Rates of Molecular Evolution and Gene Flow

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ABSTRACT

Species diversity is driven by speciation, extinction and immigration. In this thesis I explore species diversity among regions and the process of speciation via four related studies.

The latitudinal biodiversity gradient (LBG) describes the pattern of higher diversity levels towards the tropics. One of the popular hypotheses to explain the LBG is the evolutionary speed hypothesis, which suggests that species in the tropical regions are evolving faster than those in temperate regions. I demonstrate that current work on the LBG often focuses on describing the pattern as distinct from understanding the process that might drive the LBG (chapter 1). Second, I show that the most popular method used to measure differences in rate of molecular evolution between taxa from different regions, the sister-species method, does not give consistent results and estimated rates of molecular evolution can vary widely depending on outgroup selection and gene analysed. The inherent problems revealed within the current approaches raise questions as to the validity of inferences made from putative variation in rate of molecular evolution between high and low latitudinal taxa. In particular, I show that studies of the LBG that use very close sister-species pairs, rely on the most problematic datasets and should therefore be treated with considerable scepticism (chapter 2).

A phenomenon such as the LBG must derive from small-scale processes in population dynamics. Species are sometimes defined as reproductively isolated units, with hybridisation viewed as evidence of failure to speciate. But it is now increasingly acknowledged that speciation is a dynamic process during which species and populations can exchange alleles. I found relatively high levels of admixture and gene flow between morphologically distinct biological entities in two very different study systems: insects and snails. Analyses of a pair of grasshopper species provided no evidence of deviation from random mating in the region where they are sympatric. However, analysis of morphological traits provided no evidence for hybrid phenotypes between these *Sigaus* grasshoppers. I infer that some loci associated with these morphological characters are subject to strong natural

selection but neutral genetic material is being extensively shared between the two species (chapter 3). A similar situation was found in terrestrial snails that I studied, but in this case there was a clear association of neutral genetic makers with phenotype. The mtDNA haplotypes, 3,764 SNP loci and morphometric data revealed two clear genotypic clusters among New Caledonian *Placostylus*, despite strong evidence for gene flow (chapter 4). Although it is convenient and popular to define species on the basis of their reproductive isolation, a more dynamic model that allows for the possibility of gene flow is closer to reality for these taxa. Speciation can be complex and our current understanding of the process of speciation does not suggest it is limited by the genomes' rate of molecular evolution. Results from my research shows, in support of other recent research, that the process of speciation is often the product of adaptation.

PREFACE

The overall aim of this research project, *Rates of Molecular Evolution and Gene Flow*, was to examine the drivers of divergence and adaptation of populations and explore how this could drive large-scale diversity patterns. United by this common aim, the thesis is composed of four chapters, each of which can stand-alone, and a fifth, integrating chapter. Supplementary material is included for chapters 2, 3 and 4. Reading the supplementary material is not needed to understand each chapter but provides additional information to the reader that is perhaps interesting in regards to methodology and results. Although this work was a collaboration between my supervisors (Steve Trewick and Mary Morgan-Richards) and myself, all laboratory work, data analysis and initial drafts were done by myself. Steve and Mary made invaluable assistance contributions to method design, the theoretical underpinning of the work, editorial guidance and funding.

The first chapter explores the theoretical explanations for observed differences in diversity between regions. Chapter one is a detailed review of the Latitudinal Biodiversity Gradient (LBG) and the hypotheses surrounding its formation and has been published: EJ Dowle, M Morgan-Richards and SA Trewick. 2013. Molecular evolution and the latitudinal biodiversity gradient. *Heredity*: 110, 501-510. This chapter does not contain new analysis or results, rather, it suggests how the field of study around the LBG might move forward with new datasets and focuses questions. I conducted most of the background research and reviewing of the work surrounding the LBG and wrote the initial manuscript draft. Steve, Mary and I subsequently edited the draft, with input from reviewers and the journal editor.

The evolutionary speed hypothesis (chapter one), a popular hypothesis to explain the LBG, predicts that species in the tropics are diverging faster than species in temperate regions via, in part, a faster rate of molecular evolution. Chapter two surveys the ways in which rates of molecular evolution are currently measured between species using the popular sister-species method. It is currently being formatted for review. Many studies report the detection of shifts in rates of molecular evolution between species, but no one has examined the accuracy of the

methods used. Drawing on published studies, I examine whether current methods provide reliable estimates of relative rates of molecular evolution by comparing results obtained from analysis of different genes and varying outgroups. Results from this analysis have implications for numerous studies on molecular evolution rate variation. The dataset I used contains new whole mitochondrial genomes from crickets. My supervisors obtained most of the specimens but I designed the project and undertook all laboratory work for the new mitochondrial genome generation and assembled 12 of the 14 genomes. I also undertook all statistical analysis and wrote the initial draft of the manuscript. This chapter has been prepared for publication but not submitted.

Chapter three is the first of two chapters in which I study the drivers of speciation. Rate of molecular evolution might impact large-scale biodiversity patterns if speciation is largely driven by mutational speciation or if selection is limited by the variation within a species. Here I examineed two species complexes to determine what processes underlie their divergence. Chapter three is a population genetics study on two species of NZ short-horned *Sigaus* grasshoppers, which is under review with *The Journal Ecology and Evolution*. One grasshopper species with a restricted range occurs in complete sympatry with the other. Previous work had hinted at gene flow between the species from comparison of mitochondrial DNA sequences. I questioned whether this pattern extended to nuclear markers and used geometric morphometric techniques to try identify morphological hybrids. I undertook all ITS sequencing, new mtDNA haplotype sequencing, RAD-sequencing, morphometric data collection and subsequent analyses. Steve and I generated the microsatellite library, and I then conducted the genotyping across the samples and ran the analyses. I wrote the initial manuscript draft and Steve, Mary and I subsequently edited this chapter.

Chapter four is a population genetics study of *Placostylus* snails from New Caledonia, which is being formatted for review. By examining their genetics and morphology I explored whether admixing has occurred in the past and whether gene flow is ongoing in areas of sympatry. I modelled environmental variables with the genetics and morphology to determine whether components of the environment drive

morphological adaptation and genetic variation. I designed this project and undertook some of the sampling of snail tissue and shells in New Caledonia, additional material was supplied by Fabrice Brescia from his work on *Placostylus*. I generated all shell morphometric data. All DNA extractions, mtDNA sequencing, RAD-sequencing, geometric morphometric analysis and the following statistical analyses were conducted by myself, as was the initial manuscript draft. Steve, Mary and I subsequently edited the draft.

Accordingly, the main thesis falls into two sections, reflecting the understanding that large-scale diversity patterns we observe must be driven in part by small-scale population dynamics. In the final summation (Chapter five) I first give a brief overview of how we can use different techniques to improve our understanding of the process of speciation. Second I describe the drivers of speciation and their role in the formation of biogeographic processes such as the LBG.

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