# Genetic studies on prehistoric translocations of chickens in the Indo-Pacific



**Michael James Bannister Herrera** 

Bachelor of Science (Biology)

Bachelor of Science (Archaeology)

Australian Centre for Ancient DNA
School of Biological Sciences
University of Adelaide

A thesis submitted for the degree of Doctor of Philosophy at The University of Adelaide

March 2015

### **Table of Contents**

## **Contents**

Table of Contents	iii
Thesis Abstract	vii
Declaration	ix
Acknowledgements	X
List of Tables	xii
List of Figures	xii
CHAPTER 1: General introduction	1
Introduction	2
Background to the Austronesian expansion	3
The genetic landscape of the Austronesian dispersal	5
The advent of agriculture in Island Southeast Asia & the Pacific	7
The animal domesticates in ISEA and the Pacific	9
The Pig	11
The Dog	13
The domestication and translocation history chickens	14
Tracking the translocation of chickens using genetics	19
General aims of the thesis	22
Thesis outline	23
References	26
CHAPTER 2: Island Southeast Asian origin and the dispersal of Polynesian chickens indicated by mitochondrial DNA	35
Abstract	40
Keywords	41
Introduction	41
Materials and methods	44
Sampling	44
PCR amplification and sequencing	46
Phylogeny and phylogeography	47
Population variability and structure	47
Bayesian coalescent simulation	48
Results	
Classification of chicken mtDNA control region sequences and haplogroup distribu	
	Ε0

Population structure	53
Distribution and diversity of haplogroup D	56
Phylogenetic relationship of D haplotypes from ISEA and the Pacific	59
Population dynamics of chickens in ISEA and the Pacific	60
Expansion history testing using BayeSSC	62
Discussion	62
Conclusion	65
Acknowledgement	66
References	67
Supplementary information	70
CHAPTER 3: East African origins for Malagasy chickens as indicated by mi	
Abstract	80
Keywords	81
Introduction	81
Materials and Methods	84
Results	86
Mitochondrial haplogroup distribution patterns	86
Population genetic structure	87
Population genetic variability and dynamics	89
Phylogenetic relationships of Madagascan mtDNA haplotypes in East A	
Discussion	93
Conclusion	
Acknowledgement	96
References	97
Supplementary information	100
CHAPTER 4: Mitochondrial DNA genomes resolve the genetic origins of Pochickens	•
Abstract	110
Keywords	111
Introduction	111
Materials and methods	114
Sample collection, DNA extraction, library preparation, hybridisation-e sequencing	
Network analysis & phylogenetic construction	118

Population genetic statistics and structure	119
Results	119
Sequence and phylogenetic network of Gallus gallus	119
Haplogroup D in the Asia-Pacific	122
Phylogenetic reconstructions of haplogroup D mitochondrial genomes	124
Comparisons between evolutionary inferences based on WMG and mtDNA-CR	126
Discussion	128
Conclusion	131
Acknowledgement	131
References	132
Supplementary information	134
CHAPTER 5: Exploring the population history of chickens in the Asia-Pacific using genome-wide single nucleotide polymorphism	139
Abstract	144
Keywords	145
Introduction	145
Materials and methods	149
Samples and SNP genotyping array	149
Detection of loci under selection & population structure analysis	151
Results	152
Phylogenetic inference	152
Outlier loci detection & neutral population structure	154
Discussion	158
Conclusion	161
Acknowledgment	161
References	162
Supplementary information	166
CHAPTER 6: Concluding discussion	169
Aim 1: Genetic characterisation of Indo-Pacific chickens	172
Aim 2: Reconstruct the Austronesian expansion using a proxy species	177
Aim 3: Assessing the resolution of human-mediated dispersal histories of chickens u control region, whole mitochondrial genome, and genome-wide SNP based histories	•
Concluding remarks	185
References	187

#### Thesis Abstract

The study conducted in this thesis examines the genetic population history of chickens in the Indo-Pacific region in order to infer the prehistoric human-mediated translocation of chickens and investigate whether the dispersal history of chickens in this region parallels the Austronesian expansion. The research focuses on chicken populations found in Island Southeast Asia, Pacific Ocean, and Indian Ocean — regions where Austronesian languages are spoken. The islands and archipelagos found in this region are separated by vast distances of ocean, thus the dispersal of chickens within this region is mediated only through human agency. The geographic distribution of genetic variation in chickens of this region is due only to humans translocating chickens during their voyages, thus this genetic information can be utilised to examine the expansion of the Austronesian-speaking people.

A genetic survey that spans two oceans is challenging, thus the study relied mostly on modern chicken DNA and available ancient DNA to reconstruct events that transpired several millennia ago. The use of modern DNA allowed comparison with reference sequences from across the globe, whereas ancient DNA allowed population continuity to be tested – *i.e.*, whether the modern specimen still represents past populations. The phylogeographic and population genetic analyses on these chickens provided unparalleled insights into the prehistoric translocation history of chickens in the Indo-Pacific region. These have allowed us to confirm the Philippine homeland of the Polynesian chickens and find the east African proximate population source for chickens in Madagascar. Furthermore, the study supports that chickens were dispersed into the Pacific along with the Austronesian expansion, but not in the Indian Ocean. The study also revealed original insights and highlights the complex

picture about the population history and human-mediated dispersals of chickens in the Indo-Pacific. This complexity is brought by the fact that the prehistoric translocation of chickens cannot be solely attributed to one dominant human group or expansion event that occurred in the region. Therefore, it is paramount to use archaeological and linguistic narratives to explain the genetics of chickens and reach the best inference possible about their history.

This research demonstrates the usefulness of using genetic studies on chickens in elucidating the origins and routes of prehistoric translocations and Austronesian expansion in the Indo-Pacific. This study advances our knowledge about prehistoric dispersal of chickens in the Indo-Pacific region and will precipitate exciting new avenues of research.

**Declaration** 

I certify that 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. In

addition, I certify that no part of this work, in the future, be used in a submission of

any other degree or diploma in any university or other tertiary institution without

prior approval of the University of Adelaide and where applicable, any partner

institution responsible for the join-award of this degree.

I give consent to this copy of my thesis when deposited in the University Library,

being made available for loan and photocopying, subject to the provision of the

Copyright Act 1968.

I also give permission for the digital version of this thesis to be made available on the

web, via the University's digital repository, the Library catalogue and also through

web search engines, unless permission has been granted by the University to restrict

access for a period of time.

Michael James Bannister Herrera

March 2015

ix

#### Acknowledgements

Learning to become a proper researcher was indeed fraught with challenges. What is normally unbearable has become bearable because of the so many kind people who believed, supported, and encouraged me. I am forever grateful to have had the chance to work with all of you. I want to express my sincerest gratitude to them who have been a part in the creation of this work.

- DR. JEREMY AUSTIN, my PhD adviser, for his unwavering guidance and patience, unfailing support, and constant stirring during the conduct of this research. As my mentor, he was an invaluable source of inspiration. His guidance was catalytic in the fruition of this work. Thank you for believing in me, Jeremy.
- DR. VICKI THONSON, my co-supervisor, for all her able counsel and support during the conduct of this research. She patiently answered all of my questions as well as asked questions back to help me to better my work. She afforded her valuable time and guided me throughout my research. For which, I am truly thankful.
- DR. ALAN COOPER, my co-supervisor, for all the guidance and suggestions during the planning stage of my research. He has always been a source of inspiration.
- DR. JESSICA WADLEY, my mentor, for all the help and guidance that she afforded me. She patiently and carefully discussed concepts and ideas with me, which was helpful in the completion of my thesis. You have always been there, for which, I am very thankful.
- DR. PHILIP PIPER, my external co-supervisor, thank you so much for supporting me throughout my PhD journey. I am forever indebted to you for your unwavering support and guidance.
- DR. JULIEN SOUBRIER, DR. WOLFGANG HAAK, & KEIREN MITCHEL, thank you very much for providing me with technical support and assistance. I would not have been able to make it without you guys.
- DR. JAIME GONGORA, thank you very much for providing the valuable Indonesian chicken samples, for they were a critical component of the research. Thank you as well for the guidance and support.

I also express my thanks to all the people who have in part allowed me to accomplish this work; this will include museum people (American Museum of Natural History, National Museum of Natural History) and all the people who helped me during my sampling trip.

I would also like to extend my gratitude to my all friends who helped me put some cheer in my face during trying times.

Finally, I would like to express my deepest thanks to my family. Although very far, they were always there when I needed a shoulder to lean on.

## **List of Tables**

Table 2-1. Sampling locations, sample sizes, haplogroup and haplotype assignments for 6169 worldwide chicken samples
Table 2-2. Population genetic structure estimated from the analysis of molecular variance (AMOVA) based on mtDNA
Table 2-3. Population genetic summary statistics for regional mtDNA haplogroup D sequences
Table S2-1. Samples used in the study. (Excel File/ CD-ROM)
Table S2-2. Population genetic summary statistics for the study populations worldwide
Table 3-1. Population genetic structure estimated from the analysis of molecular variance (AMOVA) based on mtDNA
Table 3-2. Population pairwise (F <sub>ST</sub> ) between chicken samples from Indonesia, South Asia, East Africa, and Madagascar90
Table 3-3. Genetic diversity measures and historical demographic patterns of chickens from Indonesia, South Asia
Table S3-1. Samples used in the study. (Excel File/ CD-ROM)
Table S3-2. Genetic diversity measures for each chicken population from Indonesia, South Asia, Continental Africa
Table S4-1. Samples used in the study, reference number, description
Table S4-2. Sequencing library structure and PCR primers used for the construction of the sequencing libraries
Table 5-1. List of chicken samples successfully genotyped
List of Figures
Figure 1-1. Map of Southeast Asia and the Pacific regions showing the demarcation between Near and Remote Oceania
Figure 1-2. Worldwide map showing the regions where agriculture approximately developed (Diamond & Bellwood 2000)
Figure 2-1. Sampling localities of modern and historical chickens collected in this study from Mainland Southeast Asia

Figure 2-2. Neighbour-joining tree showing the relationships between 5 27 mtDNA control region haplotypes (201bp) from 6169
Figure 2-3. Multidimensional scaling plots (MDS) on population pairwise F <sub>st</sub> for (a) 6169 worldwide chicken samples
Figure 2-4: Frequency of mtDNA haplogroup D (green), D haplotypes containing the 4-SNP Polynesian motif (red), and other haplogroups
Figure 2-5. Median-joining network of 25 D haplotypes found in the Pacific including Polynesian haplotypes from
Figure 2-6. Mismatch distribution patterns for mtDNA control region haplogroup D samples from chickens sampled in the Philippines
Figure S2-1. Serial Coalescent Simulation and Approximate Bayesian  Computation models for the reconstruction chicken translocation
Figure S2-2. Multidimensional scaling plots (MDS) on population pairwise Fst for (a) world-wide chicken samples from haplogroup E only
Figure 3-1. Frequency distribution of chicken mitochondrial DNA haplogroup ( <i>blue</i> – haplogroup D, <i>white</i> – haplogroup E
Figure 3-2. Multidimensional scaling plots (MDS) on population pairwise F <sub>ST</sub> scores for (A) 3115 chickens from Asia ( <i>Orange</i> )
Figure 3-3A. Median-joining network depicting the relationship of D haplotypes of chickens from East Africa and Madagascar
Figure 3-3B. Median-joining (MJ) network of mtDNA-CR D haplotypes observed in Africa ( <i>blue</i> ), South Asia ( <i>brown</i> ), Indonesia
Figure S3-1. Principal Coordinate Analysis (PCoA) via covariance matrix of pairwise genetic distances of D haplotypes observed
Figure S3-2. Neighbour-joining tree of the 429 haplotypes generated by the 349 bp dataset used in the study. Haplogroup D and E
Figure S3-3. Median-joining network depicting the relationship of the E haplotypes observed in East Africa and Madagascar
Figure 4-1. Photographs of red jungle fowl (left) and green
Figure 4-2. Median-joining network of 120 chicken mitochondrial genomes (117 haplotypes). Colours represent 13 known haplogroups
Figure 4-3. Map of the Asia-Pacific region showing the geographic distribution of 120 whole mitochondrial genomes

Figure 4-4. Median-joining network of 64 haplogroup D mitochondrial genomes (62 haplotypes). Colours represent the geographic	122
Figure 4-5. PCoA based on the genetic distances of haplogroup D mitochondrial genomes of chickens from the Asia-Pacific	124
Figure 4-6. Maximum likelihood tree of 64 haplogroup D chicken mitochondrial genomes constructed using a GTR+G model	125
Figure 4-7. Phylogenetic networks of 120 chickens based on (a) whole mitochondrial genome, (b) control region, and (c) 201 bp	127
Figure S4-1: Examples of DNA map damage profiles for (A) ancient (ACAD 3896) and (B) museum (ACAD 14814) samples	134
Figure 5-1. Maximum likelihood tree of 51 chickens based on genome-wide SNPs constructed using MULTIGAMMA model	153
Figure 5-2. Examples of LOSITAN plots identifying outlier SNP loci, with blue dots representing SNPs.	154
Figure 5-3. Principal coordinate analysis performed using Eigensoft on genome-wide neutral SNPs of chickens from the	156
Figure 5-4. Principal coordinate analysis performed using Eigensoft on genome-wide neutral SNPs of chickens from the Philippines	157
Figure S5-1. Maximum likelihood tree of 51 chickens based on genome-wide SNPs constructed using MULTIGAMMA	166
Figure S5-2. Principal coordinate analysis performed using Eigensoft on genome-wide of the combined neutral and selected SNPs	167