic models (Figure 2, Table 2) including constant population size. This could either be due to an artefact in the BSP analysis or differences in the way the BSP and BayeSSC work. The BSP utilises complete sequence data while BayeSSC compares summary statistics, so is only using a subset of the available data. The decline in relative fossil abundance may reflect changes in the distribution and population density of northern heavy-footed moa with changing habitat but not necessarily a change in population size. If true, this would suggest that while the faunal composition and relative abundance data from fossil deposits can provide insights into the

age of each deposit (e.g. Worthy and Holdaway 1995, 1996), they cannot be applied to estimates of population size

In southern heavy-footed moa the palaeodemographic history inferred from the BSP and BayeSSC analyses also contradicted each other. While the BSP analysis suggested a constant population size until the Late Holocene followed by a decline, BayeSSC analysis suggested a constant population size through time. This is in contrast to southern heavy-footed moa populations from Southland, where the BSP and BayeSSC analyses suggested constant population size through time, contrary to relative fossil abundance data that suggested an increase in population size (Worthy 1998a, b; Worthy and Grant Mackie 2003). However, there are considerable taphonomic biases associated with the relative fossil abundance data for southern heavy-footed moa. This includes a paucity of Late Glacial and Pleistocene fossil localities, and accessioned material. It also includes an over abundance of swamp deposits compared to cave and sand dune deposits, which sample different aspects of the palaeofauna of southern New Zealand (Worthy 1998b). Further excavations of Late Glacial and Otiran Glacial deposits will help construct more robust relative abundance estimates of the different moa species in southern New Zealand.

Climate change affected heavy-footed moa allometry

Climate change has been linked with allometric variation in body size in many mammalian and avian species (Yom-Tov 2001; Worthy and Holdaway 2002; Millien 2004; Yom-Tov and Yom-Tov 2004; Millien *et al.* 2006; Yom-Tov *et al.* 2006). While Teplitsky *et al.* (2008) has argued that allometric size variation is the result of phenotypic plasticity, rather than a genetic response, there was not enough power in the data to fully reject a genetic response. In addition, allometric variation in body size could be the result of rapid epigenetic changes. The results from this study suggest that heavy-footed moa exhibited allometric size variation in response to temperature changes in accordance with Bergman's Rule (Bergman 1847; Figure 7, Table S4), which is an evolutionary response of taxa to cold climates to regulate body temperature. The results also corroborate the findings of Worthy and Holdaway (2002) suggesting heavy-footed moa from Canterbury exhibited allometric variation in body size through time. However, the results from this study show that the same pattern of allometric size variation is seen in two separate phylogeographic units that diverged about 500,700 years ago, suggesting that variation in body size through time is a common response in moa to climate change.

Hadly *et al.* (2004) showed that declines in population size can be concurrent with declines in mean body size, while Guthrie (2006) showed that *Equus* horses in North America went through a significant decline in body size leading up to their extinction about 12.5 Kya. The results from this study have shown that changes in population size can be concurrent with changes in mean body size. For example, in northern heavy-footed moa from North Canterbury and Marlborough, changes in population size inferred from the BSP and relative fossil abundance data are matched by changes in mean body size (Figure 8). However, the significance of these observations is uncertain, as constant population size could not be rejected in the BSP and BayeSSC analyses.

Climate change was not partly to blame for the extinction of the moa

In Holarctic species including Arctic fox (Dalen *et al.* 2007), bison (Shapiro *et al.* 2004; Drummond *et al.* 2005) and musk ox (Campos *et al.* 2010), climate change has been linked with population bottlenecks, local extinctions and the consequent loss of genetic diversity. In contrast, heavy-footed moa from New Zealand appear to have had a relatively stable population size throughout the Late Quaternary. From fossil distribution data (reviewed by Worthy and Holdaway 2002) it appears that some species of moa shifted their distributions from glacial to interglacial periods. In addition, morphological evidence presented in this study suggests that some species of moa exhibited allometric size variation. This suggests that climate change had no influence on the ultimate extinction of heavy-footed moa and did not predispose them to the over-hunting and habitat destruction by early Maori.

Acknowledgments

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References

Allen JA 1877. The influence of physical conditions in the genesis of species. Radical Review 1: 108-140.

Anderson CNK, Ramakrishnan U, Chan YL, Hadly EA 2006. Serial SimCoal: A population genetics model for data from multiple populations and points in time. Bioinformatics 21: 1733-1734.

Austin JJ, Arnold EN, Jones CG 2009. Interrelationships and history of silt-eared skinks (Gongylomorphus, Scincidae) of the Mascarene Islands, based on mitochondrial and nuclear gene sequences. Zootaxa 2153: 55-68.

Baker AJ, Huynen LJ, Haddrath O, Miller CD, Lambert DM 2005. Reconstructing the tempo and mode of evolution in an extinct clade of birds with ancient DNA: The giant moas of New Zealand. Proceedings of the National Academy of Science USA 102: 8257-8262.

Barnes I, Matheus P, Shapiro B, Jensen D, Cooper A 2002. Dynamics of Pleistocene population extinctions in Beringian brown bears. Science 295: 2267-2270.

Barnes IB, Shapiro B, Lister A, Kuznetsova T, Sher A, Guthrie D, Thomas MG 2007. Genetic structure and extinction of the woolly mammoth, *Mammuthus primigenius*. Current Biology 17: 1072-1075.

Barrell DJA, Alloway BV, Shulmeister J, Newnham RM (Eds) 2005. Towards a climate event stratigraphy for New Zealand over the past 30,000 years. Institute of Geological and Nuclear Sciences science report 2005/07, 12p. and poster.

Benton MJ, Twitchett RJ 2003. How to kill (almost) all life: the end-Permian extinction event. Trends in Ecology and Evolution 18: 358-365.

Bergman C 1847. Uber die verhaltnisse der warmeokonomie der thiere su ihrer grosse. Gottinger Studien Pt. 1: 595-708.

Bunce M, Worthy TH, Phillips MJ, Holdaway RN, Willerslev E, Haile J, Shapiro B, Scofield RP, Drummond A, Kaamp PJJ, Cooper A 2009. The evolutionary history of the extinct ratite moa and New Zealand Neogene paleogeography. Proceedings of the National Academy of Science USA 106: 20646-20651.

Burger J, Rosendahl W, Loreille OP, Hemmer H, Eriksson T, Gotherstrom A, Hiller J, Collins MJ, Wess T, Alt KW 2004. Molecular phylogeny of the extinct cave lion *Panthera leo spelaea*. Molecular Phylogenetics and Evolution 30: 841-849.

Burnham KP, Anderson DR 2002. Model selection and inference: A practical information – theoretic approach (2nd ed). Springer-Verlag, New York.

Burrows CJ, Greenland DE 1979. An analysis of the evidence for climatic change in the last thousand years: Evidence from diverse natural phenomena and from instrumental records. Journal of the Royal Society of New Zealand 9: 321-373.

Campos PF, Willerslev E, Sher A, Orlando L, Axelsson E, Tikhonov A, Aaris-Sorensen K, Greenwood AD, Kahlke RD, Kosintsev P, Krakhmalnaya T, Kuznetsova T, Lemey P, MacPhee R, Norris CA, Shepherd K, Suchard MA, Zazula GD, Shapiro B, Gilbert MTP 2010. Ancient DNA analyses exclude humans as the driving force behind late Pleistocene musk ox (*Ovibos moschatus*) population dynamics. Proceedings of the National Academy of Science USA 107: 5675-5680.

Cooper SJB, Ibrahim KM, Hewitt GM 1995. Postglacial expansion and genome subdivision in the European grasshopper *Chorthippus parallelus*. Molecular Ecology 4: 49-60.

Dalen L, Nystrom V, Valdiosera C, Germonpre M, Sablin M, Turner E, Angerbjorn A, Arsuaga SL, Gotherstrom A 2007. Ancient DNA reveals lack of postglacial habitat tracking in the arctic fox. Proceedings of the National Academy of Science USA 104: 6726-6729.

Darby J, Fordyce RE, Mark A, Probert K, Townsend C (Eds) 2004. The Natural History of Southern New Zealand. Otago University Press, Dunedin, New Zealand.

Debruyne R, Chu G, King CE, Bos K, Kuch M, Schwarz C, Szpak P, Grocke DR, Matheus P, Zazula G, Guthrie D, Froese D, Buigues B, Marliave C, Flemming C, Poinar D, Fisher D, Southon J, Tikhonov AN, MacPhee RDE, Poinar HN 2008. Out of America: Ancient DNA evidence for a New World origin of Late Quaternary woolly mammoths. Current Biology 18: 1320-1326.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7: 214.

Drummond AJ, Rambaut A, Shapiro B, Pybus OG 2005. Bayesian coalescent inference of past population dynamics from molecular sequences. Molecular Biology and Evolution 22: 1185-1192.

Excoffier L, Novembre J, Schneider S 2000. SIMCOAL: A general coalescent program for simulation of molecular data in interconnected populations with arbitrary demography. Journal of Heredity 91: 506-509.

Firestone RB, West A, Kennett JP, Becker L, Bunch TE, Revay ZS, Schultz PH, Belgya T, Kennett DJ, Erlandson JM, Dickenson OJ, Goodyear AC, Harris RS, Howard GA, Kloosterman JB, Lechler P, Mayewski PA, Montgomery J, Poreda R, Darrah T, Que Hee SS, Smith AR, Stich A, Topping W, Wittke JH, Wolback WS 2007. Evidence for an extraterrestrial impact 12,900 years ago that contributed to the megafaunal extinctions and the Younger Dryas cooling. Proceedings of the National Academy of Science USA 104: 16016-16021.

Goldstein PZ, Desalle R 2003. Calibrating phylogenetic species formation in a threatened insect using DNA from historical specimens. Molecular Ecology 12: 1993-1998.

Graham RW, Lundelius EL, Graham MA, Schroeder EK, Toomey RS, Anderson E, Barnosky AD, Burns JA, Churcher C, Grayson DK, Guthrie D, Harington CR, Jefferson GT, Martin LD, McDonald G, Morlan RE, Semken HA, Webb S, Werdelin L, Wilson MC 1996. Spatial responses of mammals to Late Quaternary environmental fluctuations. Science 272: 1601-1606.

Guthrie RD 2003. Rapid size decline in Alaskan Pleistocene horse before extinction. Nature 426: 169-171.

Guthrie RD 2006. New carbon dates link climatic change with human colonisation and Pleistocene extinctions. Nature 441: 207-209.

Hadly EA 1997. Evolutionary and ecological response of pocket gophers (*Thomomys talpoides*) to late-Holocene climatic change. Biological Journal of the Linnaean Society London 60: 277-296.

Hadly EA, Kohn MH, Leonard JA, Wayne RK 1998. A genetic record of population isolation in pocket gophers during Holocene climate change. Proceedings of the National Academy of Science USA 95: 6893-6896.

Hadly EA, Ramakrishnan U, Chan YL, Tuinen M, O'Keefe Spaeth PA, Conroy CJ 2004. Genetic response to climatic change: Insights from ancient DNA and phylochronology. PLoS Biology 2: e290.

Hewitt G 2000. The genetic legacy of the Quaternary ice ages. Nature 405: 907-913.

Hofreiter M, Serre D, Rohland N, Rabeder G, Nagel D, Conard N, Munsel S, Paabo S 2004. Lack of phylogeography in European mammals before the last glaciation. Proceedings of the National Academy of Science USA 101: 12963-12968.

Ho SYW, Larson G 2006. Molecular clocks: when times are a-changin'. Tends in Genetics 22: 79-83.

Ho SYW, Phillips MJ, Cooper A, Drummond AJ 2005. Time dependency of molecular rate estimates and systematic overestimation of recent divergence times. Molecular Biology and Evolution 22: 1561-1568.

Ibrahim KM, Nichols RA, Hewitt GM 1996. Spatial patterns of genetic divergence generated by different forms of dispersal during range expansion. Heredity 77: 282-291.

Knox GA (Ed) 1969. The natural history of Canterbury. AH and AW Reed, Wellington, New Zealand.

Lambert DM, Baker A, Huynen L, Haddrath O, Herbert PDN, Miller CD 2005. Is a large-scale DNA-based inventory of ancient life possible? Journal of Hereditary 96: 279-284.

Lessa EP, Cook JA, Patton JL 2003. Genetic footprints of demographic expansion in North America, but not Amazonia, during the Late Quaternary. Proceedings of the National Academy of Science USA 100: 10331-10334.

McGlone MS 1988. New Zealand. In Huntley B, Webb T III (Eds), Vegetation History. Kluwer Academic Publishers, Norwell, MA, USA, pp 557-599.

McGlone MS, Newnham RM, Moar NT 2010. The vegetation cover of New Zealand during the Last Glacial Maximum: Do pollen records under-represent woody vegetation? In: Haberle SG, Stevenson J, Prebble M (Eds). Altered Ecologies: Fire, Climate and Human Influence on Terrestrial Landscapes. Terra Australis 32. ANU E-Press, Australian National University, Canberra, Australia.

Millien V 2004. Relative effects of climate change, isolation and competition on body size evolution in the Japanese field mouse, *Apodemus argenteus*. Journal of Biogeography 31: 1267-1276.

Millien V Lyons K, Olson L, Smith FA, Wilson AB, Yom-Tov Y 2006. Ecotypic variation in the context of global climate change: Revisiting the rules. Ecology Letters 9: 853-869.

Moar NT 1971. Contributions to the Quaternary history of the New Zealand flora 6: Aranuiun pollen diagrams from Canterbury, Nelson, and North Westland, South Island. New Zealand Journal of Botany 9: 80-145.

Moar NT 2008. Late Quaternary Vegetation. In: Winterbourn M, Knox G, Burrows C, Marsden I (Eds.) 2008. The Natural History of Canterbury. Christchurch, Canterbury University Press.

Moar NT, Suggate RP 1973. Pollen analysis of Late Otiran and Aranuian sediments at Blue Spur Road (S51), North Westland. New Zealand Journal of Geology and Geophysics 16: 333-344.

Moar NT, Suggate RP 1979. Contributions to the Quaternary history of New Zealand flora 8: Interglacial and glacial vegetation in the Westport District South Island. New Zealand Journal of Botany 17: 361-87.

Munoz-Fuentes V, Green AJ, Negro JJ, Sorenson MD 2005. Population structure and loss of genetic diversity in the endangered white-headed duck, *Oxyura leucocephala*. Conservation Genetics 6: 999-1015.

Orlando L, Metcalf J, Alberdi MT, Telles-Antunes M, Bonhean D, Otte M, Martin F, Eisenmann V, Mashkour M, Morello F, Prado JL, Salas-Gismondi R, Shockey BJ, Wrinn PJ, Vasil-ev SK, Ovodov ND, Cherry MI, Hopwood B, Male D, Austin JJ, Hanni C, Cooper A 2009. Revising the recent evolutionary history of equids using ancient DNA. Proceedings of the National Academy of Science USA 106: 21754-21759.

Overpeck J, Witlock C, Huntley B 2002. In: Alverson K, Bradley R, Pedersen T (Eds). Paleoclimate, Global Change and the Future. Springer, Berlin. pp. 81-103.

Parmesan C, Yobe G 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature 421: 37-42.

Pounds JA, Fogden MLP, Campbell JH 1999. Biological response to climate change on a tropical mountain. Nature 398: 611-615.

Ramakrishnan U, Hadly EA 2009. Using phylochronology to reveal population histories: review and synthesis of 29 ancient DNA studies. Molecular Ecology 18: 1310-1330.

Rambaut A, Ho SYW, Drummond AJ, Shapiro B 2008. Accommodating the effect of ancient DNA damage of inferences of demographic histories. Molecular Biology and Evolution 26: 245-248.

Rohland N, Pollack JL, Nagel D, Beauval C, Airvaux J, Paabo S, Hofreiter M 2005. The population history of extant and extinct hyenas. Molecular Biology and Evolution 22: 2435-2443.

Root TL, Price JT, Hall KR, Schneider SH, Rosenzweig C, Pounds JA 2003. Fingerprints of global warming on wild animals and plants. Nature 421: 57-60.

Shapiro B, Drummond AJ, Rambaut A, Wilson MC, Matheus PE, Sher AV, Pybus OG, Gilbert MTP, Barnes I, Binladen J, Willerslev E, Hansen AJ, Baryshnikov GF, Burns JA, Davydov S, Driver JC, Froese DG, Harington CR, Keddie G, Kosintsev P, Kunz ML, Martin LD, Stephenson RO, Storer J, Tedford R, Zimov S, Cooper A 2004. Rise and fall of the Beringian Steppe Bison. Science 306: 1561-1565.

Smith FA, Betancourt JL, Brown JH 1995. Evolution of body size in the wood rat over the past 25,000 years of climate change. Science 270: 2012-2014.

Stiller M, Baryshnikov G, Bocherens H, d'Anglade G, Hilpert B, Munzel SC, Pinhasi R, Rabeder G, Rosendahl W, Trinkaus E, Hofreiter M, Knapp M 2010. Withering away – 25, 000 years of genetic decline preceded cave bear extinction. Molecular Biology and Evolution 27: 975-978.

Taberlet P, Fumagalli L, West-Saucy A, Cossons JF 1998. Comparative phylogeography and post-glacial colonisation routes in Europe. Molecular Ecology 7: 453-464.

Tennyson A, Martinson P 2006. Extinct birds of New Zealand. Te Papa Press, Wellington, New Zealand.

Teplitsky C, Mills JA, Alho JS, Warrall JW, Merila J 2008. Bergman's rule and climate change revisited: Disentangling environmental and genetic responses in a wild bird population. Proceedings of the National Academy of Science USA 105: 12492-13496.

Thomas CD, Cameron A, Green RE, Bakkenes M, Beaumont LJ, Collingham TC, Erasmus BFN, Siqueira MF, Grainger A, Hannah L, Hughes L, Huntley B, Jaarsveld AS, Midgley GF, Miles L, Ortega-Huerta MA, Perterson AT, Phillips OL, Williams SE 2004. Extinction risk from climate change. Nature 427: 145-148.

Thuiller W 2003. BIOMOD: Optimising predictions of species distributions and projecting potential future shifts under global change. Global Change Biology 9: 1353-1362.

Thuiller W 2004. Patterns and uncertainties of species' range shifts under climate change. Global Change Biology 10, 2020-2027.

Vandergoes MJ, Fitzimons SJ 2003. The Last Glacial-Interglacial Transition (LGIT) in south Westland, New Zealand: Palaeoecological insight into mid-latitude Southern Hemisphere climate change. Quaternary Science Reviews 22: 1461-1476.

Wardle P 1991. Vegetation of New Zealand. Cambridge University Press, Cambridge, UK.

Wilmshurst JM, Anderson AJ, Higham TFG, Worthy TH 2008. Dating the late prehistoric dispersal of Polynesians to New Zealand using the commensal Pacific rat. Proceedings of the National Academy of Science USA 105: 7676-7680.

Worthy TH 1993a. A review of fossil bird bones from loess deposits in eastern South Island, New Zealand. Records of the Canterbury Museum 10: 95-106.

Worthy TH 1993b. Fossils of Honeycomb Hill. Museum of New Zealand Te Papa Tongawera, Wellington, New Zealand

Worthy TH 1994. Late Quaternary changes in the moa fauna on the West Coast of the South Island, New Zealand. Records of the South Australian Museum 27: 125-134.

Worthy TH 1997. Fossil deposits in the Hodge Creek Cave System, on the northern foothills of Mt Arthur, Nelson, South Island, New Zealand. Notornis 44: 111-124.

Worthy TH 1998a. Quaternary fossil faunas of Otago, South Island, New Zealand. Journal of the Royal Society of New Zealand 28: 421-521.

Worthy TH 1998b. The Quaternary fossil avifauna of Southland, South Island, New Zealand. Journal of the Royal Society of New Zealand 28: 537-589.

Worthy TH 1999. Changes induced in the New Zealand avifauna by climate during the last glacial-interglacial period. Proceedings of the 4th International Meeting of the Society of

Avian Paleontology and Evolution (Washington, D.C., June 1996). Smithsonian Contributions to Palaeobiology 89: 111-123.

Worthy TH, Grant-Mackie JA 2003. Late-Pleistocene avifaunas from Cape Wanbrow, Otago, South Island, New Zealand. Journal of the Royal Society of New Zealand 33: 427-485.

Worthy TH, Holdaway RN 1994. Quaternary fossil faunas from caves in Takaka valley and on Takaka Hill, northwest Nelson, South Island, New Zealand. Journal of the Royal Society of New Zealand 24: 297-391.

Worthy TH, Holdaway RN 1995. Quaternary fossil faunas from caves on Mt Cookson, North Canterbury, New Zealand. Journal of the Royal Society of New Zealand 25: 333-370.

Worthy TH, Holdaway RN 1996. Quaternary fossil faunas, overlapping taphonomies, and palaeofaunal reconstruction in North Canterbury, South Island, New Zealand. Journal of the Royal Society of New Zealand 26: 275-361.

Worthy TH, Holdaway RN 2002. The lost world of the moa: Prehistoric life of New Zealand. Canterbury University Press, Christchurch, New Zealand.

Yom-Tov Y 2001. Global warming and body mass decline in Israeli passerine birds. Proceedings of the Royal Society of London Series B 268: 947-952.

Yom-Tov Y, Yom-Tov S 2004. Climate change and body size in two species of Japanese rodents. Biological Journal of the Linnaean Society London 82: 263-267.

Yom-Tov Y, Yom-Tov S, Wright J, Thorne CJR, Feu RD 2006. Recent changes in body weight and wing length among some British passerine birds. Oikos 112: 91-101.

The effect of Late Quaternary climate change on heavy-footed moa (*Pachyornis elephantopus*).

Supplementary Information

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Table S1 – Information regarding heavy-footed moa (*Pachyornis elephantopus*) samples used in this study. For AMS ^{14}C date category, 'radiocarbon' means the specimen has a finite radiocarbon date, 'stratigraphic' means the specimen is undated but has a mean stratigraphic date associated with the specimen, and 'estimate' means that there are no dates associated with the fossil locality but a broad stratigraphic date can be estimated based on geography and stratigraphy. Grey bars indicate specimens that could potentially belong to multiple haplotypes because of missing sequence data or could not be placed in a discrete time bin and were therefore excluded from the phylogeographic and/or Arlequin and BayeSSC analysis.

Sample	Moa species (DNA)	Sample location	Geographic region	Museum number*	AMS ^{14}C date	AMS ^{14}C date category
A3786	*P. elephantopus* north	Gradungula Passage	West Coast	NRS 353, in situ	19753	stratigraphic
A3719	*P. elephantopus* north	Graveyard	West Coast	MNZ S23798	19007	stratigraphic
A3721	*P. elephantopus* north	Graveyard	West Coast	MNZ S22720	19007	stratigraphic
A3728	*P. elephantopus* north	Graveyard	West Coast	MNZ S25860	19007	stratigraphic
A3749	*P. elephantopus* north	Predator Cave	NW Nelson	MNZ S32425	32230 +/- 380	radiocarbon
A3755	*P. elephantopus* north	Takaka Hill	NW Nelson	DM417E	20330 +/- 90	radiocarbon
A3756	*P. elephantopus* north	Takaka Hill	NW Nelson	DM417E	14145 +/- 60	radiocarbon
A4280	*P. elephantopus* north	Sentinel Rock	Marlborough	MNZ S28439	-	-
A3906	*P. elephantopus* north	Wairau River	Marlborough	MM 2005/22 Box AA	3806	stratigraphic
A3913	*P. elephantopus* north	Wairau River	Marlborough	MM 2005/22 Box 27	3700 +/- 29	radiocarbon
A3916	*P. elephantopus* north	Wairau River	Marlborough	MM 2005/22 Box CC (2/3)	3026 +/- 28	radiocarbon
A3917	*P. elephantopus* north	Wairau River	Marlborough	MM 2005/22 Box CC (2/3)	4694 +/- 35	radiocarbon
A3918	*P. elephantopus* north	Wairau River	Marlborough	MM 2005/22 Box CC (2/3)	3806	stratigraphic
AY299919	*P. elephantopus* north	Molesworth Station	Marlborough	CM Av16217	2277	stratigraphic
A4252	*P. elephantopus* north	Molesworth Station	Marlborough	CM Av16217	2277 +/- 27	radiocarbon
A3754	*P. elephantopus* north	Blenheim	Marlborough	DM206	995 +/- 24	radiocarbon
A3899	*P. elephantopus* north	Limestone Creek	Marlborough	KM N99-1017	663 +/- 24	radiocarbon
A3900	*P. elephantopus* north	Limestone Creek	Marlborough	KM N99-1016	668 +/- 24	radiocarbon
A2548	*P. elephantopus* north	Merino Cave West	North Canterbury	MNZ 33402.82	22690	stratigraphic
A2550	*P. elephantopus* north	Merino Cave West	North Canterbury	MNZ 33402.36	19060 +/- 90	radiocarbon
A3751	*P. elephantopus* north	Merino Cave East	North Canterbury	MNZ S33402.32	32903	stratigraphic
A2552	*P. elephantopus* north	Merino Cave West	North Canterbury	MNZ S33403.1	16861	stratigraphic
A2553	*P. elephantopus* north	Merino Cave West	North Canterbury	MNZ S33403.2	16861	stratigraphic
A2554	*P. elephantopus* north	Merino Cave West	North Canterbury	MNZ S33403.3	14655 +/- 230	radiocarbon

GU139073	*P. elephantopus* north	Glencrieff	North Canterbury	MNZ S32671.1	10848	stratigraphic
A2533	*P. elephantopus* north	Glencrieff	North Canterbury	MNZ S32670.2	10760 +/- 70	radiocarbon
A2534	*P. elephantopus* north	Glencrieff	North Canterbury	MNZ S32670.3	11490 +/- 80	radiocarbon
A2535	*P. elephantopus* north	Glencrieff	North Canterbury	MNZ S32670.9	11230 +/- 210	radiocarbon
A2536	*P. elephantopus* north	Glencrieff	North Canterbury	MNZ S32670.8	10680 +/- 70	radiocarbon
A2537	*P. elephantopus* north	Glencrieff	North Canterbury	MNZ S32670.7	11390 +/- 130	radiocarbon
A3811	*P. elephantopus* north	Glencrieff	North Canterbury	CM GC136	10848	stratigraphic
A3812	*P. elephantopus* north	Glencrieff	North Canterbury	CM GC196	10848	stratigraphic
A3814	*P. elephantopus* north	Glencrieff	North Canterbury	CM GC122	10848	stratigraphic
A3815	*P. elephantopus* north	Glencrieff	North Canterbury	CM GC170	10848	stratigraphic
A3817	*P. elephantopus* north	Glencrieff	North Canterbury	CM GC84	10848	stratigraphic
AY299915	*P. elephantopus* north	Pyramid Valley	North Canterbury	CM Av15036	3583	stratigraphic
DQ055492	*P. elephantopus* north	Pyramid Valley	North Canterbury	CM Av8389	3583	stratigraphic
GU139075	*P. elephantopus* north	Pyramid Valley	North Canterbury	CM Av8389	3583	stratigraphic
A2630	*P. elephantopus* north	Pyramid Valley	North Canterbury	CM Av151029	3583	stratigraphic
A2631	*P. elephantopus* north	Pyramid Valley	North Canterbury	CM Av8383	3583	stratigraphic
A2632	*P. elephantopus* north	Pyramid Valley	North Canterbury	CM Av8388	3583	stratigraphic
A2634	*P. elephantopus* north	Pyramid Valley	North Canterbury	CM Av8384	3583	stratigraphic
GU139072	*P. elephantopus* north	Broken River	North Canterbury	MNZ S196	1151 +/- 30	radiocarbon
A2563	*P. elephantopus* north	Broken River	North Canterbury	DM323	3800 +/- 60	radiocarbon
A2564	*P. elephantopus* north	Broken River	North Canterbury	DM196.1	2475	stratigraphic
A2565	*P. elephantopus* north	Broken River	North Canterbury	DM196.2	2475	stratigraphic
AY299916	*P. elephantopus* north	Treasure Downs	North Canterbury	CM SB301	1715	stratigraphic
AY299917	*P. elephantopus* north	Treasure Downs	North Canterbury	CM SB152	1715	stratigraphic
DQ055488	*P. elephantopus* north	Treasure Downs	North Canterbury	CM THW215	1715	stratigraphic
DQ055489	*P. elephantopus* north	Treasure Downs	North Canterbury	CM THW214	1715	stratigraphic
DQ055490	*P. elephantopus* north	Treasure Downs	North Canterbury	CM SB306	1715	stratigraphic
GU139077	*P. elephantopus* north	Treasure Downs	North Canterbury	CM SB299	1546 +/- 26	radiocarbon
A2635	*P. elephantopus* north	Treasure Downs	North Canterbury	CM Av266	1715	stratigraphic
A2646	*P. elephantopus* north	Treasure Downs	North Canterbury	CM SB143/174	1715	stratigraphic
A2650	*P. elephantopus* north	Treasure Downs	North Canterbury	CM SB144	1715	stratigraphic
GU139076	*P. elephantopus* north	Bell Hill	North Canterbury	MNZ S40024	1345	stratigraphic
GU129075	*P. elephantopus* north	Bell Hill	North Canterbury	MNZ S40023	1345	stratigraphic
A2538	*P. elephantopus* north	Bell Hill	North Canterbury	MNZ S39929.8	1345	stratigraphic
A2540	*P. elephantopus* north	Bell Hill	North Canterbury	MNZ S40220	1345	stratigraphic
A2541	*P. elephantopus* north	Bell Hill	North Canterbury	MNZ S40351	1345	stratigraphic

A2542	*P. elephantopus* north	Bell Hill	North Canterbury	MNZ S40350	1345	stratigraphic
A2543	*P. elephantopus* north	Bell Hill	North Canterbury	MNZ S40009	1345	stratigraphic
A3712	*P. elephantopus* north	Sloven's Creek	North Canterbury	AIM 5915	-	-
A2623	*P. elephantopus* north	Albury Park	South Canterbury	CM Av19320	3976 +/- 26	radiocarbon
A2655	*P. elephantopus* north	Albury Park	South Canterbury	SCM 2004/153.2	7013	stratigraphic
A4251	*P. elephantopus* north	Albury Park	South Canterbury	CM Av19307	7013	stratigraphic
DQ055493	*P. elephantopus* north	Kapua	South Canterbury	CM Av8624	2882	stratigraphic
A2625	*P. elephantopus* north	Kapua	South Canterbury	CM Av8944	2882	stratigraphic
A2626	*P. elephantopus* north	Kapua	South Canterbury	CM Av9172	2882	stratigraphic
A4257	*P. elephantopus* north	Kapua	South Canterbury	CM Av8915	2882	stratigraphic
A4258	*P. elephantopus* north	Kapua	South Canterbury	CM Av8913	2882	stratigraphic
A4259	*P. elephantopus* north	Kapua	South Canterbury	CM Av8916	2882	stratigraphic
A4260	*P. elephantopus* north	Kapua	South Canterbury	CM Av9067	2882	stratigraphic
A2657	*P. elephantopus* north	Opawa Station	South Canterbury	SCM 1995/13	2440 +/- 110	radiocarbon
A2659	*P. elephantopus* north	Opawa Station	South Canterbury	SCM 1995/13.29	2045	stratigraphic
A2643	*P. elephantopus* north	Mt Hay	South Canterbury	CM Av38939	1488 +/- 24	radiocarbon
A2644	*P. elephantopus* north	Mt Hay	South Canterbury	CM Av38940	941 +/- 27	radiocarbon
A2662	*P. elephantopus* north	Ardgowan	North Otago	NOM un-registered	Late Holocene	estimate
A2663	*P. elephantopus* north	Ardgowan	North Otago	NOM un-registered	Late Holocene	estimate
A2665	*P. elephantopus* north	Ardgowan	North Otago	NOM un-registered	Late Holocene	estimate
A2669	*P. elephantopus* north	Spring Gully	North Otago	NOM un-registered	-	-
A2670	*P. elephantopus* north	Spring Gully	North Otago	NOM un-registered	Late Holocene	estimate
A2671	*P. elephantopus* north	Spring Gully	North Otago	NOM un-registered	Late Holocene	estimate
A2672	*P. elephantopus* north	Spring Gully	North Otago	NOM un-registered	-	-
A2674	*P. elephantopus* north	Five Forks	North Otago	NOM un-registered	Late Holocene	estimate
A2675	*P. elephantopus* north	Five Forks	North Otago	NOM un-registered	Late Holocene	estimate
AY299918	*P. elephantopus* north	Enfield	North Otago	OM Av4139	2020	stratigraphic
DQ023686	*P. elephantopus* north	Enfield	North Otago	OM Av4139	2020	stratigraphic
DQ023687	*P. elephantopus* north	Enfield	North Otago	OM Av9209	2020	stratigraphic
A2618	*P. elephantopus* north	Enfield	North Otago	CM Av8955	2020	stratigraphic
A2666	*P. elephantopus* north	Enfield	North Otago	NOM un-registered	2020	stratigraphic
A2667	*P. elephantopus* north	Enfield	North Otago	NOM un-registered	2020	stratigraphic
A2689	*P. elephantopus* north	Enfield	North Otago	OM Av4139	2020	stratigraphic
A3710	*P. elephantopus* north	Kia Ora	North Otago	AM 5980	1878 +/- 29	radiocarbon
A3825	*P. elephantopus* north	Prydes Gully	North Otago	OM Av7929	-	-
A3881	*P. elephantopus* north	Shag River	North Otago	OUAD SM/A2-BM-1	673	stratigraphic

AY299920	*P. elephantopus* north	Paerau	Central Otago	CM Av9820	3946	stratigraphic
A2714	*P. elephantopus* north	Paerau	Central Otago	OM Av2452	3946	stratigraphic
A2717	*P. elephantopus* north	Paerau	Central Otago	OM Av2253	3946	stratigraphic
A2724	*P. elephantopus* north	Paerau	Central Otago	OM Av2451	3946	stratigraphic
A2725	*P. elephantopus* north	Paerau	Central Otago	OM Av2450	3946	stratigraphic
A2726	*P. elephantopus* north	Paerau	Central Otago	OM Av2435	3946	stratigraphic
A4261	*P. elephantopus* north	Paerau	Central Otago	OM Av10007	3946	stratigraphic
A4264	*P. elephantopus* north	Paerau	Central Otago	OM Av9933	3946	stratigraphic
A4265	*P. elephantopus* north	Paerau	Central Otago	OM Av9904	3946	stratigraphic
A4267	*P. elephantopus* north	Paerau	Central Otago	OM Av9905	3946	stratigraphic
A4268	*P. elephantopus* north	Paerau	Central Otago	OM Av9936	3946	stratigraphic
A4269	*P. elephantopus* north	Paerau	Central Otago	OM Av9903	3946	stratigraphic
A4270	*P. elephantopus* north	Paerau	Central Otago	OM Av9938	3946	stratigraphic
A2690	*P. elephantopus* north	Styx	Central Otago	OM Av un-registered (Styx 112)	2615	stratigraphic
A2691	*P. elephantopus* north	Styx	Central Otago	OM Av un-registered (Styx 101)	3520 +/- 95	radiocarbon
A2693	*P. elephantopus* north	Styx	Central Otago	OM Av un-registered (Styx 53)	2615	stratigraphic
A2694	*P. elephantopus* north	Styx	Central Otago	OM un-registered	2615	stratigraphic
A2729	*P. elephantopus* north	Hamilton's	Central Otago	OM Av4057	2296	stratigraphic
A2753	*P. elephantopus* north	Hamilton's	Central Otago	OM Av4069	2296	stratigraphic
A2058	*P. elephantopus* north	Ida Valley	Central Otago	OUAD FB245	2818	stratigraphic
A2059	*P. elephantopus* north	Ida Valley	Central Otago	OUAD FB419	2818	stratigraphic
GU139068	*P. elephantopus* north	Chatto	Central Otago	OM Av4064 [C542]	1641	stratigraphic
A2712	*P. elephantopus* north	Chatto	Central Otago	OM Av un-registered	1045 +/- 20	radiocarbon
A2731	*P. elephantopus* north	Lawrence	Central Otago	Om Av4041	-	-
A2781	*P. elephantopus* north	Moa Creek	Central Otago	Clyde Museum un-registered	Late Holocene	estimate
AY299921	*P. elephantopus* north	Maniototo	Central Otago	CM Av8927	-	-
DQ055491	*P. elephantopus* north	Unknown locality	Unknown locality	CM Av9142	-	-
A2656	*P. elephantopus* north	Unknown locality	Unknown locality	SCM NH015	-	-
A2660	*P. elephantopus* north	Unknown locality	Unknown locality	SCM NH013	-	-
A2580	*P. elephantopus* south	Herbert	North Otago	DM362	12565 +/- 45	radiocarbon
A3872	*P. elephantopus* south	Pleasant River	North Otago	OUAD 322	590	stratigraphic
A2698	*P. elephantopus* south	Tom Arthur's	Central Otago	OM Av10787	3790 +/- 60	radiocarbon
A2696	*P. elephantopus* south	Styx	Central Otago	OM Styx 32	1710 +/- 60	radiocarbon
GU139069	*P. elephantopus* south	Chatto	Central Otago	OM Av7941	1641	stratigraphic
A2700	*P. elephantopus* south	Chatto	Central Otago	OM Av4065	1641	stratigraphic

A2701	*P. elephantopus* south	Chatto	Central Otago	OM Av7941	1731 +/- 63	radiocarbon
A2713	*P. elephantopus* south	Chatto	Central Otago	OM Av4064	1100 +/- 70	stratigraphic
A3821	*P. elephantopus* south	Chatto	Central Otago	OM Av7942	1641	stratigraphic
A3826	*P. elephantopus* south	Chatto	Central Otago	OM Av7961	1641	stratigraphic
A3883	*P. elephantopus* south	Chatto	Central Otago	OUAD FB117	1641	stratigraphic
A4282	*P. elephantopus* south	Chatto	Central Otago	MNZ S44738	1641	stratigraphic
A2587	*P. elephantopus* south	Bannockburn	Central Otago	MNZ S38933	930 +/- 27	radiocarbon
AY261990	*P. elephantopus* south	Clutha	Central Otago	CUM 381A	Late Holocene	estimate
GU139070	*P. elephantopus* south	Nevis	Central Otago	CUM 381A	Late Holocene	estimate
AC32	*P. elephantopus* south	Hector Range	Central Otago	ACAD collection	Late Holocene	estimate
A2754	*P. elephantopus* south	West Wanaka Station	Central Otago	OM Av7488	1259 +/- 25	radiocarbon
A3884	*P. elephantopus* south	Stotburn, Maniototo	Central Otago	OUAD FB121	Late Holocene	estimate
A2776	*P. elephantopus* south	Chard Farm	Central Otago	LDM F9	1258 +/- 25	radiocarbon
A2778	*P. elephantopus* south	Chard Farm	Central Otago	LDM F25	4613 +/- 29	radiocarbon
A2779	*P. elephantopus* south	Chard Farm	Central Otago	LDM F44	2485	stratigraphic
A2777	*P. elephantopus* south	Muddy Creek	Central Otago	LDM F40	965 +/- 24	radiocarbon
A2045	*P. elephantopus* south	Daley's Flat	West Otago	OM Av10428	956	stratigraphic
A2046	*P. elephantopus* south	Daley's Flat	West Otago	OM Av10428	820 +/- 70	radiocarbon
A2047	*P. elephantopus* south	Daley's Flat	West Otago	OM Av10421	1060 +/- 70	radiocarbon
A2048	*P. elephantopus* south	Daley's Flat	West Otago	OM Av10422	990 +/- 60	radiocarbon
A2049	*P. elephantopus* south	Daley's Flat	West Otago	OM Av10426	956	stratigraphic
A2050	*P. elephantopus* south	Daley's Flat	West Otago	OM Av10430	956	stratigraphic
A2051	*P. elephantopus* south	Daley's Flat	West Otago	OM Av10430	956	stratigraphic
A2052	*P. elephantopus* south	Daley's Flat	West Otago	OM Av10421	956	stratigraphic
A2053	*P. elephantopus* south	Daley's Flat	West Otago	OM Av10424	956	stratigraphic
DQ023688	*P. elephantopus* south	Shotover River	West Otago	OM Av4068	-	-
A2775	*P. elephantopus* south	Lakes District	West Otago	LDM F72	-	-
A3886	*P. elephantopus* south	Hollyford Valley	West Otago	OUAD FC496	Late Holocene	estimate
A2687	*P. elephantopus* south	Owaka	Southland	OM Av4075	24400 +/- 110	radiocarbon
DQ023689	*P. elephantopus* south	Opio	Southland	CM Av38563	22866	stratigraphic
A2638	*P. elephantopus* south	Opio	Southland	CM Av38717	22866	stratigraphic
A2639	*P. elephantopus* south	Opio	Southland	CM Av38717	22866	stratigraphic
A2640	*P. elephantopus* south	Opio	Southland	CM Av38717	21250 +/- 80	radiocarbon
A2642	*P. elephantopus* south	Opio	Southland	CM Av38717	25780 +/- 160	radiocarbon
A2761	*P. elephantopus* south	Opio	Southland	SMAG 2003.108.1	21570 +/- 37	radiocarbon
A2756	*P. elephantopus* south	Avondale	Southland	SMAG 88.95	2885 +/- 28	radiocarbon
A2757	*P. elephantopus* south	Avondale	Southland	SMAG 88.95	12395 +/- 45	radiocarbon

A2762	*P. elephantopus* south	Eastern Bush	Southland	SMAG un-registered	10900 +/- 200	radiocarbon
A3865	*P. elephantopus* south	Hamilton's	Southland	SMAG un-registered	4869	stratigraphic
A2759	*P. elephantopus* south	Riverton	Southland	SMAG E80.13	1336 +/- 24	radiocarbon
A2760	*P. elephantopus* south	Riverton	Southland	SMAG E80.13	1336	stratigraphic
A2763	*P. elephantopus* south	Riverton	Southland	SMAG E80.13	1336	stratigraphic
A2764	*P. elephantopus* south	Riverton	Southland	SMAG E80.13	1336	stratigraphic
A2545	*P. elephantopus* south	Kauana	Southland	MNZ S34515.1	35880 +/- 660	radiocarbon
A2589	*P. elephantopus* south	Kauana	Southland	MNZ S34519.1	50200 +/- 3500	radiocarbon
A2590	*P. elephantopus* south	Kauana	Southland	MNZ S34519.2	>49500	radiocarbon
GU139071	*P. elephantopus* south	Stewart Island	Southland	OM Av4661 [C.09.29]	654 +/- 56	radiocarbon

* OM: Otago Museum; SMAG: Southland Museum and Art Gallery; OUAD: Otago University Anthropology Department; LDM: Lakes District Museum; CUM: Cambridge University Museum; DM: Dominion Museum, now Museum of New Zealand Te Papa Tongawera; NOM: North Otago Museum; SCM: South Canterbury Museum; KM: Kaikoura Museum; MM: Marlborough Museum; MNZ: Museum of New Zealand Te Papa Tongawera; CM: Canterbury Museum; ACAD; Australian Centre for Ancient DNA.

Table S2 Carbon dates and stable isotope data generated in this study. Shaded grey rows are previously published radiocarbon dates.

Sample	Moa species (DNA)	Sample location	Museum no.	Lab no.	^{14}C date	C:N ratio	δ^{15} N	δ^{13}C
A3749	*P. elephantopus* north	Predator Cave	MNZ S32425	OxA-20336	32230 +/- 380	3.39623	1.42	-20.53
A3755	*P. elephantopus* north	Takaka Hill	DM417E	OxA-20292	20330 +/- 90	3.37509	2.30	-21.67
A3756	*P. elephantopus* north	Takaka Hill	DM417E	OxA-20293	14145 +/- 60	3.37008	0.90	-21.86
A3913	*P. elephantopus* north	Wairau River	MM 2005/22 Box 27	OxA-20341	3700 +/- 29	3.3695	7.49	-25.32
A3916	*P. elephantopus* north	Wairau River	MM 2005/22 Box CC (2/3)	OxA-20342	3026 +/- 28	3.38446	7.83	-23.13
A3917	*P. elephantopus* north	Wairau River	MM 2005/22 Box CC (2/3)	OxA-20343	4694 +/- 35	3.40376	6.36	-24.01
A4252	*P. elephantopus* north	Molesworth Station	CM Av16217	OxA-20344	2277 +/- 27	3.34381	4.81	-23.06
A3754	*P. elephantopus* north	Blenheim	DM206	OxA-20345	995 +/- 24	3.43328	7.20	-23.86
A3899	*P. elephantopus* north	Limestone Creek	KM N99-1017	OxA-20339	663 +/- 24	3.34498	6.36	-22.79
A3900	*P. elephantopus* north	Limestone Creek	KM N99-1016	OxA-20340	668 +/- 24	3.35757	5.82	-22.33
A2550	*P. elephantopus* north	Merino Cave West	MNZ 33402.36	CURL-10269	19060 +/- 90	n/a	n/a	-20.4
A2554	*P. elephantopus* north	Merino Cave West	MNZ S33403.3	ANUA-38613	14655 +/- 230	n/a	n/a	-22.9
A2533	*P. elephantopus* north	Glencrieff	MNZ S32670.2	ANU-4924	10760 +/- 70	n/a	n/a	-23.8
A2534	*P. elephantopus* north	Glencrieff	MNZ S32670.3	ANU-1606	11490 +/- 80	n/a	n/a	-23.3
A2535	*P. elephantopus* north	Glencrieff	MNZ S32670.9	ANU-1607	11230 +/- 210	n/a	n/a	-23.6
A2536	*P. elephantopus* north	Glencrieff	MNZ S32670.8	ANU-7625	10680 +/- 70	n/a	n/a	-23.5
A2537	*P. elephantopus* north	Glencrieff	MNZ S32670.7	ANU-1610	11390 +/- 130	n/a	n/a	-23.0
A2563	*P. elephantopus* north	Broken River	DM323	ANU-4936	3800 +/- 60	n/a	n/a	-23.4
GU139072	*P. elephantopus* north	Broken River	MNZ S196	OxA-12432	1151 +/- 30	3.338	5.554	-23.271
GU139077	*P. elephantopus* north	Treasure Downs	CM SB299	OxA-12665	1546 +/- 26	3.3	8.0	-25.4
A2623	*P. elephantopus* north	Albury Park	CM Av19320	OxA-20365	3976 +/- 26	3.32318	5.71	-22.87
A2657	*P. elephantopus* north	Opawa Station	SCM 1995/13	ANU-1622	2440 +/- 110	n/a	n/a	-24.7
A2643	*P. elephantopus* north	Mt Hay	CM Av38939	OxA-20337	1488 +/- 24	3.35818	4.69	-22.92
A2644	*P. elephantopus* north	Mt Hay	CM Av38940	OxA-20338	941 +/- 27	3.36216	5.32	-23.28
A2691	*P. elephantopus* north	Styx	OM unregistered Styx 101	ANU1624	3520 +/- 95	n/a	n/a	-23.4
A2712	*P. elephantopus* north	Chatto	OM unregistered	CURL-10270	1045 +/- 20	n/a	n/a	-17.4
A2701	*P. elephantopus* south	Chatto	OM Av7941	NZA-5919	1731 +/- 63	n/a	n/a	-23.5
A2713	*P. elephantopus* south	Chatto	OM Av4064	ANU-4931	1100 +/- 70	n/a	n/a	-23.2
A2580	*P. elephantopus* south	Herbert	DM362	OxA-20324	12565 +/- 45	3.37677	5.76	-24.77
A2698	*P. elephantopus* south	Tom Arthur's	OM Av10787	ANU-1618	3790 +/- 60	n/a	n/a	-23.5
A2696	*P. elephantopus* south	Styx	OM Styx 32	ANU-1625	1710 +/- 60	n/a	n/a	-23.5
A2587	*P. elephantopus* south	Bannockburn	MNZ S38933	OxA-20332	930 +/- 27	3.3788	4.64	-21.83
A2776	*P. elephantopus* south	Chard Farm	LDM F9	OxA-20329	1258 +/- 25	3.23246	5.50	-23.78
A2778	*P. elephantopus* south	Chard Farm	LDM F25	OxA-20364	4613 +/- 29	3.37122	6.93	-22.71
A2779	*P. elephantopus* south	Chard Farm	LDM unregistered	OxA-20330	1586+/-24	3.24543	8.47	-21.07
A2754	*P. elephantopus* south	West Wanaka Station	OM Av7488	OxA-20328	1259 +/- 25	3.34359	4.46	-23.33
A2777	*P. elephantopus* south	Muddy Creek	LDM F40	OxA-20331	965 +/- 24	3.33006	7.26	-24.74
A2046	*P. elephantopus* south	Daley's Flat	OM Av10428	ANU-4932	820 +/- 70	n/a	n/a	n/a
A2047	*P. elephantopus* south	Daley's Flat	OM Av10421	ANU-4935	1060 +/- 70	n/a	n/a	-22.4
A2048	*P. elephantopus* south	Daley's Flat	OM Av10422	ANU-4933	990 +/- 60	n/a	n/a	-22.0
A2640	*P. elephantopus* south	Opio	CM unregistered	OxA-20334	21250 +/- 80	3.40775	5.20	-21.47
A2642	*P. elephantopus* south	Opio	CM Av38717	OxA-20335	25780 +/- 160	3.3909	4.74	-21.44
A2761	*P. elephantopus* south	Opio	SMAG 2003.108.1	ANU-1614	21570 +/- 37	n/a	n/a	-21.3

A2687	*P. elephantopus* south	Owaka	OM Av4075	OxA-20325	24400 +/- 110	3.50196	4.29	-23.09
A2756	*P. elephantopus* south	Avondale	SMAG 88.95	OxA-20326	2885 +/- 28	3.34285	2.28	-22.42
A2757	*P. elephantopus* south	Avondale	SMAG 88.95	OxA-20327	12395 +/- 45	3.39996	4.27	-22.73
A2762	*P. elephantopus* south	Eastern Bush	SMAG unregistered	ANUA-38614	10900 +/- 200	n/a	n/a	-23.0
A2545	*P. elephantopus* south	Kauana	S34515.1	CURL-1027	35880 +/- 660	n/a	n/a	-18.7
A2589	*P. elephantopus* south	Kauana	S34519.1	UCIAMS-57001	50200 +/- 3500	n/a	n/a	-22.4
A2590	*P. elephantopus* south	Kauana	S34519.2	CURL-10281	>49500	n/a	n/a	-15.5
A2759	*P. elephantopus* south	Riverton	SMAG E80.13	OxA-20333	1336 +/- 24	3.39001	3.49	-24.54
GU139071	*P. elephantopus* south	Stewart Island	OM Av4661 [C.09.29]	NZA-9069	654 +/- 56	n/a	3.18	-22.3

Table S3 Summary morphological statistics of heavy-footed moa (*Pachyornis elephantopus*) femora. Kya, thousands of years; SD, standard deviation.

Taxa	**Site**	**Age (Kya)**	**Mean femoral shaft width (mm)**	**SD**	**Mean femur length (mm)**	**SD**	**Sample size**
Northern heavy-footed moa	Merino Cave East	22-38	50.10	n/a	n/a	n/a	18
	Merino Cave West	14-20	57.50	n/a	n/a	n/a	8
	Glencrieff	10-12	53.68	3.35	312.58	4.19	19
	Albury Park	3-10	54.64	4.27	316.83	3.91	12
	Pyramid Valley	3-4	48.36	2.11	305.18	2.82	11
	Treasure Downs	1-2	47.89	4.83	289.65	4.01	23
	Bell Hill	0.9-2	46.50	4.80	293.56	4.46	16
Southern heavy-footed moa	Kauana	32-51	59.20	2.28	324.80	2.58	5
	Opio	20-26	60.00	9.25	320.00	15.14	6
	Hamilton's Winton	4-5	48.00	2.38	282.86	4.61	7
	Dart River	0.7-1	51.88	5.94	285.00	5.82	8

Table S4 Summary population genetic statistics from Arlequin for heavy-footed moa (*Pachyornis elephantopus*).

	Sequences	Length (bp)	Haplotypes	Segregating sites	Pairwise difference
Northern heavy-footed moa					
North Canterbury					
Late Holocene	24	498	19	19	4.23
Late Glacial	10	498	9	28	5.62
Otiran Glacial	5	498	5	12	3.50
Marlborough/North Canterbury					
Late Holocene	31	498	20	28	4.82
Late Glacial	10	498	9	28	5.62
Otiran Glacial	5	498	5	12	3.50
Southern heavy-footed moa					
Complete dataset					
Late Holocene	37	500	19	25	4.83
Otiran Glacial	9	500	9	14	3.42
Southland					
Late Holocene	7	500	6	11	3.62
Otiran Glacial	8	500	8	13	3.29
South of Clutha River					
Late Holocene	25	500	15	20	3.91
Otiran Glacial	8	500	8	13	3.29

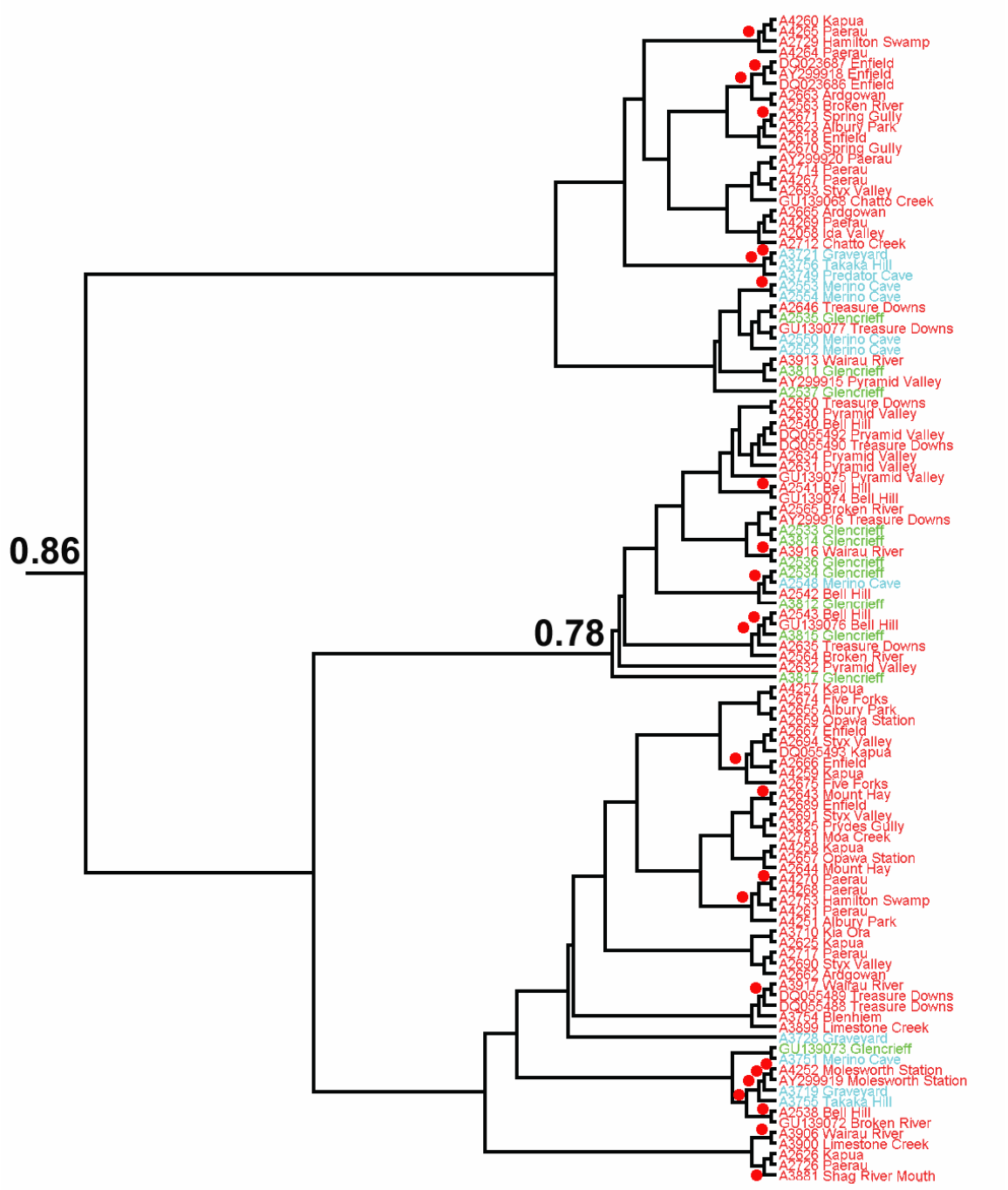

Figure S1 Maximum clade credibility phylogram of northern heavy-footed moa (*Pachyornis elephantopus*), with branch lengths proportional to the number of substitutions. Colours represent different time periods. Red = Holocene (0-10 Kya); Lime = Late Glacial (10-14 Kya); and Sky Blue = Otiran Glacial (14-70 Kya). Posterior probability support (> 0.5) is indicated at each node in black (or by red dots).

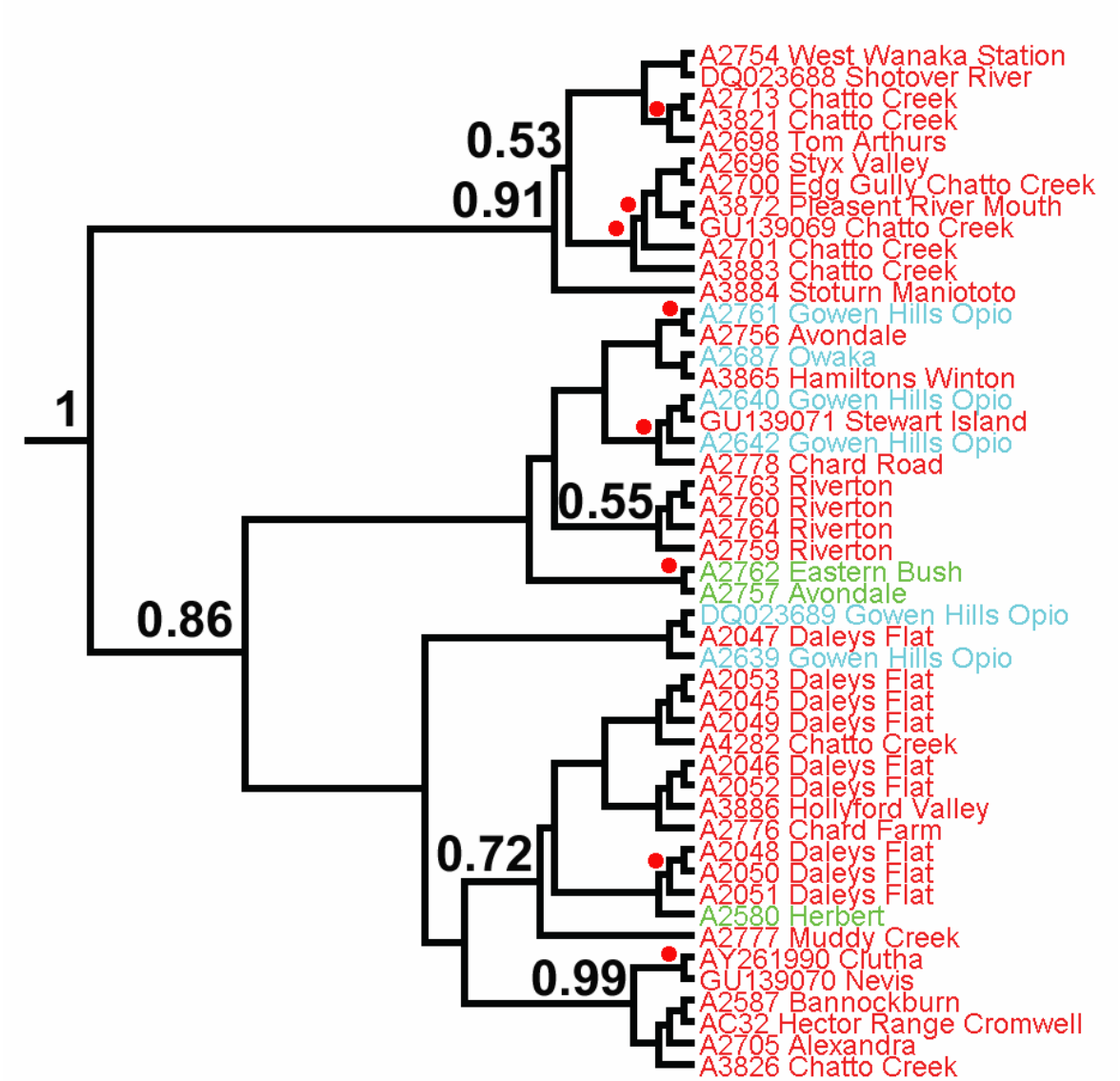

Figure S2 Maximum clade credibility phylogram of southern heavy-footed moa (*Pachyornis elephantopus*), with branch lengths proportional to the number of substitutions. Colours represent different time periods. Red = Holocene (0-10 Kya); Lime = Late Glacial (10-14 Kya); and Sky Blue = Otiran Glacial (14-70 Kya). Posterior probability support (> 0.5) is indicated at each node in black (or by red dots).

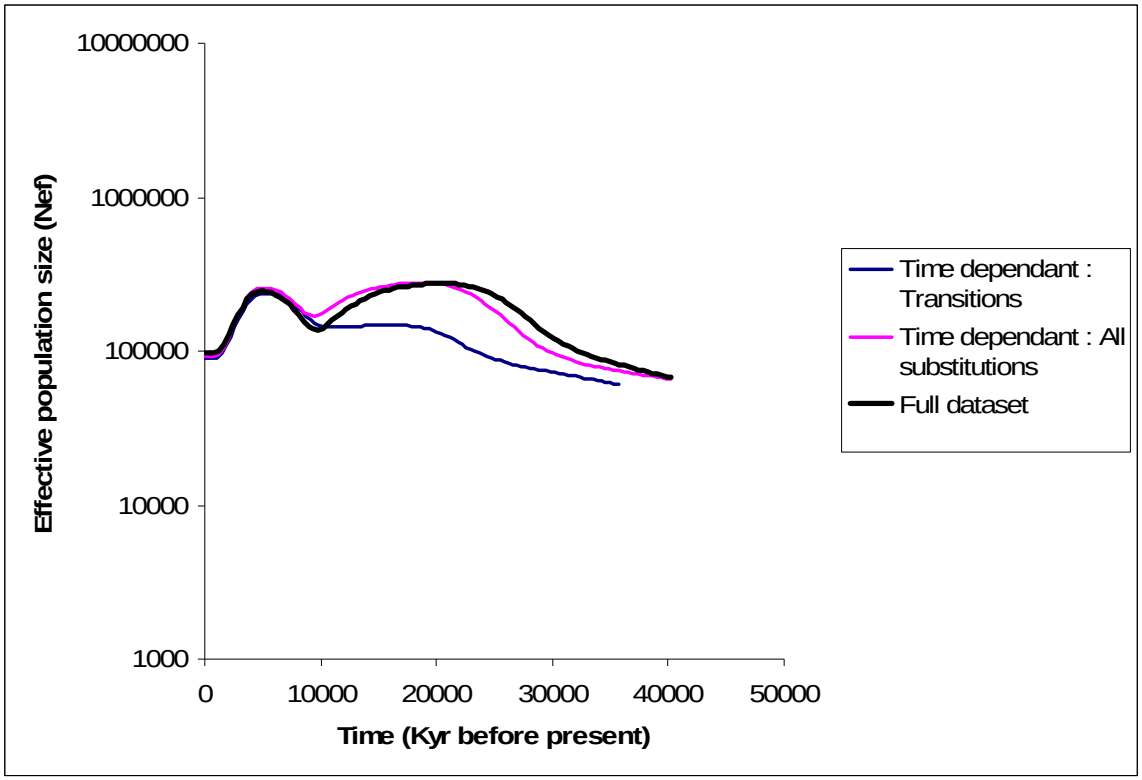

Figure S3 Effect of DNA damage on the northern heavy-footed moa (*Pachyornis elephantopus*) BSP.

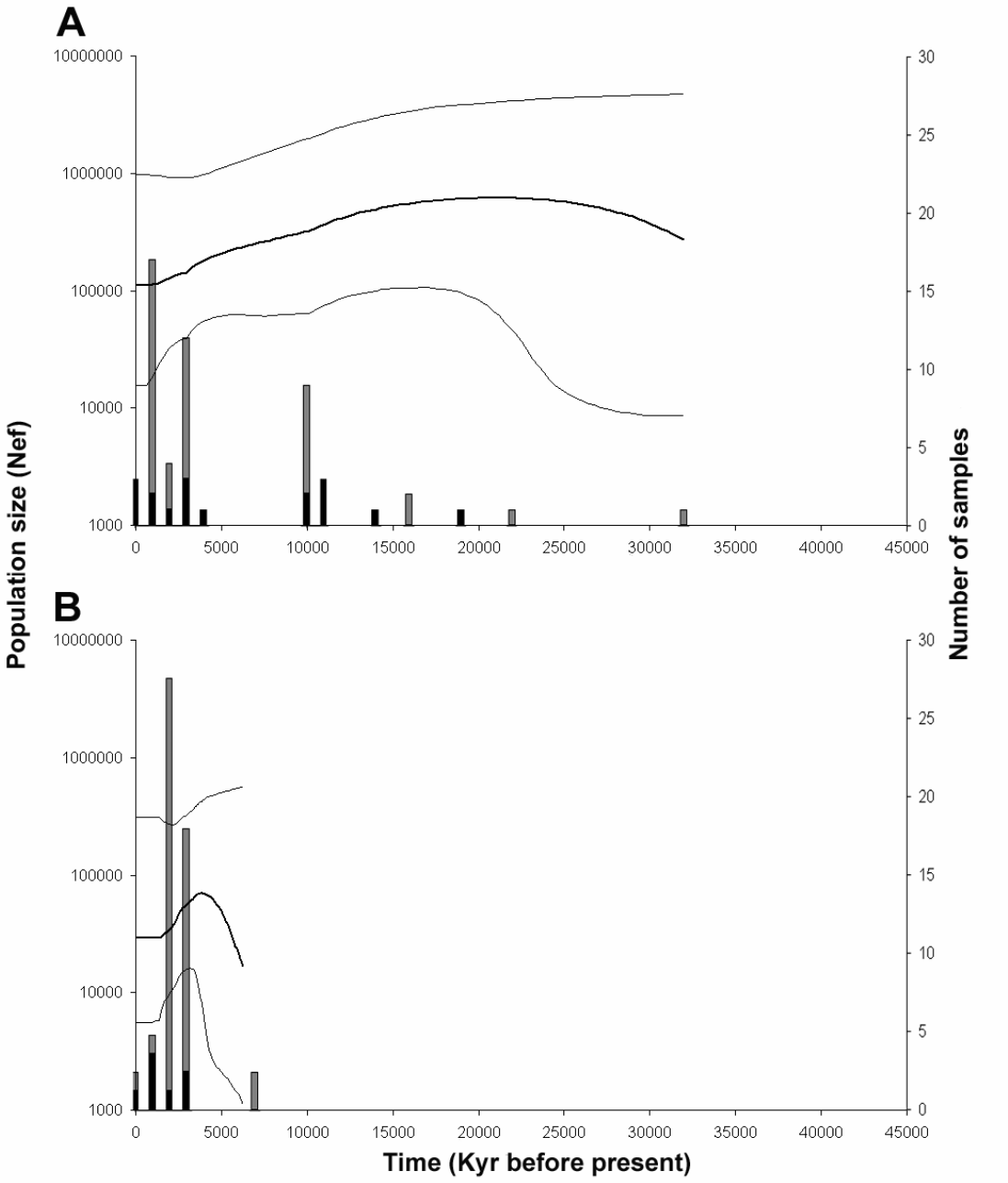

Figure S4 Bayesian Skyline Plot (BSP) of northern heavy-footed moa (*Pachyornis elephantopus*) populations in Marlborough/North Canterbury (**A**) and South Canterbury/Otago (**B**). The black line represents the median BSP, while the grey lines represent the 95% credible intervals. Each column represents the number of radiocarbon dated sequences: black, number of finite radiocarbon dates; grey, number of undated sequences with mean stratigraphic ages assigned.

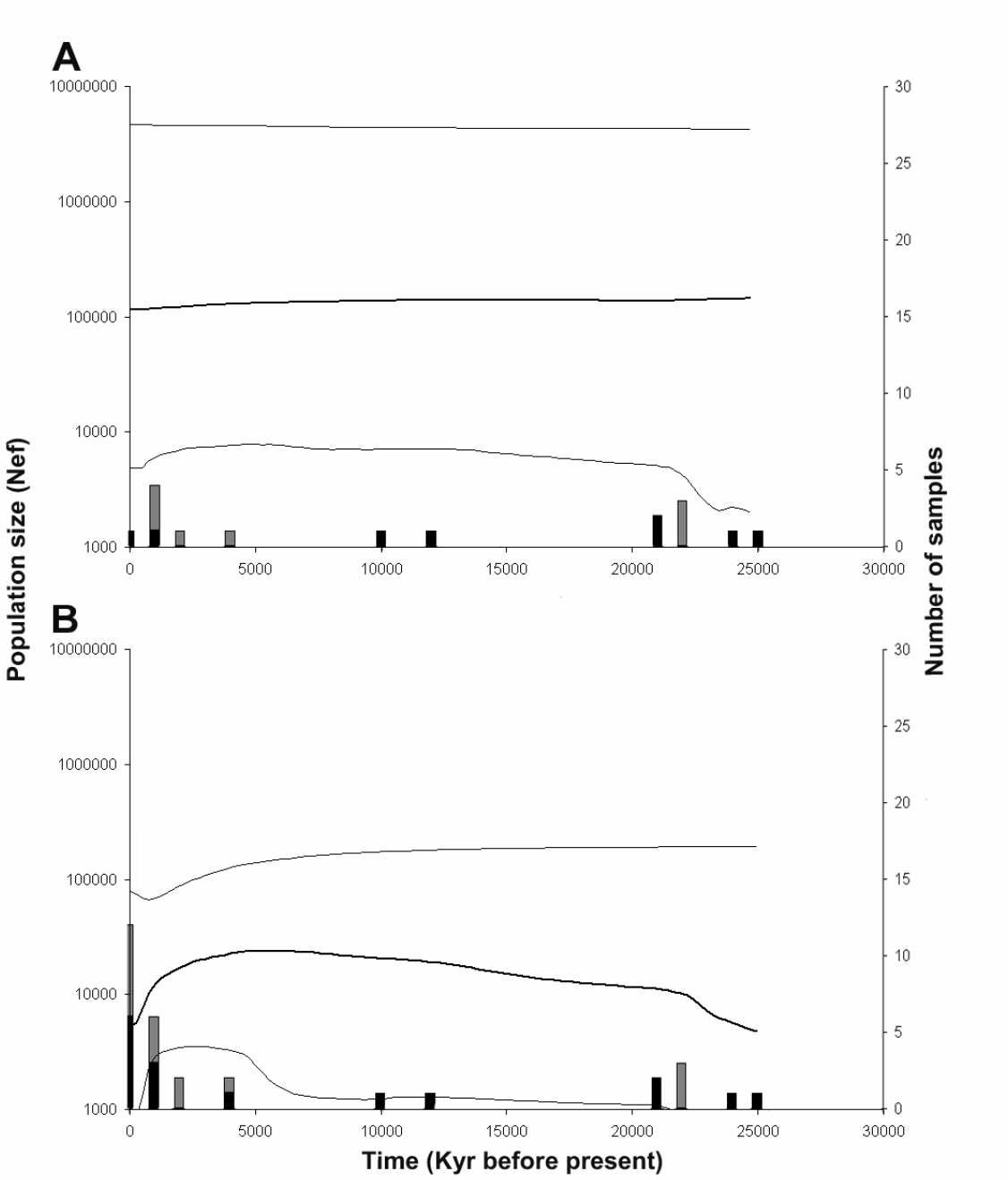

Figure S5 Bayesian Skyline Plot (BSP) of southern heavy-footed moa (*Pachyornis elephantopus*) populations from Southland (**A**) and south of Clutha River (**B**). The black line represents the median BSP, while the grey lines represent the 95% credible intervals. Each column represents the number of radiocarbon dated sequences: black, number of finite radiocarbon dates; grey, number of undated sequences with mean stratigraphic ages assigned.

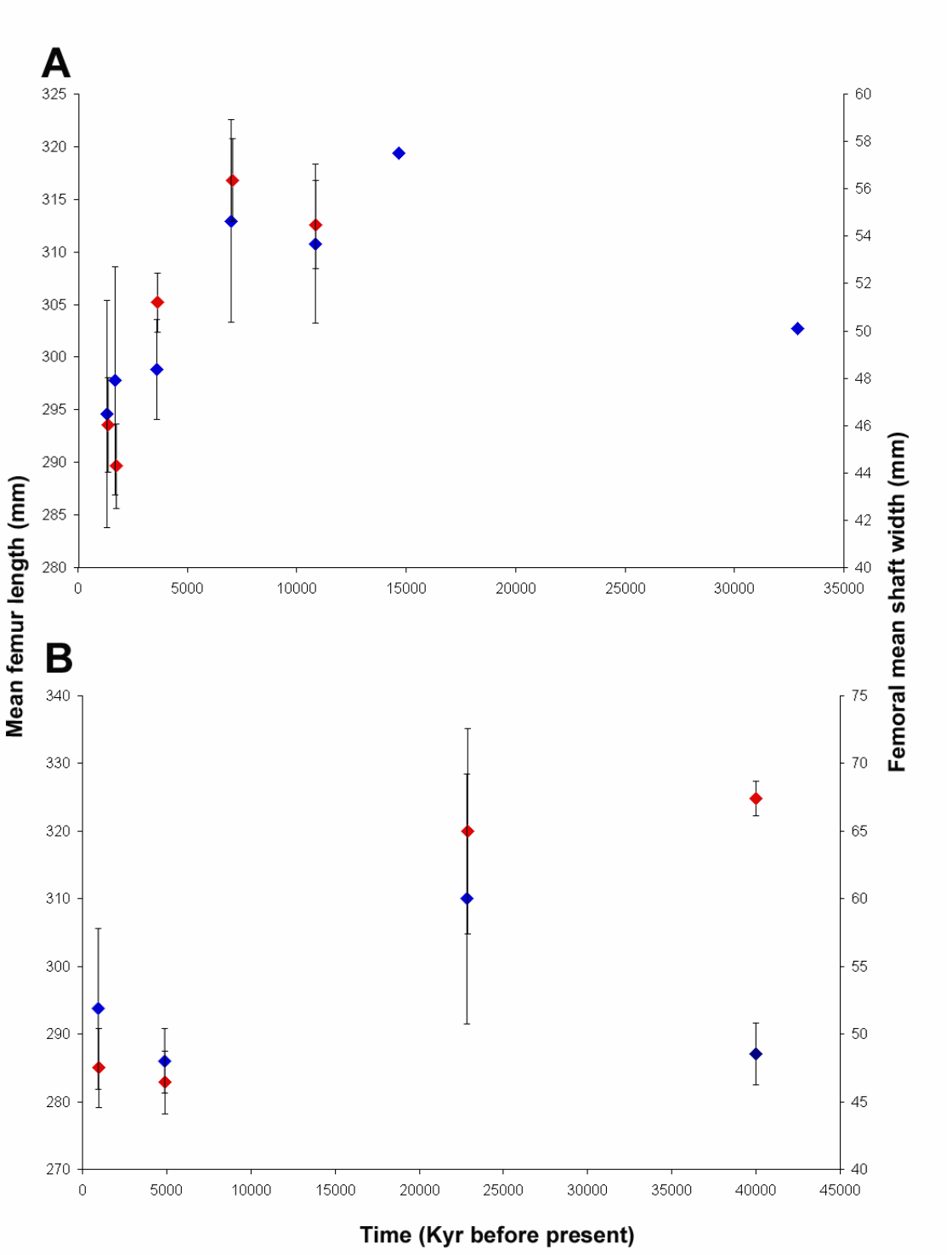

Figure S6 Temporal size variation in heavy-footed moa (*Pachyornis elephantopus*) femora from dated fossil deposits for northern (**A**) and southern (**B**) heavy-footed moa. Each data point represents the mean femoral length (red) or mean femoral shaft width (blue) of each deposit with standard deviations where possible.

CHAPTER EIGHT

Incorporating ancient DNA into Bayesian Skyline Plot analyses: the effect of biased sampling on inferences of past population demographics

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STATEMENT OF AUTHORSHIP

Chapter Eight

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Nicolas. J. Rawlence

Designed experiment, performed data analysis, interpreted data and wrote paper.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed..... Date....20/04/10

Simon Y. W. Ho

Performed simulations, interpreted data and evaluated manuscript.

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Jeremy J. Austin

Supervised development of research, evaluated manuscript and provided funding.

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Supervised development of research, evaluated manuscript and provided funding.

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Chapter Eight

Incorporating ancient DNA into Bayesian Skyline Plot analyses: the effect of biased sampling on inferences of past population demographics.

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Abstract

The Bayesian Skyline Plot (BSP) has been used to infer the palaeodemographic history of taxa by analysing time-structured ancient DNA (aDNA) datasets. However, most aDNA datasets have inherent temporal, geographic, phylogenetic and biomolecular preservation sampling biases that can potentially affect the reliability of the BSP. To date, no in-depth investigation has been conducted into the effects of these sampling biases on the BSP. To address this issue we analysed three different datasets: a non-biased simulated dataset, a Beringian Steppe Bison aDNA dataset with minimal known sampling biases and a moa (Aves: Dinornithiformes) aDNA dataset with known sampling biases. We show that biased sampling in the empirical datasets produced large variations in palaeodemographic history inferred from the BSP. We also show that when analysing contemporary genetic data, the BSP may not accurately reflect the true demographic history. Because all genetic datasets are potentially a biased sample of population history, considerable caution needs to be used when inferring palaeodemographic history from the BSP.

Introduction

Understanding the temporal population demographics of taxa relies on coalescent analyses in a Bayesian framework of time structured ancient DNA (aDNA) datasets. Recent examples include bison (Shapiro *et al.* 2004; Drummond *et al*. 2005), woolly mammoths (Barnes *et al.* 2007; Debruyne *et al*. 2008), musk ox (Campos *et al.* 2010), southern elephant seals (Bruyn *et al*. 2009), bowhead whales (Borge *et al.* 2007; Ho *et al.* 2008) and moa (Chapter Six and Seven).

These studies utilised the Bayesian Skyline Plot (BSP; Drummond *et al.* 2005; Table 1), implemented in the software programme BEAST (Drummond and Rambaut 2007). The BSP is a posterior distribution of population size change (Theta, θ) at each coalescent point within a sample of genealogies. Theta is a measure of nucleotide polymorphism and is used to represent effective population size (Beebee and Rowe 2004; Drummond *et al.* 2005). Theta is defined (for haploid, uniparental inheritance) by the equation $\theta = 2N_e\mu$, where N_e is the effective population size and μ is the mutation rate (Beebee and Rowe 2004). The BSP should only be used in cases where simple parametric models of demographic history such as constant population size or exponential growth can be rejected. However, several published analyses of aDNA datasets have not taken this into account and have used the BSP despite failing to reject constant population size (Barnes *et al.* 2007; Debruyne *et al.* 2008).

The shape of the BSP is dependant on several factors, including sampling a large enough proportion of the population to capture all the relevant coalescent events and the assumption of panmixia of individuals with a single population coalescent (i.e. a single unstructured population; Drummond *et al.* 2005). When these assumptions are met, theta approximates the effective population size. Violation of the assumption of panmixia will always result in an elevation of theta, which will not approximate effective population size, and changes to shape of the BSP (Drummond pers comm. 2009).

Most aDNA studies have some inherent sampling biases that violate the assumptions of the BSP. Sampling biases are in four broad categories: temporal, geographic, phylogenetic, and biomolecular preservation (Figure 1). Temporal biases occur when samples are not evenly distributed through time but are clustered in discrete time periods. Geographic biases result from samples being unevenly distributed across the species range (in total or during one time period). Phylogenetic biases occur when (i) unrecognised phylogeographic structure exists; (ii) samples are from two or more phylogeographic units or populations; (iii) different clades are sampled at different times (e.g. replacement events); or (iv) only one clade is sampled. Finally, biomolecular preservation biases result from non-uniform DNA preservation across all samples (e.g. some samples are represented by more or less sequence data than others or DNA is not present at all).

Table 1 Ancient DNA datasets analysed using the Bayesian Skyline Plot. Highlighted grey bars represent BSP analyses that could not reject constant population size because of wide 95% credible intervals. m, modern sequences; r, radiocarbon dated sequences; s, stratigraphic dated sequences (the mean radiocarbon age of the deposit or stratigraphic layer has been assigned to undated sequences); h, historical sequences with known collection dates; *Cyt b*, cytochrome b; CR, control region.

Taxa	Samples	Radiocarbon dates	Sequence	Length	Population Demographic signal	Preservation	Reference
Bison	191	169 r, 22 m	CR	456-685 bp	Expansion, decline and recovery	Arctic, temperate	Drummond *et al.* 2005
Musk ox	149	149 r	CR	682 bp	Expansion, decline, expansion, decline, expansion	Arctic	Campos *et al*. 2010
Southern elephant seal	320	223 r, 97 m	CR	325 bp	Constant Expansion Expansion, decrease	Antarctica	Bruyn *et al.* 2009
Mammoth	33	33 r	CR	741 bp	Decline, increase	Permafrost	Barnes *et al*. 2007
Mammoth	138	131 r, 7 s	CR	705-743 bp	Decline	Permafrost	Debruyne *et al.* 2008
Cave bear	59	25 r, 34 s	CR	251-285 bp	Decline†	Temperate	Stiller *et al*. 2010
Brown bear	40	20 r, 5 s, 15 m	CR	177 bp	Constant	Temperate	Stiller *et al*. 2010
Grey wolf	960	947 m, 13 r	CR	661 bp (m) 57 bp (m, r)	Expansion* Expansion†	Temperate	Pilot *et al.* 2010
Red-fox	260	260 h	*Cyt b*	145-354 bp	Expansion	Temperate	Aubry *et al*. 2009
Red-fox	174	174 h	CR	342 bp	Expansion	Temperate	Aubry *et al*. 2009
Long-tailed vole	29	29 s	*Cyt b*	312 bp	Constant	Temperate	Spaeth *et al*. 2009
Auroch	37	37 r	CR	360 bp	Constant	Temperate	Mona *et al.* 2010
Cariboo	91	42 r, 49 m	CR	454 bp	Constant	Arctic	Kuhn *et al*. 2010
Collared lemming	64	54 s, 10 m	CR, *Cyt b*	744 bp	Decline, expansion†	Arctic	Prost *et al*. 2010
Bowhead whale	99	99 r	CR	453 bp	Expansion	Arctic	Ho *et al*. 2008
Crested moa	62	21 r, 41 s	CR	150-500 bp	Expansion then decline	Temperate	Chapter Six
Northern heavy footed moa	115	27 r, 88 s	CR	150-500 bp	Expansion, decline, expansion, decline	Temperate	Chapter Seven
Southern heavy footed moa	48	22 r, 26 s	CR	150-500 bp	Decline	Temperate	Chapter Seven

† Bayes factor analysis (Suchard *et al.* 2001) suggested that the BSP model was a better fit to the data than constant population size, despite the wide confidence intervals in the BSP analysis.

* Bayes factor analysis suggested that constant population size was the most likely demographic model.

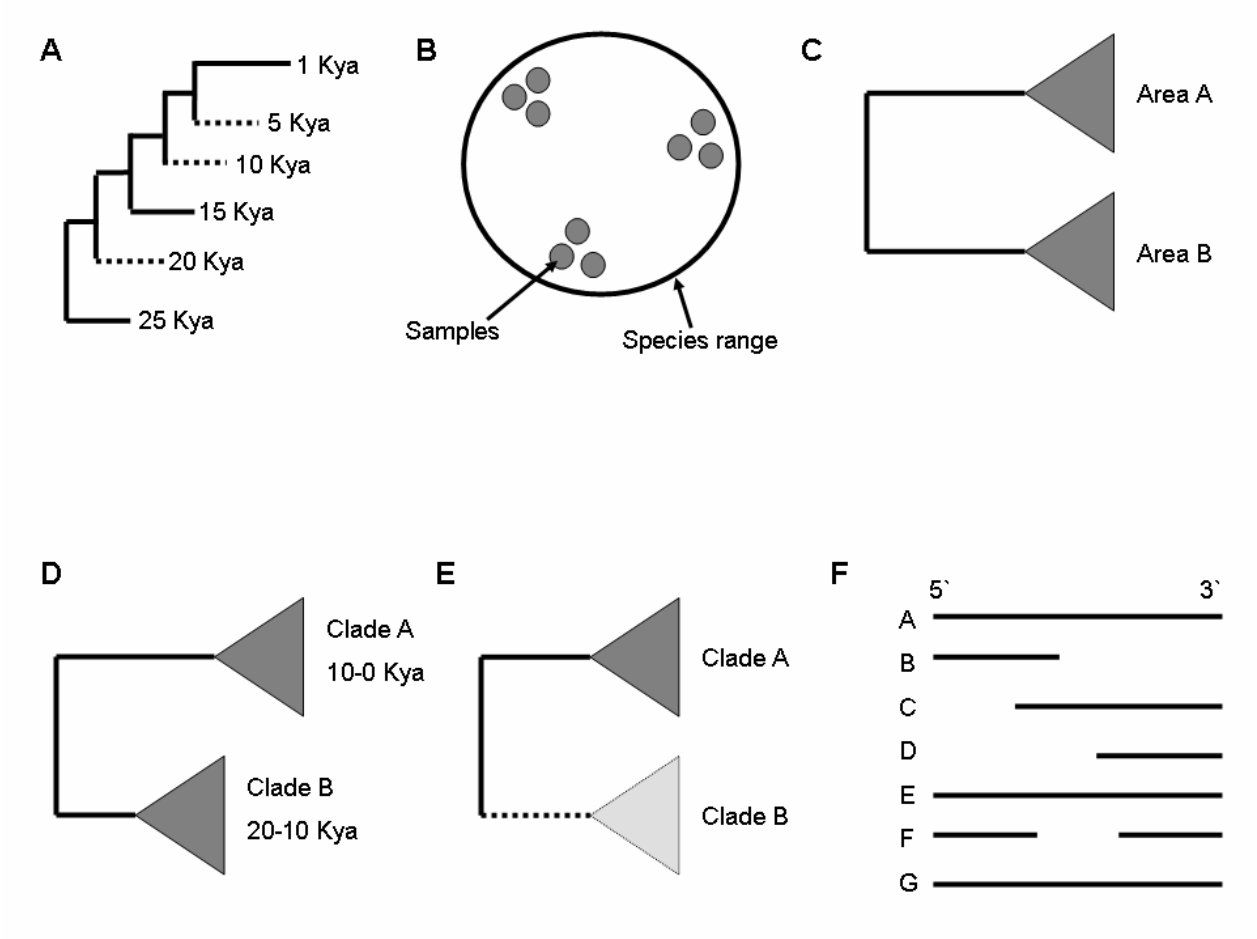

Figure 1 Sampling biases inherent in most ancient DNA datasets. (A) Temporal biases: Samples are not evenly distributed through time but are clustered in discrete time periods. Dashed branches represented un-sampled time periods. (B) Geographic biases: Samples are unevenly distributed across the species range. (C-E) Phylogenetic biases: (C) Unrecognised phylogeographic structure exists or samples are from two or more phylogeographic units or populations; (D) Different clades are sampled at different times; (E) Only one clade is sampled. Clade B is un-sampled in this example. (F) Biomolecular preservation biases: DNA preservation is not uniform across all samples (e.g. some samples are represented by more or less sequence data than others or DNA is not present at all).

Previously published studies have only conducted preliminary investigations into the effects of temporal and phylogenetic biases (Grazziotin *et al.* 2006; Crandall *et al.* 2008; Debruyne *et al*. 2008; Rambaut *et al.* 2008; Aubry *et al.* 2009; Campos *et al.* 2010; Mona *et al.* 2010; Pilot *et al.* 2010; Prost *et al*. 2010; Stiller *et al*. 2010). These studies suggest that the BSP is unaffected by temporal sampling biases, at least in Influenza (Rambaut *et al.* 2008) and cave bear (Stiller *et al.* 2010) datasets but is affected in auroch (Mona *et al.* 2010) and grey wolf (Pilot *et al*. 2010) datasets. In addition, these studies suggest the BSP is affected by phylogenetic biases including phylogeographic structuring. Different phylogeographic units or populations within a species can have different demographic signals (Grazziotin *et al.* 2006; Crandall *et al.* 2008; Campos *et al.* 2010; Chapter Six and Seven) meaning that the demographic history of one population is not indicative of the species as a whole (Scott 2009). However, in cave bears (Stiller *et al.* 2010), red fox (Aubry *et al.* 2009) and mammoth (Debruyne *et al.* 2008) datasets, different phylogeographic units or populations had the same demographic signal (e.g. decline or expansion) as the complete datasets, despite changes in overall population size. To date, no in-depth investigation has been published into how temporal, geographic, phylogenetic and biomolecular preservation biases affect the BSP.

The effect of temporal, geographic, phylogenetic and biomolecular preservation biases on the BSP was investigated using three different datasets. First, a non-biased dataset containing ancient and modern sequences was simulated using the coalescent with an inferred constant and boom-bust palaeodemographic history (Figure 2 and 3). The simulated datasets were used to construct a BSP and then each dataset was sub-sampled under different biased sampling regimes to investigate how sampling biases affect the inferred palaeodemographic history. Second, the Beringian Steppe Bison (hereafter referred to as bison) aDNA dataset (Shapiro *et al.* 2004; Drummond *et al.* 2005) was re-analysed and sub-sampled to introduce biases. The bison dataset is thought to contain minimal known sampling biases because it consisted of mostly complete sequences from well preserved bison specimens with an even temporal and geographical spread of samples (Shapiro *et al.* 2004). Finally, a moa aDNA dataset with known sampling biases (Chapters Six and Seven) was re-analysed and sub-sampled to introduce further biases. This dataset contained missing sequence data, temporal gaps, phylogeographic structuring (Baker *et al.* 2005; Lambert *et al.* 2005; Bunce *et al.* 2009; Chapters Six and Seven) and small sample sizes.

Materials and Methods

Simulations

Using the programmes BEAST (Drummond and Rambaut 2007) and Seq-Gen (Rambaut and Grassly 1997), sequence data (1000 bp) was simulated under constant and boom-bust models of population demography. Sequences were simulated according to a Jukes-Cantor model of nucleotide substitution, with rate homogeneity among sites. A boom-bust model, where the effective population size increases to a population size maximum and thereafter declines, was chosen to reflect the broad demographic history of bison (Shapiro *et al.* 2004; Drummond *et al.* 2005). The boom-bust model had exponential growth from 100 Kya reaching a peak population size of 10^6 at 25 Kya, then exponential decline until a population size of 10^5 at the present to reflect changes in the effective population size of bison. For the constant and boom-bust models two clades were simulated, each containing 50 ancient sequences (ten sequences each at 10, 20, 30, 40 and 50 Kyr) and 50 modern sequences, with a substitution rate of 2×10^{-7} substitutions per site per million years. Fifty modern and ancient sequences were chosen to minimise stochastic errors associated with small sample size. The layered sampling strategy employed for the ancient sequences was used because it performs better than a sampling strategy where sequences are spread evenly through time at 1 Kyr intervals (Ho *et al.* 2007). The substitution rate, while conservative, is a broad match to previous aDNA studies. The slow rate, while decreasing information content (that a faster rate would provide), increases the sensitivity of the simulated dataset and methods to the effects of sampling biases, allowing the performance of the BSP to be evaluated under non ideal conditions.

To generate a baseline BSP, the simulated datasets were analysed using BEAST with eight groups (number of coalescent intervals in the analysis to reduce noise from outliers in the dataset), for 50 million generations, sampling demographic parameters every 5000 generations to ensure enough genealogies are sampled. The first 10% of runs were discarded as burn-in, so that only the portions of the data that are in equilibrium are analysed. To simulate sampling biases, the analysis was repeated with the following sequence data excluded:

i) Temporal biases: sequences 20 Kyr, 30 Kyr or all ancient sequences were excluded.
ii) Biomolecular preservation biases: one third of the sequence data for half the sequences was excluded.

Each biased sampling scenario was replicated 10 times. The raw data files from each replication were visualised using Tracer v1.8 (http://beast.bio.ed.ac.uk/Tracer) to construct the BSP.

Bison BSP analysis

The bison dataset (Shapiro *et al.* 2004; Drummond *et al.* 2005) was re-analysed using the BSP under temporal, geographic and phylogenetic sampling biases. Biomolecular preservation biases were not examined because the majority of bison specimens analysed by Shapiro *et al.* (2004) had complete sequence data. To introduce temporal biases, time periods were excluded that corresponded with sampling intervals in the bison and moa datasets (Table 2). Geographic biases were simulated by analysing specific geographic regions defined by Shapiro *et al.* (2004; Table 2) that corresponded with well supported phylogeographic units within the bison dataset. The geographical regions are defined as:

i) Western Beringia: Bison from Siberia and China.
ii) Eastern Beringia: Bison from Alaska and northwest Canada, north of the Last Glacial Maximum (LGM) Laurentide and Cordilleran ice sheets.
iii) South of the ice: Bison from central North America, south of the LGM Laurentide and Cordilleran ice sheets.

Phylogenetic biases were introduced by excluding the well supported major clades in the bison phylogeny (classified as clades one to four; Shapiro *et al.* 2004). Each analysis was run using the BSP in BEAST with a group size of 15 and the HKY+I+G model of nucleotide substitution for 50 million generations (replicating Drummond *et al.* 2005 analysis). Demographic parameters were sampled every 1000 generations, with the first 10% of runs discarded as burn-in. Results were visualised using Tracer v1.8.

Moa BSP analysis

The crested moa (*Pachyornis australis*; Chapter Six), and northern and southern heavy-footed moa (*Pachyornis elephantopus*; Chapter Seven) datasets were sub-sampled to introduce temporal, geographic, phylogenetic and biomolecular preservation biases, and re-analysed using the BSP.

Table 2 Temporal and geographic biases analysed in this study. Because there was no phylogeographic structuring in crested moa (Chapter Six), the specified geographical regions were excluded from the analysis. * These biases were assessed in Chapter Seven.

	Temporal biases	**Geographic biases**
Species	**Time periods (Kya) excluded**	**Geographical regions analysed**
Bison	modern 0-1 1-2 2-3 3-4 4-7 7-9 5-10 10-18 18-30 18-26 9-13 13-18 26-30 30-40 40-50 50-60 20-60 30-60 40-60	Beringia Eastern Beringia Western Beringia South of the ice Western Beringia/South of the ice Eastern Beringia/South of the ice
Crested moa	0-5 10-12 12-17 18-20 12-30 18-30 20-30	Mount Owen Kaarst Mount Arthur Kaarst Takaka Hill/Golden Bay Kaarst Honeycomb Hill Kaarst Charleston Kaarst
Northern heavy footed moa	0-1 1-2 2-3 3-4 4-5 7-9 9-12 14-17 18-25 14-40 18-40 30-40	North Canterbury/Marlborough* South Canterbury/Otago* North Canterbury Northern heavy-footed moa excluding Central Otago Eastern South Island excluding Central Otago Marlborough/North Canterbury/South Canterbury South Canterbury and Otago (excluding Central Otago) Central Otago
Southern heavy footed moa	0-1 1-2 2-3 0-3 3-4 4-5 10-15 10-30 20-30	Southland* South of the Clutha* North of Clutha Otago

For each dataset, temporal biases were simulated by excluding time periods that corresponded with clustering of radiocarbon dated sequences (Table 2). Geographical biases were introduced by analysing or excluding discrete collections of fossil localities and/or phylogeographic units (Table 2). In southern-heavy footed moa, the clades are structured geographically (Figure S3), so geographic biases were used as a proxy for phylogenetic sampling biases.

Phylogenetic biases were simulated by excluding pre-defined clades (see Figure S1-S3) from each phylogeny. Finally, biomolecular preservation biases were introduced by analysing different levels of missing sequence data:

i) Complete mitochondrial DNA (mtDNA) hyper variable one (HVRI) sequences. Specimens that had missing sequence data were excluded from the analysis.
ii) HVRI sequences including specimens with missing sequence data.
iii) Complete HVRI and HVRII sequences. The central conserved region was excluded from the analysis because only specimens from Baker *et al.* (2005) had this region sequenced.
iv) Incomplete sequences that potentially belonged to multiple haplotypes were excluded from the analysis (see Chapter Six and Seven).

Each analysis was run with a group size of 10 and the GTR + I + G model of nucleotide substitution (replicating BSP analyses in Chapter Six and Seven) following the methodology for bison.

Results

Simulations

Sub-sampling the simulated datasets with inferred constant and boom-bust palaeodemographic histories, to introduce temporal and biomolecular preservation biases, had no obvious effect on the BSP (Figure 2 and 3). Visual inspection shows that there was increased variance when the simulated datasets were sub-sampled to introduce biases. For example when only modern sequences were analysed there was overall increased variance in the inferred demography plot compared to the combined analysis of modern and ancient sequences. This is at least partly, perhaps entirely, due to the reduction in sample size.

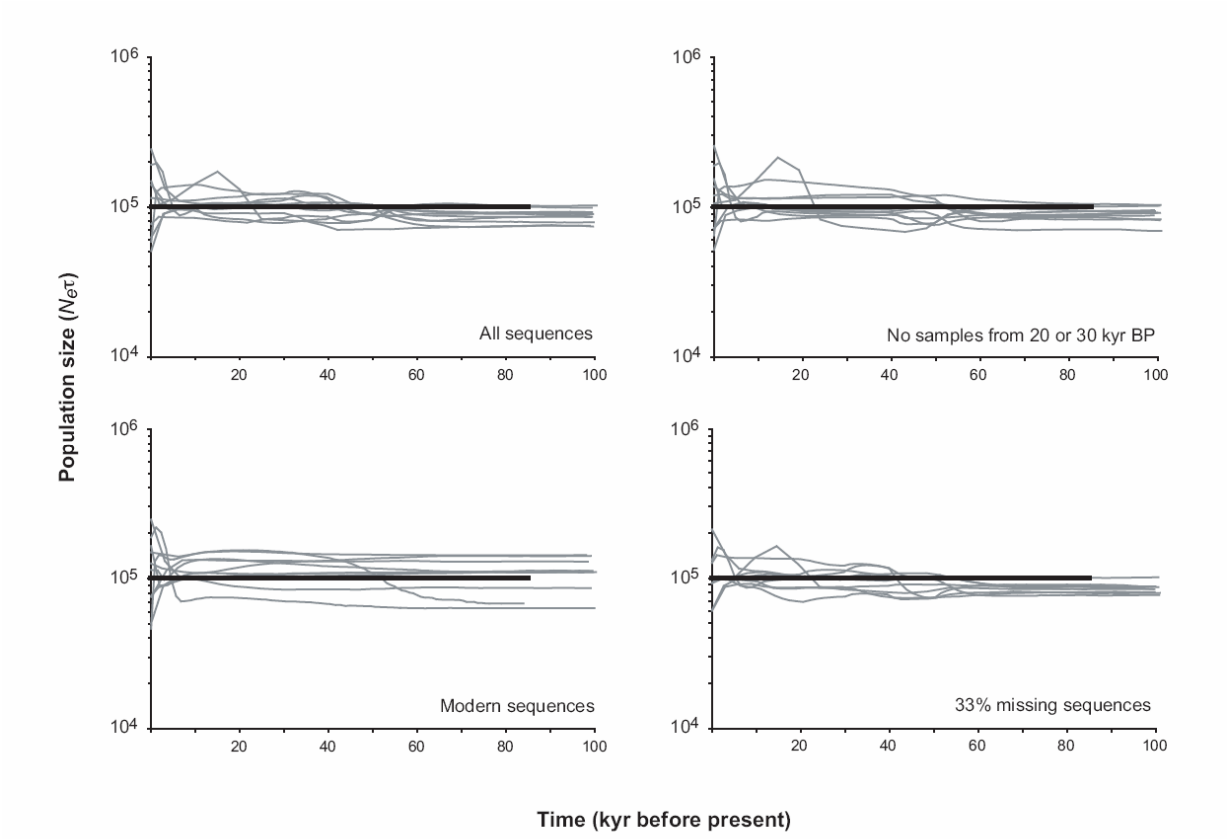

Figure 2 Bayesian skyline plots of simulated data under a constant population size demographic model analysed under different biased sampling regimes.

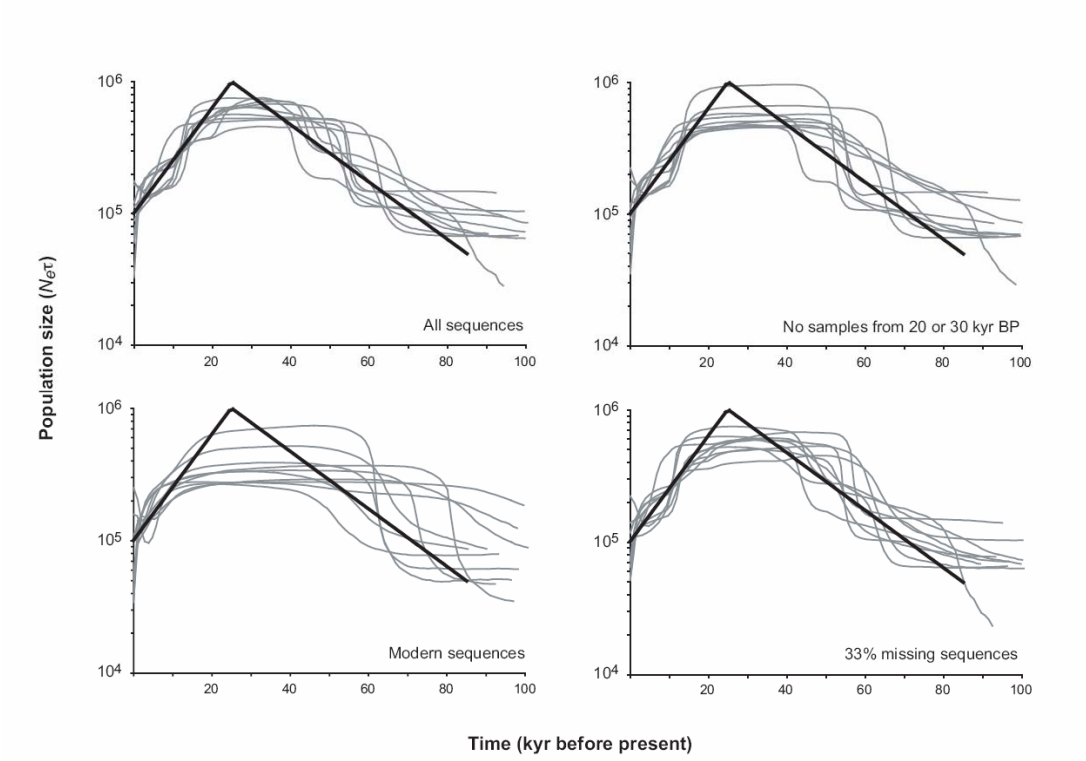

Figure 3 Bayesian skyline plots of simulated data under a boom-bust population size demographic model analysed under different biased sampling regimes.

Temporal sampling biases

Removing discrete time periods from the empirical datasets had a large effect on the BSP. In bison, BSP approximating the Drummond *et al.* (2005) BSP (Figure 4 and S4: Full Dataset) were reconstructed for the majority of analyses. However, there were some pronounced deviations in the BSP (Figure 4). The inferred decline in population size 10-12 Kya (Drummond *et al.* 2005) was not seen when sequences 10-18 Kyr were excluded. In addition, the maximum effective population size 45-30 Kya was lower when sequences 30-40 Kyr were excluded.

The largest effect occurred when sequences 20-60, 30-60, 40-60 and 50-60 Kyr were excluded from the bison dataset, resulting in a truncation of the inferred demography plot, with the tail end of the BSP progressively shifting towards the present (Figure 4). The shape of the BSP also changed, which resulted in more pronounced increases in population size and lower population size maxima. In addition, the BSP always inferred an increase in population size to a maximal point, followed by a decline. This is despite when sequences 20-60 and 30-60 Kyr are excluded from the bison dataset, the oldest sequences in the analysis are sampling from an inferred declining population in the full dataset.

The effect of temporal sampling biases was more pronounced in moa than bison. In crested moa the removal of the oldest sequences (12-30 and 18-30 Kyr) resulted in tail end of the BSP progressively shifting towards the present, changes to the shape of the BSP and the inferred magnitude of population size change compared to the full dataset (Figure 5). When sequences 12-30 Kyr were excluded the inferred palaeodemographic history was a declining population size from 12-10 Kya, followed by constant population size throughout the Holocene. In contrast, when sequences 18-30 Kyr were excluded, a near constant population size was inferred (Figure 5 and S7). In northern heavy-footed moa (Figure 6 and S11) significant deviations occurred when sequences 3-4, 9-12 and 18-25 Kyr were excluded, which resulted in pronounced increases and decreases in effective population size and changes to the shape of the BSP. For example when sequences 3-4 Kyr were excluded from the analysis, the northern heavy-footed moa population did not appear to recover from the population decline 20-10 Kya but continued to decline (Figure 6). Excluding sequences 14-40, 18-40 and 30-40 Kyr also resulted in the truncation and deviation of the inferred demographic signal (Figure 6), similar to what was seen in bison when the oldest sequences were progressively excluded (Figure 4). In comparison there was no obvious effect on the southern heavy-footed moa BSP, except when sequences 0-3 Kyr (the majority of sequences

sampled from the Holocene are restricted to 0-3 Kyr) or the oldest sequences are excluded (Figure 7 and S15). This resulted in a constant population size through time and truncation of the inferred demographic signal (the tail of the BSP moved towards the present) respectively.

Geographic sampling biases

The palaeodemographic re-analysis of the bison and moa datasets to introduce geographic sampling biases highlighted that the bison and moa datasets contained multiple geographically restricted populations, violating the assumption of panmixia of individuals (i.e. unstructured single population; Drummond *et al.* 2005). In bison, which exhibited phylogeographic structuring (Shapiro *et al.* 2004), the re-analysis of the BSP showed that the post decline recovery (Figure 4: Full dataset) was seen only in bison south of the ice (Figure 4 and S5). Bison populations in eastern Beringia did not recover from the population declines 30-10 Kya (Figure 4). Bison populations in western Beringia had a high constant population size, while bison populations south of the ice had a constant population size until about 30 Kya, and then underwent a decline and subsequent recovery (Figure 4). This is consistent with Shapiro *et al.* (2004) who suggested that the boom-bust demographic signal in bison came from the eastern Beringian population.

In crested moa, which did not exhibit phylogeographic structuring (Chapter Six), there was no pronounced effect of geographic sampling biases, except when sequences from the Mount Owen and Honeycomb Hill kaarst areas were excluded (Figure 5 and S8). When sequences from Mount Owen were excluded, it resulted in a BSP with high constant population size until 20 Kya, followed by a decline to a low constant population size. When sequences from Honeycomb Hill were excluded, it resulted in a BSP characterised by a slow increase in population size until 12 Kya, followed by a decline until 10 Kya and constant population size thereafter. In Chapter Seven, I previously argued that northern heavy-footed moa may have been separated into two different populations in Marlborough/North Canterbury and South Canterbury/Otago that had contrasting palaeodemographic histories inferred from the BSP (Figure S12). I also showed that excluding major phylogeographic clades had a large effect on the southern heavy-footed moa BSP (Figure S16). Further geographic biases are evident within the northern heavy-footed moa dataset. Excluding sequences from Central Otago from the analysis resulted in a significant elevation in effective population size (Figure 6).

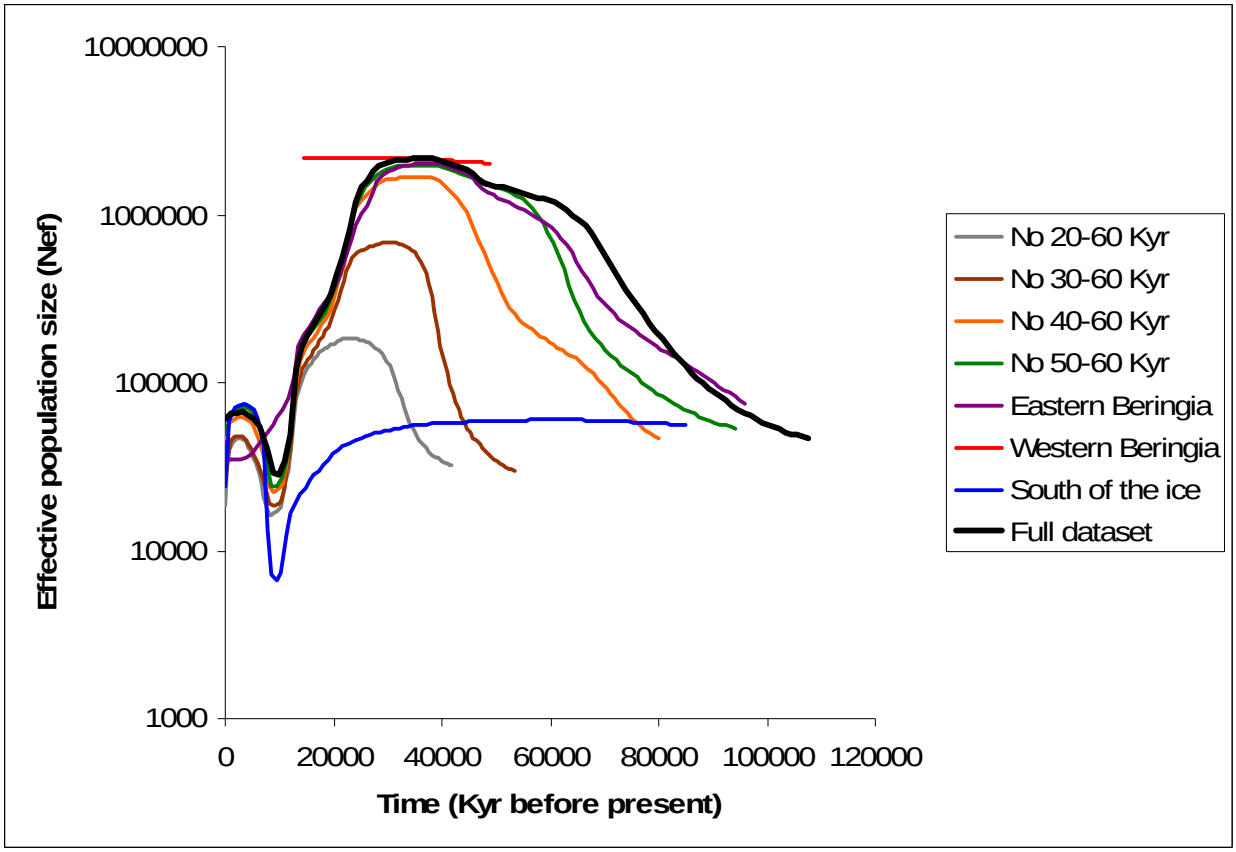

Figure 4 Effect of biased sampling on the demographic history of bison inferred from the BSP.

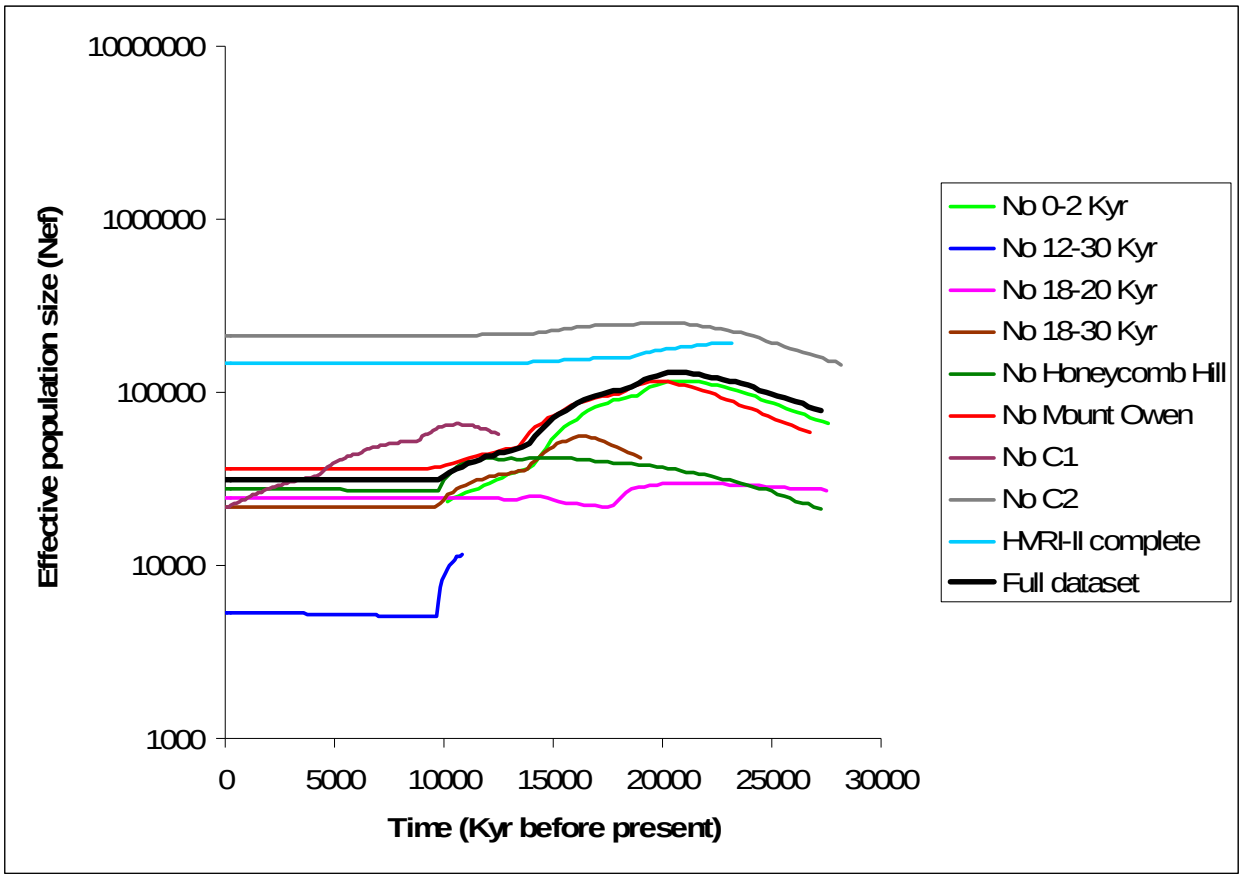

Figure 5 Effect of biased sampling on the demographic history of crested moa (*Pachyornis australis*) inferred from the BSP.

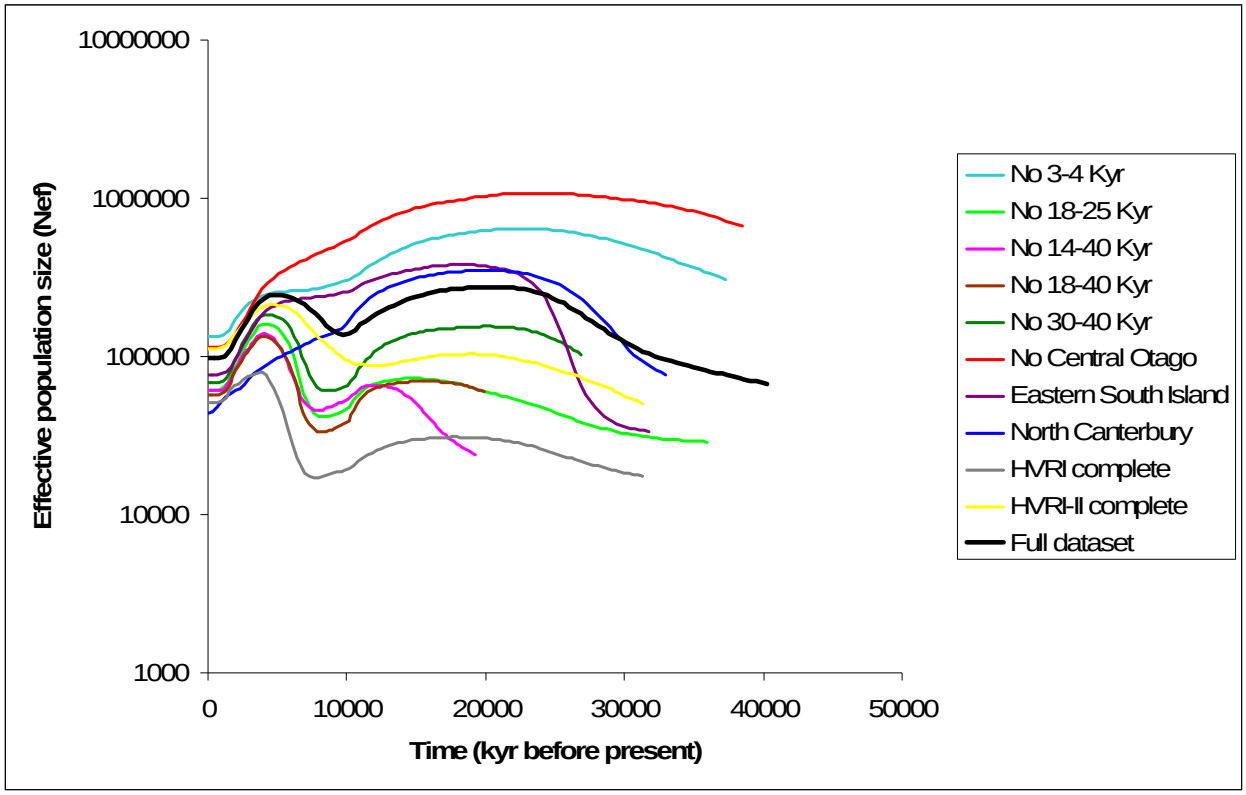

Figure 6 Effect of biased sampling on the demographic history of northern heavy-footed moa (*Pachyornis elephantopus*) inferred from the BSP.

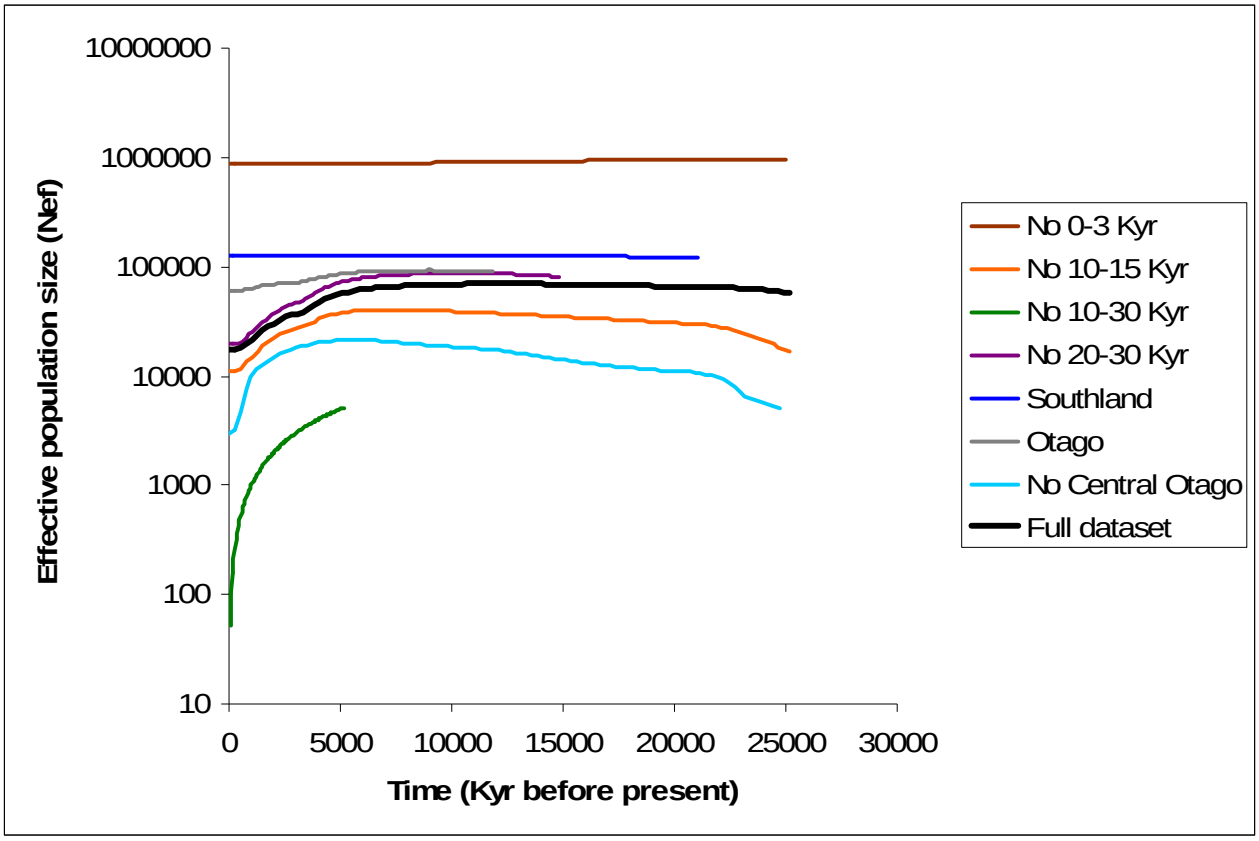

Figure 7 Effect of biased sampling on the demographic history of southern heavy-footed moa (*Pachyornis elephantopus*) inferred from the BSP.

In contrast, if sequences from the eastern South Island are analysed (excluding northwest Nelson/West Coast and Central Otago), the palaeodemographic signal inferred from the BSP is truncated and characterised by a rapid increase in population size from 30-25 Kya followed by a slow decline until 5 Kya, then a rapid decline (Figure 6).

Phylogenetic sampling biases

When phylogenetic clades were excluded from the analysis it resulted in large deviations in the palaeodemographic history inferred from the BSP, especially if the clade contained rare haplotypes or an underlying geographic signal. In bison, exclusion of clade one (defined by Shapiro *et al.* 2004) which contained bison south of the ice, resulted in no apparent Holocene population expansion (Figure S6). In contrast, the effect of excluding clades two to four from Shapiro *et al.* (2004) was minimal (Figure S6). In crested moa, only the exclusion of clades one and two had a pronounced effect on the BSP (Figure 5 and S9), characterised by a truncated demographic signal with a declining population size, and an increase in population size and smoothing of the BSP respectively. In northern-heavy footed moa, exclusion of clades from the analysis resulted in fluctuations in the population minima and maxima inferred from the BSP (Figure S13) similar to the effects of temporal sampling biases.

Biomolecular preservation biases

Biomolecular preservation biases, where DNA sequence length is not uniform across samples and is simulated by incorporating different levels of missing sequence data into the analysis, had a pronounced effect on the moa BSP. In crested moa, when complete HVRI-II sequences were analysed, a near constant population size was reconstructed (Figure 5 and S10). This is compared to northern heavy-footed moa (Figure 6 and S14) where analysis of complete HVRI (missing HVRII), and complete HVRI-II sequence data resulted in BSP similar to the effects of temporal sampling biases (excluding sequences 9-12 and 18-25 Kyr). However, analysis of HVRI sequence data (including specimens with missing sequence data) resulted in a marked deviation in the inferred demography plot, characterised by low population size 40-10 Kya, followed by a rapid increase in population size (Figure S14). By contrast, the effect of biomolecular preservation biases on the southern heavy-footed moa BSP was minimal (Figure S17), with only the overall population size 30-5 Kya affected.

Discussion

The results of this study illustrate the sensitivity of the BSP to common sampling biases inherent in most aDNA datasets (Figure 1). This can have serious implications on the interpretation of BSP from published (see Table One) and future aDNA datasets utilising the BSP. Despite the utility and importance of the Bayesian Skyline method, to date there has been no in-depth investigation into the way the BSP is affected by sampling biases (Shapiro *et al.* 2004; Drummond *et al.* 2005; Ho *et al.* 2007; Debruyne *et al.* 2008; Rambaut *et al.* 2008; Aubry *et al*. 2009; Bruyn *et al.* 2009; Campos *et al.* 2010; Mona *et al.* 2010; Pilot *et al*. 2010; Prost *et al.* 2010; Stiller *et al.* 2010). Consequently, there are a number of critical observations arising from this research that need to be addressed.

Simulations

The simulations showed that temporal and biomolecular preservation sampling biases had no obvious affect on the demographic history inferred from the BSP when sequences were modelled under constant population size and boom bust population demography (Figure 2 and 3). This is not surprising given the sequences were simulated under the coalescent, so that sub-samples of the dataset should be representative of the population as a whole. We assume the variability in the simulations is due to random noise, from the stochastic nature of the coalescent, and not an inherent bias.

Sampling biases

The simulations (Figure 2 and 3) and empirical data (Figure 4-7) indicate that aDNA datasets that have a strong demographic signal are more robust to phylogenetic and biomolecular preservation biases. The exception to this is that the bison and moa datasets were affected by geographic and temporal biases. Phylogeographic structuring within the data had a pronounced impact on the BSP. For bison, this means that Shapiro *et al.* (2004) and Drummond *et al.* (2005) sampled multiple populations (Eastern Beringia, Western Beringia and south of the ice), which violated the assumption of panmixia of individuals with a single population coalescent (Drummond *et al.* 2005). The inferred palaeodemographic histories suggest that bison in Beringia (Eastern and Western Beringia), and south of the ice had differing responses to climate and habitat change (Figure 4). The post decline recovery of bison was only seen south of the ice, while eastern Beringian bison continued to decline until their local extinction a couple of hundred years ago.

The earliest undisputed radiocarbon dates for humans in western Beringia are 27 Kya (~32 Kyr cal. years BP; Pitulko *et al.* 2004; Goebel *et al.* 2008). In contrast, the earliest undisputed dates for eastern Beringia are ~ 12 Kyr cal. years BP in central Alaska (Yesner 2001; Holmes and Crass 2003), and 12.3 Kya (~14.27-14 Kyr cal. years BP) in central North America (Gilbert *et al.* 2008). The local extinction of bison in western Beringia is coincident with the earliest dated archaeological deposits. In contrast, the decline in the population size of bison from eastern Beringia and south of the ice (Figure 4) predates the earliest undisputed evidence for humans in North America, suggesting bison populations declined in the lead up to the LGM. However, eastern Beringian populations are characterised by a second decline (Figure 4) coincident with the earliest undisputed evidence of humans in Alaska, the Younger Dryas and a hypothesised extraterrestrial bolide impact (Firestone *et al*. 2007). These factors may also have contributed to the continued decline of bison populations south of the ice until about 10 Kya (Figure 4). Bison populations south of the ice recovered (Figure 4) probably as a result of the development of the American prairies. The recent decline in bison south of the ice (Figure 4) is coincident with (i) the re-introduction of horses by the Spanish in 1492, allowing Indian populations to hunt bison more effectively (Flores 1991), and (ii) widespread culling of bison in the ninetieth century. The contrasting demographic history of bison populations in Beringia and south of the ice suggests that the demographic history of one population cannot be applied to other populations of a species (Scott 2009). The results also show that the major signal for the boom-bust nature of bison comes from Eastern Beringia, supporting Shapiro *et al.* (2004).

The effects of temporal, phylogenetic and biomolecular preservation biases in the bison and moa datasets were similar to geographic biases. This is because of the underlying phylogeographic structure within these datasets. For example, in bison, excluding clade one from the analysis (phylogenetic bias; Figure S6) was the same as excluding bison south of the ice and analysing only Beringia (geographical bias; Figure 4). Further, in northern heavy-footed moa, when complete HVRI, and HVRI-II sequences were examined (biomolecular preservation bias; Figure 6) the BSP was similar to BSP where sequences 9-12 and 18-25 Kyr were excluded (temporal bias; Figure 6 and S11). This was because sequences 9-12 and 18-25 Kyr were restricted to a single geographical region and contained higher levels of missing sequence data. These findings highlight the importance of understanding the temporal distribution of the specimens and how this affects aDNA preservation.

Finally, excluding sequences in pre-defined time periods from the bison and moa datasets showed that the BSP is prone to temporal biases, contrary to the findings of Rambaut *et al.* (2008) and Stiller *et al.* (2010). The most pronounced effect occurred when the oldest sequences in the bison (Figure 4) and moa datasets (Figure 5-7) were progressively excluded. This resulted in a significant truncation of the demographic signal with major deviations from the BSP reconstructed from the complete dataset. For example, in bison, this resulted in the tail end of the BSP progressively shifting towards the present, in turn resulting in more pronounced population increases and smaller population maxima (Figure 4). This means that bison probably had a high population size about 40 Kya, followed by a major population bottleneck and recovery (depending on geographical location). However, given the significant effects of these temporal biases, the demographic history of bison after 40 Kya is uncertain. In BSP analyses, the population size is reconstructed to the most recent common ancestor of all sequences in the analysis (Drummond *et al.* 2005). The results of this study strongly suggest that the reconstructed demographic history after the oldest radiocarbon date may not be accurate, given (i) the fluctuation in the tail end of the BSP and (ii) the fact that the BSP reconstructs an increasing population size from the most recent common ancestor to the oldest radiocarbon date, despite the fact that sequences may be sampled from a declining population overall (Figure 4). This suggests that the inferred increase in population size is probably a significant artefact of the Bayesian Skyline methodology.

Implications for aDNA studies utilising the BSP

The results of this study suggest that the accuracy of the palaeodemographic history of taxa inferred from the BSP is influenced by sampling biases inherent in most aDNA datasets. Given these findings, there are some important points to consider when building time-structured aDNA datasets for BSP analysis, to ensure sampling biases are kept to minimal levels. First, it is important to have a large number of radiocarbon dated specimens, spread evenly through time (Table 1) to sample multiple coalescent events, especially before, during and after population bottlenecks. Incorporating additional radiocarbon dates into BSP analyses will improve the accuracy of the BSP because the Bayesian Skyline methodology samples terminal nodes (i.e. the radiocarbon dated sequences) and uses the mutation rate to infer the age and effective population size at different coalescent intervals in a sample of genealogies. Small numbers of undated sequences can be incorporated into the BSP analysis but only if the sequences can be constrained to a small age range or if a prior age distribution can be chosen. Constraining the age can be

achieved by placing a stratigraphic age from well-dated fossil deposits or stratigraphic layers on undated specimens from the same deposit or layer (Drummond *et al.* 2007; Bunce *et al.* 2009; Chapter Six and Seven) or by placing a uniform prior on the age of small numbers of samples (Korsten *et al.* 2009). Stiller *et al.* (2010) showed that excluding 17 out of 21 identically dated (44,550 years BP) cave bear specimens from Pestera cu Oase Cave in Romania from the BSP analysis did not significantly change the demographic signal inferred from the BSP compared to the complete dataset. This suggests that the BSP, at least in the cave bear dataset (Stiller *et al*. 2010), is not affected by large numbers of specimens the same age.

Second, "old" sequences are needed to calibrate the genealogies and ensure an accurate evolutionary rate. The importance of this is seen in the effects of removing the oldest sequences from the BSP analysis (Figure 4). This raises two points. First, caution needs to be used when conducting BSP analyses on contemporary data (e.g. Grazziotin *et al.* 2006; Crandall *et al.* 2008; Marske *et al.* 2009). If we consider bison sequences 0-30 Kyr as "recent" and sequences 30-60 Kyr as "ancient", then a significantly different demographic signal is produced when "ancient" sequences are excluded (Figure 4). This is similar to deviations in the inferred palaeodemographic history from the simulated datasets when only modern sequences were analysed (Figure 2 and 3). Further cause for caution in conducting BSP analyses on contemporary data comes from Depaulis *et al.* (2009) and Mona *et al.* (2010). Depaulis *et al.* (2009) argued that classical population genetics algorithms assume contemporary sampling (isochronous sampling like modern DNA datasets). This study showed that when classical population genetic algorithms were used on simulated and empirical (cave bear sequence data published by Hanni *et al.* 1994; Kuhn *et al.* 2001; Loreille *et al.* 2001; Hofreiter *et al*. 2002, 2004a, b; Orlando *et al.* 2002) time-structured aDNA datasets (heterochronous sampling) it resulted in an overestimation of genetic polymorphism (theta), effective population size and support for a false palaeodemographic history. Depaulis *et al.* (2009) also showed that when a simulated panmictic population was divided into two sub-populations and assigned different sampling times (to simulate aDNA sampling strategies) the result was pronounced false population structure. This supports data from Achaz *et al.* (2004) on HIV, suggesting that heterochronous sampling can artificially strengthen real phylogeographic structure. Supporting Depaulis *et al.* (2009), Mona *et al.* (2010) argued that in heterochronous datasets, where a constant population size through time was inferred, the BSP will infer a demographic expansion, if the age of the sequences are not taken into account (isochronous sampling). Mona *et al.* (2010)

showed that for aurochs in Italy and central/northern Europe, a constant population size through time was inferred from the BSP. However, when the age of the sequences was fixed to the age of the youngest auroch specimen (2 Kya for central/northern Europe and 7 Kya for Italy), a demographic expansion was inferred from the BSP. The second point is that caution needs to be used when interpreting BSP beyond the oldest radiocarbon date as it may not be accurate because (i) there are no calibration points to anchor the genealogies when inferring population size (Stefan Prost pers comm. 2009) and (ii) there is a disturbing trend of population expansion detected in simulations (Figure 2 and 3) and truncated empirical datasets (Figure 4), suggesting that this is a serious artefact of the BSP.

Third, it is important to establish a sampling programme that avoids phylogeographic structuring. When the assumption of a single unstructured population is met (Drummond *et al.* 2005), theta is expected to approximate the effective population size. If the population has been continuous and panmictic (i.e. unstructured) over time, then most sub-samples should still be representative of the population as a whole. However, when the assumption is violated, theta is always elevated and the shape of the BSP is altered. For example, Bruyn *et al.* (2008) analysed southern elephant seal populations in Antarctica (Victoria Land) and Macquarie Island utilising the BSP, which was characterised by constant population size. However, when Bruyn *et al.* (2008) combined these two populations the BSP was characterised by an increase in population size from 15-1 Kya, followed by a marked decline 1-0 Kya. An example of a good sampling programme that avoided phylogeographic structuring was Prost *et al.* (2010). This study sampled 64 Collared Lemmings (*Dicrostonyx torquatus*) from four stratigraphic layers (21.91, 13.09, 10 Kya and modern) from Pymva Shor Cave, Northern Pre Urals, Russia. Phylogeographic analysis showed that the specimens sampled from the stratigraphic layers represented a continuous unstructured population through time (Prost *et al*. 2010).

A further constraint of BSP analyses is population continuity through time. Ancient DNA studies have clearly shown that glacial/interglacial cycles can affect the level of phylogeographic structuring and population continuity (Barnes *et al.* 2002; Hofreiter *et al.* 2004b; Hofreiter and Stewart 2009). For example, Barnes *et al.* (2002) showed that the Holocene phylogeographic pattern in brown bears was only present during the Holocene, with their demographic history characterised by multiple replacement events, with each event representing a separate population. Population continuity through time is also important when calculating mutation rates used in BSP

analyses. Hay *et al.* (2008) used modern and ancient tuatara (*Sphenodon punctatus*) sequences to calculate a substitution rate of 1.56 substitutions per nucleotide per million years (95% HPD 0.83-2.34) and concluded that this was the fastest substitution rate calculated from a vertebrate aDNA dataset. However, Miller *et al*. (2009) argued that there was a high probability that the tuatara specimens analysed by Hay *et al.* (2008) did not come from a single continuous population, and that phylogeographic structuring within tuatara had elevated the substitution rate. Miller *et al.* (2009) showed that there was marked phylogeographic structuring within and between offshore island populations of modern tuatara, and between modern island and ancient mainland populations, arguing against population continuity. To ensure that multiple populations are not sampled and the assumptions of the BSP are met, aDNA datasets must be divided into different phylogeographic or temporal units, so that each population is independent. This approach showed that the bison BSP (Drummond *et al.* 2005) is a composite of three different geographical signals (Figure 4).

Conclusion

The BSP was designed to provide a means to investigate the past population demographics of taxa using time-structured genetic datasets like aDNA. However, most aDNA datasets have inherent sampling biases including temporal, geographic, phylogenetic and biomolecular preservation biases that can potentially affect the inferred palaeodemographic history of taxa. The results of this study have shown that biased sampling will produce variations in the BSP and unless aDNA datasets are produced under conditions that minimise sampling biases, the BSP may not be an accurate representation of the past population demography history of taxa. As aDNA datasets are a biased sample of the true population demographic history, considerable caution needs to be used when interpreting inferred palaeodemographic histories from most aDNA datasets.

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Literature Cited

Achaz G, Palmer S, Kearney M, *et al.* (7 co-authors). 2004. A robust measure of HIV-1 population turnover within chronically infected individuals. Mol. Biol. Evol. 21: 1902-1912.

Albury KB, Statham MJ, Sacks BN, Perrine JD, Wisely SM. 2009. Phylogeography of the North American red fox: vicariance in Pleistocene forest refugia. Mol. Ecol. 18: 2668-2686.

Axelsson E, Willerslev E, Gilbert MTP, Nielsen R. 2008. The effect of ancient DNA damage of inferences of demographic histories. Mol. Biol. Evol. 2: 2181-2187.

Baker AJ, Huynen LJ, Haddrath O, Miller CD, Lambert DM. 2005. Reconstructing the tempo and mode of evolution in an extinct clade of birds with ancient DNA: The giant moas of New Zealand. Proc. Natl. Acad. Sci. USA. 102: 8257-8262.

Barnes I, Matheus P, Shapiro B, Jensen D, Cooper A. 2002. Dynamics of Pleistocene population extinctions in Beringian brown bears. Science 295: 2267-2270.

Barnes IB, Shapiro B, Lister A, Kuznetsova T, Sher A, Guthrie D, Thomas MG. 2007. Genetic structure and extinction of the woolly mammoth, *Mammuthus primigenius*. Curr. Biol. 17: 1072-1075.

Beebee T, Rowe G. 2004. An introduction to molecular ecology. Oxford University Press, Oxford, UK.

Borge T, Bachmann L, Bjornstad G, Wiig O. 2007. Genetic variation in Holocene bowhead whales from Svalbard. Mol. Ecol. 16: 2223-2235.

Bunce M, Worthy TH, Phillips MJ, *et al.* (11 co-authors). 2009. The evolutionary history of the extinct ratite moa and New Zealand Neogene paleogeography. Proc. Natl. Acad. Sci. USA. 106: 20646-20651.

Campos PF, Willerslev E, Sher A, *et al.* (20 co-authors). 2010. Ancient DNA analyses exclude humans as the driving force behind late Pleistocene musk ox (*Ovibos moschatus*) population dynamics. Proc. Natl. Acad. Sci. USA. 107: 5675-5680.

Crandall ED, Frey MA, Grosberg RK, Barber PH. 2008. Contrasting demographic histories and phylogeographical patterns in two Indo-Pacific gastropods. Mol. Ecol. 17: 611-626.

De Bruyn M, Hall BL, Chauke LF, Baroni C, Koch PL, Hoelzel AR. 2009. Rapid response of a marine mammal species to Holocene climate and habitat change. PLoS Genet. 5: e1000554.

Debruyne R, Chu G, King CE, *et al.* (20 co-authors). 2008. Out of America: Ancient DNA evidence for a New World origin of Late Quaternary woolly mammoths. Curr. Biol. 18: 1320-1326.

Depaulis F, Orlando L, Hanni C. 2009. Using classical population genetic tolls with heterochroneous data: time matters! PLoS One 4: e5541.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7: 214.

Drummond AJ, Rambaut A, Shapiro B, Pybus OG. 2005. Bayesian coalescent inference of past population dynamics from molecular sequences. Mol. Biol. Evol. 22: 1185-1192.

Flores D. 1991. Bison ecology and bison diplomacy: the southern plains from 1800-1850. The J. Am. Hist. 78: 465-485.

Firestone RB, West A, Kennett JP, et al. (26 co-authors). 2007. Evidence for an extraterrestrial impact 12,900 years ago that contributed to the megafaunal extinctions and the Younger Dryas cooling. PNAS 104: 16016-16021.

Gilbert MTP, Jenkins DL, Gotherstrom A, *et al.* (10 co-authors). 2008. DNA from pre-Clovis human coprolites in Oregon, North America. Science 320: 786-789.

Goebel T, Waters MR, O'Rourke D. 2008. The late Pleistocene dispersal of modern humans in the Americas. Science 319: 1497-1502.

Grazziotin FG, Monzel M, Echeverrigaray S, Bonatto SL. 2006. Phylogeography of the *Bothrops jararaca* complex (Serpentes: Viperidae): Past fragmentation and island colonisation in the Brazilian Atlantic Forest. Mol. Ecol. 15: 3969-3982.

Guthrie RD. 2003. Rapid body size decline in Alaskan Pleistocene horses before extinction. Nature 426: 169-171.

Hanni C, Laudet V, Stehelin D, Taberlet P. 1994. Tracking the origins of the cave bear (*Ursus spelaeus*) by mitochondrial DNA sequencing. Proc. Natl. Acad. Sci. USA. 91: 12336–12340.

Hay JM, Subramanian S, Millar CD, Mohandesan E, Lambert DM. 2008. Rapid molecular evolution in a living fossil. TIG. 24: 106-109.

Ho SYW, Kolokotronis SO, Allaby RG. 2007. Elevated substitution rates estimated from ancient DNA sequences. Biol. Lett. 3: 702-705.

Ho SYW, Saarma U, Barnett R, Haile J, Shapiro B. 2008. The effect of inappropriate calibration: Three case studies in molecular ecology. PLoS One 3: e1615.

Hofreiter M, Capelli C, Krings M, Waits L, Conard N, *et al.* (13 co-authors). 2002. Ancient DNA analyses reveal high mitochondrial DNA sequence diversity and parallel morphological evolution of Late Pleistocene Cave Bears. Mol. Biol. Evol. 19: 1244–1250.

Hofreiter M, Rabeder G, Jaenicke-Despres V, Withalm G, Nagel D, *et al.* (8 co-authors). 2004a. Evidence for reproductive isolation between cave bear populations. Curr. Biol. 14: 40–43.

Hofreiter M, Serre D, Rohland N, Rabeder G, Nagel D, Conard N, Munsel S, Paabo S. 2004b. Lack of phylogeography in European mammals before the last glaciation. Proc. Natl. Acad. Sci. USA. 101: 12963-12968.

Hofreiter M, Stewart J. 2009. Ecological change, range fluctuations and population dynamics during the Pleistocene. Curr. Biol. 19, R584-594.

Holmes CE, Crass BA, paper presented at the 30th annual meeting of the Alaska Anthropological Association, Fairbanks, 27-29 March 2003.

Kuhn R, Schroder W, Rottman O. 2001. Sequencing mtDNA of the cave bear (*Ursus spelaeus*) from the Bavarian Alps is feasible by nested and touchdown PCR. Acta. Theriol. 46: 61–68.

Kuhn TS, McFarlane KA, Groves P, Mooers AO, Shapiro B. 2010. Modern and ancient DNA reveal recent partial replacement of caribou in the southwest Yukon. Mol. Ecol. 19: 1312-1323.

Kumar S, Tamura K, Nei M. 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief. Bioinform. 5: 150-163.

Lambert DM, Baker A, Huynen L, Haddrath O, Herbert PDN, Miller CD. 2005. Is a large-scale DNA-based inventory of ancient life possible? J. Hered. 96, 279-284.

Loreille O, Orlando L, Patou-Mathis M, Philippe M, Taberlet P, *et al.* (6 co-authors). 2001. Ancient DNA analysis reveals divergence of the cave bear, *Ursus spelaeus*, and brown bear, *Ursus arctos*, lineages. Curr. Biol. 11: 200–203.

Marske KA, Leschen RAB, Barker GM, Buckley TR. 2009. Phylogeography and ecological niche modelling implicate coastal refugia and trans-alpine dispersal of a New Zealand fungus beetle. Mol. Ecol. 18: 5126-5142.

Miller HC, Moore JA, Allendorf FW, Daugherty CH. 2008. The evolutionary rate of tuatara revisited. TIG. 25: 13-15.

Mona S, Catalano G, Lari M, Larson G, Boscato P, Casoli A, Sineo L, Patti CD, Pecchioli D, Caramelli D, Bertorelle G. 2010. Population dynamics of the extinct European aurochs: genetic evidence of a north-south differentiation pattern and no evidence of post-glacial expansion. BMC Evol. Biol. 10: 83.

Orlando L, Bonjean D, Bocherens H, Thenot A, Argant A, *et al.* (7 co-authors). 2002. Ancient DNA and the population genetics of Cave Bears (*Ursus spelaeus*) through space and time.

Mol. Biol. Evol. 19: 1920–1933.
Pilot M, Branicki W, Jedrzejewski W, Goszczynski J, Jedrzejewska B, Dykyy I, Shkvyrya M, Tsingarska E. 2010. Phylogeographic history of grey wolves in Europe. BMC Evol. Biol. 10: 104.
Pitulko VV, Nikolsky PA, Girya EY, Basilyan AE, Tumskoy VE, Koulakov SA, Astakhov SN, Pavlova EU, Anisimov MA. 2004. The Yana RHS site: humans in the Arctic before the Last Glacial Maximum. Science 303: 52-56.
Prost S, Smirnov N, Fedorov VB, Sommer RS, Stiller M, Nagel D, Knapp M, Hofreiter M. 2010. Influence of climate warming on Arctic mammals? New insights from ancient DNA studies of the Collared Lemming *Dicrostonyx torquatus*. PLoS One 5: e10447.
Rambaut A, Grassly NC. 1997. Seq-Gen: An application for the Monte Carlo simulation of DNA sequence evolution along phylogenetic trees. Comput. Appl. Biosci. 13: 235-238.
Rambaut A, Ho SYW, Drummond AJ, Shapiro B. 2009. Accommodating the effect of ancient DNA damage of inferences of demographic histories. Mol. Biol. Evol. 26: 245-248.
Rambaut A, Pybus OG, Nelson MI, Viboud C, Taubenberger JK, Holmes EC. 2008. The genomic and epidemiological dynamics of human influenza A virus. Nature 453: 615-619.
Scott E. 2009. Extinctions, scenarios, and assumptions: Changes in latest Pleistocene large herbivore abundance and distribution in western North America. Quat. Int. 217: 225-239.
Shapiro B, Drummond AJ, Rambaut A *et al.* (26 co-authors). 2004. Rise and fall of the Beringian Steppe Bison. Science 306: 1561-1565.
Spaeth PA, Tuinen M, Chan YL, Terca D, Hadly EA. 2009. Phylogeography of *Microtus longicaudus* in the tectonically and glacially dynamic central Rocky Mountains. J. Mammal. 90: 571-584.
Stiller M, Baryshnikov G, Bocherens H, d'Anglade G, Hilpert B, Munzel SC, Pinhasi R, Rabeder G, Rosendahl W, Trinkaus E, Hofreiter M, Knapp M. 2010. Withering away – 25, 000 years of genetic decline preceded cave bear extinction. Mol. Biol. Evol. 27: 975-978.
Suchard MA, Weiss RE, Sinsheimer JS. 2001. Bayesian selection of continuous-time Markov chain evolutionary models. Mol. Biol. Evol. 18: 1001-1013.
Yesner DR. 2001. Human dispersal into interior Alaska: antecedent conditions, mode of colonisation, and adaptations. Quat. Sci. Rev. 20: 315-327.

Incorporating ancient DNA into Bayesian Skyline Plot analyses: the effect of biased sampling on inferences of past population demographics.

Supplementary Information

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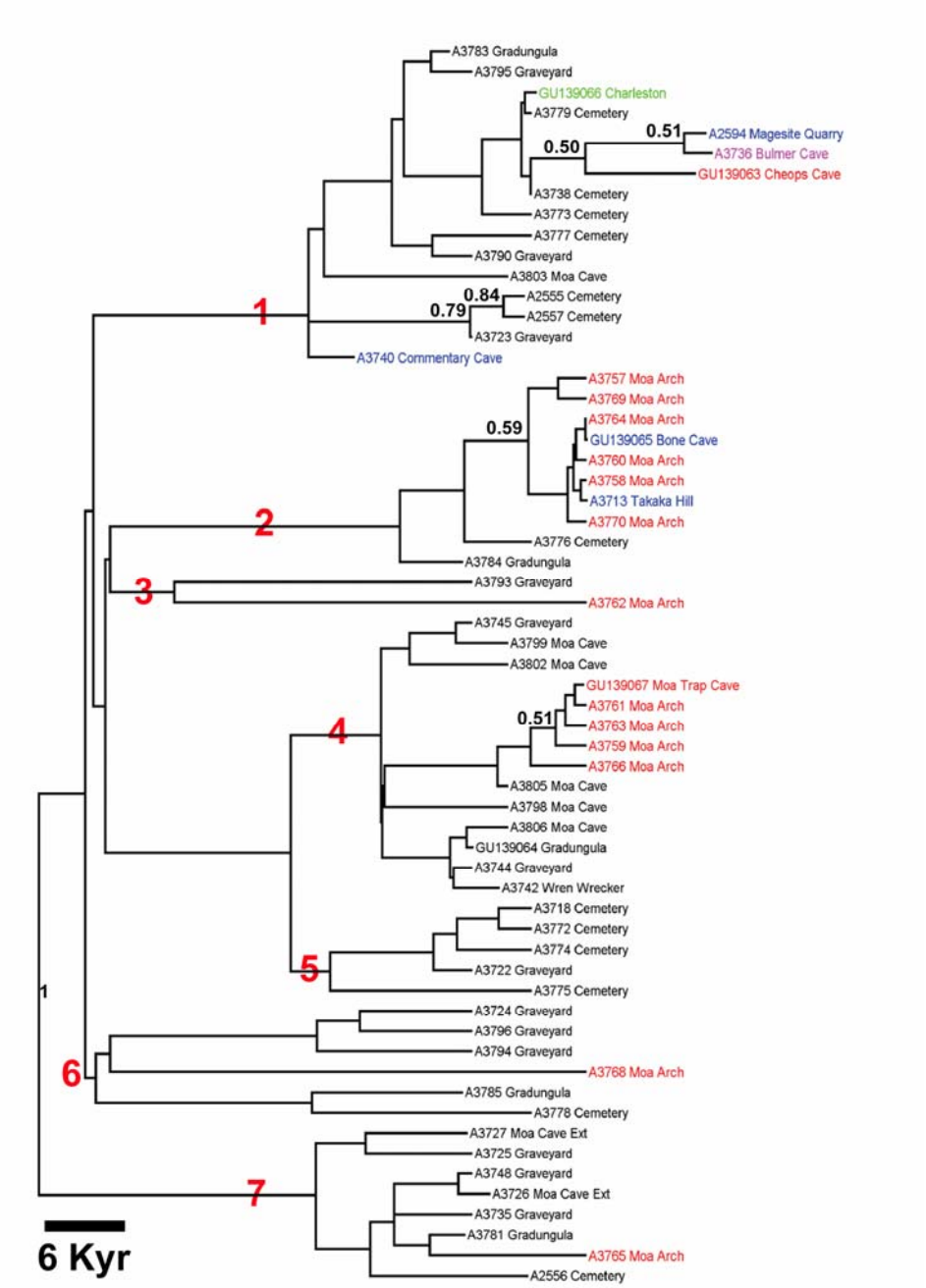

Figure S1 Maximum clade credibility phylogeny of crested moa (*Pachyornis australis*) from the Bayesian Skyline Plot (BSP) analysis, with branch lengths proportional to time. Posterior probability support (> 0.5) is indicated at each node in black. Clades excluded from the BSP analysis, to simulate phylogenetic biases, are indicated in red. Taxon labels are colour coded to represent cave systems excluded from the analysis, to simulate geographical biases: Fuchsia = Mount Owen; Red = Mount Arthur; Blue = Takaka Hill/Golden Bay; Black = Honeycomb Hill; and Lime = Charleston.

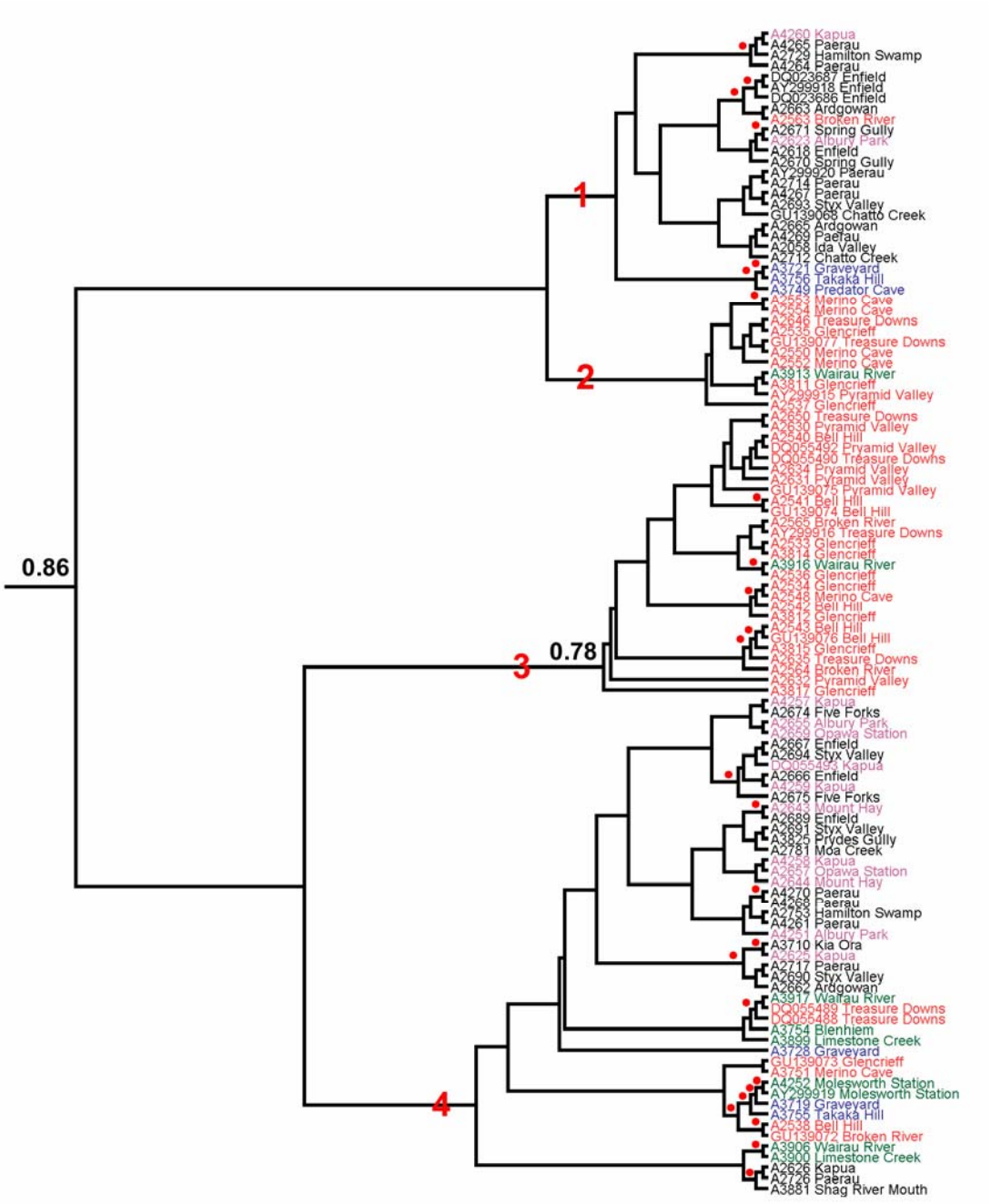

Figure S2 Maximum clade credibility phylogeny of northern heavy-footed moa (*Pachyornis elephantopus*), with branch lengths proportional to the number of substitutions (Chapter Seven). Posterior probability support (> 0.5) is indicated at each node in black (or by red dots). Clades excluded from the BSP analysis, to simulate phylogenetic biases, are indicated in red. Taxon labels are colour coded to represent geographical areas, to simulate geographical biases Blue: Northwest Nelson/West Coast; Green: Marlborough; Red: North Canterbury; Fuchsia: South Canterbury; and Black: Otago (North Otago/Central Otago).

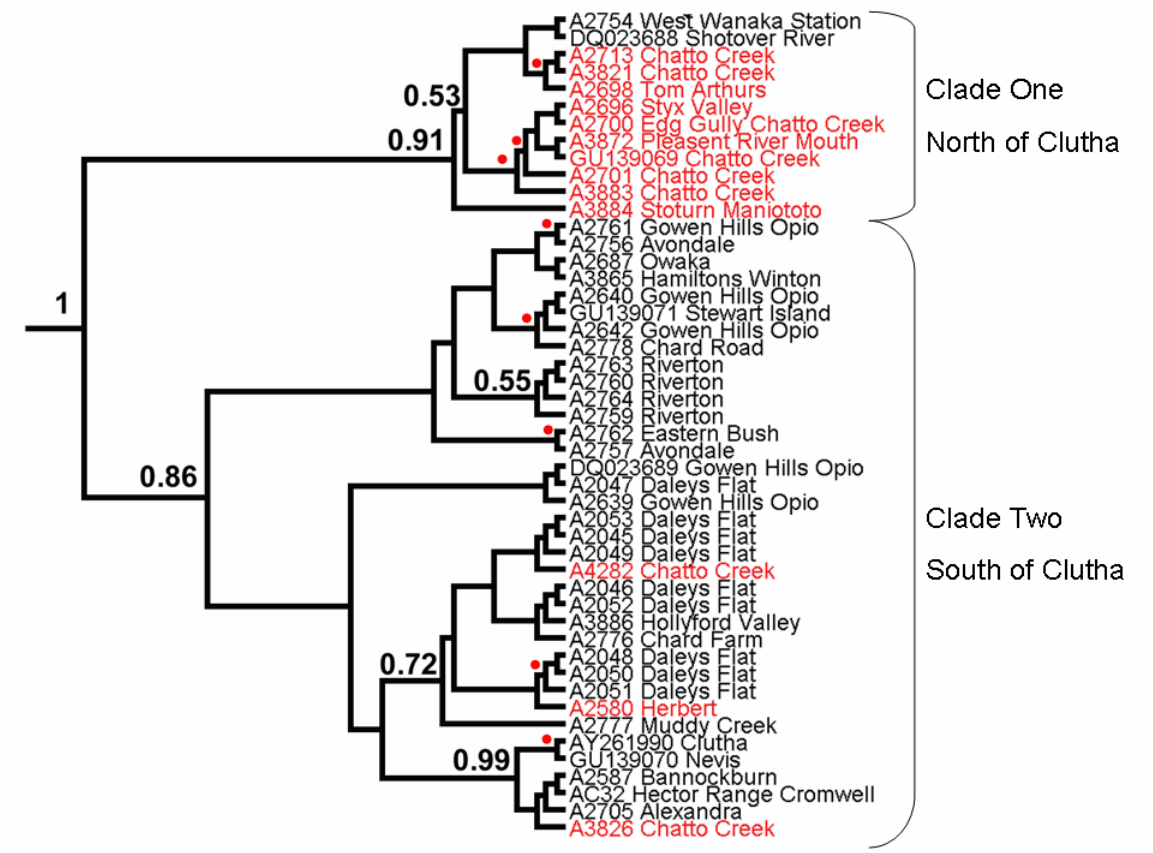

Figure S3 Maximum clade credibility phylogram of southern heavy-footed moa (*Pachyornis elephantopus*), with branch lengths proportional to the number of substitutions (Chapter Seven). Posterior probability support (> 0.5) is indicated at each node in black (or by red dots). There are two well supported clades within southern heavy-footed moa, restricted to north and south of the Clutha River. Red: north of the Clutha; Black: south of the Clutha.

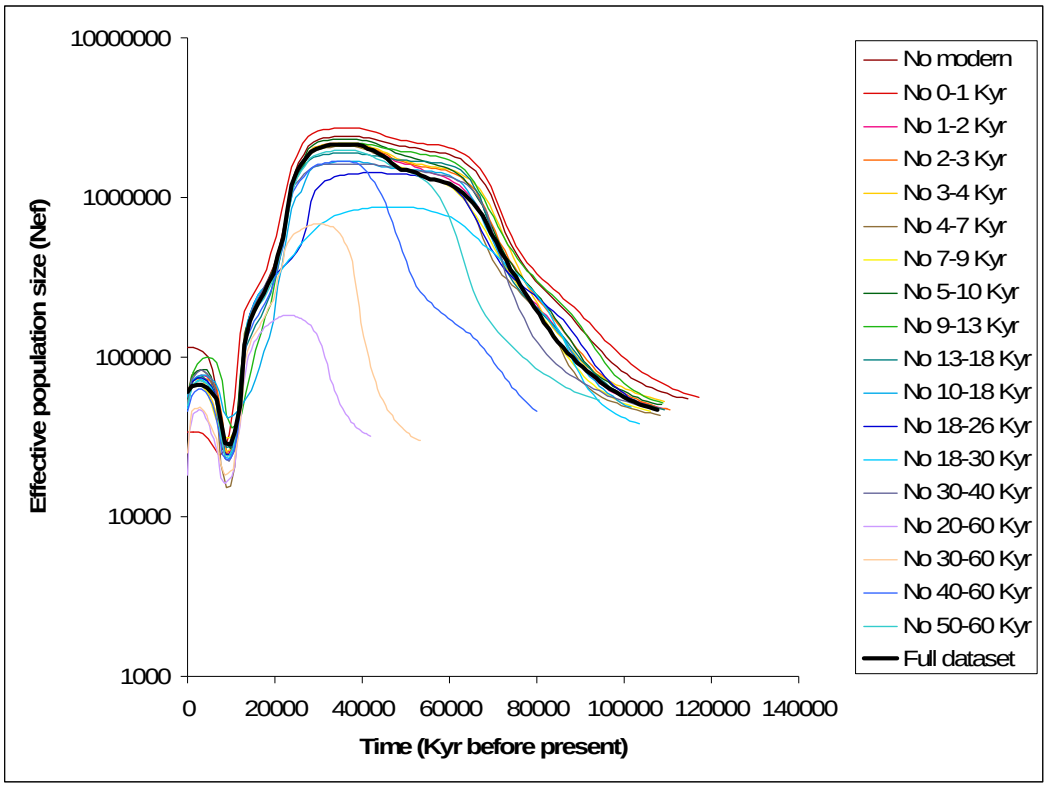

Figure S4 Effect of biased temporal sampling on the bison BSP.

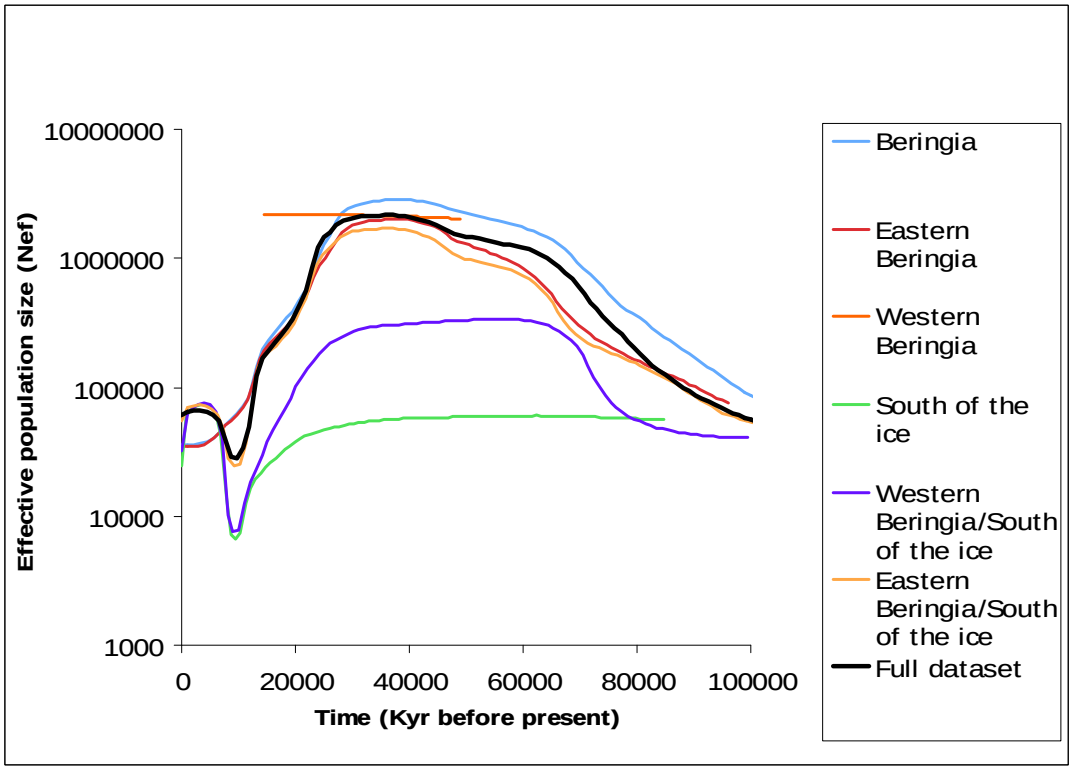

Figure S5 Effect of biased geographical sampling on the bison BSP.

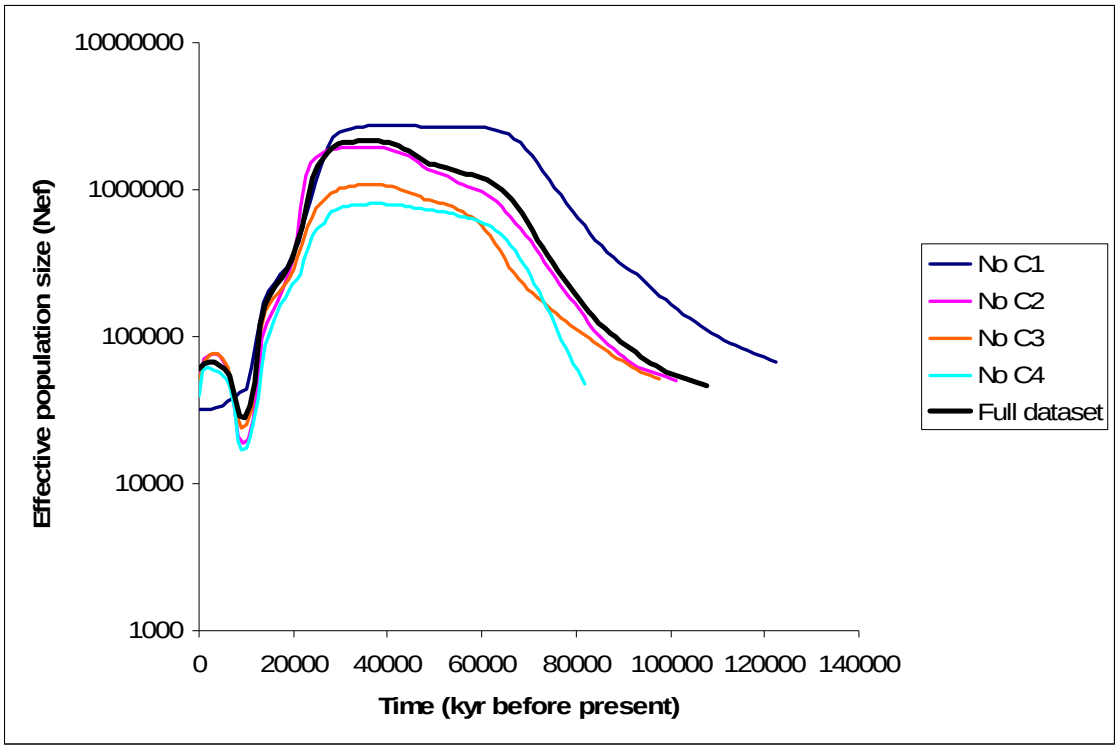

Figure S6 Effect of biased phylogenetic sampling on the bison BSP.

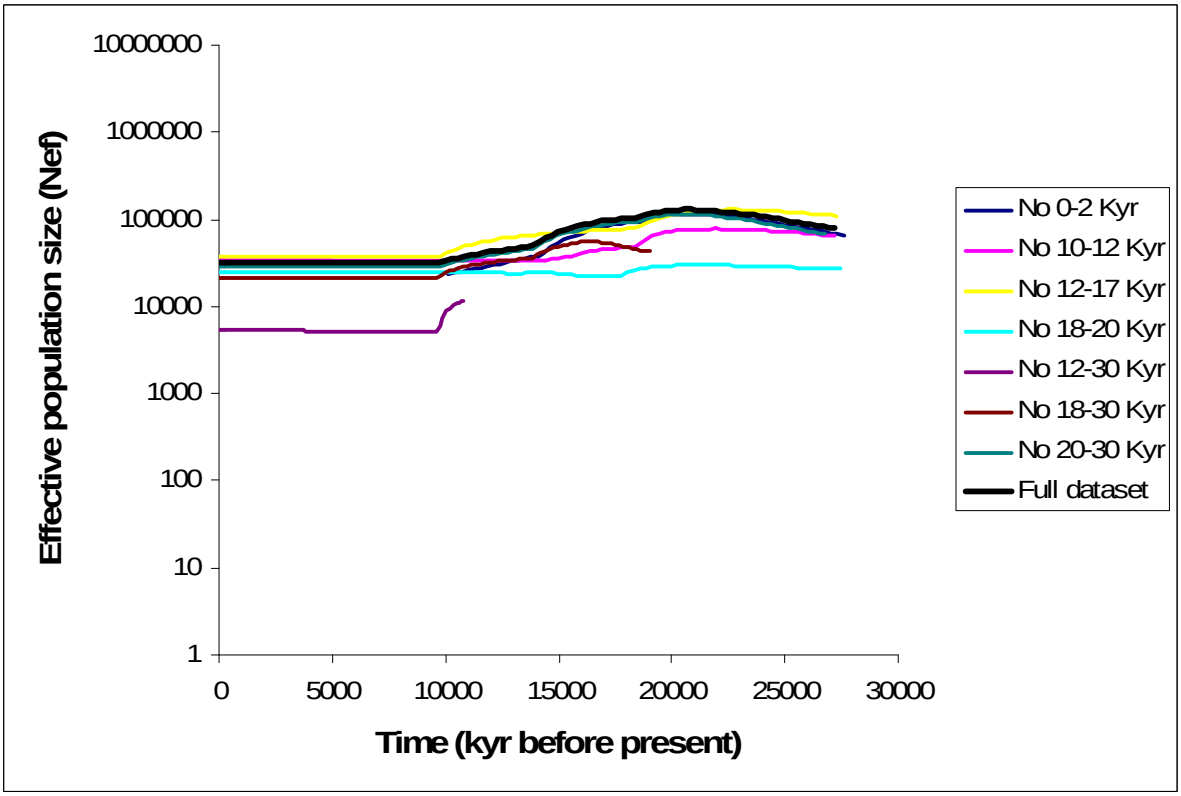

Figure S7 Effect of biased temporal sampling on the crested moa BSP.

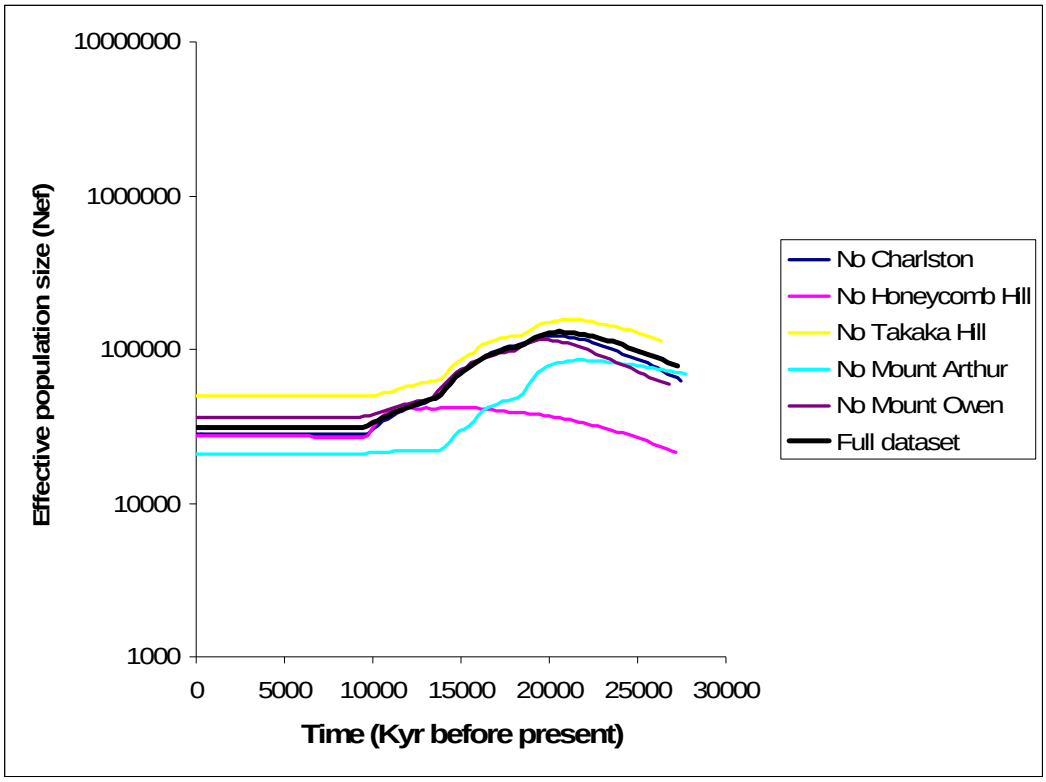

Figure S8 Effects of biased geographical sampling on the crested moa BSP.

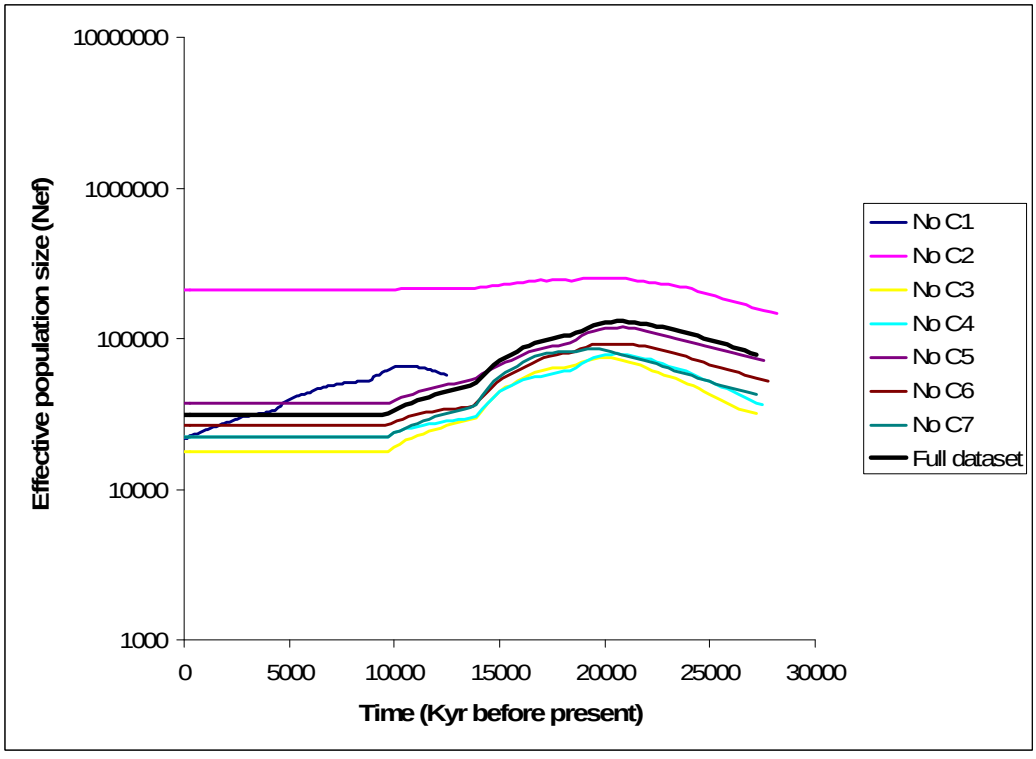

Figure S9 Effects of biased phylogenetic sampling on the crested moa BSP.

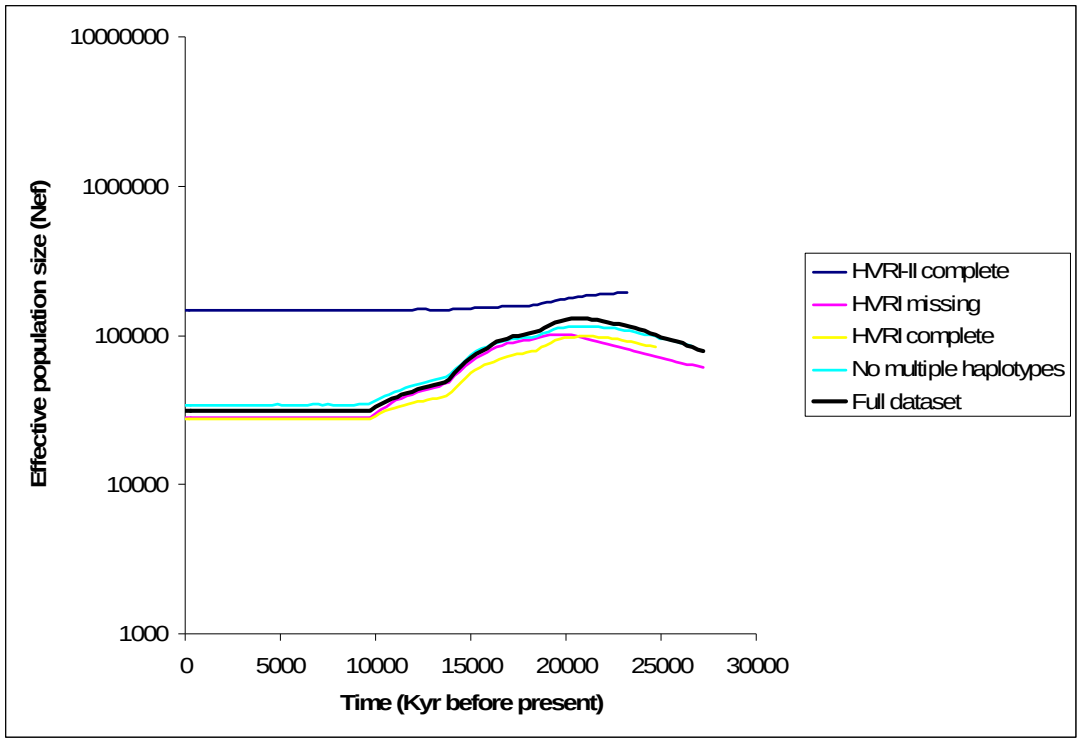

Figure S10 Effects of biomolecular preservation biases on the crested moa BSP.

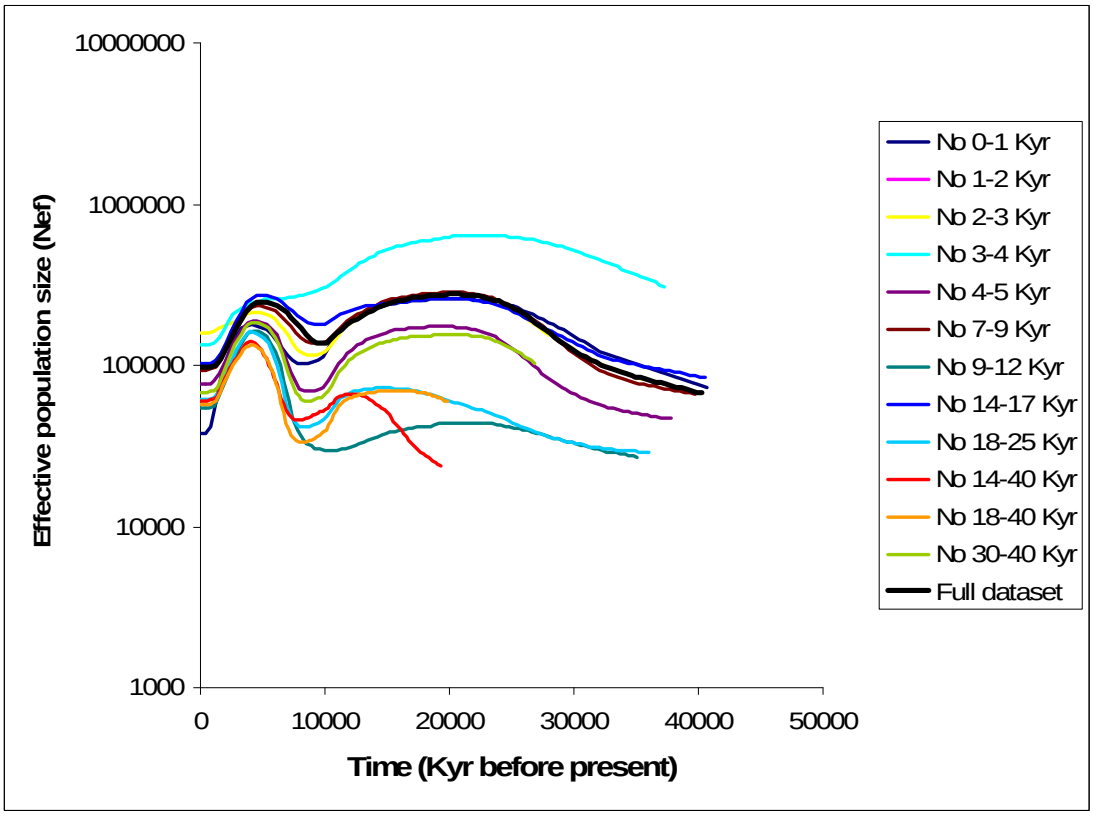

Figure S11 Effects of biased temporal sampling on the northern heavy-footed moa BSP.

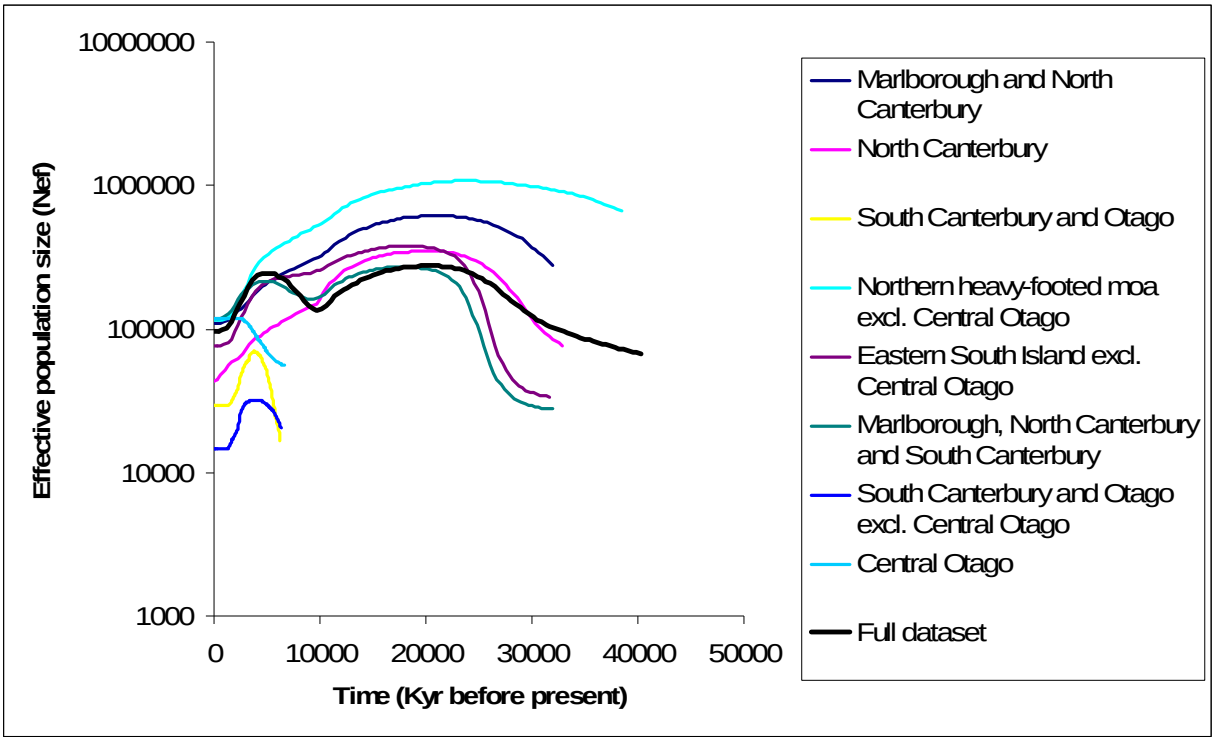

Figure S12 Effect of biased geographic sampling on the northern heavy-footed moa BSP.

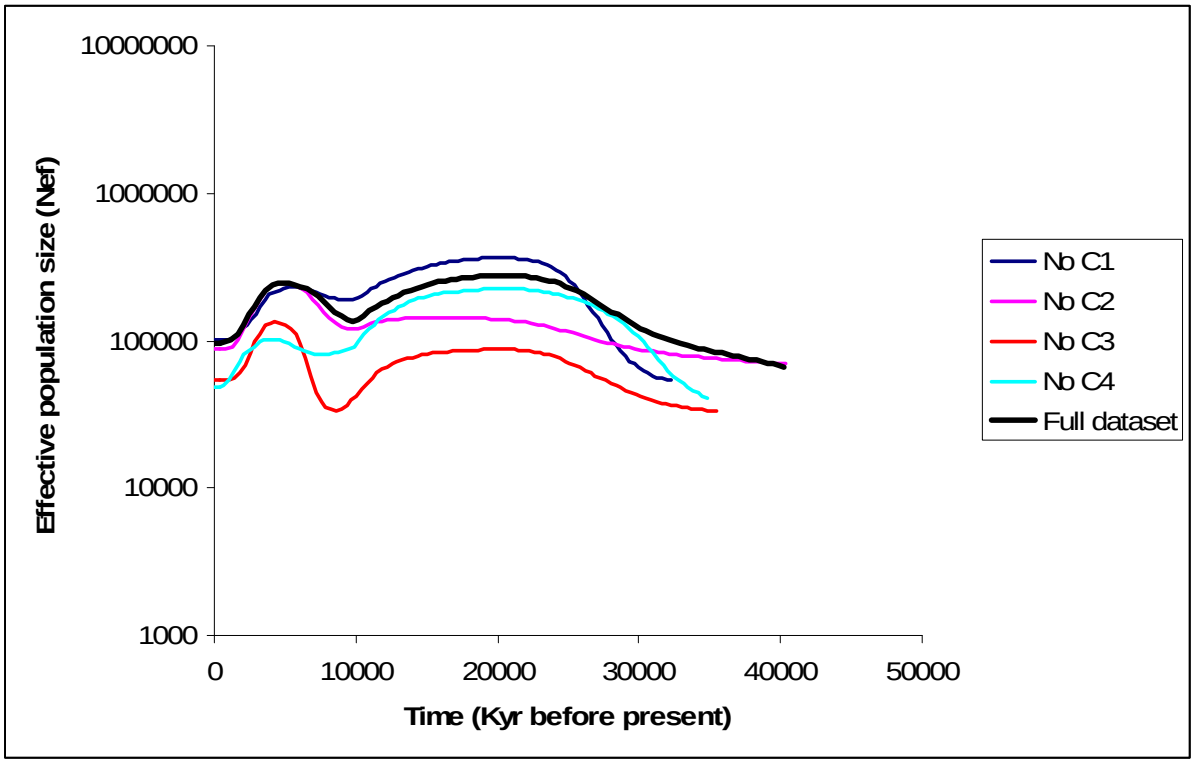

Figure S13 Effects of biased phylogenetic sampling on the northern heavy-footed moa BSP.

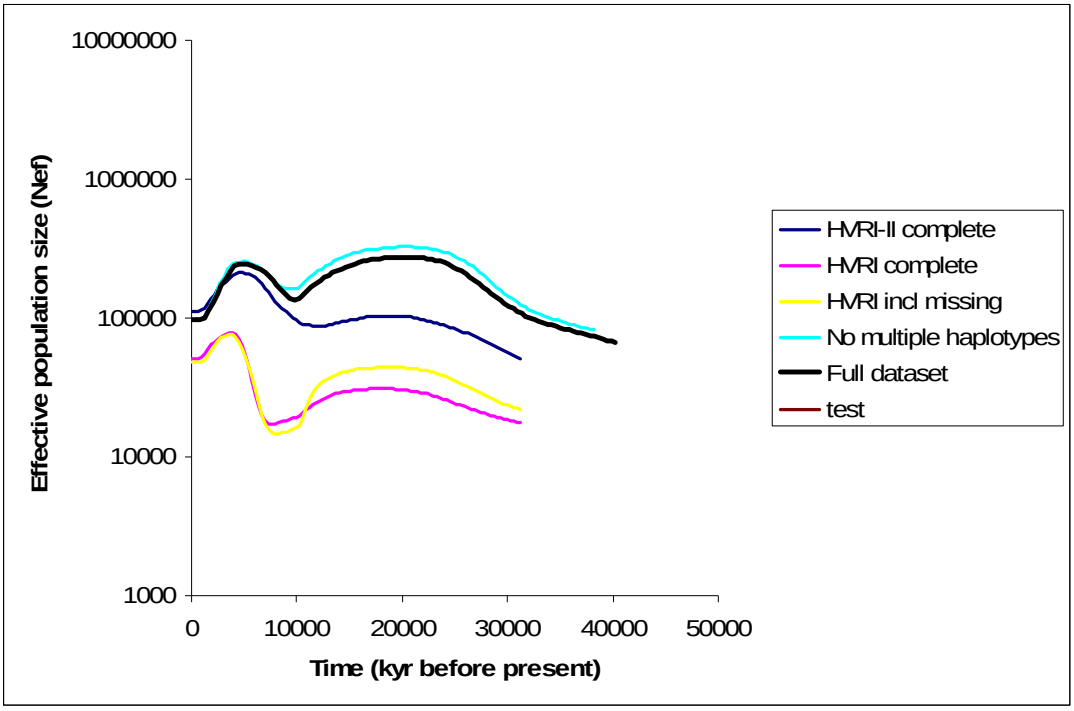

Figure S14 Effects of biomolecular preservation biases on the northern heavy-footed moa BSP.

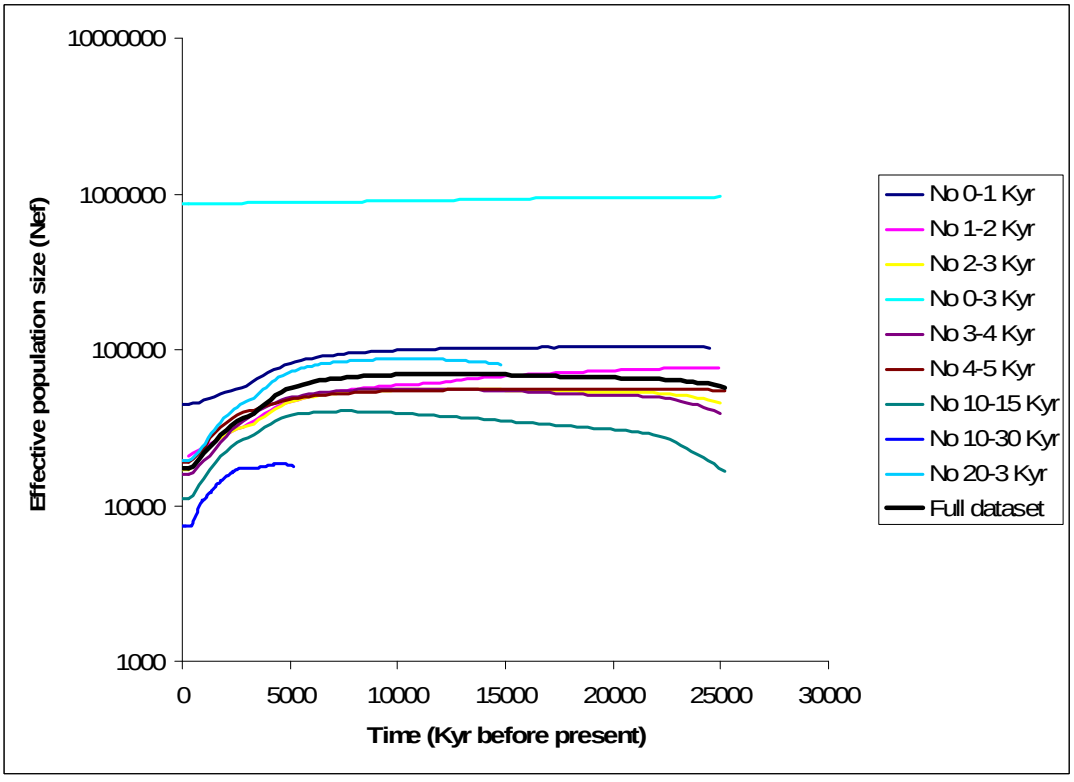

Figure S15 Effects of biased temporal sampling on the southern heavy-footed moa BSP.

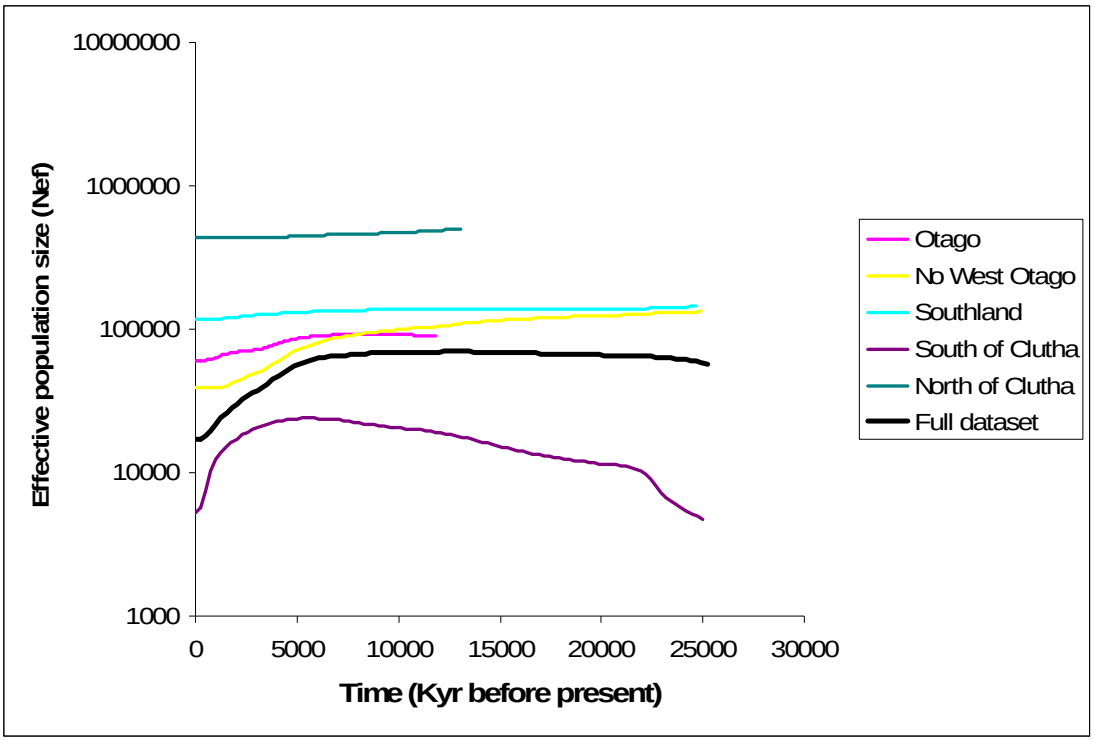

Figure S16 Effect of biased geographic sampling on the southern heavy-footed moa BSP.

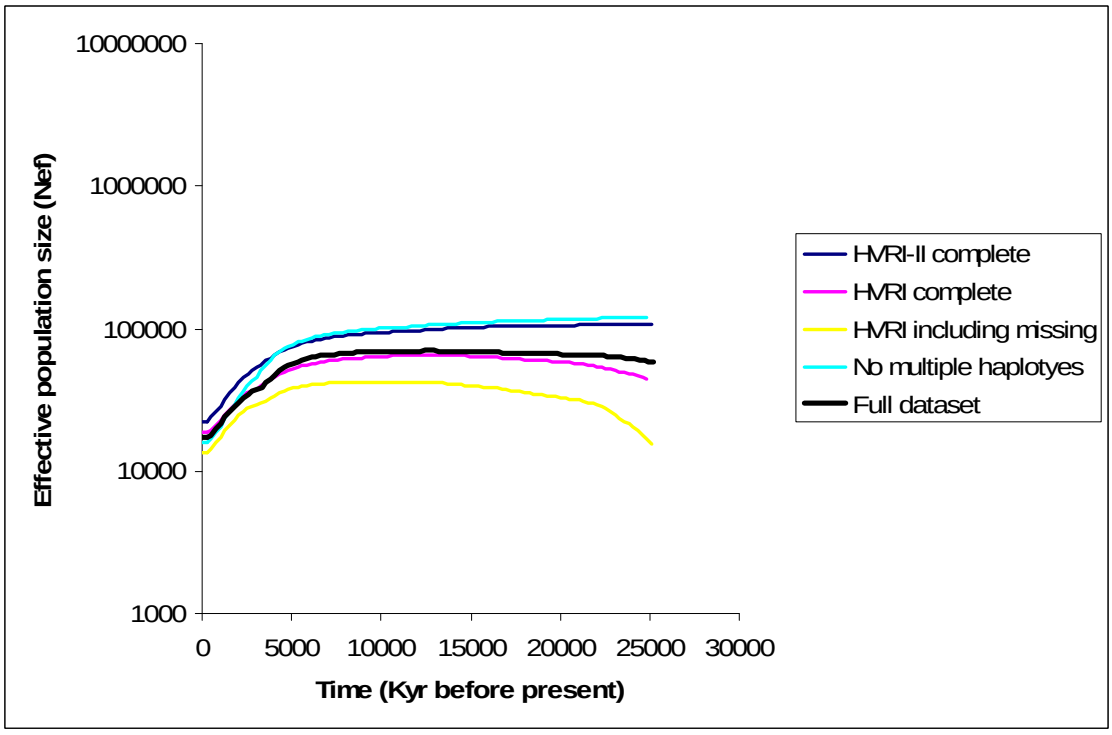

Figure S17 Effect of biomolecular preservation biases on the southern heavy-footed moa BSP.

Chapter Nine

General Discussion

1. Wider significance of the research

1.1 Taphonomic deposition mechanisms of Holocene moa swamp deposits

Historically, there has been a number of theories proposed to explain the deposition mechanisms of Holocene miring bone deposits, including stampeding during wildfires (Booth 1875; Buick 1931) or flooding (Howorth 1887; Tregear 1893; Hutton 1900). Chapter Two (Wood *et al.* 2008b) shows that the collection of individuals in miring bone deposits can be explained by periodic entrapment of moa over time, often a couple of thousand years, rather than through discrete events (as floods and stampeding would suggest). This is not surprising given that wetlands and swamps were, and still are, a natural part of the environment in New Zealand (Wilson 2004) where animals occasionally become mired.

This finding has wider significance for the interpretation of catastrophic scenarios and understanding how fossil deposits form. In Chapter Two (Wood *et al*. 2008b), we hypothesised that if miring bone deposits spanning thousands of years were compressed over geological time they may be interpreted as originating from catastrophic scenarios (Rogers 1990; Henrici and Fiorillo 1993). However, one argument against this hypothesis is that variation in the preservation of bones within bone beds has been interpreted as evidence of a time-averaged deposit (Fursich and Flessa 1991) for example an unstratified swamp, cave or predator deposit. A lack of variation in bone preservation has been argued as evidence for a catastrophic scenario (Voorhies 1969; Lyman 1994; Ryan *et al.* 2001). At Glencrieff, there was variation in bone preservation (Chapter Three), with bones lower in the stratigraphy and consequently closer to the water table better preserved than bones higher in the stratigraphy. Liebig *et al.* (2007) has argued there can be variation in the preservation of bones in deposits interpreted as originating from catastrophic scenarios. This study examined a mass stranding assemblage of false killer whales (*Pseudorca crassidens*) on the Colorado River Delta and showed there was variation in preservation within and among skeletal elements. Liebig *et al.* (2007) argued this was due to variations in bone density, bone size, sediment type and exposure (exposed versus buried). This conclusion was supported by Haynes (1988), who warned against the use of variation in bone

preservation as an indicator of a time averaged deposit on the basis of shared taphonomic characteristics between catastrophic and time averaged deposits. Shared characteristics included different preservation conditions, bite marks from scavengers or predators, multiple taxa and dense accumulations of bone (Haynes 1988).

Finally, unless scientists have a thorough understanding of how fossil deposits form, interpretations of palaeofaunal (Chapter Three) and population demographic reconstructions (Chapters Six and Seven) may be inaccurate. For example, the South Canterbury swamp deposit Albury Park (Worthy 1997a) has been dated to the Early to Late Holocene at about 3-9 Kya (Worthy 1997a; Chapter Seven) but is dominated by heavy-footed moa (*Pachyornis elephantopus*). The dominance of heavy-footed moa is similar to the Glencrieff (10-12 Kya) swamp deposit in North Canterbury (Chapter Three) and would normally be interpreted as indicating a Late Glacial (10-14 Kya) to Otiran Glacial (14-70 Kya) faunal assemblage. The similarity in palaeofauna between Albury Park and Glencrieff further suggests that the decline in relative fossil abundance with the major episode of climate and habitat change from the Late Glacial to the Holocene occurred much later in South Canterbury than North Canterbury. The late vegetation succession in South Canterbury is supported by palynological analyses that suggest that the succession from a grassland/tall shrubland mosaic to forest did not occur until about 6 Kya (Moar 1971; Worthy 1997a), indicating that the microclimate and habitat surrounding Albury Park during the time period of deposition was characteristic of the Late Glacial and Otiran Glacial. Without palynological data and radiocarbon dates from bones, determining what fossil localities are important to sample for palaeodemographic analyses would be problematic.

1.2 Palaeofaunal reconstruction of the Glencrieff moa swamp deposit

The excavations conducted at Glencrieff to determine the deposition mechanism of this fossil deposit (Chapter Two), also allowed the palaeofauna of Glencrieff to be reconstructed (Chapter Three). The palaeofaunal reconstruction suggests that moa can cope with moderate levels of climate and habitat change (e.g. from the Otiran Glacial to the Late Glacial) with only a slight decline in relative fossil abundance (Chapter Seven, 1.57 fold decline), but not large changes in climate (e.g. from the Late Glacial to the Holocene). During the Otiran and Late Glacial, heavy-footed moa dominated the Canterbury moa assemblage (Worthy 1993a; Worthy and Holdaway 1995, 1996), despite the succession from grassland to tall shrubland during the Late Glacial (Worthy and Holdaway 1996). In contrast, the transition from the Late Glacial to the Holocene

was characterised by a marked decline in the relative fossil abundance (Chapter Seven, 4.44 fold decline) of heavy-footed moa coincident with the succession from tall shrubland to forest (Moar 2008), and increasing temperature and precipitation (Williams *et al*. 2005). It is possible that secondary interactions like interspecies competition for dietary resources or suitable habitat may have increased from the Late Glacial to the Holocene, leading to declines in relative fossil abundance and changes in moa distributions. However, the demographic history of heavy-footed moa inferred from the fossil record is in contrast to the genetic results (Chapter Seven), where a constant population size through time could not be rejected. Reasons for this conflict are discussed in *Section 1.5*.

1.3 Reconstructing the diet of moa

Further excavations of rockshelters in southern New Zealand for coprolites also allowed the diet of moa to be reconstructed (Chapter Four; Wood *et al.* 2008a). Historically moa have been described as grassland grazers (Buick 1931; Oliver 1949, 1955) and, more recently, predominantly browsers of trees and shrubs (Burrows *et al.* 1981; Horrocks *et al.* 2004; Wood 2007). However, the new data from Chapter Four (Wood *et al.* 2008a) has forced a rethink of what is known about moa diet. The evidence suggests moa were both browsers and grazers, with extensive dietary overlap between species. Interestingly, the majority of plants eaten by moa in our study were less than 30 cm in height, in direct contrast to gizzard contents that suggest moa were dominant browsers of trees and shrubs (Burrows *et al*. 1981; Wood 2007). One explanation for the difference in diet between coprolites and gizzard contents may be sexual differentiation of dietary niches or moa with wide ranging distributions had broad dietary niches (Wood *et al.* 2008a). The majority of the preserved gizzard contents attributed to *Dinornis robustus* (Burrows *et al.* 1981; Wood 2007) are from females based on associated skeletal remains that were previously classified as *D. giganteus* (Burrows *et al.* 1981) and are now recognised as female *D. robustus* (Bunce *et al.* 2003; Huynen *et al.* 2003).

The palaeodietary data from Chapter Four (Wood *et al.* 2008a) shows that moa ate plants with "anti-browsing" growth characteristics, consistent with the moa anti-browsing hypothesis (Greenwood and Atkinson 1977) but does not prove that moa were the evolutionary cause of these growth characteristics. However, further data to support the moa anti-browsing hypothesis comes from additional sources, as outlined in *Section 3.3.5* of the General Introduction. Islands with large avian herbivores like Madagascar have plants with "anti-browsing" growth

characteristics (Bond and Silander 2007; Burns and Dawson 2006), while islands without large avian herbivores like the Chatham Islands do not (Fadzly *et al.* 2009). To lend support to the climate hypothesis for the origin of plants with 'anti-browsing' growth characteristics (McGlone and Webb 1981), sub-fossil or fossil deposits dating to Pleistocene glacial periods should be excavated on islands without large avian herbivores to see if plants with 'anti-browsing' characteristics were part of the glacial flora. This analytical approach would separate the avian herbivore and climate components of the debate, so climate causes could be examined in isolation.

In addition to plants with 'anti-browsing' growth strategies, there is evidence for co-evolution between moa and spring annual herbs (e.g. *Ceratocephala pungens* and *Myosurus minimus novaezelandiae*). In coprolites from Central Otago, there were abundant seeds from spring annual herbs that are now rare due to anthropogenic environmental modification and now lack seed dispersers. It is probable that moa played a significant role in their seed dispersal. Ironically, these herbs now only survive in areas of intense sheep and rabbit grazing, the one case where ungulates and exotic animals are ecological surrogates for moa (Jamie Wood pers comm. 2010). Examples of seed and fruit dispersal by vertebrates and invertebrates are common in the animal kingdom (e.g. Handel and Beattie 1990; Clark *et al.* 2001); with seed dispersal resulting in the colonisation of favorable sites and the reduction of competition between sibling and parental plants (Tiffney 2004). It is probable that moa were not the only avian taxa in the pre-human ecosystem that functioned as seed dispersers for spring annual herbs (Herrera 2002). Species that were grazers like moa include the extant takahe (*Porphyrio hochstetteri*) and kakapo (*Strigops habroptilus*; Horrocks *et al*. 2008), and the extinct giant goose (*Cnemiornis* sp.) and Finsch's duck (*Cheonetta finschi*; hypothesised to be grazers based on comparisons with their closest living Australian relatives, the Cape Barren goose (*Cereopsis novaehollandiae*) and wood duck (*Chenoetta jubata*) respectively). Continued conservation of these endangered herb species could involve transplantation of herbs into areas where takahe or kakapo are common. For takahe this includes the Murchison Mountains in Fiordland, Te Anau and Mana Island. For kakapo, this includes Codfish Island off Stewart Island.

There is also abundant data that plants that do not have defences against moa browsing are being decimated from browsing by exotic ungulates like deer and goats (Wood *et al.* 2008a; Forsyth *et al.* 2010; Lee *et al.* 2010), raising doubts over Pleistocene re-wilding (Donlan *et al*. 2005; Martin

2005). The data from Chapter Four (Wood *et al.* 2008a) shows that moa ate a high proportion of plant taxa that are avoided by exotic ungulates, and also includes some taxa that are toxic to mammals. This suggests that ungulates will not be able to function as surrogates for moa. Subsequent research has supported this theory. Forsyth *et al.* (2009) and Lee *et al*. (2009) have shown that deer and moa had different effects on the environment including:

(i) An order of magnitude difference in population density between deer and moa.
(ii) The potential for co-evolutionary role between moa and plants was greater than between deer and plants.
(iii) A greater ability of deer to process low nutrient foliage.
(iv) The foot pressure of deer is greater than moa.
(v) The importance of moa in seed dispersal compared to deer.
(vi) Forest under story species dispersed by moa are markedly reduced by deer browsing.

The unsuitability of exotic ungulates as surrogates for moa for Pleistocene re-wilding of New Zealand is not surprising. The pre-human ecosystem of New Zealand evolved in the presence of birds not large mammalian taxa. The New Zealand ecosystem cannot be restored to the pre-human condition because 40 of the 132 bird species from the mainland New Zealand (North, South and Stewart Island) Late Holocene pre-human ecosystem are now extinct (Worthy and Holdaway 2002). However, stopping the destruction of the remnants of the pre-human ecosystem by exotic ungulates can be achieved through the widespread culling of exotic ungulates. As an additional conservation measure, there is still a case for Pleistocene re-wilding in New Zealand. While extant ratites are not suitable as ecological surrogates of moa (Wood *et al.* 2008a), many extinct New Zealand bird species have Australian sister taxa that could potentially be surrogates. These include but are not limited to:

(i) The giant goose *Cnemiornis* sp.
(ii) Numerous duck species (e.g. Finsch's Duck).
(iii) Hodgen's Rail (*Gallinula hodgenorum*), whose closest living relative is the black-tailed native hen (*G. ventralis*) and the Tasmanian water hen (*G. mortierii*).

However, the validity of the Australian species as surrogates cannot be determined until dietary comparisons are conducted. Coprolites have been found in Central Otago rockshelters that are

comparable in morphology to wood duck coprolites. It is also possible that some of the coprolites attributed to moa may belong to the extinct giant goose. Sub-fossil remains of both Finsch's duck and *Cnemiornis* have been found in Central Otago (Worthy 1998a). These coprolites are currently being analysed to determine whether they belong to Finsch's duck and *Cnemiornis*.

Ecological research has shown that habitat specialists are at greater risk of extinction than generalists from habitat degradation or loss and global warming (McKinney 1997; Munday 2004; Thomas *et al.* 2004). Specialisation has been hypothesised to increase extinction risk in a range of terrestrial animals including mammals (Harcourt *et al.* 2002), birds (Owens and Bennett 2000) and reptiles (Foufopoulos and Ives 1999). For example, as outlined in *Section 4.3.1* of the General Introduction, the specialist *Genyornis newtonii* went extinct 50 +/- 5 Kya as a result of major environmental change, while the generalist emu (*Dromaius* sp.) did not go extinct (Miller *et al.* 1999, 2005). However, like Northern Hemisphere taxa it is not known what secondary interactions were also involved in the extinction of *G. newtonii* like human hunting or interspecies competition (Chapter Six). The palaeodietary data from Chapter Four (Wood *et al.* 2008a) suggests that moa were generalists with plasticity in dietary preferences, supported by the large overlap in diet between species and the large amount of variation in diet between geographical locations. This suggests that climate and associated habitat change did not significantly affect moa and is supported by palaeodemographic analyses conducted in Chapters Six and Seven. Evidence from North American megaherbivores contradicts the hypothesis that specialists are at greater risk of extinction with climate and habitat change. As outlined in Chapter Six, the dominant generalists, mammoth and *Equus* horses, were replaced by the generalist bison about 12.5 Kya (Guthrie 2006; Scott 2009). The relative fossil abundance of mammoths and horses declined after 12.5 Kya (mammoths were rare, while *Equus* horses were extinct after 12.5 Kya), while bison increased (McDonald 1981; Guthrie 2006; Scott 2009). However, Shapiro *et al.* 2004 and Drummond *et al.* 2005 argued that bison were experiencing a major bottleneck at this time.

1.4. Reconstructing the plumage of moa

Amongst the coprolites excavated from numerous rockshelters in southern New Zealand were isolated sub-fossil feathers attributed to moa that allowed the reconstruction of moa plumage (Chapter Five; Rawlence *et al.* 2009), though much remains to be studied regarding moa plumage. The results (Chapter Five) suggest that heavy-footed and upland moa (*Megalapteryx*

didinus) had both a speckled plumage and plain brown streaky plumage, while stout legged (*Euryapteryx curtus*) and South Island giant moa (*Dinornis robustus*) only had plain brown streaky plumage. The fact that all four species analysed had plain brown streaky plumage suggests that moa may have used a mate recognition system that did not rely on plumage (e.g. booming, smell or pre-mating displays). It is also possible that males and females had different plumage, suggesting that the occurrence of two plumage types in upland and heavy-footed moa may correspond to different sexes, indicating a plumage based recognition system may have been used by moa.

The occurrence of overlapping plumage types in four moa species also raises the question of what was the evolutionary cause of the overlap. In Chapter Five (Rawlence *et al.* 2009) I hypothesised that the overlap may be due to convergent evolution of drab plumage for camouflage against aerial predators. This is not surprising given the evolutionary pressure on the New Zealand avifauna to avoid predation. In the pre-human ecosystem, aerial predators, which hunt by sight, replaced mammalian predators, which generally hunt by smell. The aerial predators included the diurnal Haast's eagle (*Harpagornis moorei*), Eyles's harrier (*Circus teauteensis*), New Zealand falcon (*Falco novaeseelandiae*); and the nocturnal predators, laughing owl (*Sceloglaux albifacies*), morepork (*Ninox novaeseelandiae*) and owlet nightjar (*Aegotheles novaezelandiae*; Worthy and Holdaway 2002; Tennyson and Martinson 2006). The selection pressure on the pre-human avifauna to avoid predation was to become nocturnal, crepuscular (active at dawn and dusk) or camouflaged in an effort to decrease visibility to aerial predators (Worthy and Holdaway 2002). There are several avian taxa in New Zealand that are nocturnal or at least partly nocturnal including kiwi (*Apteryx* spp.), kakapo, kaka (*Nestor* spp.), petrels, cuckoos (*Eudynamys* spp., *Chrysococcyx* spp.*)* and brown teal (*Anas chlorotis*). Several of these nocturnal species are also camouflaged including kiwi, kakapo, kea (*Nestor notabilis*), and kaka, while camouflaged diurnal species include takahe (*Porphyrio hochstetteri*), weka (*Gallirallus australis*), the New Zealand bittern (*Ixobrychus novaezelandiae*) and moa (Worthy and Holdaway 2002; Rawlence *et al*. 2009).

Finally, Chapter Five (Rawlence *et al.* 2009) shows that feathers can be used as a new resource to investigate evolution. Previously aDNA had only been extracted from modern feathers collected in the field or from historical museum specimens (Payne and Sorenson 2002; Sefc *et al.* 2003; Horvath *et al.* 2005). However, DNA from ancient feathers can now be incorporated with DNA

from bones, coprolites, eggshell and sediment to conduct ecosystem approaches to reconstruct extinct megafaunal ecosystems. In addition, only the tips of feathers need to be sampled, leaving the rest of the feather *in situ*, as DNA can be extracted from the distal parts of the feather (that is, the rachis and barbs). This is especially important given the rarity of many museum bird specimens (Knox and Walters 1994). Recently, Oskam *et al.* (2010) commented on the research in Chapter Five, stating that feathers may be suitable for high throughput sequencing of avian mitochondrial or nuclear genomes because feathers may have a significantly lower exogenous bacterial contamination load than bone, similar to eggshell.

1.5 Responses of moa to climate change in pre-human New Zealand

There have been a number of theories proposed to explain the worldwide Late Pleistocene megafaunal extinctions including human impact (Martin 1984), climate change and associated habitat change (e.g. Axelrod 1967; Guthrie 1984), disease (McPhee 1999), and extraterrestrial bolide impact (Firestone *et al.* 2007). In New Zealand, there is extensive palaeontological evidence suggesting that climate change had an effect on moa during the Pleistocene and Early Holocene, and that Late Holocene moa populations were stable prior to the arrival of Polynesians in approximately 1280 AD (Worthy 1994, 1997a, b, 1998a, b, 1999; Worthy and Holdaway 1994, 1995, 1996; Wilmshurst *et al.* 2008). The palaeontological data suggested two broad responses to climate change. The first is that climate and habitat change resulted in changes to the relative fossil abundance of moa taxa (Worthy and Holdaway 1994, 1995, 1996; Worthy 1997a, 1998a, b). For example, in North Canterbury, the dominant moa species changed from heavy-footed and eastern moa (*Emeus crassus*) during the Otiran Glacial and Late Glacial to eastern moa and South Island giant moa during the Holocene, coincident with a well-documented vegetation succession from grassland to shrubland to forest (Worthy and Holdaway 1995, 1996; Chapter Three). The second response was that in wetter western areas like lowland northwest Nelson/West Coast, there were complete faunal replacements as a result of climate and habitat change – an example of this is the replacement of the genus *Pachyornis* by little bush moa (*Anomalopteryx didiformis*) and South Island giant moa in the Holocene (Worthy 1993b, 1994, 1999; Worthy and Holdaway 2002; Chapter Six). The difference in the two responses is due to the different nature of the vegetation successions. The succession in North Canterbury did not result in the total replacement of grassland by forest, rather the relative proportions of grassland, shrubland and forest changed (Moar 2008). In contrast, the succession in lowland northwest Nelson/West Coast resulted in the complete replacement of grassland by forest (Worthy 1999).

The data obtained during this research (Chapters Six and Seven) supports the previously published evidence that suggests both climate and habitat change had an effect of moa, but that their ultimate extinction was caused by human arrival and impact. There was debate about whether crested moa went extinct with changing climate at the end of the Pleistocene or survived until after the arrival of Polynesians in about 1280 AD (Tennyson and Martinson 2006; Wilmshurst *et al.* 2008). The results reported in Chapter Six indicate that crested moa persisted with the other moa species until their extinction after Polynesian colonisation. In addition, the results show that while the population size of crested moa declined slightly (though constant population size could not be rejected) in response to climate and habitat change, they responded to changes in the availability of the sub-alpine habitat by changing their distribution accordingly. In heavy-footed moa (Chapter Seven), climate and habitat change may have facilitated phylogeographic structuring, but had little effect on population size. The demographic signals of crested and heavy-footed moa, and possibly moa overall, were stable when Polynesians colonised New Zealand (Wilmshurst *et al.* 2008), implying that over-hunting and habitat destruction by early Maori rather than climate and habitat change was solely responsible for the extinction of moa and other avian taxa in New Zealand.

When the demographic history of crested and heavy-footed moa is compared to studies of Holarctic megafauna (Barnes *et al.* 2002; Burger *et al.* 2002; Shapiro *et al.* 2004; Drummond *et al.* 2005; Rohland *et al.* 2005; Stiller *et al.* 2010) it is evident that climate change had little effect on moa population demography. Crested and heavy-footed moa populations appear to have been relatively stable through time with minimal loss of genetic diversity coincident with major climatic events, compared to some Holarctic species like bison (Shapiro *et al*. 2004; Drummond *et al*. 2005) and cave bears (Stiller *et al.* 2010), which appear to have gone through dramatic population bottlenecks. Stiller *et al.* (2010) inferred from the Bayesian Skyline Plot (BSP) that cave bear populations were declining for 25 Kyr prior to their extinction, which was coincident with human arrival, major climate change and competition for cave sites with humans. The exception to Holarctic species going through pronounced population bottlenecks during the Late Quaternary was mammoths (Barnes *et al.* 2007; Debruyne *et al.* 2008), where constant population size could not be rejected. However, as outlined in *Section 1.3*, relative fossil abundance data suggests that mammoth populations declined markedly after 12.5 Kya (Guthrie 2006; Scott 2009). The demographic analyses of crested and heavy-footed moa also suggests that the losses of genetic diversity inferred from the BSP are comparable to mammoths (Barnes *et al.*

2007; Debruyne *et al.* 2008) and not bison (Shapiro *et al.* 2004; Drummond *et al.* 2005). Bison went through a major population bottleneck before, during and after the LGM, with a second bottleneck about 10-12 Kya coincident with human arrival in eastern Beringia, the Younger Dryas and a hypothesised extra-terrestrial bolide impact (Firestone *et al.* 2007). Finally, the BSP and BayeSSC results (Chapter Six and Seven) suggest that there was minimal to no loss of genetic diversity within crested and heavy-footed moa with declining habitat, in contrast to some Holarctic species like Arctic fox (Dalen *et al.* 2007; see Chapter Six for further discussion). However, secondary interactions like geographical barriers (Hewitt 1999) and interspecies competition (Bennett *et al*. 1991) cannot be ruled out in the extinction of Arctic fox populations.

The results and conclusions of this study (Chapters Six and Seven) suggest the possibility that the effects of climate and habitat change were not as severe on New Zealand's megafauna compared to Holarctic megafauna, despite the New Zealand fossil record suggesting large declines in the relative abundance of moa. It is possible that some megafauna like moa may have been ecologically primed to deal with climate change by tracking changes in habitat availability (Chapter Six) or exhibiting allometric size variation (Chapter Seven). However, as shown for crested and heavy footed moa, there is a large disparity between the fossil and genetic records. The same is true for mammoths (*Section 1.3*; Guthrie 2006; Barnes *et al.* 2007; Debruyne *et al.* 2008), brown bears (Barnes *et al.* 2002) and bison (Shapiro *et al*. 2004; Drummond *et al.* 2005; Guthrie 2006). Barnes *et al.* (2002), Shapiro *et al.* (2004) and Drummond *et al.* (2005) found cryptic population demographic changes not seen in the fossil record (McDonald 1981; Guthrie 2006; Scott 2009).

The research presented in Chapters Six and Seven, and previous studies (Shapiro *et al.* 2004, Drummond *et al*. 2005, Barnes *et al*. 2007, Debruyne *et al* 2008), has used mitochondrial DNA (mtDNA) to calculate the effective population size, or more correctly, the effective breeding female population size. In contrast, relative fossil abundance data tracks changes in the census population size. There are several reasons for the disparity between the genetic and fossil records. First, changes in relative fossil abundance may reflect changes in the distribution of taxa not population size (Chapter Three). For example, a constant population size could not be rejected for northern heavy-footed moa, despite a decline in relative fossil abundance. This situation could be explained by a moa population of constant size shifting distribution with changing habitat resulting in a decline in relative of fossil abundance in one fossil deposit. Second, the fossil

record may be highly biased. As outlined in *Section 3.3.8* of the General Introduction, this has been shown in New Zealand with the genetic analysis of several Late Holocene swamp deposits in North Canterbury. Allentoft *et al.* (2010) showed that there was a highly skewed sex ratio of >2 female: 1 male, suggesting increased mortality of males compared to females during juvenile and sub-adult growth stages, creating an excess of females.

Third, there may be a lag time between changes in effective population size and relative fossil abundance. There are several examples of effective population size lagging behind census population size including Chatham Island black robins (*Petroica traversi*; Butler and Merton 1992) and humans. In 1980, the population size of Chatham Islands black robins was five individuals, comprising three males and two females. Through intense conservation efforts the black robin population is now estimated at 250 individuals. In contrast, the effective population size of black robins is very small because the current population is descended from one breeding pair "Old Blue" and "Old Yellow" (Butler and Merton 1992). The other example is modern humans, where the census population size is nearly seven billion, while previous estimates of the effective population size are about 10,000-20,000 (Tenesa *et al.* 2007).

Finally, there may not be enough power in the genetic data to accurately reconstruct the demographic history of taxa. A constant population size could not be rejected for moa and several other taxa (See Table One, Chapter Eight). The use of multiple independent fast evolving loci and the extended Bayesian Skyline Plot (discussed in *Section 3.5*) may resolve this issue. The significance of contrasting demographic histories inferred from the fossil record and the BSP is that aDNA and palaeontology are telling different parts of the overall story, and should be examined together, not in isolation. In reality, the reason for the conflict between the fossil and genetic records is probably a combination of all four scenarios discussed above.

1.6 Robustness of the Bayesian Skyline Plot to biased sampling

When conducting demographic analyses utilising the BSP (Chapters Six and Seven) the assumption is that the population under analysis is panmictic with a single population coalescent (Drummond *et al*. 2005). In addition, despite the use of the BSP with modern (e.g. Grazziotin *et al.* 2006; Crandall et al. 2008; Marske *et al.* 2009) and aDNA (Drummond *et al.* 2005; Barnes *et al*. 2007; Debruyne *et al.* 2008; Ho *et al*. 2008; Bruyn *et al*. 2009; Campos *et al.* 2010) datasets,

the BSP was originally designed to analyse time series viral datasets with high mutation rates and strong demographic signals (Drummond *et al.* 2005; Lemey *et al.* 2006; Rambaut *et al*. 2008).

This research (Chapter Eight) has shown that the assumptions of the BSP are vitally important to the interpretation of results and allocation of causal theories to changes in the population demographic history of taxa. Violations of these assumptions caused by temporal, phylogenetic, geographic and biomolecular preservation biases significantly change the palaeodemographic history of taxa inferred from the BSP. For example, the re-analysis of the Shapiro *et al.* (2004) bison dataset showed that the signal for post decline recovery seen in the Drummond *et al.* (2005) BSP (Chapter Eight, Figure 3: Full dataset) was only seen in bison south of the ice. In contrast, bison in Beringia did not recover, confirming that different populations within a species will often have different responses to climate and habitat change i.e. one population is not indicative of the species as a whole (Scott 2009). This highlights:

(i) The importance of proper investigations of the genetic data to assess the impacts of sampling biases and whether the assumptions of the BSP methodology are met.
(ii) The question of how many previously published BSP reconstructions (See Chapter Eight, Table 1) are accurate given the biases inherent in most aDNA datasets, especially when there is unrecognised phylogeographic structuring and/or when phylogeographic structuring is not accounted for.

Given the results and conclusions from this research, caution needs to be exercised when designing sampling strategies so that sampling biases are minimised and the assumption of a single unstructured population is met. A good example would be analysing a single population through time from a discrete fossil locality (See Chapter Eight for a good example by Prost *et al.* 2010). In addition, a BSP that cannot reject constant population size is still informative. The BSP is either correct (i.e. there were no significant change in population size), meaning the palaeontological evidence is biased, or the magnitude of population size change combined with the mutation rate is too low to be detected by the BSP. The alternative is to use BayeSSC (Excoffier *et al.* 2000; Anderson *et al*. 2006; Limitations of this approach are discussed in *Section 2.5.3*). Prost *et al.* (2010) used BayeSSC to show that collared lemmings from northern Russia went through a population bottleneck after the LGM, declining to a minimal population size about 14.5 Kya. This corroborated the BSP analysis; despite the wide credible intervals

suggesting constant population size could not be rejected (although the 95% credible intervals of the BSP matched the demographic signal inferred from the mean demographic plot, albeit at a higher and lower overall population size). In addition, comparison of BayesFactors (Suchard *et al.* 2001) from demographic analyses of collared lemmings using constant population size and BSP demographic priors, rejected constant population size in favour of the demographic history described by the BSP.

Another major implication of the research reported in Chapter Eight is that it calls into question whether the BSP should be used to infer population demographic history from modern genetic datasets and highlights the real possibility that BSPs constructed from modern genetic data may be an artefact. Glacial and interglacial cycles can have large effects on the phylogeographic (e.g. Barnes *et al.* 2002) and demographic history (e.g. Shapiro *et al.* 2004; Drummond *et al.* 2005) of taxa. For instance, population bottlenecks during glacial cycles can wipe out genetic variation, making it difficult to reconstruct past demographic and phylogeographic events from modern genetic data (Barnes *et al.* 2002). It is for this reason that aDNA is such a powerful tool at the evolutionary and population genetics level as it allows populations to be sampled before, during and after population bottlenecks. A significant argument against using the BSP on modern datasets comes from the re-analysis of the Shapiro *et al.* (2004) bison dataset and simulations (Chapter Eight), where progressive exclusion of older sequences from the analysis resulted in truncation of the BSP signal, as well significantly altering the shape and magnitude of population size change. One pertinent question that has wide ranging ramifications for previously published and future studies utilising aDNA datasets and the BSP, is why the BSP reconstructs an expanding population size after the oldest radiocarbon date, when DNA sequences are sampling from a declining population inferred from the full dataset (Chapter Eight; Figure Three). This is potentially a serious artefact of the Bayesian Skyline methodology. There are potentially several possible explanations for this artefact, though I will only discuss two of them:

(i) Phylogeographic structuring:

Taking the bison genealogy (Shapiro *et al*. 2004) and BSP (Drummond *et al.* 2005; Chapter Eight, Figure Three: Full dataset) for example, if we work forward in time from the most recent common ancestor, the first couple of divergences in the bison phylogeny establish three of the four major phylogeographic clades (defined by Shapiro *et al.* 2004). It is possible that the Bayesian Skyline methodology assumes these divergences increase the amount of genetic

variation and hence the population size of bison. If for example, sequences 20-60 Kyr are removed from the bison dataset (Chapter Eight, Figure Three), so only sequences 0-20 Kyr are sampled from the complete dataset, the sequences are still spread throughout the major phylogeographic clades, hence the population increase, despite the fact that sequences 0-20 Kyr are initially sampling from a declining population when the full dataset is analysed.

(ii) Branching rate:

BSPs are generally characterised by either constant or increasing population size from the most recent common ancestor, not declining population size. Increases in population size may be due to numerous branching events in the genealogy just after the most recent common ancestor, while constant population size may be due to minimal branching events just after the most recent common ancestor.

As stated previously in this section, the BSP can still be used to reconstruct the population demographic history of taxa, but only if sampling biases in the dataset are fully accounted for and the BSP is not interpreted past the oldest radiocarbon date, because there are no calibration points to anchor the genealogies and artefacts in the BSP can seriously affect the interpretation of the demographic history of taxa.

2. Limitations and specific concerns of each chapter

2.1 Taphonomic deposition mechanisms of Holocene moa swamp deposits

While it does not change the results and conclusions of this chapter (Wood *et al.* 2008b), there was an absence of radiocarbon dates for the deposits analysed at the time of publication, with only three dates for Glencrieff and two dates for Bell Hill in North Canterbury, and no dates for Styx Valley in Central Otago (Wood *et al*. 2008b). Subsequently, more dates have been obtained (Chapter Three, Seven) confirming that deposition at Glencrieff dates to 10-12 Kya, while deposition at Styx Valley dates to 1-4 Kya. While we examined only leg bones (femora, tibiotarsi and tarsometatarsi), we could have included bones from additional taxa or small bones such as vertebrae to see how they behave taphonomically compared to larger bones as this can affect the accuracy of palaeofaunal reconstructions (Chapter Three). Small bones were unrepresented in the deposits analysed (Chapter Two, Three), and is a common occurrence in other New Zealand swamp deposits (Worthy 1998b) and at the Rancho La Brea tar seeps in Los Angeles (Spencer *et*

al. 2003). See Chapter Two (Wood *et al*. 2008b) for hypotheses explaining the under representation of small bones in swamp deposits.

2.2 Palaeofaunal reconstruction of the Glencrieff moa swamp deposit

The paucity of radiocarbon dates raised in *Section 2.1* also applies to the Glencrieff deposit. Despite the Late Glacial Glencrieff deposit being represented by at least five separate sub-deposits: Canterbury Museum; Eaves one and two; Worthy and Holdaway; and the 2007 sub-deposit, radiocarbon dates are only associated with one – the Worthy and Holdaway sub-deposit dated to 10-12 Kya. In Chapter Three I hypothesised that the other sub-deposits within Glencrieff formed during the Late Glacial based on the relative fossil abundance data of the different sub-deposits being characteristic of Otiran Glacial to Early Holocene faunas from the eastern South Island (Worthy and Holdaway 1995, 1996; Worthy 1997a, 1998a, b; Worthy and Grant-Mackie 2003). In addition, the presence of high levels of *Sporormiella* spores (used as an indicator of megaherbivore biomass, Burney *et al.* 2003) in sediment samples from the 2007 sub-deposit, and low levels during the Holocene from the Moar (2008) sediment core, taken from the Worthy and Holdaway sub-deposit, suggests deposition occurred during the Late Glacial (Wood; unpublished data). Palynological analysis currently being conducted on sediment samples and preserved gizzard contents from the 2007 sub-deposit are suggesting that the habitat surrounding Glencrieff was comprised of a grassland/tall shrubland mosaic (unpublished data, Rawlence), characteristic of the Late Glacial (Worthy and Holdaway 1996). However, the loss of material overseas (e.g. Eaves collection) prevents us from determining the age of the bones excavated from the Eaves one and two deposits.

These results (Chapter Three) also raise the question of whether the perceived absence of *Sporormiella* during the Holocene represents a true decline in the relative abundance of moa in the area or that moa shifted their distribution to a new area. The incorporation of *Sporormiella* spores into the deposit is very directional (Jamie Wood pers comm. 2010) with moa literally having to defecate in the swamp for *Sporormiella* to be incorporated into the deposit. There are three possible reasons for the decline in *Sporormiella* from the Late Glacial to the Holocene at Glencrieff. First, there was a decline in the population size of moa and thus the density of megaherbivores. There is some support for this from the decline in the relative fossil abundance of heavy-footed moa (Chapter Three) and the BSP analysis (Chapter Seven) where the median demographic plot of heavy-footed moa declined from the Late Glacial to the Holocene, though

constant population could not be rejected. However, the relative fossil abundance of eastern moa remained unchanged, while the abundance of South Island giant moa increased (Worthy and Holdaway 1995, 1996; Chapter Three), potentially offsetting the decline in heavy-footed moa numbers. Second, the population size of moa may have remained constant (supported by BSP and BayeSSC analyses failing to reject constant population size) but the distribution changed, which would be reflected in the decline of *Sporormiella*. The most likely reason is that the peat at Glencrieff became too deep to trap moa (Chapter Two) as no remains have been dated to the Holocene, and the palynological, *Sporormiella* and palaeofaunal composition all suggest that deposition occurred during the Late Glacial.

2.3 Reconstructing the diet of moa

The conclusions drawn from the analysis of moa diet are influenced by:

(i) Morphological differences in the skull and bill of moa.
(ii) Sex-specific differences in moa diet.
(iii) The number of sub-fossil deposits included in the analyses of moa diet.
(iv) Leaching of DNA from the moa coprolites into the surrounding matrix and vice versa.

Variation in moa diet between species would be expected given the morphological variation seen in moa skulls and bills (Atkinson and Greenwood 1989; Worthy and Holdaway 2002). However, surprisingly the analysis showed extensive dietary overlap in four species of moa despite marked differences in bill and skull morphology, with more variation in diet between geographical regions than between species (Wood *et al.* 2008a). It is probable that there were dietary differences between moa species that we did not find given the differences in bill and skull morphology. If the overlap in moa diet holds up to scientific scrutiny with additional research, it raises the question: what was the selective evolutionary force that resulted in the skull and bill differences. In Chapter Four (Wood *et al.* 2008a) we hypothesised that niche partitioning of dietary resources may not have been important until glacial periods or drought conditions where food resources were scarce.

Given the high degree of sexual dimorphism in some species of moa (Bunce *et al.* 2003; Huynen *et al.* 2003) and size differences between juveniles and adults (Anderson 1989; Worthy and Holdaway 2002), it can be hypothesised that different sexes and ages (juveniles versus adults)

had different dietary niches. As outlined in *Section 1.3* and Chapter Four (Wood *et al.* 2008a) the dietary analysis of moa gizzards (Burrows *et al.* 1981; Wood 2007) has been heavily biased towards *Dinornis* females, suggesting they were predominantly browsers of trees and shrubs, in direct contrast to the coprolite analysis (Wood *et al.* 2008a) that suggested moa were grazers. The diet inferred from coprolites could represent the male diet (Wood *et al.* 2008a), though no coprolites were genetically sexed in our study. The differences between preserved gizzard contents and coprolites may also suggest that moa had a broad dietary range adding further support to the argument that moa were generalists with dietary plasticity and were ideally adapted to respond to major episodes of climate and habitat change. The conflicting dietary reconstructions from preserved gizzard contents and coprolites also suggests that previous palaeodietary analyses (Poinar *et al.* 1998; Hofreiter *et al.* 2000, 2001; Kuch *et al.* 2002; van Geel *et al.* 2008) may reflect only a portion of the full dietary range of a species, suggesting that where possible dietary data from preserved intestinal contents (e.g. van Geel *et al.* 2008) should be analysed with data from coprolites.

While the evidence from moa coprolites greatly increases our knowledge of moa diet, the analysis was restricted to two small geographical areas: the Dart Valley and Central Otago. To date, moa coprolites have only been found in the southern South Island (Wood 2007; Wood *et al.* 2008a). In addition, there is more variation in moa diet between different geographical regions that between species, meaning the results may be specific to southern New Zealand only. Further coprolite deposits are required, preferably from the northern South Island and North Island, to extend the dietary analysis. An additional concern is the differential preservation of aDNA in coprolites from the Dart Valley and Central Otago. The Dart Valley coprolites came from cool dry conditions in caves under boulders in a temperate rainforest with 20/22 coprolites containing amplifiable aDNA, compared to Central Otago, which experiences hot conditions during summer and cold conditions during winter, where only 4/21 coprolites contained amplifiable aDNA. It is likely that the failure rate of Central Otago coprolites is due to the extreme temperature fluctuations facilitating the degradation of aDNA. Further analysis is required to determine whether the preservation conditions for the Dart Valley coprolites are the norm or represent exceptional examples.

Given the differential preservation of coprolites, the question of whether leaching of DNA from less well-preserved coprolites into others and the surrounding sediment is a concern. This was

raised by Haile *et al.* (2007) who documented in two Hawke's Bay (North Island, New Zealand) rockshelters the vertical migration of sheep DNA from European stratigraphic layers into pre Polynesian stratigraphic layers containing moa DNA. However, there was no vertical migration of moa DNA. The difference in sheep and moa DNA migration is due to the large amount of DNA rich urine produced by sheep. Because there was no vertical migration of moa DNA in the sediment column Haile *et al.* (2007) concluded that moa DNA from sediment could be used for palaeoecological reconstructions and that leaching of moa DNA was not a factor in rockshelter deposits. Willerslev *et al*. (2003), Haile *et al*. (2007) and Lydolf *et al*. (2005) hypothesised that DNA in sediment originates from the physical remains of animals or their excreta e.g. faeces, urine, feathers, skin and hair. Haile *et al*. (2007) further hypothesised that the taxonomic diversity of moa DNA in sediments from rockshelters and caves is dependant on the physical constraints of the site including the size of entrance, secondary interactions like competition between taxa for nesting sites, and the vegetation surrounding the rockshelter. For example, Haile *et al.* (2007) only found little bush moa and Mappin's moa (*Pachyornis geranoides*) DNA in sediment from Hukanui 7a rockshelter in Hawkes Bay but not North Island giant moa (*Dinornis novaezelandiae*) DNA. Little bush and Mappin's moa were the two smallest moa in the region while giant moa females were the largest of all moa. In contrast, all three taxa were found in Hukanui Pool rockshelter. The clean sequences obtained from each coprolite in our study (Chapter Four; Wood *et al.* 2008a) suggest leaching and vertical migration of moa DNA was not a concern, but must be taken into consideration.

2.4 Reconstructing the plumage of moa

The conclusions drawn from the analysis of moa plumage are influenced by:

(i) The origin and purity of ancient feather DNA.
(ii) Techniques for determining feather colour.
(iii) Whether one feather is representative of the whole bird.

Despite isolating aDNA from feathers, the origin and purity (i.e. the level of bacterial contamination) of the DNA is still unknown. Extracting DNA with minimal levels of exogenous contamination is crucial for the amplification and sequencing of whole mtDNA and nuclear genomes, especially for next generation sequencing technologies like 454 (Margulies *et al.* 2005). In 454 sequencing the DNA is fragmented into short pieces (300-800 bp), polished to remove

‘sticky’ ends and adapters are ligated onto the polished ends of the DNA fragments to serve as primer binding sites for amplification and sequencing. This method will fragment total genomic DNA including exogenous contaminants and amplify/sequence contaminants because taxon specific primers are not being used. There are three theories concerning the origin of ancient feather DNA:

(i) The DNA is within the nuclei of the keratin cells that make up feathers.
(ii) The DNA is in pockets between overlapping keratin cells on the exterior of the feather, protected from the 30 minute incubation in 1:10 bleach solution to remove potential exogenous contaminants from the exterior surface.
(iii) A combination of (i) and (ii).

There are multiple methods that could have been used to determine whether the colour of sub-fossil feathers had faded since deposition, which can have implications on how the plumage of moa is inferred. One method is to characterise the shape and alignment of melanosomes in moa feathers by scanning electron microscopy and compare this against different coloured feathers of extant avian taxa. Melanosomes are organelles inside keratin cells whose shape and alignment is directly linked with feather colour. This method has been used in several high profile papers to reconstruct the plumage of Cretaceous birds and dinosaurs (Vinther *et al.* 2008, 2009; Li *et al.* 2010; Zhang *et al.* 2010). Using this method it could be determined whether the white colour attributed to some moa feathers (Worthy and Holdaway 2002) was authentic or the result of fading. However, due to the precious nature of moa feathers in museum collections, curators are not willing to have the feathers ‘destroyed’ for scientific investigation. Characterising the shape and alignment of melanosomes involves grinding up feathers in liquid nitrogen and photographing melanosomes with a scanning electron microscope (Vinther *et al.* 2008).

Finally, when the plumage of moa was reconstructed it was assumed that one feather was representative of the entire plumage. However, as the results testify, both heavy-footed and upland moa have white tipped feathers and plain brown feathers that could mean either (i) speckled and plain brown plumages were present in different individuals of the same species (e.g. possibly males versus females or juveniles versus adults), or (ii) birds had mixed plumages. This would not be surprising as there are size (Bunce *et al.* 2003; Huynen *et al.* 2003) and potentially dietary differences (Chapter Four; Wood *et al.* 2008a) between male and female moa. Thus,

while our results (Chapter Five; Rawlence *et al.* 2009) are more accurate than previous reconstructions (Anderson 1989; Worthy 1989; Vickers Rich *et al.* 1985; Vickers-Rich *et al.* 1995; Gill and Martinson 1991; Flannery and Schouten 2001; Murray and Vickers Rich 2003; Tennyson and Martinson 2006), much remains to be learned about moa plumage.

2.5 Responses of moa to climate change in pre-human New Zealand

2.5.1 Radiocarbon dating

In palaeodemographic analyses utilising the BSP the number of radiocarbon dates is important if accurate reconstructions of past population demographics are to be obtained (Chapter Eight). For example, 99 bowhead whale (Ho *et al.* 2007), 138 mammoth (Debryne *et al.* 2008), 191 bison (Shapiro *et al.* 2004), and 320 elephant seal (Bruyn *et al.* 2008) sequences were dated in previous BSP analyses (See Table One, Chapter Eight). In comparison, the 48-115 dated moa sequences are at the lower end of the scale considering the number of specimens with stratigraphic dates (26-88 specimens).

In palaeodemographic analyses, the temporal spread of dates is also important; especially the use of 'old' dates (Chapter Eight). Old dates are required to calculate an accurate evolutionary rate and reconstruct the demographic history prior to, during and after the Last Glacial Maximum (LGM), in this case 29-19 Kya for New Zealand (Williams *et al.* 2005). While New Zealand has an extensive fossil record spanning the past 50 Kyr, only five sites older than 20 Kya had adequate biomolecule preservation to warrant aDNA analysis and radiocarbon dating. This is a concern as there is a significant bias in the New Zealand fossil record towards younger sites, and better biomolecule preservation in these sites. For example, there are only two and eight radiocarbon dated sequences prior to 20 Kya in the crested and heavy-footed moa datasets respectively, compared to 74 in bison (Shapiro *et al.* 2004). The bias towards Late Holocene fossil localities and a paucity of glacial aged localities is common in most geographical locations worldwide excluding permafrost, where bones can essentially remain frozen for millennia. This means that it may not be possible to accurately reconstruct the effects of the LGM on populations in temperate areas of the Southern Hemisphere. To assess the effects of climate change on taxa in the Southern Hemisphere, the best course of action may be to conduct studies on taxa in Antarctica or the sub-Antarctic where there is a good fossil record. Good examples include Adele penguins (Lambert *et al*. 2002; Ritchie *et al*. 2004; Shepherd *et al*. 2005; Miller *et al*. 2008), where fossils have been dated to at least 45 Kya (Emslie *et al*. 2007), or southern elephant seals

(Bruyn *et al*. 2009). Most Pleistocene sub-fossil deposits from South Africa (Hoss *et al.* 1996) and mainland Australia do not have adequate biomolecular preservation to warrant aDNA analysis, although there are a few exceptions (Orlando *et al.* 2009; Weinstock *et al.* 2009; Oskam *et al.* 2010). In contrast, sub-alpine and alpine areas of Tasmania (unpublished ACAD) and South America (Orlando *et al*. 2009; Weinstock *et al*. 2009) do have adequate biomolecular preservation and can be used to investigate the population demographic history of taxa.

Accurate palaeontological and/or population data is needed to build demographic models in BayeSSC (Excoffier *et al.* 2000; Anderson *et al*. 2006). For example, while the radiocarbon dating of crested moa remains (Worthy 1993b; 1994; Worthy and Holdaway 1994; Bunce *et al.* 2009; Chapter Six) has resolved some of the dating issues surrounding fossil deposition, there is no relative fossil abundance data for Otiran Glacial to Holocene assemblages. For example, it was not known when the remains of moa were deposited at Moa Arch in northwest Nelson (Worthy 1997b). Subsequent radiocarbon dating has shown that deposition occurred about 10.2 Kya during the Late Glacial (Chapter Six). Determining changes in the relative fossil abundance of crested moa is complicated by the extreme morphological similarity of crested and heavy-footed moa leg bones (Rawlence unpublished data). Leg bones (femora, tibiotarsi, tarsometatarsi) of *Pachyornis* moa from northwest Nelson/West Coast were previously classified as crested moa and heavy-footed moa if they were small or large respectively (Worthy pers comm. 2010). However, the majority of morphologically identified heavy-footed moa leg bones in this study (from field and museum collections) were genetically identified as crested moa (Rawlence unpublished data; Chapter Six).

2.5.2 Biomolecular preservation biases

Biomolecular preservation biases, previously defined as "*biases where DNA preservation is not uniform across all samples, so some samples are represented by more or less sequence data than others or none at all*" (Chapter Eight), are a common occurrence in aDNA studies. In this study (Chapters Six and Seven) there was a distinct bias towards increased levels of missing data in older samples. For example, in northern heavy-footed moa the PCR amplification success rate and the amount of missing sequence data from bones excavated from the Merino Cave deposit (14-40 Kya) in North Canterbury (Worthy and Holdaway 1995; Chapter Seven) was higher compared to the Late Glacial Glencreiff deposit (10-12 Kya; Worthy and Holdaway 1996; Chapter Two, Seven) and Late Holocene sites. This is an important concern considering 'old'

complete sequences are needed for the accurate estimation of mutation rates and the reconstruction of the BSP (Chapter Eight). For example when only complete sequences (HVRI or HVRI-II) from northern heavy-footed moa were analysed the same demographic signal as the full dataset was reconstructed, albeit at the lower overall effective population size (Chapter Eight). However, analysis of HVRI sequence data (including specimens with missing sequence data) resulted in a marked deviation in the inferred demography plot, characterised by low population size 40-10 Kya, followed by a rapid increase in population size (Chapter Eight).

In addition, sampling gaps in some geographical areas and temporal periods can affect the accuracy of the palaeodemographic history inferred from the BSP (Chapter Eight), for example in South Canterbury/Otago, Southland and northwest Nelson. The majority of loess deposits in South Canterbury and Otago (Worthy 1993a; Worthy and Grant-Mackie 2003), dating to the Late Glacial and Otiran Glacial, do not have adequate biomolecular preservation to warrant aDNA analysis (Chapter Seven). This was tested by DNA extraction and PCR, with PCR failing to amplify the small 50 bp fragment used for the species identification of moa coprolites (Chapter Four; Wood *et al.* 2008a). While bones preserved in loess have remarkable morphological preservation, the bones are the colour and texture of porcelain and crumble when samples are taken for aDNA analysis. Currently the BSP suggests that northern heavy-footed moa may have been separated into two different populations with contrasting demographic signals: Marlborough/North Canterbury and South Canterbury/Otago (Chapter Seven). While Marlborough and North Canterbury are represented by Otiran to Late Holocene samples, South Canterbury and Otago are represented by Late Holocene samples only. DNA sequences from the loess bones from South Canterbury and Otago are required to determine:

(i) If phylogeographic structuring is present during the Late Glacial and Otiran Glacial. This would show whether there were any geographic barriers in Canterbury that might have separated northern heavy-footed moa in Marlborough and North Canterbury from South Canterbury and Otago. There is the possibility that many of the braided rivers in Canterbury were deep and fast flowing during the Late Glacial and Otiran Glacial due to glacial runoff.

(ii) If a decline in population size from the Otiran Glacial to the Early Holocene is also seen in South Canterbury and Otago.

In Southland, heavy-footed moa were rarer than other areas along the eastern South Island (Worthy and Holdaway 1995, 1996; Worthy 1997a, 1998a, b; Chapter Three), making Late Glacial and Otiran Glacial specimens vitally important for phylogeographic and demographic analyses. In addition, further specimens of crested moa with adequate biomolecular preservation are needed from lowland northwest Nelson during the Late Glacial and Otiran Glacial, and areas above 1200 m asl. These specimens would have filled a sampling gap between the West Coast and northwest Nelson and would have helped determine whether there is any phylogeographic structuring within crested moa due to contraction of sub-alpine habitat to areas above 1200 m asl effectively creating "sky islands" that can promote phylogeographic structuring and speciation (Marlowe and Hufford 2008).

2.5.3. Population demographics

In the BSP and BayeSSC analyses a constant population size through time could not be rejected, despite relative fossil abundance data (Worthy and Holdaway 1995, 1996; Worthy 1997a, 1998a, b; Chapter Three) suggesting population declines with marked climate and habitat change. This raises the possibility that only datasets with strong demographic signals and high mutation rates, like viral datasets, can be analysed using the BSP (Chapter Eight). Of the published BSP using aDNA, only the bison (Drummond *et al.* 2005), bowhead whale (Ho *et al.* 2008), musk ox (Campos *et al.* 2010), grey wolf (Pilot *et al.* 2010) and cave bear (Stiller *et al*. 2010) datasets rejected constant population size, while the mammoth (Barnes *et al.* 2007; Debruyne *et al.* 2008), southern elephant seal (Bruyn *et al*. 2008), red-fox (Aubry *et al*. 2009), auroch (Mona *et al*. 2010), caraboo (Kuhn *et al*. 2010) and moa (Chapters Six and Seven) datasets did not reject constant population size (See Chapter Eight, Table One).

Given that some aDNA datasets do not have a strong demographic signal, dividing data into phylogeographic units to meet the unstructured single population assumption of the BSP could result in a significant loss of power in the dataset (Chapter Eight). BayeSSC (Excoffier *et al.* 2000; Anderson *et al.* 2006) provides a solution to this problem by allowing coalescent simulations of phylogeographically structured populations, and thus has the power to reject constant population size when the BSP cannot (see *Section 1.6* for example).

The conflict between the palaeontological record and demographic analyses utilising aDNA has been discussed in depth in *Section 1.5*. However, some further thoughts about the ability of the

BSP and BayeSSC to reject constant population size are warranted. Reconstructing the "true" demographic history of a population is important to determine if the conflict between the fossil record and aDNA data is real or an artefact of BSP and BayeSSC analyses. As discussed in Chapter Seven, the BSP uses a holistic approach and analyses DNA sequences to reconstruct the demographic history of taxa (Drummond *et al.* 2007), compared to BayeSSC, which compares simulated and observed population genetic summary statistics e.g. nucleotide diversity or average pairwise difference (Excoffier *et al.* 2000; Anderson *et al.* 2006). In addition, BayeSSC is hypothesis based, so more explicit hypotheses about the population demographics of taxa are needed. Also, BayeSSC requires user specified models of demographic history to be simulated. The most likely model of population demography in BayeSSC is not the best demographic model overall, just the best model of the ones tested. In comparison, the BSP and the newly released extended BSP (eBSP; Heled and Drummond 2008; see *Section 3.5* for further discussion) allows the genetic data and the coalescent to reconstruct the demographic history of taxa. In light of these points, the BSP is potentially more accurate at reconstructing the demographic history of taxa, even though constant population size cannot be rejected in some instances (See Chapter Eight, Table 1).

2.6 Robustness of the Bayesian Skyline Plot to biased sampling

Methodological components of the BSP, in addition to sampling biases discussed in *Section 1.6*, can affect the accuracy of the BSP. Methodological components include sample size, group size and the proportion of invariant sites. Sample size is defined as the number of sequences at each time point, which is intimately linked with biomolecular preservation biases. Group size is the number of coalescent intervals in the analysis to reduce noise from outliers in the dataset and act as a temporal smoothing factor of the inferred demographic history. The BSP requires the user to choose the number of groups, with the demographic history constrained with exactly *x* groups. However, it is not obvious what number of groups to choose. Choosing the wrong number of groups may result in large credible intervals (Heled and Drummond 2008). Stiller *et al.* (2010) examined the use of different group sizes (2, 4, 6, 8, and 10) in cave bears and showed that it did not have a large effect on the inference of demographic history in cave bears. Choosing the number of groups is solved by the eBSP (Heled and Drummond 2008), implemented in the latest version of BEAST v.1.5.4 where the number of groups is estimated using the genetic data (see *Section 3.5* for further discussion). However, the eBSP is only designed to work with multiple independent loci.

The proportion of invariant sites concerns the proportion of base pairs that do not change in a DNA sequence alignment. How the proportion of invariant sites affects the BSP is harder to investigate. Most population genetic datasets will have a nucleotide substitution model incorporating invariant sites, because the majority of sites in an alignment do not change. However, it is not really biologically plausible to allow for invariant sites within a species, as the proportion is always overestimated because of the large number of sites in an alignment that have not changed in the short time span covered by the aDNA data set. While we did not simulate the effect of the proportion of invariant sites on the BSP, they could potentially have a large impact on the robustness of the BSP (Simon Ho, Stefan Prost pers comm. 2010).

3. Future directions

Outlined below are ideas for future research to extend the current knowledge of the pre-human New Zealand ecosystem and to address some of the concerns and limitations about this research raised in *Section Two*.

3.1 Taphonomic deposition mechanisms of Holocene moa swamp deposits

The techniques developed in this research (Chapter Two; Wood *et al.* 2008b) are being used to examine the taphonomy of rockshelter and swamp deposits including the 3D mapping of coprolites within rockshelter sediments. In order to improve the reconstructions of miring bone deposits, small bones like vertebrae and bones from other species could be incorporated into the analysis to see how these bones behave taphonomically compared to the larger moa leg bones (femora, tibiotarsi, tarsometatarsi). As discussed in *Section 2.1*, there is a significant under representation in small bones like vertebrae, skulls and phalanges in swamps deposits in New Zealand (Worthy 1998b; Wood *et al*. 2008b). In addition, further excavations are required to determine if the sub-horizontal inclination and preferential alignment of moa leg bones is characteristic of New Zealand miring bone deposits. In Chapter Two (Wood *et al*. 2008b) we hypothesised that the sub-horizontal alignment of leg bones was due to the natural buoyancy of bones in peat during rare liquefaction events when moa became mired and raked their legs through the semi-liquid peat matrix (Wood *et al.* 2008b).

3.2 Palaeofaunal reconstruction of the Glencrieff moa swamp deposit

Two important areas of future research, which will also help address concerns raised in *Section 2.1* about the taphonomy of Glencrieff, stem from the palaeofaunal reconstruction. The first is to

radiocarbon date bones from the Canterbury Museum and 2007 sub-deposits and determine whether these sub-deposits formed at the same time as the Worthy and Holdaway sub-deposit. The second is to genetically sex the bones from the Canterbury Museum, Worthy and Holdaway and 2007 sub-deposits (heavy-footed, eastern, South Island giant and stout legged moa) to determine if the sex ratio of moa trapped in Glencrieff is comparable to Allentoft *et al.* (2010) ratio of >2 females: 1 male, suggesting increased mortality of males compared to females during juvenile and sub-adult growth stages. It would be interesting to see if the hypothesis of Allentoft *et al.* (2010) was still valid during the Pleistocene in North Canterbury, and other Holocene or Pleistocene swamp deposits in New Zealand. This will determine whether it is a general phenomenon in swamp deposits or specific to North Canterbury. In addition, sexing moa bones from cave deposits would determine whether the over abundance of females is specific to swamp deposits or is a general phenomenon in fossil deposits throughout New Zealand.

3.3 Reconstructing the diet of moa

An unexpected result from this research was the extensive dietary overlap observed between moa species (Chapter Four; Wood *et al.* 2008a). Less overlap may be observed if pollen and/or phytoliths (silicon structures within plant leaves whose shape is unique to each species) were studied. Pollen and phytoliths can survive the initial degradation of plant macrofossils like twigs, seeds and leaf cuticles. Coprolites without observable plant macrofossils or amorphous organic material can have large numbers of plant microfossils. Research is currently underway to widen this study across the South Island, New Zealand to determine whether the differences in diet between species are upheld by excavating new sites and analysing plant macrofossils, pollen, phytoliths and aDNA. It will be particularly interesting if coprolites of the little bush moa, which is the only moa species specifically adapted to forest habitats, are found, and how the diet of little bush moa compares to the other moa species. If the diet of little bush moa comprises only forest species, it would suggest that this species was a specialist. However, if the diet of little hush moa overlaps with other moa species, it would suggest this species is a generalist, raising the question, why are little bush moa remains only found in fossil deposits in areas that are or were characterised by closed canopy forest (Worthy and Holdaway 2002)?

Given the plasticity and overlap of moa diet, moa may have easily shifted their dietary preferences in response to climate and habitat change (*Section 1.3*). With this in mind it is vital to find Late Glacial (10-14 Kya) and Otiran Glacial (14-70 Kya) coprolite deposits of adequate

preservation, to test if moa changed their diet. Cave deposits of Late Glacial and Otiran Glacial age are known (Worthy 1993b, 1994, 1997b; Worthy and Holdaway 1994, 1995), but no moa coprolite deposits have been found dating to this time. It is highly probable that previous palaeontological excavations failed to recognise coprolites. Another hypothesis is that the coprolites have become disaggregated into sediment within caves and rockshelters. This has been observed in Central Otago where some rockshelters contain a layer of disaggregated coprolites and/or dense organic material in stratigraphically older layers than those containing intact coprolites (Wood *et al*. 2008a). The absence of coprolites in cave and rockshelter deposits is not unique to New Zealand. In Australia there is a conspicuous absence of Pleistocene megafaunal coprolites – while a few fossilised examples of coprolites and preserved gizzard contents are held in museum collections (Rawlence pers obs), the only records of sub-fossil coprolites are from the Late Holocene and belong to stick nest rats (Pearson *et al.* 2001), ghost bats (Medlin 1993) and barn owls (Tores *et al.* 2005).

There are several possible methodologies to test whether coprolites have disaggregated into sediment. The first methodology is to conduct aDNA extractions on sediment from caves and rockshelters. This has been done by Willerslev *et al.* (2003) and Haile *et al.* (2007) from rockshelters in New Zealand. Both these studies extracted, amplified and sequenced avian (moa, duck and parrot) and plant DNA from rockshelter sediments. While these studies showed that avian DNA was present in each stratigraphic layer, the DNA sequences do not conclusively prove that the sediment comprised disaggregated coprolites. However, Willerslev *et al*. (2003), Lydolf *et al.* (2005) and Haile *et al.* (2007) have hypothesised that a large proportion of animal DNA in sediment originates from animal excreta. This leads to the second methodological technique: Palynological analysis of sediment for *Sporormiella* fungal spores (Davis 1987; Burney *et al.* 2003; Davis and Shafer 2006; Gill *et al.* 2009; Chapter Three). In addition, because *Sporormiella* deposition is very directional (Wood pers comm. 2010; Chapter Three) high numbers of *Sporormiella* spores in sediment may indicate that the sediment comprises disaggregated coprolites.

In the absence of coprolite deposits, preserved gizzards would offer a biased insight into dietary changes over time. Moa gizzards are now known from the Late Glacial Glencrieff miring bone deposit (Chapter Three). Unfortunately, the border between preserved gizzard contents and the surrounding matrix is often indistinct (e.g. Glencrieff, Chapter Three; Scaifes Lagoon, Burrows *et*

al. 1981) compared to gizzard contents from Treasure Downs and Pyramid Valley where the border is distinct (Burrows *et al.* 1981; Wood 2007). The clarity of the border between the preserved gizzard contents and surrounding matrix will affect the reliability of palaeodietary reconstructions and whether plant macro or microfossils from the surrounding matrix have "contaminated" the preserved gizzard contents.

One result (or lack of a result) from this research was the failure rate of coprolites from Central Otago compared to the Dart Valley. There are several possible reasons for this. As outlined in *Section 2.3*, the first explanation may be temperature differences between the Dart Valley and Central Otago. The second explanation may be that the coprolites are not from moa. It is possible that the coprolites belong to the extinct adzebill (*Aptornis defossor*), extinct giant goose (*Cnemiornis calcitrans*) or the locally extinct kakapo (*Strigops habroptilus*; Wood 2007). Excluding moa, adzebill, goose and kakapo were the largest terrestrial herbivores in the pre-human ecosystem. It would be particularly interesting if the coprolites were identified as adzebill as Worthy and Holdaway (2002), based on $\delta^{13}C$ and $\delta^{15}N$ isotope signatures, hypothesised that adzebill were carnivorous. If the diet of adzebill could be reconstructed, hypotheses regarding adzebill diet could be critically assessed. Though sub-fossil remains of adzebill have not been found in Central Otago (Worthy and Holdaway 2002) it does not preclude their presence. Hofreiter *et al*. (2001) discovered a previously unrecognised species of ground sloth in the fossil record; the Cuchillo Cura ground sloth, using aDNA from coprolites. Using PCR primers for rails and anseriforms, we would attempt to amplify ralliform and anseriform DNA from Central Otago coprolites. However, this cannot rule out that the coprolites could be from moa, geese or rails but have inadequate aDNA preservation. Preliminary experiments have failed to amplify avian DNA from the majority of Central Otago coprolites (Jamie Wood pers comm. 2010).

Coprolites can now be used as additional source of aDNA along with bone, feathers, and eggshell, and can be genetically sexed. This could be used to determine whether there are differences between sexes in diet, especially relevant given the differences between moa in plumage (Chapter Five; Rawlence *et al.* 2009), diet inferred from gizzards and coprolites (Chapter Four; Wood *et al.* 2008a), and height (Bunce *et al.* 2003; Huynen *et al.* 2003). Generally coprolites are not included in demographic and population genetic studies because there are no controls in place to prevent sampling an individual more than once, which would artificially increase the amount of genetic variation in aDNA datasets.

3.4 Reconstructing the plumage of moa

One area of research that needs to be conducted is to determine the purity of ancient feather DNA and the origin of ancient feather DNA. These questions could be answered following the methodology of Oskam *et al.* (2010), who showed that ancient mitochondrial and nuclear DNA could be isolated from eggshell up to 19 Kyr, with approximately 125 fold lower exogenous bacterial contamination levels than bone. Oskam *et al.* (2010) used:

(i) Quantitative real time PCR to assess mitochondrial and nuclear DNA template number and quality in eggshell.
(ii) A real time PCR dilution assay to determine the level of inhibition present in aDNA extracts from eggshell.
(iii) A comparative real time PCR assay of endogenous avian DNA and exogenous bacterial DNA from eggshell and bone of similar ages and deposition contexts to assess levels of aDNA preservation in eggshell.
(iv) Confocal imaging with fluorescent dyes that bind to DNA (e.g. Hoechst and SYBR) to determine the location of aDNA within eggshell.

Irrespective of the origin and purity of ancient feather DNA, future research should utilise further sites (like forested habitat), additional species, and genetic sexing of feathers to investigate whether there are plumage differences between sexes, habitats, or species, and how this relates to moa palaeoecology and mate recognition systems. For example, the mummified remains of a little bush moa individual dating to the Late Holocene (Bunce *et al*. 2009) were discovered in a cave in closed canopy forest at Lake Echo in Fiordland, South Island (Forest 1987). However, only feather bases were preserved *in situ*, providing no information on external appearance. Re-excavation of the cave may result in coprolites and feathers of little bush moa being found allowing the plumage and diet of this species to be reconstructed.

Finally, there are two analytical methodologies available to validate the plumage reconstructions reported in Chapter Five (Rawlence *et al.* 2009). The first methodology is to compare the morphological reconstructions with feather colour inferred from nuclear colour gene sequence variants (like the melanocortin receptor one gene, MC1R) amplified from feather and bone DNA extracts. MC1R sequences from moa could be compared against MC1R variants in extant bird taxa. This would determine whether feather colours inferred from gene sequences are seen in sub-

fossil feathers, and what colour variants are not seen in the feathers. The second methodology is to characterise the alignment and shape of moa feather melanosomes and compare this against a database of avian melanosomes from extant species (Vinther *et al.* 2008, 2009; Li *et al.* 2010; Zhang *et al.* 2010) as outlined in *Section 2.4*. Scanning election microscopy of feathered dinosaur fossils, (without destruction of the fossil), have shown that the feathered dinosaur *Sinosauropteryx* had alternating bands of ginger and white feathers down its tail (Zhang *et al.* 2010).

3.5 Responses of moa to climate change in pre-human New Zealand

This research (Chapters Six and Seven) has focused on two species in the genus *Pachyornis*: crested moa and heavy-footed moa. A third species restricted to the North Island, Mappin's moa, was not studied due to the paucity of Late Glacial and Otiran Glacial specimens, and biomolecular preservation biases. However, the study of moa population demographics could have focused on any of the other five genera of moa: *Megalapteryx*, *Emeus*, *Dinornis*, *Euryapteryx* and *Anomalopteryx*. Each of these genera had different responses to climate and habitat change based on the fossil record and the genetic diversity present in recent phylogenetic studies (Lambert *et al.* 2005; Baker *et al.* 2005; Bunce *et al.* 2009). For example eastern moa has very little genetic variation hypothesised to be a result of repeated population bottlenecks during glacial cycles (Bunce *et al.* 2009). This is compared to upland moa, whose habitat was alpine and rocky/rugged environments (Worthy and Holdaway 2002), had four well-defined clades suggesting that niche persistence and habitat stability are important in maintaining genetic variation (Bunce *et al.* 2009).

Several challenges are present to investigating the population demographics of the other moa species, given the sampling biases inherent in most aDNA datasets that can affect the interpretations of demographic history inferred from the BSP (Chapter Eight). These include:

(i) Finding well-preserved Late Glacial (14-10 Kya) and Otiran Glacial (70-14 Kya) remains in large numbers, so that there is good sampling before, during and after the LGM.
(ii) Studying moa taxa that do not exhibit temporal phylogeographic structuring.

Finding Late Glacial and Otiran Glacial specimens is complicated by temporal and biomolecular preservation biases (Chapter Eight). Bones from loess deposits (Worthy 1993a; Worthy and

Grant-Mackie 2003) do not have adequate biomolecule preservation to warrant aDNA analysis – this restricts aDNA sampling to swamp and cave deposits. Swamp deposits of Late Glacial and Otiran Glacial age in the South Island are limited to Glencrieff (Worthy and Holdaway 1996), Herbert (Oliver 1949), Owaka (Otago Museum), Opio (Chapter Seven) and Kauana (Worthy 1998b). Of these, Owaka and Kauana are 'destroyed'; Hebert, Owaka and Opio are limited to small sample sizes, and Herbert, Owaka and Kauana have substandard biomolecule preservation. The majority of the Late and Otiran Glacial cave deposits, with the exception of Merino Cave (Worthy and Holdaway 1995; Chapter Seven) and some caves in northwest Nelson/West Coast (Worthy 1993b, 1994, 1997b; Worthy and Holdaway 1994; Chapters Six and Seven) do not have sufficient biomolecule preservation to warrant aDNA analysis. In the North Island, humid conditions and acidic volcanic ash do not promote biomolecule preservation, limiting aDNA analysis to the Holocene (Bunce *et al.* 2003; Huynen *et al.* 2003; Baker *et al.* 2005; Lambert *et al.* 2005; Haile *et al.* 2007; Bunce *et al.* 2009). The implication of all this is that further palaeodemographic analyses of moa taxa are limited to species with a long fossil record in southern New Zealand or alpine regions.

Despite the challenges involved in investigating the population demographics of moa, there are several pertinent research questions that could be addressed. The most interesting questions address the population demographics of eastern, little bush and upland moa. Eastern moa was one of the dominant species of moa in the eastern South Island during the Otiran Glacial to Late Holocene based on relative fossil abundance data (Worthy 1993a, 1997a, 1998a, b; Worthy and Holdaway 1996). However, recent genetic studies (Lambert *et al.* 2005; Baker *et al.* 2005; Bunce *et al.* 2009) show that there is no genetic variation within eastern moa, suggesting a rapid expansion during the Holocene. The challenge would be finding specimens with adequate biomolecule preservation from Late Glacial and Otiran Glacial sites (e.g. the Glencreiff swamp deposit; Chapter Three and Seven). In contrast, little bush moa is the only moa species specifically adapted to forested environments. Very rare in glacial deposits but common in Holocene deposits (Worthy and Roscoe 2003), there is deep phylogeographic structuring with a reciprocally monophyletic North and South Island clade (Bunce *et al.* 2009). It is hypothesised that during the last glacial period little bush moa retreated to sheltered, and warmer northern refugia with retreating forest in both the North and South Island. However, there are either no fossil deposits of this age within proposed refugial areas, the areas are now below sea level, or the little bush moa is not represented in cave deposits of the appropriate age. Finally, upland moa

was characterised by four well defined clades in the northwest, northeast, southwest and southeast South Island (Bunce *et al.* 2009). While the southern and north-eastern clades are represented by Holocene specimens only, it would be interesting to replicate the research conducted on crested moa (Chapter Six) on the northwest clade to see if upland moa responded to changes in the availability of sub-alpine habitat by changing its distribution accordingly.

The research questions concerning the population demographics of eastern, little bush and upland moa also raise some general research questions as follows:

(i) In the absence of secondary interactions like interspecies competition (Bennett *et al*. 1991) and geographic barriers (Hewitt 1999), do taxa with the same habitat niche inferred from the fossil record, have similar responses to climate and habitat change? This is pertinent to Guthrie (2006) and Scott (2009) who hypothesised that the decline of mammoth populations and the extinction of *Equus* horses about 12.5 Kya was due to competition for habitat with bison, whose numbers rapidly increased about 12.5 Kya. Studying species responses to changing habitat in the absence of secondary interactions would also help elucidate the causes of the Late Pleistocene megafaunal extinctions.

(ii) Do declines in the amount of habitat result in the loss of genetic diversity?
(iii) Do changes in relative fossil abundance match changes in effective population size?

The demographic power of aDNA datasets is also essential in constructing a BSP (Chapter Eight). As outlined above, it is essential to carefully choose taxa to maximise the power of the data and minimise the risk of violating the assumptions of the BSP. In the meantime, multiple analytical approaches to the study of population demographics have been subsequently developed that can be used as independent verification of the BSP, such as the extended BSP (eBSP; Heled and Drummond 2008) and the Bayesian Skyride Plot (BSRP; Minin *et al.* 2008). The eBSP builds upon the BSP (Drummond *et al.* 2005) in at least two important ways:

(i) Multiple genetic loci can be analysed and combined to reconstruct a shared population demographic history.

(ii) The number of groups (number of coalescent intervals in the analysis to reduce noise from outliers in the dataset) is estimated based on the genetic data rather than fixing the number of groups in the BSP.

Simulations have shown that the eBSP can reconstruct the demographic history of taxa through multiple population bottlenecks (Heled and Drummond 2008). However, given that different genetic loci can have different phylogenetic and geographic signals, it does not solve the problem of phylogeographic structuring in BSP analyses.

In contrast to the BSP and eBSP, the BSRP uses a temporal smoothing method to construct a posterior distribution of population size change at each coalescent point, and is a superior method to the BSP (Minin *et al*. 2008). Unlike the BSP, the BSRP does not require strong prior decisions like fixing the number of groups, which controls the smoothness of the inferred population demography (Minin *et al.* 2008). However, given that datasets with strong demographic signals are more robust to sampling biases than datasets with weak demographic signals, and can reject constant population size (Chapter Eight), it is not known how weak datasets would perform under the BSRP.

3.6 Robustness of the Bayesian Skyline Plot to biased sampling

Many aDNA studies will find phylogeographic structuring at some time period within the study species. Thus it is vital to determine the level of phylogeographic structuring that will not violate the assumption of panmixia with a single population coalescent (Drummond *et al*. 2005). However, there are some advances in analytical methodology that could potentially help address these issues.

Ho *et al.* (2007) developed a method utilising BEAST (Drummond and Rambaut 2007) that constructs multi-species genealogies, where each "taxa" has a different demographic prior (constant, expansion or logistic growth). Ho *et al.* (2007) used this methodology to examine the effects of climate change and domestication on bovid evolution. It is not known whether the Ho *et al.* (2007) method could be extended to the BSP, so that the BSP can accommodate multiple populations across a species range with their own "BSP demographic priors" to construct an overall BSP. If this approach could be developed, it would negate the division of aDNA datasets into single populations or phylogeographic units to meet the assumptions of the BSP, which

reduces the power of the data. Currently, BayeSSC (Excoffier *et al.* 2000; Anderson *et al.* 2006) is the only coalescent-based programme that can accommodate species with multiple population coalescent events. However, there are serious limitations to BayeSSC as discussed in *Section 2.5*. Ideally, if BEAST could accommodate diverging and coalescing populations like BayeSSC, then BSP (and eBSP) analyses would be a superior analytical method. Currently, however, scientists are restricted to using the BSP and either dividing aDNA datasets into multiple distinct populations for analysis (Grazziotin *et al.* 2006; Crandall *et al.* 2008; Debruyne *et al*. 2008; Campos *et al.* 2010; Mona *et al.* 2010; Chapter Six and Seven) or ignoring the assumption of panmixia (Shapiro *et al.* 2004; Drummond *et al.* 2005; Bruyn *et al.* 2009).

Secondly, the methodology of Campos *et al.* (2010), who used aDNA to investigate the population demographic history of the musk ox (*Ovibos moschatus*), may be applicable to future studies. Campos *et al.* (2010) conducted a combined temporal and geospatial demographic analysis (Lemey *et al.* 2009) in BEAST, where each sequence (tip) was assigned a different geographical sampling location. Posterior probabilities of the probable geographic location of internal nodes were then estimated, along with a co-estimated BSRP incorporating a post mortem DNA damage model (Rambaut *et al.* 2008). The BSRP for each sample of geographical tips/nodes was combined into an overall BSRP to describe the demographic history of musk ox and the contribution of each geographical locality to the overall BSRP. However, this geospatial methodology is currently not available in the latest version of BEAST. In addition, it is still unclear whether the approach of Campos *et al.* (2010) resolves the issue of phylogeographic structuring. The phylogenetic analysis showed that musk ox was phylogeographically structured into three well supported clades. Each of the three clades contained sequences from multiple geographic localities. For example, clade one was predominantly found in north east Siberia but was also present on the Taimyr Peninsula, clade two was almost exclusively from the Taimyr Peninsula and the Urals, Russia, and clade three was predominantly from North America and Greenland (Campos *et al*. 2010). However, the geospatial demographic analysis constructed BSRP for each geographical location, for example the Taimyr Peninsula, despite sequences from the Taimyr Peninsula belonging to two different phylogeographic units, violating the assumption of panmixia.

Finally, it is vital to analyse simulated and empirical datasets to investigate how methodological components of the BSP (e.g. sample size, group size and proportion of invariant sites) affect interpretations of the demographic history of taxa inferred from the BSP. The effects of sample size can be investigated by simulating samples sizes of 10, 20, 30 ancient sequences at 10, 20, 30 Kyr. Small samples sizes can result in artificial phylogeographic structuring, that will violate the assumption of panmixia (Goldstein and Desalle 2003; Munoz-Fuentes *et al.* 2005; Austin *et al*. 2009). The effects of group size, which controls the number of discrete changes in population size/coalescent intervals and the smoothness of the BSP can be investigated by re-analysing the simulated and empirical datasets with a group size of 1 (constant population size), 5, 10, 15, and 20. It has been recommended that group sizes of 5-10 are more biologically plausible than 15 (Simon Ho pers comm. 2010), which was used by Drummond *et al.* (2005) in his re-analysis of the Shapiro *et al.* (2004) bison aDNA dataset. Finally, the effect of the proportion of invariant sites can be investigated by simulating different datasets with varying degrees of invariant sites, and by re-analysing the empirical datasets using nucleotide substation models with and without invariant sites. This is currently being investigated (Simon Ho pers comm. 2010).

4.0 Conclusion

"With regard to general problems of biogeography, the biota of New Zealand has been, perhaps, the most important of any in the world. It has figured prominently in all discussions of austral biogeography, and all notable authorities have felt obliged to explain its history: explain New Zealand and the world falls into place around it." – Nelson (1975). While Nelson (1975) was talking about the study of biogeography, this statement is especially pertinent to the results and conclusions of this thesis and the ongoing debate into the causes of the Late Pleistocene megafaunal extinctions.

In this thesis New Zealand was chosen to provide a unique geographical setting where the effects of climate change and human impact on a megafaunal palaeoecosystem could be separated. Within the course of this study, considerable amounts of new data have been accumulated from the fossil record of New Zealand. The reliability of this information has been critically assessed and then used to analyse several aspects of the pre-human ecosystem of New Zealand and the effects of climate change on that ecosystem. This study has established New Zealand as a new model system for analysing the relative contributions of climate change and human impact in the Late Pleistocene megafaunal extinctions. This thesis has also demonstrated the power and utility

of multidisciplinary research involving palaeontology, palynology, aDNA, morphology, geography, geology and ecology to investigate scientific questions. This approach has demonstrated how it is possible to reconstruct aspects of the palaeoecosystem of New Zealand before the fateful day in 1280 AD when Polynesians colonised New Zealand and this unique ecosystem was tragically destroyed.

5.0 References

Allentoft ME, Bunce M, Scofield RP, Hale ML, Holdaway RN 2010. Highly skewed sex ratios and biased fossil deposition of moa: Ancient DNA provides new insights on New Zealand's extinct megafauna. Quaternary Science Reviews 29: 753-762.

Anderson A 1989. Prodigious birds. Cambridge University Press, Cambridge, UK.

Anderson CNK, Ramakrishnan U, Chan YL, Hadly EA 2006. Serial SimCoal: A population genetics model for data from multiple populations and points in time. Bioinformatics 21: 1733-1734.

Atkinson IAE, Greenwood RM 1989. Relationships between moas and plants. New Zealand Journal of Ecology 12 (supplement): 67–96.

Austin JJ, Arnold EN, Jones CG 2009. Interrelationships and history of silt-eared skinks (Gongylomorphus, Scincidae) of the Mascarene Islands, based on mitochondrial and nuclear gene sequences. Zootaxa 2153: 55-68.

Axelrod DI 1967. Quaternary extinctions of large mammals. Geological Science 74: 1-42.

Baker AJ, Huynen LJ, Haddrath O, Miller CD, Lambert DM 2005. Reconstructing the tempo and mode of evolution in an extinct clade of birds with ancient DNA: The giant moas of New Zealand. Proceedings of the National Academy of Science USA 102: 8257-8262.

Barnes I, Matheus P, Shapiro B, Jensen D, Cooper A 2002. Dynamics of Pleistocene population extinctions in Beringian brown bears. Science 295: 2267-2270.

Barnes IB, Shapiro B, Lister A, Kuznetsova T, Sher A, Guthrie D, Thomas MG 2007. Genetic structure and extinction of the woolly mammoth, *Mammuthus primigenius*. Current Biology 17: 1072-1075.

Bennett KD, Tzedakis PC, Willis KJ 1991. Quaternary refugia of north European trees. Journal of Biogeography 18: 103-115.

Bond WJ, Silander WJ 2007. Springs and wire plants: anachronistic defences against Madagascar's extinct elephant birds. Proceedings of the Royal Society Series B 274: 1985–1992.

Booth BS 1875. Description of the Moa Swamp at Hamilton. Transactions and Proceedings of the Royal Society of New Zealand 7: 123-138.

Bruyn M, Hall BL, Chauke LF, Baroni C, Koch PL, Hoelzel AR 2009. Rapid response of a marine mammal species to Holocene climate and habitat change. PLoS Genetics 5: e1000554.

Buick TL 1931. The mystery of the moa. Thomas Avery & Sons Limited, New Plymouth, New Zealand.

Bunce M, Worthy TH, Ford T, Hoppitt W, Willerslev E, Drummond A, Cooper A 2003. Extreme reversed sexual size dimorphism in the extinct New Zealand moa *Dinornis*. Nature 425: 172–175.

Bunce M, Worthy TH, Phillips MJ, Holdaway RN, Willerslev E, Haile J, Shapiro B, Scofield RP, Drummond A, Camp PJJ, Cooper A 2009. The evolutionary history of the extinct ratite moa

and New Zealand Neogene paleogeography. Proceedings of the National Academy of Science USA 106: 20646-20651.

Burger J, Rosendahl W, Loreille OP, Hemmer H, Eriksson T, Gotherstrom A, Hiller J, Collins MJ, Wess T, Alt KW 2004. Molecular phylogeny of the extinct cave lion *Panthera leo spelaea*. Molecular Phylogenetics and Evolution 30: 841-849.

Burney DA, Robinson GS, Pigott Burney L 2003. *Sporormiella* and the late Holocene extinctions in Madagascar. Proceedings of the National Academy of Science USA 100: 10800-10805.

Burns KC, Dawson JW 2006. A morphological comparison of leaf heteroblasty between New Caledonia and New Zealand. New Zealand Journal of Botany 44: 387–396.

Burrows CJ, McCulloch B, Trotter MM 1981. The diet of moas based on gizzard contents samples from Pyramid Valley, North Canterbury, and Scaife's Lagoon, Lake Wanaka, Otago. Records of the Canterbury Museum 9: 309–336.

Butler D, Merton D 1992. The Black Robin: Saving the world's most endangered bird. Oxford University Press, Oxford, UK.

Campos PF, Willerslev E, Sher A, Orlando L, Axelsson E, Tikhonov A, Aaris-Sorensen K, Greenwood AD, Kahlke RD, Kosintsev P, Krakhmalnaya T, Kuznetsova T, Lemey P, MacPhee R, Norris CA, Shepherd K, Suchard MA, Zazula GD, Shapiro B, Gilbert MTP 2010. Ancient DNA analyses exclude humans as the driving force behind late Pleistocene musk ox (*Ovibos moschatus*) population dynamics. Proceedings of the National Academy of Science USA 107: 5675-5680.

Clark CJ, Poulsen JR, Parker VT 2001. The role of arboreal seed dispersal groups on the seed rain of a lowland tropical forest. Biotropica 33: 606-620.

Crandall ED, Frey MA, Grosberg RK, Barber PH 2008. Contrasting demographic histories and phylogeographical patterns in two Indo-Pacific gastropods. Molecular Ecology 17: 611-626.

Dalen L, Nystrom V, Valdiosera C, Germonpre M, Sablin M, Turner E, Angerbjorn A, Arsuaga SL, Gotherstrom A 2007. Ancient DNA reveals lack of postglacial habitat tracking in the arctic fox. Proceedings of the National Academy of Science USA 104: 6726-6729.

Davis OK 1987. Spores of the dung fungus *Sporormiella*: Increased abundance in historic sediments and before Pleistocene megafaunal extinction. Quaternary Research 28: 290-294.

Davis OK, Shafer DS 2006. *Sporormiella* fungal spores, a palynological means of detecting herbivore density. Palaeogeography, Palaeoclimatology, Palaeoecology 273: 40-50.

Debruyne R, Chu G, King CE, Bos K, Kuch M, Schwarz C, Szpak P, Grocke DR, Matheus P, Zazula G, Guthrie D, Froese D, Buigues B, Marliave C, Flemming C, Poinar D, Fisher D, Southon J, Tikhonov AN, MacPhee RDE, Poinar HN 2008. Out of America: Ancient DNA evidence for a New World origin of Late Quaternary woolly mammoths. Current Biology 18: 1320-1326.

Donlan J, Greene HW, Berger J, Bock CE, Bock JH, Burney DA, Estes JA, Foreman D, Martin DS, Roemer GW, Smith FA, Soule ME 2005. Rewilding North America. Nature 436: 913–914.

Drummond AJ, Ho SYW, Rawlence N, Rambaut A 2007. A rough guide to BEAST 1.4. Unpublished user manual, University of Auckland.

Drummond AJ, Rambaut A 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7: 214.

Drummond AJ, Rambaut A, Shapiro B, Pybus OG 2005. Bayesian coalescent inference of past population dynamics from molecular sequences. Molecular Biology and Evolution 22: 1185-1192.

Emslie SD, Coats L, Licht K 2007. A 45,000 yr record of Adelie penguins and climate change in the Ross Sea, Antarctica. Geology 35: 61-64.

Excoffier L, Novembre J, Schneider S 2000. SIMCOAL: a general coalescent program for simulation of molecular data in interconnected populations with arbitrary demography. Journal of Heredity 91: 506-509.

Fadzly N, Jack C, Schaefer HM, Burns KC 2009. Ontogenetic colour changes in an insular tree species: signalling to extinct browsing birds? New Phytologist 184: 495-501.

Firestone RB, West A, Kennett JP, Becker L, Bunch TE, Revay ZS, Schultz PH, Belgya T, Kennett DJ, Erlandson JM, Dickenson OJ, Goodyear AC, Harris RS, Howard GA, Kloosterman JB, Lechler P, Mayewski PA, Montgomery J, Poreda R, Darrah T, Que Hee SS, Smith AR, Stich A, Topping W, Wittke JH, Wolback WS 2007. Evidence for an extraterrestrial impact 12,900 years ago that contributed to the megafaunal extinctions and the Younger Dryas cooling. Proceedings of the National Academy of Science USA 104: 16016-16021.

Flannery T, Schouten P 2001. A gap in nature: discovering the world's extinct animals. Australia: The Text Publishing Company.

Forest RM 1987. A partially mummified skeleton of *Anomalopteryx didiformis* from Southland. Journal of the Royal Society of New Zealand 17: 399-408.

Forsyth DM, Wilmshurst SM, Allen RB, Coomer DA 2010. Impacts of introduced deer and extinct moa on New Zealand ecosystems. New Zealand Journal of Ecology 34: 48-65.

Foufopoulos J, Ives AR 1999. Reptile extinctions on land-bridge islands: Life-history attributes and vulnerability to extinction. American Naturalist 153: 1-25.

Fursich FT, Flessa KW 1991. The origin and interpretation of Bahia la Choya (northern Gulf of California) taphocoenoses: implications for palaeoenvironmental analysis. Zitteliana 18: 165-169.

van Geel B, Zazula GD, Schwegerc CE 2007. Spores of coprophilous fungi from under the Dawson tephra (25,300 14C years BP), Yukon Territory, northwestern Canada. Palaeogeography, Palaeoclimatology, Palaeoecology 252: 481-485.

Gill B, Martinson P 1991. New Zealand's extinct birds. New Zealand: Random Century.

Gill JL, Williams JW, Jackson ST, Lininger KB, Robinson GS 2009. Pleistocene megafaunal collapse, novel plant communities, and enhanced fire regimes in North America. Science 326: 1100-1103.

Goldstein PZ, Desalle R 2003. Calibrating phylogenetic species formation in a threatened insect using DNA from historical specimens. Molecular Ecology 12: 1993-1998.

Grazziotin FG, Monzel M, Echeverrigaray S, Bonatto SL 2006. Phylogeography of the *Bothrops jararaca* complex (Serpentes: Viperidae): Past fragmentation and island colonisation in the Brazilian Atlantic Forest. Molecular Ecology 15: 3969-3982.

Greenwood RM, Atkinson IAE 1977. Evolution of divaricating plants in New Zealand in relation to moa browsing. New Zealand Ecological Society Proceedings 24: 21-23.

Guthrie RD 1984. Mosaics, Allelochemics and Nutrients. In: Martin PS, Klein RG (Eds) Quaternary extinctions: A prehistoric revolution. The University of Arizona Press, USA. Pp. 259-298.

Guthrie RD 2006. New carbon dates link climatic change with human colonisation and Pleistocene extinctions. Nature 441: 207-209.

Haile J, Holdaway R, Oliver K, Bunce M, Gilbert MTP, Nielsen R, Munch K, Ho SYW, Shapiro B, Willerslev E 2007. Ancient DNA chronology within sediment deposits: Are palaeobiological reconstructions possible and is DNA leaching a factor? Molecular Biology and Evolution 24: 982–989.

Hamilton A 1894. On the feathers of a small species of Moa (*Megalapteryx*) found in a cave at the head of the Waikaia River, with a notice of a moa-hunters camping place on the Old Man Range. Transactions and Proceedings of the New Zealand Institute 27: 232–238.

Handel SN, Beattie AJ 1990. Seed dispersal by ants. Scientific American 263: 76-83.

Harcourt AH, Copeton SA, Parks SA 2002. Rarity, specialisation and extinction risk in primates. Journal of Biogeography 29: 445-456.

Haynes G 1988. Mass deaths and serial predation: Comparative taphonomic studies of modern large-mammal death sites. Journal of Archaeological Science 15: 219-235.

Heled J, Drummond AJ 2008. Bayesian inference of population size history from multiple loci. BMC Evolutionary Biology 8: 289.

Henrici AC, Fiorillo AR 1993. Catastrophic death assemblage of *Chelomophrynus bayi* (Anura, Rhinophrynidae) from the middle Eocene Wagon Bed Formation of Central Wyoming. Journal of Paleontology 67: 1016-1026.

Herrera CM 2002. Seed dispersal by vertebrates. In: Herrera CM, Pellmyr O (Eds), Plant–Animal Interactions: An Evolutionary Approach. Blackwell, Oxford, pp. 185–208.

Hewitt GM 1999. Post-glacial re-colonisation of European biota. Biological Journal of the Linnaean Society 68: 87-112.

Ho SYW, Heupink TH, Rambaut A, Shapiro B 2007. Bayesian estimation of sequence damage in ancient DNA. Molecular Biology and Evolution 24: 1416-1422.

Ho SYW, Saarma U, Barnett R, Haile J, Shapiro B 2008. The effect of inappropriate calibration: Three case studies in molecular ecology. PLoS One 3: e1615.

Hofreiter M, Betancourt JL, Sbriller AP, Markgraf V, McDonald 2001. Phylogeny, diet and habitat of an extinct ground sloth from Cuchillo Cura, Neuquen Province, southwest Argentina. Quaternary Research 59: 364-378.

Hofreiter M, Poinar HN, Spaulding WG, Bauer K, Martin PS, Possnert G, Paabo S 2000. A molecular analysis of ground sloth diet through the last glaciation. Molecular Ecology 9: 1975–1984.

Horrocks M, D'Costa D, Wallace R, Gardner R, Kondo R 2004. Plant remains in coprolites: Diet of a sub alpine moa (Dinornithiformes) from southern. New Zealand. Emu 104: 149–156.

Horrocks M, Salter J, Braggins J, Nichol S, Moorhouse R, Elliot G 2008. Plant microfossil analysis of coprolites of the critically endangered kakapo (*Strigops habroptilus*) parrot from New Zealand. Review of Palaeobotany and Palynology 149: 229–245.

Horvoth MB, Martinez-Cruz B, Negro JJ, Kalmar L, Godoy JA 2005. An overlooked DNA source for non-invasive genetic analysis in birds. Journal of Avian Biology 36: 84-88.

Hoss M, Jaruga P, Zastawny TH, Dizdaroglu, Paabo S 1996. DNA damage and DNA sequence retrieval from ancient tissues. Nucleic Acids Research 24: 1304-1307.

Howorth HH 1887. The Mammoth and the Flood: An Attempt to Confront the Theory of Uniformity with the Facts of Recent Geology. S. Low, Marston, Searle, & Rivington, London.

Hutton FW 1900. The Geological History of New Zealand. Transactions and Proceedings of the New Zealand Institute 32: 159-183.

Huynen L, Millar CD, Scofield RP, Lambert DM 2003. Nuclear DNA sequences detect species limits in ancient moa. Nature 425: 175–178.

Janzen DH 1984. Dispersal of small seeds by big herbivores: Foliage is the fruit. The American Naturalist 123: 338–353.

Knox AG, Walters MP 1994. Extinct and endangered birds in the collections of the Natural History Museum. British Ornithologists Club Occasional Publications, UK.

Korsten M, Ho SYW, Davidson J, Pahn D, Wulla E, Roht M, Tumanov IL, Kojola I, Andersone-Lilley Z, Ozolins J, Pilot M, Mertzanis Y, Giannakopoulos A, Vorobiev AA, Markov NI,

Saveljev A, Yapunova EA, Abramov AV, Mannil P, Valdmann H, Pazetnov SV, Pazetnov VS, Rokov AM, Saarma U 2009. Sudden expansion of a single brown bear maternal lineage across northern continental Eurasia after the last ice age: a general demographic model for mammals? Molecular Ecology 18: 1963-1979.

Kuch M, Rohland N, Betancourt JL, Latorre C, Steppan C, Poinar HN 2002. Molecular analysis of a 11,700-year-old rodent midden from the Atacama Desert, Chile. Molecular Ecology 11: 913-924.

Kuhn TS, McFarlane KA, Groves P, Mooers AO, Shapiro B 2010. Modern and ancient DNA reveal recent partial replacement of caribou in the southwest Yukon. Molecular Ecology 19: 1312-1323.

Lambert DM, Baker A, Huynen L, Haddrath O, Herbert PDN, Miller CD 2005. Is a large-scale DNA-based inventory of ancient life possible? Journal of Hereditary 96: 279-284.

Lambert DM, Ritchie PA, Miller CD, Holland B, Drummond AJ, Baroni C 2002. Rates of evolution in ancient DNA from Adelie penguins. Science 295: 2270-2273.

Lee WG, Wood JR, Rogers GM 2010. Legacy of avian-dominated plant-herbivore systems in New Zealand. New Zealand Journal of Zoology 34: 28-47.

Lemey P, Rambaut A, Drummond AJ, Suchard MA 2009. Bayesian phylogeography finds its roots. PLoS Computational Biology 5(9): e1000520.

Lemey P, Rambaut A, Pybus OG 2006. HIV evolutionary dynamics within and among hosts. AIDS Reviews 8: 124-40.

Leonard JA, Wayne RA, Cooper A 2000. Population genetics of ice age brown bears. Proceedings of the National Academy of Science USA 97: 1651-1654.

Liebig PM, Flessa KW, Taylor TSA 2007. Taphonomic variation despite catastrophic mortality: Analysis of a mass stranding of false killer whales (*Pseudorca crassidens*), Gulf of California, Mexico. Palaios 22: 384-391.

Li Qm Gao KQ, Vinther J, Shawkey MD, Clarke JA, Alba LD, Meng Q, Briggs DEG, Prum RO 2010. Plumage colour patterns of an extinct dinosaur. Science 327: 1369-1372.

Lydolph M, Jacobsen J, Arctander P, Gilbert M, Gilichinsky D, Hansen A, Willerslev E, Lange L 2005. Beringian paleoecology inferred from permafrost-preserved fungal DNA. Applied Environmental Microbiology 71:1012–1017

Lyman RL 1994. Vertebrate Taphonomy. Cambridge University Press, Cambridge, UK.

McDonald JN 1981. North American bison, their classification and evolution. University of California Press, Berkeley, USA.

McGlone MS, Webb CJ 1981. Selective forces influencing the evolution of divaricating plants. New Zealand Journal of Ecology 4: 20-28.

McKinney ML 1997. Extinction vulnerability and selectivity: Combining ecological and palaeontological views. Annual Review of Ecology and Systematics 28: 495-516.

MacPhee RDE 1999. Extinctions in Near Time: Causes, Contexts and Consequences. Kluwer Academic/Plenum, New York.

Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen YJ, Chen Z, Dewell SB, Du L, Fierro JM, Gomes XV, Goodwin BC, He W, Helgesen S, Ho CH, Irzyk GP, Jando SC, Alenquer MLI, Jarvie JP, Jirage KB, Kim JB, Knight JR, Lanza JR, Leamon JH, Lefkowitz SM, Lei M, Li J, Lohman KL, Lu H, Makhijani VB, McDade KE, McKenna MP, Myers EW, Nickerson E, Nobile JR, Plant R, Puc BP, Ronan MT, Roth GT, Sarkis GJ, Simons JF, Simpson JW, Srinivasan M, Tartaro KR, Tomasz A, Vogt KA, Volkmer GA, Wang SH, Wang Y, Weiner MP, Yu P, Begley RF, Rothberg JM 2005. Genome sequencing in open micro fabricated high density picoliter reactors. Nature 437: 376-380.

Marlowe K, Hufford L 2008. Evolution of *Synthyris* sect. *Dissecta* (Plantaginaceae) on sky islands in the Northern Rocky Mountains. American Journal of Botany 95: 381-392.

Marske KA, Leschen RAB, Barker GM, Buckley TR 2009. Phylogeography and ecological niche modelling implicate coastal refugia and trans-alpine dispersal of a New Zealand fungus beetle. Molecular Ecology 18: 5126-5142.

Martin PS 1984. Prehistoric overkill: A global model. In: Martin PS, Klein RG (Eds) Quaternary Extinctions: A prehistoric revolution. University of Arizona Press, Tuscon, USA, pp 354-403.

Martin PS 2005. Twilight of the mammoths: Ice age extinctions and the rewilding of America. University of California Press, Berkeley, USA.

Medlin GC 1993. Field guide to Chambers Gorge, Flinders Ranges. South Australian Museum, Adelaide, Australia.

Miller CD, Dodd A, Anderson J, Gibb GC, Ritchie PA, Baroni C, Woodhams MD, Hendy MD, Lambert DM 2008. Mutation and evolutionary rates in Adelie penguins from the Antarctic. PLoS Genetics 4: e10000209.

Miller GH, Fogel ML, Magee JW, Gagan MK, Clarke SJ, Johnson BJ (2005) Ecosystem collapse in Pleistocene Australia and a human role in megafaunal extinction. Science 309: 287-290.

Miller GH, Magee JW, Johnson BJ, Fogel ML, Spooner NA, McCulloch MT, Ayliffe LK 1999. Pleistocene extinction of *Genyornis newtoni*: Human impact on Australian megafauna. Science 283: 205-208.

Minin VN, Bloomquist EW, Suchard MA 2008. Smooth skyride through a rough skyline: Bayesian coalescent-based inference of population dynamics. Molecular Biology and Evolution 25: 1459-1471.

Moar NT 1971. Contributions to the Quaternary history of the New Zealand flora 6: Aranuiun pollen diagrams from Canterbury, Nelson, and North Westland, South Island. New Zealand Journal of Botany 9: 80-145.

Moar NT 2008. Late Quaternary Vegetation. In: Winterbourn M, Knox G, Burrows C, Marsden I (Eds) 2008. The Natural History of Canterbury. Christchurch, Canterbury University Press.

Mona S, Catalano G, Lari M, Larson G, Boscato P, Casoli A, Sineo L, Patti CD, Pecchioli D, Caramelli D, Bertorelle G 2010. Population dynamics of the extinct European aurochs: genetic evidence of a north-south differentiation pattern and no evidence of post-glacial expansion. BMC Evolutionary Biology 10: 83.

Munday PL 2004. Habitat loss, resource specialisation, and extinction on coral reefs. Global Change Biology 10: 1642-1647.

Munoz-Fuentes V, Green AJ, Negro JJ, Sorenson MD 2005. Population structure and loss of genetic diversity in the endangered white-headed duck, *Oxyura leucocephala*. Conservation Genetics 6: 999-1015.

Murray P, Vickers-Rich P 2003. Magnificent mihirungs: The colossal flightless birds of the Australian dreamtime. USA: Indiana University Press.

Nelson G 1975. Book Review: Kuschel G (Ed) 1975, Biogeography and ecology of New Zealand. W. Junk, The Hague. Systematic Zoology 24, 494-495.

Oliver WRB 1949. The moas of New Zealand and Australia. Wellington, Dominion Museum.

Oliver WRB 1955. New Zealand birds, 2nd edn. Wellington, New Zealand: Reed.

Orlando L, Metcalf J, Alberdi MT, Telles-Antunes M, Bonhean D, Otte M, Martin F, Eisenmann V, Mashkour M, Morello F, Prado JL, Salas-Gismondi R, Shockey BJ, Wrinn PJ, Vasil-ev SK, Ovodov ND, Cherry MI, Hopwood B, Male D, Austin JJ, Hanni C, Cooper A 2009. Revising the recent evolutionary history of equids using ancient DNA. Proceedings of the National Academy of Science USA 106: 21754-21759.

Oskam CL, Haile J, McLay E, Ribgy P, Allentoft ME, Olsen ME, Bengtsson C, Miller GH, Schwenninger JL, Jacomb C, Walter R, Baynes A, Dortch J, Parker-Pearson M, Gilbert MTP, Holdaway RN, Willerslev E, Bunce M 2010. Fossil avian eggshell preserves ancient DNA. Proceedings of the Royal Society of London Series B 277: 1991-2000.

Owens IPF, Bennett PM 2000. Ecological basis of extinction in birds: habitat loss versus human persecution and introduced predators. Proceedings of the National Academy of Science USA 97: 12144-12148.

Payne RB, Sorenson MD 2002. Museum collections as sources of genetic data. Bonner Zoologische Beitrage 51: 97-104.

Pearson SG, Baynes A, Triggs BE 2001. The record of fauna, and accumulating agents of hair and bone, found in middens of stick-nest rates (Genus *Leporillus*) (Rodentia: Muridae). Wildlife Research 28: 435-444.

Pilot M, Branicki W, Jedrzejewski W, Goszczynski J, Jedrzejewska B, Dykyy I, Shkvyrya M, Tsingarska E 2010. Phylogeographic history of grey wolves in Europe. BMC Evolutionary Biology 10: 104.

Poinar HN, Hofreiter M, Spaulding WG, Martin PS, Stankiewicz BA, Bland H, Evershed RP, Possnert G, Paabo S 1998. Molecular coproscopy: Dung and diet of the extinct ground sloth *Nothrotheriops shastensis*. Science 218: 402–406.

Prost S, Smirnov N, Fedorov VB, Sommer RS, Stiller M, Nagel D, Knapp M, Hofreiter M 2010. Influence of climate warming on Arctic mammals? New insights from ancient DNA studies of the Collared Lemming *Dicrostonyx torquatus*. PLoS One 5: e10447.

Rambaut A, Pybus OG, Nelson MI, Viboud C, Taubenberger JK, Holmes EC 2008. The genomic and epidemiological dynamics of human influenza A virus. Nature 453: 615-619.

Rambaut A, Ho SYW, Drummond AJ, Shapiro B 2009. Accommodating the effect of ancient DNA damage of inferences of demographic histories. Molecular Biology and Evolution 26: 245-248.

Rawlence NJ, Wood JA, Armstrong KN, Cooper A 2009. DNA content and distribution in ancient feathers and potential to reconstruct the plumage of extinct avian taxa. Proceedings of the Royal Society of London Series B 276: 3395-3402.

Ritchie PA, Miller CD, Gibb G, Baroni C, Lambert DM 2004. Ancient DNA enables the timing of the Pleistocene origin and Holocene expansion of two Adelie penguin lineages in Antarctica. Molecular Biology and Evolution 21: 240-248.

Rogers RR 1990. Taphonomy of three dinosaur bone beds in the upper Cretaceous Two Medicine Formation of Northwestern Montana: Evidence for drought-related mortality. Palaios 5: 394-413.

Rohland N, Pollack JL, Nagel D, Beauval C, Airvaux J, Paabo S, Hofreiter M 2005. The population history of extant and extinct hyenas. Molecular Biology and Evolution 22: 2435-2443.

Ryan MJ, Russel AP, Eberth DA, Currie PJ 2001. The taphonomy of a *Centrosaurus* (Ornithischia: Certopsidae) bone bed from the Dinosaur Park Formation (Upper Campanian), Alberta, Canada, with comments on cranial ontogeny. Palaios 16: 482-506.

Scott E 2009. Extinctions, scenarios, and assumptions: Changes in latest Pleistocene large herbivore abundance and distribution in western North America. Quaternary International, 217: 225-239.

Sefc KM, Payne RB, Sorenson MD 2003. Microsatellite amplification from museum feather samples: Effects of fragment size and template concentration on genotyping errors. Auk 120: 982-989.

Shapiro B, Drummond AJ, Rambaut A, Wilson MC, Matheus PE, Sher AV, Pybus OG, Gilbert MTP, Barnes I, Binladen J, Willerslev E, Hansen AJ, Baryshnikov GF, Burns JA, Davydov

S, Driver JC, Froese DG, Harington CR, Keddie G, Kosintsev P, Kunz ML, Martin LD, Stephenson RO, Storer J, Tedford R, Zimov S, Cooper A 2004. Rise and fall of the Beringian Steppe Bison. Science 306: 1561-1565.

Shepherd LD, Miller CD, Ballard G, Ainley DG, Wilson PR, Haynes GD, Baroni C, Lambert DM 2005. Microevolution and mega-icebergs in the Antarctic. Proceedings of the National Academy of Science USA 102: 16717-16722.

Spencer LM, Van Valkenburgh B, Harris JM 2003. Taphonomic analysis of large mammals recovered from the Pleistocene Rancho La Brea tar seeps. Paleobiology 29: 561-575.

Stiller M, Baryshnikov G, Bocherens H, d'Anglade G, Hilpert B, Munzel SC, Pinhasi R, Rabeder G, Rosendahl W, Trinkaus E, Hofreiter M, Knapp M 2010. Withering away – 25, 000 years of genetic decline preceded cave bear extinction. Molecular Biology and Evolution 27: 975-978.

Suchard MA, Weiss RE, Sinsheimer JS 2001. Bayesian selection of continuous-time Markov chain evolutionary models. Molecular Biology and Evolution 18: 1001-1013.

Tenesa A, Navarro P, Hayes BJ, Duffy DL, Clarke GM, Goddard ME, Visscher PM 2007. Recent human effective population size estimated from linkage disequilibrium. Genome Research 17: 520-526.

Tennyson A, Martinson P 2006. Extinct birds of New Zealand. Te Papa Press, Wellington, New Zealand.

Thomas CD, Cameron A, Green RE, Bakkenes M, Beaumont LJ, Collingham TC, Erasmus BFN, Siqueira MF, Grainger A, Hannah L, Hughes L, Huntley B, Jaarsveld AS, Midgley GF, Miles L, Ortega-Huerta MA, Perterson AT, Phillips OL, Williams SE 2004. Extinction risk from climate change. Nature 427: 145-148.

Tiffney BH 2004. Vertebrate dispersal of seed plants through time. Annual review of ecology, evolution and systematics 35: 1-29.

Tores M, Motro Y, Motro U, Yom-Tov Y 2005. The barn owl – a selective opportunist predator. Israel Journal of Zoology 51: 349-360.

Tregear E 1893. The Extinction of the Moa. Transactions and Proceedings of the New Zealand Institute, 25: 413-426.

Vickers-Rich P, van Tets GF, Knight F 1985. Kadimakara: Extinct vertebrates of Australia. USA: Princeton University Press.

Vickers-Rich P, Trusler P, Rowley MJ, Cooper A, Chambers GK, Bock WJ, Millener PR, Worthy TH, Yaldwyn JC 1995. Morphology, myology, collagen and DNA of a mummified moa, *Megalapteryx didinus* (Aves: Dinornithiformes) from New Zealand. Tuhinga Records of the Museum of New Zealand Te Papa Tongarewa 4: 1–26.

Vinther J, Briggs DEG, Clarke J, Mayr G, Prum RO 2009. Structural colouration in a fossil feather. Biology Letters 6: 128-131.

Vinther J, Briggs DEG, Prum RO, Saranathan V 2008. The colour of fossil feathers. Biology Letters 4: 522–525.

Voorhies MR 1969. Taphonomy and population dynamics of an Early Pliocene vertebrate fauna, Knox County, Nebraska. University of Wyoming Contributions to Geology, Special Paper no. 1: 1-69.

Weinstock J, Shapiro B, Preito A, Marin JC, Gonzalez BA, Gilbert MTP, Willerslev E 2009. The Late Pleistocene distribution of vicunas (*Vicugna vicugna*) and the 'extinction' of the gracile llama ('*Lama gracilis*'): New molecular data. Quaternary Science Reviews 28: 1269-1373.

Willerslev E, Hansen AJ, Binladen J, Brand TB, Gilbert MTP, Shapiro B, Bunce M, Wiuf C, Gilichinsky DA, Cooper A 2003. Diverse plant and animal genetic records from Holocene and Pleistocene sediments. Science 300: 791-795.

Williams PW, King DNT, Zhao JX, Collerson KD 2005. Late Pleistocene to Holocene composite speleotherm ^{18}O and ^{13}C chronologies from South Island, New Zealand – did a global Younger Dryas really exist? Earth and Planetary Science Letters 230: 301-317.

Wilmshurst JM, Anderson AJ, Higham TFG, Worthy TH 2008. Dating the late prehistoric dispersal of Polynesians to New Zealand using the commensal Pacific rat. Proceedings of the National Academy of Science USA 105: 7676-7680.

Wilson KJ 2004. Flight of the Huia: Ecology and conservation of New Zealand's frogs, reptiles, birds and mammals. Canterbury University Press, Christchurch, New Zealand.

Wood JR 2006. Subfossil kakapo (*Strigops habroptilus*) remains from near Gibraltar Rock, Cromwell Gorge, Central Otago, New Zealand. Notornis 53: 191-193.

Wood JR 2007. Moa gizzard content analyses: further information on the diets of *Dinornis robustus* and *Emeus crassus*, and the first evidence for the diet of *Pachyornis elephantopus* (Aves: Dinornithiformes). Records of the Canterbury Museum 21: 27–39.

Wood JR, Rawlence NJ, Rogers GM, Austin JJ, Worthy TH, Cooper A 2008a. Coprolite deposits reveal the diet and ecology of the extinct New Zealand megaherbivore moa (Aves: Dinornithiformes). Quaternary Science Reviews 27: 2593-2602.

Wood JR, Worthy TH, Rawlence NJ, Jones SM, Read SE 2008b. A deposition mechanism for Holocene miring bone deposits, South Island, New Zealand. Journal of Taphonomy, 6: 1-20

Worthy TH 1989. Mummified moa remains from Mt Owen, northwest Nelson. Notornis 36: 36–38.

Worthy TH 1993a. A review of fossil bird bones from loess deposits in eastern South Island, New Zealand. Records of the Canterbury Museum 10: 95-106.

Worthy TH 1993b. Fossils of Honeycomb Hill. Museum of New Zealand Te Papa Tongawera, Wellington, New Zealand.

Worthy TH 1994. Late Quaternary changes in the moa fauna on the West Coast of the South Island, New Zealand. Records of the South Australian Museum 27: 125-134.

Worthy TH 1997a. Quaternary fossil fauna of South Canterbury, South Island, New Zealand. Journal of the Royal Society of New Zealand 27: 67-162.

.Worthy TH 1997b. Fossil deposits in the Hodges Creek Cave System, on the northern foothills of Mount Arthur, Nelson, South Island, New Zealand. Notornis 44: 111-124.

Worthy TH 1998a. Quaternary fossil faunas of Otago, South Island, New Zealand. Journal of the Royal Society of New Zealand 28: 421-521.

Worthy TH 1998b. The Quaternary fossil avifauna of Southland, South Island, New Zealand. Journal of the Royal Society of New Zealand 28: 537-589.

Worthy TH 1999 Changes induced in the New Zealand avifauna by climate during the last glacial-interglacial period. Proceedings of the 4th International Meeting of the Society of Avian Paleontology and Evolution (Washington, D.C., June 1996). Smithsonian Contributions to Palaeobiology 89: 111-123.

Worthy TH, Grant-Mackie JA 2003. Late-Pleistocene avifaunas from Cape Wanbrow, Otago, South Island, New Zealand. Journal of the Royal Society of New Zealand 33: 427-485.

Worthy TH, Holdaway RN 1994. Quaternary fossil faunas from caves in Takaka valley and on Takaka Hill, northwest Nelson, South Island, New Zealand. Journal of the Royal Society of New Zealand 24: 297-391.

Worthy TH, Holdaway RN 1995. Quaternary fossil faunas from caves on Mt Cookson, North Canterbury, New Zealand. Journal of the Royal Society of New Zealand 25: 333-370.

Worthy TH, Holdaway RN 1996. Quaternary fossil faunas, overlapping taphonomies, and palaeofaunal reconstruction in North Canterbury, South Island, New Zealand. Journal of the Royal Society of New Zealand 26: 275-361.

Worthy TH, Holdaway RN 2002. The lost world of the moa: Prehistoric life of New Zealand. Canterbury University Press, Christchurch, New Zealand.

Worthy TH, Roscoe D 2003. Takaka Fossil Cave – a stratified Late Glacial to Late Holocene deposit from Takaka Hill, New Zealand. Tihinga 14: 41-60.

Zhang F, Kearns SL, Orr PJ, Benton MJ, Zhou Z, Johnson D, Xu X, Wang X 2010. Fossilised melanosomes and the colour of Cretaceous dinosaurs and birds. Nature 463: 1075-1078.

APPENDIX ONE

A rough guide to BEAST 1.4

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Unpublished user manual. Available for download from http://beast.bio.ed.ac.uk/.

APPENDIX TWO

Indo-European and Asian origins for Chilean and Pacific chickens revealed by mtDNA

and

Reply to Storey *et al*.: More DNA and dating studies needed for ancient El-Arenal-1 chickens

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Indo-European and Asian origins for Chilean and Pacific chickens revealed by mtDNA

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European chickens were introduced into the American continents by the Spanish after their arrival in the 15th century. However, there is ongoing debate as to the presence of pre-Columbian chickens among Amerindians in South America, particularly in relation to Chilean breeds such as the Araucana and Passion Fowl. To understand the origin of these populations, we have generated partial mitochondrial DNA control region sequences from 41 native Chilean specimens and compared them with a previously generated database of ≈1,000 domestic chicken sequences from across the world as well as published Chilean and Polynesian ancient DNA sequences. The modern Chilean sequences cluster closely with haplotypes predominantly distributed among European, Indian subcontinental, and Southeast Asian chickens, consistent with a European genetic origin. A published, apparently pre-Columbian, Chilean specimen and six pre-European Polynesian specimens also cluster with the same European/Indian subcontinental/Southeast Asian sequences, providing no support for a Polynesian introduction of chickens to South America. In contrast, sequences from two archaeological sites on Easter Island group with an uncommon haplogroup from Indonesia, Japan, and China and may represent a genetic signature of an early Polynesian dispersal. Modeling of the potential marine carbon contribution to the Chilean archaeological specimen casts further doubt on claims for pre-Columbian chickens, and definitive proof will require further analyses of ancient DNA sequences and radiocarbon and stable isotope data from archaeological excavations within both Chile and Polynesia.

Araucana chicken | *Gallus gallus* | pre-Columbian chicken | control region

The presence of chickens in the American continents has traditionally been attributed to the Spanish introduction of European chickens after their arrival in the 15th century. However, there is still debate about whether Amerindians in South America possessed chickens before the arrival of the Spanish, particularly in relation to several unusual Chilean breeds. For example, the Araucana breed is thought to be descended from indigenous Amerindian chickens and is unusual in that it lays blue/green-shelled eggs and possesses distinctive plumage patterns. Three groups or variants of Araucana are currently recognized: ketros (ear-tufted and normal tailed), kolloncas (nontufted and rumpless), and kollonca de aretes (ear-tufted and rumpless). Because these features are also found among Asian rather than Mediterranean chickens, it has been hypothesized that the Araucana breed might have an Asian origin. A similar origin has been suggested for another historic Chilean breed, the Passion Fowl.

Potential sources for the pre-Columbian introduction of chickens include early Polynesian or Dutch traders on the Pacific coast of South America (1–3). However, there has been no detailed genetic analysis of these so-called "Amerindian" chickens to investigate the potential contribution of Asian or European breeds, although a short mitochondrial DNA control region (mtDNA CR) sequence was recently reported from two feathers of potential Araucana origin along with a Chilean archaeological specimen (El Arenal-1) apparently dating to a time immediately preceding the Columbian period Cal AD 1304–1424 (4). The latter sequence was linked to other chicken sequences from several Polynesian archaeological sites in an analysis that included only a limited number of other comparative sequences and was used as support of pre-Columbian trading contact between Polynesians and South American Amerindians.

A previous phylogenetic study of the chicken mtDNA CR suggested that the Indochinese Red Junglefowl subspecies *Gallus gallus gallus* is the primary maternal ancestor of the domestic chicken (*Gallus gallus domesticus*) and that Southeast Asia was a likely center of domestication (5). A subsequent study analyzed an expanded mtDNA CR dataset in which nine divergent haplogroups were identified, seven of which consisted of both domestic chicken and Red Junglefowl subspecies (6). In contrast to the earlier study, this pattern suggested multiple centers of domestication across both Southeast Asia and the Indian subcontinent, consistent with archaeological studies (7, 8).

Three of the nine haplogroups or clusters [A, B, and E of Liu *et al.* (6)] contain mtDNA CR haplotypes from all over Eurasia, whereas the other haplogroups contain haplotypes mainly from southern and Southeast Asia. Haplogroups A, B, C, and E are relevant for this study because they contain the ancient and modern Chilean chicken sequences or provide insights into the mtDNA signatures of ancient Pacific chickens. Haplogroup E is predominant among Indian, Middle Eastern, and European chickens and is an indication that the roots of European chickens lie within the Indian subcontinent. The primary haplotype of this haplogroup, E1, is the single most-common chicken haplotype found around the world. Haplogroups A and B predominate among South and eastern Chinese and Japanese chickens as well as wild Red Junglefowl. Consequently, although chicken

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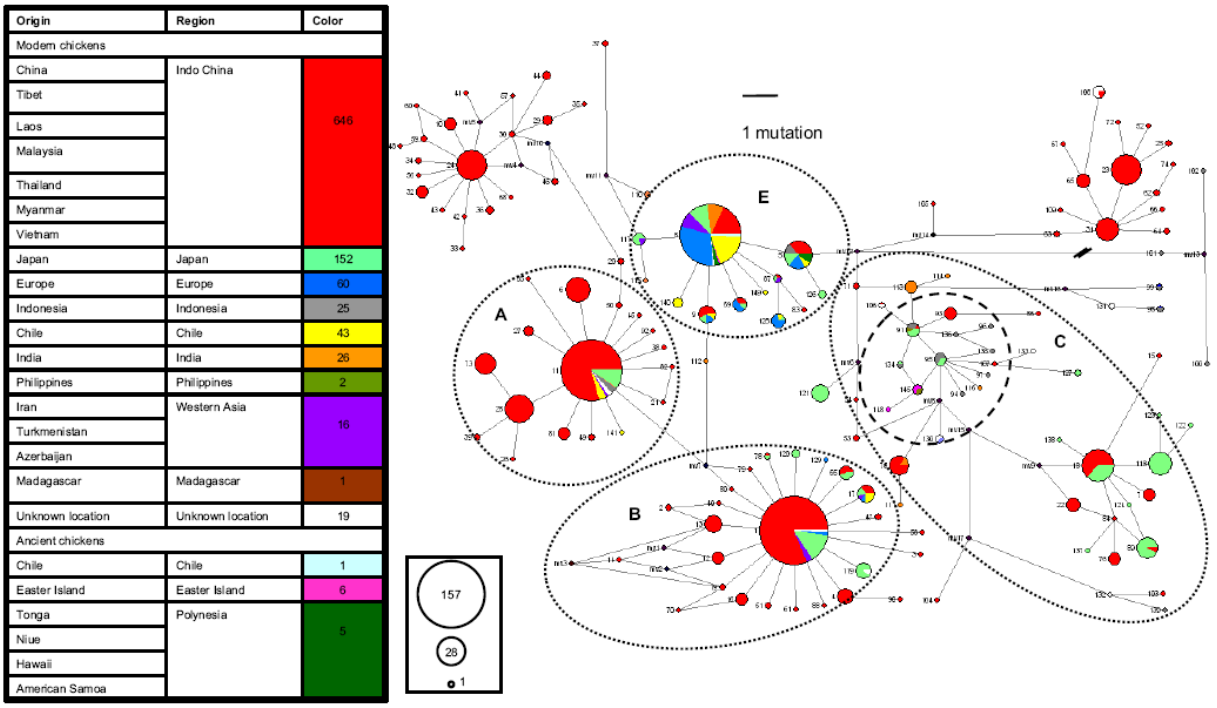

Fig. 1. MJNs showing the relationships and clustering the mtDNA CR (205 bp) from worldwide, Chilean Araucana, pre-Columbian, and ancient Pacific/Polynesian chickens. Haplotype numbers are shown next to nodes, the geographical locations of samples are given in color, and node size is proportional to the frequency of the corresponding haplotypes as shown in the circles with numbers on the left. Branch lengths are proportional to the number of mutations except for two: the branch leading from median vector (mv) 12 to haplotype 101 (17 mutations) and the branch leading from mv 3 to haplotypes 13 and 19 (one mutation), for visual clarity. A, B, C, and E correspond to the major haplogroups identified by Liu *et al.* (6). Most of the modern and ancient Chilean chickens analyzed in this study cluster with Indian subcontinental/European/Chinese chickens (nodes 5, 8, 9, 128, and 140), with the remainder clustering with nodes predominant in South and eastern Chinese/Japanese/Indonesian chickens (nodes 11, 17, and 141). The pre-Columbian Chilean sequence falls within haplotype 8, which is the single most common chicken haplotype found around the world. Six ancient Polynesian sequences (Tonga Mele Havea and Ha'ateiho, Samoa Fatuma Futi, Hawaii Kualoa, Niue Paluki, and one from Easter Island) also cluster within the same haplogroup. Five of the ancient chicken sequences from Easter Island cluster with haplotypes 145 and 148 (dashed circle within haplogroup C), which are part of an uncommon group comprising mostly Indonesian chickens. The Easter Island sequences may represent mtDNA signatures of early Polynesian chicken transport.

mtDNA sequences do not show the strict phylogeographic patterns observed in other domestic animals in the region, such as pigs (9), the majority of haplogroups do exhibit some form of restricted distribution on subcontinental scales. Specific haplotypes within these haplogroups however, such as E1 and A1 (equivalent to haplotypes 8 and 11, respectively, in Fig. 1) are present at high frequencies within chicken breeds and populations all over the world. Haplogroup C is found mainly in Japanese and southeastern Chinese chickens (6) and contains a subcluster of southern Japanese natives from the island of Okinawa found to be closely related to Indonesian native chickens (10).

To further investigate the potential Polynesian–Chilean genetic links, we generated mtDNA CR sequences from 41 modern Chilean chickens and analyzed their phylogenetic relationships with a large database of ≈1,000 sequences, including those described by Liu *et al.* (6) and available in ancient mtDNA CR sequences from Chile and the Pacific islands (4). In addition, we modeled the potential impact of marine carbon contribution on the calibrated age of the pre-Columbian chicken bone from El Arenal-1, an analysis not undertaken by Storey *et al.* (4). It is important to note that this specimen was from a near-coastal site and dated only 70 years before initial Spanish contact and that samples including material derived from marine environments can exhibit older apparent radiocarbon ages because of the uptake of carbon that has already undergone radioactive decay through long residence times in the deep ocean. Studies of marine reservoir effects (ΔR) for Chile are limited, so we calibrated the age under several scenarios of marine carbon input.

Results

Phylogenetic Clustering of Modern Chilean Chickens. Seventeen polymorphic sites and eight haplotypes were identified among the modern Chilean chicken 530-bp CR sequences [supporting information (SI) Figs. S1 and S2], with 16 of these sites falling within the 205-bp region (Fig. S3). In contrast, Storey *et al.* (4) report only nine polymorphic sites within ancient and modern sequences across the same region because of the very limited number of sequences analyzed. Chilean haplotypes were named by using the haplotype numbers of the median-joining network (MJN, Fig. 1) analysis of the 205-bp sequence alignment (Fig. S3), followed by "CH" for Chile, as follows: 5-CH (number of samples, $n = 2$), 8-CH ($n = 25$), 9-CH ($n = 1$), 11-CH ($n = 5$), 17-CH ($n = 2$),128-CH ($n = 1$), 140-CH ($n = 4$), and 141-CH ($n = 1$). Araucana chickens ($n = 28$) possess seven of the eight observed haplotypes, indicating a high genetic diversity, whereas in contrast, the same single haplotype (8-CH) observed in 15 of these Araucana specimens is also shared by Passion Fowl ($n = 4$), Creole ($n = 5$), and Chilean/Japanese Long Tail ($n = 1$) (Table S1). MJN analyses (Fig. 1) show that five of the Chilean chicken haplotypes (5-CH, the very common 8-CH, 9-CH,

Table 1. Effect of increasing proportions of marine-derived carbon on calibration of directly dated El Arenal-1 chicken bone

ΔR	Marine C, %	Calibration dataset	Radiocarbon on chicken bones from the El Arenal-1 (n =622 ± 35)
0 ± 0	0	SHCal04	**AD1304–1424**
61 ± 50*	10	Mixed marine SoHem	**AD1322–1445**
61 ± 50	20	Mixed marine SoHem	**AD1395–1459**
61 ± 50	30	Mixed marine SoHem	AD1407–1501
61 ± 50	40	Mixed marine SoHem	AD1430–1619
61 ± 50	50	Mixed marine SoHem	AD1455–1630

*ΔR of 61 ± 50 based on single bivalve determination from Valparaiso, Chile (12). This value is considered a conservative estimate of depletion in these waters. All calibrated dates are reported at 2σ. Bold type denotes pre-Columbian values.

128-CH, and 140-CH) cluster within a geographically widespread major haplogroup consisting of European, Middle Eastern, Indian, and Chinese domestic chickens (equivalent to haplogroup E, Liu *et al.*) (6). Of the other three Chilean haplotypes, 11-CH and 141-CH are found mainly in domestic chickens from South and eastern China, including wild Red Junglefowl and some Japanese and Indonesian chickens. The last haplotype, 17-CH, recovered from one Araucana and one creole chicken, is a sequence found mainly in Chinese and Japanese chickens. One of the two modern Araucana feather sequences generated by Storey *et al.* (4) also belonged to haplotype 17. The other feather sequence corresponds to a unique haplotype 149. However, there was insufficient information to assess whether these two specimens indeed originated from the Araucana breed.

Maximum parsimony (MP) and MJN analyses gave similar topologies [Fig. 1, and Figs. S4–S7], with minor differences from Liu *et al.* (6) because of the different phylogenetic approaches and because several sequences collapsed into single haplotypes within the shorter (205 bp) alignment.

Phylogenetic Position of Archaeological Pacific and Pre-Columbian Chickens. The single ancient sequences reported from Tonga Mele Havea and Ha'ateiho, Samoa Fatuma Futi, Hawaii Kualoa, Niue Paluki, one sequence from Easter Island Anakena (early settlement phase Cal AD 1270–1400), and the putatively pre-Columbian (Cal AD 1304–1424) Chilean El Arenal-1 sequence belong to haplotypes 8 and 5 within haplogroup E (Fig. 1). Haplotype 8 equates to the E1 haplotype reported in Liu *et al.* (6), which is ubiquitous worldwide and 100% identical to haplotypes from European Barred Plymouth Rock, White Plymouth Rock, White Leghorn. and New Hampshire as well as native chicken sequences from Africa, India, Central Asia, and China. In contrast, five of the other contemporaneous archaeological chicken sequences from Easter Island cluster with haplotypes 145 (n = 4) and 148 (n = 1), which are part of an uncommon group comprising mostly Indonesian chickens within haplogroup C (Fig. 1). Ancient Easter Island haplotype 145 is identical to one sequence of Red Junglefowl from the Philippines (11). Within other modern chickens, the closest related sequences have been recorded from Lombok and Java in Indonesia and the Philippines. Given their unique phylogenetic position and their pre-European contact dates, haplotypes 145 and 148 presumably represent a record of early Polynesian chicken transport, potentially overwritten subsequently in the western Pacific. The noticeably less star-like pattern of haplogroup C, centered on the less frequent haplotypes 91 and 95, is likely to be an artifact of incomplete sampling or a different population history.

Marine Carbon Contribution to Calibrate the Date of a Chicken Bone from El Arenal-1. The directly dated chicken bone from El Arenal-1 reported by Storey *et al.* (4) was calibrated by using different estimates of marine reservoir effects for the Chilean coast (12–14). A conservative value of ΔR = 61 ± 50 and a slightly more depleted ΔR = 154 ± 131 (which represents the weighted mean of the ΔR = 61 ± 50 from Valparaiso and ΔR = 243 ± 49 from northern Peru) were used to calibrate the date (15, 16), along with different proportions of marine-derived carbon. When either ΔR value is used, the carbon content would need to be <20% derived from marine pathways to securely date it before AD 1500 (Table 1 and Table S2). However, a marine carbon contribution of >20% would be consistent with a post-Columbian date.

Discussion

mtDNA Signatures Do Not Support a Polynesian Origin for Pre-Columbian Chicken. The position of the single Chilean pre-Columbian chicken sequence within the worldwide distributed haplotype 8 removes any genetic support for a Polynesian introduction of this haplotype to South America. This haplotype is likely to have been common in chickens introduced by the Spanish in the 15th century as well as European breeds transported during subsequent colonial era trade.

Five of the six archaeological Easter Island sequences are part of an uncommon, closely related cluster of haplotypes within haplogroup C, which is centered in the Indonesian islands (eastern and western Java, West Sumatra, and Lombok Island) with a few representatives in Japan and the Philippines, but which has not yet been detected elsewhere in Polynesia (4). The ancient Easter Island specimens are clearly pre-European according to both radiocarbon dating and stratigraphy, indicating that this haplotype must form part of the original "Polynesian/Pacific" chicken dispersal, which has potentially been erased across the western Polynesian islands by replacement or introgression by haplotypes 5 and 8 (Fig. 1). Because Easter Island is commonly suggested as the key site to facilitate contact between Polynesian and South American cultures, it is significant that the common Easter Island haplotypes do not appear to have reached South America or have not survived into the modern day if they did so.

This scenario is analogous to the history of domestic pigs in Europe (17) and bottle gourds in the New World (18). In the first study, Neolithic domestic pigs in Europe were shown to be derived from Near Eastern wild boar, but were subsequently replaced by domestic pigs derived from European wild boar (17). In the case of the bottle gourd, although all modern American samples demonstrated a genetic affinity with Africa (19), DNA from ancient samples revealed that the first bottle gourds to enter the Americas did so, not across the Atlantic, but via Asia (18). This shifting temporal pattern indicates that the original lineages of domestic plants and animals introduced into a new area can often be replaced by later introductions of the same domestic species with a different genetic heritage, thus erasing

the evidence of the initial dispersal. Depending on the degree to which the legacy of the initial introductions has been erased, studies of modern samples may be capable of detecting only the most recent introduction.

In this context, archaeological sequences from Chile, Hawaii, Niue, Tonga, and American Samoa fall within at least two of the widespread European/Indian subcontinental/Chinese haplogroups, suggesting that at least four haplotypes (Fig. 1, haplotypes 5, 8, 145, and 148) can be considered part of Pacific/Polynesian chicken dispersals. It is possible that these dispersals were not contemporaneous, in which case the European/Indian subcontinental/Chinese haplotypes may have formed a more recent wave of dispersals, overwriting and removing the earlier Indonesian sequences across western Polynesia but failing to do the same in distant Easter Island. However, it is also important to note that standard European/Indian subcontinental/Chinese haplotypes such as 8 would result if either the specimens or laboratory procedures were contaminated with any amount of modern chicken DNA. The contamination of laboratory consumables is a well known problem when working with ancient samples of domestic species (20), which tend to be widespread but not genetically diverse.

Marine Carbon Input and the Presence of Pre-Columbian Chickens. As a consequence of the phylogenetic analyses, the evidence for pre-Columbian chickens in South America depends on the quality of the radiocarbon dates and calibration of the El Arenal-l specimen (Cal AD 1304–1424) reported in Storey *et al.* (4). Importantly, this date was not corrected for possible marine carbon offset, presumably because of the high $\delta^{13}C$ value (21%) (4). Although it is known that $\delta^{13}C$ values for chickens, like other birds, are strongly controlled by diet, the relationship between carbon isotopic fractionation and the metabolic process in chickens is poorly understood (21, 22), masking the potential contributions of marine and terrestrial sources to the isotopic signature of chicken bones (23). As omnivores, chickens consume a wide range of materials available in their local environment, including fish, shellfish, and other marine-derived materials in coastal areas. Marine shell grit, for example, is often ingested by chickens and other birds to assist digestion and provide minerals to the diet (24, 25). In the case of El Arenal-1, consumption of marine foods is especially likely because of the close proximity of the coast (<3 km) and the apparent stratigraphic association of recovered chicken remains with marine shellfish, fish, and marine birds, suggesting the ready availability of materials deriving from marine environments to the chicken diet (26).

On average, the surface ocean reservoir (<200 m) has an apparent ^{14}C age ≈400 years older than the atmospheric reservoir (27). Variation in ^{14}C activity in marine environments, although related to changes in atmospheric activity, depends greatly on local and regional factors, such as rates of mixing of surface waters with upwelled ^{14}C-depleted deep ocean waters, hinterland geology, tidal flushing, and terrestrial water input. Such factors are highly variable and can introduce uncertainties of up to several hundred years into dates obtained on marine samples in some parts of the world. Although accurate measurements for El Arenal-1 are lacking, recent estimates of the marine reservoir effect along the Chilean coast are conservatively estimated at $\Delta R = 61 \pm 50$ and the site itself is characterized by large shell middens (A.J.A., unpublished work).

Consequently, if the diet of the El Arenal-1 chicken included a marine carbon contribution of >20%, the calibrated age would be post-Columbian. Examination of a large number of specimens of several species at the site, including studies of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$, will be necessary to evaluate the contribution of marine carbon to the omnivorous chicken diet (28, 29). It is also worth noting that the radiocarbon-dating laboratory used to date the El Arenal-1 sample has recently been suggested to have overestimated the age of zooarchaeological bones elsewhere in the Pacific (30). The uncertainty of the marine carbon contribution and accuracy of the date indicates that further analyses are needed to definitively identify them as pre-Columbian.

Multiple mtDNA Origins of the Modern Araucana Chicken. The high percentage of European chickens (>91%) sharing haplotypes with the Indian subcontinent suggests that the latter was the main source for chickens introduced into Europe. As a result, we consider the clustering of the modern Chilean chicken sequences with haplotypes predominant in Europe to indicate the contribution of Spanish introduced chickens. In the light of our analyses, it would be controversial to use the single pre-Columbian sequence (or indeed any haplotype from the European/Indian subcontinental/ Chinese haplogroups) as evidence for a Southeast/eastern Asian or Pacific origin for the Araucana, as claimed by Storey *et al.* (4).

At present, there is certainly no evidence to support an ancient Asian Pacific route for the introduction of Indian subcontinental/European/Chinese chicken haplotypes into Chile. It is possible that the few Araucana sequences that cluster with haplotypes predominant in southern and eastern China, Indonesia, and Japan could result from a pre-Columbian Asian introduction, but it would appear much more likely to represent modern introgression of Asian mtDNA genes into the South American populations. If ancient Pacific/pre-Columbian chickens were the ancestors of the Araucana, traits such as blue/green-shells, ear-tufts, and/or rumples might be expected to occur in some modern Pacific Island chickens. However, we are unable to find any reports that this is the case.

In conclusion, the archaeological Pacific and Chilean pre-Columbian chicken sequences fall among a widespread European/Indian subcontinental/Chinese haplogroup and provide no support for Polynesian-South American contact. Four haplotypes have been detected in Polynesian archaeological material, but the only unique sequences are those from Easter Island that appear to originate in Island Southeast Asia. None of these Easter Island haplotypes have been detected in either ancient or modern South American breeds. Three major maternal lineages have contributed to the modern Chilean chicken breeds, mainly from the European/Indian subcontinental region but also in low frequency from the South Chinese/Indonesian/Japanese area. In addition, the lack of information about the marine carbon input for the chicken bones from El Arenal-1 leaves the pre-Columbian date uncertain. The identification of diagnostic markers for Asian and Mediterranean traits in combination with mtDNA, female-specific W chromosome and microsatellite markers will be required to resolve the timing and nature of introductions, modern diversity and regional adaptation of local chicken breeds in the Americas and Island South East Asia. Of particular interest will be chickens kept by some indigenous communities in the Amazon forest, the origins of which remain unclear.

Materials and Methods

Chicken Samples, PCR, and Sequencing. Blood samples were obtained from 41 modern Chilean chickens including 28 Araucanas (ketros, kolloncas, kollonkas de aretes), 7 creole, 1 Japanese Long Tail and 5 Passion Fowl from 12 locations in Chile. The authenticity of the Araucana specimens was assessed by J.A.A., an international authority on this breed. The voucher information and identification number of the DNA samples used in this study are supplied in Table S1. DNA was precipitated and extracted by using a salting-out method (31) at the Pontificia Universidad Catolica de Chile. PCR and sequencing procedures were conducted at the International Livestock Research Institute (Kenya). Primer pairs (5′-AGGACTACGGCTTGAAAAGC-3′ and 5′-ATGTGCCTGACCGAGGAACCAG-3′) were used to amplify ≈600 bp of the mitochondrial hypervariable 1 region. PCR was performed in 30-μl reaction volumes containing 50 mM KCl, 10 mM Tris·HCl (pH 8.3), 0.1% Triton X-100, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.1 μM concentrations of each primer, 1.25 units of TaqDNA polymerase (Pro-

mega), and 100–200 ng of template DNA. PCR conditions included an initial denaturation at 94°C for 2 min, followed by 35 cycles of 25 s at 94°C, 35 s at 58°C, and 1 min 10 s at 72°C, and a final extension for 10 min at 72°C. PCR products were purified by using the QIAQuik PCR purification kit (Qiagen) and directly sequenced by using two internal primers CR for (5′-TCTATATTCCACATTTCTC-3′) and CR-Rev (5′-GCGAGCATAACCAAATGG-3′) on an ABI 3730. Forward and reverse sequences were overlapped to obtain a consensus sequence of 530 bp for each sample, after excluding primer sequences. To avoid PCR artifacts, two independent PCRs were used to amplify the mtDNA sequence from all specimens.

Phylogenetic Analyses. In the Storey *et al.* (4) study, a phylogenetic analysis was not performed, and 9 SNPs were simply contrasted between specimens. Furthermore, the analysis did not include extensive mtDNA CR datasets available on GenBank from native and modern chicken breeds and Red Junglefowl. To perform a detailed phylogenetic analysis, ClustalW (32) was used to align the 41 Chilean chicken mtDNA CR sequences with a large mtDNA CR dataset of ≈950 sequences including those from Liu *et al.* (6) and Oka *et al.* (10). The alignment included sequences from European Barred Plymouth Rock (GenBank accession no. AB007719), White Plymouth Rock (AP003318), White Leghorn (AP003317 and D82920), and New Hampshire chickens (AY235571) (33). Two aligned datasets were generated; the first consisted of 534 bp (990 sequences) and the second a truncated version of 205 bp (1,003 sequences) including indels (insertions/deletions). The shorter dataset was limited to the sequence lengths of the ancient Pacific and pre-Columbian chickens that were 169–201 bp [not ≈400 bp as stated in Storey *et al.* (4)] to facilitate phylogenetic comparisons of these specimens. The two aligned datasets were reduced to unique haplotypes by using FaBOX (www.birc.au.dk/fabox), exported as aligned FASTA files, and converted into Nexus format by using MEGA 3.1 (34) for network and phylogenetic analyses.

MJN analyses were performed by using Network 4.1.1.2 (35). The original 534-bp aligned dataset was used to generate a multistate alignment rdf (Roehl data format) file of variable positions along with the frequency per haplotype and phylogenetic weight for each variable position (Figs. S2 and S3), which was imported into Network 4.1.1.2. The impact of down weighting sites that are apparent mutational hotspots or that exhibit high levels of conflict within Network analyses was investigated. Sites observed to mutate >10 times within a network were given a weighting score of 0.0 (ignored in the analysis) in the rdf file, whereas sites that mutated more than five times were down weighted to 0.2, by using standard settings within Network 4.1.1.2. This rdf multistate alignment file was star contracted (with a threshold connection limit of five) to further collapse very closely related sequences (e.g., star phylogenies/polytomies) into haplotypes. Network analysis was also performed on the shorter 205-bp alignment as described above by using down weighting of the same sites and without weighting or star contraction.

Phylogenetic analyses were attempted by using ML with the 534-bp datasets, but the very large number of closely related sequences generated multiple polytomies and unreliable bootstrap analyses. Consequently, MP was performed by using PAUP 4.0b10 (36), because MP is better suited than ML to missing sequence data/different length sequences in large datasets (37, 38). A strict consensus MP tree (Fig. S6) was constructed by using 20 independent runs with random starting points, the maximum number of trees saved set at 10,000, a random addition of sequences, and the collapsing of all branches with a minimum length of zero to decrease internal branch rearrangements within star phylogenies. Two Red Junglefowl sequences (*Gallus gallus gallus*, AB007720, and *Gallus gallus bankiva*, AB007718) were used as outgroups.

Approximately 200,000 MP trees were generated, and the 30,276 most-parsimonious trees were used to construct a strict consensus tree. This was used to investigate the phylogenetic position of the short ancient Polynesian and Chilean chicken sequences by repeating the analysis but including these sequences and by using the strict consensus tree topology as a fixed phylogenetic constraint, with the random addition of sequences and collapsing branches with a minimum length of zero. Approximately 24,300 most-parsimonious trees were produced and used to construct a 50% majority-rule consensus tree. Bootstrap analysis was impractical because of the large dataset of very closely related sequences.

A description of polymorphic sites, haplogroups, and network analyses are included in Tables S1 and S3 and Fig. S1. The full 534-bp alignment contained 125 polymorphic sites, whereas the truncated alignment (205 bp) contained just 81 polymorphic sites, and the number of haplotypes correspondingly decreased from 213 to 149. The haplotype information for both alignments and the MP and MJN analyses of the 534-bp alignment are described in Figs. S1–S7.

Marine Reservoir Effects and the Calibration of Direct Dates from El Arenal-1. Marine reservoir effects are routinely expressed as a ΔR value, which is the difference between the conventional radiocarbon age of a sample of known age from a specific locality and the equivalent age predicted by the global modeled marine calibration curve (39). Eight ΔR values are available for the west coast of South America between the equator and Cape Horn (Table S4) to calibrate the 622 ± 35 date for the El Arenal-1 specimen. Unfortunately, all but one of these values can be shown to be problematic for calibration purposes (Table S4), so we follow Reimer and Reimer's (13) recommendation when pooling multiple ΔR values, and take the ΔR uncertainty as the larger of the standard deviation and weighted mean measurement error. For each ΔR value, we calibrated the date for the El Arenal-1 specimen with variable marine-derived carbon content from 0 to 50% (Table 1 and Table S2) based on Ingram and Southon's (12) single bivalve determination from Valparaiso, Chile. Conventional radiocarbon ages were calibrated by using CALIB (v.5.0.1) (15, 16) and the Southern Hemisphere calibration dataset (SHCal04) (40) and the marine calibration model dataset (Marine04) (27). The ''Mixed Marine SoHem'' option was used to calibrate dates with marine and terrestrial carbon pathways.

Figs. S1–S7 and Tables S1–S4 and Table S5 and Table S6 are available in the SI.

1. Carter GF (1975) The chicken in America. *Anthropol J Can* 13:25–26.
2. Errázuriz J (2000) *Cuenca del Pacífico: 4.000 Años de Contactos Culturales.* Serie Divulgación. Santiago (Universidade de Santiago de Compostela, Santiago, Chile).
3. Ramirez JMA (2006) Cronologia y fuentes de la historia de Rapa Nui: 1722–1966. *Rev Arch Hist Patrim Viña del Mar* 7:185–209.
4. Storey AA, *et al.* (2007) Radiocarbon and DNA evidence for a pre-Columbian introduction of Polynesian chickens to Chile. *Proc Natl Acad Sci USA* 104:10335–10339.
5. Fumihito A, *et al.* (1996) Monophyletic origin and unique dispersal patterns of domestic fowls. *Proc Natl Acad Sci USA* 93:6792–6795.
6. Liu YP, *et al.* (2006) Multiple maternal origins of chickens: Out of the Asian jungles. *Mol Phylogenet Evol* 38:12–19.
7. West B, Zhou B-X (1988) Did chickens go north? New evidence for domestication. *J Archaeol Sci* 15:515–533.
8. Zeuner FE (1963) *A History of Domesticated Animals* (Hutchinson, London).
9. Larson G, *et al.* (2005) Worldwide phylogeography of wild boar reveals multiple centers of pig domestication. *Science* 307:1618–16121.
10. Oka T, *et al.* (2007) Analysis of mtDNA sequences shows Japanese native chickens have multiple origins. *Anim Genet* 38:287–293.
11. Nishibori M, Shimogiri T, Hayashi T, Yasue H (2005) Molecular evidence for hybridization of species in the genus *Gallus* except for *Gallus varius*. *Anim Genet* 36:367–375.
12. Ingram BL, Southon JR (1996) Reservoir ages in Eastern Pacific coastal and estuarine waters. *Radiocarbon* 38:573–582.
13. Reimer P, Reimer R (2008) *Marine Reservoir Correction Database.* http://calib.qub.ac.uk/marine/, accessed February 10, 2008.
14. Taylor RE, Berger R (1967) Radiocarbon content of marine shells from the Pacific coasts of Central and South America. *Science* 158:1180–1182.
15. Stuiver M, Reimer PJ (1993) Extended ^{14}C data base and revised CALIB 3.0 ^{14}C age calibration program. *Radiocarbon* 35:215–230.
16. Stuiver M, Reimer PJ, Reimer R (2005) *CALIB Manual* http://calib.qub.ac.uk/calib/manual/, accessed March 31, 2006.
17. Larson G, *et al.* (2007) Ancient DNA, pig domestication, and the spread of the Neolithic into Europe. *Proc Natl Acad Sci USA* 104:15276–15281.
18. Erickson DL, Smith BD, Clarke AC, Sandweiss DH, Tuross N (2005) An Asian origin for a 10,000-year-old domesticated plant in the Americas. *Proc Natl Acad Sci USA* 102:18315–18320.
19. Decker-Walters DS, Staub JE, Lopez-Sese A, Nakata E (2001) Diversity in landraces and cultivars of bottle gourd (*Lagenaria siceraria*; Cucurbitaceae) as assessed by random amplified polymorphic DNA. *Genet Resources Crop Evol* 48:369–378.
20. Leonard JA, *et al.* (2006) Animal DNA in PCR reagents plagues ancient DNA research. *J Archaeol Sci* 34:1361–1366.
21. Sakamoto N, *et al.* (2002) Concentrations of radiocarbon and isotope compositions of stable carbon in food. *J Nucl Sci Technol* 39:323–328.
22. Hobson KA, Clark RG (1992) Assessing avian diets using stable isotopes I: Turnover of ^{13}C in tissues. *Condor* 94:181–188.
23. Hobson KA, Clark RG (1992) Assessing avian diets using stable isotopes II: Factors influencing diet-tissue fractionation. *Condor* 94:189–197.
24. Buckner GD, Martin JH, Pierce WC, Peter AM (1922) Calcium in egg-shell formation. *J Biol Chem* 51:51–54.
25. Fritz JC (1937) The effect of feeding grit on digestibility in the domestic fowl. *Poult Sci* 16:75–79.
26. Contreras L, Quiroz D, Sanchez M, Caballero C (2005) Ceramios, maices y ranas. . . un campamento El Vergel en las costas de Arauco. *Actas del XVI Congreso Nacional de Arqueología Chilena* (Museo de Historia Natural de Concepcion, Direccion de Bibliotecas, Archivos y Museos, Sociedad Chilena de Arqueologia, Concepcion, Chile), pp 357–367.
27. Hughen KA, *et al.* (2004) MARINE04 marine radiocarbon age calibration, 0–26 cal kyr BP. *Radiocarbon* 46:1059–1086.
28. Athfield BN, Green RC, Craig J, McFadgen B, Bickler S (2008) Influence of marine sources on ^{14}C ages: Isotopic data from Watom Island, Papua New Guinea inhumations and pig teeth in light of new dietary standards. *J R Soc N Z* 38:1–23.

29. Leach BF, Quinn C, Morrison J, Lyon G (2003) The use of multiple isotope signatures in reconstructing prehistoric human diet from archaeological bone from the Pacific and New Zealand. *N Z J Archaeol* 23:31–98.
30. Wilmshurst JM, Anderson AJ, Higham TFG, Worthy TH (2008) Dating the late prehistoric dispersal of Polynesians to New Zealand using the commensal Pacific rat. *Proc Natl Acad Sci USA* 105:7676–7680.
31. Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 16:1215.
32. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 24:4876–4882.
33. Fumihito A, *et al.* (1994) One subspecies of the red junglefowl (*Gallus gallus gallus*) suffices as the matriarchic ancestor of all domestic breeds. *Proc Natl Acad Sci USA* 91:12505–12509.
34. Kumar S, Tamura K, Nei M (2004) MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* 5:150–163.
35. Forster P, Harding R, Torroni A, Bandelt H-J (1996) Origin and evolution of Native American mtDNA variation: A reappraisal. *Am J Hum Genet* 59:935–945.
36. Swofford DL (2007) *PAUP. Phylogenetic Analysis Using Parsimony* (Sinauer, Sunderland, MA), Version 4.
37. Philippe H, *et al.* (2004) Phylogenomics of eukaryotes: impact of missing data on large alignments. *Mol Biol Evol* 21:1740–1752.
38. Weins JJ (2006) Missing data and the design of phylogenetic analyses. *J Biomed Inform* 39:34–42.
39. Stuiver M, Kra RS (1986) Calibration issue, Proceedings of the 12th International ^{14}C Conference. *Radiocarbon* 28:805–1030.
40. McCormac FG, *et al.* (2004) SHCAL04 southern hemisphere calibration, 0–11.0 cal kyr BP. *Radiocarbon* 46:1087–1092.

Correction

ANTHROPOLOGY. For the article "Indo-European and Asian origins for Chilean and Pacific chickens revealed by mtDNA," by Jaime Gongora, Nicolas J. Rawlence, Victor A. Mobegi, Han Jianlin, Jose A. Alcalde, Jose T. Matus, Olivier Hanotte, Chris Moran, Jeremy J. Austin, Sean Ulm, Atholl J. Anderson, Greger Larson, and Alan Cooper, which appeared in issue 30, July 29, 2008, of *Proc Natl Acad Sci USA* (105:10308–10313; first published July 28, 2008; 10.1073/pnas.0801991105), the authors note that in the Abstract, line 20, "China" should be replaced with "the Philippines." The corrected sentence should read: "In contrast, sequences from two archaeological sites on Easter Island group with an uncommon haplogroup from Indonesia, Japan, and the Philippines and may represent a genetic signature of an early Polynesian dispersal." Additionally, on page 10310, left column, second full paragraph, line 11, the phrase "native chicken sequences from Africa, India, Central Asia, and China" should instead read: "native chicken sequences from India, Central Asia, and China." These errors do not affect the conclusions of the article. Finally, the authors wish to include Nicolas J. Rawlence, Olivier Hanotte, and Sean Ulm among those credited with writing the paper; Sean Ulm among those credited with performing research; and Alan Cooper among those credited with analyzing data. The corrected author contributions footnote appears below.

Author contributions: J.G. and J.A.A. designed research; J.G., V.A.M., H.J., J.A.A., J.T.M., O.H., and S.U. performed research; N.J.R. and S.U. contributed new reagents/analytic tools; J.G., N.J.R., V.A.M., H.J., O.H., J.J.A., and A.C. analyzed data; and J.G., N.J.R., O.H., C.M., S.U., A.J.A., G.L., and A.C. wrote the paper.

www.pnas.org/cgi/doi/10.1073/pnas.0807512105

Supporting Information

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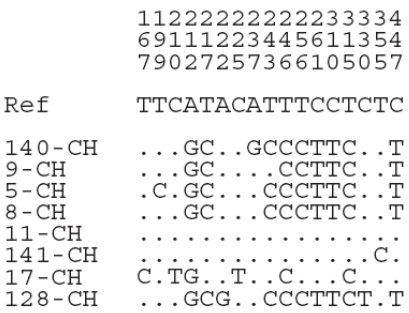

Fig. S1. Haplotypes identified among Chilean chickens. Numbers indicate the site position of the variable sites. Dots indicate identity with the reference sequence (GenBank accession no. AB098668) (1), and different base letters denote substitution.

1. Komiyama T, Ikeo K, Gojobori T (2003) Where is the origin of the Japanese gamecocks? *Gene* 317:195–202.

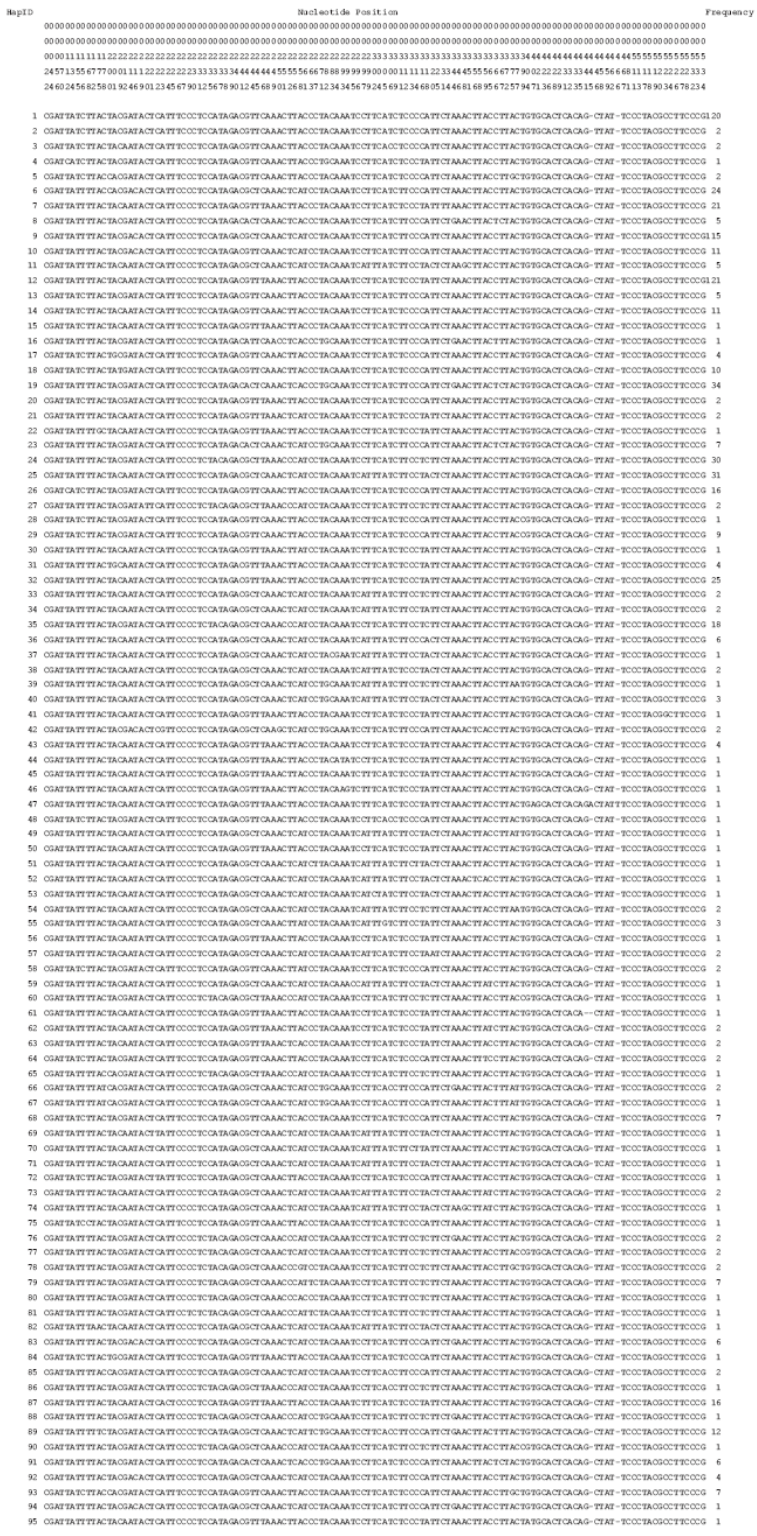

Fig. S2a. Haplotypes and frequencies used for networks analyses from the 534-bp alignment including indels. Because of the differences in the number of polymorphic sites, it was not possible to keep the same haplogroup numbers for both the complete and truncated alignment analyses.

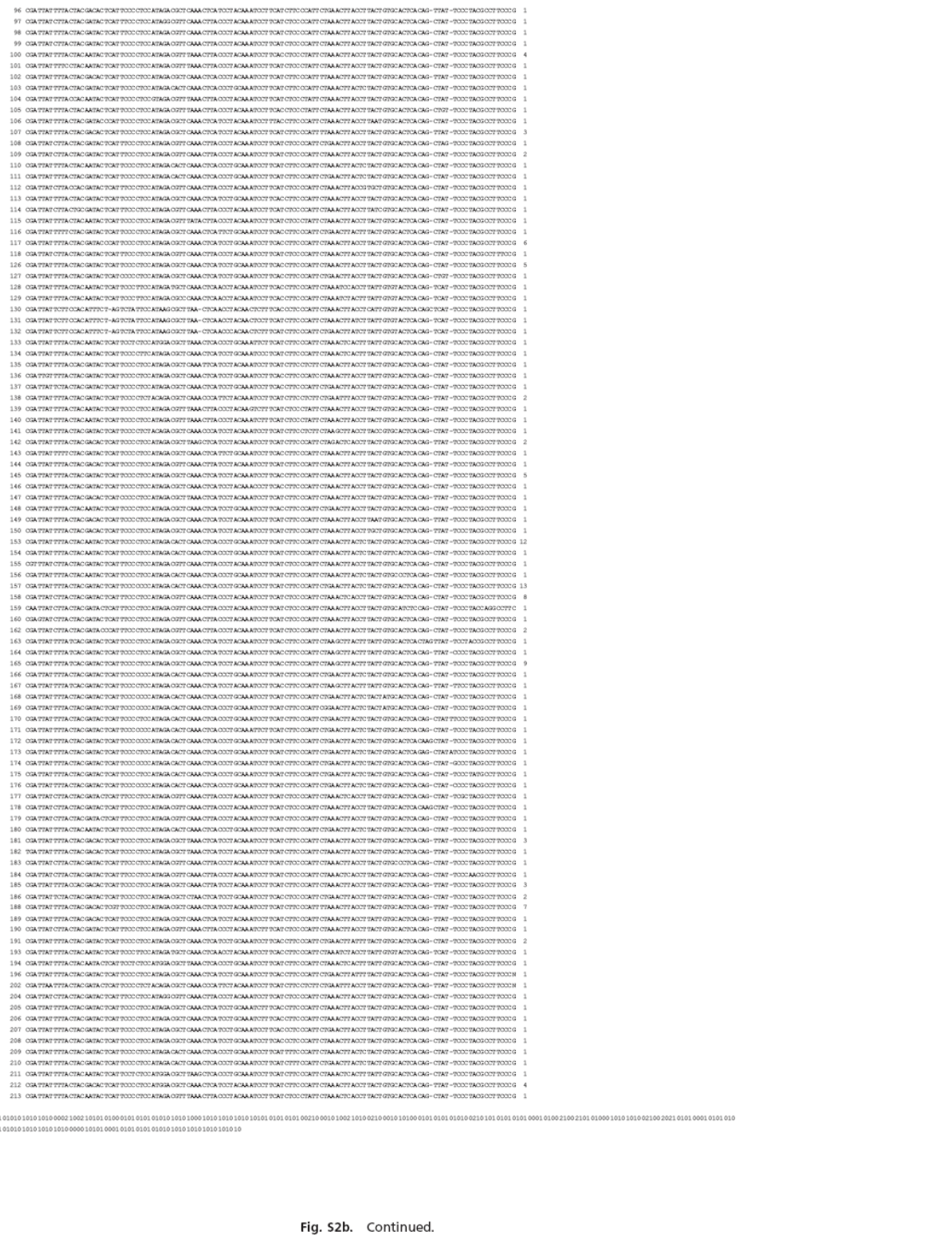

Fig. S2b. Continued.

HapID	Nucleotide Position	Frequency
	000000000000000000000000000000000000000000000000000000000000000000000000000000000	
	000000000000000000000000000000000000000000000000000000000000000000000000000000000	
	000000000000000000000000000000000000000000011111111111111111111111111111111111122	
	000133444555555556666666677777777788888899900111222233333344444455566777888999900	
	148134257923456789023458901234578912345914604567678902578924567913847179149128905	
1	CTTACTACGATACTCATTTCCCTCCATAGACGTTCAAACTTACCCTACAAATCCTTCATCTCCCCATTCGTAAACTTACCT	157
2	CTTACTACAATACTCATTTCCCTCCATAGACGTTCAAACTTACCCTACAAATCCTTCACCTCCCCATTCGTAAACTTACCT	2
3	CTTACTACGATACTCATTTCCCTCCATAGACGTTCAAACTTACCCTGCAAATCCTTCATCTCCCTATTCGTAAACTTACCT	1
4	CTTACCACGATACTCATTTCCCTCCATAGACGTTCAAACTTACCCTACAAATCCTTCATCTCCCCATTCGTAAACTTACCT	9
5	TTTACCACGACACTCATTCCCCTCCATAGACGCTCAAACTCATCCTACAAATCCTTCATCTTCCCATTCGTAAACTTACCT	29
6	TTTACTACAATACTCATTCCCCTCCATAGACGTTTAAACTTACCCTACAAATCCTTCATCTCCCTATTTGTAAACTTACCT	21
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8	TTTACTACGACACTCATTCCCCTCCATAGACGCTCAAACTCATCCTACAAATCCTTCATCTTCCCATTCGTAAACTTACCT	124
9	TTTACTACGACACTCATTCCCCTCCATAGACGTTCAAACTCATCCTACAAATCCTTCATCTTCCCATTCGTAAACTTACCT	12
10	TTTACTACAATACTCATTCCCCTCCATAGACGCTCAAACTCATCCTACAAATCATTTATCTTCCTACTCGTAAGCTTACCT	5
11	TTTACTACAATACTCATTCCCCTCCATAGACGTTTAAACTTACCCTACAAATCCTTCATCTCCCTATTCGTAAACTTACCT	130
12	CTTACTACGATACTCATTTCCCTCCATAGACGTTCAAACTTACCCTACAAATCCTTCATCTTCCCATTCGTAAACTTACCT	5
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Fig. S3a. Haplotypes and frequencies used for networks analyses from the 205-bp alignment including the archaeological DNA sequences. Because of the differences in the number of polymorphic sites, it was not possible to keep the same haplogroup numbers for both the complete and truncated alignment analyses.

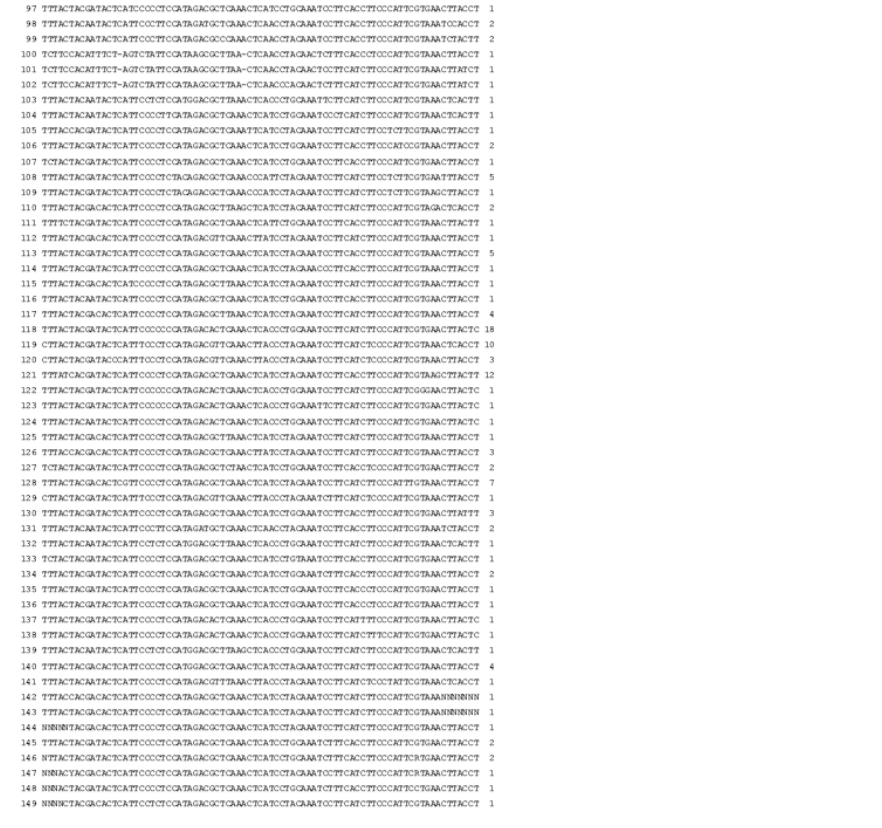

Fig. S3b. Continued.

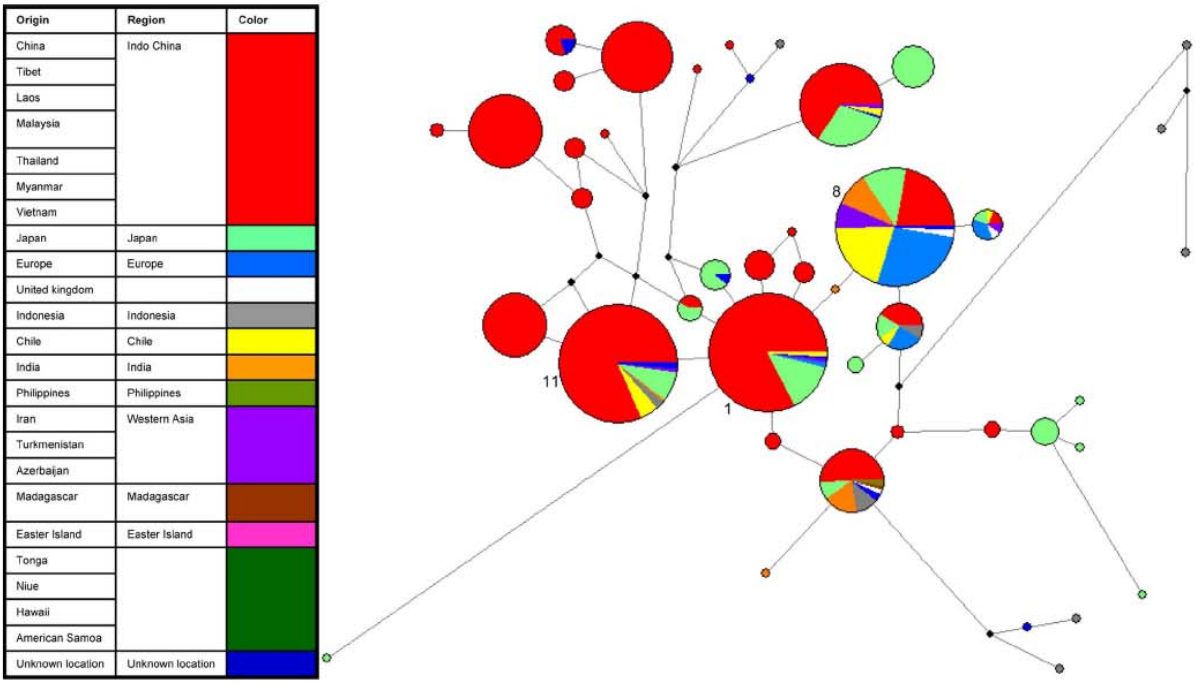

Fig. S4. Median-joining network start showing the position of 41 Chilean modern chickens (yellow) among native and wild junglefowl from Asia, Africa, and Europe. Only major and relevant networks are presented here by using 534 bp of the mtDNA CR. Circle size is proportional to the frequency of the corresponding haplotypes, and the geographical origin of the haplotypes are distinguished by use of color codes described in the table next to the figure. Most of the Chilean chickens cluster with Indian subcontinental/European/Chinese chickens, whereas other Chilean chickens cluster with haplogroups predominant of South and eastern Chinese/Japanese/Indonesian chickens.

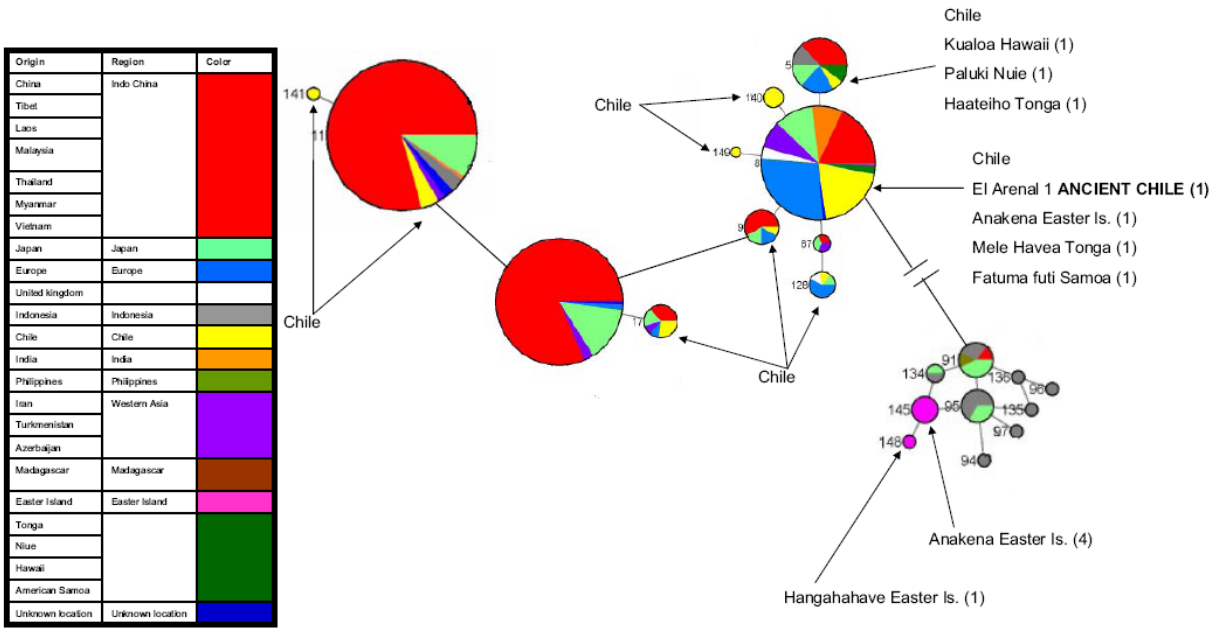

Fig. S5. Simplified median-joining networks showing the relationships and clustering of 205 bp of the mtDNA control region from worldwide, Chilean Araucana, pre-Columbian, and ancient Pacific/Polynesian chickens. The numbers next to the circles correspond to the haplotype clade number. The area of each circle is proportional to the frequency of the corresponding haplotypes, and the geographical origins of the haplotypes are distinguished by use of color codes described in the table next to the figure. Most of the Chilean chickens cluster with Indian subcontinental/European/Chinese chickens, more specifically with haplotype numbers 5, 8, 9, 128, and 140. The other Chilean chickens cluster with haplotypes predominant in South and eastern Chinese/Japanese/Indonesian chickens (haplotypes numbers 11, 17, and 141). Pre-Columbian sequence clusters with haplotype number 8. A more complete description of this MJN is presented in Fig. 1. Certain closely related haplotypes described in this and other figures have collapsed into others because of ambiguities and different length sequences.

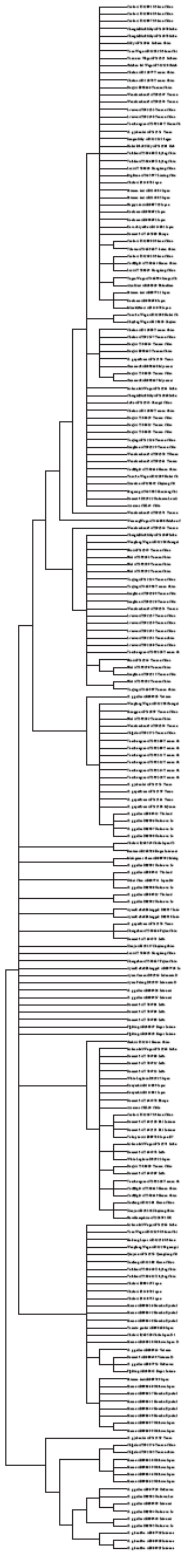

Fig. S6. Strict consensus MP tree. It was constructed from 30,276 trees left as described in *Materials and Methods*.

Fig. S7. MJN showing eight haplotypes of 41 modern Chilean chickens based by using 530 bp of the mtDNA CR sequences. The area of each circle is proportional to the frequency of the corresponding haplotypes described in supporting information (SI) Table S1. Clustering of these sequences among native chickens and wild junglefowl from Asia, Africa, and Europe is shown in yellow in Fig. 1 and Figs. S4 and S5.

Table S1. Voucher information of modern Chilean chicken samples collected from latitudes 33° to 40° S

Sample ID	Breed or type	Flock	Sex (♀/♂)	Chilean haplotype name	Haplotype no. as indicated in network analyses
CHL1	Ketro	A Pirque 1, Santiago (Lat 33° 40′ S, Long 70° 28′ W)	♂	11-CH	11
CHL2	Kollonca		♂	8-CH	8
CHL3	Kollonca		♀		
CHL4	Kollonca		♀		
CHL5	Creole/naked neck		♂	17-CH	17
CHL6	Kollonca		♀	11-CH	11
CHL7	Creole	B Pirque 2, Santiago (Lat 33° 39′ S, Long 70° 27′ W)	♂	8-CH	8
CHL8	Kollonca	C Antiquina, Arauco (Lat 38° 11′ S, Long 73° 25′ W)	♂	8-CH	8
CHL9	Kollonca		♂	8-CH	8
CHL10	Ketro		♀	140-CH	140
CHL11	Kollonca de Aretes		♀	8-CH	8
CHL12	Kollonca		♀	8-CH	8
CHL13	Kollonca		♂	8-CH	8
CHL14	Creole	D Malihue, Valdivia (Lat 39° 44′ S, Long 72° 38′ W)	♀	8-CH	8
CHL15	Creole		♀	8-CH	8
CHL16	Kollonca	G Cañete, Arauco (Lat 37° 47′ S, Long 73° 23′ W)	♂		
CHL17	Ketro		♂	11-CH	11
CHL18	Kollonca		♂	8-CH	8
CHL19	Creole	E Ninhue, Chillán (Lat 36° 21′ S, Long 72° 23′ W)	♀	8-CH	8
CHL20	Creole	F Yumbel, Chillán (Lat 37° 02′ S, Long 72° 32′ W)	♀	140-CH	140
CHL21	Kollonca	G Cañete, Arauco (Lat 37° 47′ S, Long 73° 23′ W)	♂	8-CH	8
CHL22	Ketro		♀	140-CH	140
CHL23	Ketro		♀	5-CH	5
CHL24	Kollonca		♀	9-CH	9
CHL25	Ketro		♀	8-CH	8
CHL26	Kollonca		♀	5-CH	5
CHL27	Kollonca		♀	8-CH	8
CHL28	Ketro		♂	8-CH	8
CHL29	Ketro		♀	140-CH	140
CHL30	Kollonca		♀	8-CH	8
CHL31	Kollonca	B Pirque 2, Santiago (Lat 33° 39′ S, Long 70° 27′ W)	♂	17-CH	17
CHL32	Kollonca		♀	128-CH	128
CHL33	Ketro		♀	8-CH	8
CHL34	Japanese Long Tail ancestry		♂	8-CH	8
CCHL35	Kollonca		♂	8-CH	8
CHL36	Kollonca		♀	11-CH	11
CHL37	Ketro		♂	11-CH	11
CHL38	Creole		♀	8-CH	8
CHL39	Passion fowl		♀	8-CH	8
CHL41	Passion fowl	H Viña del Mar, Valparaíso (Lat 32° 59′ S, Long 71° 33′ W)	♂	8-CH	8
CHL40	Passion fowl		♂	8-CH	8
CHL42	Passion fowl		♀	8-CH	8
CHL43	Ketro	J Melipilla, Santiago (Lat 33° 42′ S, Long 71° 13′ W)	♂	8-CH	8
CHL44	Passion fowl/Sebright ancestro	I El Monte, Santiago (Lat 33° 42′ S, Long 70° 58′ W)	♀	141-CH	141

Creole translates from ''criollo'' and stands for an unselected smallholder chicken typical of the countryside. DNA extraction precedures failed for samples 3, 4, and 16.

Table S2. Calibration of one direct date on chicken bone with increasing proportion of marine-derived carbon

ΔR	Marine C, %	Calibration dataset	Radiocarbon on chicken bones from the El Arenal-1, Chile (n = 622 ± 35)
0 ± 0	0	SHCal04	**AD1304–1424**
154 ± 131	10	Mixed marine SoHem	**AD1387–1449**
154 ± 131	20	Mixed marine SoHem	**AD1395–1492**
154 ± 131	30	Mixed marine SoHem	AD1412–1620
154 ± 131	40	Mixed marine SoHem	AD1440–1644
154 ± 131	50	Mixed marine SoHem	AD1439–1796

ΔR of 137 ± 114 based on Ingram and Southon's (1) single bivalve determination from Valparaiso, Chile combined with Taylor and Berger's (2) gastropod date from approximately the same location. Given the problems inherent with the use of gastropods for characterizing marine reservoir effects, the derived ΔR value is used simply for illustrative purposes. All calibrated dates are reported at 2σ. Bold type denotes pre-Columbian values.

1. Ingram BL, Southon JR (1996) Reservoir ages in Eastern Pacific coastal and estuarine waters. *Radiocarbon* 38:573–582.
2. Taylor RE, Berger R (1967) Radiocarbon content of marine shells from the Pacific coasts of Central and South America. *Science* 158:1180–1182.

Table S3. Frequency of modern Chilean chicken haplotypes

Haplotype	Frequency
17-CH	2
11-CH	5
141-CH	1
140-CH	4
5-CH	2
9-CH	1
8-CH	25
128-CH	1
Total	41

Table S4. ΔR values for the west coast of South America (after Reimer and Reimer 2008)

Longitude	Latitude	ΔR	Location	Ref	Collection year	Reservoir age	^{14}C age	Lab no.	Taxa	Diet
−80.00	−3.00	−216 ± 37	Guayaquil, Ecuador*	10	1927	85 ± 38	235 ± 37	UCIA-1249A	Cerithidea valida	Deposit feeder
−80.00	−3.00	84 ± 45	Guayaquil, Ecuador*	10	1927	386 ± 46	536 ± 45	UCLA-1249B	Thais biserialis	Carnivore
−80.00	−10.00	243 ± 49	Northern Peru*	10	1935†	544 ± 50	700 ± 49	UCLA-1282	Strombus peruvianus	Herbivore/omnivore
−78.00	−14.00	670 ± 44	Peru*	10	1935†	971 ± 45	1127 ± 44	UCLA-1279	Oliva peruviana	Unknown
−70.00	−24.00	175 ± 34	Antofagasta, Chile*	10	1925	477 ± 35	626 ± 34	UCLA-1277	Concholepas concholepas	Carnivore
−72.00	−33.00	313 ± 76	Valparaiso, Chile*	10	1935†	614 ± 77	770 ± 76	UCLA-1278	Tequla aler	Unknown
−71.80	−33.10	61 ± 50	Valparaiso, Chile	6	1939‡	370 ± 51	520 ± 50	CAMS-17919/1	Mytilus californianus	Suspension feeder
−72.65	−51.70	221 ± 40	Puerto Natales, Chile	6	1939‡	530 ± 41	680 ± 40	CAMS-17918	Mytilus californianus	Suspension feeder

Marine reservoir effects for Chile are poorly resolved. There are eight ΔR values published for the west coast of South America between the equator and Cape Horn. All but one of these values can be shown to be problematic. Six of these values were published by Taylor and Berger (10) on the basis of dating of gastropods. Over the last 20 years, several studies have indicated that detrital feeders are potentially problematic because ingested organic carbon from diverse sources can become incorporated into shell structures through metabolic action (5, 9). These effects have been found to be particularly problematic in limestone-dominated areas (1, 4). It is for this reason that the use of suspension feeders, herbivores, and omnivores is recommend in ΔR research. Taylor and Berger's sample contains two carnivores, a deposit feeder, and two other gastropods for which dietary information is lacking. Only the determination on *Strombus peruvianus* from northern Peru is unproblematic as a herbivore/omnivore, resulting in $\Delta R = 243 \pm 49$. In Ingram and Southon's (6) more recent study focused on California included two samples of the suspension-feeding bivalve *Mytilus californianus* from Chile. The sample from Puerto Natales is an estuarine reservoir value, potentially influenced by terrestrial runoff and incomplete exchange with the open ocean and therefore may not reflect open water reservoir conditions (11). This leaves the determination from Valparaiso in central Chile, resulting in $\Delta R = 61 \pm 50$. This single ΔR value provides the single reliable estimate for marine reservoir effect in near-shore open waters in southern South America. However, this value is likely to underestimate ΔR in the region because of heavily depleted Antartic source waters brought to the Chilean coastline by the Antartic circumpolar current. Seven values are reported in the Marine Reservoir Database for northern Antartica (7) giving a combined $\Delta R = 871 \pm 176$ (see refs. 2, 3, 8). We believe, therefore, that the $\Delta R = 61 \pm 50$ from Valparaiso should be treated as a conservative estimate of open water marine reservoir effect in Chile.

*Approximate location.

†Mid-point. Collected between 1930–1940 (10).

‡Ingram and Southon (1996:574) state that "*n most cases, it is uncertain whether these specimens were collected live or not.*"

1. Anderson AT, Higham FG, Wallace R (2001) The radiocarbon chronology of the Norfolk Island archaeological sites. *Rec Australian Mus* 27(Supplement):33–42.
2. Berkman PA, Forman SL (1996) Pre-bomb radiocarbon and the reservoir correction for calcareous marine species in the Southern Ocean. *Geophys Res Lett* 23:363–366.
3. Bjorck S, Hjort C, Ingolfsson O, Skog G (1991) Radiocarbon dates from the Antarctic peninsula region—problems and potential. *Radiocarbon Dating: Recent Applications and Future Potential*, ed Lowe JJ (Quaternary Research Association, Cambridge, UK), Quaternary Proceedings 1, pp 55–65.
4. Dye T (1994) Apparent ages of marine shells: Implications for archaeological dating in Hawaii. *Radiocarbon* 36:51–57.
5. Hogg AG, Higham TFG, Dahm J (1998) ^{14}C dating of modern marine and estuarine shellfish. *Radiocarbon* 40:975–984.
6. Ingram BL, Southon JR (1996) Reservoir ages in Eastern Pacific coastal and estuarine waters. *Radiocarbon* 38:573–582.
7. Reimer P, Reimer R (2008) Marine reservoir correction database. http://calib.qub.ac.uk/marine, accessed February 10, 2008.
8. Peck LS, Brey T (1996) Bomb signals in old Antarctic brachiopods. *Nature* 380:207–208.
9. Tanaka N, Monaghan MC, Rye DM (1986) Contribution of metabolic carbon to mollusk and barnacle shell carbonate. *Nature* 320:520–523.
10. Taylor RE, Berger R (1967) Radiocarbon content of marine shells from the Pacific coasts of Central and South America. *Science* 158:1180–1182.
11. Ulm S (2002) Marine and estuarine reservoir effects in central Queensland, Australia: Determination of ΔR values. *Geoarchaeology* 17:319–348.
12. Taylor RE, Berger R (1967) Radiocarbon content of marine shells from the Pacific coasts of Central and South America. *Science* 158:1180–1182.

Other Supporting Information Files

Table S5 (XLS)
Table S6 (XLS)

LETTER

Pre-Columbian chickens, dates, isotopes, and mtDNA

Recently Gongora *et al*. (1) stated that their analyses of chicken mtDNA and potential offsets for dietary marine carbon cast doubt on "claims for pre-Columbian chickens" in the Americas. We present additional data supporting the interpretation of Storey *et al*. (2) showing that evidence for pre-Columbian chickens at the site of El Arenal, Chile, is secure.

Gongora *et al*. (1) analyzed mtDNA of modern chickens only. They gave no consideration to the fact that both European and prehistoric Pacific chickens are ultimately Asian-derived and thus may be expected to share lineages. European stocks were further influenced by the 19th-century import of Chinese chickens to develop commercial and show breeds (3). The authors also imply that the Indian/Asian/European mtDNA signature identified in our ancient Pacific and Chilean samples would not have been available for dispersal to the prehistoric Pacific. This is refuted by linguistic, archaeological, and ethnohistoric evidence (4).

Ultimately, the question rests on the antiquity of the El Arenal chickens. We have directly dated and sequenced two additional chicken bones from the site, which is not a shell midden as claimed (1). Stable isotope determinations (δ ^{13}C, δ ^{15}N, and δ ^{34}S) further confirm a terrestrial dietary signature; thus, no marine calibration of the dates is required (Table 1). All dates obtained from the site are securely pre-Columbian (even at 2σ), consistent with the stratigraphic and artifactual evidence. Therefore, the most parsimonious explanation continues to be that chickens were first introduced to South America by Polynesian voyagers as part of a well-documented eastward expansion.

Table 1. Radiocarbon and isotope data for archaeological chicken bones and associated thermoluminescence dates obtained from pottery from the El Arenal-1 site in Chile

Sample no.	Lab no.	Material	Date	Calibrated age (2σ)	δ ^{13}C, ‰	δ ^{15}N, ‰	δ ^{34}S, ‰	P, Gy	D, Gy/year
CHLARA001	NZA 26115	Chicken bone	622 ± 35 BP	AD 1304–1424	−20.9	ND	ND		
CHLARA003	NZA 28271	Chicken bone	510 ± 30 BP	AD 1427–1459	−19.85	2.6	2.16		
CHLARA004	NZA 28272	Chicken bone	506 ± 30 BP	AD 1426–1457	−19.45	3.5	ND		
EA1-001	UCTL 1617	Pottery	650 ± 65 BP	AD 1285–1415				1.14 ± 0.11	1.76×10^{-3}
EA1-002	UCTL 1618	Pottery	610 ± 55 BP	AD 1335–1445				0.96 ± 0.11	1.58×10^{-3}

All ^{14}C dates were calibrated with CALIB (5) by using the Southern Hemisphere atmospheric curve (6). P, Paleodose; D, dose rate.

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1. Gongora J, *et al*. (2008) Indo-European and Asian origins for Chilean and Pacific chickens revealed by mtDNA. *Proc Natl Acad Sci USA* 105:10308–10313.
2. Storey AA, *et al*. (2007) Radiocarbon and DNA evidence for a pre-Columbian introduction of Polynesian chickens to Chile. *Proc Natl Acad Sci USA* 104:10335–10339.
3. Crawford RD (1984) in *Evolution of Domesticated Animals*, ed Mason IL (Longman, London), pp 298–311.
4. Bellwood P (2007) *Prehistory of the Indo-Malaysian Archipelago* (Univ of Hawaii Press, Honolulu).
5. Stuiver M, Reimer PJ (1993) Extended 14C data base and revised CALIB 3.0 14C Age calibration program. *Radiocarbon* 35:215–230.
6. McCormack FG, *et al*. (2004) SHCal04 Southern Hemisphere calibration, 0–1000 cal BP. *Radiocarbon* 46:1087–1092.

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LETTER

Reply to Storey *et al*.: More DNA and dating studies needed for ancient El Arenal-1 chickens

In their letter, Storey *et al*. (1) concede that there is no direct genetic support for Polynesian–South American contact. However, they claim that linguistic, archaeological, and ethnohistoric evidence supports Polynesia as the most likely source of the El Arenal-1 chickens. We disagree on two grounds. First, such indirect evidence is conjectural, documents no eastward expansion to South America, and says nothing about the prehistoric availability of particular mtDNA haplotypes. Second, our central point was that analyses of all available ancient (2) and modern chicken mtDNA data reveal that the El Arenal-1 chicken carries a worldwide genetic signature potentially available to any of the possible introduction routes via Europe, Asia, and Polynesia (3). In contrast, none of the unusual genetic signatures from Easter Island chickens have been reported from South America (3).

The argument rests entirely on the radiocarbon dates. Current isotopic data indicate a fully terrestrial dietary signature (1). However, contrary to Storey *et al*. (1), El Arenal-1 is indeed a midden where chicken bones were found associated with marine organisms (4), and there are no local isotopic standards available to confirm the relationship between diet and isotopic signatures. Any marine input for the two new dates (1) would be consistent with a post-Columbian chronology. A region-specific set of isotopic standards and radiocarbon and stable isotope determinations for a large number of specimens of several species at the site are required as a matter of priority including dating additional chicken bones in independent laboratories to ensure reliable radiocarbon measurements (5).

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1. Storey A, *et al*. (2008) Pre-Columbian chickens, dates, isotopes, and mtDNA. *Proc Natl Acad Sci USA* 105:E99.
2. Storey AA, *et al*. (2007) Radiocarbon and DNA evidence for a pre-Columbian introduction of Polynesian chickens to Chile. *Proc Natl Acad Sci USA* 104:10335–10339.
3. Gongora J, *et al*. (2008) Indo-European and Asian origins for Chilean and Pacific chickens revealed by mtDNA. *Proc Natl Acad Sci USA* 105:10308–10313.
4. Contreras L, Quiroz D, Sanchez M, Caballero C (2005) *Actas del XVI Congreso Nacional de Arqueologia Chilena*, Ceramios, maices y ranas. . . un campamento El Vergel en las costas de Arauco (Museo de Historia Natural de Concepcion, Direccion de Bibliotecas, Archivos y Museos, Sociedad Chilena de Arqueologia, Concepcion, Chile), pp 357–367.
5. Wilmshurst JM, Anderson AJ, Higham TFG, Worthy TH (2008) Dating the late prehistoric dispersal of Polynesians to New Zealand using the commensal Pacific rat. *Proc Natl Acad Sci USA* 104:7676–7680.

Author contributions: J.G., N.J.R., V.A.M., H.J., J.A.A., J.T.M., O.H., C.M., J.J.A., S.U., A.J.A., G.L., and A.C. wrote the paper.

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APPENDIX THREE

Evolutionary history of the Falklands wolf

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study is now required to address several open questions. Most pertinently: how do ants encode skyline information? And, how do ants derive a movement direction from the comparison of their current skyline view and their remembered view? Recent experiments, also with *M. bagoti* [10], have addressed the first question. By masking different portions of the visual panorama, it was possible to show that being able to view prominent skyline features at high retinal elevations was neither necessary nor sufficient for successful orientation. This suggests that *M. bagoti* derive information from a broad range of azimuthal directions rather than a small set of the most prominent features.

Supplemental Data
Supplemental data are available at http://www.cell.com/current-biology/supplemental/S0960-9822(09)01585-1

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References

1. Wehner, R., Michel, B., and Antonsen, P. (1996). Visual navigation in insects: coupling of egocentric and geocentric information. J. Exp. Biol. *199*, 129–140.
2. Collett, T.S., Graham, P., and Durier, V. (2003). Route learning by insects. Curr. Opin. Neurobiol. *13*, 718–725.
3. Collett, T.S., Graham, P., Harris, R.A., and Hempel-De-Ibarra, N. (2006). Navigational memories in ants and bees: memory retrieval when selecting and following routes. Adv. Stud. Behav. *36*, 123–171.
4. Fukushi, T. (2001). Homing in wood ants, *Formica japonica*: use of the skyline panorama. J. Exp. Biol. *204*, 2063–2072.
5. Collett, T.S., Graham, P., and Harris, R.A. (2007). Novel landmark-guided routes in ants. J. Exp. Biol. *210*, 20–25.
6. Towne, W.F., and Moscrip, H. (2008). The connection between landscapes and the solar ephemeris in honeybees. J. Exp. Biol. *211*, 3729–3736.
7. Collett, M., Collett, T.S., Bisch, S., and Wehner, R. (1998). Local and global vectors in desert ant navigation. Nature *394*, 269–272.
8. Möller, R. (2002). Insects could exploit UV-green contrast for landmark navigation. J. Theor. Biol. *214*, 619–632.
9. Stürzl, W., and Zeil, J. (2007). Depth, contrast and view-based homing in outdoor scenes. Biol. Cyber. *96*, 519–531.
10. Graham, P., and Cheng, K. (2009) Which portion of the natural panorama is used for view-based navigation in the Australian desert ant? J. Comp. Physiol. A. *195*, 681–689.

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Evolutionary history of the Falklands wolf

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After visiting the Falkland Islands during the voyage of the Beagle, Charles Darwin remarked on the surprising presence of a wolf-like canid unique to the islands [1]. One hundred and forty years after its extinction [2], the evolutionary relationships of this unusual canid remain unresolved. Here, we present a phylogenetic analysis based on nuclear and mtDNA sequence data from the extinct Falklands wolf and find that its closest extant relative is the South American maned wolf. Molecular dating analyses suggest that the Falklands wolf and several extant South American canid lineages likely evolved in North America, prior to the Great American Interchange. The Falklands wolf was the sole representative of a distinct South American canid lineage that survived the end-Pleistocene extinctions on an island refuge.

The Falklands wolf (*Dusicyon australis*) was the only endemic terrestrial mammal inhabiting the Falkland Islands until its extinction in 1876 [1,2]. Since its description by Darwin, the occurrence of this large, wolf-like canid on a pair of islands 480 km from the eastern coast of Argentina has remained a mystery [1–4]. Although formerly abundant on the islands [1,4], few museum specimens of the species have been preserved. The Falklands wolf presents a puzzling combination of craniodental characteristics, resulting in controversial taxonomic alliances with domestic dogs (*Canis familiaris*) [2], the North American coyote (*Canis latrans*) [5], and South American foxes (*Pseudalopex* and *Cerdocyon*) [5,6]. Hypotheses for its presence on the islands, which have never been connected to the South American mainland [7], have ranged from dispersal by ice or logs [3,7], to domestication and subsequent transport by Native Americans [2].

We sequenced the complete *cytochrome oxidase II* mitochondrial gene and part of the *cytochrome b* gene from five museum specimens of Falklands wolf, including one specimen collected by Darwin himself (see Supplemental data published with this article online for details). We also sequenced four nuclear loci containing phylogenetically informative polymorphisms. Phylogenetic analyses of these datasets using neighbour joining, maximum likelihood and Bayesian inference do not recover a close relationship between the Falklands wolf and South American foxes (*Cerdocyon* and *Pseudalopex*) or *Canis* species, as suggested by morphological data [2,5,6]. In contrast, we found a surprising and well-supported sister group relationship to the South American maned wolf (*Chrysocyon brachyurus*) (Figure 1). Given our limited sequence data, we were unable to recover a monophyletic South American canid clade, or a clade comprising the maned wolf and bush dog (*Speothos venaticus*) in our unconstrained analyses. However, a topology constrained to include these well-documented relationships is not significantly less likely than the tree produced by unconstrained analyses (see Supplemental data). Furthermore, the monophyly of both the South American canids and the maned wolf/Falklands wolf/bush dog clade are independently supported by nuclear polymorphisms (Figure 1). Although the maned wolf is the closest extant relative of the Falklands wolf, a morphologically similar species, *Dusicyon avus*, survived in South America until the mid-Holocene [7]. This species may have an even closer relationship with the Falklands wolf and warrants further investigation.

Both Darwin [1] and Fitzroy [3] were surprised by the striking differences between the Falklands wolf and the canids of the South American mainland. We estimated the divergence time between the Falklands wolf and maned wolf as 6.7 million years ago (mya) (Figure 1; 95% highest probability density (HPD) = 4.2–8.9 mya). Such a long divergence time, coupled with subsequent ecological isolation on the Falkland Islands may account for the pronounced morphological

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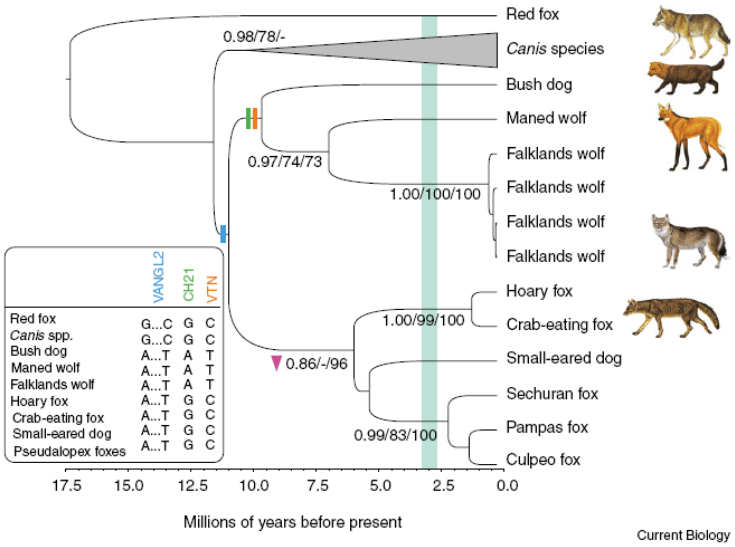

Figure 1. Chronogram showing phylogenetic relationships of the Falklands wolf.

Colour bars indicate phylogenetically informative polymorphisms shared by the Falklands wolf. The Falklands wolf also lacks an insertion (purple arrowhead) specific to the South American foxes. The shaded green bar indicates formation of the Panamanian isthmus. Node support values are Bayesian inference/maximum likelihood/neighbour-joining. Values < 70% are indicated by a hyphen. Canid images with permission from D.W. MacDonald and P. Barrett (1999). Mammals of Europe (London: Harper Collins); J.F. Eisenberg and K.H. Redford (1999). Mammals of the Neotropics, Volume 3 The Central Neotropics: Ecuador, Peru, Bolivia, Brazil (Chicago: Chicago University Press); and St. G. Mivart (1890). A Monograph of the Canidae: Dogs, Jackals, Wolves, and Foxes (London: Porter).

divergence between these taxa. Furthermore, based on our dating analyses and fossil evidence, we suggest that most South American canid lineages originated in North America [7,8]. At least six exclusively South American canid lineages, including the Falklands wolf, originated prior to the formation of a Panamanian land bridge approximately 3 mya (Figure 1). Canids are not recorded in the South American fossil record until the late Pliocene (Uquian, 2.5–1.5 mya [7]), while three South American canid lineages are recorded in the North American fossil record before this time (*Cerdocyon,* 6–5 mya; *Chrysocyon,* 5–4 mya; *Theriodictis,* 5–4 mya; [9]). The South American canids probably evolved from the fossil taxon *Eucyon,* which was widespread in North America during the late Miocene [9]. The ultimate extinction of South American canid lineages in North America may have resulted from resource competition with *Canis*, which immigrated to the New World during the late Pliocene [7,9].

Based on mtDNA sequence analysis, we estimated the age of the most recent common ancestor of our Falklands wolf samples to be 330 thousand years ago (kya) (Figure 1; 95% HPD = 70–640 kya). Genetic and archaeological evidence suggests that humans first arrived in the New World no earlier than 20–15 kya [10], implying that a human-mediated origin of the Falklands wolf is unlikely [3,9]. The Falklands wolf may have reached the islands by rafting or dispersing over glacial ice [3,9] during the late Pleistocene and was probably able to survive into the recent past by subsisting on a rich diet of penguins, geese and pinnipeds [1,4]. Unfortunately, by the time Darwin described the species, its exploitation for the fur trade was well underway. Forty years later, the Falklands wolf was extinct, ending a long evolutionary process of the kind central to the development of Darwin's theories.

Supplemental Data

Supplemental data are available at http://www.cell.com/current-biology/supplemental/S0960-9822(09)01695-9.

Acknowledgments

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References

1. Darwin, C. (1838). The zoology of the voyage of H. M. S. Beagle, under the command of Captain Fitzroy, R. N., during the years 1832 to 1836 (London: Smith, Elder and Co, London).
2. Clutton-Brock, J., Corbet, G.G., and Hills, M. (1976). A review of the family Canidae, with a classification by numerical methods. Bull. Brit. Mus. Nat. Hist. *29*, 119–199.
3. FitzRoy, R. (1839). Narrative of the surveying voyages of His Majesty's Ships Adventure and Beagle between the years 1826 and 1836, describing their examination of the southern shores of South America, and the Beagle's circumnavigation of the globe. Proceedings of the second expedition, 1831-36, under the command of Captain Robert FitzRoy, R.N. (London: Henry Colburn).
4. Gallagher, R.E. (1964). Byron's Journal of his Circumnavigation 1764–1766 (Cambridge: Cambridge University Press).
5. Pocock, R.I. (1913). The affinities of the Antarctic wolf (Canis antarcticus). Proc. Zool. Soc. Lond. 382–393.
6. Tedford, R.H., Taylor, B.E., and Wang, X. (1995). Phylogeny of the Caninae (Carnivora: Canidae): the living taxa. Am. Mus. Nov. *3146*, 1–37.
7. Berta, A. (1987). Origin, diversification, and zoogeography of the South American Canidae. Fieldiana: Zoology, *39*, 455–471.
8. Wayne, R. K., Geffen, E., Girman, D. J., Koepfli, K.-P. Lau, L.M. and Marshall, C.R. (1997). Molecular systematics of the Canidae. Sys. Biol. *46*, 622–653.
9. Wang, X., Tedford, R.H. and Anton, M. (2008). Dogs: Their Fossil Relatives and Evolutionary History (New York: Columbia University Press).
10. Jobling, M. A., Hurles, M.E., and Tyler-Smith C. (2004). Human Evolutionary Genetics: Origins, Peoples and Disease (New York: Garland Science).

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Supplemental Data:

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Supplemental Methods

Sample details:

We obtained samples (bone, tissue scrapings and toe pads) from Falklands wolf specimens in the following museum collections:

1. BMNH 37.3.15.47 (skull and mandible), collected by C. Darwin. Adult Male, East Falkland Island.
2. BMNH 69.2.24.3 (skull), purchased from E. Gerrard Jr. Young adult, sex unknown, locality unknown.
3. ANSP 588 (skull, mandible, metatarsus and skin), collected by Capt. Burnsee. Adult, sex unknown, locality unknown.
4. D.557 (partial skull), bequeathed by 13th Earl of Derby. Adult, sex unknown, locality unknown.
5. VT2369, (mount) origins unknown.

(ANSP = Academy of Natural Sciences, Philadelphia; BMNH = Natural History Museum, London; D = World Museum, Liverpool; VT = Otago Museum)

DNA extraction and amplification

Approximately 100 mg of soft tissue or bone sample from each specimen was ground into a fine powder and DNA was extracted and amplified using appropriate ancient DNA methods and controls in laboratories dedicated to ancient DNA handling at UCLA and the University of Adelaide. Specific extraction and PCR parameters are given below. PCR products were directly sequenced using a capillary sequencer according to the manufacturer's instructions. Cloning experiments were performed on PCR products produced at UCLA as detailed in Table S1. Sequence electropherograms were inspected manually and final alignments were assembled using the program Geneious Pro v.4.0.4 (http://www.geneious.com). Sequences were deposited in Genbank (accession numbers GQ485608-GQ485615)

Extraction and amplification methods, UCLA.

DNA extraction was performed according to [S1] with the modification that 25mM NaCl was substituted with 300mM $NaC_2H_3O_2$ (pH 5.2) in the binding buffer. Five µl of extract, together with mock extractions and negative PCR controls, were amplified in a 25 µl PCR containing: 1xAmpliTaq Gold buffer (Applied Biosystems), 1.5mM $MgCl_2$, 0.4mM primer (primer sequences available upon request), 0.2mM each dNTP (Applied Biosystems), 1.5U AmpliTaq Gold (Applied Biosystems), 0.4% BSA (Sigma-Aldrich) and sterile H_2O. PCRs were run on a Mastercycler EP Gradient S (Eppendorf, Germany) under the following conditions: initial denaturation at 94°C for 10 min; 60 cycles of denaturation at 94°C for 20 sec; primer annealing at respective temperature for 30 sec; elongation at 72 °C for 45 sec; a final elongation step at 72 °C for 30 min. PCR products were visualized under UV light on a 2.0% agarose gel stained with ethidium bromide. Successful amplifications were subjected to both direct sequencing and cloning using the TOPO TA cloning kit (Invitrogen). A minimum of eight clones were reamplified in a colony PCR with M13 primers [S2]. Subsequent to PCR and colony PCR all products were purified using an EXO/SAP digestion protocol (USB Corporation) and cycle sequenced with the respective forward and reverse primers. All PCR products were processed on a 3730 DNA Analyzer (Applied Biosystems).

Extraction and amplification methods, U. Adelaide

DNA extractions were performed using a Qiagen DNeasy Blood and Tissue Kit according to manufacturer's instructions, with the following modifications. Tissue samples were rehydrated in 1 ml of 10mM Tris pH 8.0 overnight, before extraction. Double volumes of buffer ATL, Prot. K and AL were used; carrier RNA (1 ug) was added prior to addition of buffer AL and DNA was eluted twice with 100 ul of buffer AE. One ul of extract, together with extraction controls and negative PCR controls, were amplified in a 25 ul PCR containing: 1x Platinum *Taq* High Fidelity Buffer (Invitrogen), 2 mM $MgSO_4$, 0.4 mM each primer, 0.25 mM each dNTP, 0.5 U Platinum *Taq* DNA Polymerase High Fidelity, 1mg/ml RSA (Sigma-Aldrich) and sterile H_20. PCRs were run on a Palmcycler (Corbett Research) under the following conditions: initial denaturation at 94°C for 1 min; 50 cycles of denaturation at 94°C for 15 sec; primer annealing at 55°C for 15 sec; elongation at 68°C for 30 sec; a final elongation step at 68°C for 10 min. PCR products were visualized under UV light on a 3.5% agarose gel stained with ethidium bromide. Successful amplifications were purified using Ampure (Agencourt) according to manufacturer's instructions and sequenced directly using Big Dye chemistry and an ABI 3130XL Genetic Analyzer (Applied Biosystems).

Phylogenetic analysis.

Phylogenetic analyses were performed on a concatenated matrix of 1824 bp for 16 canid taxa. Trees were rooted with the red fox (*Vulpes vulpes)*. The five Falklands wolf samples were represented by sequences of variable length (Table S1).

Neighbor joining analyses were performed in the program MEGA v.4 [S3] using the Maximum Composite Likelihood substitution model, and the pair-wise deletion option to account for missing data. 1000 bootstrap replicates were performed.

We conducted Maximum likelihood (ML) analyses in the program GARLI v.0.951 [S4]. We used a GTR+I+G model of sequence evolution, based on analysis using Modeltest v.3.7 [S5] under the AIC criterion. ML analyses were conducted three times to ensure adequate sampling across all possible trees. The consistency of results was verified using visual inspection of tree topologies and log likelihood scores of the best trees for each run. We performed 1000 bootstrap replicates to assess node support.

Bayesian analyses were performed with the program MrBayes v.3.1.2. [S6] using the GTR+I+G model, as above, and default priors. We ran two simultaneous analyses, each with four chains (three heated and one cold) for 20 million generations, sampling every 1000 generations, The analysis was set stopped when the average deviation of the split frequencies indicated that the chains had converged (<0.001). The first 25% of trees were discarded as burn-in. We assessed support for clades using posterior probabilities.

Dating

We estimated the age of the divergence between the Falkland Island and maned wolf lineages using a relaxed molecular clock analysis of the concatenated mitochondrial dataset (1824 bp), with the program BEAST v.1.4.8 [S7]. A GTR+I+G model of sequence evolution was assumed (above) along with a Yule process of cladogenesis. We used four fossil calibration points [S8] as log-normal priors, as follows: 1) The most recent common ancestor of the wolf-like canids (*Canis, Lycaon,* and *Cuon*) was assigned a minimum age of 9 million years, based on the fossil taxon *Canis cipio*; 2) The most recent common ancestor of *Cerdocyon*, *Atelocynus*, *Pseudalopex*, and *Lycalopex* was was assigned a minimum age of 5 million years, based on fossil remains of *Cerdocyon* sp. from North America; 3) A minimum age of 4 million years was assumed for the most recent common ancestor of *Chrysocyon* and *Dusicyon* based on fossil remains of *Chrysocyon* from North America; and 4) we assigned a minimum age for the root (*Vulpes* + all other canids) of 9 million years, based on the first occurrence of the fossil taxon *Vulpes stenognathus*. For the first three dates, we applied a 95% confidence limit of 10 million years, based on fossil remains of the North American stem canine *Eucyon*. The calibration point for the root age was assigned a 95% confidence limit of 32 million years based on the split between Caninae and Borophaginae [S8].

Supplemental Results

All phylogenetic analyses recovered a sister group relationship between the Falklands wolf and the maned wolf. Although the maned wolf is clearly the most closely related extant taxon, it is possible that the Falklands wolf was more closely related to extinct mainland South American canids, such as *Dusicyon avus*. Attempts are curently being made to obtain DNA from this species and other taxa.

We did not recover a clade comprising (bush dog + maned wolf), as has been found in previous phylogenetic analyses based on morphology [S9], and mitochondrial [S10] and nuclear [S11] sequence data. Rather, these taxa fell as sequential sister taxa to the wolf-like canid clade, rendering the South American canids paraphyletic in the process (Figure S1). To test whether this topology was significantly preferred over a monophyletic South American canid clade with the bush dog and maned wolf as sister taxa, we performed an additional set of analyses in GARLI with the topology constrained to produce a clade comprising the bush dog, maned wolf and Falklands wolf. This topological constraint alone was also sufficient to produce a monophyletic South American canid clade. Shimodaira-Hasegawa (SH) test and Approximately Unbiased (UA) tests implemented in the program CONSEL [S12] revealed that this topology is not significantly less likely than the topology recovered based on an unconstrained analysis (SH test, p= 0.299; AU test, p=0.273). In contrast, a topology constrained to produce a clade comprising the *Pseudalopex* foxes and the Falkland Island wolf, a relationship that has previously been suggested by morphological data [S13], was rejected as significantly less likely than the topology producing a clade comprising the bush dog, maned wolf and Falkland Island wolf (SH test, p = 0.017; AU test, p = 0.008). Therefore, based on the results of previous analyses [S9-11] and the presence of diagnostic nuclear polymorphisms reported in this study that support the monophyly of the South American canids and a clade comprising the bush dog, maned wolf and Falkland Island wolf, we enforced these topological constraints in our dating analysis.

We ran our analysis for 20 million generations in BEAST, sampling every 1000 generations. We used the program TRACER v.1.4.1. (http://tree.bio.ed.ac.uk/software/tracer/) to check that convergence had been reached (effective sample size >500). The first 25% of trees were discarded as burn-in using the program Tree Annotator v.1.4.8. (http://beast.bio.ed.ac.uk/#Downloads). Mean divergence dates and 95% HPDs are shown in Table S2.

Supplemental References

S1. N. Rohland, and Hofreiter, M. (2007). Ancient DNA extraction from bones and teeth, Nature Protocols *2*, 1756-1762.

S2. C. Kilger, Krings, M., Poinar, H., and Paabo S. (1997). Colony sequencing: direct sequencing of plasmid DNA from bacterial colonies, Biotechniques *22*, 412-418.

S3. K. Tamura, Dudley J., Nei, M., and Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software Version 4.0. Mol. Biol. Evol. *24*, 1596-1599.

S4. D.J. Zwickl. (2006). Genetic Algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. PhD. thesis, University of Texas at Austin.

S5. D. Posada, and Crandall, K.A. (1998). MODELTEST: testing the model of DNA substitution, Bioinformatics *14*, 817-818.

S6. F. Ronquist, and Huelsenbeck, J.P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models, Bioinformatics *19*, 1572-1574.

S7. A.J. Drummond, and Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees, BMC Evol. Biol. *7*, 214-221.

S8. X. Wang, Tedford, R.H., and Anton, M. (2008). Dogs: Their Fossil Relatives and Evolutionary History, (New York: Columbia University Press).

S9. D.V. Ivanoff. (2007). Unlocking the ring: Occurrence and development of the uninterrupted intrabullar septum in Canidae, Mamm. Biol. *72,* 145-162.

S10. R. K. Wayne, Geffen, E., Girman, D. J., Koepfli, K.-P., Lau, L. M., and Marshall, C. R. (1997). Molecular systematics of the Canidae, Sys. Biol. *46,* 622-653.

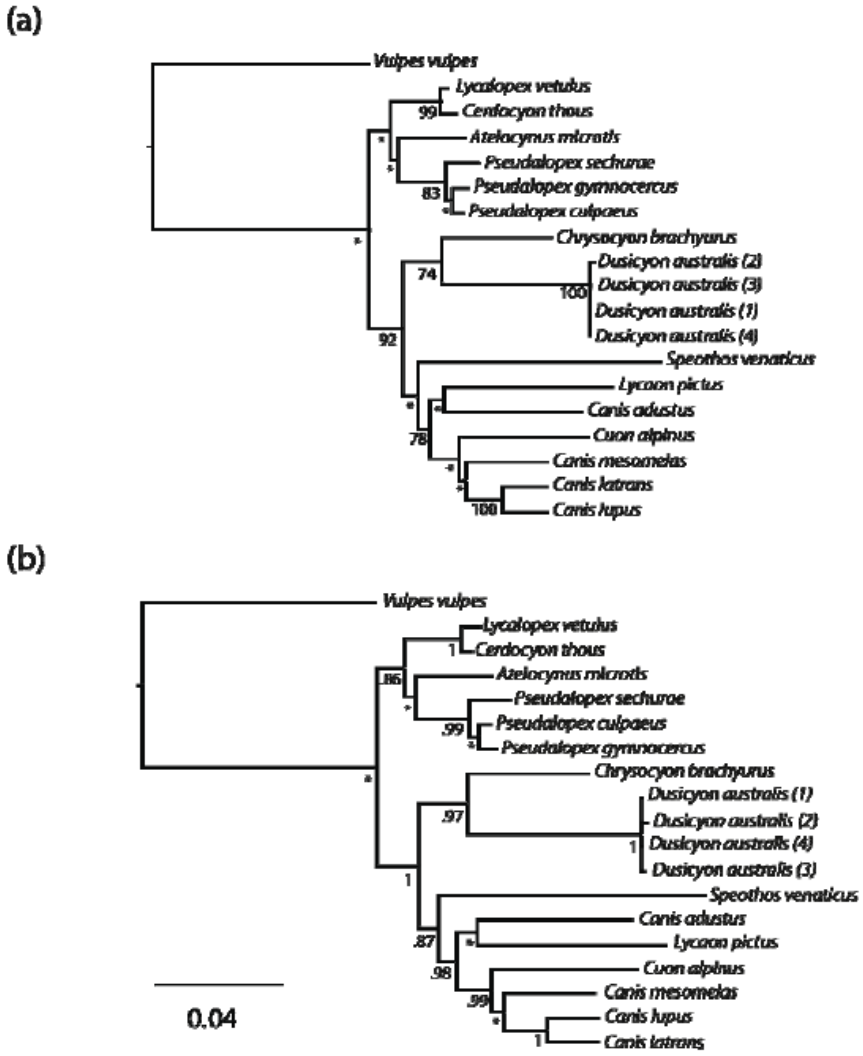
S11. Lindblad-Toh, K., Wade, C. M., Mikkelsen, T. S., Karlsson, E. K., Jaffe, D. B., Kamal, M., Clamp, M., Chang, J. L., Kulbokas, E. J., Zody, M. C., *et al*.. (2005). Genome sequence, comparative analysis and haplotype structure of the domestic dog. Nature *438*, 803-819.

S12. H .Shimodaira, and Hasegawa, M. (2001). CONSEL: for assessing the confidence of phylogenetic tree selection, Bioinformatics *17*, 1246-1247.

S13. J. Clutton-Brock. (1977). Man-made dogs, Science *197*, 1340-1342.

Supplemental Figures

Figure S1: Phylogenetic tree topologies based on (a) maximum likelihood, and (b) Bayesian inference. Both of these analyses were unconstrained. Asterixes indicate bootstrap values lower than 70% (a) and posterior probabilities lower than 0.85 (b).



Supplemental Tables

Table S1: Segments of each gene (mtDNA) or locus (nuclear DNA) sequenced and cloned for each individual of *Dusicyon australis*. Specimen numbers correspond to those given in the Supplementary Information above. We sequenced the complete *COXII* gene for two individuals, but we were only able to amplify 3 segments of *Cytochrome b*. Succesfully sequenced segments are indicated by x. Unsuccessfully sequenced and/or cloned segments are indicated by -.

COXII

Specimen	segment 1		segment 2		segment 3 gap1		segment 4		segment 5 gap2		Total
	Seq	Cloned	Seq	Cloned	Seq	Cloned	Seq	Cloned	Seq	Cloned	(bp)
1	x	8	x	8	x	16	x	8	x	8	684
2	x	8	x	5	-	-	x	8	-	-	374
3	x	-	x	-	-	-	x	-	-	-	342
4	x	8	x	8	x	8	x	6	x	8	684
5	x	-	-	-	x	-	-	-	x	-	153

Cytb

Specimen	segment 1		segment 6		segment 7		Total
	Seq	Cloned	Seq	Cloned	Seq	Cloned	(bp)
1	x	5	x	5	x	8	385
2	x	3	x	8	x	8	385
3	x	-	x	-	x	-	274
4	x	5	x	3	x	8	385
5	x	-	x	-	x	-	120

Specimen	VANGL		VTN(indel)		CH21		VTN(SNP)	
	Seq	Cloned	Seq	Cloned	Seq	Cloned	Seq	Cloned
1	-	-	-	-	-	-	-	-
2	x	-	-	-	-	-	-	-
3	x	8	-	-	-	-	-	-
4	x	-	x	8	x	4	x	5
5	x	-	x	-	x	-	x	-

Table S2: Time of the most recent common ancestor (tMRCA) for clades recovered in the BEAST analysis. Dates are given as mean age, plus the range for the 95% HPD

tMRCA	Mean age (mya)	95% HPD
(*Vulpes* (South American+wolf-like canids))	17.04	10.99-23.05
South American+wolf-like canids	11.31	10.21-12.65
Wolf-like canids	10.00	9.99-10.01
South American canids	10.71	9.03-12.35
South American foxes	5.71	5.04-6.72
Crab-eating fox+Hoary fox	1.02	0.47-1.67
Small-eared dog+*Pseudalopex* foxes	5.10	-
Pseudalopex foxes	1.95	1.13-2.85
(Bush dog (Maned wolf+Falkland Island wolf))	9.40	7.11-11.50
Maned wolf+Falkland Island wolf	6.71	4.30-8.92
Falkland Island wolves	0.33	0.07-0.64