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**Evolutionary Genetics and the Major
Histocompatibility Complex of New Zealand Robins
(Petroicidae)**

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Molecular BioSciences at Massey University, New Zealand
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Photo: Don Merton

The founding black robin pair,
Old Blue (above), and Old
Yellow (right)

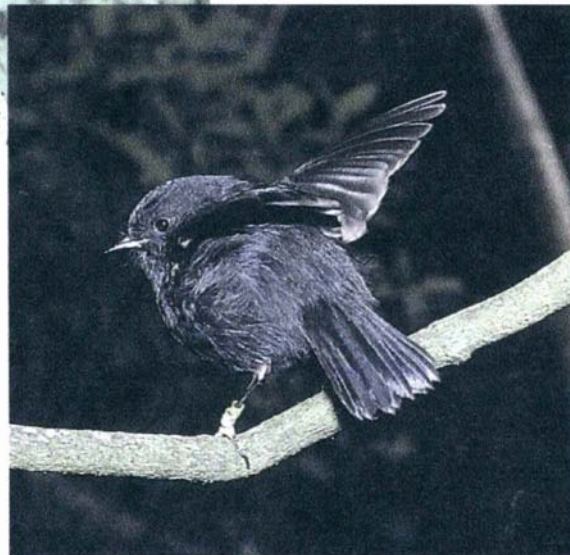


Photo: Rod Morris



The South Island robin
Photo: J. Kendrick (DOC)

Abstract

The genes of the major histocompatibility complex (MHC) are highly polymorphic and play a direct role in disease resistance. Loss of variation at MHC loci may increase extinction risk in endangered species, due to an inability to combat a range of pathogens. In this thesis, the evolution of class II B MHC genes is investigated, and levels of variation at these loci are measured in two species of New Zealand robin, the endangered Chatham Island black robin (*Petroica traversi*), and the non-endangered South Island robin (*Petroica australis australis*). Transcribed class II B MHC loci from both black robin and South Island robin were characterised prior to analysis of MHC variation. To this end, a non-lethal protocol for isolation of transcribed sequences from blood using 3'RACE and RT-PCR was developed. Four class II B cDNA sequences were isolated from black robin, and eight sequences were isolated from the South Island robin, indicating there are at least four class II B loci. RFLP analysis indicated that all class II MHC loci were contained in a single linkage group. Analysis of 3'untranslated region sequences enabled orthologous loci to be identified in the two species, and indicated that multiple rounds of gene duplication have occurred. A partial genomic DNA sequence of a putative pseudogene was also isolated from the black robin. Evolution of MHC genes in New Zealand robins appears to be influenced by gene conversion and balancing selection, resulting in loss of orthologous relationships in the coding region, and a highly diverse peptide-binding region. In order to assess the effect of population bottlenecks on MHC variation, levels of variation in the extant black robin population, which is descended from a single breeding pair, were compared with artificially bottlenecked populations of South Island robin and their respective source populations. Both RFLP and sequence analysis indicated that the black robin is monomorphic at class II B loci, while both source and bottlenecked populations of South Island robin have retained moderate to high levels of variation. Comparison of MHC variation with minisatellite DNA variation in each population indicated that genetic drift was the predominant force determining MHC diversity in bottlenecked populations in the short-term. Despite its lack of MHC variation, the black robin population appears to be viable under existing conditions. The evolutionary history of New Zealand's *Petroica* species, investigated by phylogenetic analysis of mitochondrial DNA sequences, is also discussed.

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Thesis Structure and Format

This thesis is written as a series of papers, with a general introduction and final summary drawing together the main themes of the thesis. The general introduction (Chapter one) is a literature review providing background information on aspects of MHC genetics relevant to this thesis, and enabling the specific topics covered in later chapters to be put into the wider context. Some of this information is reiterated in reduced form in the later chapters, but the introduction provides additional detail that would not be appropriate in a scientific paper. An outline of the major aims of the study is also given.

Chapters two to six are data chapters and are written in the format of scientific papers. Each chapter is written to stand alone as an independent unit, which results in some repetition, particularly in the introduction and reference sections. This format, however, allows each aspect of the study to be considered as a whole, in preparation for publication. Chapters two and four are written in the format of short communications, with the results and discussion sections combined. A modified version of Chapter two (An evaluation of methods of blood preservation for RT-PCR from endangered species) has been accepted for publication as a technical note in *Conservation Genetics*, and a reprint is included in Appendix D. The remaining chapters are being prepared for publication. The second paper in Appendix D was also published during the course of this work. This paper also involved analysis of genetic variation in an endangered species, but is not directly relevant to main themes of this thesis.

Each of the data chapters includes a discussion, which covers important aspects of the empirical data presented and places the results in the context of existing work. The final chapter summarises the main findings of the study and outlines possible areas of future research. Appendices A and B consist of the raw sequence data from Chapters five and six, respectively, and a full list of samples used in this study is given in Appendix C.

Note on nomenclature: In chapter six, the term “New Zealand robin” refers specifically to *Petroica australis*, however in the remainder of the thesis “New Zealand robins” is used in more general terms for simplicity, and includes the Chatham Island black robin *Petroica traversi* as well as *Petroica australis*.

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