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The Evolution of the Mitochondrial DNA Control Region in the Adélie Penguins of Antarctica

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The Adélie Penguins of Antarctica

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Abstract

Rates of evolution have been important components of many phylogenetic and population genetic studies. In Antarctica, underlying active and abandoned Adélie penguin (*Pygoscelis adeliae*) rookeries, are well preserved subfossil bones. This thesis aimed to estimate a rate of mitochondrial (mt) DNA evolution by sampling sequences from serially preserved subfossil bones through time, and compare these to sequences from modern populations. During this study four field expeditions were undertaken in Antarctica during the austral summers, resulting in the collection of over 400 blood and 329 subfossil bone samples. This thesis research showed that the Adélie penguin mtDNA control region is unusually long (1768 b.p.) and contains a repeat complex at the 3'-end. A phylogeny of 17 penguin species (suborder Sphenisciformes) was constructed from *rns* and *rnl* mtDNA gene sequences. The characteristics (i.e. heteroplasmy and length variation) of control region sequences from penguin species were plotted onto this phylogeny as a mechanism for investigating their evolution. DNA sequence variation from a total of 381 modern Adélie penguin samples revealed the presence of two distinct maternal lineages (7.1% net sequence difference). One lineage is present in all locations around Antarctica (*A*) sampled and the other was recorded only in Ross Sea populations (*RS*). The phylogeographic pattern of the *A* and *RS* lineages suggests Adélie penguins were restricted to distinct ice-age refugia during the last glacial cycle. Ancient DNA was extracted from 80 subfossil bones (¹⁴C dates ranged from 310-6082 years before present) from 16 locations on the coast of the Ross Sea. The ancient DNA from these frozen subfossil Adélie penguins is extraordinarily well preserved. Using both modern and ancient DNA sequences a rate of mtDNA control region evolution was determined. This estimated rate is five times higher than previous estimates for the avian mtDNA control region. This rate was then used to time the divergence of the two lineages *A* and *RS*, and showed they split 83 kyr BP during the last glacial cycle. Adélie penguins appear to have endured dramatic changes to their habitat during the ice-ages of the Late Pleistocene.

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Table of Contents

	<i>Page</i>
Chapter One	
<i>Rates of Mitochondrial DNA Evolution</i>	1
1.1 Estimating Substitution Rates	
1.2 Ancient DNA: The Opportunity to Measure Evolution	
1.3 Antarctica: the 'Ideal Situation'	
1.4 Thesis Outline	
1.5 References	
Chapter Two	
<i>The Mitochondrial Genome: Maternal Inheritance and Mutations</i>	12
2.1 Introduction	
2.2 Maternal Inheritance	
2.3 Mutations in the Mitochondrial Genome	
2.4 Summary Remarks	
2.5 References	
Chapter Three	
<i>A Repeat Complex in the Mitochondrial Control Region of Adélie Penguins</i>	26
3.1 Introduction	
3.2 Materials and Methods	
3.3 Results and Discussion	
3.4 References	
Chapter Four	
<i>The Evolution of the Hypervariable Region in Penguins</i>	41
4.1 Introduction	
4.2 Materials and Methods	
4.3 Results	
4.4 Discussion	
4.5 References	
Chapter Five	
<i>Two Maternal Lineages of Adélie Penguins: An Ice-Age Legacy</i>	66
5.1 Introduction	
5.2 Materials and Methods	
5.3 Results	
5.4 Discussion	
5.5 References	
Chapter Six	
<i>The Ancient DNA of Cryopreserved Adélie Penguins from Antarctica</i>	93
6.1 Introduction	
6.2 Materials and Methods	

- 6.3 Results
- 6.4 Discussion
- 6.5 References

Chapter Seven

Conclusions: Modern and Ancient Adélie Penguins 114

- 7.1 Introduction
- 7.2 Measurably Evolving Lineages
- 7.3 The Ice-Ages of Antarctica
- 7.4 Concluding Remarks
- 7.5 References

Appendix A

Field Diaries from Antarctica 132

Appendix B

DNA Sequences and Analyses 138

Appendix C

Radiocarbon Dating of Subfossil Bones 153

Appendix D

Manuscripts 162

List of Figures

	<i>Page</i>
1.1	2
1.2	4
1.3	6
2.1	19
2.2	20
3.1	30
3.2	31
3.3	34
3.4	36
4.1	48
4.2	48
4.3	51
4.4	52
4.5	52
4.6	53
4.7	55
4.8	56
4.9	58
5.1	69
5.2	73
5.3	75
5.4	76
5.5	78
5.6	79
5.7	81
5.8	81
6.1	96
6.2	97
6.3	101
6.4	102
6.5	106
6.6	107
6.7	109
7.1	115
7.2	116
7.3	125
7.4	126

List of Tables

	<i>Page</i>	
2.1	The DNA repair pathways in the mitochondria	16
4.1	Penguin species sampled for <i>rns</i> , <i>rnl</i> and CR sequences	43
4.2	Pairwise comparison of sequence difference for <i>rns</i> and <i>rnl</i>	49
4.3	Primers using to amplify the CR from penguins	55
4.4	Pairwise comparison of sequence difference for the CR	57
5.1	Summary statistics of the HVR-1 in Adélie penguins	72
5.2	Pairwise comparisons of ϕ_{ST} and their significance test	74
5.3	Contingence χ^2 -tests and lineage frequencies	77
5.4	Maximum likelihood estimates of demographic models	80
6.1	Radiocarbon dates of subfossil bones	102
6.2	Assigned ages of each subfossil bone	103-4
7.1	Estimated evolutionary rates for the mtDNA	120

List of Abbreviations

A	adenine
A	Antarctic (mtDNA lineage type I)
AMS	accelerator mass spectrometry
BSA	bovine albumin serum
BER	base excision repair
b.p.	base pairs
C	cytosine
¹⁴ C	carbon-14
°C	degree Celsius
CR	control region
<i>d</i>	DNA sequence distance
D	dextro
g	grams
G	guanine
<i>h</i>	haplotypic diversity
H	heavy
HVR-1	hypervariable region I
HVR-2	hypervariable region II
kb	kilo bases
L	light (mtDNA) or levo (amino acids)
<i>L</i>	likelihood distances
LRT	likelihood ratio test
m	metres
M	moles
ML	maximum likelihood
MP	maximum parsimony
mt	mitochondrial
mtDNA	mitochondrial DNA
<i>n</i>	sample size
π	nucleotide diversity
NJ	neighbor-joining
<i>P</i>	probability
RRT	relative rate test
RS	Ross Sea (mtDNA lineage type II)
s/s/Myr	substitutions/site/million years
T	thymine
ti	transition
tv	transversion
μ l	microlitres
UPGMA	unweighted paired-group method with arithmetic means
UV	ultra-violet
yr	years
yr BP	years before present

Animal Ethics and Antarctic Permits

All blood sampling of Adélie penguins from Antarctica was performed under approval of the Massey University Ethics Committee, protocol numbers 96/146 and 99/160. Approval to restrain, take blood from Adélie penguins, and to enter Specially Protected Areas (SPA) and Sites of Special Scientific Interest (SSSI) was given by *Antarctica New Zealand* permit numbers 96/4, 97/5, 98/7, 99/9.