Microsatellite Evolution and Population Genetics of Ancient and Living Adélie penguins in Antarctica.

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Errata

Page	
24	**Table 2.1** The length of the clone sequences that are known are as follows: TP500 121 bp RM3 223 bp RM6 169 bp FhU2 75 bp
78	**Table 4.4** Sequences are listed 5' to 3'
79	**Table 4.5** Sequences are listed 5' to 3'

Abstract

Microsatellites are widely used as genetic markers for examining a variety of biological questions. Despite their widespread use, little is known about the processes by which they evolve. An accurate understanding of these processes is essential for their correct use as population genetic markers. In this study, microsatellite loci from both living and cryopreserved (AMS ^{14}C dated at up to 6424 years BP ±80) Antarctic Adélie penguins (*Pygoscelis adeliae*) were examined in order to gain insights into temporal population genetics and the evolution of microsatellite loci.

Firstly, ancient DNA extracted from Adélie penguin subfossil bones was found to be extremely well-preserved and readily allowed the amplification of single-copy nuclear microsatellite DNA. Genotyping six microsatellite loci in ancient and living samples from three populations of Adélie penguins in the Terra Nova Bay region allowed a comparison of genetic change over time. Although the ancient sample sizes were limiting, several statistical tests indicated that the ancient and living populations from Inexpressible Island were genetically distinct. In addition, differentiation was also inferred between the three ancient populations that were examined, which is in contrast to the lack of differentiation found between the living populations. These genetic changes may be a result of population expansion out of ice-age refugia since the Last Glacial Maximum.

To study microsatellite evolution over a substantial time period, up to 500 living and 100 cryopreserved Adélie penguins were genotyped at six microsatellite loci. No novel electromorph alleles were detected in the ancient samples. Numerous alleles were sequenced from four of these loci in both Adélie penguins and several other species of penguin (Spheniscidae). Analysis of these sequences provided an insight into the mutational processes occurring at these loci. In particular, these allele sequences revealed extensive size homoplasy, both within Adélie penguins and between penguin species. At one locus, variation in the flanking region allowed discrimination between the mechanisms proposed for length change at microsatellite loci. Slippage was the most plausible mechanism for length change. In this same locus, instability was observed in the

region bordering the repeat tract with a transversional bias predominating. This bias may be a caused by inaccurate DNA replication resulting from structural features of DNA.

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This project could not have been done without assistance from a large number of people - so thanks to everyone who contributed to this project, for example by collecting samples or extracting DNA. These contributions are outlined in the following preface.
Lastly, I want to thank Leon Perrie, my parents and George for all the encouragement and assistance they have given me whilst doing this thesis. Your support is greatly appreciated (although I may not always show it!).

Preface

The research undertaken for this thesis was part of a much larger research project studying Adélie penguins. Other research being undertaken includes an examination of population structure in living Adélie penguins (Roeder et al., in press), calculation of the mutation rate of the mitochondrial control region using ancient and living samples (Ritchie, 2001) and the development of nuclear and mitochondrial genetic markers for penguins (Roeder et al., submitted). Consequently much of the work discussed in this thesis was carried out in collaboration with others.

The contributors to various aspects of this project are given below.

Sample collection

Adélie blood samples and subfossil bones were collected by Peter Ritchie, Paul Barrett (Massey University) and Craig Miller (University of Auckland). Subfossil bones were also collected by Carlo Baroni (University of Pisa). Blood or tissue samples from other penguin species were provided by the following people: Graeme Elliot, Kath Walker and Peter Moore (Department of Conservation); Boris Culik (Institut für Meereskunde an der Universität Kiel, Germany); Allan Baker (Royal Ontario Museum); Cindy Hull (University of Tasmania); Corey Bradshaw (Otago University); Janier González, Gerry Kooyman (Scripps Institution of Oceanography); John Darby and Ian Mclean (Otago Museum).

DNA extractions

The Adélie blood samples were extracted by Richelle Marshall, Peter Ritchie, Amy Roeder and Sarah Eyton (Massey University). DNA was extracted from the Adélie subfossil bone samples, PE7 to PE136, by Peter Ritchie. I extracted samples PE137 to PE196. The tissue and blood samples from other species were extracted by Amy Roeder and Peter Ritchie (Massey University), and Kerri-Anne Edge (Otago University). I re-extracted DNA from the Chinstrap and Emperor samples used in this thesis.

Microsatellite Genotyping

540 genotypes from living Adélie penguin are referred to in this thesis. I genotyped the 98 living samples from the Terra Nova Bay region (Inexpressible Island, Northern Foothills and Edmonson Point). I also genotyped all of the ancient samples. The remaining samples were genotyped by Richelle Marshall, Amy Roeder (Massey University), Amanda Mitchelson and Helen McPartlan (Victorian Institute of Animal Sciences).

DNA Sequencing

I did all the microsatellite DNA sequencing in both Adélie and other penguin species.

Data Analysis

I conducted all the analyses reported in this thesis.

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List of Abbreviations

A	Adenine
AMS	Accelerator mass spectrometry
bp	base pairs
BSA	bovine serum albumin
C	cytosine
^{14}C	Carbon-14
DNA	Deoxyribonucleic acid
G	guanine
IAM	infinite allele model
indel	insertion/deletion
KAM	K-allele model
kb	kilobases
LGM	last glacial maximum
M	moles
n	number of samples
p	Probability
SSCP	Single-stranded conformation polymorphism
SMM	stepwise mutation model
T	thymine
TPM	two phase model
UCO	unequal crossing-over
yrs BP	years before present