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**Molecular ecology of the endangered
Gouldian Finch *Erythrura gouldiae***

Thesis submitted by:

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For the degree of Doctor of Philosophy
In Conservation Biology
Within the School of Marine and Tropical Biology
James Cook University
Townsville, Queensland, Australia
July 2008

*A mis padres:
Graciela y Víctor*

STATEMENT OF ACCESS

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STATEMENT OF CONTRIBUTION OF OTHERS

All data chapters of this thesis include collaborative work with my supervisors Prof. Ross H. Crozier and Prof. Chris N. Johnson.

Collection of blood samples from the Yinberrie Hills locality, used in chapters 2 to 4, was done in collaboration with Milton Lewis.

Collection of blood samples from Mornington Wildlife Sanctuary, used in chapters 2 to 4, was done in collaboration with Sarah Legge and Steve Murphy.

Blood samples from the captive population in Mareeba, QLD, used in chapters 2 and 3 were provided by the Mareeba Wetlands Foundation.

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Analyses for the reconstruction of phylogenies used in Chapter 4 were carried out using the High Performance Computing Facility at JCU (JCU-HPC).

DECLARATION OF ETHICS

The research presented and reported in this thesis was conducted within the guidelines for research ethics outlined in the *National Statement on Ethics Conduct in Research Involving Human* (1999), the *Joint NHMRC/AVCC Statement and Guidelines on Research Practice* (1997), the *James Cook University Policy on Experimentation Ethics. Standard Practices and Guidelines* (2001), and the *James Cook University Statement and Guidelines on Research Practice* (2001). The proposed research methodology received clearance from the James Cook University Experimentation Ethics Review Committee (approval numbers A810 and A969).

.....
Rodrigo Esparza-Salas (Date)

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GENERAL ABSTRACT

The Gouldian Finch (*Erythrura gouldiae*) is a species endemic to the Australian tropical savannas that has experienced drastic population declines in recent times. Population declines have been largely attributed to uncontrolled fire regimes, cattle grazing and disease. The decline of *E. gouldiae* populations has raised concerns about the state of conservation of tropical savannah habitats across northern Australia. There are a number of current conservation efforts aimed at understanding the biology of this species, and at using such knowledge to implement effective management for the recovery of its populations. An in depth understanding of the molecular ecology of *E. gouldiae* is important to complement the current knowledge of the species biology and will contribute to the implementation of management practices for its recovery.

I used molecular genetic techniques to resolve aspects of the ecology of *E. gouldiae* that are relevant to the management and recovery of their populations. I contrasted some of the patterns of genetic variation with those of the Long-tailed Finch (*Poephila acuticauda*); another tropical savannah species that is comparatively abundant and has not experienced the kind of population declines that have affected Gouldian finch populations. The research in my thesis has three main components:

1. Phylogeography of *E. gouldiae* and its relation to historical habitat changes in the Australian tropical savannas.

I investigated the geographical distribution of *E. gouldiae* lineages using mitochondrial DNA (mtDNA) control region sequences from extant populations and museum specimens. Results show a lack of lineage isolation among different regions. Patterns of mismatch distribution between mtDNA haplotypes are consistent with range expansions, dating from the Holocene period, approximately 7000 to 3600 years before present. Analyses of samples from different geographical regions suggest that population expansion events took place at different times: earlier at the easternmost localities and more recently at the westernmost parts of the species distribution. This proposed scenario of gradual colonisation of northern Australia can be explained by well documented climatic and socio-cultural changes during the Holocene. Change of

the landscape into the present drier habitats with grassy open vegetation would have favoured the expansion of *E. gouldiae* populations.

2. Genetic variability, population structure and migration in *E. gouldiae* and *P. acuticauda* populations.

I used microsatellite genotypes to investigate genetic variation, population structure and gene flow in *E. gouldiae* and *P. acuticauda* populations. Heterozygosity seems slightly higher in *P. acuticauda* than in *E. gouldiae*. I found no evidence of recent genetic bottlenecks or severe inbreeding in wild populations, although there is significantly lower heterozygosity and evidence for a recent population bottleneck in a captive stock that is part of a reintroduction programme for *E. gouldiae*. The indicators of intra-population inbreeding (F_{IS}) were relatively low in both species and there was no evidence for severe inbreeding. Inter-population differentiation (F_{ST}) is moderate for both species, which is consistent with their dispersal capabilities. Assignment tests show evidence of structuring between *Poephila acuticauda* populations, whereas such structuring is absent between individuals of *E. gouldiae* from the same locations. Migration rates appear to be higher for *E. gouldiae* than for *P. acuticauda*. These differences can partly explain the absence of population structure in *E. gouldiae*. However, mitochondrial DNA analyses have suggested the possibility of incomplete lineage sorting, which might potentially compromise this interpretation.

3. Variability, gene conversion and natural selection at Major Histocompatibility Complex (MHC) genes of *E. gouldiae* and *P. acuticauda*.

I amplified exon 2 of the MHC class II β gene in *E. gouldiae* and *P. acuticauda* to test for the presence of gene conversion and to search for evidence of positive selection. As previously found in other passerine bird species, the number of alleles per individual was high (up to 10 in *E. gouldiae* and up to 12 in *P. acuticauda*). The total number of haplotypes and nucleotide diversity are higher in *P. acuticauda*, although this difference is not significant. The observed individual allele diversity may be driven partly by gene conversion, which appears to occur more frequently in *E. gouldiae* than in *P.*

acuticauda. Analyses of MHC codon substitution show evidence of positive selection at 9 codons in each species, most of which are located at or adjacent to the predicted peptide binding amino acids in human MHC. There are differences in the positively selected amino-acid sites between both species. It is likely that the observed higher rates of gene conversion and positive selection have been comparably more important in the maintenance of MHC diversity in *E. gouldiae* as a result of lower genetic variability in this species. This suggests *E. gouldiae* may be subject to higher selective pressure from pathogens, consistent with documented evidence on differences in parasite loads between the two species studied.

In summary, the present distribution of *E. gouldiae* populations appears to be relatively recent in origin. This may have resulted in the reduced levels of genetic variability, as is expected under a scenario of population expansion following a genetic bottleneck. Results from this research indicate *E. gouldiae* have a high dispersal capability, which is reflected by the lack of genetic structure between populations and high levels of gene flow. Genetic variability does not appear to be of immediate concern for this species, as there is no evidence of severe inbreeding or recent population bottlenecks in wild populations. However, monitoring of genetic diversity can be critical in the success of future captive breeding and reintroduction programs. Although MHC gene diversity seems to have been maintained (in terms of allele richness) in spite of past and present population reductions in *E. gouldiae*, it is still not known if the present diversity confers resistance to pathogens similar to that for other species whose populations have been more stable, particularly given that a high proportion of the observed MHC variants in this species appear to be of recombinant origin. Future research in this topic will help to determine the importance of gene conversion and selection on the maintenance of MHC diversity and parasite resistance in small and declining populations.

TABLE OF CONTENTS

STATEMENT OF ACCESS	i
STATEMENT OF SOURCES	ii
STATEMENT OF CONTRIBUTION OF OTHERS.....	iii
DECLARATION OF ETHICS.....	iv
ACKNOWLEDGEMENTS	v
GENERAL ABSTRACT.....	vii
TABLE OF CONTENTS	x
LIST OF TABLES	xv
LIST OF FIGURES	xvii
CHAPTER ONE: General Introduction	1
Genetic diversity and population viability.....	1
Molecular genetics in ecological research.....	2
<i>Resolution of taxonomic uncertainties.....</i>	<i>2</i>
<i>Calculation of genetic diversity.....</i>	<i>2</i>
<i>Phylogeography.....</i>	<i>3</i>
<i>Resolution of population structure and gene flow.....</i>	<i>4</i>
<i>Resolution of mating systems.....</i>	<i>4</i>
<i>Adaptive variation</i>	<i>5</i>
Conservation genetics	5
Study species.....	8
<i>Population trends and current threats.....</i>	<i>8</i>

<i>Conservation status and current conservation measures</i>	9
Research aims	10
CHAPTER TWO: Historical phylogeography of the endangered Gouldian Finch	
<i>Erythrura gouldiae</i>: evidence of gradual population expansion during the	
Holocene	12
Abstract	12
Introduction	13
Methods	17
<i>Sampling</i>	17
<i>DNA extractions</i>	17
<i>Amplification of mtDNA control region</i>	18
<i>Genetic diversity</i>	19
<i>Analysis of Molecular Variance and Median-joining network</i>	20
<i>Mismatch distributions</i>	20
<i>Tests of neutrality</i>	21
<i>Relative dating of population expansions</i>	21
Results	22
<i>Genetic Diversity</i>	22
<i>Genetic structure</i>	24
<i>Mismatch distributions and tests of neutrality</i>	26
<i>Estimated times of population expansion</i>	28
Discussion	28
<i>Population structure</i>	29
<i>Population expansion and gradual colonisation of northern Australia</i>	30
<i>Conservation implications</i>	34

CHAPTER THREE: Microsatellite variation, genetic structure and migration in Gouldian Finch *Erythrura gouldiae* and Long-tailed Finch *Poephila acuticauda* populations. 36

Abstract.....	36
Introduction.....	37
Methods.....	40
<i>Sample collection and DNA extraction.....</i>	<i>40</i>
<i>Cross-species amplification of microsatellite loci.....</i>	<i>41</i>
<i>Evaluation of microsatellite loci.....</i>	<i>43</i>
<i>Analyses of recent population bottlenecks.....</i>	<i>43</i>
<i>Genetic diversity and genetic structure</i>	<i>44</i>
<i>Assignment of individuals to populations</i>	<i>45</i>
<i>Migration rates</i>	<i>45</i>
Results	46
<i>Cross-species microsatellite loci amplification.....</i>	<i>46</i>
<i>Bottleneck analyses.....</i>	<i>47</i>
<i>Genetic variability, genetic differentiation and inbreeding.....</i>	<i>47</i>
<i>Assignment tests.....</i>	<i>49</i>
<i>Migration rates</i>	<i>50</i>
Discussion	50
<i>Patterns of genetic diversity</i>	<i>50</i>
<i>Recent population bottlenecks</i>	<i>51</i>
<i>Population structure and migration</i>	<i>52</i>
<i>Management implications.....</i>	<i>52</i>

CHAPTER FOUR: Gene conversion and natural selection in MHC class II β genes of Gouldian Finch *Erythrura gouldiae* and Long-tailed finch *Poephila acuticauda* 55

Abstract.....	55
Introduction.....	56

Methods.....	59
<i>Sampling</i>	<i>59</i>
<i>PCR + 1 cloning of MHC class IIβ genes</i>	<i>59</i>
<i>SSCP gel electrophoresis.....</i>	<i>61</i>
<i>Sequence analyses.....</i>	<i>62</i>
<i>Phylogeny reconstruction</i>	<i>62</i>
<i>Detection of gene conversion.....</i>	<i>63</i>
<i>Detection of positive selection</i>	<i>63</i>
Results	65
<i>Patterns of allele variation</i>	<i>65</i>
<i>Gene conversion</i>	<i>67</i>
<i>Positive selection at MHC class IIβ genes.....</i>	<i>73</i>
Discussion	76
<i>Maintenance of MHC class II diversity</i>	<i>76</i>
<i>Considerations on statistical methodologies</i>	<i>78</i>
CHAPTER FIVE: General discussion	80
General summary	80
Summary of key findings	81
Conservation implications of results	82
Overall conclusions	84
Future research directions	85
<i>Comparative phylogeography in Australian Tropical Savannas</i>	<i>85</i>
<i>Population structure</i>	<i>86</i>
<i>Evolution and variation of MHC genes</i>	<i>86</i>
REFERENCES.....	89

APPENDICES.....	103
Appendix I Posterior means and 95% credible intervals (CI) of panmictic heterozygosity (Hs), f and θ^I for <i>E. gouldiae</i> and <i>P. acuticauda</i> under three different models.....	104
Appendix II Assignment of <i>E. gouldiae</i> and <i>P. acuticauda</i> individuals to populations under hypothetical scenarios of two to six populations (K).....	105
Appendix III Amino-acid sequences of MHC class IIβ haplotypes	106

LIST OF TABLES

Table 2.1 Localities of <i>E. gouldiae</i> samples used in this study.....	18
Table 2.2 Variable sites and geographic distribution of the 20 mtDNA control region haplotypes (HVRI, 208bp) in <i>E. gouldiae</i>	23
Table 2.3 Haplotype and Nucleotide diversity of mtDNA control region HVRI for different <i>E. gouldiae</i> sample groupings.....	24
Table 2.4 Analyses of molecular variance, fixation indexes and significance for mtDNA HVRI of <i>E. gouldiae</i>	24
Table 2.5 Mismatch distribution parameters and tests of neutrality for mtDNA control region HVRI sequences of <i>E. gouldiae</i>	27
Table 2.6 Pairwise comparisons between mismatch distributions of different geographic regions.	28
Table 3.1 Primer sequences, number of alleles and optimal annealing temperatures for microsatellite loci used in this study.....	42
Table 3.2 Evaluation of microsatellite loci used in this study.....	46
Table 3.3 Wilcoxon test and M ratio tests for recent population bottlenecks in <i>E. gouldiae</i> and <i>P. acuticauda</i> populations	47
Table 3.4 Summary of population parameters H_s (panmictic heterozygosity), f and θ^I , and 95% credible intervals (CI) calculated for <i>E. gouldiae</i> and <i>P. acuticauda</i> populations	48
Table 3.5 Model fit parameters calculated using the program Hickory.....	49
Table 3.6 Migration rates and 95% confidence intervals (CI) for <i>E. gouldiae</i> and <i>P. acuticauda</i> populations, obtained with the program BayesAss	50
Table 4.1 Diversity of MHC class II β alleles in <i>E. gouldiae</i> and <i>P. acuticauda</i>	65

Table 4.2 Hierarchical Analyses of Molecular Variance (AMOVA) for MHC class IIβ alleles in <i>E. gouldiae</i> and <i>P. acuticauda</i>	66
Table 4.3 Tests of neutrality for MHC class IIβ alleles in <i>E. gouldiae</i> and <i>P. acuticauda</i>	67
Table 4.4 Detected gene conversion events in MHC class IIβ haplotypes of <i>Erythrura gouldiae</i>.....	68
Table 4.5 Detected gene conversion events in MHC class IIβ haplotypes of <i>Poephila acuticauda</i>	70
Table 4.6 Amino-acid positions detected under positive selection	74

LIST OF FIGURES

Figure 2.1 Map of Northern Australia showing the distribution of <i>E. gouldiae</i>	15
Figure 2.2 Median-joining network of <i>E. gouldiae</i> mtDNA control region HVRI haplotypes.....	25
Figure 2.3 Mismatch distributions of <i>E. gouldiae</i> mtDNA control region HVRI sequences.....	26
Figure 2.4 Timeline of some climatic and socio-cultural events during the Holocene (above box), and approximate predicted times of <i>E. gouldiae</i> population expansions (below box).....	33
Figure 3.1 Map of northern Australia showing the distribution of <i>E. gouldiae</i> and <i>P. acuticauda</i>	39
Figure 3.2 Assignment of <i>E. gouldiae</i> and <i>P. acuticauda</i> individuals to two hypothetical populations	49
Figure 4.1 Bayesian phylogenetic trees of MHC class II β alleles	71
Figure 4.2 Frequency of gene conversions detected between MHC class II β haplotypes of <i>E. gouldiae</i> and <i>P. acuticauda</i> , relative to nucleotide positions.....	73
Figure 4.3 Ratios of d_N/d_S per amino-acid site in MHC class II β genes	75