GENETIC STRUCTURE OF *PLEUROBRANCHAEA MACULATA* IN NEW ZEALAND

A thesis presented in partial fulfilment of the
requirements for the degree of **Doctor of Philosophy** (**PhD**) in Genetics

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2016

ACKNOWLEDEGEMENTS

I have a long list of people to acknowledge, as my PhD project would not have been possible without their support.

Firstly, I would like to thank my supervisor, Professor Paul B. Rainey (New Zealand Institute of Advanced Study, Massey University), for providing me with the opportunity to join his research group. He gave me constant support, insightful guidance, valuable input, and showed me how to think like a scientist. I would also like to thank my co-supervisor, Dr Craig D. Millar, who welcomed me into his genetics laboratory at the Department of Biological Sciences at the University of Auckland whenever I needed help. He guided me patiently right from the beginning of my PhD project, was generous with his time, encouraging, and taught me how to troubleshoot where necessary. I would like to extend a special mention to Selina Patel, a very talented technician at Dr Millar's Lab who shared her extensive technical and theoretical knowledge with me generously, but also allocated considerable time to help me progress with my research. My study was kicked off by Selina Patel and Dr Millar's willingness to sequence my study organism's genome in the facilities of the "Centre for Genomics, Proteomics and Metabolomics" at the at the University of Auckland. Thank you also to my co-supervisor, Professor Marti Anderson (Massey University), for her guidance, her helpful contributions, for not only teaching me how to analyse my data but also for being involved in the analysis herself, and patiently answering my very naive questions about statistical methods. Her explanations helped me to understand that these very sophisticated methods are not something to be scared of – they are actually fun. Many thanks to my other co-supervisor, Professor Nigel French, (Massey University), for supporting me whenever I asked for help, and encouraging and backing me in the Allan Wilson Centre meetings.

My project was very much dependent on the divers and researchers who collected *Pleurobranchaea maculata* samples for me. I am grateful to Dr Susanna Wood and Dr David Taylor (Cawthron Institute, Nelson) for supplying me with numerous sea slug samples, sharing their extensive knowledge of *P. maculata*, discussing my findings, providing access to unpublished manuscripts, and introducing me to the people who

helped me to obtain more samples. I would have been lost without them. I would like to acknowledge the following people and/or institutes who either collected samples, put me into contact with people who provided samples or helped with the shipment of the samples: Mike McMurtry (Auckland Regional Council); Lauren Salvitti, Dudley Bell, Warrick Powrie and David Culliford (University of Waikato, Hamilton); Richard Huges and Bakhti Patel (Leigh Marine Laboratory, the University of Auckland); Severine Hannam and Dr Wilma Blom (Auckland Museum); Shane Genage (Victoria University of Wellington), Steve Journee (The Dive Guys, Wellington); Don Morrisey, Matthew Smiths and Stephen Brown (The National Institute of Water and Atmospheric Research); Dr Mark Stevens (South Australian Museum) and Te Papa Museum (Wellington).

Dr Paul McNabb (Cawthron Institute) conducted the tetrodotoxin assay for some of the samples. Serena Khor (The University of Waikato) extracted tetrodotoxin from some samples for the toxicity assay. I thank them both.

I would like to extend my gratitude to everyone who helped me with the data analyses and interpretation. I need to mention the contribution of my supervisors and Selina Patel again in this area. Virginia Moreno-Puig (Massey University), who has just submitted her thesis in which she utilised a population genetics tool, was a fount of knowledge and took a great deal of time to reflect on my questions with me. I would like to thank Dr Peter Ritchie (Victoria University of Wellington) and my colleagues Dr Stephen Ritchie, Jenny Herzog, Christina Straub, Dr Katrin Hammerschmidt, Dr Libby Liggins, Dr Peter Deines, Oliver Hannaford and Luca Bütikofer for their help and suggestions.

Many people helped me while I was writing my thesis. Many thanks once more to my main supervisor Professor Rainey for his patience while reading my thesis, his guidance, his outstanding comments and his corrections. I am immensely grateful to Dr Honour McCann (NZIAS, Massey University) as she critically reviewed, proofread and edited Chapter 1. I thank Dr Gayle Ferguson (INMS, Massey University) as she edited Chapter 2, but also Jacque Mackenzie (Institute of Veterinary, Animal & Biomedical Sciences, MU) as she proofread an earlier version of the same chapter. Thank you to Adam Bedford, Tatyana Pichugina (the University of Auckland), and my colleagues

Chhavi Chawla and Elena Colombi for editing and proofreading some parts of my thesis. I am very grateful to Joanna Niederer as well for copy-editing my whole thesis.

On a more personal level, I thank my mother Çakır Ayşe Selçuk and my brother Övgün Yıldırım, who have never failed to support me from the other side of the world. I know that I will always have a warm home waiting for me. I am very grateful to Adam Bedford, who encouraged me and gave me sound advice whenever I fell into despair. He was the one who suffered my whims, but he was also at my side during the many happy days with lots of laughs and adventures. I would also like to thank Rashmi Ramesh Iyer and Setareh Mokhtari, who were my first flatmates and my first friends in New Zealand. I would not have been able to survive in New Zealand without their support. I am grateful to Jenny Herzog for her friendship and cheerfulness, her wise observations and encouragement. My special thanks goes to one of the most wonderful and sophisticated people I have ever met, Marleen Bailing. She has always been there without hesitation whenever I needed help. She also showed me many tricks to improve my climbing skills. Thank you to Chhavi Chawla for being such a kind-hearted, sincere, wise and supportive friend. I wish you had joined our lab earlier. Thank you to my office mates and friends Yuriy Pichugin and Yunhao Liu. They always listened to my complaints, understood me very well and offered me practical advice and valuable opinions on my work. Thank you to all the Rainey Lab members as well as to Claire-Marie Loudon, Tatyana and Luca for making my life enjoyable. I have to stop here otherwise this list will never end.

My study was funded by the Allan Wilson Centre, Institute for Advanced Study at Massey University, and the Auckland Regional Council. I am very grateful to these institutes, as this research could not have happened without their support.

ABSTRACT

AIMS

The grey side-gilled sea slug (*Pleurobranchaea maculata*), which is native to the western and south Pacific, is known to contain high concentrations of tetrodotoxin (TTX). *P. maculata* populations around New Zealand exhibit individual, spatial and temporal differences in TTX concentration, but the origin of TTX in *P. maculata* is not fully understood. The main goal of my PhD project was to examine the genetic structure and demographic history of *P. maculata* populations from different regions in New Zealand and to clarify whether there is a correlation between variability in TTX concentrations and genetic structure.

METHODS

A sample of 146 *P. maculata* individuals were collected from three populations from the north-eastern North Island (Ti Point-TP, Auckland-AKL and Tauranga-TR), one population from the southern North Island (Wellington-WL) and one population from the northern South Island (Nelson-NL). TP, AKL and TR were designated the "Northern cluster", whereas the WL and NL population were labelled as the "Southern cluster" due to the relative geographical locations of these clusters. Twelve nuclear microsatellite markers that were developed based on shotgun sequence were obtained from the genome of *P. maculata*. The markers were used to analyse the genetic structure of *P. maculata* populations. The mitochondrial cytochrome c oxidase subunit I (1153 bp) and cytochrome b (1060 bp) genes were also partially sequenced in *P. maculata* individuals.

RESULTS AND MAIN CONCLUSIONS

The microsatellite data reveal high genetic diversity and lead to the rejection of the hypothesis of panmixia: populations from the Northern cluster are highly connected but significantly differentiated from the Sothern cluster. A weak differentiation was also observed between the WL and NL populations. The two populations correlate with regional variations in TTX concentrations: the Northern cluster populations contain highly toxic individuals, whereas the Southern cluster (WL and NL) populations

harbour either slightly toxic or non-toxic populations. The disjunction between the Northern and Southern clusters can be explained by biogeographical barriers specific to New Zealand but also with a stepping stone model. The geographical gap between the sampling locations made it impossible to draw firm conclusions as to the origin of the disjunction. The mtDNA sequence data reveal high haplotype diversity, low nucleotide diversity and a star-shaped haplotype network. These data can be explained by a population expansion dating back to the Pleistocene era. All the sampling locations are significantly differentiated from each other according to mtDNA data. Given that microsatellite and mitochondrial sequences evolve at different rates, incomplete linkage sorting is expected to be completed for mtDNA before, which should be reflected in a more pronounced structure for mtDNA markers where members of the populations have diverged recently. Although this may explain the geographical conflict between the microsatellite and mtDNA data, it is necessary to consider the possibility that the discordance between microsatellite markers and mtDNA may be in part attributable to the relatively small sample size.

TABLE OF CONTENTS

List of Figures

List of Tables