Comparative phylogeography of two niche-differentiated Australian funnel web spiders

Amber S. Beavis

A thesis submitted for the degree of Doctor of Philosophy of The Australian National University
DECLARATION

The research presented in this thesis is my own original work except where indicated in the Preface. Due acknowledgement has been made in the text to all other material used. In all cases I am the principal author and contributor to the work presented. No part of this thesis has been submitted for any previous degree.

Amber Skye Beavis

May, 2008
FOR MY MENTORS,
WHO IMBUED ME WITH SCHOLARLY TENDENCIES:

DR. S.G. BEAVIS

&

PROF. F.C. BEAVIS

(1924 – 2006)
PREFACE

The Australian funnel web spiders are a neglected taxon group. Consequently, this PhD project included a substantial period of laboratory troubleshooting. As a direct outcome, the pilot study for this project is the sole publication at the time of submission. This publication is included in the Appendix. To facilitate prompt publication of my research findings I have prepared this thesis as a collection of papers. I also felt that this format was appropriate given the fact that I have addressed a series of very targeted research questions. One consequence of this approach is that there is some repetition amongst chapters. Where repetition occurs, it is due to the fact that some information needs to be given within the context specific to that chapter’s research questions.

This thesis comprises four manuscripts in preparation for publication as follows:

Chapter 2: Target Journal – Molecular Ecology

Chapter 3: Target Journal – Evolution
Beavis AS, Is the Pleistocene the one and only? Discerning the effects of past climate and current environment on the phylogeography of an Australian funnel web spider.

Chapter 4: Target Journal – Parasitology
Beavis AS & Rowell DM Parasite phylogenies elucidate patterns of host phylogeographic structure.

Chapter 5: Target Journal – Molecular Phylogenetics and Evolution
Beavis AS, Rowell DM, Sunnucks P, Comparative phylogeography elucidates significant biological idiosyncrasies separating two species of Australian funnel web spider.

Specific contributions include the following:
DM Rowell executed the analysis of behavioural data presented in Chapter 3; Large datasets for analysis in MrBayes (Chapter 2 and Chapter 4) and MIGRATE (Chapter 5) were carried out by using the resources of the Computational Biology Service Unit from Cornell University which is partially funded by Microsoft Corporation.
A NOTE ON THE TEXT

Sample labels in this thesis may be interpreted as per the following two examples:

Example 1) OZ13_1.2_A

- Z13: refers to the site number. Sites were localised areas of no more that 25m\(^2\). GPS readings were taken according to site. Site locations are provided in Map 2.1 and Map 3.1. Further details of each site are given in Appendix 1.
- 1: is the log identifier
- .2: is the sample (spider) identifier.
- A: identifies the species

Example 2) Z14.1_H

- This sample label format is used primarily for *Hadronyche* Sp. 1 samples that were collected in 2003. This format is used less often in this study.
- Z14: refers to the site number. One log was sampled per site. Sites were localised areas of no more that 10m\(^2\). GPS readings were taken according to site. Site locations are provided in Map 2.1 and Map 3.1. Further details of each site are given in Appendix 1.
- 1: is the sample (spider) identifier.
- H: identifies the species

All figures and maps included in this thesis may be interpreted without reference to sample numbers. Enlarged copies of all figures and maps in which sample numbers are shown are included in the Insert section at the back of the thesis.
THESIS ABSTRACT

This thesis is primarily concerned with the phylogeography of two niche-separated species of Australian funnel web spider in the Tallaganda region of South-Eastern New South Wales, Australia. Chapter 1 provides a general overview of the field of phylogeography, Australian funnel web spiders and the context provided by the broader Tallaganda comparative phylogeography project. Each of the four data chapters presented in my thesis addresses a distinct set of questions arising from this broad topic. Chapter 2 addresses the question of whether the saproxylic *Hadronyche* Sp. 1 has been affected by Pleistocene glaciation similarly to other saproxylic invertebrates at Tallaganda. Chapter 3 asks whether historical climate change or ongoing environmental factors, such as underlying geology, have produced the phylogeographic structure detected in the ground dwelling *Atrax* Sp. 1. Chapter 4 presents the phylogeography of a Laelapid mite that is parasitic upon funnel web spiders, asking whether this co-phylogeny elucidates aspects of funnel web spider demographic history. Chapter 5 is a comparative phylogeographic study of both *Hadronyche* Sp. 1 and *Atrax* Sp. 1 that aims to elucidate previously unknown elements of the biology of both taxa, via their different responses to a common environmental history. Chapter 6 recaps the key findings of this PhD project and highlights future directions that have emerged from this body of work.

The first research component of my thesis (Chapter 2) presents the phylogeography of the saproxylic *Hadronyche* Sp. 1 in the Tallaganda region. Pleistocene glacial-interglacial cycling has been a significant determining factor in the phylogeographic structure of extant taxa. An ongoing comparative phylogeographic study of log-dwelling – or saproxylic – invertebrate taxa in the Tallaganda region has detected remarkably fine-scale spatial structure that is broadly congruent across multiple species and is congruent with the hypothetical locations of historical refugia. The saproxylic *Hadronyche* Sp. 1 is extremely specialised to the log-habitat, potentially more so than any other invertebrate studied at Tallaganda. Consequently, this study aimed to detect whether the response of this log-dwelling mygalomorph spider to historical climate change had been consistent with that of other saproxylic taxa. I found that *Hadronyche* Sp. 1 displayed phylogeographic structure congruent with a recent range expansion into Tallaganda from the Great Dividing Range. There was no evidence that this taxon had
survived through Pleistocene glaciation within Tallaganda, the findings of this study being congruent with *Hadronyche* Sp. 1 becoming locally extinct as a consequence of historical climate change.

The focus of Chapter 3 is upon determining whether historical and intermittent, or contemporary and ongoing factors, have produced the phylogeographic structure observed in Tallaganda populations of the ground-dwelling funnel web *Atrax* Sp. 1. *Atrax* Sp. 1 occupies a specialised habitat: ground burrows constructed directly into the soil substrate. This is of significance given the underlying geology of the Tallaganda region: the area is a spur of metasediments that is surrounded and isolated by granite. Given that Tallaganda has been subject to the effects of glacial-interglacial cycling, the region presents both a temporally and geographically variable habitat for *Atrax* Sp. 1. Therefore this chapter aimed to investigate historical and contemporary factors that may have shaped the phylogeographic structure of this taxon. I found that the *Atrax* Sp. 1 phylogeny displays phylogeographic structure at two distinct levels: ancient structure divides the taxon into clades that have distinct and non-overlapping geographic ranges; whilst more shallow structure divides clades into granitic and metasedimentary groups. Ancestral state reconstruction indicated that a preference for granite was ancestral. A behavioural study that investigated spider preferences for soil type showed clear evidence that: a) spiders display an overall preference for granite soil; b) spiders from granitic areas prefer granite, whilst spiders from metasedimentary areas show no preference for metasediments; c) spiders from granitic areas prefer granite from their own geographic region. Overall, these findings support the hypothesis that both historical climate change and underlying geology have driven population differentiation in *Atrax* Sp. 1. Furthermore, the degree of genetic differentiation amongst groups is sufficient to suggest that these factors may be driving the speciation process.

Chapter 4 presents a phylogeographic study of Laelapid mites that are parasitic upon *Atrax* Sp. 1 and *Hadronyche* Sp. 1 in Tallaganda. This study investigated whether a parasite phylogeny could elucidate the patterns of mygalomorph phylogeography. Overall this study found little evidence of host-defined mite population differentiation, however, it did appear that mite populations reflected the phylogeography of the host. This study found that mite phylogeography was consistent with *Atrax* Sp. 1 having a
long history in the region. Furthermore, mite phylogeography was consistent with the extant Tallaganda population of *Hadronyche* Sp. 1 being the outcome of a recent range expansion into the region.

Chapter 5 is a comparative phylogeographic study of the saproxylic *Hadronyche* Sp. 1 and the ground-burrowing *Atrax* Sp. 1. This chapter aimed to use the phylogeographic differences that separate *Hadronyche* Sp. 1 and *Atrax* Sp. 1 as a means of inferring biological differences in migration capacity. This approach as a means of inferring key biological characteristics is of particular significance to neglected taxa, such as mygalomorph spiders. Where few natural history studies exist and there is little baseline data available on taxon-biology, this approach permits the elucidation of key biological traits that would otherwise underpin any phylogenetic research on that group. I found evidence of markedly different dispersal abilities and effective population sizes amongst the two groups.
ACKNOWLEDGEMENTS

In these final months of my PhD candidature, a sign has hung above my desk that bears the best advice ever given to a PhD student:

"Keep Calm and Carry On"

A great many people have provided me with their wisdom, their council and their support over the past five years. By doing so they have helped me to put this advice into practice, with the consequence that this PhD has been a glorious adventure.

My supervisory panel have mentored me through my transition from Classics major undergraduate to enquiring scientist. First and foremost, I count my primary supervisor Dave Rowell as a formative influence. He has taught me how to ask questions and, as a scientist, I cannot express sufficient gratitude for this. Paul Sunnucks has been instrumental in teaching me how to best express and communicate my research outcomes, and to do so with determination. Scott Keogh has – since my undergraduate years – taught me how to make myself heard and how to make my research visible. In doing so, he has been instrumental in helping me to develop confidence in my own opinions and ideas. Together these three academics have given me the wherewithal to develop into an independent researcher, to my excitement and glee!

There are a number of researchers who have provided me with more informal mentoring. They have been integral to my development into a scientist. Julian Ash, Pat Backwell, Andrew Cockburn, David Ellis, David Morris and Rod Peakall from ANU; Lyn Cook, from the University of Queensland; Andrew Austin, from the University of Adelaide; Robert Raven, from the Queensland Museum; Kevin Omland, from the University of Maryland Baltimore County; and Rosie Gillespie, from University of California Berkeley. Together these researchers have provided me with models of academia to hold up as guidelines for my own approach to scientific enquiry.

This PhD project included a substantial period of troubleshooting. Simply put, it seems that the DNA of Australian funnel web spiders degrades within 24 hours of extraction. Chris Hayes was an ongoing source of advice in the laboratory. Quite simply, I could
not have developed the protocol that permitted me to conduct this research project without her.

An integral component of the PhD has been “small game hunting”, otherwise known as fieldwork. Kate Hodges, Mel Twidale, Noel Tait, Chester Sands, Ryan Garrick, Christina Schmucki, Dan Edwards, Jody Taylor and Sherryn Ciavaglia were my brave and dedicated companions in the field.

During this PhD my learning has been facilitated by attendance at three workshops. The opportunities provided by these workshops for discussions with students and faculty were invaluable. I am deeply appreciative of the learning environments provided by:

- The Workshop on Molecular Evolution, Marine Biological Laboratory, Woods Hole, MA, USA.
- The Bodega Bay Phylogenetics Workshop, UC Davis, CA, USA.
- The GenAlEx Workshop on Population Genetics, The Australian National University, ACT.

I am extremely grateful for the financial support received from the following organizations and funding bodies: The Australian Research Council, The Linnean Society of NSW, The Ecological Society of Australia, The Royal Zoological Society of NSW, ANU Fieldwork Fund, The Society for the Study of Evolution, ANU Michael White Travel Award, ANU Vice-Chancellor’s Travel Grant.

In the final year of my PhD candidature (in the wake of my scholarship) I have worked for ANU Student Administrative Services, first with the Research Students’ Office and later with Enrolments. My sincerest thanks go to all my colleagues in SAS for their support during this time. In particular Ros Taylor, Gay Kennedy, Marian Irvine, Helen Wong, Wendy Slater and Diana Ilchef have provided me with ongoing understanding.

And now for my friends and my family…

I often think that those who know me must be perplexed by my choice of study species. Nobody would think that “danger” could be my middle name. But my people are understanding of my deep interest in the most venomous of all spiders: they accept my insistence that the spiders are “merely misunderstood”; they revel in any discussion of my work; and they have been exceptionally kind to me throughout my PhD candidature. For this, I thank them.
My BoZo associates have been ongoing sources of advice, camaraderie and, on occasion, frivolity. Suzi Morrison was my sounding-board for a cornucopia of issues; Mitzy Pepper talked shop with me to my delight; Andrew Thornhill helped me to network better than ever before; Andy Leigh was my fellow iconoclast; Tom Sapienza came to my rescue at the final charge of the light brigade.

Luke Ferguson, Nady Kraljevic and little Elliot have been wonderful friends for the duration.

Lauretta Grasso, Natalie Craig, Bridget Maidment, Nicholas Johnson and Will Tse have been endlessly supportive of me, both personally and professionally, come hell or high water.

My sister, Summer Beavis, and her partner, Matt Bosworth, dropped everything to join me in the field after I had experienced a dangerous run-in with some local hillbillies. They became my protectors without question, and I cannot thank them enough.

My sister, Fern Beavis, overcame her geological tendencies to join me in a spot of small game hunting. She inspires me with her example of everything that a hard-core field scientist can be.

My mother, Sara Beavis, has been a field-assistant, a co-author and a mentor throughout my PhD. When I was very small and she was completing her own PhD research in the field of geology I “helped” her by carrying rocks. To have her assistance in my own research endeavours has been an honour.
CHAPTER 1: INTRODUCTION

THE FIELD OF RESEARCH: PHYLOGEOGRAPHY
Phylogeography: bridging population genetics and phylogenetics
Trends and fashions in phylogeography
Benefits of the comparative approach

THE STUDY SITE: TALLAGANDA
The Tallaganda project
The saproxylic habitat and its occupants
The Tallaganda region
Geology of Tallaganda
Climate variation and its effects
Current progress in Tallaganda

THE TAXON: AUSTRALIAN FUNNEL WEB SPIDERS
Taxonomy of Australian funnel web spiders
Biology and its influences
Funnel web spiders at Tallaganda
Niche separation
Previous research

THE PRESENT STUDY
Neglected taxa and their inherent challenges

AIMS AND OBJECTIVES

SUMMATION

REFERENCES

CHAPTER 2: SURVIVING THE PLEISTOCENE

INTRODUCTION
Hadronyche in Tallaganda
The Tallaganda region
Pleistocene climate variation in Australia
The Tallaganda model

SUMMATION

METHODS
Sample collection
DNA extraction and sequencing
Sequence analysis
Phylogenetic analysis
Population genetic analysis

RESULTS
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequence diversity</td>
<td>39</td>
</tr>
<tr>
<td>Tests of neutrality</td>
<td>41</td>
</tr>
<tr>
<td>Phylogenetic analysis</td>
<td>41</td>
</tr>
<tr>
<td>Population genetics analysis</td>
<td>44</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>45</td>
</tr>
<tr>
<td>Overview</td>
<td>45</td>
</tr>
<tr>
<td>Inferred population history</td>
<td>45</td>
</tr>
<tr>
<td>Phylogeography and the influences of historical climate change</td>
<td>46</td>
</tr>
<tr>
<td>Hadronyche Sp. 1 at Tallaganda</td>
<td>48</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>49</td>
</tr>
<tr>
<td>CHAPTER 3: IS THE PLEISTOCENE THE ONE AND ONLY?</td>
<td>55</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>55</td>
</tr>
<tr>
<td>Phylogeographic fingerprints of past climate and current environment</td>
<td>55</td>
</tr>
<tr>
<td>The study species: Atrax Sp. 1</td>
<td>56</td>
</tr>
<tr>
<td>The Tallaganda region's historical environment: Pleistocene glaciation</td>
<td>57</td>
</tr>
<tr>
<td>The Tallaganda region's current environment: Geology</td>
<td>58</td>
</tr>
<tr>
<td>Local adaptation in Atrax Sp. 1</td>
<td>60</td>
</tr>
<tr>
<td>METHODS</td>
<td>61</td>
</tr>
<tr>
<td>The Approach: Addressing Prior Expectations</td>
<td>61</td>
</tr>
<tr>
<td>Sample collection</td>
<td>61</td>
</tr>
<tr>
<td>DNA extraction and sequencing</td>
<td>63</td>
</tr>
<tr>
<td>Sequence analysis</td>
<td>64</td>
</tr>
<tr>
<td>Phylogenetic analysis</td>
<td>65</td>
</tr>
<tr>
<td>Ancestral state reconstruction</td>
<td>65</td>
</tr>
<tr>
<td>Phylogeographic analysis</td>
<td>66</td>
</tr>
<tr>
<td>Behavioural assessment of local adaptation</td>
<td>67</td>
</tr>
<tr>
<td>RESULTS</td>
<td>67</td>
</tr>
<tr>
<td>Sequence Diversity</td>
<td>67</td>
</tr>
<tr>
<td>Tests of neutrality</td>
<td>68</td>
</tr>
<tr>
<td>Phylogenetic analysis</td>
<td>69</td>
</tr>
<tr>
<td>Ancestral state reconstruction</td>
<td>73</td>
</tr>
<tr>
<td>Phylogeographic analysis</td>
<td>73</td>
</tr>
<tr>
<td>Behavioural analysis</td>
<td>76</td>
</tr>
<tr>
<td>Summation</td>
<td>76</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>81</td>
</tr>
<tr>
<td>Summation</td>
<td>81</td>
</tr>
<tr>
<td>Influence of Pleistocene glacial-interglacial cycling</td>
<td>81</td>
</tr>
<tr>
<td>Influence of underlying geology</td>
<td>82</td>
</tr>
<tr>
<td>Underlying geology and local adaptation</td>
<td>84</td>
</tr>
<tr>
<td>Summation</td>
<td>85</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>86</td>
</tr>
<tr>
<td>CHAPTER 4: PARASITE PHYLOGENY ELUCIDATES HOST PHYLOGEOGRAPHY</td>
<td>94</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>94</td>
</tr>
<tr>
<td>Tallaganda</td>
<td>95</td>
</tr>
<tr>
<td>Funnel web spiders and Laelapidae mites</td>
<td>96</td>
</tr>
<tr>
<td>Study aims</td>
<td>97</td>
</tr>
<tr>
<td>METHODS</td>
<td>97</td>
</tr>
<tr>
<td>Sample collection</td>
<td>97</td>
</tr>
<tr>
<td>DNA extraction and sequencing</td>
<td>97</td>
</tr>
<tr>
<td>Summary Statistics</td>
<td>98</td>
</tr>
<tr>
<td>Phylogenetic Analysis</td>
<td>99</td>
</tr>
<tr>
<td>Phylogeographic Analysis</td>
<td>99</td>
</tr>
<tr>
<td>RESULTS</td>
<td>100</td>
</tr>
<tr>
<td>Sequence diversity and summary statistics</td>
<td>100</td>
</tr>
<tr>
<td>Phylogenetic analysis</td>
<td>100</td>
</tr>
<tr>
<td>Population genetics analysis</td>
<td>102</td>
</tr>
</tbody>
</table>
**List of Figures**

**Figure 2.1** Hadronyche Sp. 1 statistical parsimony network ............................................. 41

**Figure 2.2** Hadronyche Sp. 1 Bayesian phylogeny .......................................................... 42

**Figure 3.1.a** Atrax Sp. 1 statistical parsimony network (phylogeography) .................... 68

**Figure 3.1.b** Atrax Sp. 1 statistical parsimony network (underlying geology) .............. 69

**Figure 3.2** Atrax Sp. 1 Bayesian phylogeny ................................................................. 70

**Figure 3.3** Atrax Sp. 1 ancestral state reconstruction ...................................................... 72

**Figure 3.4** Overall preference for soil type ........................................................................... 75

**Figure 3.5.a** Spiders from granitic sites tend to prefer granite ........................................... 76

**Figure 3.5.b** Spiders from metasedimentary sites do not prefer home soil ....................... 76

**Figure 3.6.a** Spiders from site A1 preferred A1 (granitic) soil ........................................... 77

**Figure 3.6.b** Spiders from site B1 preferred B1 (granitic) soil ........................................... 77

**Figure 4.1** Laelapid mite Bayesian phylogeny ................................................................. 98

**Figure 5.1** Atrax Sp. 1 and Hadronyche Sp.1 Bayesian phylogeny ................................. 122
LIST OF TABLES

Table 2.1 Genetic diversity indices of Hadronyche Sp.1.................................39
Table 2.2 AMOVA tests of genetic subdivision.............................................43
Table 3.1 Genetic diversity indices of Atrax Sp. 1........................................66
Table 3.2 AMOVA tests of genetic subdivision.............................................73
Table 4.1 Genetic diversity indices for H. barbarae mites..............................97
Table 4.2 AMOVA tests of genetic subdivision.............................................99
Table 5.1 Genetic diversity indices for Hadronyche Sp. 1 and Atrax Sp. 1........120
LIST OF MAPS

Map 1.1 The location of Tallaganda in South-East NSW, Australia.................. 8

Map 1.2 Tallaganda region hydrological catchments and underlying geology........ 11

Map 2.1 Hadronyche Sp. 1 sample distribution map........................................ 34

Map 3.1 Atrax Sp. 1 sample distribution map.................................................. 60
LIST OF PLATES

Plate 1.1 A view of the eastern side of the Tallaganda range, January 2007............9

Plate 1.2 A view of the western side of the Tallaganda range, January 2007............9

Plate 1.3 An adult female Hadronyche Sp. 1.........................................................16

Plate 1.4 An adult female Atrax Sp. 1.................................................................16

Plate 1.5 Hadronyche Sp. 1 burrow entrance......................................................17

Plate 1.6 Atrax Sp. 1 burrow entrance.................................................................17
CHAPTER 1
Phylogeography is a multidisciplinary field that unites disparate fields into a single research area (Arbogast & Kenagy 2001). In its most elemental form, phylogeography is driven by questions and concepts that are both intuitive and elegant. However, the practice of the field is complicated by the fact that phylogeography is a young and rapidly developing research field (Avise 1998). Additionally, data interpretation in this field suffers from the impacts of numerous confounding factors such as stochasticity (Carstens et al. 2005) and multiple, overlapping, historical barriers to gene-flow (Knowles & Maddison 2002). To date there has been no real consensus regarding which analytical approaches are the most effective for interpreting geographically structured genetic variation (Carstens et al. 2005). Consequently the field is arguably in a hydra-headed state of confusion: the tools to be used have not yet been established; the methodology to be applied is not concrete; and the hypotheses to be addressed have barely been phrased. This state of affairs is not entirely unexpected given that the field is focused upon an ambiguous phase of the evolution of species, not to mention the fact that the word we use to name this field – “phylogeography” – is only 20 years old (Avise et al. 1987). The consequence of these challenges is that the thoughtful and logical application of the available tools and methods is of paramount importance to any meaningful body of research. This is a broad statement but it has particular relevance to this thesis, which is focused upon the phylogeography of two genera of Australian funnel web spider, a neglected taxon-group that, prior to this study, has long suffered from a dearth of targeted research.

This thesis presents the findings of a phylogeographic study of two niche-separated taxa in sister genera of Australian funnel web spider. The story that has emerged from this project is somewhat unusual, both in the clarity of its phylogeographic signal and the complexity of those factors that may have shaped the evolution of populations – and perhaps species – in the Tallaganda region of South-Eastern Australia. Overall, a major issue that has affected the interpretation of results emerging from this study is the fact that funnel web spiders constitute, by any definition, a neglected taxon. The taxonomy
of funnel web spiders is in need of revision and basic natural-history observations are infrequent. As a consequence, research efforts in this area are hampered by an incomplete understanding of species biology. This study has sought to disregard these facts as necessary precursors to a detailed survey of Hadronyche and Atrax. Instead this thesis aims to address interesting questions about the evolution of funnel web spider populations, placing them in the context of the landscape these spiders inhabit and the history they share with multiple saproxylic invertebrates at Tallaganda. In the process, this project has collected basic biological data upon which future research endeavours may be based.

The Field of Research: Phylogeography

Phylogeography: bridging population genetics and phylogenetics

Phylogeography is broadly known as the discipline that bridges the gap between phylogenetics and population genetics (Avise et al. 1987), or the field that permits us to “see genes in space and time” (Hewitt 2001). In its most basic form, phylogeography seeks to reconstruct the biogeographic history of species and to infer the processes that have produced the spatial structuring of phylogenies observed in extant taxa (Hugall et al. 2002; Knowles & Maddison 2002). The traditional phylogeographic approach was to examine the geographic structure of gene lineages (Arbogast & Kenagy 2001), generally at and around the species boundary (Avise 1987). This approach was implemented by sampling individuals from throughout the geographic range of interest, inferring multiple gene-trees for this group, and providing a geographic context for each individual included in the process. By this method, one could elucidate the geographical origins of distinct clades (Arbogast & Kenagy 2001). More recently, there have been a series of research ‘movements’ that have proposed a suite of specific aims and approaches for phylogeographic research. These goals vary according to whether the research invoking them has a theoretical, empirical or applied focus. Theoretical research has chiefly focused upon moving away from descriptive phylogeography by investigating methods of addressing hypotheses with a degree of statistical certainty (eg. Knowles & Maddison 2002). This has been one of the most rapidly changing areas of phylogeographic research and has centred upon accounting for stochasticity as well as methods of tree inference (Carstens et al. 2005; Steele et al. 2005; Spellman & Klicka
2006). Empirical studies have focused upon investigating the effects of historical events on phylogenies, in particular, the climate change that characterised the Pleistocene (Knowles 2001; Galbreath & Cook 2004; Omland et al. 2006). Applied phylogeography studies have chiefly focused upon establishing conservation priorities, for example, via identification of Operational Taxonomic Units in a given system (Moritz & Faith, 1998; Moritz et al. 2001). These separate but overlapping branches of phylogeography have all experienced a common trend: the old, clearly delineated boundaries that separated disciplines such as phylogenetics, systematics, population genetics, and genomics have been blurred such that a phylogeographic research project may make use of methods from all these fields. In addition, phylogeography is a field of study whose genetic findings require the context provided by palaeoclimatic reconstructions (Hugall et al. 2002), underlying geology (Pepper et al. 2006), species distribution modelling (Kidd & Ritchie 2006) and multitudinous other areas across numerous disciplines. Hence, it is becoming clear that an environmental context is crucial to understanding the factors driving the evolution of populations and species.

**Trends and fashions in phylogeography**

Phylogeography has undergone numerous changes since its inception 20 years ago. In part, these changes have occurred in response to advances in technology. Phylogeography arguably emerged as an outcome of the analytical potential of mitochondrial sequence data. As new methods have become available phylogeographic research co-opts them to address pertinent questions, for example, the move from mitochondrial DNA sequence data to nuclear DNA sequences has been a recent major development (Hoelzer 1997). Whilst a sizeable proportion of advances in phylogeographic research have emerged as a result of technological advances, the driving impetus could be in part due to the troublesome nature of phylogeography itself. Phylogeography uses the evidence provided by DNA sequence evolution as a means of inferring historical demographic change. This historical aspect of the discipline lends itself particularly well to the “narrative” approach, that is, accounts of a series of inferred events drawn from factual evidence. Although the word “narrative” has a less than positive reputation within the field of phylogeography, the term accurately describes a number of phylogeography’s key goals. For example, the definition of a “narrative” is “an account of a series of events, facts, etc., given in order and with the
Chapter 1: Introduction

establishing of connections between them” (OED). It is possible to argue that phylogeography is not intuitively a hypothesis-driven discipline and whilst there are significant benefits to be gained by framing phylogeographic questions as testable hypotheses, this may not always be the most appropriate approach for a given question. The overall goal of any given phylogeographic study is to determine how historical populations responded to demographic change, and thus the aims of phylogeographic research are, by their very nature, narrative in their outcomes.

Whilst the descriptive approach was the most common method adopted in phylogeographic research during the earlier years of the discipline, the last decade has been characterised by a move away from the narrative. In 1995 Templeton published his series of papers on Nested Clade Analysis as a means of inferring historical demographic change with a degree of statistical certitude (Templeton et al. 1995; Templeton 1998; Templeton 2008). In 2002 Knowles and Maddison were at the forefront of the rise of Statistical Phylogeography, a hypothesis-driven approach that made significant use of coalescence theory (Knowles & Maddison 2002). However there have been distinct difficulties in eliminating the narrative approach from phylogeographic methods. Currently, phylogeographic research arguably may be on the cusp of a major shift in thinking, driven by conflict between the ideas that a purely descriptive approach is open to the excesses of “storytelling” (Humphries 2000) whilst strictly hypothesis-driven methods do not make full use of the information available (McDowall 2004; Kidd & Ritchie 2006; Diniz-Filho et al. 2008). The recent exchange of views on the validity of nested clade analysis as a phylogeographic approach is an example of one such discussion (Garrick et al. 2008; Petit 2008; Templeton 2008). Given the trends emerging in recent publications, it is a distinct possibility that the discipline will equalise at an approach that makes use of methods from both schools of thought.

Benefits of the comparative approach

Phylogeography is widely used to elucidate the responses of a single taxon to historical environmental change. However, single-taxon studies are limited in their potential to provide insights into landscape evolution, broad-scale environmental change or ecological-niche-specific evolution. In contrast, comparisons of phylogeographic
structure across multiple taxa can detect broad-scale processes and the factors that have driven them (Avise 2000; Crawford et al. 2007). The appeal of a comparative approach to phylogeography is articulated by Robert Zink who expounded upon the benefits of the comparative approach, stating that “one could test whether co-distributed taxa have congruent phylogeographic patterns of genetic variation, which might be predicted if a given area has but a single history” (Zink 1996). A rapidly increasing number of comparative phylogeographic studies have utilised the capabilities of the comparative method to infer the effects of historical environmental change on populations (Bermingham & Moritz 1998). This approach has permitted the development of increasingly complex and theory-driven research questions.

The Study Site: Tallaganda

The Tallaganda project

The Tallaganda region of South-Eastern New South Wales, Australia has been the site of an ongoing comparative phylogeographic study of saproxylic – or log-dwelling – invertebrate taxa. This project has been investigating the phylogenetic outcomes of Pleistocene glacial-interglacial cycling in multiple co-distributed taxon-pairs that represent the velvet worms, springtails, terrestrial flatworms and water skinks (Garrick et al. 2004; Beavis & Rowell 2006; Sunnucks et al. 2006; Garrick et al. 2007; Hodges et al. 2007). This thesis reports on the phylogeography of funnel web spiders. Each taxon was represented by two species (or genera where taxonomy was uncertain) that displayed some ecological or biological disparity in social structure, migration capabilities or habitat specificity. The two funnel web spider taxa that are the focus of this thesis display the greatest habitat disparity of all invertebrates surveyed in Tallaganda to date.

The saproxylic habitat and its occupants

The saproxylic component of biodiversity is both taxonomically diverse and ecologically threatened. Saproxylic species account for approximately 20% of forest insects and contain representatives of all major insect orders (Grove 2002a; Grove 2002b; Grove 2002c). Saproxylic invertebrates are susceptible to habitat disturbances and the group includes a disproportionately high number of threatened species. A large number of saproxylic taxa are susceptible to desiccation, with the result that many log-
dwelling species are sessile, occupying narrow and specialised habitat niches (Hammond 1984; Yee et al. 2001). This combination of characteristics causes saproxylic invertebrates to be sensitive to changes in their local habitat or broader environment (Garrick et al. 2004; Grove, 2002a; Grove 2002b; Grove 2002c). Consequently, saproxylic taxa are vulnerable to environmental change, with the result that extant populations retain clear phylogenetic signals of past events, for example, historical climate variation (eg. Moritz et al. 2001).

The Tallaganda region
The Tallaganda region (35°24′S – 36°12′S and 149°28′E – 149°37′E) is a 100km long transect of continuous, temperate sclerophyll forest located in South-East New South Wales (NSW), comprising sections of both State Forest and National Park (Map 1.1). Tallaganda is situated along the ridge of the Gourock Range and connects to the Great Dividing Range (GDR) in the south at “Badja”. Otherwise the Tallaganda region is surrounded on all sides by low-lying grasslands and pasture that effectively isolate it from the escarpment, the only point of contact with the GDR being at Badja (State Forests of NSW 1995). The forest is primarily sclerophyll forest, ranging from wet to dry types dependent upon altitude, aspect and local climate (State Forests of NSW 1995). Local climate variation includes the orographic influence of the GDR, causing the eastern slopes (Plate 1.1) to be subject to high orographic rainfalls (wet sclerophyll forest and rainforest), whilst the western slopes (Plate 1.2) are subject to a rain-shadow effect (dry sclerophyll forest). The region has been subject to regular but selective logging since the 1890s and currently selective logging practices are predominantly implemented, with some clear-felling (State Forests of NSW 1995). This has produced an environment in which there is a preponderance of saproxylic habitat available.
Map 1.1 The location of Tallaganda (and Badja) in South-East NSW, Australia, with respect to the Great Dividing Range (GDR), and showing the location of sampling points outside of the Tallaganda region.
Plate 1.1 A view of the eastern side of the Tallaganda range, January 2007

Plate 1.2 A view of the western side of the Tallaganda range, January 2007
Geology of Tallaganda

Tallaganda is predominantly composed of metasedimentary and granitic (igneous intrusive) lithology, though igneous extrusives and alluvial sediments also occur within the region (Map 1.2). Metasediments dominate the spine of the multiple ridges that collectively form the Tallaganda range. Igneous intrusives, predominantly granite, occur on the eastern and western flanks of Tallaganda, the ridge lines of the Tuross Gorge and the Badja region and occur as intrusions within otherwise metasedimentary regions (State Forests of NSW 1995). In essence, Tallaganda is a spur of metasediments surrounded and intruded by granitic material, which isolates the region from a predominantly metasedimentary section of the Great Dividing Range (GDR). The Tallaganda region has been geologically stable for some time and the lithology defines the soil landscapes: metasediments found in the region date from the Ordovician period (490-443 mybp), whilst the granitic material and other igneous intrusives date from the Upper Silurian/ Lower Devonian (443-354mybp) (State Forests of NSW 1995).

Climate variation and its effects

Unlike Northern Hemisphere land masses, Australia did not undergo widespread glaciation during the Quaternary, with only the Kosciuszko Massif (2228m asl) and the West Coast Ranges of Tasmania being covered by ice (Singh & Geissler 1985; Barrows et al. 2001). Instead, the majority of the continent experienced significant changes in temperature, sea level and the geographical extent and composition of vegetational classes. Of relevance to the present study are the significant impacts of Pleistocene glacial-interglacial cycling upon the composition of South-Eastern Australian vegetation (Hope et al. 2004). In broad terms, glacial periods were characterised by dominance of grasses and by herbaceous and shrub-like Asteraceae. In contrast, interglacials were characterised by forest and woodland taxa, predominantly Eucalyptus, but including Nothofagus and tree ferns (Hope et al. 2004). The overall picture that has been built up of the most recent glacial-interglacial cycle in Australia shows that temperature and rainfall decreased from approximately 30 000 – 20 000 ybp, until glacial maxima at approximately 15 000 ybp. At approximately 25 000 ybp, sea levels had declined to the extent that a land bridge formed between the mainland and Tasmania (Dodson & Ono 1997; Hope et al. 2004). Opinion is divided as to whether Eucalypt forests were restricted to coastal areas or whether they survived in gully
Map 1.2 Tallaganda region hydrological catchments and underlying geology
(See Insert for enlarged copy of map)
refugia throughout South-Eastern NSW, but it is generally accepted that this region was predominantly herbaceous grassland at glacial maxima (Hope et al. 2004; Williams et al. 2006; Kershaw et al. 2007). Between 15 000 – 10 000 ybp sea levels increased as the continent began to experience the current interglacial, and by 10 000 ybp current forest patterns were established, albeit to a lesser extent than present (Dodson & Ono 1997).

The Tallaganda region is situated immediately south of Lake George, the site of a palaeorecord spanning the entirety of the last glacial cycle. Lake George is 673m above sea level (asl) and the lake catchment extends into the northernmost limits of Tallaganda itself. Lake George is one of the large, hydrologically sensitive, terminal, closed intermittent water bodies of internal drainage in Australia. At present Eucalyptus dominates the overall vegetation profile and vegetation within the Lake George catchment ranges from warm temperate dry-sclerophyll forests through to Eucalypt woodlands and grasslands. Some regions exclusively contain flora peculiar to coastal heaths, swamps and treeless frost-hollows. Above the treeline, alpine herbaceous taxa are dominant (2000m asl). The palaeorecord is derived from an 18m core that covers the period 730 000 – 0 ybp and contains pollen, spore, algae and charcoal particles (Singh & Geissler 1985). The conclusions of studies based on this type of data operate under the assumption that changes in temperature will produce elevational changes in vegetation groups and therefore, movements in and out of the Lake George catchment. The palaeorecord indicates that altitudinal shifts of vegetation occurred synchronously with glacials, cool-temperate periods and interglacials. Singh & Geissler (1985) infer that the upper treeline decreased from present levels by 1200 – 1500m during glacials and 300 – 600m during cool-temperate periods. These drops in the treeline are calculated to represent decreases in the average temperature of 8 – 10°C and 2 – 4°C at glacial maxima and cool-temperate periods respectively. At glacial maxima (23 000 – 16 000 ybp) the palaeorecord indicates a “total disappearance” of Eucalypt sclerophyll forests and their replacement with open herbland (Singh & Geissler, 1985). The record shows evidence for the presence of the following: tree vegetation is limited to two samples of the conifer Phyllocladus; tall groundcover occurs in low densities and consists of Cyathea, Pteris, Gleichenia, Asperula, Bursaria and ferns; herbaceous taxa make up the bulk of species present and primarily consists of Lycopodium, Liliaceae and Poaceae (Singh & Geissler, 1985). The two glacials that preceded the most recent
glacial maxima display equivalent vegetational patterns. During the present interglacial, *Eucalypts* resumed their earlier inter-glacial dominance in the landscape.

The reconstruction of the Lake George palaeorecord (Singh & Geissler 1985) is congruent with those from other sites in Eastern Australia. The severity of the most recent glacial, which appears to exclude *Eucalypt* forest from the region, is mirrored in other locations on the continent. For example, a similar phenomenon is observed in the palaeorecord from the Caledonia Fen (1280m asl) in the South-Eastern highlands of Victoria (Kershaw *et al.* 2007). Whilst *Eucalyptus* does not completely disappear from the record, *Eucalypt* values declined to approximately 5% and herbaceous taxa predominated. At the beginning of the current interglacial, *Eucalypt* values increased from 5% to 60%, reaching 85% at the most recent sampling point (Kershaw *et al.* 2007). Kershaw *et al.* suggest that *Eucalyptus* species survived in the region at low densities in shrub form. The present study submits that the repeated observations of an absence of *Eucalypts* is too widespread to be ignored, whilst taking into account that the absence of pollen in the palaeorecord could be equally congruent with extremely low densities of *Eucalypt* within sheltered locations. However, I note that the distinction between these two possibilities has far-reaching implications for the present study, which is focused on saproxylic invertebrates that inhabit fallen *Eucalypt* logs, and their ability to persist in Tallaganda throughout glacial maxima.

**Current progress in Tallaganda**

The Tallaganda study has focused upon inferring the responses of saproxylic taxa to Pleistocene climate variation. The phylogeographic structure displayed by log-dwelling invertebrates in Tallaganda has been found to be broadly congruent with the *a priori* regions defined by Garrick *et al.* (2004), which correspond to the boundaries separating hydrological catchments. However, each taxon displays unique deviations from these expectations. For example, two unnamed species of neanurid collembolan (Garrick *et al.* 2004; Garrick *et al.* 2007) displayed strong phylogeographic structure supporting five populations in the first species (Garrick *et al.* 2004) and six populations in the other (Garrick *et al.* 2007), including distinct Badja and Deua groups. Two terrestrial flatworms, *Artioposthia lucasi* and *Caenoplanca coerulea* (Sunnucks *et al.* 2006), were defined into six and five distinct populations respectively, whilst a saproxylic species of
skink, *Eulamprus tympanum*, displayed evidence for distinct Tallaganda and Badja populations (Hodges *et al*. 2007). Overall, the phylogeographic boundaries detected across these taxon-specific studies are broadly congruent; that is, some phylogeographic breaks are common to all taxa. Together these studies constitute a body of evidence that supports the hypothesis that saproxylic invertebrates have responded similarly to the glacial interglacial cycling that characterised the Pleistocene.

**The Taxon: Australian funnel web spiders**

**Taxonomy of Australian funnel web spiders**

Australian funnel web spiders (Mygalomorphae: Hexathelidae) are divided into two reciprocally monophyletic sister-genera, *Hadronyche* and *Atrax* (Rowell 1980). The current taxonomy dates from 1988 and describes 13 valid species and 35 characterised species within these two genera (Gray 1988). According to this taxonomy, *Hadronyche* contains 32 species that are separated into six species groups (*infensa*, *cerberea*, *illawarra*, *modesta*, *lamington* and *adelaidensis*) whilst the remaining three species belong to the genus *Atrax* (Gray 1988). However, the current taxonomy is in need of review: since 1988, large numbers of undescribed species have continued to be identified throughout the range of the group (Wilson 2001). An early publication stemming from the current project identified numerous candidates for species status (Beavis & Rowell 2006) that appear to be cryptic and, as such, were not considered in the 1988 taxonomy. Consequently it is possible that the current taxonomy is unrepresentative of actual species diversity.

**Biology and its influences**

All members of the Mygalomorphae (or “primitive” spiders) are thought to be restricted in their dispersal capabilities and distribution due to a propensity for desiccation, which occurs as an outcome of one of the defining characters of the infraorder: two pairs of book-lungs that expose a large surface area of tissue for gas-exchange (Main 1982). The distribution of mygalomorph spiders is chiefly restricted to habitats where opportunities for water-loss are reduced (Main 1982). Consequently, the distributions of *Atrax* and *Hadronyche* are restricted to more humid environments, both genera displaying high levels of habitat fidelity to ecological niches where water loss is less likely to occur (Main 1982). This effect of biology upon species distributions is obvious in the range of
Australian funnel web spiders: *Atrax* and *Hadronyche* occupy a range that is essentially limited to South-Eastern Australia, extending approximately 150km inland from South-Eastern Queensland to Adelaide, and also including Tasmania (Wilson 2001).

**Funnel web spiders at Tallaganda**

The Tallaganda region provides habitat to high densities of unnamed species of *Hadronyche* and *Atrax*. The taxonomy and systematics of Tallaganda populations of these two genera were unstudied prior to this project. Consequently, funnel web spider taxa found at Tallaganda will be referred to as *Hadronyche* Species 1 (*Hadronyche Sp.1; Plate 1.3*) and *Atrax* Species 1 (*Atrax Sp.1; Plate 1.4*) throughout the text.
Chapter 1: Introduction

Plate 1.3 An adult female *Hadronyche* Sp. 1 (Picture courtesy of Dr Julian Ash, ANU)

Plate 1.4 An adult female *Atrax* Sp. 1
Plate 1.5 *Hadronyche* Sp. 1 burrow entrance

Plate 1.6 *Atrax* Sp. 1 burrow entrance
Chapter 1: Introduction

Niche separation

*Hadronyche* Sp.1 exclusively inhabits decomposing logs (Plate 1.5). In contrast, *Atrax* Sp.1 constructs silk-lined ground burrows on the forest floor (Plate 1.6). Both species are common within Tallaganda, albeit with patchy distributions. Female individuals within both *Hadronyche* Sp. 1 and *Atrax* Sp. 1 are long-lived, with a maximum lifespan of 20 years (Levitt 1961), and are thought to display generally low levels of vagility after an initial period of juvenile dispersal (Woodman *et al.* 2006). Males also disperse as juveniles and remain sessile until they moult to adulthood at 5 years, after which they are vagrant until they experience almost 100% mortality after 9 months (Levitt 1961; Wishart 1993). Tallaganda *Hadronyche* Sp. 1 are extremely specialised to the saproxylic habitat. *Hadronyche* Sp. 1 spiderlings are thought to undergo a once-off dispersal event at an early age. At this initial dispersal event, individuals excavate burrows in the crevices of decomposing wood – generally within 5m of the maternal burrow but with some long-range dispersal – and remain there for the duration of their lives (or, if male, until they molt to maturity), enlarging the burrow as they grow (Woodman *et al.* 2006). Burrows are lined with a double layer of silk and may be up to 3m long (Rowell 1980; Woodman *et al.* 2006) with a single entrance from which silk trip-lines radiate (Woodman *et al.* 2006). In contrast, *Atrax* Sp. 1 appear to disperse freely throughout their lifetime. Individuals construct ground burrows that may extend to lengths of up to 30-50cm. The burrow entry is surrounded by a network of silk-defined entries and is generally found beneath logs, rocks, leaf-litter and occasionally in open ground. The burrow itself is lined with a single layer of silk. Spiders may be found in a chamber at the end of this burrow or in loose soil at the end of the chamber.

The specific parameters of *Atrax* Sp. 1 dispersal capabilities have not been quantified. However, a phylogeographic pilot study for this thesis produced both genetic and anecdotal evidence suggesting that whilst widespread dispersal is limited due to the propensity of mygalomorphs to desiccation, local migration may be common (Beavis & Rowell 2006). In the course of field collections for the present study I have encountered burnt sites where there are large *Atrax* Sp. 1 burrows containing the decomposing carcase and exoskeleton of large individuals. These large burrow spaces almost always contained a smaller *Atrax* Sp. 1 individual that had constructed the regular silk ‘stocking’ burrow lining, appropriate to its own size, and fastened it at intervals to the
far larger burrow cavity left by the previous inhabitant (Pers. Obs.). Furthermore, areas
damaged by forestry practices were found to contain *Atrax* Sp. 1, but not *Hadronyche*
Sp. 1. *Atrax* Sp. 1 individuals located in such sites were found in burrows constructed
above bark debris that had accumulated only as a process of logging. These
observations suggest that although widespread migration of *Atrax* Sp. 1 individuals is
restricted due to their tendency to desiccate, migration and gene-flow may be
uninhibited within localised regions. In contrast, there have been suggestions that
*Hadronyche* Sp. 1 undergoes widespread migration, despite the fact that dispersal
occurs as a once-off event (Woodman *et al.* 2006). Woodman *et al.* (2006) described
how logs that provided habitat to *Hadronyche* Sp. 1 were separated from nearest-
neighbour logs by up to 23m, suggesting that juveniles were capable of dispersing
equivalent distances. In addition, the vagrant life-stage of adult male *Hadronyche* Sp. 1
provides opportunities for dispersal that far exceed the range of juvenile individuals.

**Previous research**
Although previous research on *Hadronyche* and *Atrax* species within the Tallaganda
region has been limited, that which has been conducted is deeply relevant to the present
across both latitudinal and longitudinal gradients, finding that the distribution and
abundance of *Hadronyche* Sp. 1 responded to a series of variables including the degree
of log decay and longitudinal effects. Woodman *et al.* (2006) also found that logs in a
state of advanced decay were favoured over hard, fallen wood, possibly due to a higher
moisture content. In addition, southern sites with high rainfall were found to contain the
highest population densities, though northern sites containing logs in a state of
advanced decay provided habitat to comparable population densities (Woodman *et al.*
2006). An earlier study by Rowell (1980) investigated the systematics of unnamed
species of *Atrax* and *Hadronyche* in Monga State Forest, situated north of the
Tallaganda region. Rowell’s work established the reciprocal monophyly of *Hadronyche*
and *Atrax* and identified a suite of diagnostic phenotypic characters.
The Present Study

Neglected taxa and their inherent challenges

The definition of “neglected taxa” is subjective, but a reasonable consensus might agree that “neglected taxa” are any taxonomic group for whose members there is an absence of data, due to practical grounds (e.g. difficulties in specimen collection or preservation; technological challenges such as a lack of appropriate molecular genetics protocols and tools) or lack of research interest and an absence of funding. In essence these are taxa that have not been studied in depth, which further dissuades future research because there no starting point exists (Blaxter et al 2004). Organisms that are often referred to as “neglected taxa” include tardigrades and other invertebrate taxa (Blaxter et al 2004). There are common impediments to studying neglected taxa, one of the most challenging of which is the identification of appropriate molecular markers that provide sufficient resolution for phylogenetic inferences to be made. A more subtle difficulty is the lack of base-line data available for little-studied taxa, such as information on dispersal capabilities and ecological requirements. This means that it is challenging to knowingly meet the assumptions implicit to basic phylogenetic and population genetic methods. To address these knowledge gaps, it is essential to take a multi-faceted approach to obtaining and interpreting data.

Aims and Objectives

There is very little known about the basic biology, ecology and behaviour of Australian funnel web spiders. Consequently, this project has taken a multidisciplinary approach with the aim of placing the phylogeography of *Atrax* Sp. 1 and *Hadronyche* Sp. 1 within a biologically meaningful context by addressing specific aims as explored in the following chapters:

Chapter 2

This chapter is focused upon investigating the phylogeographic structuring of the log-dwelling *Hadronyche* Sp. 1 and inferring the historical processes responsible for that structure. Three key questions will be addressed in this chapter:

- What is the inferred population history for Tallaganda populations of *Hadronyche* Sp. 1?
Chapter 1: Introduction

- Is the geographic structuring of the phylogeny consistent with the effects of glacial-interglacial cycling, either in its patterns or timing?

- Has the response of *Hadronyche* Sp. 1 to historical climate variation been consistent with that of other log-dwelling taxa?

Chapter 3

This chapter addresses a single overriding question: are the barriers that occurred as a result of Pleistocene glacial-interglacial cycling the sole determining factors for the structuring of Tallaganda *Atrax* Sp. 1 populations, or has underlying geology also acted to provide an ongoing barrier to gene-flow?

Chapter 4

This chapter aims to use the phylogeographic patterns of Laelapidae mites to elucidate the population history of *Hadronyche* Sp. 1 and *Atrax* Sp. 1 in Tallaganda with the primary aim of addressing the following questions:

- Is there evidence of mite population differentiation according to host genus?

- Is there evidence of shared responses to historical demographic changes amongst Laelapidae mites and the host population with reference to host phylogeographic structure?

- Does the Laelapidae phylogeny support the inferred genus histories for *Atrax* Sp. 1 and *Hadronyche* Sp. 1?
  
  - *Hadronyche* Sp. 1: local extinction within Tallaganda and subsequent recolonisation
  
  - *Atrax* Sp. 1: a long history within Tallaganda with population subdivision

Chapter 5

By comparing the phylogeographic structure of two related mygalomorph taxa (one saproxylic and one ground-dwelling) with the previous findings of the Tallaganda
study, this chapter aims to elucidate the role of habitat in defining invertebrate responses to historical climate change. This chapter addresses the following questions:

- Are the patterns and timing of population divergences congruent amongst spider genera?
- Are the patterns and timing of population divergences congruent amongst mygalomorph spiders and the saproxylic taxa included within the Tallaganda project?
- Are the findings of this study congruent with the region’s inferred climate history?

**Summation**

The overall aim of this thesis is to investigate the processes that have contributed to the phylogeographic structure displayed by extant Australian funnel web spider species in the Tallaganda region of South-Eastern NSW; with a view to elucidating the historical, environmental and biological factors that have influenced the structure of extant populations. In doing so, this project will investigate population genetic, phylogenetic and behavioural aspects of Australian funnel web spider biology.
References


Grove SJ (2002b) Tree basal area and dead wood as surrogate indicators of saproxylic insect faunal integrity: a case study from the Australian lowland tropics. *Ecological Indicators*, 1, 171-299.


Templeton AR, Routman E, Phillips CA (1995) Separating population structure from population history: a cladistics analysis of the geographical distribution of


CHAPTER 2
CHAPTER 2

SURVIVING THE PLEISTOCENE: DOES HISTORICAL CLIMATE CHANGE DICTATE BARRIERS TO GENE-FLOW IN A SAPROXYLIC SPECIES OF FUNNEL WEB SPIDER?

Introduction

The Quaternary has come to be acknowledged as a period characterised by frequent climatic oscillations that have had a dramatic impact on the evolution of populations (Hewitt 2000; Hewitt 2004; Knowles & Richards 2005; Schonswetter et al. 2005). Phylogeographic techniques have emerged as a means of investigating the impacts of Quaternary glacial-interglacial cycling on extant biota and to infer climate-defined barriers to dispersal in historical populations (Bermingham & Moritz 1998; Beheregaray et al. 2001; Garrick et al. 2007). Phylogeography is classically referred to as the bridge between phylogenetics and population genetics, focusing as it does around and below the species level (Avise 1987; Avise 1998), however, identifying species that retain the genetic imprint of historical climate change has emerged as a dilemma central to the field. The saproxylic environment (i.e., decomposing logs on the forest floor) provides habitat to a suite of invertebrate species that are both sessile and long-lived (Grove & Stork 1999; Grove 2002a; Garrick et al. 2004). These traits lend log-dwelling invertebrate species to investigating phylogeographic principles. This is chiefly due to the fact that saproxylic taxa experience their environment at a fine scale, display limited long-range dispersal and often have long generation times, the combination of which conserves the detail of the phylogenetic signal (Moritz et al. 2001; Hugall et al. 2002, Garrick et al. 2004).

An ongoing study based in the Tallaganda region of South-Eastern Australia has been investigating the phylogeography of saproxylic invertebrates and its relation to the glacial-interglacial cycling that characterised the Pleistocene (Garrick et al. 2004; Beavis & Rowell 2006; Sunnucks et al. 2006; Garrick et al. 2007; Hodges et al. 2007). To date, multiple invertebrate taxon-pairs (including velvet worms, springtails, terrestrial flatworms and water skinks) have displayed phylogeographic patterns
congruent with the hypothetical locations of historical refugia. To distinguish between the phylogeographic effects of habitat (e.g., Baker & Bradley 2006), two niche-separated genera of funnel web spider – one saproxylic and one ground-dwelling – were added to the study. This taxon-pair showed the greatest disparity of habitat type of any others included in the Tallaganda project (Beavis & Rowell 2006), and was key to investigating whether the saproxylic habitat was indeed responsible for the phylogeographic patterns (and, therefore, responses to Quaternary climate change) found amongst saproxylic invertebrates in Tallaganda. This chapter investigates the phylogeography of a log-dwelling funnel web spider from the genus *Hadronyche*, and interprets these findings within the context provided by the other saproxylic invertebrates studied at Tallaganda.

*Hadronyche* in Tallaganda

This study examined the phylogeographic structuring of an unnamed species of log-dwelling funnel web spider belonging to the genus *Hadronyche* (Mygalomorphae: Hexathelidae). Henceforth this species will be referred to as *Hadronyche* Species 1 (*Hadronyche* Sp. 1). The group is common within Tallaganda and exclusively inhabits decomposing logs. Very little is known about the biology of this group, however, there are some documented characteristics. Female individuals are long-lived, with a life-span of up to 20 years (Levitt 1961), and display low vagility after an initial period of juvenile dispersal (Woodman *et al.* 2006). Males also disperse as juveniles and remain sessile until they moult to adulthood at 5 years, after which they are vagrant until they experience almost 100% mortality after 9 months (Levitt 1961; Wishart 1993). At the initial dispersal event individuals excavate burrows in the crevices of decomposing wood – generally within 5–23m of the maternal burrow but with some long-range dispersal – and remain there for the duration, enlarging the burrow as they mature (Woodman *et al.* 2006). Burrows are lined with a double layer of silk and may be up to 3m long with a single entrance from which silk trip-lines radiate (Rowell 1980; Woodman *et al.* 2006). *Hadronyche* Sp. 1 is extremely specialised to the saproxylic habitat and it was hypothesised that the species would display congruence with the phylogeographic patterns found in other saproxylic taxa studied in Tallaganda.
Chapter 2: Surviving the Pleistocene

The Tallaganda region
The Tallaganda region is a 100km long transect of continuous, temperate sclerophyll forest located in South-Eastern New South Wales (NSW), comprising sections of both State Forest and National Park (State Forests of NSW 1995). Tallaganda is an isolated spur that extends out from the Great Dividing Range (GDR). The Tallaganda region connects to the GDR in the south, but is otherwise surrounded on all sides by low-lying grasslands and pasture that effectively isolate it from the escarpment. The Tallaganda region includes a portion of the Lake George catchment. Lake George is the major source of data for vegetation history reconstruction in South-Eastern New South Wales. In 1985 Singh and Giessler reported on the results of pollen, spore, algal and charcoal particle analyses from an 18m soil core sample from Lake George. From this data the climate history of the region has been reconstructed for the period 730 000 years before present (ybp) to the present day. Consequently, there is a comprehensive record of climate variation in the region immediately adjacent to Tallaganda.

Pleistocene climate variation in Australia
The last 2.4 million years (Myr) have been punctuated by 20 glacial-interglacial periods occurring on a 100 000 year cycle, the most recent glacial ending 10 000 years ago (Hewitt 1996; Hewitt 2004). At the last glacial maxima (LGM) temperatures appear to have been as much as 7 – 15°C cooler than at present (Hewitt 2004), causing glaciation in the Northern hemisphere and extensive climate modification in the Southern hemisphere (Hewitt 1996). In Australia, glacial periods resulted in high levels of aridity (estimates suggest that precipitation was reduced by 50%), sea levels dropped by 120m and fossilised pollen records (the palaeorecord) suggest that the range of Eucalypt forest contracted to the coastal regions. The Eucalypts were virtually replaced by expanding alpine and grassland systems (Dodson & Ono 1997). Thus, from the perspective of flora and dependent fauna, the Quaternary consisted of episodic contractions of forests to refugia during glacials, and subsequent expansion during the more temperate interglacials. In short, climate change during this period alternately established and removed barriers to dispersal and gene-flow, impacting on the evolution of populations to the extent that the imprint of this series of climatic changes may be found in extant taxa.
The Tallaganda model

Garrick et al. (2004) defined five a priori microbiogeographic zones in Tallaganda. The parameters of these zones were primarily defined by the ridge-line boundaries that separate hydrological catchments. The Tallaganda project has subsequently been based upon the a priori prediction that these microbiogeographic regions had been the locations of glacial refugia (Garrick et al. 2004). I predicted that phylogeographic structuring of the saproxylic Hadronyche Sp. 1 would correspond with these five microbiogeographic regions on the basis of two key assumptions. First, this species is saproxylic and consequently the prediction of this study was that the taxon would respond to glacial cycling in a similar fashion to the other log-dwelling invertebrates under study. Second, this species is a habitat-specialist and appears to undergo a once-off dispersal event as a spiderling, remaining in the burrow it establishes at this time for the remainder of its lifespan (Woodman et al. 2006). Therefore, one would predict that the distribution of individuals within Tallaganda would be sensitive to any demographic change in the Eucalypt population, for example, the reduction of tree-line to lower elevations during the glacials. In addition, mygalomorph spiders are long-lived, sessile terrestrial invertebrates (Levitt 1961): these are traits that, across multiple taxa, result in an increased retention of the genetic signals of older landscape events (Moritz et al. 2001).

Summation

This study is aimed at investigating the phylogeographic structuring of the log-dwelling Australian funnel web spider genus Hadronyche Sp. 1 and inferring the historical processes responsible for that structure. Consequently, this paper aims to address three key questions:

- what is the inferred population history for Tallaganda Hadronyche Sp. 1;
- is the geographic structuring of the phylogeny consistent with the effects of glacial-interglacial cycling, either in its patterns or timing;
- has the response of Hadronyche Sp. 1 to historical climate variation been consistent with that of other log-dwelling taxa?
Chapter 2: Surviving the Pleistocene

Methods

Sample collection
Samples were collected from throughout the Tallaganda region and from adjacent mountain ranges between 2002 – 2005 (Map 2.1). Hadronyche Sp. 1 individuals were extracted from their burrows in decomposing logs. Where possible, I attempted to sample between 2–6 individuals per sampling site (Map 2.1; Appendix 1.1). One whole leg was taken from samples – prior to preservation of the individual in 70% ethanol – and kept at -20°C.
Map 2.1 Hadronyche Sp. 1 sample distribution map
(see Appendix 1.1 for sample details; see Insert for enlarged copy of map)
DNA extraction and sequencing

This project has found that Australian funnel web spider DNA can be extremely unstable, sometimes degrading completely within 24-48 hours after extraction using a standard C-TAB-Chloroform protocol. The following extraction protocol and PCR conditions were developed as the most effective means of obtaining sufficient PCR product for sequencing. Genomic DNA was isolated from 1mm³ muscle tissue using QIAGEN DNeasy Tissue Kits. To obtain template DNA robust to degradation, it was essential to dissect muscle tissue from inside a leg segment and to prevent excess contact between the external cuticle and the extraction buffer. Sequence was obtained from 94 individuals at the mitochondrial locus of interest. From these, a subset of 32 individuals was selected for sequencing at a nuclear intron locus. Samples included in this subset were selected to represent the major mitochondrial clades and the full geographic range of Tallaganda. A 700 base pair (bp) fragment of mitochondrial sequence was amplified at the gene Cytochrome Oxidase Subunit I (COI) using the universal primer pair Lco1490: 5’ – GGTCACAACATCATAAGATATTGG and Hco2198: 5’ – TAAACTTCAGGCTGAACAAAAATCA (Folmer et al, 1994). Amplifications were as follows: 1 cycle of 94°C for 3 min, 65°C for 30 s, 72°C for 45 s; 2 cycles of 94°C for 30 s, 60°C for 20 s, 72°C for 45 s; 2 cycles of 94°C for 45 s, 55°C for 20 s, 72°C for 45 s; 3 cycles of 94°C for 45 s, 50°C for 20 s, 72°C for 45 s; 2 cycles of 94°C for 45 s, 45°C for 20 s, 72°C for 45 s; 40 cycles of 94°C for 45 s, 50°C for 20 s, 72°C for 45 s; 1 cycle of 72°C for 3 min; rest at 4°C. Amplification reactions consisted of 2µL template DNA; 2U Platinum Taq PCRx DNA Polymerase (Invitrogen); 4µL 10X PCR Buffer, minus Mg; 2.4µL 50mM MgCl₂; 1.6µL 5mM dNTPs; 1µL each 10mM primer; in 40µL total volume. A 400 bp fragment of nuclear intron sequence from the gene Histone 3 (H3) was amplified using the Colgan et al. (2000) primer pair H3F: 5’ – ATGGCTCGTACCAAGCAGACVGC and H3R: 5’ – ATATCCTTRGGCATRATRGTGAC. Amplifications were as follows: 1 cycle of 94°C for 3 min, 65°C for 30 s, 72°C for 45 s; 2 cycles of 94°C for 30 s, 60°C for 20 s, 72°C for 45 s; 2 cycles of 94°C for 45 s, 55°C for 20 s, 72°C for 45 s; 3 cycles of 94°C for 45 s, 50°C for 20 s, 72°C for 45 s; 40 cycles of 94°C for 45 s, 50°C for 20 s, 72°C for 45 s; 1 cycle of 72°C for 3 min; rest at 4°C. Amplification reactions consisted of 2µL
template DNA; 2.5 U Platinum Taq PCRx DNA Polymerase (Invitrogen); 4 µL 10X PCRx Buffer; 2.4 µL 50 mM MgSO₄; 1.6 µL 5 mM dNTPs; 1 µL each 10 mM primer; in 40.5 µL total volume. Polymerase chain reaction (PCR) products were purified using 3M NaAc with an ethanol precipitation. PCR products were sequenced in both directions using Big Dye Terminator (Applied Biosystems) and run on an ABI3100 Genetic Analyser.

Sequences were edited and aligned in SEQUENCHER (version 3.0, Gene Codes Corporation, Mi). Ambiguous end regions were removed such that all individuals within each genus were analysed over the same sequence length. The protein coding sequences (COI) were translated to identify the codon reading frame for the fragment, and to validate that the target was consistent with being functional mtDNA. After alignment and cropping, a 551 bp fragment for COI and a 324 bp fragment for H3 remained. The model of nucleotide substitution that best explained the data set was determined using a hierarchical likelihood ratio test (hLRT) and Akaike Information Criterion (AIC), implemented in Modeltest v.3.7.0 (Posada & Crandall 1998) and PAUP* v.4.0 b10 (Swofford 2001). A partition homogeneity test was implemented in PAUP* to test for significant congruence of the genetic signal between the two loci. A test for saturation was implemented in DnaSP v.4.10.9 (Rozas et al. 2003) by plotting the relative proportions of transitions and transversions against genetic distance under a model of nucleotide evolution selected by Modeltest as the simplest model that best fit the data. Unique haplotypes were identified for each locus using GenAlEx v.6.1 (Peakall & Smouse 2001).

Sequence analysis
Summary statistics were calculated for the purposes of describing the variation contained within the data, testing the neutrality of the markers used and investigating the dynamics of the populations under consideration. Using the program DnaSP the number of unique haplotypes (h), haplotype diversity (Hd), nucleotide diversity (π) and the average number of nucleotide differences (k) were calculated separately for each locus. In order to address a key assumption behind inferring phylogeographic history from mtDNA sequences, namely, that sequences evolve in a selectively neutral fashion (Zink et al. 2006) I performed a series of neutrality tests. Tajima’s D and Fu’s Fs were
used to assess whether sequences conformed to neutral expectations, that is, whether they had been subject to selection. In the absence of selection, significant deviations from neutral expectation for Tajima’s D and Fu’s Fs statistics are indicative of historical population expansion (Dean & Ballard 2004). In order to separate the putative effects of selection from those of historical demographic processes, I conducted the MK-test (McDonald & Kreitman 1991), to test solely for selection (Ballard & Whitlock 2004).

Phylogenetic analysis

Multiple approaches to phylogenetic analysis were employed in this study as follows. Statistical parsimony networks were constructed using the program TCS v.1.21 (Clement et al. 2000). Networks constructed using these methods have two main advantages over tree-based methods: first, statistical parsimony implements a 95% confidence interval on the network produced (Avila et al, 2006); and second, this method does not artificially impose a tree-like structure on the phylogeny. Bayesian searches of tree space were conducted using MrBayes v.3.1.2 (Huelsenbeck & Ronquist 2001) as follows: 2 runs, each with four Metropolis Coupling Markov Chain Monte Carlo (MCMCMC) chains for 10 000 000 generations, sampling trees every 100 000 generations (test runs indicated that convergence occurred after 5 000 000 generations, regardless of chain temperature or model parameters). A 10% burn-in was implemented and those trees discarded accordingly. MrBayes runs were conducted using the MODELTEST selected model of nucleotide substitution – the General Time Reversible + a proportion of invariant sites + gamma distribution model (GTR + I + G) – and the default uniform priors. Each run was started using a random tree. I assessed convergence of the separate runs by examining the plot of generation vs. log probability of the data (ie. log likelihood values) produced by MrBayes, looking for evidence of stationarity.

Population genetic analysis

In order to test for significant population structure a number of analyses of molecular variance (AMOVA) were implemented in GenAlEx v.6.1 (Peakall & Smouse 2001). AMOVA (\(\phi_{ST}\)) performs hierarchical analyses of genetic differentiation with tests of significance via random permutation. I tested the null hypothesis of no significant population structure against a series of alternative hypotheses as follows:
Chapter 2: Surviving the Pleistocene

- H1: populations defined according to \textit{a priori} expectations, that is, the boundaries that distinguish hydrological catchments and sub-catchments as per Garrick \textit{et al} (2004);
- H2: populations defined as Tallaganda vs. Badja vs. the GDR.

AMOVA generates a number of haplotype correlation measures referred to as $\phi$-statistics, which are analogous to F-statistics, however the $\phi_{ST}$ statistic has advantages over F$_{ST}$ for the present study. F-statistics calculate the degree of variation explained by \textit{a priori} population parameters according to absolute differences amongst individuals, whereas $\phi$-statistics make use of the degree of variation contained within the sequences themselves. The 95\% confidence interval was calculated via 9999 bootstrap replicates (with replacement).

\textbf{Results}

\textbf{Sequence diversity}

Thirty-three mtCOI unique haplotypes were identified from 94 individuals. 45 individuals were represented by a single, widespread, mtDNA haplotype that was found across the full longitudinal extent of the Tallaganda region. This widespread haplotype was not recovered from outside Tallaganda, however, of 25 private haplotypes 18 individuals represented Tallaganda specimens and the remaining 7 samples were sourced from outside Tallaganda. Those private haplotypes recovered from within Tallaganda were closely related to common haplotypes, whereas private haplotypes recovered from the GDR were reasonably distinct from other haplotypes (See Phylogenetic Analysis section for details). Nucleotide and haplotype diversity values from the whole region were 0.024 (COI)/ 0.013 (H3) and 0.763 (COI)/ 0.569 (H3) respectively (Table 2.1). Thus, at both loci, a pattern of high haplotype diversity and low nucleotide diversity was detected: this pattern is indicative of rapid expansion from a small population.

Analysis of sequence diversity for the coding mtDNA locus, COI, revealed 181 variable sites, 105 of which were parsimony informative. No unexpected stop codons were found within the open reading frame. Third codon positions were most variable (74.03\% of variable sites), followed by first (21.00\%) and then second (4.97\%) codon
positions. A test for sequence saturation (including 3rd codon positions) found that transversions outnumbered transitions at approximately 18% sequence divergence, indicating the degree of sequence divergence at which saturation would impact on the amount of information encoded by the sequences. As the maximum uncorrected p-distance found was 15%, sequence saturation was unlikely to bias any parsimony analysis. Analysis of sequence diversity of 324bp from the nuclear intron H3 revealed 18 variable sites, 14 of which were parsimony informative, and 9 unique haplotypes. A partition homogeneity test indicated no evidence for phylogenetic incongruence between the two loci of interest. Therefore I concatenated the sequences using MacClade v.4.07 resulting in a 875bp length of sequence. All individuals found within a single mitochondrial haplotype could be unambiguously nested within a single nuclear haplotype. Consequently, the concatenated sequence data haplotypes were defined as per mtDNA haplotypes, which provided more resolution when examining phylogenetic relationships amongst taxa. The concatenated COI and H3 fragments produced an 875bp fragment containing 216 variable characters, 148 of which were parsimony informative.

**Table 2.1: Genetic diversity indices of Hadronyche Sp. 1 (total sample and Tallaganda sample).**

Table 2.1 shows N, number of individuals; M, number of haplotypes; π, nucleotide diversity; h, haplotype diversity; k, average number of nucleotide differences.

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>COI</th>
<th>H3</th>
<th>M</th>
<th>COI</th>
<th>H3</th>
<th>h</th>
<th>COI</th>
<th>H3</th>
<th>k</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>94</td>
<td>32</td>
<td>33</td>
<td>9</td>
<td>0.024</td>
<td>0.013</td>
<td>0.763</td>
<td>0.569</td>
<td>13.259</td>
<td>4.196</td>
</tr>
<tr>
<td>Tallaganda</td>
<td>80</td>
<td>23</td>
<td>25</td>
<td>6</td>
<td>0.012</td>
<td>0.008</td>
<td>0.690</td>
<td>0.395</td>
<td>6.816</td>
<td>0.474</td>
</tr>
</tbody>
</table>

40
Tests of neutrality

At the mitochondrial locus of interest, neutrality tests supported a recent expansion in population size. Significant deviations from the null hypothesis were detected using Tajima’s D (-2.43984; p < 0.01), Fu & Li’s D (-3.896; p < 0.02) and Fu and Li’s F (-3.92241; p < 0.02). Fu’s F statistic did not significantly deviate from neutral expectations (-1.818; p > 0.01). The significantly negative neutrality test values are indicative of either the impact of selection or population expansion. The McDonald-Kreitman test was implemented as a means of separating the effects of selection from those attributable to demographic change. An MK test (significance estimated via a G-test) showed that the data set did not significantly deviate from neutral expectations (Neutrality Index: 0.938; p < 0.953). Therefore, these findings supported the hypothesis that Tallaganda populations of Hadronyche Sp. 1 had experienced a recent population expansion event. At the nuclear locus of interest, neutrality tests produced no values significantly different from 0, however, H3 showed little sequence variation and is unlikely to be informative at a population scale.

Phylogenetic analysis

Overall, the phylogenies obtained using maximum parsimony (Figure 2.1) and Bayesian methods (Figure 2.2) produced equivalent topologies. Bayesian approaches show strong support for those clades that represent geographic populations external or peripheral to Tallaganda, namely those found in Deua (100), Mt Fairy (99) and the Gudgenby Range (96). The Tallaganda clade itself has high node support (96) but is unresolved (ln L = -3516.33). Both the maximum parsimony network and the Bayesian phylogeny show that there is virtually no spatial genetic structure within Tallaganda, the phylogeny being shallow and characterised by multiple polytomies. The section of the phylogeny that represents haplotypes sourced from within Tallaganda displays evidence of a recent and rapid radiation, that is the phylogeny displays a classic broom topology (Figure 2.2) whilst the network forms a star-burst pattern (Figure 2.1).
Chapter 2: Surviving the Pleistocene

Figure 2.1 Hadronyche Sp. 1 statistical parsimony network

Statistical parsimony network for *Hadronyche* Sp. 1 (Blue = Tallaganda samples; Green = external to Tallaganda). (See Insert for enlarged copy of network)
Figure 2.2 Hadronyche Sp. 1 Bayesian phylogeny

Bayesian MCMC phylogeny for Hadronyche Sp. 1, using a GTR + \gamma + \lambda model of nucleotide evolution (ln L = -3516.33). Clade credibility values are shown for major nodes. All taxa occur within Tallaganda, except where indicated by the key. Scale shows the number of inferred changes per site. (See Insert for enlarged copy of phylogeny)
Population genetics analysis

An Analysis of Molecular Variance (AMOVA) detected no significant genetic structure under the assumption of populations defined according to *a priori* expectations, that is, hydrological catchments (H1). However, this test detected significant genetic structure under the assumption of a phylogeographic break separating Tallaganda from Badja and the GDR (Table 2.2). This finding confirms the importance of the Badja/ Tallaganda break – which is common to multiple saproxylic taxa in Tallaganda – to the system of interest.

**Table 2.2 AMOVA tests of genetic subdivision**

Table 2.2 shows AMOVA results. Populations were defined according to H1 and H2 (see methods). The probability of observations was assessed via 9999 permutations of the data. Table 2 shows the following measures of population structure: n, number of individuals; N, number of populations; df, degrees of freedom; SS, value for the sum of squares based on pairwise distances; Var., the variance component; %, percentage of variance; \( \phi_{ST} \); p-value.

<table>
<thead>
<tr>
<th>Hypothesised population parameters</th>
<th>Source of Variation</th>
<th>n</th>
<th>N</th>
<th>df</th>
<th>SS</th>
<th>Var</th>
<th>%</th>
<th>( \phi_{ST} )</th>
<th>p &lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1. <em>a priori</em> regions</td>
<td>Among populations</td>
<td>78</td>
<td>7</td>
<td>6</td>
<td>41 502</td>
<td>0.460</td>
<td>17%</td>
<td>0.175</td>
<td>0.102</td>
</tr>
<tr>
<td></td>
<td>Within populations</td>
<td>71</td>
<td>154</td>
<td>395</td>
<td>2.175</td>
<td>83%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H2. Tallaganda/ Badja/ GDR</td>
<td>Among populations</td>
<td>89</td>
<td>4</td>
<td>3</td>
<td>106.192</td>
<td>2.009</td>
<td>29%</td>
<td>0.294</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>Within populations</td>
<td>85</td>
<td>415</td>
<td>605</td>
<td>4.899</td>
<td>71%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Chapter 2: Surviving the Pleistocene

Discussion

Overview

Tallaganda Hadronyche Sp. 1 displayed no significant population structuring nor was the Tallaganda population a repository of high levels of genetic diversity, rather subdivision of the phylogeny was shallow and geographically unstructured. The five \textit{a priori} regions defined by Garrick \textit{et al.} (2004) were not reflected in the Hadronyche Sp. 1 phylogeny, however, there was evidence that the Tallaganda/ GDR boundary acted as a significant barrier to gene-flow. The topology of the phylogeny suggested that a recent radiation had occurred, multiple recent polytomies forming the "broom-like" pattern that is often indicative of a rapid radiation (eg. Walton \textit{et al.} 2000). A series of neutrality tests produced significantly negative values, suggesting that these populations had recently and rapidly recovered from a reduction in population size (Ballard & Whitlock 2004). Overall, these population traits are characteristic of a vicariance event followed by an increase in population size.

Inferred population history

Features of the Hadronyche Sp. 1 phylogeny and population parameters suggest that this genus has recently colonised Tallaganda, an event that could have occurred in one of two possible ways. First, the current Tallaganda Hadronyche Sp. 1 population may represent the first time this taxon has inhabited the region. Second, Hadronyche Sp. 1 may have become locally extinct in the region at a previous point in time, only recently recolonising the area. It is impossible to distinguish between these two hypotheses using the phylogenetic evidence alone, however, one may investigate the colonisation strategies that have resulted in the observed phylogeographic structure of Hadronyche Sp. 1. Overall, statistical parsimony and Bayesian approaches produced the classic "star-burst" topology that is indicative of a recent and rapid increase in haplotype diversity (Slatkin & Hudson 1991; Althoff & Pellmyr 2002; Carsten & Knowles 2007). This feature of the dataset is also reflected in measures of nucleotide diversity and tests of neutrality. The relatively low nucleotide diversity and high haplotype diversity found within Tallaganda populations of Hadronyche Sp. 1 was detected at both the mitochondrial and nuclear loci of interest and is consistent with rapid expansion from a small population (Hewitt 2000; Heilveil & Berlocher 2006). Of particular significance
to the present study is that low haplotype diversity is a feature typical of taxa from recently deglaciated regions (Heilveil & Berlocher 2006). Tests of neutrality also provided evidence that the *Hadronyche* Sp. 1 population numbers have rapidly expanded to current levels. This study considers a recent radiation – perhaps following the colonisation of Tallaganda – to be a probable cause of the phylogeographic patterns observed in *Hadronyche* Sp. 1.

Given the findings of the present study that *Hadronyche* Sp. 1 may have recently colonised the Tallaganda region, the phylogenetic relationship between Tallaganda and GDR samples is of significant interest. The close relationship between Tallaganda *Hadronyche* Sp. 1 and samples taken from multiple locations along the south-east coastline of NSW suggests that the GDR – in conjunction with overall low nucleotide diversity – provided the source population for a founder event resulting in the extant *Hadronyche* Sp. 1 population at Tallaganda (eg. Anderson *et al.* 2006). A similar pattern was also detected in *Eulamprus heatwolei*, a log-associated water skink, at Tallaganda by Hodges *et al.* (2007). Similarly to *Hadronyche* Sp. 1, *E. heatwolei* displayed evidence of a recent radiation in the form of a “star” phylogeny. Interestingly, the ecological requirements of the two species share distinct similarities. *E. heatwolei* displays a distinct preference for more mesic habitats, that is, sites with a higher moisture content (Hodges *et al.* 2007), whilst mygalomorph spiders are extremely susceptible to desiccation due to the large tissue surface area exposed to the effects of water-loss via book lungs (Foelix 1996). This ecological similarity combined with the congruent phylogeographic structure displayed by *Hadronyche* Sp. 1 and *E. heatwolei* suggests that the dry conditions – perhaps those that occurred during glacial periods – influenced the persistence of these species in Tallaganda.

**Phylogeography and the influences of historical climate change**

Whilst the saproxylic habitat niche occupied by *Hadronyche* Sp. 1 acts as a buffer to external conditions (Sunnucks *et al.* unpublished data) and provides a means of avoiding desiccation, Pleistocene glaciation may have resulted in the fragmentation – or even absence – of this refuge, thus exposing *Hadronyche* Sp. 1 to the potentially devastating effects of historical climate change. The importance of continuous habitat availability – both in space and time – for the saproxylic community has been firmly
established in a conservation context (Grove 2002b). Northern hemisphere studies have proposed that the absence of continuous habitat acts as a major limiting factor for sessile saproxylic invertebrates (Grove 2002b). This paper proposes that an absence of either temporally or spatially continuous habitat may be responsible for the phyleogeographic patterns observed in *Hadronyche* Sp. 1. Given this context, this study suggests three arenas in which the environment experienced by *Hadronyche* Sp. 1 was not continuous: prey availability, habitat lifespan and generational recruitment. The broader Tallaganda project has been concerned with the effects of Pleistocene glacial-interglacial cycling upon saproxylic invertebrates, and has argued that the cold and dry conditions associated with glacial periods acted to fragment the forest. Forest fragmentation would unavoidably produce a fragmented saproxylic landscape and the literature emerging from the Tallaganda project suggests that this was indeed the case (Garrick *et al.* 2004; Beavis & Rowell 2006; Sunnucks *et al.* 2006; Garrick *et al.* 2007, Hodges *et al.* 2007). However, *Hadronyche* Sp. 1 is exclusively log-dwelling and cannot re-establish a burrow after an initial juvenile dispersal stage (Beavis & Rowell 2006). Therefore, this paper proposes that the following outcomes of being exclusively log-dwelling during glacial periods are peculiar to *Hadronyche* Sp. 1. First, *Hadronyche* Sp. 1 feeds upon larger invertebrates and small vertebrate species including lizards and frogs (Rowell 1980). The data emerging from the Tallaganda project indicates that invertebrate species other than spiders experienced contractions in their geographic range during glacials (Garrick *et al.* 2004; Beavis & Rowell 2006; Sunnucks *et al.* 2006; Garrick *et al.* 2007). The recent research interest in the effects of Pleistocene glacial-interglacial cycling has shown that a contraction in a species’ geographic range is a common and widespread response to climate variation (Carsten & Knowles 2007). Consequently, it is likely that *Hadronyche* Sp. 1’s prey availability altered during colder and drier periods. Second, *Hadronyche* Sp. 1 inhabits burrows built within decomposing logs, a habitat item that has a finite lifespan of approximately 50 years (Barclay *et al.* 2000). In Australia during the last glacial maxima, the geographic range of *Eucalyptus* is thought to have been reduced to low-lying coastal regions. Under this assumption, habitat availability would have not persisted the full duration of glaciation. Thirdly, *Hadronyche* Sp. 1 are thought to disperse via a once-off juvenile migration event. Hostile (ie. cold and dry) conditions would act to increase the already high juvenile
mortality rate, functionally increasing the extent of habitat fragmentation experienced by *Hadronyche* Sp. 1.

**Hadronyche Sp. 1 at Tallaganda**

The initial expectation of this study was that *Hadronyche* Sp. 1 would provide the clearest phylogeographic evidence – of all the saproxylic invertebrates studied at Tallaganda – that pockets of suitable habitat provided refuges for populations during glacial periods. This expectation was grounded upon the biological assumption that *Hadronyche* Sp. 1 individuals inhabit a single log after an initial juvenile dispersal event. Consequently, the present study hypothesised that this dependence on a single log as habitat made *Hadronyche* Sp. 1 more log-dependent than the other saproxylic invertebrates at Tallaganda that display evidence of migration – albeit restricted – amongst logs (Garrick *et al.* 2004; Beavis & Rowell 2006; Sunnucks *et al.* 2006; Garrick *et al.* 2007; Hodges *et al.* 2007). However, the results produced by this study appear to support a recent population expansion across the extent of Tallaganda, possibly from a GDR source population, since which time there have been no significant barriers to gene-flow. This is in stark contrast to other log-dwelling invertebrates studied in the Tallaganda region, for example, terrestrial flatworms (Sunnucks *et al.* 2006), Collembolons (Garrick *et al.* 2004; Garrick *et al.* 2007), and Onychophora (Sands *et al.*, unpublished data), all of which display evidence for having persisted in the area throughout the Pleistocene. Furthermore, the lack of phylogeographic structure displayed by *Hadronyche* Sp. 1 suggests a capability to disperse broadly, if not with some habitat restrictions. If the phylogeography of *Hadronyche* Sp. 1 has been influenced by the Pleistocene glacialis, as have many saproxylic invertebrates, then the findings of this study suggests that *Hadronyche* Sp. 1 has been remarkably sensitive to the effects of historical climate variation.
Chapter 2: Surviving the Pleistocene

References


Beheregaray LB, Sunnucks P, Briscoe DA (2001) A rapid fish radiation associated with the last sea-level changes in southern Brazil: the silverside Odontesthes perugiae


Grove SJ (2002b) Tree basal area and dead wood as surrogate indicators of saproxylic insect faunal integrity: a case study from the Australian lowland tropics. *Ecological Indicators*, 1, 171-299.


terrestrial flatworms to past environmental climatic fluctuations at Tallaganda in montane southeastern Australia. *Molecular Ecology*, 15 (14), 4513-4531.


CHAPTER 3
CHAPTER 3

IS THE PLEISTOCENE THE ONE AND ONLY?
DISCERNING THE EFFECTS OF PAST CLIMATE AND CURRENT ENVIRONMENT ON THE PHYLOGEOGRAPHY OF AN AUSTRALIAN FUNNEL WEB SPIDER.

Introduction

Phylogeographic fingerprints of past climate and current environment

Distinguishing the evolutionary effects of historical processes from those of contemporary ones is an ongoing challenge in phylogeographic enquiry (cf. Jerry et al. 1998; Whiteman et al. 2007). In its purest form, phylogeography is aimed at inferring the demographic history of taxa from the geographic distribution of extant lineages, and is chiefly concerned with discerning the evolutionary effects of historical barriers to gene-flow (Avise 1998). However, the phylogeographic patterns displayed by extant taxa are equally as likely to have been influenced by ongoing and contemporary gene-flow restrictions, as they are intermittent and historical ones (Sork et al. 1999; Carini & Hughes 2004; Dyer & Nason 2004; Mardulyn & Milinkovitch 2005). Consequently, any taxonomic group may display the – not necessarily complementary – signatures of both historical and contemporary barriers to gene-flow. This characteristic of phylogeographic data is a potentially confounding factor for the inference of population histories, a commonly encountered example of which is the effect of sex-biased dispersal (Bond et al. 2001; Forister et al. 2004). Where sex-biased dispersal occurs, the phylogenetic signal may be confounded by the presence of two – not necessarily congruent – restrictions upon gene-flow, that is male vs. female dispersal. This situation is frequently addressed in the literature by the use of multiple nuclear loci in addition to the standard use of mitochondrial genes (eg. Sanetra & Crozier 2003; Forister et al. 2004; Criscione & Blouin 2007). A key point of note is that the addition of nuclear loci controls for the effects of sex-biased dispersal (ie. the separation of the signal of conflicting restrictions upon gene-flow) because it allows us to separate female-only gene-flow from gene-flow originating from both sexes. Where loci cannot be so easily partitioned according to the manner of their transmission it becomes more challenging to discern the driving forces behind conflicting genetic signals.
In the vast majority of scenarios, untangling the relative contributions of historical and contemporary gene-flow barriers will not be directly explored because there are no direct means of quantifying the relative effects that multiple factors have had upon gene-flow (Sork et al. 1999). In addition, where barriers to gene-flow have been in effect at multiple time-scales there is an increased chance that the assumptions underlying population genetic and phylogenetic methods will be violated, thus producing an unreliable result (Congdon et al. 2000). For example, population genetic analysis of a taxon-lineage containing both deep and shallow structure may violate the assumption of equilibrium conditions (i.e. there is no phylogenetic history) that underlies many population genetic analyses (Slatkin & Barton 1989; Whitlock & McCauley 1999; Drummond & Hamilton 2007). Conversely, analysis of the same dataset using tree-based methods may violate the assumption that the phylogenetic history of the taxon is tree-like, that is, there is no contemporary gene-flow (Dean & Ballard 2004). Whilst it is currently not possible to attribute proportions of phylogeographic variation to one restricting factor or another directly, it is possible to evaluate whether multiple factors have produced the phylogeographic structure detected in extant populations by taking a multidisciplinary approach (e.g. Congdon et al. 2000; Mardulyn & Milinkovitch 2005). In this way, One can build a detailed profile of the evolutionary processes potentially responsible for the phylogeographic structuring of extant populations. This paper seeks to investigate whether barriers to gene-flow other than those that occurred as a result of Pleistocene glacial-interglacial cycling have impacted on the phylogeographic structuring of spiders from the ground-dwelling genus Atrax (Hexathelidae: Mygalomorphae) in the Tallaganda region of South-Eastern New South Wales, Australia.

The study species: Atrax Sp. 1
Australian funnel web spiders (Mygalomorphae: Hexathelidae), sometimes referred to as the Atraceae (Rowell 1980), consists of two reciprocally monophyletic sister genera: Hadronyche and Atrax. The current taxonomy recognises 13 valid and 35 characterised species within these two genera (Gray 1988). However, it is widely recognised that the taxonomy of the Australian funnel web spiders is in need of review, as large numbers of undescribed species have continued to be identified throughout the range of the group since the publication of the current taxonomy in 1988 (Wilson 2001). Therefore, this
project will use the species signifier *Atrax* Sp. 1 to encompass all *Atrax* referred to in this study (ie. collected from Tallaganda and its immediate geographic surrounds). *Atrax* Sp. 1 is specialised to the forest floor habitat, constructing ground-burrows on the forest floor. Like other mygalomorph taxa (eg. Tarantulas and trapdoor spiders), *Atrax* Sp. 1 displays a suite of characteristics that make members of this taxon particularly appropriate and informative for phylogeographic studies. In general, terrestrial invertebrate taxa display both low vagility and habitat-specificity and consequently they experience their environment at fine spatial scales, thus acquiring and retaining the phylogenetic signals of past demographic change to a higher degree than vertebrate taxa (Moritz et al, 2001). Funnel web spiders, in particular, fit this profile, individuals being long-lived, sessile and occupying specific habitat niches. Female individuals are long-lived, reaching up to 20 years of age whilst males are thought to enjoy a shorter lifespan (Levitt, 1961; Main 1982). All members of the Mygalomorphae (or “primitive” spiders) are restricted in their dispersal capabilities and distribution due to a propensity for desiccation, which occurs as an outcome of one of the defining character of the infraorder: two pairs of book-lungs that expose a large surface area of tissue for gas-exchange (Main 1982). Consequently, the distribution of mygalomorph spiders are chiefly restricted to habitats where opportunities for water-loss are reduced (Main 1982). The distribution of *Atrax* Sp. 1 is restricted to the comparatively humid east coast, and the genus displays high levels of habitat fidelity to ecological niches where water-loss is less likely to occur (Woodman et al. 2006). *Atrax* Sp. 1 constructs silk-lined ground burrows on the forest floor and is common within Tallaganda, albeit with a patchy distribution. Individuals construct ground burrows that may extend to lengths of up to 30-50cm in depth. The burrow entry is surrounded with a network of silk-defined entries and is generally found beneath small – medium logs, rocks, leaf-litter and occasionally in open ground. The burrow itself is lined with a single layer of silk. Spiders may be found in a chamber at the end of this burrow or in loose soil found at the end of this chamber.

The Tallaganda region's historical environment: Pleistocene glaciation
The Tallaganda region is the site of an ongoing comparative phylogeography study that has been investigating the impacts of Pleistocene glaciations on a suite of log-dwelling invertebrates. A detailed description of the region and its history may be found in
Chapter 4, Hodges (2007) and Garrick (2004), however this paper will provide a précis of the characteristics and history of Tallaganda. The Tallaganda region is situated in South-East New South Wales (NSW), Australia and occurs as a narrow, 100km-long mountainous spur that extends from the Great Dividing Range (GDR). The region is surrounded by low-lying grasslands and is isolated from other mountainous, forested areas on all sides, except at its southernmost point (Badja) where it connects to the GDR (State Forests of NSW 1995). Tallaganda is adjacent to Lake George, one of the major large intermittent Australian water bodies. Lake George has been the source of much of the data for vegetation history reconstruction in South-Eastern NSW and, consequently, there are detailed climate reconstructions for the region extending back through the Pleistocene, to approximately 730 000 years before present (ybp) (Singh & Geissler 1985). Unlike Northern hemisphere land-masses, the Australian continent did not experience widespread glaciation during the Pleistocene (Barrows et al. 2001). Rather, glacial periods were characterised by a decrease of average temperatures that produced changes of vegetation composition according to elevation, for example, the upper tree-line in high-elevation areas decreased (Singh & Geissler 1985; Dodson & Ono 1997; Williams et al. 2006; Kershaw et al. 2007). It appears that this reduction of the distribution of vegetation may have restricted gene-flow amongst extant patches of available habitat. To date, a series of studies have found that multiple co-distributed log-dwelling invertebrates display phylogeographic structuring that is congruent with the locations of putative glacial refugia (Garrick et al. 2004; Beavis & Rowell 2006; Sunnucks et al. 2006; Garrick et al. 2007; Hodges et al. 2007). Research emerging from the Tallaganda project convincingly suggests that historical climate variation restricted gene-flow amongst glacial refuges, resulting in geographical structuring of genetic variation in animals that inhabit the saproxylic habitat.

The Tallaganda region's current environment: Geology

Whilst the Pleistocene glaciations are an obvious factor in restricting gene-flow amongst Tallaganda Atrax Sp. 1 populations, the specialised habitat-niche occupied by Atrax Sp. 1 may also impact on migration in this system (eg. Vandergast et al. 2004). Atrax Sp. 1 individuals exclusively occupy ground-burrows that they construct by digging directly into the soil substrate. The physical and chemical characteristics of soils encountered across Tallaganda vary according to a number of factors, for example,
the amount of organic material present in the soil, the degree of weathering the parent material has experienced, and ultimately the underlying geology of the geographic region in question (State Forests of NSW 1995; Beavis & Beavis, in prep.). This paper proposes that it is unrealistic to expect that the phylogeographic structure of *Atrax* Sp. 1 should have emerged solely as a result of Pleistocene glacial cycling, when the ongoing environment experienced by the taxon may have been limiting dispersal and restricting gene-flow for as long as *Atrax* Sp. 1 has been present in the region. Whilst it may be possible to detect the effects of both historical barriers to gene-flow and ongoing, contemporary, barriers, the results of this approach may be confounded by multiple – possibly interacting – factors. This study aims to assess whether geological barriers have acted – and continue to act – as barriers to gene-flow by investigating two elements of *Atrax* Sp. 1 phylogeography: first, whether there is evidence for partitioning of the phylogeny according to the barriers defined by underlying geology; and second, whether there is evidence for an evolved preference for soil type, that is for local adaptation.

Tallaganda is predominantly composed of metasedimentary and granitic (Igneous intrusive) lithology, though igneous extrusives and alluvial sediments also occur within the region (State Forests of NSW 1995). Metasediments dominate the spine of the multiple ridges that collectively form the Tallaganda range. Igneous intrusives, predominantly granite, occur on the Eastern and Western flanks of Tallaganda, the ridge lines of the Tuross Gorge and the Badja region and occur as intrusions within otherwise metasedimentary regions (State Forests of NSW 1995). In short, Tallaganda is a spur of metasediments surrounded and intruded by granitic material, which isolates it from a predominantly metasedimentary section of the Great Dividing Range (GDR). Despite the fact that the Tallaganda region is bisected by a number of fault-lines, the area has experienced little significant seismic activity during the Pleistocene and Holocene (State Forests of NSW 1995). Furthermore, the region has been geologically stable with the result that the lithology of the Tallaganda region defines the soil landscapes: metasediments found in the region date from the Ordovician period (490-443 mybp), whilst the granitic material and other igneous intrusives date from the Upper Silurian/Lower Devonian (443-354mybp) (State Forests of NSW 1995). Therefore, Tallaganda provides a temporally and geographically variable and patchy habitat for *Atrax*.  

59
Local adaptation in *Atrax* Sp. 1

The environmental and temporal variability encountered by *Atrax* Sp. 1 presents a rarely-addressed quandary: the interpretation of any phylogeographic signal occurring as a result of Pleistocene glacial-interglacial cycling may be confounded by the phylogeographic signal that occurs as an outcome of ongoing environmental barriers to gene-flow (i.e., geology). At best, this scenario may produce ambiguous or only partially congruent phylogeographic patterns; at worst, the phylogeographic fingerprint of gene-flow barriers both past and present may directly conflict. In either case, the inferences drawn from the genetic evidence will be compromised. To address this problem, the present study has quantified the behavioural ‘phenotype’ of *Atrax* Sp. 1 individuals by investigating evidence for local adaptation to refugia regions (as defined by the phylogeny) and soil type, thus providing an independent measure of the influence of history versus underlying geology on the phylogeography of *Atrax* Sp. 1. Local adaptation is said to have occurred when individuals from a local population (or deme) display increased fitness within their own habitat (or local environmental conditions) relative to non-local individuals (Kawecki & Ebert 2004; Ellis & Weis 2006; Knight *et al.* 2006; Sambatti & Rice 2006; Margraf *et al.* 2007). In Tallaganda *Atrax* Sp. 1 persist within both granitic and metasedimentary regions and, consequently, one can surmise that *Atrax* Sp. 1 can persist in both environments. However, the physical properties of these soils are very different and it is possible that underlying geology and local adaptation to soil type may be acting to limit gene-flow across Tallaganda. By quantifying local adaptation, this study aims to separate the phylogeographic influences of history from those of landscape, a question that cannot be addressed via an exclusively phylogenetic approach.

It is imprudent to ignore the potential for multiple factors to restrict gene-flow on either historical or contemporary scales: to do so may produce confounded or wholly incorrect phylogeographic inferences. This project takes a multi-stranded approach to addressing the quandary at hand. Overall, this study asks a single question: are the barriers that occurred as a result of Pleistocene glacial-interglacial cycling the sole determining factors for the structuring of Tallaganda *Atrax* Sp. 1 populations, or has underlying geology also acted to provide an ongoing barrier to gene-flow.
Methods

The Approach: Addressing Prior Expectations

This study predicts that *Atrax* Sp. 1 will display evidence of having survived Pleistocene glacial-interglacial cycling via phylogeographic congruence with the five *a priori* regions defined by Garrick *et al.* (2004), that is, with hydrological catchment boundaries. This paper aims to investigate the following specific prior expectations:

- *Atrax* Sp 1 occupies a specialised habitat: ground burrows constructed directly into the soil substrate. Consequently, I expect that the taxon will also display evidence of restricted migration and/or gene-flow amongst geologically distinct regions.

- Given the variation across Tallaganda of the underlying geology and the resulting soil profiles, I predict that individuals from a given geological region may show evidence of local adaptation.

- Overall, I expect that Tallaganda *Atrax* Sp. 1 will display evidence of gene-flow having been restricted by historical and intermittent barriers to gene-flow as well as ongoing and contemporary ones.

Sample collection

Spiders for the genetic component of this study were collected from throughout the Tallaganda region between 2002-2007 (Map 3.1) by locating burrow entrances beneath rocks and logs and excavating these burrows to extract the resident spider. Where possible, 2-6 individuals per site were sampled. One whole leg was taken from samples – prior to preservation of the individual in 70% ethanol – and kept at -20°C. Spiders for the behavioural component of this study were collected in January 2007. 18 spiders were sampled per site and were kept at 18°C in day/night conditions prior to the experimental period.
Map 3.1 Atrax Sp. 1 sample distribution map
(see Appendix 1.2 for sample details; see Insert for enlarged copy of map)
DNA extraction and sequencing

This project has found that Australian funnel web spider DNA can be extremely unstable, sometimes degrading completely within 24-48 hours after extraction using a standard C-TAB-Chloroform protocol. The following extraction protocol and PCR conditions were developed as the most effective means of obtaining sufficient PCR product for sequencing. Genomic DNA was isolated from 1mm³ muscle tissue using QIAGEN DNeasy Tissue Kits. To obtain template DNA robust to degradation, it was essential to dissect muscle tissue from inside a leg segment and to prevent excess contact between the external cuticle and the extraction buffer. Sequence was obtained from 143 individuals at the mitochondrial locus of interest. From these, a subset of 70 individuals was selected for sequencing at a nuclear intron locus. Samples included in this subset were selected to represent the major mitochondrial clades and the full geographic range of Tallaganda. A 700 base pair (bp) fragment of mitochondrial sequence was amplified at the gene Cytochrome Oxidase Subunit I (COI) using the universal primer pair Lco1490: 5’ – GGTCACCAAATCTAAAGATATTGG and Hco2198: 5’ – TAAACTTCAGGGTGACCAAAAAATCA (Folmer et al, 1994). Amplifications were as follows: 1 cycle of 94°C for 3 min, 65°C for 30 s, 72°C for 45 s; 2 cycles of 94°C for 30 s, 60°C for 20 s, 72°C for 45 s; 2 cycles of 94°C for 45 s, 55°C for 20 s, 72°C for 45 s; 3 cycles of 94°C for 45 s, 50°C for 20 s, 72°C for 45 s; 2 cycles of 94°C for 45 s, 45°C for 20 s, 72°C for 45 s; 40 cycles of 94°C for 45 s, 45°C for 20 s, 72°C for 45 s; 1 cycle of 72°C for 3 min; rest at 4°C. Amplification reactions consisted of 2μL template DNA; 2U Platinum Taq PCRx DNA Polymerase (Invitrogen); 4μL 10X PCR Buffer, minus Mg; 2.4μL 50mM MgCl₂; 1.6μL 5mM dNTPs; 1μL each 10mM primer; in 40μL total volume. A 400 bp fragment of nuclear intron sequence from the gene Histone 3 (H3) was amplified using the Colgan et al. (2000) primer pair H3F: 5’ – ATGGCTCGTACCAAGCAGAC and H3R: 5’ – ATATCCCTTRGGCATRATRGTGAC. Amplifications were as follows: 1 cycle of 94°C for 3 min, 65°C for 30 s, 72°C for 45 s; 2 cycles of 94°C for 30 s, 60°C for 20 s, 72°C for 45 s; 2 cycles of 94°C for 45 s, 55°C for 20 s, 72°C for 45 s; 3 cycles of 94°C for 45 s, 50°C for 20 s, 72°C for 45 s; 3 cycles of 94°C for 45 s, 45°C for 20 s, 72°C for 45 s; 40 cycles of 94°C for 45 s, 45°C for 20 s, 72°C for 45 s; 1 cycle of 72°C for 3 min; rest at 4°C. Amplification reactions consisted of 2μL
template DNA; 2.5U Platinum Taq PCRx DNA Polymerase (Invitrogen); 4µL 10X PCRx Buffer; 2.4µL 50mM MgSO₄; 1.6µL 5mM dNTPs; 1µL each 10mM primer; in 40.5µL total volume. Polymerase chair reaction (PCR) products were purified using 3M NaAc with an ethanol precipitation. PCR products were sequenced in both directions using Big Dye Terminator (Applied Biosystems) and run on an ABI3100 Genetic Analyser.

Sequences were edited and aligned in SEQUENCHER (version 3.0, Gene Codes Corporation, Mi). Ambiguous end regions were removed such that all individuals within each genus were analysed over the same sequence length. The protein coding sequences (COI) were translated to identify the codon reading frame for the fragment. After alignment and cropping, a 566bp fragment for COI and a 320bp fragment for H3 remained. I determined the model of nucleotide substitution that best explained the data set using a hierarchical likelihood ratio test (hLRT) and Akaike Information Criterion (AIC), implemented in Modeltest v.3.7.0 (Posada & Crandall 1998) and PAUP* v.4.0 b10 (Swofford 2001). A partition homogeneity test was implemented in PAUP* to test for significant congruence of the genetic signal between the two loci. A test for saturation was implemented in DnaSP v.4.10.9 (Rozas et al. 2003) by plotting the relative proportions of transitions and transversions against genetic distance under a model of nucleotide evolution selected by Modeltest as the simplest model that best fit the data. Unique haplotypes were identified for each locus using GenAlEx v.6.1 (Peakall & Smouse 2001).

**Sequence analysis**

In order to directly and quantitatively compare the sequence diversity contained within *Atrax* Sp. 1 this study calculated a suite of summary statistics (Table 3.1) as follows: the number of unique haplotypes (h), haplotype diversity (Hd), nucleotide diversity (π) and the average number of nucleotide differences (k). To determine whether the loci of interest were subject to selection pressures I performed several tests of neutrality, namely Tajima’s D and Fu’s F statistics. In order to address a key assumption behind inferring phylogeographic history from mtDNA sequences, namely, that sequences evolve in a selectively neutral fashion (Zink et al. 2006) I performed a series of neutrality tests. Tajima’s D and Fu’s Fs were used to assess whether sequences
conformed to neutral expectations, that is, whether they had been subject to selection. In the absence of selection, significant deviations from neutral expectation for Tajima’s D and Fu’s Fs statistics are indicative of historical population expansion (Dean & Ballard 2004). In order to separate the putative effects of selection from those of historical demographic processes, I also conducted the MK-test (McDonald & Kreitman 1991), which tests solely for selection (Ballard & Whitlock 2004).

Phylogenetic analysis

A pilot study for this thesis indicated that haplotypes across the taxa of interest were separated by both shallow and deep divergences (Beavis & Rowell 2006). Therefore, I analysed the data set using statistical parsimony and Bayesian methods. I estimated mtDNA haplotype networks for *Atrax* Sp. 1 using the software package TCS 1.13 (Clement et al. 2000). TCS uses the statistical parsimony method to generate an unrooted network of haplotypes from a pairwise matrix of absolute haplotype differences (Clement et al. 2000). The program is run with a 95% limit of parsimony (Templeton et al. 1992). A phylogeny was constructed for the *Atrax* Sp. 1 concatenated COI-H3 sequence dataset using a Bayesian approach, as implemented in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). I conducted 2 runs, each with four Metropolis Coupling Markov Chain Monte Carlo (MCMCMC) chains for 10 000 000 generations, sampling trees every 100 000 generations, with a 25% burn-in defined. MrBayes runs were implemented using a General Time Reversible + a proportion of invariant sites + gamma distribution model of nucleotide substitution (as selected by MODELTEST) and the default uniform priors. Each run was started using a random tree. I assessed convergence of the separate runs by examining the plot of generation vs. log probability of the data (ie. log likelihood values) produced by MrBayes.

Ancestral state reconstruction

To reconstruct ancestral character states for *Atrax* Sp. 1 according to underlying geology (ie. granitic or metasedimentary parent material), I used the tree produced via a Bayesian analysis as the basis for ancestral character state reconstruction as implemented in Mesquite v.1.12 (Maddison & Maddison 2004). Ancestral states were assessed by importing a binary character matrix (granite vs. metasediments) into Mesquite and implementing the ML reconstruction criteria.
Phylogeographic analysis

In order to test for significant phylogeographic structure I performed several analyses of molecular variance (AMOVA) as implemented in GenAlEx v.6.1 (Peakall & Smouse). AMOVA ($\phi_{ST}$) performs hierarchical analyses of genetic differentiation with tests of significance via bootstrap replicates. I tested the null hypothesis of no significant population structure against a series of alternative hypotheses as follows:

- H1) populations defined according to \textit{a priori} expectations;
- H2) populations defined as per Atrax refugia regions;
- H3) populations defined as per the findings of Hodges \textit{et al.} (2007) that significant population structure in the skink \textit{Eulamprus} was found between the Tallaganda vs. Baja populations;
- H4a) populations defined according to underlying geology (one continuous metasedimentary population and 7 geographically disjunct granitic populations);
- H4b) populations defined according to underlying geology (granitic populations only);
- H5) a hierarchically-nested AMOVA that sought to test for phylogeographic structure under the assumption of geological populations nested within refugia regions.

AMOVA generates a number of haplotype correlation measures referred to as $\phi$-statistics, which are somewhat analogous to F-statistics (Matocq \textit{et al.} 2000). However, the $\phi_{ST}$ statistic has advantages over $F_{ST}$ for the present study. F-statistics calculate the degree of variation explained by \textit{a priori} population parameters according to absolute differences amongst individuals, whereas $\phi$-statistics make use of the degree of variation contained within the sequences themselves. Consequently, I considered $\phi$-statistics to be more appropriate to the present study, in that there are high levels of sequence divergence. The sample used to test H2, H3 and H5 was modified to exclude three samples as they were thought to be examples of long-distance dispersal, being most closely related to taxa sampled from outside Tallaganda.
Chapter 3: Is the Pleistocene the One and Only?

Behavioural assessment of local adaptation

Source populations for the study were selected with respect to the phylogeny of *Atrax* in Tallaganda. The phylogeny is robust: Maximum Parsimony and Bayesian methods produce the same topology with good node support. Two regions (major clades) were selected for study: *A* in the North (corresponding to *Atrax* Refugia Region 2) and *B* in the south (corresponding to *Atrax* Refugia Region 4) of Tallaganda. Each region contained one granitic (*A1* and *B1*) and one metasedimentary (*A2* and *B2*) site (sub-clade). Sixteen individuals were collected from each site. In the laboratory, individual spiders were presented with a series of three soil choice trials. Each soil-choice trial lasted 48 hours. Spiders were subjected to three trials in total, which were separated by a 24-hour resting period. A preference for one soil sample over another was recorded if a spider had built a retreat in that soil. Where spiders had not constructed a retreat (i.e., were vagrant) a “non-choice” was recorded and was not included in the analysis. $\chi^2$ contingency tables were constructed for analysis of the following questions:

1) Overall, regardless of region and site origin, do spiders show a preference for soil type?

2) Within site (i.e. granite or metasediments), do spiders show a preference for soil type?

3) Within site (i.e. granite or metasediments), do spiders show a preference for site?

Results

Sequence Diversity

Sequence diversity for a coding region of the mtDNA locus COI was as follows (Table 3.1). From 143 individuals, 82 unique haplotypes were identified. Of 566 sites, 176 were variable. I identified no unexpected stop codons within the open reading frame. Both nucleotide and haplotype diversity were relatively high at COI, reaching values of 0.701 (s.d. = 0.00001) and 0.983 (s.d. = 0.00001) respectively. At the nuclear intron locus, H3, 9 unique haplotypes were identified from 70 individuals. Of 320 sites, 9 were both variable and parsimony informative. Haplotype diversity was calculated as 0.472 (s.d. = 0.005) and nucleotide diversity as 0.002 (s.d. = 0.0000). At COI all but two
individuals could be defined into haplotype groups, occurring exclusively within one discrete geographical region.

I tested for sequence saturation by plotting the Ti:Tv ratio against p-distance, as implemented in DAMBE (Xia & Zie 2001). I included 3rd codon positions in this analysis. I found that saturation would impact on the information-content of the sequences at p-distances of approximately 18% and as the maximum p-distance was below this level (15%), I discounted saturation as an important influence in our parsimony analyses. A partition homogeneity test found no evidence for phylogenetic incongruence between COI and H3 and consequently I concatenated the sequences using MacClade (v.4.07) to produce a sequence of 886bp length.

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>M</th>
<th>(\pi)</th>
<th>(h)</th>
<th>(k)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COI H3</td>
<td>143</td>
<td>70</td>
<td>0.070</td>
<td>0.983</td>
<td>0.472</td>
</tr>
<tr>
<td>COI H3</td>
<td>82</td>
<td>9</td>
<td>0.002</td>
<td>0.472</td>
<td>39.697</td>
</tr>
<tr>
<td>All</td>
<td>0.070</td>
<td>0.983</td>
<td>0.472</td>
<td>39.697</td>
<td>0.661</td>
</tr>
<tr>
<td>Tallaganda</td>
<td>137</td>
<td>70</td>
<td>0.065</td>
<td>0.981</td>
<td>0.472</td>
</tr>
<tr>
<td>Granite</td>
<td>70</td>
<td>37</td>
<td>0.002</td>
<td>0.519</td>
<td>36.478</td>
</tr>
<tr>
<td>Metasediments</td>
<td>66</td>
<td>22</td>
<td>0.001</td>
<td>0.385</td>
<td>0.403</td>
</tr>
<tr>
<td>COI H3</td>
<td>36.672</td>
<td>0.661</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metasediments</td>
<td>32.911</td>
<td>0.730</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3.1 Genetic diversity indices of *Atrax Sp. 1*

Table 3.1 shows genetic diversity indices for the total, Tallaganda, granite and metasediments samples. N, number of individuals; M, number of haplotypes; \(\pi\), nucleotide diversity; \(h\), haplotype diversity; \(k\), average number of nucleotide differences.

Tests of neutrality

A suite of tests of neutrality were conducted to determine: primarily whether the sequences of interest were evolving neutrally; and secondarily whether Tallaganda populations of *Atrax Sp. 1* displayed evidence of having undergone significant historical demographic change. An MK test showed that the data set did not deviate significantly from neutral expectations (Neutrality Index: 1.137; \(p > 0.90320\)), indicating that the loci of interest were selectively neutral, independently of the effects of demographic change. At COI Tajima’s D (-0.289; \(p < 0.10\)) and Fu’s Fs (0.000; \(p < 0.461\)) also corresponded
to neutral expectations. H3 sequences also corresponded to neutral expectations, but the low degree of sequence variation at this locus was unlikely to produce any meaningful result at this scale.

Phylogenetic analysis
The topology of the mtDNA statistical parsimony networks (Figure 3.1.a & Figure 3.1.b) and the combined mtDNA and nDNA Bayesian tree (Figure 3.2) were congruent. The *Atrax* Sp. 1 Bayesian phylogeny displays reciprocal monophyly according to distinct and non-overlapping geographic regions. Furthermore, the phylogeny displays structure within these “refugia regions”, which is defined by the underlying geology of the Tallaganda region. Three of the six geographically-defined clades are divided into a paraphyletic group of individuals sourced from granite soils, within which is nested a monophyletic clade of individuals sourced from metasedimentary soils. The remaining three clades contain either granite or metasedimentary individuals. The posterior probabilities for the majority of clades and groups are strong, for both “refugia regions” (posterior probability = 100) and geologically defined groups (posterior probability > 71). The phylogeny does not display monophyly within the *a priori* microbiogeographic regions defined by Garrick et al (2004), which are based upon the boundaries that separate hydrological catchments. Similarly to the Bayesian phylogeny, the mtDNA statistical parsimony network displays strong phylogeographic structure of haplotypes according to geography, which is further subdivided by structure congruent with underlying geology.
Figure 3.1.a *Atrax* Sp. 1 statistical parsimony network

Statistical parsimony network for *Atrax* Sp. 1 with reference to phylogeographic region of origin.

(See Insert for enlarged copy of network)
Figure 3.1.b *Atrax* Sp. 1 statistical parsimony network

Statistical parsimony network for *Atrax* Sp. 1 with reference to underlying geology (Granite = Orange; Metasediments = Pink). (See Insert for enlarged copy of network)
Figure 3.2 *Atrax* Sp. 1 Bayesian phylogeny

Bayesian MCMC phylogeny for *Atrax* Sp. 1, using a GTR + $\gamma + \lambda$ model of nucleotide evolution ($ln \ L = -4736.91$). Clade credibility values are shown for major nodes. Non-colour-coded samples originate from outside Tallaganda. Scale shows the number of inferred changes per site. (See insert for enlarged copy of tree)
Chapter 3: Is the Pleistocene the One and Only?

Ancestral state reconstruction

Ancestral character state reconstruction (Figure 3.3) revealed predominately unambiguous ancestral states suggesting that the Tallaganda population of *Atrax* Sp. 1 may have originated from a granitic environment. The shift from granitic to metasedimentary habitats has occurred multiple times, which is consistent with the *Atrax* Sp. 1 presence within metasedimentary material being a more derived trait.

Phylogeographic analysis

The non-random geographic distribution of haplotypes displayed by the phylogeny was confirmed by a series of AMOVAs, which supported significant differentiation amongst the major groups of interest under all assumptions of population structure defined by this study (Table 3.2). However, the magnitude of observed $\phi_{ST}$ values were highly variable according to the population definitions being imposed upon the data. Within the population-only analyses (H1 – H4), the largest proportion of spatial genetic structure could be explained under the assumption of *Atrax* refugia regions (H2: $\phi_{ST} = 0.639; p < 0.000$) and granite-only underlying geology (H4.b: $\phi_{ST} = 0.517; p < 0.000$). The hierarchically nested AMOVA (H5) – that defined populations according to underlying geology, which were nested within regions delineated according to *Atrax* refugia regions – explained the highest proportion of spatial genetic structure ($\phi_{ST} = 0.799; p < 0.00001$). These results clearly indicate that the Tallaganda population of *Atrax* Sp. 1 is highly structured, however, it appears that granitic soils and some deeper division (referred to in this study as *Atrax* refugia regions) are responsible for the larger portion of phylogeographic partitioning.
Figure 3.3 *Atrax* Sp. 1 ancestral state reconstruction

Ancestral state reconstruction for underlying geology preferences in *Atrax* Sp. 1.

(See Insert for enlarged copy of tree)
Table 3.2 AMOVA tests of genetic subdivision

Table 3.2 shows the AMOVA results for populations defined according to H1 – H5 (see methods). The probability of observations was assessed via 9999 permutations of the data. Table 2 shows the following measures of population structure: n, number of individuals; N, number of populations; df, degrees of freedom; SS, value for the sum of squares based on pairwise distances; Var., the variance component; %, percentage of variance; $\phi_{ST}$; p-value.

<table>
<thead>
<tr>
<th>Hypothesised population parameters</th>
<th>Source of Variation</th>
<th>n</th>
<th>N</th>
<th>df</th>
<th>SS</th>
<th>Var</th>
<th>%</th>
<th>$\phi_{ST}$</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1: a priori regions</td>
<td>Among populations</td>
<td>136</td>
<td></td>
<td>9</td>
<td>502 967</td>
<td>3 371</td>
<td>18%</td>
<td>0.178</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Within populations</td>
<td>127</td>
<td></td>
<td></td>
<td>1980 349</td>
<td>15 593</td>
<td>82%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H2: Atrax refugia regions</td>
<td>Among populations</td>
<td>133</td>
<td></td>
<td>6</td>
<td>1405 534</td>
<td>12 964</td>
<td>64%</td>
<td>0.639</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Within populations</td>
<td>127</td>
<td></td>
<td></td>
<td>929 587</td>
<td>7 320</td>
<td>36%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H3: Tallaganda/ Badja</td>
<td>Among populations</td>
<td>133</td>
<td></td>
<td>2</td>
<td>185 954</td>
<td>6 023</td>
<td>27%</td>
<td>0.269</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Within populations</td>
<td>131</td>
<td></td>
<td></td>
<td>2149 166</td>
<td>16 406</td>
<td>73%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H4 a: Underlying Geology</td>
<td>Among populations</td>
<td>136</td>
<td></td>
<td>8</td>
<td>715 993</td>
<td>6 596</td>
<td>32%</td>
<td>0.323</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Within populations</td>
<td>128</td>
<td></td>
<td></td>
<td>1767 323</td>
<td>13 807</td>
<td>68%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H4 b: Underlying Geology (Granite only)</td>
<td>Among populations</td>
<td>70</td>
<td></td>
<td>7</td>
<td>553 650</td>
<td>9 880</td>
<td>52%</td>
<td>0.517</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Within populations</td>
<td>63</td>
<td></td>
<td></td>
<td>581 792</td>
<td>9 235</td>
<td>48%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H5: Underlying Geology (populations) &amp; Atrax refugia regions (regions)</td>
<td>Within regions</td>
<td>133</td>
<td></td>
<td>6</td>
<td>1405 534</td>
<td>9 362</td>
<td>46%</td>
<td>0.464</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Among populations</td>
<td>14</td>
<td></td>
<td>8</td>
<td>445 919</td>
<td>6 756</td>
<td>33%</td>
<td>0.799</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Within populations</td>
<td>119</td>
<td></td>
<td></td>
<td>483 668</td>
<td>4 064</td>
<td>21%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Chapter 3: Is the Pleistocene the One and Only?

Behavioural analysis

Part 1) Overall, regardless of region and site origin, do spiders show a preference for soil type?

This study found that spiders display an overall preference for granitic soil over metasedimentary material, regardless of the individual spider’s “home” site soil type or of phylogeographic region (Figure 3.4), with 63.64% (N = 70) of spiders choosing granite and 36.36% (N = 40) choosing metasediments ($\chi^2 = 8.18; \text{d.f.} = 1; p < 0.005$). No choice for soil type was made in 10 trials (total number of trials = 120).

Part 2) Within site (ie. granite or metasediments), do spiders show a preference for soil type?

These experiments found that spiders sourced from granitic sites sometimes preferred granitic soils (Figure 3.5.a). Spiders from the granitic site B1 showed a significant preference for granite soil, regardless of which phylogeographic region the soil was sourced from. B1 spiders chose granitic material in 76.67% (N = 23) of the choice-trials and metasedimentary material in 23.33% (N = 7) of trials ($\chi^2 = 4.0; \text{d.f.} = 1; p < 0.05$). No choice for soil type was made in 2 trials (total number of trials = 32). Spiders from the granitic site A1 displayed a trend towards a preference for granite soil with spiders choosing granite in 59.38% (N = 19) of trials, whereas spiders chose metasediments in 40.62% (N = 13) of trials. However this finding was not significant, possibly due to sample-sizes ($\chi^2 = 1.125; \text{d.f.} = 1; \text{N.S.}$). Non choices did not occur in this experiment.

This study found that spiders sourced from metasedimentary soils (sites A2 and B2) showed no significant preference for metasediments over granite (Figure 3.5.b). Spiders from site A2 chose metasediments in 61.54% (N = 16) of trials and granite in 38.46% (N = 10) of trials ($\chi^2 = 0.692; \text{d.f.} = 1; \text{N.S.}$). No choice for soil type was made in 4 trials (total number of trials = 30). Spiders from site B2 chose metasediments in 54.55% (N = 12) of trials and granite in 45.45% (N = 10) of trials ($\chi^2 = 0.091; \text{d.f.} = 1; \text{N.S.}$). No choice for soil type was made in 4 trials (total number of trials = 26)

Part 3) Within site (ie. granite or metasediments), do spiders show a preference for site?

This study found that spiders from both granitic sites showed a significant preference for granite from their own site over granite from the other site surveyed. A1 spiders (Figure 3.6.a) chose A1 granite in 75% (N = 22) of choice trials and B1 granite in 25% (N = 8) of choice trials ($\chi^2 = 4.0; \text{d.f.} = 1; p < 0.05$). Non choices did not occur in this experiment. B1 spiders (Figure 3.6.b) chose B1 granite in 80% (N = 24) of choice trials
and A1 granite in 20% (N = 6) of choice trials ($\chi^2 = 5.4$; d.f. =1; p < 0.05). No choice for soil type was made in 2 trials (total number of trials = 32). Because spiders taken from metasedimentary sites showed no preference for metasediments, regardless of site, this study does not present the findings of whether metasedimentary spiders showed a preference for site here (nb. spiders from metasedimentary sites showed no significant preference for site).

**Do spiders display an overall preference for either granite or metasediments, regardless of home site soil type?**

![Bar chart showing soil choice preference](chart)

**Figure 3.4 Overall preference for soil type**

Regardless of home-site soil type, individuals showed a significant overall preference for granite.
Do spiders from granitic sites prefer granite?

![Graph showing spider preference for granite and metasediments](image)

Figure 3.5.a Spiders from granitic sites tend to prefer granite

Spiders sourced from granitic soils showed an overall trend towards preferring granite.

Do spiders from metasediments sites prefer metasediments?

![Graph showing spider preference for granite and metasediments](image)

Figure 3.5.b Spiders from metasedimentary sites do not prefer home soil

Atrax Sp. 1 individuals sourced from metasedimentary sites displayed no significant preference for metasediments over granite.
Chapter 3: Is the Pleistocene the One and Only?

Do spiders from site A1 (granite) prefer A1 soil?

![Bar chart showing spider preference for A1 soil](image)

**Figure 3.6.a Spiders from site A1 preferred A1 (granitic) soil**

_Atrax_ Sp. 1 individuals from site A1 showed a significant preference for A1 soil.

Do spiders from site B1 (granite) prefer B1 soil?

![Bar chart showing spider preference for B1 soil](image)

**Figure 3.6.b Spiders from site B1 preferred B1 (granitic) soil**

_Atrax_ Sp. 1 individuals from site B1 showed a strong, significant preference for B1 soil.
Summation

Tallaganda *Atrax* displays phylogeographic structure at two distinct levels within the phylogeny. Shallow structure is congruent with boundaries defined by underlying geology. Structure occurring deeper in the phylogeny is congruent with the parameters of distinct, non-overlapping geographic regions: these regions will subsequently be referred to as *Atrax* “Refugia Regions”. The *a priori* regions defined by Garrick et al (2004) are supported by an AMOVA, however the phylogeny does not display monophyly according to either hydrological catchments or sub-catchments. Behavioural soil-choice experiments indicate evidence for local adaptation to a granitic substrate. In addition, soil-choice experiments showed an overall preference for granite soil, regardless of the underlying geology of an individual’s habitat.
Chapter 3: Is the Pleistocene the One and Only?

Discussion

Summation

The initial expectation of this study was that *Atrax* Sp. 1 would show congruence with both underlying geology and the *a priori* regions defined for the broader Tallaganda project. However, the results of this project suggest that the structure contained within Tallaganda populations of *Atrax* Sp. 1 may be the product of processes other than those previously investigated at Tallaganda, that is, the effects of glacial-interglacial cycling (Garrick *et al.* 2004; Sunnucks *et al.* 2006; Garrick *et al.* 2007; Hodges *et al.* 2007). In contrast to these earlier studies of saproxylic taxa, the present project has found that *Atrax* Sp. 1 phylogeographic structure reflects both ancient population partitioning and underlying geology. These findings are particularly significant given that the divergences amongst *Atrax* Sp. 1 refugia regions are large enough to suggest the presence of multiple cryptic species, the phylogeny suggesting that *Atrax* Sp. 1 population has either recently undergone speciation or is currently in the process of speciating (cf. Bond *et al.* 2001; Bond 2004; Hendrixson & Bond 2005). These key findings have focused the aims of this study, namely towards assessing the evidence that supports Pleistocene climate variation and/or underlying geology as factors that may have restricted gene-flow, and therefore have acted as the driving forces behind speciation in this system.

Influence of Pleistocene glacial-interglacial cycling

There is mixed evidence to suggest that Pleistocene glacial-interglacial cycling has produced the phylogeographic structure displayed by *Atrax* Sp. 1. The *Atrax* Sp. 1 phylogeny displays reciprocal monophyly for clades that unequivocally correspond with distinct and non-overlapping geographic regions. The reciprocal monophyly displayed by these “refugia regions” is indicative of long-standing – and potentially ancient – barriers to gene-flow amongst clades, as opposed to divisions that have a Pleistocene origin (Waters *et al.* 2001; Zink 2002). In contrast, there is mixed evidence in support of the central Tallaganda hypothesis that high-elevation ridge-lines (ie. those that delimit hydrological catchments) have acted as significant barriers to gene-flow during glacial periods (cf. Garrick *et al.* 2004; Sunnucks *et al.* 2006; Garrick *et al.* 2007; Hodges *et al.* 2007). *Atrax* Sp. 1 individuals from each of the *a priori* regions defined by Garrick *et
al. (2004) are spread across all major clades, indicating that cross-boundary migration has not been uncommon. Whilst there is some evidence to support the influence of hydrological sub-catchments upon the Atrax Sp. 1 phylogeny (Table 3.2), it should be noted that the $\phi_{ST}$ values produced under the assumption of the a priori regions (H1: $\phi_{ST} = 0.178$; $p < 0.000$) are much lower than those detected under the assumption of underlying geology (H4.b: $\phi_{ST} = 0.517$; $p < 0.000$). Therefore, it is important to be cautious in attributing the observed phylogeography to the influence of the Pleistocene.

These findings do not exclude Pleistocene glaciation as the causative agent for Atrax Sp. 1 phylogeography. However, this study does suggest that saproxylic and non-sapropxylic invertebrates in Tallaganda have had different responses to a shared history. In the first instance, the geographic barriers experienced by the non-sapropxylic Atrax Sp. 1 may be different from those that have been significant for multiple saproxylic species in Tallaganda. Alternatively, taxa may have responded differently to the same physical barriers (eg. Gaston 2003; Forister et al. 2004). Regardless of the locations of barriers to Atrax Sp. 1 gene-flow, the data suggests that Tallaganda populations have experienced subdivision at some point in their history. Distinct groups located within each Atrax Sp. 1 refugia region display features that are characteristic of having survived historical climate change (Hewitt 1996; Hewitt 2000; Hewitt 2004). For example, individual clades display the star-burst pattern that is characteristic of the group having undergone a rapid increase in population size (Avila et al. 2006). These findings, combined with the deep phylogeographic structure displayed by the phylogeny, suggest that Atrax Sp. 1 has experienced historical demographic change. However, without a more comprehensive molecular dataset it would be presumptuous to attempt to identify the historical processes or the specific habitat characteristics that may have prevented migration amongst the refugia regions identified.

**Influence of underlying geology**

Phylogeography has been chiefly concerned with the effects of historical climate variation upon extant taxa (Hewitt 1996; Knowles 2001; Hewitt 2004; Knowles & Richards 2005). However, there is a growing body of literature that is focused upon the effects of underlying geology and landscape processes on the phylogeographic structure of current populations (Heaney et al. 2005; Brown et al. 2006; Lindell et al. 2006).
Chapter 3: Is the Pleistocene the One and Only?

Geological processes affecting the phylogeography of diverse taxa include volcanic activity and lava flows (Vandergast et al. 2004); debris avalanches (Brown et al. 2006); tectonic processes (Lindell et al. 2006); and the effects of sea-level on shore-lines (Heaney et al. 2005). However, geological upheaval is unlikely to be the proximal cause of phylogeographic structure in this system. The Tallaganda region has been geologically stable as of approximately 354 – 490 mybp (State Forests of NSW 1995). Given that Mygalomorph spiders split from the Araneomorphae approximately 400 mybp (King 2004), it is extremely improbable that either the underlying geology or the soils of different areas within Tallaganda have altered substantially since Mygalomorph spiders colonised the region, or the Australian funnel web spiders themselves arose as a taxonomic entity. Consequently this paper holds that, upon colonising the region, *Atrax* Sp. 1 were faced with a geologically variable environment containing multiple habitat niches that were not equally attractive to individual spiders. This view is supported by the findings of this project, there being strong evidence that underlying geology has played a key role in the development of extant *Atrax* Sp. 1 phylogeography.

As discussed previously, the *Atrax* Sp. 1 phylogeny is divided at a deeper level into monophyletic refugia region clades. However, more shallow phylogeographic structure separates refugia regions containing both soil types into distinct granitic and metasedimentary groups. In these three cases, groups sourced from granitic soils are paraphyletic to a monophyletic metasediments clade (Figure 3.2). These groups exclusively contain individuals sourced from either granitic or metasedimentary soils, with no overlap. A conspicuous feature that occurs repeatedly within the *Atrax* Sp. 1 phylogeny are the polytomies displayed by metasedimentary clades, particularly those which are nested within a paraphyletic granitic group. Neither mtDNA or nDNA displays evidence of saturation, suggesting that these polytomies have arisen via a rapid range expansion and subsequent radiation (eg. Reeves & Bermingham 2006). In addition, population analyses indicate that underlying geology explains up to 52% of the variation contained across populations (Table 3.2). Overall, the partitioning found throughout the *Atrax* Sp. 1 phylogeny suggests that underlying geology has played a significant role in the diversification of the taxon. In particular, the placement of metasedimentary clades within paraphyletic granitic groups suggests that spiders currently found in metasedimentary soil have recently expanded their range, migrating...
from neighbouring granitic regions (Omland et al. 2006). The import of this phylogenetic finding is highlighted by the results of this study’s tests for evidence of soil type preferences.

**Underlying geology and local adaptation**

Experiments aimed at detecting local adaptation to soil type and to refugia regions permit this study to begin to separate the effects of historical climate variation from that of the ongoing environmental conditions. This study has found evidence suggesting that *Atrax* Sp. 1 is both locally adapted to granitic soil overall, and to local granitic soils. Local adaptation may be demonstrated when individuals display improved fitness within – or as a proxy, a significant preference for – its own habitat relative to ‘foreign’ individuals (Kawecki & Ebert 2004; Margraf et al. 2007). In long-lived invertebrate taxa (such as mygalomorph spiders where individuals may live for up to 20 years) it is not practical to demonstrate improved individual fitness under local conditions. However, it is possible to test individual habitat preferences. Soil choice experiments clearly indicated there was an overall significant preference for granite soil, regardless of the underlying geology of an individual’s home-site. Furthermore, these experiments indicated that *Atrax* individuals collected from granite sites were able to distinguish amongst granite soils from different refugia regions, unequivocally preferring soil from their own site. Given the results of the ancestral state reconstruction, indicating that occupying a granitic substrate was ancestral, the findings of the behavioural component of the present study provide real insight into the processes that may be driving ongoing population differentiation. These findings are suggestive of local-adaptation, and raise the question of whether underlying geology may have acted to drive population divergence of *Atrax* Sp. 1.

Underlying geology – specifically soil type – has been shown to drive local-adaptation and speciation in plant species, however it is rare for local adaptation to soil type (i.e. granite) to be demonstrated in an animal taxon. Sambatti & Rice (2006) demonstrated local adaptation to serpentine soils in sunflowers, whilst Ellis & Weis (2006) demonstrated local adaptation to soil microenvironments amongst quartz specialist and a generalist species of *Argyroderma* (a Southern African member of the Aizoaceae). However, neither of these studies provided a phylogenetic framework for their
transplant experiments, as these studies were working upon known species and were focused upon among-species differences. The present study differs from these in the respect that the taxonomy of the Australian funnel web spiders is currently incomplete (Wilson 2001). Whilst this study has referred to *Atrax* Sp. 1 as the sole representative of the genus *Atrax* within Tallaganda, the high degree of mitochondrial sequence divergence amongst geographically distinct groups is more than sufficient to warrant investigating the possibilities that these are cryptic species (Beavis & Rowell 2006).

**Summation**

This study has evaluated the indications that both historical and ongoing barriers to gene-flow have influenced the phylogeny of extant *Atrax* Sp. 1 in Tallaganda. The phylogenetic structure displayed by the phylogeny suggests that there has been a relatively recent radiation of individuals into metasedimentary regions, a hypothesis that is further supported by the evidence for local adaptation to granitic soils. This study highlights the need for consideration of ongoing barriers to gene-flow, as well as those resulting from intermittent historical processes.
Chapter 3: Is the Pleistocene the One and Only?

References


Chapter 3: Is the Pleistocene the One and Only?


Chapter 3: Is the Pleistocene the One and Only?


CHAPTER 4

PARASITE PHYLOGENIES ELUCIDATE PATTERNS OF HOST PHYLOGEOGRAPHIC STRUCTURE

Introduction

There is a small but expanding body of evidence suggesting that parasite phylogenies can act to elucidate host phylogeography and population history (e.g. Nieberding et al. 2004; McCoy et al. 2005; Noël et al. 2005; Nieberding & Oliveri 2006; Criscione & Blouin 2007; Waltari et al. 2007). Increasingly, parasites are being used to provide an additional taxon group – and therefore an additional perspective – within a traditional comparative phylogeography context (Koblmüller et al. 2006). One emerging approach is the use of parasites as proxies for host phylogeny (Nieberding & Oliveri 2006).

Comparative phylogeographic approaches are conventionally used to infer population history across co-distributed taxa that, by virtue of their sympatric distribution, have experienced a common set of environmental conditions (Avise 2000). However, the inclusion of host-parasite systems within broader comparative phylogeography studies has only recently emerged as a means of elucidating elements of host and/or parasite demographic history (McCoy et al. 2005), for example, dispersal (Criscone & Blouin 2007), cryptic diversity (Nieberding et al. 2004), and host-shifts (Picard et al. 2007).

The recent emergence of this approach is perhaps surprising given that the host-parasite interaction provides a unique system within which to investigate the phylogeography of multiple taxa. Where comparative phylogeography investigates the impacts of a shared history upon otherwise independent taxa, a phylogeographic study of host-parasite systems examines ecologically linked taxa (Nieberding et al. 2004). In fact, congruence in the phylogeographic structure displayed by host-parasite taxon pairs increases with the strength of the obligate nature of the host-parasite relationship (Hafner et al. 2003; Nieberding et al. 2004). Therefore, the inclusion of a parasitic group may provide an additional avenue for elucidating details of host phylogeography.

A multi-taxa phylogeographic study based in the Tallaganda region of South-Eastern New South Wales, Australia has been investigating the effects of Pleistocene glacial-interglacial cycling on a suite of log-dwelling (or saproxylic) invertebrates. To date
multiple single-taxon studies, conducted within the broader comparative phylogeographic project, have revealed congruent spatial genetic structure that roughly corresponds to the putative locations of glacial refugia (Garrick et al. 2004; Beavis & Rowell 2006; Sunnucks et al. 2006; Garrick et al. 2007; Hodges et al. 2007). However, two genera of Australian funnel web spider have produced phylogenies that deviate from the Tallaganda project’s *a priori* expectations (Beavis & Rowell 2006). The log-dwelling *Hadronyche* Sp. 1 shows no congruence with the structure of other saproxylic taxa, rather the genus displays low levels of genetic diversity and no geographic structure whatsoever (see Chapter 2). In contrast, the ground-dwelling genus *Atrax* Sp. 1 shows deep divergences amongst geographically distinct clades, characteristics that are indicative of a long history in Tallaganda and long-standing barriers to gene-flow amongst geographic regions (see Chapter 3). One interpretation of these findings is that *Hadronyche* Sp. 1 did not survive the glacial-interglacial cycling that characterised the Pleistocene and became locally extinct in the Tallaganda region, recolonising the area from the Great Dividing Range (GDR) in the 10 000 years since the most recent glacial maxima (Beavis & Rowell 2006). The present study aims to use the phylogeography of a Laelapid mite, *Hypoaspis barbarae* (Strong 1995) to elucidate the demographic history of associated funnel web spider species. By adding this extra layer of information to a comparative phylogeographic study, this project aims to accumulate evidence for differing responses of host taxa to a single, shared demographic event, that is, to the Pleistocene glacials.

**Tallaganda**

The study site for this project is the Tallaganda region of NSW, a 100km long mountainous spur of State Forest and National Park that extends from the Great Dividing Range (GDR). Tallaganda connects to the GDR in the south, but is otherwise isolated within a matrix of low-lying grasslands (State Forests of NSW 1995). The region was subject to the effects of glacial-interglacial cycling that occurred on 100 000 year cycles throughout the Pleistocene, resulting in repeated changes to the geographic range of forests and associated fauna (Singh & Geissler 1985; Hewitt 1996; Garrick et al. 2004). The outcomes of glacial cycles in Tallaganda appear to have been typical of Pleistocene climate change in the Southern Hemisphere, that is, there was no actual glaciation (Hewitt 1996). Pleistocene glacial-interglacial cycling in Australia had the
effect of lowering the upper tree line to lower elevations, thus fragmenting the landscape into glacial refugia (Macphail & Colhoun 1985; Dodson & Ono 1997). Tallaganda is adjacent to Lake George, one of the major large intermittent Australian water bodies. Lake George has been the source of much of the data for vegetation history reconstruction in South-Eastern NSW and, consequently, there are detailed climate reconstructions for the region extending back through the Pleistocene, to approximately 730 000 years before present (ybp) (Singh & Geissler 1985). Detailed descriptions of the region, its climatic history and the phylogeography of the invertebrates found there can be found in Beavis et al. (in prep, see Chapter 5), Hodges et al. (2007) and Garrick et al. (2004).

Funnel web spiders and Laelapidae mites

Two genera of Australian funnel web spider are found in high densities throughout Tallaganda: the log-dwelling *Hadronyche* Sp. 1 and the ground-dwelling *Atrax* Sp. 1 (Beavis & Rowell 2006). The two genera have a sympatric distribution across the forest, however they exhibit niche-partitioning in their choice of habitat. *Hadronyche* Sp. 1 is exclusively saproxylic, excavating burrows within decomposing logs. In contrast, *Atrax* Sp. 1 constructs ground burrows directly into the soil substrate, the entrances to which are found beneath logs, rocks and in open ground. Otherwise, the two genera are reasonably ecologically similar: they are both top-level predators, feeding opportunistically upon invertebrates and small vertebrates; they are of a similar size; and they are long-lived, females reaching ages of up to 20 years (Levitt 1961). Both *Hadronyche* Sp. 1 and *Atrax* Sp. 1 individuals are closely associated with *Hypoaspis barbarae*, a Laelapidae mite that is found – sometimes at extremely high loads – upon the carapace of individual spiders (Strong 1995; Pers. Obs.). Laelapid mites have a well-documented association with mygalomorph spiders, several genera in this family having been found to be obligate parasites of spiders (Strong 1995). There is no evidence that *H. barbarae* exists in such close association with either *Atrax* Sp. 1 or *Hadronyche* Sp. 1: individuals are mobile upon the carapace of their host, and it seems that members of this species can live and reproduce independently of their host genera (Strong 1995).
Chapter 4: Parasite Phylogeny Elucidates Host Phylogeography

Study aims
This study aimed to use the phylogeographic patterns of Laelapidae mites to elucidate the population history of *Hadronyche* Sp. 1 and *Atrax* Sp. 1 in Tallaganda with the primary aim of addressing the following questions:

- Is there evidence of mite population differentiation according to host genus?
- Is there evidence of shared responses to historical demographic changes amongst Laelapidae mites and the host population with reference to host phylogeographic structure?
- Does the Laelapidae phylogeny support the inferred genus histories for *Atrax* Sp. 1 and *Hadronyche* Sp. 1?
  - *Hadronyche* Sp. 1: local extinction within Tallaganda & subsequent recolonisation
  - *Atrax* Sp. 1: a long history within Tallaganda with population subdivision.

Methods
Sample collection
Mites were collected from the cuticle surface of *Hadronyche* Sp. 1 and *Atrax* Sp. 1 specimens that had been preserved in 70% EtOH. Spiders had been collected from throughout the Tallaganda region between 2002-2006. Mite samples were selected to represent the geographic range and the phylogenetic structure identified within both genera of spider.

DNA extraction and sequencing
Genomic DNA was isolated from a single, whole, individual using QIAGEN DNeasy Tissue Kits. Sequence was obtained from 87 individuals at the mitochondrial locus of interest. A 450 base pair (bp) fragment of mitochondrial sequence was amplified at the gene Cytochrome Oxidase Subunit I (COI) using the universal primer pair Lco1490: 5’ – GGTCACAAATCATAAACAGATATTGG and Hco2198: 5’ – TAAACTTCAGGTTGACCAAAAAATCA (Folmer et al, 1994). Amplifications were
as follows: 1 cycle of 94°C for 3 min, 65°C for 30 s, 72°C for 45 s; 2 cycles of 94°C for 30 s, 60°C for 20 s, 72°C for 45 s; 2 cycles of 94°C for 45 s, 55°C for 20 s, 72°C for 45 s; 3 cycles of 94°C for 45 s, 50°C for 20 s, 72°C for 45 s; 2 cycles of 94°C for 45 s, 45°C for 20 s, 72°C for 45 s; 40 cycles of 94°C for 45 s, 45°C for 20 s, 72°C for 45 s; 1 cycle of 72°C for 3 min; rest at 4°C. Amplification reactions consisted of 2µL template DNA; 2U Platinum Taq PCRx DNA Polymerase (Invitrogen); 4µL 10X PCR Buffer, minus Mg; 2.4µL 50mM MgCl₂; 1.6µL 5mM dNTPs; 1µL each 10mM primer; in 40µL total volume. Polymerase chain reaction (PCR) products were purified using 3M NaAc with an ethanol precipitation. PCR products were sequenced in both directions using Big Dye Terminator (Applied Biosystems) and run on an ABI3100 Genetic Analyser. Sequences were edited and aligned in SEQUENCHER (version 3.0, Gene Codes Corporation, Mi). Ambiguous end regions were removed such that all individuals within each genus were analysed over the same sequence length. The protein coding sequences (COI) were translated to identify the codon reading frame for the fragment. After alignment and cropping, a 403bp fragment for COI remained. I determined the model of nucleotide substitution that best explained the data set using a hierarchical likelihood ratio test (hLRT) and Akaike Information Criterion (AIC), implemented in Modeltest v.3.7.0 (Posada & Crandall 1998) and PAUP* v.4.0 b10 (Swofford 2001). A partition homogeneity test was implemented in PAUP* to test for significant congruence of the genetic signal between the two loci. Unique haplotypes were identified using GenAIEx 6.1 (Peakall & Smouse 2001).

**Summary Statistics**

I calculated summary statistics with the aim of detecting evidence for vicariance events and/or evidence for increases in mite population size. Summary statistics were calculated for the entire mite sample in addition to mites sampled from either *Hadronyche* Sp. 1 or *Atrax* Sp. 1 respectively. Equivalent values were calculated for *Hadronyche* Sp. 1 and *Atrax* Sp. 1 and are presented here as a point of comparison. I calculated the number of unique haplotypes (h), haplotype diversity (Hd), nucleotide diversity (π) and the average number of nucleotide differences (k). To determine whether the loci of interest were subject to selection pressures I performed several tests of neutrality, namely Tajima’s D and Fu’s F statistics. In order to address a key
assumption behind inferring phylogeographic history from mtDNA sequences, namely, that sequences evolve in a selectively neutral fashion (Zink et al. 2006) I performed a series of neutrality tests. Tajima’s D and Fu’s Fs were used to assess whether sequences conformed to neutral expectations, that is, whether they had been subject to selection. In the absence of selection, significant deviations from neutral expectation for Tajima’s D and Fu’s Fs statistics are indicative of historical population expansion (Dean & Ballard 2004). All tests were implemented in DnaSP (Rozas et al. 2003).

**Phylogenetic Analysis**

I conducted Bayesian searches of tree space using MrBayes v.3.1.2 (Huelsenbeck & Ronquist 2001). I conducted 2 runs, each with four Metropolis Coupling Markov Chain Monte Carlo (MCMCMC) chains for 1 000 000 generations, sampling trees every 1000 generations. A 25% burn-in was defined and those trees were discarded accordingly. MrBayes runs were conducted using a General Time Reversible + proportion of invariant sites + gamma distribution model of nucleotide substitution and the default uniform priors. Each run was started using a random tree. I assessed convergence of the separate runs by examining the plot of generation vs. log probability of the data (ie. log likelihood values) produced by MrBayes, looking for evidence of stationarity.

**Phylogeographic Analysis**

I assessed the congruence of host-parasite phylogeographic structure using analyses of molecular variance (AMOVA) under the assumption of host population structure (H1a & b) and under the assumption of putative glacial refugia (H2a & b), the boundaries of which are defined as per hydrological catchments (Garrick et al, 2004). These a priori regions have been the grounding hypothesis for multiple studies of log-dwelling invertebrates in Tallaganda (Garrick et al. 2004; Beavis & Rowell 2006; Sunnucks et al. 2006; Garrick et al. 2007; Hodges et al. 2007). In addition, I tested for non-random spatial structuring of haplotypes under the assumption of differentiation according to host.
Chapter 4: Parasite Phylogeny Elucidates Host Phylogeography

Results

Sequence diversity and summary statistics

From 87 individuals I identified 56 unique haplotypes. Of 403 sites, 69 were variable. Haplotype diversity and nucleotide diversity were calculated to be 0.975 (s.d. =0.0001) and 0.041 (s.d. = 0.0000) respectively. Separating the sample according to host genus produced two groups that displayed extremely similar levels of sequence diversity across all major summary statistics calculated (Table 4.1). Tests of neutrality for pooled (and host-specific) samples produced non-significant Tajima’s D values (0.227; p > 0.10), and Fu & Li’s Fs statistic (-20.280; p > 0.10).

<table>
<thead>
<tr>
<th>Table 4.1 Genetic diversity indices for H. barbara mites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 4.1 shows genetic diversity indices for the total sample, specimens collected from a Hadronyche Sp. 1 host, and specimens collected from an Atrax Sp. 1 host. N, number of individuals; M, number of haplotypes; π, nucleotide diversity; h, haplotype diversity; k, average number of nucleotide differences.</td>
</tr>
<tr>
<td>Sample</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>All</td>
</tr>
<tr>
<td>Hadronyche</td>
</tr>
<tr>
<td>Atrax</td>
</tr>
</tbody>
</table>

Phylogenetic analysis

The topology of the combined mtDNA Bayesian tree displayed a complete absence of phylogeographic structure and phylogenetic resolution (ln = -1576.767). The tree did not display any reciprocal monophyly of mites sourced from an Atrax Sp. 1 vs. a Hadronyche Sp. 1 host. The topology reveals two major groupings that contain mites collected from both Atrax Sp. 1 and Hadronyche Sp. 1 individuals.
Unrooted Bayesian MCMCMC phylogeny for mites collected from *Atrax* Sp. 1 (red), and *Hadronyche* Sp. 1 (blue), using a GTR + $\gamma + \lambda$ model of nucleotide evolution ($\ln L = -1576.767$). Internal nodes are indicated in black.
Population genetics analysis

An Analysis of Molecular Variance (AMOVA) detected a small amount of significant population structure amongst host-differentiated mite samples ($\Phi_{PT} = 0.049, p < 0.024$). Both host-associated mite samples displayed significant phylogeographic structure congruent with the \textit{a priori} regions defined by Garrick \textit{et al.} (2004) that correspond with hydrological sub-catchment boundaries (\textit{Hadronyche} Sp. 1-associated mites: $\Phi_{ST} = 0.125, p < 0.012$; \textit{Atrax} Sp. 1-associated mites: $\Phi_{ST} = 0.268, p < 0.008$). The \textit{Hadronyche} Sp. 1-associated mite sample alone showed no significant spatial genetic structure under the assumption of a Tallaganda/ Badja phylogeographic break ($\Phi_{ST} = 0.000, p < 0.360$), whilst the \textit{Atrax} Sp. 1 associated sample showed congruence with \textit{Atrax} refugia regions ($\Phi_{ST} = 0.375, p < 0.008$) (Table 4.2).

Table 4.2 AMOVA tests of genetic subdivision

Table 4.2 shows the AMOVA results for populations defined according to H1 – H2 (see methods). The probability of observations was assessed via 999 permutations of the data. Table 4.2 shows the following measures of population structure: $n$, number of individuals; $N$, number of populations; $df$, degrees of freedom; SS, value for the sum of squares based on pairwise distances; Var., the variance component; $\%$, percentage of variance; $\Phi_{ST}$; $p$-value.

<table>
<thead>
<tr>
<th>Hypothesised population parameters</th>
<th>Source of Variation</th>
<th>$n$</th>
<th>$N$</th>
<th>$df$</th>
<th>SS</th>
<th>Var.</th>
<th>$%$</th>
<th>$\Phi_{ST}$</th>
<th>$p &lt;$</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1 a. Host population structure (\textit{Atrax} \text{ x} \text{c} \textit{Atrax} \text{ refugia regions})</td>
<td>Among populations</td>
<td>47</td>
<td>5</td>
<td>4</td>
<td>126 736</td>
<td>3 441</td>
<td>38%</td>
<td>0.375</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>Within populations</td>
<td>42</td>
<td>2</td>
<td>1</td>
<td>237 690</td>
<td>5 659</td>
<td>62%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1 b. Host population structure (\textit{Hadronyche} \text{ x} \text{e} \text{c} \textit{Tallaganda/ Badja})</td>
<td>Among populations</td>
<td>42</td>
<td>2</td>
<td>1</td>
<td>7 981</td>
<td>1 000</td>
<td>0%</td>
<td>0.000</td>
<td>0.360</td>
</tr>
<tr>
<td></td>
<td>Within populations</td>
<td>40</td>
<td>1</td>
<td>0</td>
<td>319 114</td>
<td>7 978</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H2 a. \textit{a priori} regions (\textit{Atrax} \text{ parasite})</td>
<td>Among populations</td>
<td>46</td>
<td>7</td>
<td>6</td>
<td>117 898</td>
<td>2 258</td>
<td>27%</td>
<td>0.268</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>Within populations</td>
<td>39</td>
<td>3</td>
<td>2</td>
<td>241 124</td>
<td>6 183</td>
<td>73%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H2 b. \textit{a priori} regions (\textit{Hadronyche} \text{ parasite})</td>
<td>Among populations</td>
<td>42</td>
<td>6</td>
<td>5</td>
<td>69 381</td>
<td>1 029</td>
<td>13%</td>
<td>0.125</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>Within populations</td>
<td>36</td>
<td>5</td>
<td>4</td>
<td>257 714</td>
<td>7 159</td>
<td>87%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion

The present study was focused upon investigating the utility of Laelapid mites in clarifying the evolution of funnel web spider populations in Tallaganda. The use of parasites as quasi-proxies for host phylogeography is of particular relevance when addressing phylogenetic questions in “neglected taxa” – such as mygalomorph spiders – where very little is known about dispersal, habitat specificity or other elements of ecology that directly affect phylogeography (Blaxter et al. 2004). In systems such as these it is possible to elucidate aspects of host biology (eg. dispersal capabilities) or demographic history by investigating the phylogeography of the parasite (Nieberding et al. 2004; Criscione et al. 2005; Waltari et al. 2007). The present study aimed to investigate both mite phylogeography as a component of the broader Tallaganda project, and to use these findings to elucidate elements of funnel web spider population history and ecology.

Phylogeography of Laelapidae mites

Overall, the present study detected two levels of significant phylogeographic structure in *H. barbarae*: first, host-defined interspecific variation; and second, intraspecific variation congruent with host-genus phylogeographic structure. In the first instance, Laelapid mites found on funnel web spiders in Tallaganda show some limited evidence of gene-flow restrictions amongst host-genera. While mites from both *Atrax* Sp. 1 and *Hadronyche* Sp. 1 hosts appear to be drawn from the same overall population – displaying virtually identical levels of sequence diversity – there is a small degree of significant population partitioning between mite populations according to host. Whist the degree to which gene-flow is restricted under the assumption of host-defined populations is minimal, it does highlight the possibility of rejecting the null hypothesis of panmixia (Wilson et al. 2007). However, at this point it is important to consider the ecology of *H. barbarae*. Laelapidae mites inhabit a broad range of host species – as well as soil and leaf-litter – and are thought to be highly mobile within a localised area (Strong 1995). Therefore, one would expect to see evidence for non-random mating (ie. to reject panmixia) purely on the basis that broad scale dispersal is limited (eg. Forbes & Boyd 1997). The fact that *H. barbarae* displays such low levels of non-random mating suggests that mites may achieve broader-range dispersal via host movement. Very little is known about the ecology, life-cycle and host-specificity of Laelapid mites,
however parasite dispersal is often closely linked with that of its host. Separate studies of *Hadronyche* Sp. 1 (Chapter 2) and *Atrax* Sp. 1 (Chapter 3) have found evidence that *Hadronyche* Sp. 1 disperses broadly across large distances, while *Atrax* Sp. 1 may be an effective local disperser, but does not appear to engage in long-range migration. Given these host-traits, the evidence this study presents for a small—but significant—degree of population partitioning in mites may be an artefact of host, and thus mite, migration capabilities.

In the second instance, this study has found evidence that mite population structure reflects the phylogeography both of their host and of the established Tallaganda saproxylic invertebrate phylogeography. *Hadronyche* Sp. 1 associated mites displayed no evidence of significant spatial structure under the assumption of H1.b) *Hadronyche* Sp. 1 phylogeographic structure, however, displayed some congruence with H2.b) Tallaganda *a priori* regions (Garrick *et al.* 2004). This suggests that mites found upon *Hadronyche* Sp. 1 are undergoing gene-flow restrictions independently of their host species. In contrast, *Atrax* Sp. 1 mites displayed significant spatial structure under the assumption of H1.a) *Atrax* Sp.1 phylogeographic structure, and H2.a) Tallaganda *a priori* microbiogeographic regions. These patterns are congruent with the deep divergences displayed by the *Atrax* Sp. 1 phylogeography, and also reflect restrictions upon gene-flow congruent with saproxylic species in Tallaganda. It is not unusual for the obligate nature of the host-parasite interaction to be reflected in the phylogeny of both parties (eg. McCoy *et al.* 2001; Hafner *et al.* 2003), and overall the findings of the present study appear to indicate broad-scale parasite congruence with host phylogeography. However, this study has also detected congruence with the *a priori* regions defined by Garrick *et al.* (2004) as reflecting the impacts of glacial-interglacial cycling upon saproxylic invertebrates in Tallaganda. This is informative for our interpretation of mite biology: from these findings one may infer that Laelapid mites are dependent upon their hosts for broad-scale dispersal but that they are mobile on a local scale.

Laelapid mites as proxies for funnel web spiders

In addition to the findings of the present study regarding mite phylogeography, these results also permit us to use the parasite phylogeny to provide insight into host
population histories (eg. Nieberding et al. 2004; Criscione et al. 2005; Waltari et al. 2007). Despite the possible role of the host as a barrier to gene-flow, descriptive summary statistics indicate strong similarities amongst the two host-defined mite populations. This paper proposes that the absence of any concordance with the limited degree of phylogeographic structure displayed by *Hadronyche* Sp. 1, combined with the strong congruence displayed by *H. barbarae* and its *Atrax* Sp. 1 host, is indicative of differing host histories in Tallaganda. In short, these findings are consistent with *Atrax* Sp. 1 having been present in Tallaganda – in tandem with its resident mites – for a longer period of time. In contrast, extant Tallaganda *Hadronyche* Sp. 1. may have acquired a diverse mite population more recently, the high dispersal capabilities of both *Hadronyche*, and the potential absence of *H. barbarae* host-specificity, producing the observed absence of host-specific phylogeographic structure and evidence of a recent range expansion in *H. barbarae*. These findings are consistent with *Hadronyche* Sp. 1 having recently colonised Tallaganda, that is, during the current interglacial.

**Summation**

Overall, this study has found evidence indicating that host-specific phylogeographic structure may evolve as an outcome of host-migration and parasite dispersal opportunities. Whilst the available data in such studies of neglected taxa is – by its very nature – restricted in its scope, such studies provide an essential investigatory step towards a more complete understanding of previously understudied species.
References


CHAPTER 5
CHAPTER 5

COMPARATIVE PHYLOGEOGRAPHY ELUCIDATES SIGNIFICANT BIOLOGICAL IDIOSYNCRASIES SEPARATING TWO SPECIES OF AUSTRALIAN FUNNEL WEB SPIDER

Introduction
Phylogeographic inferences are founded upon biological assumptions, which underlie virtually all means adopted to interpret the distributions of extant lineages. For example, at a broader scale biological assumptions underlie entire approaches: population genetic methods assume that there has been no effect of historical demographic change upon the distribution of current populations, whereas phylogenetic methods assume the absence of contemporary gene-flow. In turn, biological assumptions are the basis of explanatory models. For instance, an island model of migration assumes there is no mutation and that each subpopulation has the same effective population size, $N_e$ (Wright 1931; Balloux & Lougon-Moulin 2002). In particular, biological knowledge informs individual studies of specific taxa. For example, the 2006 phylogeographic study by Arnedo & Gillespie investigated species diversification patterns in the Hawaiian archipelago. Within this study, Arnedo & Gillespie calibrated rates of molecular change in *Tetragnatha* by setting the approximate date of hot-spot island formation as the maximum time for the colonisation of that island. Implicit to this approach was the assumption (amongst others) that the divergence of sister taxa did not significantly pre-date the formation of the younger island (Arnedo & Gillespie 2006). As such, phylogeographic research is dependent upon accurate biological assumptions, formed with reference to a relatively comprehensive knowledge of the taxa of interest.

Where a taxon has previously suffered from a dearth of research effort – producing a general lack of information on the group’s taxonomy, biology and ecology – it becomes near impossible to ensure that implicit biological assumptions have not been violated. Therefore, these neglected taxa present unique challenges for phylogeographic research. Given that the number of undescribed species is estimated at approximately 90% of the total biomass (Blaxter *et al*. 2004) the aforementioned challenges are...
commonly encountered, and when encountered they hinder research efforts further still, thus presenting a quandary that is circular in its effects and outcomes. However, the dilemma presented by neglected taxa may be addressed via the use of comparative phylogeography to elucidate biological differences amongst related taxa. A key underlying assumption implicit to comparative phylogeography is that co-distributed taxa have responded to a shared habitat history (Avise 1998; Bernatchez & Wilson 1998; Moritz & Faith 1998). By adopting this assumption, one can use phylogeographic congruence amongst multiple taxa as a basis for supporting or rejecting hypotheses regarding the responses of multiple taxa to a diverse suite of historical factors. However, when the species of interest are taxonomically, ecologically or physiologically diverse, the likelihood increases that these taxa have experienced differing effects of a common set of conditions (Ronquist 1997). Under these circumstances, it becomes possible to infer significant – but obscure – biological differences via the effects they have had upon phylogeography (eg. Heuertz et al. 2001).

The null hypothesis of such an endeavour is that sister taxa will display similar phylogeographic structure as a result of, first, their systematic similarity and, second, a common environmental history. If this null hypothesis is rejected, one can infer that there are biological differences separating the taxa, which have produced differential responses to a common environmental history. Thus the comparative phylogeography of related taxa has the potential to answer questions regarding those biological idiosyncrasies that distinguish the groups of interest. This study adopts comparative phylogeographic methods, not necessarily to infer species history, but to infer biological characteristics of neglected taxa. By doing so, this project identifies a means of intercepting the circularity of research effort that has resulted in so little research upon so many taxa. This approach offers an approach for studying those species that have not benefited from historical research interest for reasons of accessibility, visibility or charisma, and consequently remain neglected.

The present study is focused upon a neglected taxon group: the Australian funnel web spider (Mygalomorphae: Hexathelidae) in the Tallaganda region of South-Eastern New South Wales. Despite the group’s notoriety as one of the world’s most venomous spiders, the taxonomy of the group is both incomplete and uncertain. The Tallaganda region contains unnamed species within two niche-separated genera of mygalomorph
spider: the log-dwelling *Hadronyche* Sp. 1 and the ground-dwelling *Atrax* Sp. 1. Whilst the two taxa inhabit distinct ecological niches, very little is known regarding more subtle elements of the group’s biology. This study aims to investigate the phylogeography of these two niche-differentiated, but sympatrically-distributed, sister species. By taking a comparative approach this project aims to elucidate aspects of species biology, namely those characteristics that accompany niche-separation, such as migration capacity and environmental resilience.

**Australian funnel web spiders**

Australian funnel web spiders (Mygalomorphae: Hexathlidae) occupy a distribution that extends approximately 150km inland from the southern coastline of Australia, extending from South-Eastern Queensland to Southern Victoria, and including Tasmania (Raven 2000). The group, sometimes referred to as the Atraceae (Rowell 1980), consists of two reciprocally monophyletic sister genera: *Hadronyche* and *Atrax*. The current taxonomy recognises 13 valid and 35 characterised species within these two genera (Gray 1988). *Hadronyche* contains 32 species, which are separated into six species groups: *infensa, cerberea, illawarra, modesta, lamington* and *adelaidensis*. The remaining three species belong to the genus *Atrax* (Gray 1988). However, it is widely recognised that the taxonomy of the Australian funnel web spiders is in need of review, as large numbers of undescribed species have continued to be identified throughout the range of the group since the publication of the current taxonomy in 1988 (Wilson 2001). Furthermore, a phylogenetic pilot study for the present project identified numerous candidates for species status that appeared to be morphologically cryptic, further complicating the state of the current taxonomy (Beavis & Rowell 2006). Undescribed representatives of both genera of Australian funnel web spider are common throughout the Tallaganda region, but are not represented in the current taxonomy. Therefore, this project will use a species signifier for these groups: *Hadronyche* Sp. 1 and *Atrax* Sp. 1.

**Biology of Australian Mygalomorphae**

Very little is known regarding the natural history of Australian funnel web spiders, however, there are a number of traits that are characteristic of the majority of the Mygalomorphae. In general, mygalomorph spiders are long-lived, females reaching a maximum life-span of 20 years (Levitt 1961; Main 1982); and sessile, individuals
adopting a “sit and wait” prey capture strategy, feeding opportunistically on invertebrates or small vertebrates (Main 1982). In addition, the group is reasonably sedentary, juveniles undergoing an early dispersal event after which they establish themselves in a burrow, remaining there for the duration of their lives (Main 1982). Only male individuals leave their nest when, upon molting to maturity, they adopt a vagrant strategy in order to mate (Main 1982). Furthermore, widespread dispersal – whilst possible – is thought to be limited by the taxon’s predisposition to desiccation (Main 1982). All members of the Mygalomorphae possess two pairs of book-lungs that expose a large surface area of tissue for gas-exchange (Main 1982). Consequently, mygalomorph spiders are susceptible to desiccation and populations are chiefly restricted to humid geographic regions. In addition, members of this group display high levels of habitat fidelity to ecological niches where water loss is less likely to occur, for example decomposing logs, trees and ground burrows (Main 1982).

Known biology of Tallaganda Hadronyche and Atrax

Whilst funnel web spiders in the Tallaganda region appear to display the traits common to most Mygalomorphae, there are a small number of known differences that distinguish representatives of the taxa of interest. Both genera share a sympatric distribution, however, they occupy distinct ecological niches: the exclusively saproxylic Hadronyche Sp. 1 inhabits burrows within decomposing logs; whilst Atrax Sp. 1 constructs ground burrows directly into the soil substrate. Hadronyche Sp. 1 is thought to conform to the Mygalomorph model of a once-off juvenile dispersal event (Main 1982; Woodman et al. 2006). Dispersing juvenile individuals are thought to make use of unoccupied pre-existing cracks, knotholes or weaknesses within the saproxylic environment, that is within dead Eucalyptus wood. Individuals that successfully establish a burrow appear to occupy it for life (or, if male, until they molt to maturity), enlarging the burrow as they grow (Woodman et al. 2006; Pers. Ob.). In contrast, Atrax Sp. 1 constructs ground burrows directly into the soil substrate. The dispersal capabilities of Hadronyche Sp. 1 and Atrax Sp. 1 had not been investigated prior to the present body of research. However, a pilot study for this project (Beavis & Rowell 2006) – together with separate phylogeographic studies of both Hadronyche Sp. 1 (Chapter 2) and Atrax Sp. 1 (Chapter 3) – have revealed evidence indicating that there are marked differences between the patterns and rates of migration displayed by the taxa of interest.
Furthermore, observations collected in the course of field collecting suggest that *Atrax* Sp. 1 is capable of establishing burrows as an adult, which is in contrast to the once-off dispersal event displayed by *Hadronyche* Sp. 1. Adult *Atrax* Sp. 1 were frequently found within sites that had previously been disturbed by forestry practices. For example, a site that had been burned 1 year previous to these observations contained large *Atrax* burrows, within which was found the decomposing carcase and exoskeleton of large individuals. I observed that these large burrow-spaces almost always contained a smaller *Atrax* Sp. 1 individual that had constructed the regular silk ‘stocking’ burrow lining, appropriate to its own size, and fastened it at intervals to the far larger burrow cavity left by the previous inhabitant (Pers. Obs.).

*Hadronyche* Sp. 1 and *Atrax* Sp. 1 are morphologically distinct (Rowell 1980). *Hadronyche* Sp. 1 is physically powerful, displaying large paturons (the muscle that exerts the striking power) and chelicerae (i.e. fangs). In contrast, *Atrax* Sp. 1 displays smaller paturons and chelicerae, the chelicerae themselves displaying a finely graduated tip. When found in local sympatry, these characteristics are significantly exaggerated and are thought to reflect prey preferences (Rowell 1980). Rowell’s 1980 study of *Hadronyche* and *Atrax* species across the Monga, Tallaganda and Tinderry regions examined the prey remains found directly outside and within individual burrows. This study found that *Hadronyche*-associated remains consisted of larger, hard-bodied cockroaches and beetles. In contrast, *Atrax*-associated remains consisted of smaller beetles and ants (Rowell 1980). Overall, this material forms the bulk of the known natural history and biology of the species of interest. Whilst it is suggestive of a number of species characteristics, it does not provide us with detailed assessments of dispersal ability or environmental preferences. It is not surprising, given the 20-year life-span of the Australian funnel web spiders and the challenges involved in husbandry, that there is only one long-term natural history study of this taxon group in existence (Levitt 1961). Consequently, it is the goal of this study to infer the biological idiosyncrasies that separate these two genera of funnel web spider.

**The Pleistocene at Tallaganda**

The Tallaganda region of South-Eastern NSW occurs as an isolated mountain spur that extends out from the GDR in the south (at Badja), but is embedded on its remaining
three sides within low-lying grasslands. The entire region was subject to the effects of glacial-interglacial cycling during the Pleistocene, and has been the site of an ongoing comparative phylogeographic project that has investigated the effects of historical climate variation upon log-dwelling invertebrates. Unlike Northern-Hemisphere landmasses, Australia did not undergo widespread glaciation during the Quaternary, with only the Kosciuszko Massif (2228m asl) and the West Coast Ranges of Tasmania being covered with ice (Singh & Geissler 1985; Barrows et al 2001). Instead, the majority of the continent experienced significant changes in temperature, sea-level and the geographical extent and composition of vegetational classes. Of relevance to the present study are the significant impacts of Pleistocene glacial-interglacial cycling upon the composition of South-East Australian vegetation (Hope et al. 2004). In broad terms, glacial periods were characterised by dominance of grasses and by herbaceous and shrub-like Asteraceae. In contrast, interglacials were characterised by forest and woodland taxa, predominantly *Eucalyptus*, but including Nothofagus and tree ferns (Hope et al. 2004). The overall picture that has been built up of the most recent glacial-interglacial cycle in Australia is that from approximately 30 000 – 20 000 ybp temperature and rainfall decreased until glacial maxima at approximately 15 000 ybp. At approximately 25 000 ybp sea levels had declined to the extent that a landbridge formed between the mainland and Tasmania (Dodson & Ono 1997; Hope et al. 2004). Opinion is divided as to whether *Eucalypt* forests were restricted to coastal areas or whether they survived in gully refugia throughout South-Eastern NSW, but it is generally accepted that this region was predominantly herbaceous grassland at glacial maxima (Hope et al. 2004; Williams et al. 2006; Kershaw et al. 2007). Between 15 000 – 10 000 ybp sea levels increased as the continent began to experience the current interglacial, and by 10 000 ybp current forest patterns were established, albeit to a lesser extent than at present (Dodson & Ono 1997).

The Tallaganda region is situated immediately south of Lake George, the site of a palaeorecord spanning the entirety of the last glacial cycle. Lake George is 673m above sea level (asl) and the lake catchment extends into the northernmost limits of Tallaganda itself. Lake George is one of the large, hydrologically sensitive, terminal, closed intermittent water bodies of internal drainage in Australia (Singh & Geissler 1985), which has the effect of making it the site of one of the most complete existing
palaeorecords for South-Eastern Australia. At present *Eucalyptus* dominates the overall vegetation profile and vegetation within the Lake George catchment, however, the catchment also contains vegetation ranging from warm temperate dry-sclerophyll forests through to *Eucalypt* woodlands and grasslands. Some regions exclusively contain flora peculiar to coastal heaths, swamps or treeless frost-hollows. Above the treeline (2000 asl), alpine herbaceous taxa predominate the landscape (Singh & Geissler 1985). The Lake George palaeorecord is derived from an 18m core that covers the period 730 000 – 0 years before present (ybp) and contains pollen, spore, algae and charcoal particles (Singh & Geissler 1985). The conclusions of studies based on this type of data operate under the assumption that changes in temperature will produce elevational changes in vegetation groups and, therefore, movements in and out of the Lake George catchment. Given this assumption, the palaeorecord indicates that altitudinal shifts of vegetation occurred synonymously with glacial, cool-temperate periods and interglacials. Singh & Geissler (1985) infer that the upper treeline decreased from present levels by 1200 – 1500m during glacials and 300 – 600m during cool-temperate periods. These drops in the treeline are calculated to represent decreases in the average temperature of 8 – 10°C and 2 – 4°C at glacial maxima and cool-temperate periods respectively. At glacial maxima (23 000 – 16 000 ybp) the palaeorecord indicates a “total disappearance” of *Eucalypt* sclerophyll forests and their replacement with open herbland (Singh & Geissler, 1985). The record shows evidence for the presence of the following: tree vegetation is limited to two samples of the conifer *Phyllocladus*; tall groundcover occurs in low densities and consists of *Cyathea, Pteris, Gleichenia, Asperula, Bursaria* and ferns; herbaceous taxa makeup the bulk of species present and primarily consists of *Lycopodium, Liliaceae* and *Poaceae* (Singh & Geissler, 1985). The two glacials that preceded the most recent glacial maxima display equivalent vegetational patterns. During the present interglacial, *Eucalypts* resumed their earlier inter-glacial dominance in the landscape.

The reconstruction of the Lake George palaeorecord (Singh & Geissler 1985) is congruent with those from other sites in Eastern Australia (REF). The severity of the most recent glacial, which appears to exclude *Eucalypt* forest from the region, is mirrored in other locations on the continent. For example, a similar phenomenon is observed in the palaeorecord from the Caledonia Fen (1280m asl) in the South-Eastern
highlands of Victoria (Kershaw et al 2007). Whilst *Eucalyptus* does not completely disappear from the record, *Eucalypt* values declined to approximately 5% and herbaceous taxa predominated. At the beginning of the current interglacial *Eucalypt* values increased from 5% to 60%, reaching 85% at the most recent sampling point (Kershaw et al 2007). Kershaw *et al.* (2007) suggest that *Eucalyptus* species survived in the region at low densities in shrub form, not unlike the phenotype that may be currently observed at high elevations.

The present study submits that the widespread observation of an absence of *Eucalyptus* is too widespread to be ignored, whilst taking into account that the absence of pollen in the palaeorecord could be equally congruent with extremely low densities of *Eucalyptus* within sheltered locations. However, this study notes that the distinction between these two possibilities has far-reaching implications for the present study, which is focused on a saproxylic invertebrate that inhabits fallen *Eucalypt* logs, and its ability to persist in Tallaganda throughout glacial maxima.

**Comparative phylogeography of saproxylic invertebrates in Tallaganda**

The Tallaganda region has been the site of an ongoing comparative phylogeographic study of saproxylic – or log-dwelling – invertebrate taxa. This study has aimed to investigate whether Pleistocene glacial-interglacial cycling differentially affected the phylogeographic structure of taxa based upon habitat specificity, primarily to the saproxylic habitat (Garrick *et al.* 2004; Beavis & Rowell 2006; Sunnucks *et al.* 2006; Garrick *et al.* 2007; Hodges *et al.* 2007). Saproxylic invertebrates are particularly informative for phylogeographic research because they experience their landscape at fine spatial scales, and therefore tend to retain the phylogenetic signal of historical events (Moritz *et al.* 2001; Garrick *et al.* 2004). In a field such as phylogeography – that depends on the ability of taxa to preserve the genetic imprint of past demographic change (Crawford *et al.* 2007) – the close link between log-dwelling invertebrates and the saproxylic habitat is invaluable. Consequently, the Tallaganda project has focused upon saproxylic taxa as a means of inferring the effects of Pleistocene glacial interglacial cycling in the region.

The Tallaganda project is grounded upon the *a priori* expectation that Tallaganda forests responded to Pleistocene glacial-interglacial cycling as per the accepted model
of Southern Hemisphere "glaciation" (Garrick et al. 2004). The expectations of the Tallaganda project were that log-dwelling invertebrates would display phylogeographic structure that reflected episodic restrictions on gene-flow amongst five microbiogeographic regions. These regions were defined a priori according to the boundaries separating hydrological catchments, under the assumption that the high-elevation ridgelines that separated catchments would have been devoid of forests at glacial maxima, thus restricting gene-flow (Garrick et al. 2004). The Tallaganda project aimed to test the hypothesis that "glacial-interglacial cycling differentially affected the genetic structure of taxa based on habitat specificity" (Hodges et al. 2007). Thus the driving hypothesis of the Tallaganda project has been that forests – and therefore the saproxylic niche – responded to glacial periods by contracting to comparatively warmer, lower-lying gullies. Consequently, extant saproxylic invertebrates are expected to display spatial genetic structure that reflects the ongoing barriers to gene-flow caused by the disjunct nature of these refugia (Garrick et al. 2004; Beavis & Rowell 2006; Sunnucks et al. 2006; Garrick et al. 2007; Hodges et al. 2007).

Summation

The current paper aims to conduct a comparative phylogeographic study of Tallaganda representatives of two niche-separated genera of Australian funnel web spider: the saproxylic Hadronyche and the ground-dwelling Atrax. Whilst members of the two genera clearly occupy distinct ecological niches, other biological characteristics of the two genera are unknown. The taxa of interest have a sympatric distribution and this study is based on the assumption that Hadronyche and Atrax have experienced a common set of historical environmental conditions. Given that the response of other Tallaganda saproxylic invertebrates to Quaternary climate variation (ie. Pleistocene glacial-interglacial cycling) has been thoroughly studied, this paper uses the established Tallaganda a priori regions as a starting point. Overall this study aims to conduct a comparative phylogeographic study of unnamed species within two niche-differentiated – and therefore potentially biologically distinct – funnel web spiders: the saproxylic Hadronyche and the ground-dwelling Atrax. To do so, the following questions will be addressed:

- Do Hadronyche and Atrax display congruent phylogeographic structure?
• What is the extent of incongruence amongst *Hadronyche* and *Atrax* as measured by population-level and phylogenetic parameters?

• Therefore, this paper aims to use comparative phylogeographic methods to investigate and infer the biological idiosyncrasies that accompany the niche-separation of *Hadronyche* and *Atrax*.

**Methods**

**Sample collection**

*Atrax* and *Hadronyche* specimens for this study were collected from the Tallaganda region between 2002-2006. *Hadronyche* individuals were extracted from their burrows in decomposing logs. *Atrax* specimens were collected by locating burrow entrances beneath rocks and logs and excavating these burrows to extract the resident spider. Where possible, I attempted to sample between 2–6 individuals per site. One whole leg was taken from samples – prior to preservation of the individual in 70% ethanol – and kept at -20°C.

**DNA extraction and sequencing**

This project has found that Australian funnel web spider DNA can be extremely unstable, sometimes degrading completely within 24-48 hours after extraction using a standard C-TAB-Chloroform protocol. The following extraction protocol and PCR conditions were developed as the most effective means of obtaining sufficient PCR product for sequencing. Genomic DNA was isolated from 1mm³ muscle tissue using QIAGEN DNeasy Tissue Kits. To obtain template DNA robust to degradation, it was essential to dissect muscle tissue from inside a leg segment and to prevent excess contact between the external cuticle and the extraction buffer. Sequence was obtained from 94 individuals at the mitochondrial locus of interest. From these, a subset of 32 individuals was selected for sequencing at a nuclear intron locus. Samples included in this subset were selected to represent the major mitochondrial clades and the full geographic range of Tallaganda. A 700 base pair (bp) fragment of mitochondrial sequence was amplified at the gene Cytochrome Oxidase Subunit I (COI) using the universal primer pair Leo1490: 5’ − GGTCACAAATCATAAAGATATTGG and Hco2198: 5’ − TAAACTTCAGGGTGACCAAAAAATCA (Folmer et al, 1994).
Amplifications were as follows: 1 cycle of 94°C for 3 min, 65°C for 30 s, 72°C for 45 s; 2 cycles of 94°C for 30 s, 60°C for 20 s, 72°C for 45 s; 2 cycles of 94°C for 45 s, 55°C for 20 s, 72°C for 45 s; 3 cycles of 94°C for 45 s, 50°C for 20 s, 72°C for 45 s; 2 cycles of 94°C for 45 s, 45°C for 20 s, 72°C for 45 s; 40 cycles of 94°C for 45 s, 45°C for 20 s, 72°C for 45 s; 1 cycle of 72°C for 3 min; rest at 4°C. Amplification reactions consisted of 2μL template DNA; 2U Platinum Taq PCRx DNA Polymerase (Invitrogen); 4μL 10X PCR Buffer, minus Mg; 2.4μL 50mM MgCl₂; 1.6μL 5mM dNTPs; 1μL each 10mM primer; in 40μL total volume. A 400 bp fragment of nuclear intron sequence from the gene Histone 3 (H3) was amplified using the Colgan et al. (2000) primer pair H3F: 5’ – ATGGCGTCGACACGACGACGACGACG and H3R: 5’ – ATATCCCTTRGGCATRATRGTGAC.. Amplifications were as follows: 1 cycle of 94°C for 3 min, 65°C for 30 s, 72°C for 45 s; 2 cycles of 94°C for 30 s, 60°C for 20 s, 72°C for 45 s; 2 cycles of 94°C for 45 s, 55°C for 20 s, 72°C for 45 s; 3 cycles of 94°C for 45 s, 50°C for 20 s, 72°C for 45 s; 40 cycles of 94°C for 45 s, 50°C for 20 s, 72°C for 45 s; 1 cycle of 72°C for 3 min; rest at 4°C. Amplification reactions consisted of 2μL template DNA; 2.5U Platinum Taq PCRx DNA Polymerase (Invitrogen); 4μL 10X PCRx Buffer; 2.4μL 50mM MgSO₄; 1.6μL 5mM dNTPs; 1μL each 10mM primer; in 40.5μL total volume. Polymerase chain reaction (PCR) products were purified using 3M NaAc with an ethanol precipitation. PCR products were sequenced in both directions using Big Dye Terminator (Applied Biosystems) and run on an ABI3100 Genetic Analyser. Sequences were edited and aligned in SEQUENCHER (version 3.0, Gene Codes Corporation, Mi). Ambiguous end regions were removed such that all individuals within each genus were analysed over the same sequence length.

As a preliminary measure to investigate whether the sequence data provides an unbiased record of historical barriers to migration and gene-flow, I assessed the following. First, I determined the model of nucleotide substitution that best explained the data set using a hierarchical likelihood ratio test (hLRT) and Akaike Information Criterion (AIC), implemented in Modeltest v.3.7.0 (Posada & Crandall 1998) and PAUP* v4.0 b10 (Swofford 2001). A test for saturation was implemented in DnaSP (Rozas et al. 2003) by plotting the relative proportions of transitions and transversions against genetic distance under a Modeltest-selected model of nucleotide (including 3rd codon positions).
The degree of pairwise sequence divergences at which transitions outnumber transversions is the point at which saturation will begin to affect a parsimony analysis. I tested for congruence of the mitochondrial and nuclear gene-trees using a partition homogeneity test, implemented in PAUP*. A Likelihood Ratio Test as implemented in PAUP* was used to assess whether the sequences were evolving in a clock-like manner.

**Sequence analysis**

In order to directly and quantitatively compare the sequence diversity contained within *Hadronyche* Sp. 1 vs. *Atrax* Sp. 1 I calculated a suite of summary statistics, separately for each species and locus as follows: the number of unique haplotypes (h), haplotype diversity (Hd), nucleotide diversity (π) and the average number of nucleotide differences (k). To determine whether the loci of interest were subject to selection pressures I performed several tests of neutrality, namely Tajima’s D and Fu’s F statistics. In order to address a key assumption behind inferring phylogeographic history from mtDNA sequences, namely, that sequences evolve in a selectively neutral fashion (Zink *et al.* 2006) I performed a series of neutrality tests. Tajima’s D and Fu’s Fs were used to assess whether sequences conformed to neutral expectations, that is, whether they had been subject to selection. In the absence of selection, significant deviations from neutral expectation for Tajima’s D and Fu’s Fs statistics are indicative of historical population expansion (Dean & Ballard 2004). In order to separate the putative effects of selection from those of historical demographic processes I also conducted the MK-test (McDonald & Kreitman 1991), which tests solely for selection (Ballard & Whitlock 2004).

**Phylogenetic analysis**

I constructed a phylogeny for the combined *Atrax* and *Hadronyche* concatenated COI-H3 sequence dataset using a Bayesian approach, as implemented in MrBayes v.3.1.2 (Huelsenbeck & Ronquist 2001). I conducted 2 runs, each with four Metropolis Coupling Markov Chain Monte Carlo (MCMCMC) chains for 10 000 000 generations, sampling trees every 100 000 generations. I defined a 25% burn-in and discarded those trees accordingly. I conducted MrBayes runs using a General Time Reversible + a proportion of invariant sites + gamma distribution model of nucleotide substitution (as selected by MODELTEST) and the default uniform priors. Each run was started using a
random tree. I assessed convergence of the separate runs by examining the plot of generation vs. log probability of the data (ie. log likelihood values) produced by MrBayes.

**Coalescent-based estimates of demographic change**

This study used coalescent-based processes (as implemented in MIGRATE and LAMARC) to estimate measures of demographic change, namely effective population size (Ne), migration (M) and population growth (G). I aimed to determine whether these parameters differed between Tallaganda and GDR populations. Estimates of migration amongst all populations were calculated using MIGRATE. MIGRATE estimates (directional) migration using Monte Carlo, Markov chain, maximum-likelihood method. MIGRATE was used to produce coalescent estimates of the effective population size (θ = 2Nₑμ), and pairwise migration (M) for the total samples. The search strategy used was as follows: 10 short chains with 500 sampled genealogies each, and 3 long chains with 5000 sampled genealogies. In order to investigate population dynamics amongst geological pairs of *Atrax* Sp. 1 populations, I used LAMARC to produce coalescent estimates of the exponential growth rates (g), which is estimated from θ(t) = θ(present) exp(-gt), where t is the time before the present. Starting values of θ were calculated via Watterson’s estimate. The search strategy used was as follows: 10 short chains each with a total of 1000 genealogies and a sampling increment of 20 genealogies and two final chains with 20,000 sampled genealogies and sampling increment of 20 genealogies.

**Results**

Preliminary analysis

Modeltest selected the following models of nucleotide evolution under the hLRT. *Hadronyche* Sp. 1 COI data was best explained by the GTR +G model whilst H3 data conformed to the JC + G model of nucleotide evolution. The best fit for *Atrax* Sp. 1 COI data was the TrN + I + G model whilst H3 data was best explained by the JC model. A test for sequence saturation (including 3rd codon positions) found that transversions outnumbered transitions at approximately 18% sequence divergence, indicating the degree of sequence divergence at which saturation would impact on the amount of information encoded by the sequences. As the maximum uncorrected p-
distance found was 15%, sequence saturation was unlikely to bias the parsimony analysis. A partition homogeneity test indicated no evidence for phylogenetic incongruence between the two loci of interest. Consequently I concatenated the sequences using MacClade (v.4.07) resulting in a 875bp length of sequence. All individuals found within a single mitochondrial haplotype could be unambiguously nested within a single nuclear haplotype. Consequently, the concatenated sequence data haplotypes were defined as per mtDNA haplotypes, which provided more resolution when examining phylogenetic relationships amongst taxa. A likelihood ratio test showed that all sequences were evolving in a clock-like manner.

**Sequence analysis**

Measures of sequence diversity revealed clear differences separating *Hadronyche* Sp. 1 and *Atrax* Sp. 1 (Table 5.1). The *Hadronyche* Sp. 1 mtDNA (COI) sample of 94 individuals contained 33 unique haplotypes. 45 individuals, sourced from across the full geographic range of Tallaganda, belonged to a single mtDNA haplotype. The nDNA sample (H3) of 32 individuals was represented by 9 haplotypes. The *Atrax* Sp. 1 MtDNA (COI) sample of 143 individuals contained 82 unique haplotypes. The nDNA sample (H3) of 70 individuals was represented by 9 haplotypes. Nucleotide and haplotype diversity values for *Hadronyche* Sp. 1 from the whole region were 0.024 (COI)/ 0.013 (H3) and 0.763 (COI)/ 0.569 (H3) respectively. In *Atrax* Sp. 1 nucleotide diversity for the Tallaganda region was 0.070 (COI)/ 0.002 (H3), whilst haplotype diversity was 0.983 (COI)/ 0.472 (H3).

**Table 5.1 Genetic diversity indices for *Hadronyche* Sp. 1 & *Atrax* Sp. 1**

Table 5.1 shows genetic diversity indices for the total sample, specimens collected from *Hadronyche* Sp. 1 and *Atrax* Sp. 1. N, number of individuals; M, number of haplotypes; $\pi$, nucleotide diversity; $h$, haplotype diversity; k, average number of nucleotide differences).

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>M</th>
<th>$\pi$</th>
<th>$h$</th>
<th>$k$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>COI</td>
<td>H3</td>
<td>COI</td>
<td>H3</td>
<td>COI</td>
</tr>
<tr>
<td><em>Hadronyche</em></td>
<td>94</td>
<td>32</td>
<td>33</td>
<td>9</td>
<td>0.024</td>
</tr>
<tr>
<td><em>Atrax</em></td>
<td>143</td>
<td>70</td>
<td>82</td>
<td>9</td>
<td>0.070</td>
</tr>
</tbody>
</table>
Phylogenetic analysis

The Bayesian analysis of a combined *Hadronyche* and *Atrax* dataset strongly supports reciprocal monophyly of the two genera (Figure 5.1). Furthermore, this analysis reveals striking differences in the evolutionary history of the two lineages as follows. The *Atrax* Sp. 1 phylogeny displays two levels of phylogenetic structure, which appears to reflect both ancient and more recent barriers to dispersal. In contrast, the *Hadronyche* Sp. 1 phylogeny contains very little structure, resulting from low levels of variability. *Hadronyche* Sp. 1 currently extant in Tallaganda appear to have experienced a long period of stasis, followed by a recent and rapid radiation.
Figure 5.1 Atrax Sp. 1 and Hadronyche Sp. 1 Bayesian phylogeny

Bayesian MCMC phylogeny for Atrax Sp. 1, and Hadronyche Sp. 1 with an Araneomorphae outgroup, using a GTR + $\gamma$ + $\lambda$ model of nucleotide evolution (ln $L = -4736.91$).
Demographic change
A suite of neutrality statistics and statistics derived from coalescent analyses revealed clear differences separating *Hadronyche* Sp. 1 and *Atrax* Sp. 1, primarily showing evidence that *Hadronyche* Sp. 1 had undergone a historical population expansion, while *Atrax* Sp. 1 appeared to have maintained a constant population size. *Hadronyche* Sp. 1 displayed a significantly negative Tajima’s D value (-2.65104; \( p < 0.001 \)), but Fu’s Fs was not significant (-1.818; \( p > 0.343 \)). In contrast, I were not able to reject neutrality for *Atrax* Sp. 1 values of Tajima’s D (-0.28889; \( p > 0.10 \)) or Fu’s Fs (0.000; \( p > 0.461 \)). This study was unable to reject the null-hypothesis of neutral evolution using the McDonald-Kreitman (MK) test for either *Hadronyche* Sp. 1 or *Atrax* Sp. 1. Therefore I concluded that the negative Tajima’s D value displayed by *Hadronyche* Sp. 1 was an outcome of historical demographic change, rather than the effects of selection. Estimates of Theta (\( \Theta \)) derived from the coalescent indicated that effective population size was higher in the Tallaganda *Atrax* Sp. 1 population (\( \Theta = 0.035519 \)) than in the Tallaganda *Hadronyche* Sp. 1 population (\( \Theta = 0.013289 \)), as would be expected in the case of a *Hadronyche* Sp. 1 founder event. Estimates of population growth (\( g \)) derived from the coalescent indicated that population growth in the Tallaganda population of *Hadronyche* Sp. 1 was much higher (\( g = 212.0931 \)) than the small degree of population growth (ie. constant population size) displayed by *Atrax* Sp. 1 (20.80265).

Discussion
Central to this study’s approach is the assumption that a sympatric geographic distribution has imposed a shared set of historical environmental experiences upon the taxa of interest. In the first instance, this paper will assess congruence of the phylogeography of these mygalomorph spiders with the suite of saproxylic invertebrates previously studied at Tallaganda. By doing so, this paper will discuss whether shared geography has provoked a shared response to historical climate change. As a whole, the Tallaganda study has focused upon inferring the responses of saproxylic taxa to Pleistocene climate variation. The phylogeographic structure displayed by log-dwelling invertebrates in Tallaganda has been found to be broadly congruent with the putative locations of glacial refugia, thus supporting the hypothesis that sympatrically distributed invertebrates in Tallaganda have responded in tandem to historical climate change.
Whilst each taxon displays unique deviations from these expectations, one particular phylogeographic break appears as a constant across virtually all taxa studied, including a saproxylic species of skink. The Tallaganda-Badja break has been identified in two unnamed species of neanurid collembolan (Garrick et al 2004; Garrick et al 2007); two terrestrial flatworms, *Artioposthia lucasi* and *Caenoplana coerulea* (Sunnucks et al 2006); and both *Hadronyche* Sp. 1 (Chapter 2) and *Atrax* Sp. 1 (Chapter 3). Therefore, it is reasonable to suggest that *Hadronyche* Sp. 1 and *Atrax* Sp. 1 share a common environmental history. Consequently, the broader findings of the Tallaganda project may be used as a point of comparison for mygalomorph spiders at Tallaganda, when considering whether phylogeographic structure displayed by the taxa of interest has resulted from commonly experienced historical demographic events or species-specific biological traits.

**Inferred demographic history**

The present study has detected markedly different patterns and timing of population evolution in two niche-separated species of funnel web spider, *Hadronyche* Sp. 1 (Chapter 2) and *Atrax* Sp. 1 (Chapter 3), suggesting that habitat niche and species biology has influenced the responses of these species to glacial-interglacial cycling (cf Bell *et al.* 2007). The saproxylic *Hadronyche* displayed evidence of a recent history in Tallaganda and no phylogeographic structure within the region. The absence of phylogeographic structure in *Hadronyche* Sp. 1 appears to have resulted from a recent colonisation event: low levels of nucleotide diversity combined with relatively high levels of haplotype diversity suggest rapid expansion from a small population (eg. Eberhard & Bermingham 2004; Alexio 2006); and analyses of the spatial genetic structuring occurring within the Tallaganda population show no deviation from random expectations. This study hypothesises that *Hadronyche* Sp. 1 has colonised Tallaganda in the 11 000 years since the most recent glacial maxima as a founder event from the GDR (Chapter 1). It is possible that the genus either did not survive the most recent glacial maxima in Tallaganda, or had been absent in the region prior to the present day. One cannot differentiate between these two scenarios because the phylogeographic fingerprint of a recolonisation is identical to that of a new invasion. However given the palaeorecord evidence for the presence of *Eucalypts* throughout the region in periods prior to the last glacial maxima (Singh & Geissler 1985) *Hadronyche* Sp. 1 very well
may have been present at Tallaganda and become locally extinct during the coldest period of the most recent glacial. This pattern of phylogeographic structure is in stark contrast to that displayed by other saproxylic taxa in Tallaganda.

In contrast, the ground-dwelling *Atrax* Sp. 1 displayed evidence of a long history in the region and survival throughout the Pleistocene. *Atrax* Sp. 1 displays strong phylogeographic structure, the phylogeny displaying two levels of structure corresponding to divergences at two different time scales (Chapter 2). A separate study has found that the phylogeny displays reciprocal monophyly of clades deep in the phylogeny that correspond with distinct, non-overlapping geographic regions (or *Atrax* “refugia regions”). In contrast, recent divergence events appear to be associated with soil type (Chapter 2). These findings are consistent with *Atrax* Sp. 1 having persisted within Tallaganda throughout the Pleistocene (Paulo *et al.* 2001). However, it seems unlikely that the most recent glaciation is responsible for any phylogeographic structure present in extant *Atrax* Sp. 1 populations (Wares & Cunningham 2005). However, ostensibly *Atrax* Sp. 1 shares some similarities in its phylogeographic structure with the saproxylic taxa previously studied at Tallaganda.

**Inferred biological differences**

The niche separation displayed by *Hadronyche* Sp. 1 and *Atrax* Sp. 1 is an obvious biological difference that distinguishes the two species. However, whilst the differences in habitat displayed by the taxa of interest may account for persistence or local extinction in Tallaganda throughout glacial periods, they do not necessarily explain the extent of phylogeographic incongruence found in this system. Species-specific differences in historical population growth and estimates of migration are also likely to have come about via significant biological differences, for example in migration and dispersal capabilities. This study has detected clear differences in these parameters, which separate the taxa of interest, and I propose that these differences may be the outcome of biological differences.

The phylogenetic parameters measured by this study suggest that there are distinct differences separating *Hadronyche* Sp. 1 and *Atrax* Sp. 1 migration capacity and environmental resilience. Estimates of $\theta$ and measures of demographic change provide evidence that *Hadronyche* Sp. 1 has recently undergone a broad-scale migration event,
possibly accompanied by a recent range expansion into Tallaganda. Given these findings, it is reasonable to surmise that this saproxylic specialist is capable of broad-scale dispersal. The ability to disperse broadly is in fact relatively common amongst niche-specialised species because habitat availability acts as a limiting factor (eg. Jung & Croft 2001). Despite evidence indicating that *Hadronyche* Sp. 1 is a highly effective disperser, it also appears that this species is susceptible to environmental change. Previous research on the phylogeography of *Hadronyche* Sp. 1 (Chapter 2) has found evidence congruent with this species having become locally extinct in Tallaganda, possibly as a result of Pleistocene glaciation, which would be consistent with the extreme habitat specialisation displayed by *Hadronyche* Sp. 1. Chapter 2 presents the hypothesis that *Hadronyche* Sp. 1 has colonised the Tallaganda region in the 11 000 years since the most recent glacial period. This is further supported by the findings of this study, which indicate that Tallaganda *Hadronyche* Sp. 1 has undergone population growth. These genetic signatures of demographic change are in stark contrast to the majority of other saproxylic species studied at Tallaganda, which have displayed evidence of surviving through periods of historical climate change. From these findings, one could infer that *Hadronyche* Sp. 1 requires a stable set of environmental conditions to persist. An additional factor that may have contributed to this set of responses is that *Hadronyche* Sp. 1 individuals are unable to re-establish burrows as adults, and occupy a single log for life (Woodman et al. 2006). The findings of the present study support this type of dependence upon an individual habitat item and an individual inability to migrate in response to changing conditions.

The phylogeographic patterns displayed by *Atrax* Sp. 1 provide a stark contrast to those discussed above. Unlike *Hadronyche* Sp. 1, the ground dwelling *Atrax* Sp. 1 displays evidence of being resilient to environmental change, on both temporal and spatial scales. This taxon displays no evidence of recent range expansions or demographic change, however the phylogeny contains deep structure that a separate study had found corresponded with non-overlapping geographic locations and underlying geology (Chapter 3). This would suggest that *Atrax* Sp. 1 individuals are able to survive changes to their local environment to the extent that they were able to persist through Pleistocene glacial-interglacial cycling. However, of particular interest is the finding that growth of the Tallaganda population is low (ie. it displays evidence of a constant
population size) and migration appears to be negligible. In short, this species does not appear to display broad-scale dispersal. Natural history observations have indicated that this species is capable of localised migration, dispersing small distances easily. This ability to respond to local environmental changes may have contributed to the persistence of this species in Tallaganda throughout the Pleistocene, and to permit increasing levels of differentiation amongst populations (and species).

**Summation**

This comparative phylogeographic study of two species of Australian funnel web spider has highlighted the previously unappreciated aspects of species biology. This type of study is essential in neglected taxa, and is a more viable alternative to traditional natural history studies. By taking this inference-based approach to elucidating the biology of under-studied species, it becomes possible to address taxonomic inequalities in research efforts.
References


Chapter 5: Comparative Phylogeography Elucidates Biological Idiosyncrasies


Wares JP, Cunningham CW (2005) Diversification before the most recent glaciation in *Balanus glandula*. *Biological Bulletins*, 208, 60-68.


CHAPTER 6
CHAPTER 6

AN AFTERWORD: WHAT HAVE I LEARNT?

This thesis began as a component of the broader comparative phylogeographic study of saproxylic invertebrates at Tallaganda (NSW). As the final PhD student to commence working in this region, my brief was to investigate the phylogeography of a pair of mygalomorph spiders: one saproxylic (*Hadronyche* Sp. 1) and one non-saproxylic (*Atrax* Sp. 1). My initial expectation was that *Hadronyche* Sp. 1 would display phylogeographic structure congruent with that displayed by other log-dwelling invertebrates studied at Tallaganda, that is, clear evidence of having been restricted to refugia at glacial maxima. In contrast, I expected that the ground-dwelling *Atrax* Sp. 1 would show relatively little phylogeographic structure. This hypothesis was based upon the assumption that the habitat of *Atrax* Sp. 1 was readily available and unaffected by Pleistocene glacial-interglacial cycling. Instead, my PhD study found that the complete opposite was the case. *Hadronyche* Sp. 1 shows evidence of having become locally extinct during the most recent glacial, colonising Tallaganda from the Great Dividing Range in the 11,000 years since glacial maxima (Chapter 2; Chapter 4). Meanwhile, *Atrax* Sp. 1 has clearly had a long history in the Tallaganda region, and the group’s phylogeographic structure reflects the influence of underlying geology and possibly ancient climate change (Chapter 3; Chapter 4). Furthermore, there are clearly key biological differences (apart from habitat niche) that have affected the genetic structuring of extant funnel web spiders in the Tallaganda region (Chapter 5).

Overall – and in line with prior expectations – the findings of this PhD project raise more questions than they have has answered. Most significantly, my project has indicated the presence of multiple, previously unidentified, cryptic species of funnel web spiders within the 100km transect of forest that makes up Tallaganda. Given the influence of underlying geology upon this system, my project has shown that Pleistocene glaciation – an influence that has been the focus of phylogeographic research in recent years – is not the sole contributing factor to the phylogeography of extant *Atrax* Sp. 1 in Tallaganda. In fact, as has been raised in the literature from time to time, there are many factors that act to restrict the migration of taxa such that
populations and even species become distinct entities. It is my view that by taking a
multi-disciplinary approach one can more fully appreciate and identify the diverse
factors that have influenced biological entities. It is this approach that will drive
research proceeding from the findings of this thesis.

There are a number of areas for future research that have emerged from my PhD
project. First, I am intending to use the phylogenies produced within this thesis as a
means of testing the current Australian funnel web spider taxonomy (Australian
Biological Resources Study grant application, in review). This collaborative project will
use the phylogenies I have produced during this PhD, and the putative species
boundaries they suggest, as the basis for testing the validity of the current taxonomy.
Through the use of phylogenetic, traditional taxonomic and chemotaxonomic (the use of
venom-profiles as a taxonomic character) methods, the team involved in the proposed
research will attempt to formally delimit species that have been identified by my PhD
research. Secondly, the finding of my PhD project that underlying geology has
influenced *Atrax* phylogeography places the previous findings of the Tallaganda project
in a new light. It will be important that the overall synthesis paper that will emerge from
this project take full account of the key role underlying geology has played in restricting
gene-flow. Thirdly, this project has found that underlying geology has influenced
phylogeography and that spiders show evidence of local adaptation to (and a preference
for) granitic soil. However, it is unclear what physical or chemical characteristics of
granitic soil have produced this phylogenetic pattern. A paper in preparation (though not
included in this thesis) is investigating this aspect of spider soil preferences from a
geological perspective.

Overall this thesis highlights and underscores the fact that neglected taxa –
predominantly invertebrates – are rich sources for evolutionary enquiry. Whilst the field
of invertebrate research suffers from a dearth of base-line biological data, it is possible
to circumvent the challenges one faces when there is no real knowledge regarding the
ecological, genetic or behavioural nuances of a given taxon. By taking a multi-
disciplinary approach this thesis has been able to achieve just that, by elucidating
aspects of the biology and evolution of an iconic but – by any definition – neglected
taxon group, the Australian funnel web spider.
## Appendix 1.1: *Hadronyche* Sp. 1 Sample Details

<table>
<thead>
<tr>
<th>Site</th>
<th>Catchment</th>
<th>Sub Catchment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A46</td>
<td>Shoalhaven</td>
<td>Upper Shoalhaven</td>
</tr>
<tr>
<td>B14</td>
<td>Tuross</td>
<td></td>
</tr>
<tr>
<td>B19</td>
<td>Tuross</td>
<td></td>
</tr>
<tr>
<td>B20</td>
<td>Tuross</td>
<td></td>
</tr>
<tr>
<td>B28</td>
<td>Tuross</td>
<td></td>
</tr>
<tr>
<td>B30</td>
<td>Tuross</td>
<td></td>
</tr>
<tr>
<td>B36</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
</tr>
<tr>
<td>B38</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
</tr>
<tr>
<td>B39</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
</tr>
<tr>
<td>B58</td>
<td>Murrumbidgee</td>
<td>Queanbeyan</td>
</tr>
<tr>
<td>B60</td>
<td>Murrumbidgee</td>
<td>Queanbeyan</td>
</tr>
<tr>
<td>B62</td>
<td>Tuross</td>
<td></td>
</tr>
<tr>
<td>B63</td>
<td>Tuross</td>
<td></td>
</tr>
<tr>
<td>B72</td>
<td>Tuross</td>
<td></td>
</tr>
<tr>
<td>B78</td>
<td>Tuross</td>
<td></td>
</tr>
<tr>
<td>B89</td>
<td>Shoalhaven</td>
<td>Upper Shoalhaven</td>
</tr>
<tr>
<td>B95</td>
<td>Shoalhaven</td>
<td>Upper Shoalhaven</td>
</tr>
<tr>
<td>B98</td>
<td>Shoalhaven</td>
<td>Upper Shoalhaven</td>
</tr>
<tr>
<td>C01</td>
<td>Shoalhaven</td>
<td>Upper Shoalhaven</td>
</tr>
<tr>
<td>C05</td>
<td>Shoalhaven</td>
<td>Upper Shoalhaven</td>
</tr>
<tr>
<td>C15</td>
<td>Deua</td>
<td></td>
</tr>
<tr>
<td>C16</td>
<td>Deua</td>
<td></td>
</tr>
<tr>
<td>C17</td>
<td>Shoalhaven</td>
<td>Upper Shoalhaven</td>
</tr>
<tr>
<td>C23</td>
<td>Shoalhaven</td>
<td>Lower Shoalhaven</td>
</tr>
<tr>
<td>C30</td>
<td>Shoalhaven</td>
<td>Lower Shoalhaven</td>
</tr>
<tr>
<td>C34</td>
<td>Shoalhaven</td>
<td>Lower Shoalhaven</td>
</tr>
<tr>
<td>C39</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
</tr>
<tr>
<td>C40</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
</tr>
<tr>
<td>C41</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
</tr>
<tr>
<td>J1</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
</tr>
<tr>
<td>J2</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
</tr>
<tr>
<td>J6</td>
<td>Shoalhaven</td>
<td>Upper Shoalhaven</td>
</tr>
<tr>
<td>JA10</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
</tr>
<tr>
<td>JA11</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
</tr>
<tr>
<td>JA23</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
</tr>
<tr>
<td>JA24</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
</tr>
<tr>
<td>JA3</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
</tr>
<tr>
<td>JA36</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
</tr>
<tr>
<td>JA37</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
</tr>
<tr>
<td>JA39</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
</tr>
<tr>
<td>JA4</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
</tr>
<tr>
<td>JA70</td>
<td>Tuross</td>
<td></td>
</tr>
<tr>
<td>JA73</td>
<td>Tuross</td>
<td></td>
</tr>
<tr>
<td>JA76</td>
<td>Murrumbidgee</td>
<td>Queanbeyan</td>
</tr>
<tr>
<td>JA80</td>
<td>Murrumbidgee</td>
<td>Queanbeyan</td>
</tr>
<tr>
<td>JA83</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
</tr>
<tr>
<td>JA84</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
</tr>
<tr>
<td>JA85</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
</tr>
<tr>
<td>JA86</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
</tr>
<tr>
<td>JA87</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
</tr>
<tr>
<td>SH5</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
</tr>
<tr>
<td>W5</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
</tr>
<tr>
<td>W7</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
</tr>
<tr>
<td>W8</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
</tr>
<tr>
<td>Z09</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
</tr>
<tr>
<td>Z10</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
</tr>
<tr>
<td>Z16</td>
<td>Murrumbidgee</td>
<td>Queanbeyan</td>
</tr>
<tr>
<td>Z23</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
</tr>
<tr>
<td>Z29</td>
<td>Tuross</td>
<td></td>
</tr>
<tr>
<td>Z33</td>
<td>Shoalhaven</td>
<td>Upper Shoalhaven</td>
</tr>
<tr>
<td>Z34</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
</tr>
<tr>
<td>Z36</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
</tr>
<tr>
<td>Z39</td>
<td>Shoalhaven</td>
<td>Upper Shoalhaven</td>
</tr>
<tr>
<td>Z41</td>
<td>Murrumbidgee</td>
<td>Numeralla</td>
</tr>
<tr>
<td>Z42</td>
<td>Tuross</td>
<td></td>
</tr>
<tr>
<td>Z46</td>
<td>Tuross</td>
<td></td>
</tr>
</tbody>
</table>
## Appendix 1.2: *Atrax* Sp. 1 Sample Details

<table>
<thead>
<tr>
<th>Site</th>
<th>Catchment</th>
<th>Sub Catchment</th>
<th>Underlying Geology</th>
</tr>
</thead>
<tbody>
<tr>
<td>A01</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
<td>Metasediments</td>
</tr>
<tr>
<td>A02</td>
<td>Shoalhaven</td>
<td>Ballalaba</td>
<td>Metasediments</td>
</tr>
<tr>
<td>A03</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
<td>Metasediments</td>
</tr>
<tr>
<td>A04</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
<td>Metasediments</td>
</tr>
<tr>
<td>A05</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
<td>Metasediments</td>
</tr>
<tr>
<td>A06</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
<td>Metasediments</td>
</tr>
<tr>
<td>A12</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
<td>Metasediments</td>
</tr>
<tr>
<td>A13</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
<td>Metasediments</td>
</tr>
<tr>
<td>A14</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
<td>Metasediments</td>
</tr>
<tr>
<td>A23</td>
<td>Shoalhaven</td>
<td>Upper Shoalhaven</td>
<td>Metasediments</td>
</tr>
<tr>
<td>A27</td>
<td>Shoalhaven</td>
<td>Ballalaba</td>
<td>Granite</td>
</tr>
<tr>
<td>A28</td>
<td>Shoalhaven</td>
<td>Ballalaba</td>
<td>Granite</td>
</tr>
<tr>
<td>A30</td>
<td>Shoalhaven</td>
<td>Lower Shoalhaven</td>
<td>Basalt</td>
</tr>
<tr>
<td>A32</td>
<td>Shoalhaven</td>
<td>Lower Shoalhaven</td>
<td>Granite</td>
</tr>
<tr>
<td>A37</td>
<td>Shoalhaven</td>
<td>Ballalaba</td>
<td>Metasediments</td>
</tr>
<tr>
<td>A45</td>
<td>Shoalhaven</td>
<td>Ballalaba</td>
<td>Metasediments</td>
</tr>
<tr>
<td>A46</td>
<td>Shoalhaven</td>
<td>Upper Shoalhaven</td>
<td>Metasediments</td>
</tr>
<tr>
<td>A47</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
<td>Granite</td>
</tr>
<tr>
<td>A49</td>
<td>Shoalhaven</td>
<td>Lower Shoalhaven</td>
<td>Granite</td>
</tr>
<tr>
<td>A50</td>
<td>Shoalhaven</td>
<td>Lower Shoalhaven</td>
<td>Granite</td>
</tr>
<tr>
<td>A52</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
<td>Granite</td>
</tr>
<tr>
<td>B89</td>
<td>Shoalhaven</td>
<td>Upper Shoalhaven</td>
<td>Metasediments</td>
</tr>
<tr>
<td>B90</td>
<td>Shoalhaven</td>
<td>Upper Shoalhaven</td>
<td>Metasediments</td>
</tr>
<tr>
<td>B91</td>
<td>Shoalhaven</td>
<td>Upper Shoalhaven</td>
<td>Metasediments</td>
</tr>
<tr>
<td>B98</td>
<td>Shoalhaven</td>
<td>Upper Shoalhaven</td>
<td>Granite</td>
</tr>
<tr>
<td>C01</td>
<td>Shoalhaven</td>
<td>Upper Shoalhaven</td>
<td>Metasediments</td>
</tr>
<tr>
<td>C24</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
<td>Granite</td>
</tr>
<tr>
<td>C26</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
<td>Metasediments</td>
</tr>
<tr>
<td>C35</td>
<td>Shoalhaven</td>
<td>Ballalaba</td>
<td>Granite</td>
</tr>
<tr>
<td>C41</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
<td>Granite</td>
</tr>
<tr>
<td>H33</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
<td>Granite</td>
</tr>
<tr>
<td>OZ53</td>
<td>Lake George</td>
<td>~</td>
<td>Granite</td>
</tr>
<tr>
<td>OZ01</td>
<td>Lake George</td>
<td>~</td>
<td>Granite</td>
</tr>
<tr>
<td>SH3</td>
<td>Shoalhaven</td>
<td>Ballalaba</td>
<td>Metasediments</td>
</tr>
<tr>
<td>VM6</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
<td>Metasediments</td>
</tr>
<tr>
<td>Z03</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
<td>Granite</td>
</tr>
<tr>
<td>Z04</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
<td>Granite</td>
</tr>
<tr>
<td>Z05</td>
<td>Shoalhaven</td>
<td>Lower Shoalhaven</td>
<td>Granite</td>
</tr>
<tr>
<td>Z09</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
<td>Granite</td>
</tr>
<tr>
<td>Z11</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
<td>Granite</td>
</tr>
<tr>
<td>Z12</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
<td>Granite</td>
</tr>
<tr>
<td>Z15</td>
<td>Murrumbidgee</td>
<td>Queanbeyan</td>
<td>Metasediments</td>
</tr>
<tr>
<td>Z16</td>
<td>Murrumbidgee</td>
<td>Queanbeyan</td>
<td>Metasediments</td>
</tr>
<tr>
<td>Z18</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
<td>Granite</td>
</tr>
<tr>
<td>Z20</td>
<td>Shoalhaven</td>
<td>Ballalaba</td>
<td>Granite</td>
</tr>
<tr>
<td>Z21</td>
<td>Murrumbidgee</td>
<td>Molonglo</td>
<td>Metasediments</td>
</tr>
<tr>
<td>Z23</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
<td>Granite</td>
</tr>
<tr>
<td>Z27</td>
<td>Tuross</td>
<td>~</td>
<td>Granite</td>
</tr>
<tr>
<td>Z28</td>
<td>Tuross</td>
<td>~</td>
<td>Granite</td>
</tr>
<tr>
<td>Z33</td>
<td>Shoalhaven</td>
<td>Upper Shoalhaven</td>
<td>Metasediments</td>
</tr>
<tr>
<td>Z35</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
<td>Metasediments</td>
</tr>
<tr>
<td>Z36</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
<td>Metasediments</td>
</tr>
<tr>
<td>Z37</td>
<td>Shoalhaven</td>
<td>Jerrabattgulla</td>
<td>Granite</td>
</tr>
<tr>
<td>Z44</td>
<td>Murrumbidgee</td>
<td>Numeralla</td>
<td>Granite</td>
</tr>
<tr>
<td>Z45</td>
<td>Tuross</td>
<td>~</td>
<td>Granite</td>
</tr>
</tbody>
</table>
PHYLOGEOGRAPHY OF TWO AUSTRALIAN SPECIES OF FUNNEL WEB SPIDER (ARANEAE: MYGALOMORPHAE: HEXATHELIDAE) IN TALLAGANDA STATE FOREST, NEW SOUTH WALES

Amber S. Beavis and David M. Rowell

Abstract—Decomposing logs are habitat for invertebrate species occupying a range of ecological niches. A collaborative research project is examining patterns of genetic endemism among saproxylic (dependent on decaying wood) invertebrates across the Tallaganda region of New South Wales, Australia. An earlier study of an unnamed species of 'giant' Collembolon revealed strong population structuring suggesting that the saproxylic habitat has been a major factor influencing the evolution and speciation of some invertebrates. Two species of funnel web spiders, one in each of the genera Hadronyche and Atrax, are found throughout Tallaganda. Although ecologically similar, the Hadronyche species is log-dwelling whereas the Atrax species is ground burrowing. The niche partitioning of these species allows the separation of species and habitat as contributing factors to the patterns of local endemism found in this system. Hence, the addition of funnel web spiders to this study of log-dwelling invertebrates is central to determining whether the saproxylic habitat induces consistent and predictable outcomes in the population structuring of diverse taxa. The resolution of this issue will permit the establishment of informed conservation priorities for invertebrates in Tallaganda State Forest. The phylogeography of the Hadronyche and Atrax species was investigated using mitochondrial sequence data. We found high levels of sequence divergence (average = 0.088) in Atrax accompanied by spatial structuring of haplotypes, whilst Hadronyche displayed low sequence divergence (0.014) and an absence of any spatial structuring of haplotypes. These findings indicate differential responses of the two species to Quaternary (<0.4Mybp) glacial-interglacial cycling, namely, that Atrax persisted in gully refuges during glacial periods whilst Hadronyche became locally extinct during glacial periods and recolonised the region from outside Tallaganda during the most recent inter-glacial. The differential persistence of Atrax and Hadronyche will require the implementation of management interventions appropriate to each species.

INTRODUCTION

Decomposing logs on the forest floor, which constitute a part of the saproxylic environment, provide habitat for a wide array of invertebrate taxa. The saproxylic component of biodiversity, though little-studied, is diverse, containing representatives of all major insect orders (Grove and Stork 1999, Key 1993), including a disproportionately high number of threatened invertebrate species (Jonsson and Kruys 2001). In addition, many saproxylic invertebrates appear to be poor dispersers (Hammond 1984) and are ecologically specialized, occupying specific and narrow niches (Yee and others 2001). This is of conservation concern given that the saproxylic habitat is also susceptible to the impacts of current forest practices such as clearfelling, regeneration burning and intensive harvesting of coarse woody debris (Grove 2002). Consequently there is a clear need to establish conservation priorities and to implement management strategies for the saproxylic fauna. It is apt, then, that the same suite of characteristics which make saproxylic invertebrates vulnerable also cause their own population histories to be closely linked with that of their environment, thus providing a system well suited to phylogeographic analysis and subsequent establishment of informed conservation strategies.

Phylogeography is the study of the biogeographical and evolutionary processes which have determined the distributions of extant genealogical lineages (Avise 1998, 2000; Bossart and Prowell 1998; Garrick and others 2005; Moritz and others 2001). As a discipline, phylogeography has the unique power to investigate more recent evolutionary events occurring around and below the species level (Avise 1998). It has been argued that this focus upon relatively recent history has the effect that invertebrate taxa may be particularly informative study species; Moritz and others (2001) proposed that taxa that experience their environment at a fine spatial scale display the effects of long-acting environmental changes, such as glacial inter-glacial cycling, to a greater degree than taxa that experience their environment on a broader scale. For example, during the Quaternary, vertebrate taxa may have been driven to local extinction by climatic oscillations, and have subsequently recolonised from separate populations (Moritz and others 2001). In contrast, invertebrate taxa are often characterized by a restricted geographic range and low vagility. As such it is likely that, when exposed to climatic oscillations, many species of invertebrate survived in smaller, local, refugia, thus retaining the spatial genetic structuring which forms the basis of phylogeographic studies (Keyghobadi and others 1999, Moritz and others 2001). We propose that the restricted dispersal and habitat-specificity characteristic of many log-dwelling invertebrates provides a model system for inferring landscape history.

The Tallaganda Model

The present phylogeographic study of two unnamed species of funnel web spider is one component of a broader study encompassing a suite of saproxylic invertebrates (described in Garrick and others 2004). Focusing on the Tallaganda region (Tallaganda State Forest, Gourock National Park and

1 Amber S. Beavis, PhD candidate, Division of Botany & Zoology, Australian National University, ACT, 0200, and David M. Rowell, Australia; Reader in Evolutionary Genetics.

Badja State Forest; collectively referred to as 'Tallaganda'), in south-eastern New South Wales, Australia, this study aims to investigate whether the saproxylic habitat has induced consistent and predictable outcomes in the population structuring of diverse taxa. To date, the spatial structuring of a saproxylic 'giant' springtail at Tallaganda has revealed congruence with five *a priori* microbiogeographic regions previously defined with reference to the topography and hydrology of Tallaganda in addition to an assessment of the probable palaeoclimatological history of the region (Garrick and others 2004).

**Tallaganda**

The Tallaganda region (35°35'S-35°44'S and 149°28'E-149°30'E) is a 100km long (north/south) by 3-20km wide (east/west) section of continuous, temperate, forest situated on the spine of the Gourock Range. The forest connects to the Great Dividing Range via Deua National Park in the south, but is otherwise ecologically and topographically isolated, being surrounded by the low-lying Southern Tablelands. The following information is taken from: CSIRO 1989, Barclay and others 1987, Heatwole 1987, Hope 1994, Singh 1982, State Forests of NSW 1995, White 1990, White 1994. The region is categorized into a three-tier landscape: the low-lying and geologically recent coastal plain (altitude 600m), an escarpment which has been ecologically stable over the last 20 my (altitude 1000-1500m), and the ancient (<50-70 myo) Great Dividing Range. The forest is primarily sclerophyll forest, ranging from wet to dry types dependent upon altitude, aspect and local climate. Tallaganda is strongly affected by the orographic influence of the Great Dividing Range. The eastern, coastal, slopes are subject to high orographic rainfalls and, consequently, are typified by wet sclerophyll forest, while the western slopes are subject to a rain shadow effect and are typified by dry sclerophyll forest. The region has been subject to logging on a regular basis since the 1890's. Prior to 1949 Tallaganda State Forest was harvested using selective logging techniques, where only trees suitable for commercial use are taken. After 1949 clear-felling techniques were implemented at Tallaganda. Clearfelling is "the localized removal of most or all trees followed by burning of debris" (State of the Environment Advisory Council 1996). At Tallaganda, however, debris is left on the forest floor rather than burnt.

The Tallaganda region has been subject to a series of climate change events: the Oligocene Refrigeration (25-36 mybp), the Mid-Miocene and Terminal Miocene cooling events (7-15 mybp), a period of extended cooling and drying in the Pliocene (3-5 mybp) and, of particular relevance here, glacial/interglacial cycling during the Pleistocene (over the last 2.5 my). During the Pleistocene, glacial/inter-glacial cycling had a distinct impact on Australian flora and fauna. At glacial maxima temperatures were 9°C cooler than modern temperatures. The cooler climate resulted in altitudinal shifts in vegetation, an occurrence documented in fossil pollen data taken from Lake George (NSW) which is adjacent to Tallaganda. Forests contracted, with the upper tree line decreasing by 1200-1500m, with the result that low-lying areas such as gullies became the location of forest fragments in a matrix of treeless steppe. It is hypothesized that these isolating events affected saproxylic invertebrates, impacting on historical gene flow to the extent that the effects may be observed in the spatial structuring of modern genealogical lineages (Bowler 1982; CSIRO 1969; Frakes and others 1987; Heatwole 1987; Hope 1994; Singh 1982; State Forests of NSW 1995; White 1990, 1994).

**Funnel Web Spider Species: *Hadronyche* and *Atrax***

Two undescribed species of funnel web spider (*Mygalomorphae: Hexathelidae*) are found in very high densities at Tallaganda: *Hadronyche* sp. 1 and *Atrax* sp. 1 (hereafter referred to as *Hadronyche* and *Atrax*). These two species are ecologically similar: both are top-level predators, feeding opportunistically on invertebrates and small vertebrates; they are of a similar size (adult females of both species having carapace widths ranging between 7-11 mm); they are long-lived, females having a maximum lifespan of approximately 20 years (Levitt 1961); both are sessile following juvenile dispersal, although males wander for a short period following maturity (Wishart 1993); they construct burrows lined with silk and with trip lines extending from their entrance (Hickman 1964, Levitt 1961). The two species exist sympatrically, however, they exhibit niche partitioning in their choice of habitat. *Hadronyche* is exclusively saproxylic, occupying burrows within decomposing logs. In contrast, *Atrax* builds ground burrows directly into the soil, the entrances to which may be found beneath logs, rocks or in open ground. Thus, *Hadronyche* appears to specialize in the saproxylic habitat, with the result that individuals are restricted to dispersing between logs, presumably resulting in a patchy distribution. In contrast, *Atrax* appears to specialize in the forest-floor habitat with the result that individuals may disperse along a gradient resulting in a continuous distribution across the forest floor. Given the ecological differences between the two funnel web spider species under study, we predicted that the population genetic structure of *Hadronyche* would reflect the species' dependence upon the log habitat and produce a phylogeny that was highly structured according to spatial parameters. In contrast, we predicted that *Atrax*, being less restricted by habitat availability, would show high gene flow and be more genetically homogeneous across its range.

The addition of funnel web spiders to this comparative phylogeographic study of saproxylic invertebrates is key to distinguishing what factors have been responsible for the patterns of genetic structuring observed in saproxylic invertebrates to date (Garrick and others 2004). *Atrax* and *Hadronyche* species show the greatest disparity of habitat type within a pair of related taxa compared to the other organisms under study (Garrick and others 2004). The inclusion of a saproxylic and a ground-dwelling mygalomorph species in this study allows us to address the question of whether the patterns of spatial structuring found in saproxylic invertebrates to date are due to the saproxylic habitat or are a factor of being a forest-floor dwelling invertebrate of low vagility.

We investigated and compared the patterns of spatial structuring for *Hadronyche* and *Atrax* using mitochondrial DNA (mtDNA) sequence from Cytochrome Oxidase subunit I (COI). MtDNA is particularly suited to intraspecific phylogeographic studies as it has a relatively high mutation rate and does not recombine (reviewed in Avise 1998). We aimed to test the prediction that the spatial distribution of genetic diversity in the log-dependent species would display distinct structure reflecting the non-continuous nature of the log habitat, while the forest-floor dwelling species would be more homogeneous across the forest, as potential burrowing sites are essentially continuous.
MATERIALS AND METHODS

Taxon Sampling
Between 2002-2004 samples were collected from throughout the Tallaganda region. Hadronyche individuals were extracted from their burrows in logs, and Atrax were collected opportunistically from around log sites. Tissue samples were stored at -20°C.

Amplification and Sequencing of Mitochondrial DNA
Sequence was collected from 51 Hadronyche individuals and 18 Atrax individuals. Genomic DNA was isolated from 1mm² muscle tissue using a QIAGEN DNeasy Tissue Kit. A 700 base pair (bp) region of the mitochondrial cytochrome oxidase subunit I (COI) gene was amplified using the universal primer pair Lco1490: 5’—GGTCAAGAATCATAAGATATTGG and Hco2198: 5’—TAAACTCAGGTTGACCAAATCA (Folmer and others 1994). Using a thermal cycler, amplifications were as follows: 1 cycle of 94°C for 3 min, 65°C for 30s, 72°C for 45s; 2 cycles of 94°C for 30s, 60°C for 20s, 72°C for 45s; 2 cycles of 94°C for 45s, 55°C for 20s, 72°C for 45s; 3 cycles of 94°C for 45s, 50°C for 20s, 72°C for 45s; 45°C for 45s, 45°C for 20s, 72°C for 45s; 40 cycles of 94°C for 45s, 45°C for 20s, 72°C for 45s; 72°C for 3min. Amplification reactions consisted of 2μL of template DNA; 2U Platinum TaqPCRx DNA Polymerase (Invitrogen); 4μL 10X PGR Buffer, Minus Mg; 2.4μL 50mM MgCl₂; 1.6μL 5mM dNTPs; 1μL each 10μM primer; in 40μL total volume. Polymerase chain reaction (PCR) products were purified using 3M NaAc with an ethanol precipitation. PCR products were sequenced in both directions using Big Dye Terminator (Applied Biosystems) and run on an ABI3100 Genetic Analyzer. Sequences were aligned in SEQUENCER (version 3.0, Gene Codes Corporation, Mi). Ambiguous end regions were removed so that all individuals within each genus were analysed over the same sequence length. No stop codons were found within the reading frame. After alignment and cropping, a 500bp segment was analysed for Hadronyche and a 600bp segment was analysed for Atrax. Unique haplotypes were determined using a pairwise distance matrix.

Analysis
Sequence variation was calculated using the general time-reversible (GTR) model (Tavaré 1986) with corrected p values (using PAUP* V.4.0). The GTR model assumes that the rate of change from base a to b is the same as from base b to a. Phylogenetic relationships amongst mtDNA COI sequences were estimated for both species via a haplotype network based on statistical parsimony (Templeton and others 1992) using the program TCS V.1.13 (Clement and others 2000). Genealogical relationships were estimated for Atrax and Hadronyche separately from one another via a statistical parsimony haplotype network. This technique uses a parsimony-based algorithm to estimate the minimum number of base substitutions between haplotypes. TCS fails to connect haplotypes which differ by more than 10 substitutions. According to coalescence theory, the most common and widespread haplotype is most likely to be the ancestral haplotype (Posada and Crandall 2001). An important feature of this technique is its adherence to a 95 percent confidence limit for the acceptance of hypothetical networks.

RESULTS
Both species were abundant across Tallaganda. Comprehensive sampling was conducted for Hadronyche, however, Atrax sampling was more patchy. Fifteen haplotypes were detected for both Hadronyche (n = 51) and Atrax (n = 18). Mean corrected sequence divergence between pairs of sequences (GTR model) was 0.014 for Hadronyche and 0.088 for Atrax.

Haplotypes for Atrax (see fig. 1) and Hadronyche (see fig. 2) were constructed using a parsimony algorithm. The haplotype 'network' showing the relationships between Atrax individuals was disjointed, connecting very few of the haplotypes (one group of four haplotypes; three groups of two haplotypes; five unconnected haplotypes). All connected haplotypes were found to be located at adjacent sites. The disjointed nature of the Atrax network may be due to two factors. First, it may be indicative of deep structure, extant haplotypes being relics from ancient population contractions or extinctions. Second, it may be due to the continued presence of extant but unsampled haplotypes.

In contrast, a haplotype network for Hadronyche indicated that the majority of samples represented a single haplotype. The most common haplotype formed the central hub of the network with 12 of 15 haplotypes being connected to it by a maximum of seven steps. The most common haplotype was also the most geographically widespread, occurring across the full range of the forest.

DISCUSSION
The primary aim of this preliminary study was to compare the patterns of genetic diversity over a similar spatial scale for two species of funnel web spider: Atrax (forest-floor dwelling) and Hadronyche (exclusively log-dwelling). The secondary

![Figure 1—Haplotype network for Atrax. Networks were created in the program TCS version 1.13 using a maximum parsimony algorithm. Sampled haplotypes are numbered as an identifier and missing haplotypes are represented as black dots. Rectangles represent the "ancestral hub" of the network according to coalescence theory (Posada and Crandall 2001). Larger rectangles and circles represent more common haplotypes. The network for Atrax is disjointed with very few haplotypes being connected. Connected haplotypes were found to be located at adjacent sites.](image-url)
aim of this study was to use this set of preliminary results to look for congruence between the extent of phylogeographic structuring displayed by these two species and other taxa of saproxylic organisms examined in the same model system at Tallaganda. The addition of two funnel web spider genera with different levels of log-dependence (log-dwelling versus forest-floor-dwelling) allowed us to ask whether the patterns found amongst log-dwelling invertebrates are due to taxonomic factors. The data set discussed here is preliminary and, as such, is focused upon determining the parameters for the ongoing study.

All analyses indicated that we should unequivocally reject our initial hypothesis that the spatial distribution of genetic diversity in Hadronyche would be highly structured, while Atrax would be more homogeneous. Rather, the opposite was found: Hadronyche is surprisingly uniform across the forest whilst Atrax displays phylogeographic genetic structure. A key finding of this study was the order of magnitude difference between the mean sequence divergence of the two species. Typically, low sequence divergence is indicative of recent population expansion whilst high sequence divergence is congruent with an ancient origin (Hewitt 2000, Riddle 1996, Steiner and Catzeflis 2004, Zink and others 2000). Given the habitat preferences observed, this finding was unexpected and invites further investigation into the historical demographic processes responsible for the phylogeographic patterns displayed by the taxa. The patterns displayed by Hadronyche are characteristic of Type IV trees recognized by Avise and others (1987), namely, a shallow, unstructured, tree which is typical of a recent range expansion. The low sequence divergence (mean one percent GTR), homogeneous geographic structuring of haplotypes and the connection of 12 (of a possible 15) haplotypes to a central network hub, are consistent with recent population expansion and a lack of isolating barriers (Avise and others 1987, Zink 2002). In contrast, the deep sequence divergence (mean nine percent GTR) and the disjunct haplotype network, displayed by Atrax (Type I: deep haplotype trees showing geographic structuring) is congruent with a long history in the region and the presence of isolating barriers (Avise and others 1987, Zink 2002).

The repercussions of these finding within the context of conservation are twofold. First, the high sequence divergence (8.8 percent) within Atrax, combined with the disjointed nature of the haplotype network for this taxon, suggests that this species may be highly genetically structured over the Tallaganda region. If so, identification of evolutionarily significant units (ESUs) and management units (MUs) within Atrax will be necessary to ensure the conservation of genetic diversity within this species. In addition, the 8.8 percent sequence divergence within Atrax raises the question of whether unidentified species within this genus are present in Tallaganda. The genetic species concept has been applied to taxa displaying four percent sequence divergence (Holder and others 1999) and genetic distances of between two percent and 11 percent have been recognized as carrying a high probability of being indicative of species status (Ballard and others 2002, Bradley and Baker 2001). Given the presence of spatial structuring of Atrax haplotypes—which is congruent with the species being a poor disperser—and the high level of genetic variation occurring within the species, there is a clear need for an extensive study into whether current logging practices in Tallaganda have the potential to adversely impact on ground dwelling funnel web spiders both directly and via habitat fragmentation. Clearfelling, for example, where all trees in a localized area are felled in a single operation, results in extensive disturbance of soil by forestry machinery. Recorded impacts of forestry machinery include soil displacement, soil compression and a decrease in soil permeability (Horn and others 2004). Soil displacement caused by the wheel tracks of machinery may occur to depths of 56 centimetres (Horn and others 2004), which encompasses the habitat of Atrax. As such, the damage caused by logging practices to Atrax habitat may have impacts at the level of the individual and the population; however, there are currently no empirical data in this area.

Figure 2—Haplotype network for Hadronyche. Hadronyche shows connectivity amongst haplotypes which radiate from a single common haplotype (haplotype 1). The most common haplotype was found to occur across the full range of Tallaganda.
Second, the unexpectedly low sequence divergence and high connectivity of the haplotype network found in *Hadronyche* raises questions about the history of this species in Tallaganda. The phylogeographic patterns are consistent with recent population expansion and a lack of isolating barriers to dispersal. Widespread dispersal can be a characteristic of organisms with highly specialized and localized habitat requirements, as individuals must disperse further to find an appropriate habitat (Jung and Croft 2001). Given that *Hadronyche* has extremely specific habitat requirements, the homogeneous spatial structuring of the species over the area of Tallaganda suggests that the species has specialized habitat requirements to the extent that widespread dispersal is required. There is observational evidence that large *Hadronyche* juveniles and adults are unable to reestablish a burrow once disturbed. A one-off dispersal event, which potentially occurs in this system, would result in the confinement of an individual to its first choice of log habitat with the result that the fate of *Hadronyche* individuals would be intrinsically linked to the fate of the log they inhabit, that is, to their local environment. Consequently, *Hadronyche* individuals may be highly sensitive to any environmental change or disturbance as is typical of habitat specialists (Bolger and others 2000, Gascon and others 1999, Lynam 1997, Vandergast and others 2004). The shallow structure of the haplotype network for *Hadronyche* supports the species’ probable life history strategy, being indicative of a recent range expansion followed by a rapid radiation.

We hypothesise that the glacial—interglacial cycling affected *Hadronyche* and *Atrax* differentially according to their habitat (Hewitt 1996, Hugall and others 2002, Schoswetter and others 2002, Tribsch and others 2002). *Atrax* individuals are not dependent upon logs, however, all mygalomorph species are susceptible to desiccation due to their two pairs of book lungs which present a large surface area for water-loss (Foelix 1982, Levi 1967, Schmitz and Perry 2000) Consequently, the geographic range of *Atrax* is intrinsically linked to climate and is therefore associated with overall patterns of forestation. When, during glacial periods, conditions became drier and forests contracted to gully refuges or were locally extinguished, *Atrax* individuals would persist only in gully refuges. In contrast, because *Hadronyche* is exclusively dependent on the log habitat and individuals are confined to the one log within their lifetime, glacial events have the potential to drive *Hadronyche* to negligible population densities or to local extinction. The shallow structure of the *Hadronyche* phylogeny suggests that the taxon has re-established Tallaganda—potentially from the adjacent areas of the Queanbeyan-Shoalhaven area, ACT and NSW. Land Research Series. Number 24. CSIRO, Melbourne.

The results of this study have two implications. First, it appears that there is some congruence between the type of phylogeographic structuring of *Atrax* and that of Collembola (Garrick and others 2004). Second, a particularly striking finding of this study is that *Hadronyche*, an exclusively log-dwelling species, exhibits different patterns of phylogeographic structuring to other saproxylic taxa, namely Collembola (Garrick and others 2004). These preliminary findings suggest that the patterns exhibited by log-dwelling taxa are not unique to the log habitat, but rather are more likely to be associated with being forest-floor invertebrates of low vagility. Whilst Collembola species and other log-dwelling taxa may be log-associated, *Hadronyche* may be the only exclusively log-dwelling species which is also unable to move between logs under unfavourable environmental conditions. Thus, it seems that being a log-associated species of low vagility results in local endemism, so the identification of appropriate conservation units should be a priority. For exclusively saproxylic species, such as *Hadronyche*, the implications of being exclusively saproxylic may be extreme and carry a very real risk for local extinction if their habitat is not adequately catered for through appropriate management.

**ACKNOWLEDGMENTS**

We thank Simon Grove and Jim Hanula for organising the Insect Biodiversity and Dead Wood symposium at the XXII International Congress of Entomology, Brisbane, 2004. The input provided by Paul Sunnucks is also integral to the Tallaganda project. Chester Sands and Ryan Garrick provided guidance in the phylogenetic analysis and interpretation of this data. This manuscript was improved by comments from Paul Sunnucks, Simon Grove and Jim Hanula. The Tallaganda comparative phylogeography project is supported by the Australian Research Council (grant DP-0211156 to Paul Sunnucks and Dave Rowell).

**LITERATURE CITED**


Map 1.2 Tallaganda region hydrological catchments and underlying geology
Map 2.1 Hadronyche Sp. 1 sample distribution map

(see Appendix 1.1 for sample details)
Figure 2.1 *Hadronyche* Sp. 1 statistical parsimony network

Statistical parsimony network for *Hadronyche* Sp. 1 (Blue = Tallaganda samples; Green = external to Tallaganda)
Figure 2.2 Hadronyche Sp. 1 Bayesian phylogeny

Bayesian MCMCMC phylogeny for Hadronyche Sp. 1, using a GTR + γ + λ model of nucleotide evolution (ln L = -3516.33). Clade credibility values are shown for major nodes. All taxa occur within Tallaganda, except where indicated by the key.
Map 3.1 Atrax Sp. 1 sample distribution map

(see Appendix 1.2 for sample details)
Figure 3.1.a Atrax Sp. 1 statistical parsimony network

Statistical parsimony network for Atrax Sp. 1 with reference to phylogeographic region of origin.
Figure 3.1.b Atrax Sp. 1 statistical parsimony network

Statistical parsimony network for *Atrax* Sp. 1 with reference to underlying geology (Granite = Orange; Metasediments = Pink).
Bayesian MCMC phylogeny for *Atrax* Sp. 1, using a GTR + γ + λ model of nucleotide evolution (ln L = -4736.91). Clade credibility values are shown for major nodes.
Figure 3.3 *Atrax* Sp. 1 ancestral state reconstruction

Ancestral state reconstruction for underlying geology preferences in *Atrax* Sp. 1.
Figure 4.1 Laelapid mite Bayesian phylogeny

Unrooted Bayesian MCMC phylogeny for mites collected from *Atrax* Sp. 1 (red), and *Hadronyche* Sp. 1 (blue), using a GTR + $+$ model of nucleotide evolution ($\ln L = -1576.767$). Internal nodes are indicated in black.
Figure 5.1 *Atrax* Sp. 1 and *Hadronyche* Sp. 1 Bayesian phylogeny

Bayesian MCMC phylogeny for *Atrax* Sp. 1, and *Hadronyche* Sp. 1 with an Araneomorphae outgroup, using a GTR + $\gamma + \lambda$ model of nucleotide evolution ($\ln L = -4736.91$)