PHYLOGEOGRAPHY OF TWO AUSTRALIAN SPECIES OF FUNNEL WEB SPIDER (ARANEAE: MYGALOMORPHAE: HEXATHELIDAE) IN TALLAGANDA STATE FOREST, NEW SOUTH WALES

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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’ Collembolus 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 most 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
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 1969, Barclay and others 2000, Bowler 1982, Frakes 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 Miocene and Terminal Miocene cooling events (7-15 mybp), a period of extended cooling and drying in the Pliocene the Mid-Miocene and Terminal Miocene cooling events (7-15 mybp) and, of particular relevance here, glacial/inter-glacial cycling during the Pleistocene (over the last 2.5 my). During the Pleistocene, glacial/inter-glacial cycling had a significant 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 1mm³ 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’—GGTCAACAAATCATAAGATTTGG and Hco2198: 5’—TAAACTTCAAGGGTGACCAAAAAATCA (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; 94°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 PCR 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.

Haplotype networks 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)
The 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 recolonised Tallaganda—potentially from the adjacent areas of the Great Dividing Range—since the end of the previous glacial period.

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.

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LITERATURE CITED


