The Major Histocompatibility Complex (MHC) of the Kiwi (Apteryx spp.).

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Erratum

p iii line 19 The sentence starting "A result.." should read "The result in the rowi sample is more consistent with a remnant population."

p 15 line 15 "it's" should be "its"

p 23 line 25 The sentence starting " While a study..." should read "A study on the inbred human Hutterite population showed an increased foetal loss rate for couples with 16 matching loci"

p 25 line 22 "maybe" should be "may be"

p 28 line 10 "is" should be "are"

p 29 line 5 "Salmonella" should be "*Salmonella*"

p 29 line 23 "adapts" should be "adapt"

p 30 line 19 The sentence starting " Although Hoelzel..." should read " A study by Hoelzel et al (Hoelzel et al 1999), showed a high level of diversity in the Southern Elephant seal, not a low level in the MHC."

p 35 line 24 insert a comma after "individual"

p 36 line 1 "it's" should be "its"

p 40 line 9 insert "is" after "now"

p 47 line 20 "it's" should be "its"

p 54 3.1.1. "as it is" should be "as they are"

p 55 line 25 The sentence starting "Remove as much..." should read " Ethanol was removed, and the precipitate resuspended in ~30µl of milliQ water and stored at 4° C overnight."

p 56 line 22 "were" should be "was"

p 58 line 3 "was" should be "were"

p 67 line 28 "is" should be "was"

p 71 line 31 "is" should be "was"

p 77 line 1 "nomenculture" should be "nomenclature"

p 79 line 3 "was" should be "were"

p 79 line 13 "was" should be "were"

p 79 line 19 "was" should be "were"

p 84 line 18 "locus" should be "loci"

p 84 line 21 The sentence starting "It is hoped..." should read "It will require more work on the kiwi genome to elucidate the organisation of MHC class I and II."

p 84 line 26 "artefacts" should be "artefact"

p 84 line 29 "loci" should be "locus"

p 86 line 23 "encode" should be "encodes"

p 90 line 14 "epidemics" should be "epizootics" repeats

p 90 line 14 "lead" should be "led"

missing bits

p 96 line 16 The sentence starting "These were ..." should read" The guidelines were: to find the earliest division of the NJ tree where any given bird did not have more than two alleles at a given loci and to do this with minimum the number of loci."

p 96 line 26 The sentence starting "Interestingly the ..." should read " Figure 5.4 shows a NJ tree with the most basal division between the two sizes of loci, i.e. 281bp and 284bp."

p 98 – 100 In Figures 5.2, 5.3, and 5.4: the numbers across the top refer to the samples from different birds in that species. The boxes with ticks indicate in that bird (sample) the loci was present.

p 102 line 15 remove "are"

p 102 line 18 "is" should be "are"

p 110 line 11 "loci" should be "locus"

p 121 line 21 "... and the sample size of thie study may not be enough to allow one to readily make an inference about the mode of evolution." should be added to the end of the sentence.

p 123 line 27 The sentence starting "Although the ..." should read" Figures 5.5 and 5.6 show the basal split in the kiwi alleles tends to be between 264bp and 267bp, but this is not absolute."

p 125 line 16 "it's" should be "its"

p 126 line 16 "Westerdahl" should be "Westerdahl's"

p 129 line 23 "principals" should be "principles"

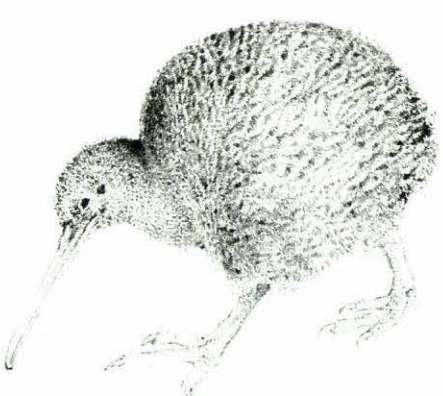
p 131 line 31 "populations" should be "population's"

p 175 line 23 Simkova et al should be on p 183

p 185 line 26 van Oosterhout et al should include the title "Balancing selection, random genetic drift, and genetic variation at the major histocompatibility complex in two wild populations of guppies (poecilia reticulata)"

Rowi (*Apteryx rowi*)

North Island Brown Kiwi (*Apteryx mantelli*)



Little Spotted Kiwi (*Apteryx owenii*)

drawn by Vivian Ward.

Abstract

This thesis investigates the polymorphism of the Major Histocompatibility Complex (MHC) in the threatened New Zealand Kiwi (*Apteryx spp.*). The MHC genes are usually highly polymorphic and play a direct role in disease resistance. A lack of MHC polymorphism may affect the ability of a population to respond to continuously evolving pathogens. The Kiwi is a unique bird, endemic to New Zealand, but despite being considered taonga (a treasure) all five kiwi species are threatened and require active management to sustain current population levels. The role of infectious diseases in the kiwi's past and future survival is currently only a matter of conjecture. To analyse the kiwi MHC and its polymorphism, a PCR and primers were designed that amplified the MHC Class II B exon 2, a protein binding region (PBR) and a site where polymorphism is expected. Feather samples from three different kiwi species, the North Island Brown (*Apteryx mantelli*), the Little Spotted Kiwi (*Apteryx owenii)*, and the Rowi (*Apteryx rowi)* were used as a non-invasive source of DNA. The MHC results for eight Little Spotted Kiwi from Red Mercury Island showed almost no variation in the form of different alleles between birds. Four putative alleles were shared by all birds, each bird having some or all of the alleles. Rowi are only found in Okarito and are a small population of 250 birds. The 18 birds tested showed a greater range of diversity than expected from a bottled necked population with 14 putative alleles and three pseudogenes. A result more consistent with a remnant population. The twelve North Island Brown birds showed a range of polymorphism: 11 putative alleles and two pseudogenes. Analysis of the Kiwi MHC supports the suggestion that avian MHC sequences evolved by concerted evolution and genetic conversion.

Acknowledgements

This work has indeed been accomplished by "standing on the shoulders of giants". I wish to express my sincere gratitude to the many people without whom this project would not be possible.

I am indebted to my mother, Noela Binney, for her help and support throughout my life. Sadly this thesis project began with helping my mother fight one cancer and ended helping her fight another cancer. The support we got, especially from the Whangaporoa Hospice staff and volunteers, for my mother and myself while I was caregiver and simultaneously finishing this thesis was precious to both of us.

Distinguished Professor David Lambert, for the wonderful opportunity and all support he gave me to do this project. Dr Leon Huynen, without whose skill and expertise I might still be in the Lab chasing down dead ends. Lara Shepherd was generous with her maps and work on ancient kiwi DNA. Vivian Ward was generous with her help on making my diagrams better and wonderful kiwi illustrations. Dr Jennie Yanamura was also helpful and supportive throughout.

I also have a special thanks to my lab buddies at the Allan Wilson Centre who helped me on a myriad of levels, Andrew Dodd, Tamara Sirey, Betty Adams, Jarod Young, and Jennifer Anderson, Charlie Gao, and Isabella Cheung for their help in sequencing

Many thanks to the dedicated kiwi wranglers at Rainbow Springs lead by Claire Travis and at Otorohanga lead by Eric Fox and the staff at the Auckland Zoo Kiwi house and Veterinarians Richard Jacob-Hoff and John Potter.

The DOC staff that helped collect samples including: Chrissy Wicks and her team at Franz Josef looking after the Rowi. Kelly Stevens for her help with the DOC paper work. The Te Runanga o Makaawhio iwi for their support that allowed access to Rowi feather samples. Dr Karen Nutt who while at Auckland University collaborated on access to Rowi samples.

Preface

The research undertaken for this thesis had contributions from other researchers.

Sample Collection.

The feather samples were collected by DOC staff and Dr Karen Nutt currently at Waikato University. The samples are from a population of North Island Brown kiwi (NIB) near Whangarei, and a population of Little Spotted Kiwi (LSK) on Red Mercury Island and Rowi a Brown Kiwi species found at Okarito.

The blood sample used for cDNA was collected by the veterinary staff at Auckland Zoo during a routine examination of a resident bird. I performed the rest of the processing of this sample.

DNA extraction.

Dr Karen Nutt and her staff extracted DNA from some of the rowi samples and I extracted the rest. I extracted the DNA from the NIB and LSK feather.

Primer Design.

Initially seven primers designed and published by other researchers were used; they are acknowledged and listed in Table 3.1. Dr Leon Huynen designed two primers, chMHCIIex1F and chMHCIIex3R, this is acknowledged in Table 3.2. The remaining primers I designed, either by eye or using Prime3 online software.

MHC PCR, Cloning, Sequencing and Data Analysis.

I performed the PCR's on the Kiwi DNA, cloned, performed the sequencing reaction and analysed the resulting data. The sequencing with the ABI 3730 Genetic Analyzer was mainly performed by Isabella Cheung. The selection factor identifying the degree of selection on the exon 2 site was analysed with MEGA 4 software with the help of Dr Sankar Subramanian.

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List of Abbreviations used.

A	Adenine
APC	Antigen Presenting Cell.
C	Cytosine
cDNA	complementary DNA (cDNA)
DNA	Deoxyribose Nucleic Acid
DOC	The New Zealand Department of Conservation.
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytidine triphosphate.
dGTP	Deoxyguanosine triphosphate
dNTP	A generic term referring to the four deoxyribonucleotides: dATP, dCTP, dGTP and dTTP.
dTTP	Deoxythymidine triphosphate.
G	Guanine.
HLA	Human Leukocyte Antigen.
Is	Island
mRNA	messenger Ribose Nucleic Acid
MHC	Major Histocompatibility Complex genes
NZ	New Zealand
PBR	Protein Binding Region
PCR	Polymerase Chain Reaction
RNA	Ribose Nucleic Acid
T	Thymine

Thesis Structure and Format.

Chapter 1 discusses the Major Histocompatibility Complex (MHC) which is important to the adaptive immune system for the identification of self from non-self. This chapter examines not only the structure and function of MHC but the importance of MHC polymorphism, and its role in combating disease. Later in Chapter 5, the relationship of MHC polymorphism to conservation of endangered species like the Kiwi is discussed.

Chapter 2 examines the endangered New Zealand Kiwi (*Apteryx spp.*). In particular, emphasis is given to diseases of the kiwi and how disease can impact on conservation of the kiwi and other species.

Details of the methods and materials used at each stage are reported separately in Chapter 3.

Chapter 4 explains how the project progressed from starting with non specific degenerate primers to finally developing a specific pair of primers for kiwi MHC. A flowchart is present to outline the stages involved. The steps taken to reduce the generation of laboratory artefacts at each stage are also discussed.

Chapter 5 shows the amount of MHC polymorphism found in three different populations of kiwi and compares the results to those of other avian and mammalian MHC.

Chapter 6 summarises the results found in this thesis and their implications for future research.

The Appendixes contain various data to support the thesis.