

UNIVERSITY OF ADELAIDE

DOCTORAL THESIS

Climate-Driven Ecological Changes Through The Last Glacial Period

*Innovations in Plant Ancient DNA and Stable Isotope
Palaeoecology*

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*A thesis submitted in fulfillment of the requirements
for the degree of Doctor of Philosophy*

in the

Australian Centre for Ancient DNA
Department of Biological Sciences

September 17, 2017

Declaration of Authorship

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Abstract

School of Genetics and Evolution

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Doctor of Philosophy

Climate-Driven Ecological Changes Through The Last Glacial Period

by Mark Timothy RABANUS-WALLACE

The impact of climate-driven ecological changes can be understood by reconstructing the effects of past climate variation on the flora and fauna. This thesis develops and applies new methods for inferring the history of the graminoid-dominated steppes of the northern Holarctic and Patagonia as they declined during the end of the Last Glacial Period (25,000–10,000 years ago). Stable nitrogen isotope data are used to argue for the pivotal role that landscape moisture played in the decline of the Pleistocene megafauna, and a new method for inferring relative changes in plant-available moisture from herbivore collagen isotopic measurements is developed. Experimental methods for working with botanical ancient DNA are presented, tested, and used to explore the taxonomy and evolutionary histories of three ancient plant species, ultimately yielding the two oldest known draft chloroplast genome sequences, dating to between 50,000 and 80,000 years ago. The results confirm the strongly-reticulated phylogenies characteristic of plants evolved to employ great plasticity as an adaptive ability, even with minimal sexual reproduction. All new genetic methods are tested with the aid of a newly designed program SimWreck, which simulates sequence data with the known characteristics of ancient DNA.

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Supplementary Material

A digital Data Supplement (DS) is provided with this thesis, consisting of the following files:

DS_Readme.txt	Column-by-column descriptions of the contents of the data-files.
DS_Samples.csv	A list of herbarium specimens used in the study, with metadata on their collection location, source, etc. Referred to in text as DS:Samples.
DS_Data.csv	Metadata on libraries sequenced during the study, including read counts, quality summaries, etc. The summary stats are produced for libraries at different stages of the read-processing pipeline described in chapter 4 (raw, merged, trimmed, etc.) Referred to in text as DS:Data.
DS_Code.txt	Programs, scripts, and commands used in the analysis, with numbered sections. Referred to in text as DS:Code:Section Number.

Abbreviations

The following abbreviations are used in the thesis (commercial reagent names are omitted):

AB	Alberta
ACAD	Australian Centre for Ancient DNA
ACRF	Australian Cancer Research Foundation
AFLP	Amplified Fragment Length Polymorphism
AGRF	Australian Genome Research Facility
ATP	Adenosine TriPhosphate
BC	British Columbia
BLAST	Basic Local Alignment Search Tool
BWA	Burrows Wheeler Aligner
CDS	Coding DNA Sequences
CI	Confidence Interval
CTAB	Cetyl Trimethyl Ammonium Bromide
DNA	DeoxyriboNucleic Acid
DS	Data Supplement
EB	East Beringia
EDTA	EthyleneDiamineTetraacetic Acid
HTS	High Throughput Sequencing
IPCC	Intergovernmental Panel on Climate Change
IUPAC	International Union of Pure and Applied Chemistry
LGIT	Last Glacial Interglacial Transition
LGM	Last Glacial Maximum
LSC	Long Single Copy
NATO	North American Treaty Organisation
NCBI	National Centre for Biotechnology Information
NEB	New England Biolabs
NW	NorthWest
PCA	Principal Components Analysis
PCR	Polymerase Chain Reaction
PEG	PolyEthylene Glycol
PEU	Paired End Untrimmed
PGSB	Plant Genome and Systems Biology
PNK	PolyNucleotide Kinase
PVP	PolyVinylPyrrolidone
QBI	Queensland Biology Institute
QT	Quality Trimmed
RAD	Restriction site Associated DNA
RE	Repetitive Element
RNA	RiboNucleic Acid
RO	Reverse Osmosis

RSA	Rabbit Serum Albumen
SBS	Sequencing By Synthesis
SE	SouthEast
SET	Single End Truncated
SM	Supplementary Materials
SNP	Single Nucleotide Polymorphism
TAIR	The Arabidopsis Information Resource
USA	United States of America
UV	UltraViolet
WA	Washington
WB	West Beringia
YPP	Yukon Palaeontology Program
YT	Yukon Territory

Conventions

All times given are in absolute years (c.f. radiocarbon years) unless otherwise stated. The units used are kiloyears (ky; 1,000 years) and kiloannum (ka; 1,000 years before present).

Unless otherwise specified, logs are given in base e .

References to material in the digital data supplement are explained in the relevant section of the front matter, and prefixed with "DS:".

All original scripts written by the author are prefixed with MTRW, and are available in DS:Code.

Where necessary, samples are identified by their partial or whole filenames, which all have the following format for parsing and globbing convenience:

```
Genus_{species|nest ID}_[mod_] [enr_] libXXX###_extYYY###\
_MoreInformation.extension
```

Where the genus is followed by a species name and the identifier "mod" for herbarium specimens, and a nest ID code for permafrost specimens. The identifier "enr" is present for enriched samples. "XXX" and "YYY" are mnemonic 2-3 letter codes for libraries and extractions respectively, and "###" are unique number identifiers for samples in library prep or extraction. "MoreInformation" may contain barcodes, or details about the processing pipeline such as the reference genome if the file is a mapped reads file.

Each batch of extractions, batch of library preparations, and sequencing run is identified with a 2–3 letter identification code corresponding to the metadata available in DS:Data. The identity codes correspond to simple mnemonics: 'tan' <=> "tangled", 'dar' <=> "Darwin", 'tun' <=> "tundra", 'nd' <=> "second", 'pow' <=> "powder", 'kmg' <=> "Kendrick Marr/Geraldine Allen" (see acknowledgements), 'kw' <=> "knotweed", 'fw' <=> "fireweed", 'cm' <=> "checkmate", 'bm' <=> "Bistorta modern", 'mm' <=> "modern mix", 'ms' <=> "modern slam", 'mst' <=> "modern slam two", 'lon' <=> "longer", 'cmp' <=> "compare", 'ste' <=> "steppe", 'tc' <=> "tube crack", 'Dhiseq' <=> "Draba HiSeq", 'cmb' <=> "checkmate/bears", 'cet' <=> "chloroplast enrichment test", 'fe' <=> "fireweed enriched", 'lf' <=> "longer/fireweed". Identity codes are occasionally reused for, say, both an extraction batch and a library preparation batch.

Acknowledgements

The work presented here would not have been possible without many collaborators, colleagues, and friends. I am hugely grateful to the following people:

- For their mentorship in all things Quaternary: Matthew Wooller, Grant Zazula, Elizabeth Hall, and Sue Hewitson.
- For their hospitality: The Zazula family.
- For their enthusiastic support in the field: Families Johnson, Schmidt, and Christie, and all the Yukon goldmining community.
- Various for brainstorming sessions, trading ideas, training, sharing protocols, offering explanations, collaboration, laboratory services, proof reading, professional opportunities, and occasional debugging: All the staff at ACAD, in particular Maria Lekis, Corinne Callegari, Steve Richards, Julian Soubrier, Kieren Mitchell, Oli Wooley, Graham Gower, and Pere Bover Arbos. From elsewhere, Eric DeChaine, Ingrid Jordon-Thaden, Chris Turney, Chris Helgen, Tyler Faith, Fred Longstaffe, Natalie Betts, Geoff Fincher, Iain Searle, John Conran, Rosalie Kenyon, Joel Geoghegan, Andreas Schreiber, Janette Edson, Marc Hew-Jones, Adam Croxford, Jono Tuke, Ben Rohrlach, Alice Gorman, Cathy Miller, Steve Pedersen, Thomas Windram, Denis Sjostrom, and Jake Parker. I also owe many thanks to the staff of Cibo Kurralta Park (long black with a dash of skim) for keeping me awake, and to Lauren White for keeping me together.
- The samples used in this study are listed in the data supplement. DNA samples for modern *Bistorta vivipara* were kindly provided by Geraldine A. Mulligan and Kendrick L. Marr from the University of Victoria, B.C., Canada. Bruce Bennett of the Yukon Department of Environment, Y.T., Canada generously provided access to his personal herbarium of Yukon plants. John Conran at the University of Adelaide helpfully provided a sample of *Ranunculus repens*.

Many authors and scientists who were pivotal in developing and communicating the ideas that underlie this work. Those to whom I am most indebted include R. Dale Guthrie, Evelyn Pielou, Richard Harington, Robert Whittaker, Richard Dawkins, David Attenborough, Les Cwynar, Grant Zazula, Duane Froese, Scott Elias, Daniel Mann, Warren Evans, Gregory Grant, Larry Wall, and many more.

Finally, I am most grateful to my supervisors for their invaluable support. In particular I thank Jimmy Breen and Bastien Llamas, who helped me most closely day-to-day throughout the entire project. I greatly appreciate the freedom they gave me to think creatively and try new things, and it is a privilege to have been given the opportunity to follow the examples set by such talented scientists and supportive friends.

*To my parents, Angela Rabanus and Allan Wallace—for your love
and support, and for encouraging me to be a curious person...*