

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

Genetics of migration timing in bar-tailed godwits



A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

in

Zoology

at Massey University, Manawatū, New Zealand.

Ángela María Parody Merino

2018

Dedicado a mis padres;



vosotros me disteis alas para volar.



Preface

In this thesis, I used molecular and genomic techniques to give insight on the genetic elements behind migratory timing behaviour in a very suitable natural population bird system: The New Zealand godwit. This thesis is structured as a series of related and connected manuscripts with exception of the introduction, which provides the terminology, background and founding of my thesis: Each chapter is a stand-alone piece of work, therefore, there will be some unavoidable repetition between them and the introduction. This thesis contains three main chapters (see listed below), which are intended to be published.

- Chapter 2: Parody-Merino A.M., Battley P.F., Verkuil Y. I., Conklin J.R., Prosdocimi F., Potter, M.A., Fidler A.E. Population genetic structure of New Zealand-overwintering bar-tailed godwits (*Limosa lapponica baueri*) in relation to breeding geography and migration timing.
- Chapter 3: Parody-Merino A.M., Battley P.F., Conklin J.R., Fidler A.E. No evidence for an association between *Clock* gene allelic variation and migration timing in a long-distance migratory shorebird (*Limosa lapponica baueri*).
- Chapter 4: Parody-Merino A.M., Battley P. F., Fidler A.E., Prosdocimi F., Lima N., Conklin J.R., Potter M.A., Cox M.P. Polygenic architecture underlies departure time in a long-distance migratory bird.

As part of a Marsden Grant project I developed the research questions addressed in this thesis, as well as most of the work (i.e. experiments, molecular data collection, analysis and writing), which was performed by me with the guidance of supervisors and some of the co-authors. The godwit behavioural data from previous years were collected principally by my supervisor Phil Battley and co-author Jesse Conklin; those from 2013 onwards were collected by Jesse Conklin (Manawatu), Tom Burns and Ian Southey (Miranda) and Peter Langlands (Catlins). I have collaborated in fieldwork and helped in the collection of behavioural data (in the Catlins) whenever I could. Yvonne Verkuil has been an important contributor to Chapter 2 by guiding in analyses and subsequent interpretation of results. Francisco Prosdocimi and Nicholas C. B. Lima were in charge of the bioinformatic process of the godwit genome assembly and annotation, which was crucial for Chapter 2 and Chapter 4. My co-author Murray Cox supervised and guided

me for the analyses (i.e. bioinformatic steps) of Chapter 4, as well as helping resolve computational needs. He also made substantial contributions to the manuscript. All supervisors provided comments and contributed to framing of all the manuscripts.

Synopsis

“A hidden drive at the right time”

Ferdinand Johann Adam von Pernau

Bird migration is one of the most amazing behaviours observed in nature and it fascinates because of its diversity and its – still – unclear reasons and origin. My thesis converges primarily two big areas of research in biology: ecology and genetics/genomics. The ecology part is the bird system (the godwit), which has been extensively studied and whose individual departure date has been proven to be exceptionally consistent. Studies of this kind are not possible without a suitable system, and longitudinal behavioural records of the same individuals are not easy to obtain. The genetics and genomics are, without doubt, the biggest part of this thesis. What can we learn about how DNA encodes biological rhythms in natural populations taking godwits as an example?

There is a huge amount of literature on chronobiology, mainly based on experiments with model organisms or caged individuals. Thanks to these experiments the elements (i.e. proteins and genes) involved in the biological clock have been identified and the characteristics of circadian and circannual rhythms have been described. However, it remains unclear how these elements link to an individual's timing behaviour in nature. Extensive research on the migration timing of bar-tailed godwits (*Limosa lapponica baueri*) in New Zealand gave rise to an intriguing observation: individual's departure dates seemed to be primarily driven by an 'internal signal', and this 'internal signal' was quite consistent across the years, while the population showed a departure-span of approximately a month. This raised questions such as: Are there elements of the internal clock that determines such within species (population) diversity and such intra-individual consistency? In my thesis research I first assessed the population genetic structure of godwits related to migration time, a step that is necessary when trying to link phenotypes with genotypes. Then I used genomic approaches and integrated behavioural data to understand whether elements related to the 'internal clock' are associated to individuals' migratory departure time in godwits.

I found evidence of slight population genetic structure between northern and southern breeders as well as between earlier and later migrants departing from the stop-

over in Asia, but not between earlier and later migrants from New Zealand. Detailed analyses of migration timing in relation to polymorphisms in *clock* (a gene implicated in other studies as potentially influencing migration timing) found no support for *clock* having any role in godwit migration timing. Analysis of variation in 120 genes associated with the internal clock, photoreception, the physiology the Hypothalamic-Pituitary-Gonadal Axis – which modulates the internal clock –, and fat metabolism and storage indicates that individual migration timing of godwits has a genetic basis to some degree, but differences between individuals seem to be associated with large numbers of genes of small effect rather than a few genes of large influence. Godwit migration timing therefore appears to be a complex trait in which genetic differences between individuals explain some of the variation timing, but a large amount of the variation observed is not explained by the suite of genes studied. It is possible that key genes exist that were not studied, and/or that non-genetic factors may be influencing an individual's decision to migrate on a given day above and beyond the genetic influence. In general, this thesis contributes to the understanding of the nature of behaviours (i.e. genes-behaviour link) in natural populations, specifically in the area of chronobiology.

Te Reigna – rarangi mai ra te rangai kuaka/ kia tau hikohiko he pai tu waho

Flocks of godwits are gathering/ moving restlessly on the seaward cliffs

Part of a saying composed by Tumatahina of Te Aupouri.

“Kuaka” is the Māori name for the godwit.

Taken from: <http://www.hekuaka.co.nz/>



Acknowledgements

First, I want to thank many people whose contribution to my research, because although this thesis is a result of many hours of work on my own, there are a bunch of people that, without their help, I would not have accomplished and fulfilled my goals. My supervisors, Andrew Fidler and Phil Battley, I cannot be more grateful that you trusted on me to carry on this project: I want to thank you for all the help and support to progress, improve and complete this thesis. Andrew: for all what I learnt about the ‘magical’ world of the molecular laboratory procedures and being there to discuss about my thesis every time I needed. Not least, for always caring about me, as a person, not only as a student; for introducing me in New Zealand for the first time and making me easier to get used to the everything-new life here. Phil: for telling me all about godwits. I am immensely grateful for giving me the opportunity to be in the field, which made me feel like a complete biologist: field and lab (well, and office). Being close to the godwits, observing their movements for hours, surviving the 4-seasons – in 4-minutes – typical from New Zealand when transitioning to the winter, and waiting for some individuals to show me their second – and hidden – leg to check their colour bands. For welcoming me when I had to move to Palmy and for your incredibly useful help and support during the last years of my doctorate. To Murray Potter, for your support and your contributions and good ideas to improve the chapters. I really appreciate your support on providing me the opportunity to take the online course on “bioinformatic tools for genomic data”, which helped me tremendously to clarify many of the bioinformatic work done by collaborators/co-authors, as well as to perform the main analyses in Chapter 4. Enormous thanks to Jesse Conklin, whose invaluable work on godwits’ behaviour and ecology has been the foundation of my thesis. And of course, for all your contribution, comments, ideas and explanations that helped me during the progress of my thesis.

Yvonne Verkuil, I am so grateful for that day we met and during our conversation I suddenly I found out you were the author of one important publication of my molecular experimental design. I cannot be more grateful to you for having me guided through the world of population genetic analysis. All the comments and guidance were essential for me to improve this chapter and to learn a lot. Thanks for the invitation to the University of Groningen in 2014, and for all those delicious dinners you invited me for during my stay. To Murray Cox, thanks so much for your valuable guidance and support, which were

essential to accomplish one of the most difficult of the challenges I encountered during my PhD – and also my most wished professional and personal accomplishment : to feel confident performing bioinformatic analyses. There is a long way of learning, but now I feel I am prepared for it. Thanks to the numerous people helped to collect data and samples in the field: David Melville, Adrian Riegen, Rob Schuckard, Peter Langlands, Ian Southey, Tom Burns...etc and a long list of volunteers!

I cannot forget about the people that contributed most to my well-being: My family and friends. This thesis is especially dedicated to my parents, who have always been with me, helping me to every step that I needed to make in order to reach my goals. To my friends, the ones from overseas and the ones I met in New Zealand. They all have been an enormous support as well. In particular, I want to thank Hugo, for all the support and good moments during the initial years of my PhD in which I was based in Nelson (South Island). Thanks to Andrea and David for the numerous enriching and enjoyable conversations we had during lunch (or coffee) breaks; I hope this continues! I want also to thank to friends that made me discover new sports and activities in New Zealand, which gave me some balance during my PhD life: Anika for the African dances, Chifuyu and Amy for Body combat, and Hugo and Alan for those games in Squash. Thanks to those who have accompanied me to camping and tramping adventures around this beautiful country. Last – and not least but most! – to Jorge, who followed me to this adventure. I would never be able to thank you enough for all your unconditional support.



Myself checking banded godwits in a beautiful day at the Catlins Coast (March 2014).

Table of Contents

Preface.....	I
Synopsis	III
Acknowledgements	VII
Chapter 1: General introduction	1
<i>Time-programed lives</i>	<i>2</i>
<i>Chronobiology: Daily and annual rhythms</i>	<i>2</i>
<i>How is time measured?</i>	<i>4</i>
<i>Understanding the genetics of migration of birds in the Era of genomics</i>	<i>7</i>
<i>Approaches to the study of genetics of migration</i>	<i>12</i>
<i>Linking genes to behaviour</i>	<i>13</i>
<i>Bar-tailed godwits: An excellent candidate model organism</i>	<i>14</i>
<i>Key questions and chapters overview</i>	<i>18</i>
<i>Overview of the techniques and technologies used in this thesis.....</i>	<i>20</i>
<i>Glossary</i>	<i>25</i>
Chapter 2: Population genetic structure of New Zealand-overwintering bar-tailed godwits (<i>Limosa lapponica baueri</i>) in relation to breeding geography and migration timing.....	33
Chapter 3: No evidence for an association between <i>Clock</i> gene allelic variation and migration timing in a long-distance migratory shorebird (<i>Limosa lapponica baueri</i>)	49

Chapter 4: Polygenic architecture underlies departure time in a long-distance migratory bird	67
Chapter 5: Synthesis	85
<i>Introduction</i>	86
<i>The first godwit genome</i>	87
<i>Population structure background in godwits and its relationship with migration departure time</i>	87
<i>ClockpolyQ polymorphism in godwits</i>	89
<i>The genetics of migration timing in bar-tailed godwits</i>	91
<i>Issues & challenges</i>	92
<i>Implications for understanding timing of migration</i>	97
<i>If not genetic, then what (or what else)?</i>	98
<i>Future directions</i>	98
<i>Final remarks</i>	100
Appendices	103
References	183