Ecological differentiation and the evolution and maintenance of fynbos diversity

Jasper Alexander Slingsby

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Supervisors:
G. A. Verboom, M. D. Cramer and J. J. Midgley

This thesis was typeset using KOMA-Script and LaTeX 2_ε.

To my family and friends, without whom this would never have been possible.

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Jasper Slingsby, March 2011.

Acknowledgements

This may take a while so bear with me. If you are primarily interested in the science, read no further and skip to the next section. Better yet, wait for the papers and spare yourself some of the more boring bits.

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A high altitude meadow dominated by *Tetraria* species, Crystal Pools, Cederberg.

Abstract

Preserving the structure and functioning of ecosystems and the benefits they provide to society depends on our understanding of how biodiversity influences ecosystem function and which processes determine the composition and diversity of biological assemblages. Ecological, evolutionary and biogeographical processes interact to determine the genesis, maintenance and spatio-temporal arrangement of biodiversity. Here I investigate these processes in the Cape fynbos of South Africa by examining the diversification, biogeography and community assembly of a clade of reticulate-sheathed *Tetraria* species (Cyperaceae: Schoeneae). My thesis is that ecological specialization and niche differentiation play a critical role in determining the manner in which species coexist, with implications for the spatial arrangement of phylogenetic diversity and species' functional attributes at a range of scales, and for rates of lineage diversification. Based on a new dated molecular phylogeny, diversification statistics indicate that diversification in *Tetraria* has declined as a function of increasing species number, consistent with the hypothesis that niche space is finite and limits diversification as it becomes increasingly saturated. Exploration of species' traits and microhabitat preferences across a set of plots on one mountainside provides further support for this hypothesis, revealing that local coexistence of *Tetraria* species is maintained by classical niche partitioning along soil moisture and nutrient gradients. I proceed to demonstrate that closely-related species tend either to have allopatric distributions or are differentiated in morphology and/or environmental preference, and that the decline in diversification rates is less severe when species ranges are narrower and more strongly allopatric. Taken together, my results suggest that the high functional and phylogenetic diversity observed in regional *Tetraria* assemblages is the result of allopatric speciation combined with convergence in form among distant relatives between regions and character displacement between close relatives within regions. Local assemblages display similar functional and phylogenetic structure because close relatives have contrasting microhabitat preferences but are generally conserved in form. Biogeographic and ecological processes may interact to affect lineage diversification rates and the spatial arrangement of diversity across the CFR by constraining the propensity for allopatric speciation between geographically isolated regions which vary

in their levels of ecological saturation and connectivity to other regions. Understanding of the role of niche differentiation, ecological saturation and biogeographical processes in structuring the diversity of the Cape flora is essential for the development of effective conservation and management strategies.

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1 General Introduction

Understanding the factors that determine the composition, structure and function of assemblages of organisms at various spatial and temporal scales has been a long standing goal of the biological sciences. The dual realisation that our survival depends on these assemblages and that we are impacting on them in negative ways at grand scales increases the urgency with which we need to improve this understanding (Doak & Mills 1994, Rozdilsky *et al.* 2001, Mooney 2010, May 2010). There is a large body of evidence that biodiversity strongly influences the structure and functioning of ecosystems, through its impact on soil properties, biomass production, transfer of nutrients between trophic levels, biogeochemical cycling, water quality and availability, disturbance regime and climate to name but a few (Chapin *et al.* 1997, Loreau & Hector 2001, Hooper *et al.* 2005, Balvanera *et al.* 2006, Cardinale *et al.* 2006). There is also much evidence that this influence is driven largely by the functional characteristics of organisms rather than by the sheer numbers of species alone (Tilman *et al.* 1997, Hooper & Vitousek 1997, Diaz & Cabido 2001, Naeem & Wright 2003, Eviner & Chapin 2003, Hooper *et al.* 2005, Hillebrand & Matthiessen 2009). The goal of developing a better understanding of the effect of biodiversity on ecosystem function and the consequences of environmental change thus depends on our understanding of the processes responsible for the generation and maintenance of species and associated functional variation (Hooper *et al.* 2005, Cavender-Bares *et al.* 2009, Loreau 2010).

The number of species that occur within an assemblage and the functional and phylogenetic relationships between them are the result of complex interactions between ecological, evolutionary and stochastic processes that operate at a range of spatial and temporal scales (Hubbell 2001, Ricklefs 2004, Cavender-Bares *et al.* 2009). These processes can be considered in terms of three interrelated themes that fall within various fields of study; the genesis of diversity [speciation and trait evolution (Foote 1997, Schluter 2001, Coyne & Orr 2004, Losos & Mahler 2010)], the maintenance of diversity [coexistence theory (Hutchinson 1957, MacArthur & Levins 1967, Chesson 2000, Leibold & McPeek 2006)], and the spatial and temporal arrangement of this diversity [the geography of diversity (Whittaker 1972), macroecology (Brown & Maurer 1989), biogeography (MacArthur &

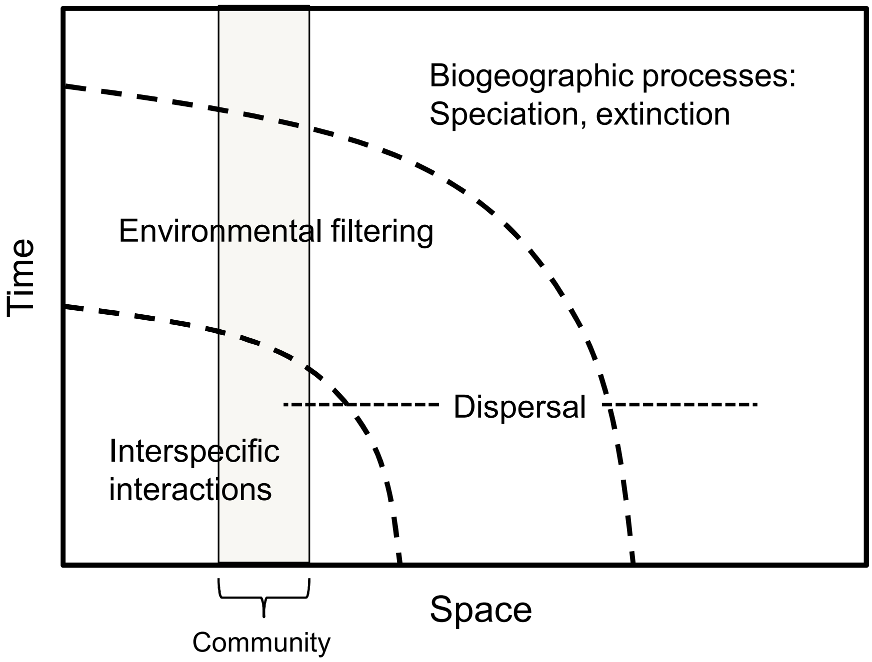


Figure 1.1: Processes that affect the assembly of communities at different spatial and temporal scales. Finer-scale processes are nested within broader scale processes. Adapted from Weiher & Keddy (1999), Cavender-Bares *et al.* (2009).

Wilson 1967, Hubbell 2001)]. It is the interaction between these themes that together determines the assembly and functional and phylogenetic composition of communities at various spatial and temporal scales (Weiher & Keddy 1999, Webb *et al.* 2002, Ricklefs 2004, Emerson & Gillespie 2008, Cavender-Bares *et al.* 2009).

The kinds of processes that affect species number and the functional and phylogenetic relationships within species assemblages at different spatial and temporal scales are illustrated in Figure 1.1 (Weiher & Keddy 1999, Cavender-Bares *et al.* 2009). The processes that affect a community depend on the scale at which it is sampled. Interspecific interactions including competition, predation, commensalism, mutualism and parasitism are potentially important at fine scales, but rapidly decrease in importance with increasing scale. Dispersal dynamics and environmental filtering, the process by which species are denied membership of an assemblage because they are not suitably adapted to the environmental conditions, are important from fine to intermediate scales. Biogeographic processes, speciation and extinction typically operate at broader levels and affect the composition of assemblages at all scales. While making a distinction between the species pools at different scales is somewhat arbitrary (Weiher & Keddy 1999, Hubbell 2001, Ricklefs 2004, 2008) it facilitates conceptual thinking and discussion of community assembly theory and allows one to consider independently the processes that operate at each scale. A simplistic representation of our current understanding of how communities are assembled is illustrated in Figure 1.2, providing theoretical distinctions between 'global', 'regional' and 'local' species pools. The global species pool is the sum of all components and processes at finer scales and can be subdivided into regional species pools (e.g. biomes, bioregions, mountains, islands) whose membership is constrained by dispersal limitation between pools due to biogeographic barriers, distance, or availability of suitable habitat. The regional pools are in turn divided into 'fundamental' pools of species which have the potential to colonize local communities. The 'fundamental' pool is a theoretical construct and is never observed, consisting of the set of species which can disperse into the local community and find the appropriate abiotic conditions in terms of resource availability and natural disturbance necessary for their survival. The actual 'realised' local communities are then subsets of the 'fundamental' species pools which have the ability to coexist, either stably or unstably. This schematic also illustrates that, as with the hierarchy of processes in Figure 1.1, each pool is a subset of the broader pool in which it is nested. This indicates that the composition of each pool (local or regional) depends on structure in the broader pool, and that the composition of the broader pool ultimately depends on the structure of its component pools.

1.1 Factors which influence the functional and phylogenetic composition of regional pools

The number, identity and spatial arrangement of species in regional pools are governed predominantly by the balance of immigration, speciation and extinction (MacArthur & Wilson 1967, Hubbell 2001, Emerson & Gillespie 2008). The relative importance of each of these processes depends largely on the properties of the region in which the pool resides. Where the region is large or has greater connectivity to other regions, much of the species pool will be comprised of colonists. With increasing isolation a greater proportion of the species pool is likely to have originated via in situ speciation, with the rate of speciation being higher in larger or more heterogeneous regions. Larger regions are also expected to have lower extinction rates because they should be able to sustain larger populations. Together, the expectation is that larger regions with greater connectivity should support larger species pools. A spin-off of greater connectivity, however, is that there will be greater

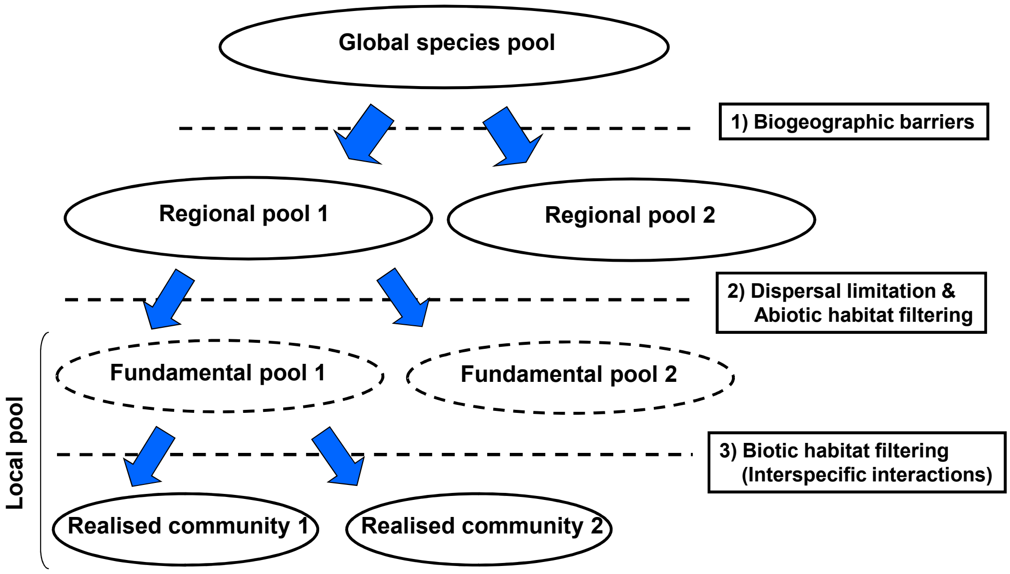


Figure 1.2: Community assembly and the distinction between global, regional and local species pools, indicating the dominant community assembly processes at each scale.

homogeneity among regional species pools, resulting in lower combined diversity in the global pool.

The mode and nature of speciation have an important influence on both the spatial arrangement and the functional similarity of species within and between regional pools (Gittenberger 1991, Schluter 2001, Hardy & Senterre 2007, Emerson & Gillespie 2008, Graham & Fine 2008). Where speciation is predominantly allopatric, with lineages diverging in geographic isolation between regions, close-relatives will be segregated into different regions (Figure 1.3). This results in regional pools being comprised of species that are more distantly-related than would be expected if they were randomly sampled from the global pool (Hardy & Senterre 2007, Emerson & Gillespie 2008, Graham & Fine 2008). By the same token, where speciation is predominantly sympatric, with lineages diverging within the same geographic region, the regional species pools should consist of closely-related species while species in different regional pools should be distantly-related. This signal of phylogenetic relatedness in regional pools will slowly become eroded with time, as the species disperse between regions (Losos & Glor 2003). The signal of functional similarity within and between regions depends on the extent to which speciation events are associated with ecological divergence. Where speciation is non-ecological, i.e. not associated with adaptive divergence among lineages (Gittenberger 1991, Rundell & Price 2009), there may be little functional disparity between close relatives. On the other hand, where speciation is associated with adaptive divergence, as is expected under models of ecological speciation (Schluter 2001) or where species are subject to divergent selective pressures or niche or character displacement post speciation (Brown & Wilson 1956), close relatives are expected to exhibit large functional differences. The interplay between the geographical mode of speciation and the extent to which speciation events are associated with ecological divergence creates a range of predictions for the expected degree of phylogenetic relatedness and functional similarity among species within and between regional species pools (Table 1.1, Figure 1.4). Under a scenario in which there is little or no ecological divergence associated with speciation the functional similarity of species within or between regional species pools should reflect their phylogenetic relatedness (Figure 1.4A). Sympatric speciation will result in regional pools comprised of functionally similar close relatives with species between pools being distantly-related and potentially functionally divergent. Allopatric speciation will result in pools comprised of more dissimilar distant relatives with little difference in relatedness and functional similarity between pools. Where speciation is associated with ecological divergence, however, the predicted pattern of functional diversity depends on whether close relatives are merely different (divergent), with little functional similarity among distant relatives, or whether distant relatives have converged to similar functional forms. If close relatives are ecologically divergent but there is little or no convergence among distant relatives, regional pools will be comprised of functionally dissimilar species with little functional similarity between pools irrespective of the mode of speciation (Figure 1.4B). Convergent evolution can modify the functional relationships among species within and between pools in two ways. Firstly, convergence in form within regions will lower the degree of functional dissimilarity among species within each region (Figure 1.4C). Secondly, convergence in form among species between regions will result in the regional pools showing greater functional similarity (Figure 1.4D). Subsequent dispersal of species into sympatry should not alter these predictions, but with time will eventually erode the signal.

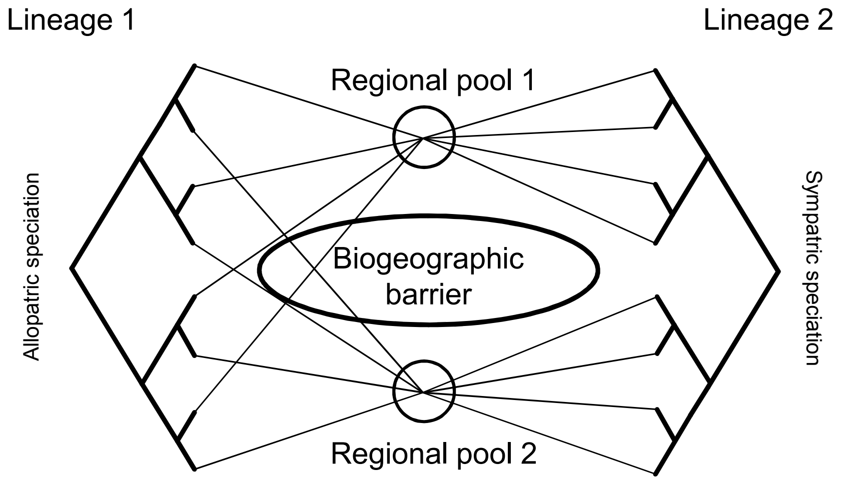
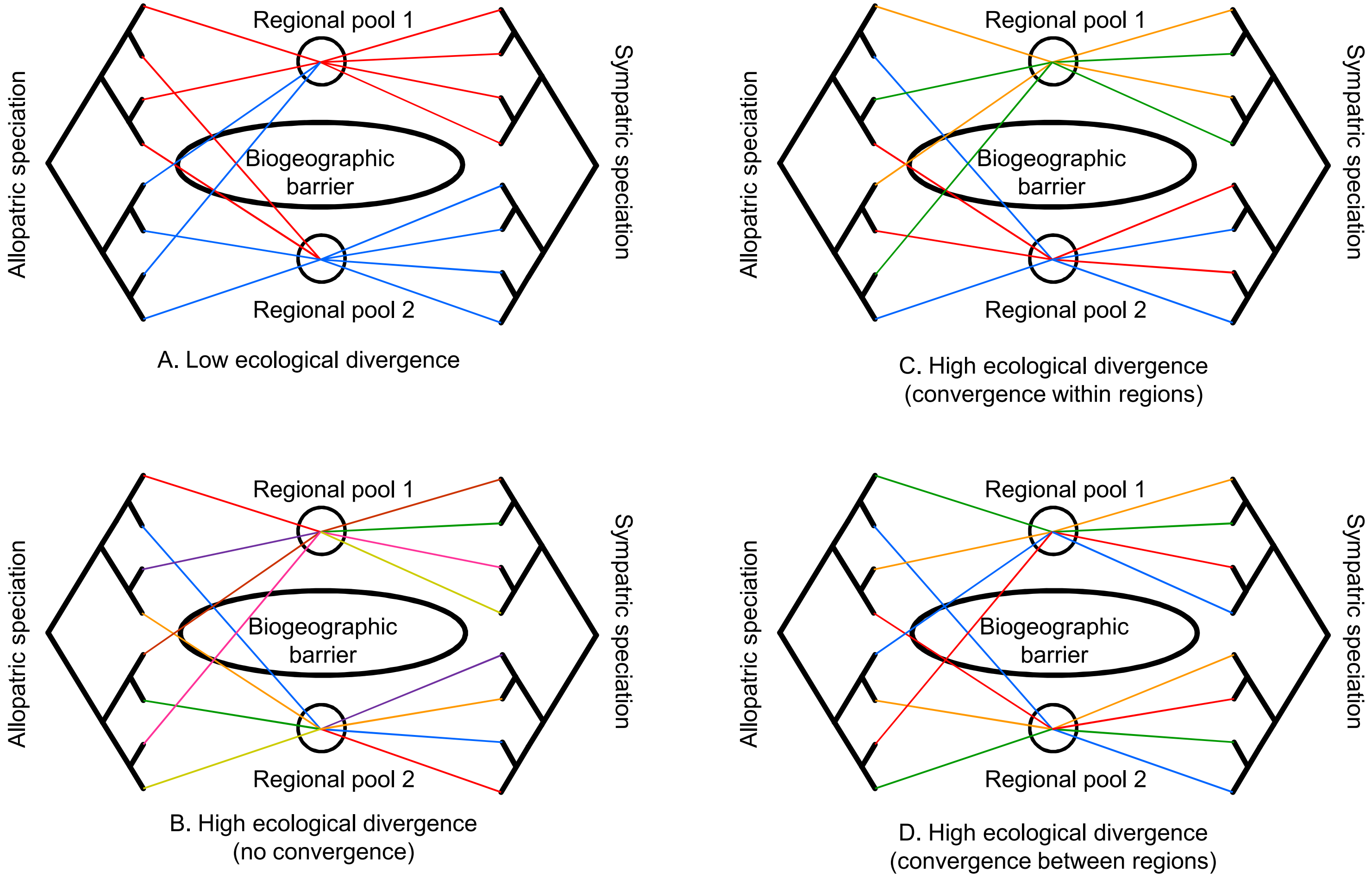


Figure 1.3: The influence of the geography of speciation on phylogenetic relatedness among species within and between regional species pools. Sympatric speciation will result in co-occurring sister species (phylogenetic clustering) while allopatric speciation will result in close relatives being segregated between regions (phylogenetic evenness).

The combination of immigration, speciation and ecological divergence thus provide multiple processes by which similar patterns of functional diversity can be produced. For example, allopatric or sympatric speciation with convergence in functional form between regions and allopatric speciation with low divergence between species all produce a pattern of high functional variation within but low variation between regional pools (Table 1.1). In this case examination of the degree of phylogenetic relatedness among species within pools and the degree to which close relatives are similar in functional form allow identification of the processes which have led to the observed pattern (Webb *et al.* 2002, Emerson & Gillespie 2008). A number of studies have adopted this and similar approaches to examine the extent to which species pools are assembled via ecologically conservative dispersal (allopatry + low ecological divergence) versus in situ speciation (sympatry + high ecological divergence; (Moen *et al.* 2009, Crisp *et al.* 2009)), although the approach hasn't been explicitly used to examine functional variation within and between pools.

Extinction undoubtedly has an important influence on functional and phylogenetic diversity, but it is difficult to infer anything from or develop heuristic predictions for extant species pools because past extinction events and their impacts can rarely be detected (Rabosky 2010, Quental & Marshall 2010). While examination of fossil records can be very useful in this regard (Foote 1997, Quental & Marshall 2010), there are few if any known fossils for most lineages. Extinction reduces functional and phylogenetic diversity simply by virtue of reducing species number, but where extinction is random with respect to functional form or phylogenetic history it should not affect the amount of functional variation or degree of phylogenetic relatedness among extant taxa. Where extinction is selective, however, eliminating species that represent particular functional forms, functional variation may be substantially reduced. Under this scenario, phylogenetic diversity will also be reduced if functional variation reflects phylogenetic history.

Among the many insights that the study of extinction in the fossil record has provided, one of the most interesting is the tendency for extinction events to be followed by rapid lineage and morphological diversification (Foote 1997). The implication of this pattern is that lineage and morphological diversification are ecologically constrained and that the opening of ecological space allows rapid diversification. The advent of methods to produce well-sampled molecular phylogenies relatively quickly has triggered new interest in exploring the signal of ecological constraint on the radiation (Pybus & Harvey 2000, Rabosky 2006*b*, Rabosky & Lovette 2008*a*, McPeek 2008) and morphological diversification (Harmon *et al.* 2003, 2010, O'Meara *et al.* 2006) of extant lineages. Where lineage diversification is constrained by available ecological space the rate of diversification is expected to decrease as a function of the number of taxa extant at a particular time (i.e. show a density-dependent decline), a pattern which has been found for a number of lineages (Rabosky & Lovette 2008*a*, Phillimore & Price 2008, McPeek 2008). On the other hand, where a lineage is diversifying into a vacant adaptive zone, the expectation is that there will be a rapid early burst of morphological diversification, slowing as the vacant ecological space is filled (Simpson 1953). A recent meta-analysis of a number of animal lineages has, however, found little evidence for such 'early bursts' (Harmon *et al.* 2010).



A. Low ecological divergence

C. High ecological divergence (convergence within regions)

B. High ecological divergence (no convergence)

D. High ecological divergence (convergence between regions)

Figure 1.4: The influence of the geography of speciation and the nature of trait evolution on phylogenetic relatedness and functional similarity among species within and between regional species pools. Coloured lines indicate functional similarity among species.

Table 1.1: The degree of functional variation and phylogenetic distance among species within and between regional pools under different modes of speciation and with differing degrees of ecological divergence.

		Ecological divergence							
		Low divergence		High divergence					
				No convergence		Convergence within regions		Convergence between regions	
Speciation mode		Within	Between	Within	Between	Within	Between	Within	Between
Sympatric									
	Functional	Low	High	High	High	Intermediate	High	High	Low
	Phylogenetic	Low	High	Low	High	Low	High	Low	High
Allopatric									
	Functional	High	Low	High	High	Intermediate	High	High	Low
	Phylogenetic	High	Low	High	Low	High	Low	High	Low

Notes: ‘Convergence’ relates to species. Thus ‘Convergence within regions’ should be read ‘Convergence among species within regions’.

1.2 Factors which influence the functional and phylogenetic composition of local pools

There is a multitude of factors which affect the composition and structure of local communities. It is useful to consider first the pool of species or individuals that could potentially occur in a local community in the absence of interspecific interactions (the 'fundamental' pool) and the processes which influence this pool (Figure 1.2). The primary constraint on this pool is dispersal. Where species are separated from the focal site by large distances or are rare in the regional pool they are unlikely to be able to disperse into the local community. The importance of dispersal in determining local community composition is highlighted by the power of neutral ecological models to predict species occurrence and relative abundance (Hubbell 2001, Allouche & Kadmon 2009). These models contend that the composition of local species pools is purely the result of dispersal limitation, with the probability of species colonizing the local community proportional to their relative abundance in the regional pool, and ignore interactions between organisms and between organisms and their environments (Hubbell 2001). By contrast, niche-based models contend that various species attributes are important for colonization and extinction potential and thus affect the composition of the local pool (Westoby & Wright 2006, McGill *et al.* 2006, Shipley *et al.* 2006). Here colonization is dependent both on dispersal and the ability of the species to tolerate the local environmental conditions. A species may have good dispersal abilities and high regional abundance, but if it is unable to tolerate the local abiotic environment or access necessary biotic interactions (e.g. mutualists, hosts or prey) it is denied membership of the local species pool. This process of environmental filtering limits local pool membership to ecologically similar species, thus reducing functional variation within the community (Diamond 1975, Keddy 1992, Cornwell *et al.* 2006). Because niche-based processes affect the relative abundance of species in local pools they also affect relative abundance in the regional species pool, and this may in turn generate a feedback loop affecting the probability of species dispersing into local communities. This can be particularly important when one considers life-history strategies (e.g. Westoby *et al.* 2002). A species that has adopted a generalist or colonizer life-history strategy is likely to be abundant and well dispersed in the regional pool and can tolerate a broad range of environmental conditions, these attributes making it more likely to disperse into any particular local community. Alternatively, a specialist or competitor life-history strategy should result in lower abundance and greater isolation in the regional pool and a narrower tolerance of environmental conditions, resulting in a lower probability of colonization. This feedback loop would likely lead to the extinction of specialist species, were it not for the improved performance and competitive ability of specialists in the habitats to which they are specialized, and/or the stochastic effects of disturbance.

The ‘realised’ pool of species which form a local community, and their respective functional attributes, is the subset of the ‘fundamental’ species pool which has the ability coexist and is determined by the mechanisms by which coexistence is maintained. There is a large number of potential mechanisms that enable species to coexist (see Wilson 1990, Chesson 2000, Hubbell 2001, Amarasekare 2003 for review), most of which fall on a continuum between classical niche theory and neutral theory (Holt 2006, Gravel *et al.* 2006, Leibold & McPeek 2006). Classical niche theory predicts that there should be little functional equivalence among co-occurring species because competition for similar resources should lead to exclusion (MacArthur & Levins 1967). Competitive exclusion may lower diversity by reducing the number of species that can coexist, but it should also ensure that those species which do coexist are ecologically differentiated. Neutral theory, by contrast, assumes that all individuals of all species are functionally equivalent and interspecific interactions can be ignored, resulting in communities which largely reflect the regional species pool but with membership constrained by dispersal limitation (Hubbell 2001). Where dispersal rates are low, landscapes are expected to be patchy, with high turnover between local communities, whereas high dispersal rates result in relatively homogeneous landscapes (Hubbell 2001, Latimer *et al.* 2005).

A recent synthesis of coexistence theory (Chesson 2000) suggests that competitive exclusion can result in greater functional differences among co-occurring species in some scenarios, and smaller functional differences in others (Mayfield & Levine 2010). According to this model, coexistence is maintained by a balance between niche differences (preferred environment) and competitive/fitness differences (growth and demographic rates). Where species occupy the same niche, only species with similar competitive abilities may coexist because differences in competitive ability rapidly lead to the exclusion of the weaker competitor. This results in the coexistence of functionally similar species. Where species are specialized to different niches, however, even large differences in competitive ability do not lead to competitive exclusion, resulting in co-occurrence of functionally different species. Most mechanisms invoked to explain species coexistence involve modifications of one or both of niche differences (a ‘stabilizing’ mechanism) or competitive differences (‘equalizing’ mechanisms) between species (Chesson 2000). For example, resource competition theory (the R* rule, Tilman 1982) suggests that species with the ability to grow and reproduce rapidly enough to maintain a population at a lower resource level (the species' R*) will be competitively dominant when the resource becomes limiting. If the environment is homogenous, i.e. there is only one resource acquisition niche, then coexistence can only be maintained if species have the same R* (fitness or competitive ability). Alternatively, in patchy environments with multiple potentially limiting resources, trade-offs in species' R* for each resource (niche differences) prevent any species from being the dominant competitor in all environments, facilitating coexistence in the landscape. Coexistence mechanisms which minimize competitive differences, or increase niche differences along an axis that is not always readily observable (e.g. the 'regeneration' niche (Grubb 1977), frequency dependent predation or parasitism (Janzen 1970, Connell 1971), fluctuating environments in space and time (Chesson & Warner 1981), or stochastic niche theory (Tilman 2004)) can potentially produce an emergent pattern of neutrality (Hubbell 2005, Holt 2006, Gravel *et al.* 2006, Leibold & McPeek 2006).

The 'local' processes of dispersal limitation, environmental filtering and competition (or coexistence mechanism) thus provide a set of heuristic predictions for the degree of functional variation that one would expect to see among species within and between local communities (Table 1.2). Of course, the pattern observed in real communities is likely to be some combination of these predictions because most communities will be affected by multiple local processes and coexistence mechanisms. The degree of phylogenetic relatedness among the species within and between communities is further determined by the degree to which functional similarity reflects phylogenetic history (Webb *et al.* 2002, Mayfield & Levine 2010). While exploring the degree of functional similarity or the degree of phylogenetic relatedness and the signal of trait evolution allows us to infer the predominance of one ecological process over others (Webb *et al.* 2002), this approach does not allow us to discriminate between the effects of environmental filtering as opposed to coexistence via equivalent fitness (Mayfield & Levine 2010).

Table 1.2: The degree of functional variation and phylogenetic distance among species within and between local pools.

		Trait Evolution			
		Conserved		Convergent	
Dominant ecological process		Within	Between	Within	Between
Dispersal limitation (Neutral)	Functional	Reflects regional pool	*	Reflects regional pool	*
	Phylogenetic	Reflects regional pool	*	Reflects regional pool	*
Environmental filtering	Functional	Low	High	Low	High
	Phylogenetic	Low	High	High	Low
Coexistence via niche segregation	Functional	High	Low	High	Low
	Phylogenetic	High	Low	Random/Low	Random/High
Coexistence via equivalent fitness	Functional	Low	High	Low	High
	Phylogenetic	Low	High	High	Low

Note: Coexistence can potentially be maintained by a balance between niche segregation and fitness equivalence in which case the degree of functional similarity or phylogenetic relatedness could be clustered, even or random. * The degree of functional or phylogenetic similarity between communities determined by dispersal limitation depends on the degree of limitation. Low dispersal rates result in patchy landscapes with high turnover between local communities, whereas high dispersal results in relatively homogeneous landscapes. Table adapted from Webb *et al.* (2002) and Mayfield & Levine (2010).

1.3 Functional and phylogenetic diversity in fynbos

Per unit area, the Cape Floristic Region (CFR) on the southern tip of Africa supports one of the most diverse floras in the world with approximately 9000 indigenous species, nearly 3% of global vascular plant species diversity (Myers *et al.* 2000, Goldblatt & Manning 2002). Fynbos vegetation, which accounts for ca. 80% of the species in the CFR, is characterized by many locally abundant, narrow-endemic species (Goldblatt & Manning 2000, 2002, Linder 2003, Latimer *et al.* 2005) resulting in moderately rich local communities (1-1000m^2) with very high turnover in species composition between habitats and between similar habitats in different regions (Bond 1983, Cowling & Campbell 1984, Cowling 1990, Cowling *et al.* 1992). By contrast, turnover in growth form and taxonomic diversity is very low, with most communities containing multiple representatives of each of the four major growth forms; shrubs, sub-shrubs, graminoids and geophytes (Cowling & Campbell 1984, Cowling 1990). The resulting vegetation thus exhibits high structural uniformity (Campbell 1985) and supports similar ecosystem processes in terms of productivity, nutrient cycling, hydrology and fire regime across its range (Richardson *et al.* 1995). While this suggests that many fynbos species are highly redundant and of comparatively low conservation value with respect to the maintenance of ecosystem function (Walker 1992), the high diversity of post-fire regeneration strategies within each growth form is often cited as a form of backup in the event of stochastic recruitment failures, thus ensuring long-term stability in ecosystem function (Cowling *et al.* 1994, Richardson *et al.* 1995, Bond 1997).

The CFR is isolated from the rest of Africa by a sharp transition from low to higher nutrient soils and from winter or year-round rainfall to a summer rainfall system. The flora of the region reflects this insular nature, with high levels of specific (69%) and generic (16%) endemism and almost half the diversity concentrated in just 33 lineages (Goldblatt & Manning 2000, Linder 2003). While most lineages have relatives which occur in other regions, phylogenetic evidence indicates that the majority of the floristic diversity of the CFR arose in situ via multiple recent radiations (Linder 2003, 2005*a*, Linder & Hardy 2004, Bergh & Linder 2009, Sauquet *et al.* 2009, Verboom *et al.* 2009). This is further supported by the clustering of close relatives within fynbos when compared to neighbouring biomes (Proches *et al.* 2006, Forest *et al.* 2007*a*). The high diversity and insular nature of the CFR thus make it highly amenable for the study of the processes which generate species diversity and functional variation and facilitate their maintenance in assemblages from fine to broad scales (Barraclough 2006).

The radiation of fynbos lineages is believed to have been adaptive in nature (Cowling *et al.* 1992, Linder 2003, 2005*a*, van der Niet & Johnson 2009), driven by strong disruptive selection on fire survival strategy (Schutte *et al.* 1995) and edaphic (Verboom *et al.* 2004), climatic (Linder & Vlok 1991, Verboom *et al.* 2003, 2009), pollinator (Johnson 1996, Johnson *et al.* 1998, Waterman *et al.* 2011) and microhabitat specialization. Phenological differences (Warren *et al.* 2011), low dispersal rates (Slingsby & Bond 1985, Schurr *et al.* 2005) and sterility barriers would have aided cladogenesis across these divergent selective environments by limiting gene flow. Geographic isolation has also been implicated as a major factor aiding the genesis of fynbos plant diversity (Goldblatt 1977, Linder 2001, 2003). A number of clades that have radiated within the CFR have coincident areas of endemism consistent with isolated mountain blocks (Weimarck 1941, Oliver *et al.* 1983, Goldblatt & Manning 2000, Linder 2001, Moline & Linder 2006), implicating a central role for allopatry in speciation. That almost 30% of all vascular plant species in the CFR are endemic to one of the six phytogeographic areas recognised by Goldblatt & Manning (2000, 2002) suggests strong geographic structuring of the flora as a whole. Recent molecular evidence indicates that geographic isolation between these mountain blocks may have performed a critical role in generating fynbos diversity via non-ecological speciation in allopatry (Britton 2010, Prunier & Holsinger 2010). The CFR may thus harbour a large number of unrecognised, allopatrically-distributed cryptic species because insufficient time has passed for them to diverge to the extent that they are morphologically diagnosable (Britton 2010). Morphological disparity among species that diverged in allopatry would have accumulated gradually via drift or selection within slightly different allopatric environments rather than rapidly as expected by an adaptive divergence model (Simpson 1953, Rundell & Price 2009, Losos & Mahler 2010). Where species subsequently dispersed into sympatry, rapid ecological divergence may have occurred via competitive character displacement (Brown & Wilson 1956).

The frequent co-occurrence of high numbers of functionally similar species in fynbos creates a conundrum for understanding the mechanism by which this high diversity is maintained and suggests that classical niche theory is of limited heuristic value in this system (Richardson *et al.* 1995). The general belief is that coexistence is facilitated by lottery models or a spatio-temporal storage effect whereby variability in the fire regime favours the differential recruitment of species with differing regeneration strategies at different times, preventing any species from becoming dominant and competitively excluding others (Grubb 1977, Cowling 1987, Yeaton & Bond 1991, Bond *et al.* 1992, Kilian & Cowling

1992, Laurie & Cowling 1995, Laurie *et al.* 1997, Cowling *et al.* 1997*a*, Higgins *et al.* 2000*b*, Thuiller *et al.* 2007). This may allow the co-occurrence of species of similar functional form and results in 'neutral-like' community dynamics with weak interspecific interactions slowly driving species to extinction (Leibold & McPeek 2006, Thuiller *et al.* 2007). In spite of a large literature supporting these models, there is some evidence to suggest that classical niche dynamics are also at play in fynbos communities. Firstly, Cody (1986) demonstrated that leaf size and shape are partitioned among co-occurring Proteaceae species, while Slingsby & Verboom (2006) demonstrated that co-occurring species from a clade of schoenoid sedges were more distantly related and functionally dissimilar than expected on the basis of chance. Secondly, the species diversity of local communities is relatively constant across the CFR despite much lower regional richness in the East (Richardson *et al.* 1995). This discordance between local and regional pools suggests that the diversity of local communities is constrained by local processes, consistent with classical niche theory (Ricklefs 2004).

There is a limited set of regional and local processes that could have generated the observed patterns of species, functional and phylogenetic diversity within and among fynbos communities. Local communities are moderately rich in species and contain much functional variation and phylogenetic history with high turnover in species composition but low functional and phylogenetic turnover between habitats and between similar habitats in different regions (Bond 1983, Cowling & Campbell 1984, Cowling 1990, Cowling *et al.* 1992, Richardson *et al.* 1995). At the regional scale this suggests that most species evolved in allopatry with little ecological divergence between them, or that species in different regions converged to similar functional forms (Table 1.1). The resultant pattern of species, functional and phylogenetic diversity could then be reflected within local communities within regions due to neutral processes, with high dispersal limitation explaining the high turnover in species composition between habitats (Table 1.2; Latimer *et al.* 2005). Alternatively, and more consistent with the findings of Cody (1986), Richardson *et al.* (1995) and Slingsby & Verboom (2006), this pattern could be a manifestation of local coexistence mediated by niche partitioning. If niche processes are important at the local scale it has implications for processes at the regional scale because it implies that most species within a regional pool are ecologically differentiated to the extent that they prefer different habitats, or that they are able to partition resources within habitats. Valente *et al.* (2010) and Linder & Hardy (2004) found phylogenetic evidence for declining diversification rates in Protea and Restionaceae respectively, consistent with the niche-based hypothesis that as the ecological space becomes saturated, diversification rates decrease (Simpson 1953, Foote 1997, Pybus & Harvey 2000). While the predominance of low ecological divergence versus convergence in form among species can potentially be teased apart by examining the degree to which functional similarity reflects phylogenetic history (Table 1.1), it is highly likely that both processes have contributed to the observed pattern. For example, where species are allopatric there is little reason for them to diverge ecologically because they do not have to share habitats within regions. Where these species disperse into sympatry, competitive interference is likely to cause them to diverge ecologically or be segregated at fine spatial scales (niche or character displacement; Brown & Wilson 1956, Dayan & Simberloff 2005). If close relatives are completely excluded from the same niche rather than partitioning resources within sites they have to specialize to new habitats, potentially converging to a similar niche and functional form as a more distant relative. There is thus much evidence to suggest that ecological specialization is a common phenomenon in fynbos (Cody 1986, Richardson *et al.* 1995, Slingsby & Verboom 2006, Linder & Hardy 2004, Valente *et al.* 2010).

1.4 Statement of thesis

It is my thesis that ecological specialization is a critical factor influencing the genesis and maintenance of fynbos functional and phylogenetic diversity and that the manner in which a lineage diversifies and specializes to different ecological conditions and strategies has a profound impact on the patterns of functional and phylogenetic diversity observed at all spatial scales. The questions I aim to answer are thus: Is there evidence to suggest that the accumulation of species diversity in fynbos lineages has been constrained by ecological limits on diversification rates? [Chapter 4]; Are close relatives with overlapping ranges ecologically differentiated while close relatives that occur in allopatry show little sign of ecological divergence? [Chapter 5]; Can coexistence of closely-related species within local communities be explained by specialization to subtly different microhabitats in accordance with the principles of classical niche theory? [Chapter 6]; And finally, does a predominance of speciation in allopatry and specialization of closely-related species to contrasting environmental conditions result in a pattern of high species turnover but low functional or phylogenetic turnover between regions and between habitats within regions? [Chapter 7]. As addressing these questions depends on exploring patterns of functional and phylogenetic diversity, this thesis starts with a consideration of the measures used to quantify functional and phylogenetic diversity [Chapter 2] and the methods used to test for significant pattern [Chapter 3].

1.5 Some notes on the study group

This thesis focuses on a clade of schoenoid sedges (Cyperaceae: Schoeneae) currently circumscribed within the genus *Tetraria* P. Beauv. Recent molecular evidence has demonstrated *Tetraria* to be polyphyletic, however, with the South African members falling into two distinct and phylogenetically disparate clades (Verboom 2006). The focal clade for this study is characterized by the presence of noded culms and fine reticulation at the base of each tiller caused by fraying of the leaf sheaths (Plate 1E). The type species of the genus, *Tetraria compar* (L.) Lestib., does not fall within the focal 'reticulate-sheathed' *Tetraria* clade, and the latter is to be assigned a new generic epithet (Verboom et al. *In preparation*). In the absence of a new generic epithet, however, and to avoid constant repetition of the 'reticulate-sheathed' descriptor I take the pragmatic approach of referring to this group simply as the genus *Tetraria* throughout. This should cause little confusion to the reader as the 'non-reticulate-sheathed' *Tetraria* species form no part of this thesis other than the cameo appearance of *T. crassa* Levyns and *T. compar* as outgroup taxa for phylogeny inference in Chapter 4.

In addition to the confusion generated by the lack of a new generic epithet, the existing taxonomy for the group is largely obsolete. A forthcoming revision will effect a number of taxonomic changes, raising the number of species from 28 to 46 (Verboom et al. *In preparation*). I have chosen to base this thesis on the forthcoming taxonomy because it reflects more accurately the true biology of the group. In the absence of appropriate epithets for a number of the 'new' species I have followed a protocol of referring to them as new species similar to the existing species which they most closely resemble, e.g. *Tetraria sp. nov. cf. maculata*, but have largely circumvented these cumbersome working titles by referring to species using unique three letter name codes (Chapter 5, Appendix 5.1). *Tetraria* is a morphologically diverse group that forms a common, often dominant, component of most fynbos plant communities (Plate 1). The genus accounts for most of the schoenoid sedges native to the Cape Floristic Region (Goetghebeur 1998) and forms one of the 33 Cape floral clades' which have diversified in and are largely restricted to the region (Linder 2003). Previous examination of phylogenetic relationships among 15 of the 46 species, using a maximum likelihood analysis of one nuclear ribosomal and two chloroplast DNA regions, provided strong bootstrap support for two clades and weak support for a third within the group (Slingsby & Verboom 2006). The *thermalis-bromoides* clade consists of a set of five broad-leaved species that are typically large in stature and consist of few-to-many florets of leaves, or tillers (Plate 1A), the *microstachys-burmannii* clade comprises three species that are both small in stature and consist of few tillers (Plate 1B), and the weakly supported *fasciata-flexuosa* clade consists of five species with small to medium body sizes, individuals of which are often composed of large numbers of tightly-packed tillers (Plate 1C).

Tetraria individuals are made up of multiple tillers which stem from a common rhizome (Plate 1, Figure 1.5). Each tiller is comprised of a rosette of leaves emanating from a basal corm from which a single, central, protruding culm, terminating in a paniculate inflorescence bearing multiple spikelets, develops (Figure 1.5, Plate 1). Rhizomes typically do not spread, but very old individuals may become diffuse as the centre of the plant dies leaving a ring of live tillers or splitting into isolated genetically identical individuals. Individuals appear to have long life-spans, surviving through multiple fire events, and often dominate post-fire environments and frequently burnt areas. Tillers resprout rapidly from a basal meristem after burning and each tiller will flower once. This results in plants flowering profusely in the first or second year after fire, with fewer, newly developed tillers flowering in later years. Inflorescences appear to take most of a year to develop and will flower for up to a month. Flowers are wind pollinated and most species flower for two to three months in either spring or autumn, the precise timing for populations within species varying with geographical location and aspect. Seed size varies between species by an order of magnitude and undifferentiated, presumably passively-dispersed, seeds are released a few months after flowering as soon as they are fully developed. Preliminary investigation indicates that *Tetraria* species produce dauciform roots (Plate 1) to enhance soil nutrient acquisition, and staining assays have revealed no evidence of mycorrhizal associations (Heidi Hawkins *pers. comm.*). Evidence of herbivory on *Tetraria* species is rare.

A number of features of *Tetraria* make it highly appropriate for a study of this nature. The apparent absence of mutualists in the form of pollinators, seed dispersers or mycorrhizal associations excludes the potentially complex effects of mutualisms on species occurrence and coexistence (Sargent & Ackerly 2008, Waterman *et al.* 2011). Also, the predominance of these species in the landscape means that they are likely to interact ecologically, while their longevity and ability to survive multiple fire events allows time for any negative interspecific interactions to run their course.

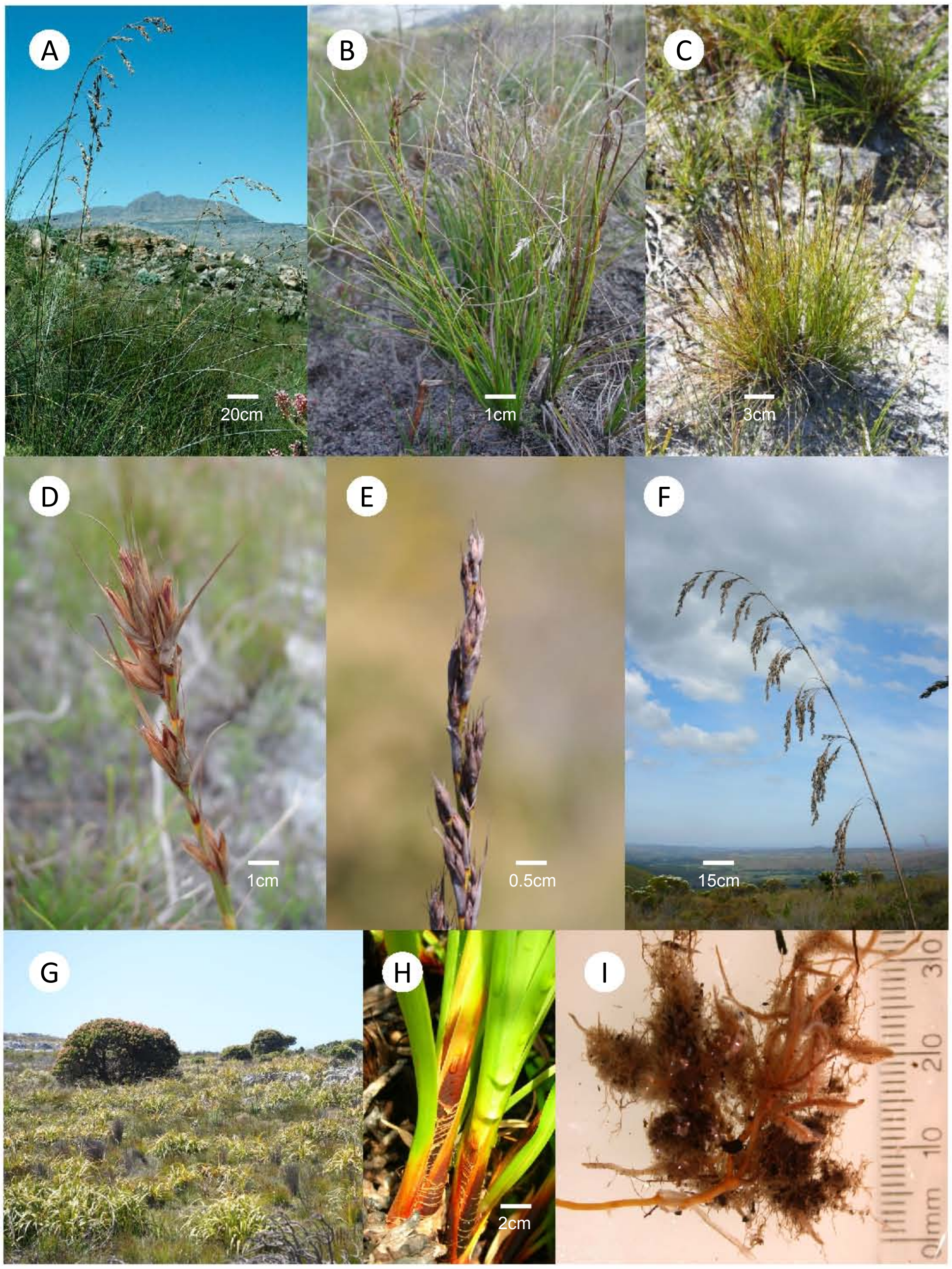


Plate 1: A) *Tetraria involucrata*, B) *T. microstachys*, C) *T. fasciata*, D) *T. eximia* inflorescence, E) *T. flexuosa* inflorescence, F) *T. involucrata* inflorescence, G) A slope dominated by *T. thermalis* and *T. fasciata*, Silvermine, Table Mountain National Park, H) the base of *T. involucrata* tillers illustrating the 'reticulate-sheathing', I) dauciform roots of *T. bromoides*.

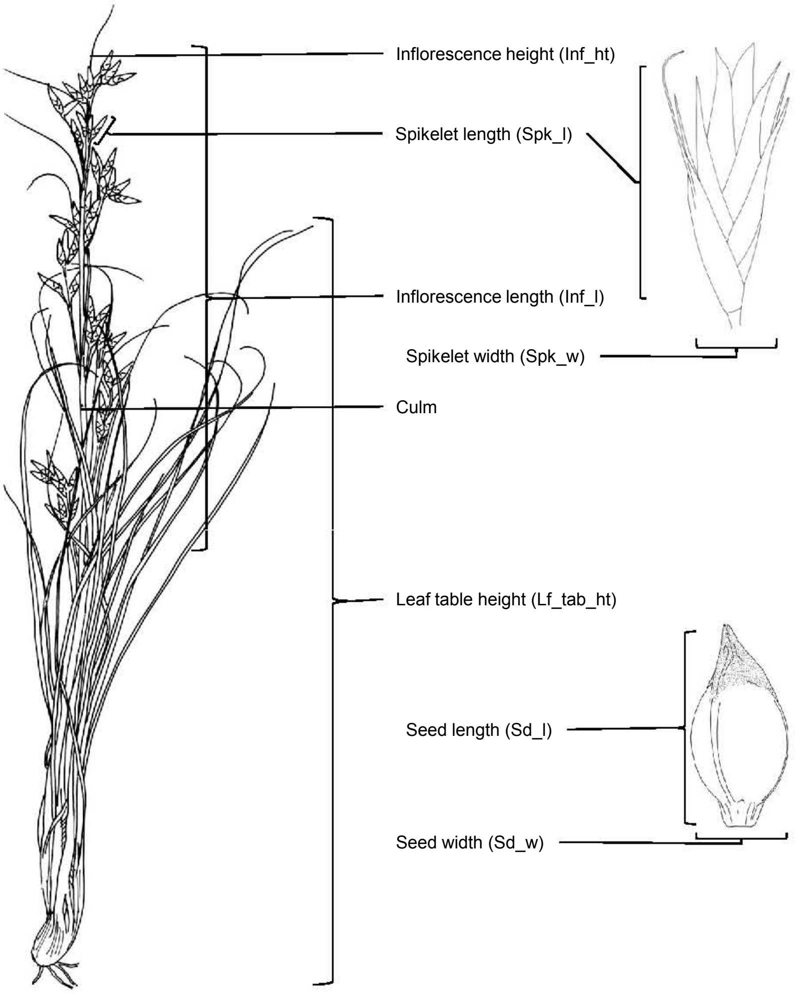


Figure 1.5: Habit diagram of a *Tetraria microstachys* tiller indicating morphological features. Leaf length (Lf_l), leaf width (Lf_w) and seed mass (Sd_m) are not indicated. Artwork: G.A. Verboom

2 Measuring the components of functional and phylogenetic diversity

2.1 Abstract

The recent development of functional and phylogenetic diversity measures allows testing of explicit hypotheses relating biodiversity to ecosystem function and deciphering the ecological and evolutionary processes that determine the assembly of communities. Unfortunately, there is some confusion in the literature because the large number of existing functional and phylogenetic diversity measures each quantify subtly different components of diversity, rarely indicating which component they quantify or how they relate to other measures. I attempt to provide some clarity by reviewing the range of available measures, classifying them by their methodological approach (categorical, hierarchical, pairwise or multidimensional) and the diversity component they quantify (richness, dispersion, evenness or redundancy). I propose new hierarchical and pairwise measures of functional or phylogenetic evenness and redundancy. I hope that clarification of the relationships between diversity measures will encourage communication between disciplines and aid the unification of ecological, evolutionary and conservation theory.

2.2 Introduction

The past two decades have seen major advances in the measurement of biological diversity (Magurran 2004). This is most clearly illustrated by the shift in focus from mere counts of species numbers and measures of relative abundance to the quantification of species' functional differences and unique evolutionary histories (Humphries *et al.* 1995, Petchey & Gaston 2002, 2006). These advances have largely been driven by the diverse and distinct interests and needs of conservation biologists (Faith 1992, Humphries *et al.* 1995), community ecologists (Weiher & Keddy 1999, Webb *et al.* 2002, Cavender-Bares *et al.* 2009), functional biologists, ecosystem ecologists (Diaz & Cabido 2001, Loreau *et al.*

2001, Hillebrand & Matthiessen 2009, Reiss *et al.* 2009) and economists (Costanza *et al.* 1997) among others. Unfortunately, this wide range of needs has led to a confounding plethora of methods that attempt to quantify various components of the functional or phylogenetic diversity of biological assemblages (Petchey & Gaston 2006, Hardy 2008, Schweiger *et al.* 2008). In this chapter, I review the literature in an attempt to make sense of the toolkit of available methods. I do this by defining the major components of diversity and identifying the approaches used to quantify them. I then review and categorize the range of measures according to the approach used and the component they quantify. I propose new measures where gaps exist and compare the relationships between all measures when applied to a common, simulated dataset. I hope that by unifying our understanding of diversity measures we will improve collaboration and communication between disciplines and develop a holistic understanding of biological assemblages and ecosystems that allows the development of effective, well-guided conservation and management practices.

2.3 Measuring functional and phylogenetic diversity in assemblages

2.3.1 What do we want to measure?

2.3.1.1 Identity versus diversity

A central question in biodiversity research is the importance of the identity versus the diversity of entities in an assemblage. Ecosystem function is often dependent on the trait values of the dominant species in the assemblage (Garnier *et al.* 2004, Diaz *et al.* 2007). Identity is also important in niche theory because particular traits and a limited range of trait values are required to survive in a particular environment (Weiher & Keddy 1999). The traits found in an assemblage may diverge from the ‘optimum’ values for a number of reasons including competition, disturbance or limitation on the dispersal and colonization of ‘optimal’ species. Alternatively, or in conjunction with optimal trait values (trait identity), greater diversity in trait values may enhance ecosystem processes by allowing more efficient and complete use of available resources, reducing negative interspecific interactions through resource partitioning, and by creating positive interactions such as facilitation (Loreau & Hector 2001). Questions of community assembly and the designation of conservation priorities are similarly concerned with both the identity of species and their trait values and the range and diversity of species and traits (Rebelo & Siegfried 1992, Weiher & Keddy

1999). Here I focus on measures of diversity, but caution that actual trait values and species identities are often the major drivers of ecosystem processes (Diaz *et al.* 2007) or have greater conservation value (e.g. Red data listed or keystone species) and should not be ignored.

2.3.1.2 The components of diversity

There is a large variety of functional and phylogenetic diversity measures (see Petchey & Gaston 2006, Hardy 2008, Schweiger *et al.* 2008 for review). Before a method can be used appropriately, however, it is important to identify which component of diversity is of interest to the study at hand (Mason *et al.* 2005, Cadotte *et al.* 2010). Species diversity is traditionally divided into two components: richness, the count of species number, and evenness, the parity in the relative abundance of the set of species (Magurran 2004). Various measures have been developed which quantify richness or evenness in the functional or phylogenetic diversity of an assemblage (Table 2.1). In addition to richness and evenness, many workers are interested in the degree of dispersion in function or evolutionary relatedness (Weiher & Keddy 1999, Webb *et al.* 2002), and the degrees of redundancy (Schmera *et al.* 2009*b*) and complementarity (Humphries *et al.* 1995, Petchey 2003) within or between assemblages (Table 2.2).

Functional or phylogenetic *richness* is the total amount of unique functional variation or evolutionary history in an assemblage of species or entities (Table 2.2). It is dependent on the number of species in the assemblage and the unique functional or phylogenetic differences between them, but independent of their relative abundance. A fundamental requirement of any richness index is that it is ‘set monotone’, increasing or remaining the same when a new species is added to the assemblage (Mason *et al.* 2003).

Dispersion is a measure of the unique functional or phylogenetic differences between species in an assemblage, or that component of richness unexplained by species number. It is a measure of how functionally or phylogenetically different species within an assemblage are from one another relative to those in other assemblages.

Evenness is a measure of parity in the distribution of abundance of species or entities with different trait values or phylogenetic position. It is independent of functional or phylogenetic richness or dispersion among species. Contrary to traditional measures of species evenness, where evenness is maximized when all species have the same abundance, an assemblage should become more functionally or phylogenetically even as the relative abundance of species or entities approaches the proportion of unique functional variation or evolutionary history that they contribute to the assemblage. Thus greater functional or phylogenetic evenness is achieved when species that represent a greater proportion of unique evolutionary history or functional variation occur in greater relative abundance. It is important to note that evenness does not express the actual abundance or biomass of species in the assemblage. This 'bulk' determines many ecosystem properties and processes but is often overlooked because biomass is typically considered an ecosystem function rather than a diversity component (Reiss *et al.* 2009).

Redundancy describes the overlap in function or shared evolutionary history of a group of entities or species in an assemblage. It can be measured in a multitude of ways because it can be expressed for each individual or species in an assemblage, for the assemblage as a whole, or for each function or piece of evolutionary history. Redundancy in an assemblage increases with species number and decreased functional or phylogenetic dispersion because this results in a greater number of entities that are more closely related or share similar traits.

Complementarity is a term often used by biologists, but it has taken on many meanings (Humphries *et al.* 1995, Petchey 2003). Complementarity in any of its forms is not a unique measurable component of diversity, being quantifiable using measures of richness, dispersion, evenness or redundancy. Conservation biologists consider two or more assemblages to be highly complementary if they have high combined functional or phylogenetic richness (Faith 1992, Humphries *et al.* 1995) and use it as a criterion for reserve selection. Studies of the importance of biodiversity for ecosystem function consider complementarity (or 'niche complementarity'), to be a mechanism by which increased diversity improves the functioning of ecosystems (Loreau & Hector 2001). The 'niche complementarity' effect occurs when the set of species in an assemblage improve ecosystem function because they either exhibit greater niche differentiation, partitioning resources between them and reducing the negative effects of competition, or greater facilitation between species, increasing the productivity or efficiency of the assemblage.

While many measures of diversity combine two or more components, it is essential to acknowledge that in reducing them into a single measure much information is lost (Purvis & Hector 2000, Leps *et al.* 2006, Melo 2008). For example, traditional measures of species diversity or 'heterogeneity' such as Shannon's H' (Shannon & Weaver 1963) or Simpson's D (Simpson 1949) incorporate both richness and evenness, but vary in the weighting apportioned to each (Magurran 2004). There is as yet no accepted objective way in which any two components of diversity can be combined and their relative importance weighted accurately (Leps *et al.* 2006, Melo 2008). How many individuals of one species is an additional species worth? Indeed, if combined measures of diversity are used it is best to include an exploration of the data using each component independently too.

2.3.2 How can we measure functional and phylogenetic diversity?

Functional or phylogenetic diversity can be quantified using discrete or continuous measures. Discrete measures such as species or functional group richness are based on counts of a set of predefined functional or taxonomic groups (categorical approaches), ignoring relationships between categories and treating them as equal. Functional groups can be defined subjectively, on the basis of expert opinion, or objectively, by scoring the presence/absence of a particular trait or traits, or by performing a cluster analysis based on measurements of each species or individual and grouping entities at a particular height on the dendrogram (Petchey & Gaston 2006). Categorical measures represent the simplest approach to measuring functional and phylogenetic diversity but they suffer from subjectivity, misclassification and loss of resolution. Subjective classification of functional groups by expert opinion is rarely repeatable by different experts, making it difficult or inappropriate to compare different datasets. Similarly, despite much effort taxonomic units are typically subjectively defined and one species or genus does not represent the same amount of evolutionary history as another (Avise & Johns 1999). In addition, taxonomic classifications are constantly under revision and the results of studies are sensitive to these changes (Agapow *et al.* 2004). Categorical measures simplify the continuous functional and phylogenetic variation in assemblages into discrete categories, losing much information and potentially sacrificing the ability to detect or explain pattern.

Continuous measures (Figure 2.1) are calculated using 1) pairwise distances between species or individuals calculated using the traits/attributes of interest and an appropriate distance measure, or based on phylogenetic relationships (pairwise matrix approaches); 2) branch length or patristic distance (number of nodes) from a tree depicting the functional or phylogenetic relationships between individuals or species (hierarchical approaches); or 3) the space or volume encompassing the positions and abundances of species or individuals in n-dimensional trait space (uni- and multidimensional approaches; Table 2.1).

Phylogenetic diversity measures are based on the evolutionary relationships between species depicted by a taxonomic classification or an ultrametric morphological or molecular phylogeny (Faith 1992, Webb *et al.* 2002). Much effort is concerned with the construction of a reliable, appropriate tree and ideally one works with the best available ultrametric molecular phylogeny with branch lengths proportional to time (Webb *et al.* 2002). Phylogenetic pairwise approaches derive the matrix of pairwise distances between species from a phylogeny. I make the distinction between pairwise and hierarchical approaches because pairwise measures allow the same branch length to be counted multiple times, whereas all branch lengths included in a hierarchical measure are only ever counted once.

Quantifying functional diversity using continuous approaches (Figure 2.1) requires measurement of one or more morphological or physiological traits, or products, that relate to the function of interest (Petchey & Gaston 2006). Pairwise matrix approaches use distance measures, such as Euclidean or Gower's distance, to summarize functional differences between entities represented on multiple trait axes into a single measure. Hierarchical approaches require the further step of clustering the distance matrix into a functional dendrogram using a linkage method such as group average (UPGMA). This approach causes some distortion of the relationships between species, but has the advantage that it represents only unique functional variation, eliminating most redundancy in the measurement of differences between entities, a major criticism of pairwise measures (Petchey & Gaston 2006).

There has been much discussion about how traits should be transformed, standardized or weighted and which distance measures or linkage algorithms should be used when calculating functional diversity using the various approaches (Podani & Schmera 2006, Petchey & Gaston 2007, Mouchet *et al.* 2008). These decisions can significantly affect results (Poos *et al.* 2009). Similarly, the inclusion of highly correlated traits (e.g. due to co-dependence on body size) can result in over-inflation of the influence of what is essentially one axis of functional variation. This collinearity can be reduced by excluding redundant traits, using the residuals from a regression between a redundant trait and a trait that is already included in the analysis, or ordinating (e.g. using principal components analysis) the redundant traits and including the standardized axes as independent traits (Quinn & Keough 2002). Multidimensional measures of functional diversity require $n + 1$ entities (or points) and orthogonal axes to calculate area or volume in n-dimensional space (Villeger *et al.* 2008, Laliberte & Legendre 2010) and commonly use ordination analyses to help meet these requirements, because they force axes to be orthogonal and allow the number of axes used in the analysis to be reduced while minimizing the amount of information lost. Different methods of transforming, standardizing or weighting traits, use of different distance measures, linkage algorithms or ordination analyses should be explored in all functional diversity studies, with preference for one set of methods over others requiring justification.

2.3.3 Including intraspecific variation in continuous measures of functional diversity

Knowledge of the abundance distribution of each species in niche space is desirable when measuring functional diversity (Mason *et al.* 2005). Multimodal variation within species due to factors such as ontogeny, sexual dimorphism, geographic variation or phenotypic plasticity can be included in most approaches by deriving a mean for each intra-specific group and treating each as a separate entity. Variation within species can also be included in continuous approaches by measuring all individuals and treating individuals, rather than species, as separate entities (Petchey & Gaston 2006, Cianciaruso *et al.* 2009). Unfortunately, logistical constraints favour the use of species means. Using species means is best justified by demonstrating that most of the functional variation lies between species rather than between individuals within species using an analysis such as a nested ANOVA. Even where intraspecific variation is appreciable, however, estimates of the *FD* measure (sensu Petchey & Gaston 2002) using species means closely reflects *FD* based on trait measurements for all individuals. Cianciaruso *et al.* (2009) demonstrated that even when the coefficient of variation in individual trait measurements around the species mean is as much as 500%, *FD* values based on a single trait calculated using individual values are strongly correlated to those calculated on the basis of species means, with an average R^2 of 0.8 (range = 0.4-1). This relationship becomes dramatically stronger if calculations are based on three or five traits, with R^2 = 0.97 (0.88-1) and R^2 = 0.98 (0.93-1) respectively. Unidimensional methods allow the use of species ranges based on a sample of all individuals by considering species as probability density functions or kernels (Mouillot *et al.* 2005). This approach is similar to using 95% confidence intervals and could conceivably be extended to multidimensional measures on two or more axes (Cornwell *et al.* 2006, Villeger *et al.* 2008), allowing quantification of gaps in the range or volume of functional variation, a property that cannot be measured directly using any of the other techniques.

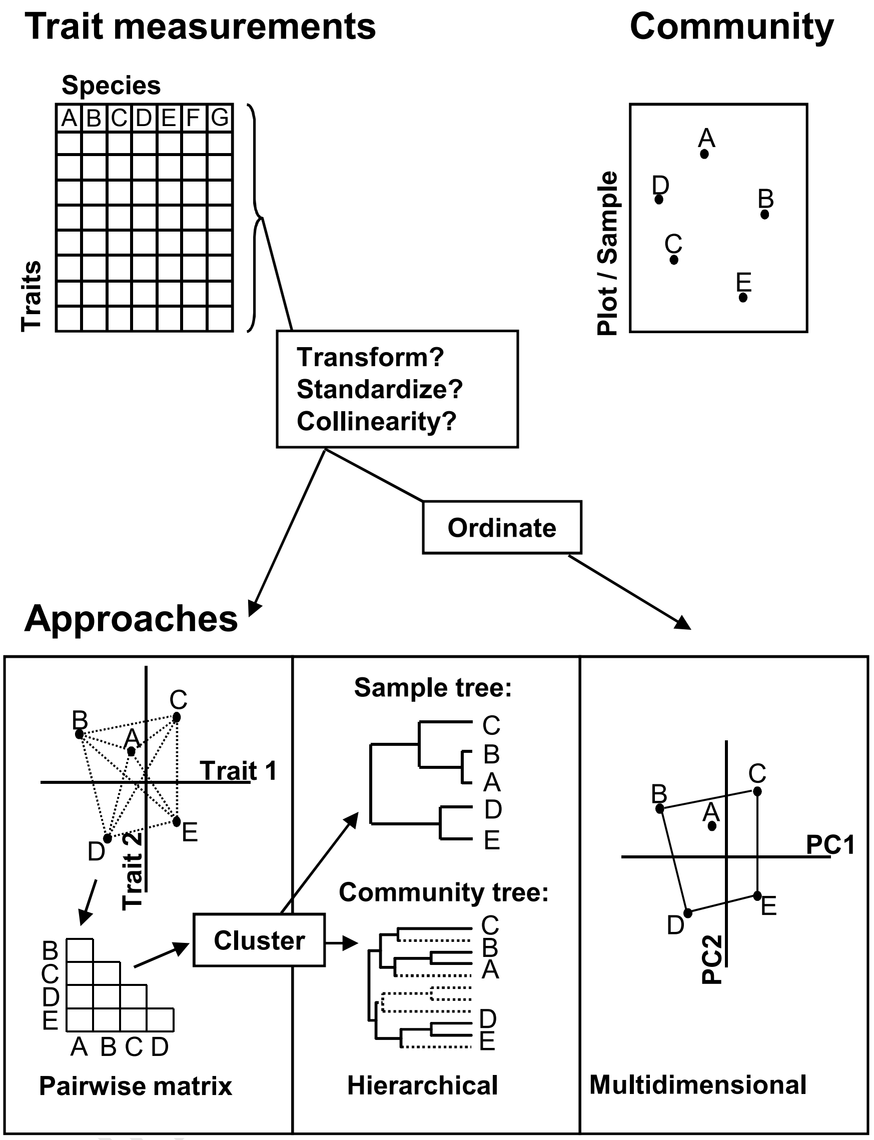


Figure 2.1: Illustration of hierarchical, pairwise and multidimensional approaches to measuring functional diversity. A set of traits are measured from a number of individuals representative of each of the species under study. These traits are then transformed, standardized and/or corrected for collinearity depending on requirement and preference (see text for details). A functional diversity measure for a community or sample is calculated using the pairwise matrix approach by summarizing the matrix of multivariate distances between each pair of species. Hierarchical measures summarize the relationships between the species on a dendrogram generated by clustering the pairwise distance matrix using a linkage method. Multidimensional measures are based on the volume encompassing the set of species in multivariate space. Multivariate measures often use axes derived from an ordination axes to ensure that axes are uncorrelated and to allow reduction in the number of axes used because n+1 entities are required to calculate volume from n axes.

Table 2.1: Functional and phylogenetic diversity measures

Measure	Basis	Component	Weakness	Reference
Categorical				
Functional group richness	Count of functional groups.	functional richness	Does not take relationships between groups into account.	
Taxon richness	Count of taxa (eg. number of species or genera represented).	phylogenetic richness	Does not take relationships between groups into account.	
Hierarchical				
Functional diversity (*FD*)	Sum of the minimum branch length required to join all taxa in the community on the functional tree for all species in the study.	functional richness	Clustering species into a tree distorts the pairwise functional differences between them.	Petchey & Gaston 2002
Functional diversity (*FDs*)	Sum of the minimum branch length required to join all taxa in the community on a functional tree for only the species in the sample.	functional richness	Clustering species into a tree distorts the pairwise functional differences between them. May not be set monotone.	Podani & Schmera 2006, Petchey & Gaston 2007
Phylogenetic diversity (*PD*)	Sum of the minimum branch length required to join all taxa in the community on the phylogenetic tree.	phylogenetic richness		Faith 1992
Abundance weighted evolutionary distinctiveness (H_{AED})	Calculated using the formula for Shannon's *H'* substituting the abundance of each species and the total community abundance with the abundance weighted evolutionary distinctness of each species and *PD* (excluding the root) respectively.	∼phylogenetic richness and evenness	Shows poor correlation with other measures of richness.	Cadotte *et al.* 2010
Equity of abundance weighted evolutionary distinctiveness (E_{AED})	Calculated using the formula for Pielou's *J*, substituting Shannon's *H'* with H_{AED} and total community abundance with community *PD*.	phylogenetic evenness		Cadotte *et al.* 2010
Imbalance of abundances at the clade level (*IAC*)	Deviation from the null expectation of phylogenetically balanced abundances across node splits, relative to the number of nodes.	phylogenetic evenness	Does not take branch length into account.	Cadotte *et al.* 2010
Phylogenetic-abundance evenness (*PAE*)	Phylogenetic evenness of the abundance distribution scaled by branch lengths.	phylogenetic evenness	Scaled to traditional species evenness rather than phylogenetic evenness making comparison between communities difficult.	Cadotte *et al.* 2010

Table 2.1: (continued on next page)

Table 2.1: (continued)

Measure	Basis	Component	Weakness	Reference
Hierarchical functional evenness (*hFEve*)	The degree to which the relative abundance of entities represents the proportion of unique functional variation (branch length) they contribute to the assemblage, expressed as a proportion of the maximally dominant community.	functional evenness	Clustering species into a tree distorts the pairwise functional differences between them.	This study
Hierarchical phylogenetic evenness (*hPEve*)	The degree to which the relative abundance of entities represents the proportion of unique evolutionary history they contribute to the assemblage, expressed as a proportion of the maximally dominant community.	phylogenetic evenness		This study
Hierarchical functional redundancy (*hFRed*)	The sum of each of the redundant branch lengths multiplied by the number of entities that share them, expressed as a proportion of itself plus the total length of unique branches.	functional redundancy	Clustering species into a tree distorts the pairwise functional differences between them.	This study
Hierarchical phylogenetic redundancy (*fPRed*)	The sum of each of the redundant branch lengths multiplied by the number of entities that share them, expressed as a proportion of itself plus the total length of unique branches.	phylogenetic redundancy		This study
Pairwise matrix				
Functional attribute diversity (*FAD2*)	Total pairwise distance between all species in a community.	∼functional richness	Increases roughly exponentially with the addition of new entities. Includes redundant variation.	Walker *et al.* 1999
Modified functional attribute diversity (*MFAD*)	Average pairwise distance between all species in a community.	∼functional dispersion	Is not set monotone. Decreases in range with increasing numbers of species. Includes redundant variation.	Schmera *et al.* 2009*a*
Mean functional dissimilarity (*MFD*)	Average pairwise distance between all taxa in a community.	∼functional dispersion	Is not set monotone. Decreases in range with increasing numbers of species. Includes redundant variation.	Heemsbergen *et al.* 2004
Average functional distinctness (*AvFD*)	Average pairwise distance between all taxa in a community weighted by abundance.	∼functional dispersion + evenness	Is not set monotone. Decreases in range with increasing numbers of species. Includes redundant variation.	Somerfield *et al.* 2008
Mean nearest functional entity distance (*MNFD*)	Average of the shortest distance to any other taxon in multivariate functional space for each species in the community.	∼functional dispersion ∼functional redundancy	Is not set monotone. Decreases in range with increasing numbers of species. Includes redundant variation.	Webb *et al.* 2002

Table 2.1: (continued on next page)

Table 2.1: (continued)

Measure	Basis	Component	Weakness	Reference
Mean nearest taxon distance (*MNTD*)	Average of the shortest distance to any other taxon on the community phylogenetic tree for each species in the community.	$\sim$phylogenetic dispersion $\sim$phylogenetic redundancy	Is not set monotone. Decreases in range with increasing numbers of species. Includes redundant phylogenetic branch length.	Webb *et al.* 2002
Mean pairwise distance (*MPD*)	Average pairwise phylogenetic distance between all taxa in a community.	$\sim$phylogenetic dispersion	Is not set monotone. Decreases in range with increasing numbers of species. Includes redundant phylogenetic branch length.	Webb *et al.* 2002
Phylogenetic species variability (*PSV*)	Expected variability in trait values between taxa in a community based on a Brownian motion model of evolution along branches.	$\sim$phylogenetic dispersion	Is not set monotone. Decreases in range with increasing numbers of species. Includes redundant phylogenetic branch length.	Helmus *et al.* 2007*a*
Rao's Quadratic Entropy (*QE*)	Pairwise distance between all taxa in a community weighted by abundance (functional or phylogenetic).	$\sim$functional richness $\sim$phylogenetic richness	Is not set monotone. Includes redundant phylogenetic branch length.	Rao 1982, Hardy & Senterre 2007
Average taxonomic distinctness (*AvTD*)	Average pairwise distance between all taxa in a community weighted by abundance.	$\sim$phylogenetic dispersion	Is not set monotone. Includes redundant phylogenetic branch length. Decreases in range with increasing numbers of species.	Clarke & Warwick 1998
Net relatedness index (*NRI*)	The standardised effect size of *MPD* x -1.	$\sim$phylogenetic dispersion	Includes redundant phylogenetic branch length.	Webb *et al.* 2002
Nearest Taxon Index (*NTI*)	The standardised effect size of *MNND* x -1.	$\sim$phylogenetic dispersion	Includes redundant phylogenetic branch length.	Webb *et al.* 2002
Pairwise functional evenness (*pFEve*)	The degree to which the relative abundance of entities represents the average proportion of functional distance between them and all other entities in the assemblage, expressed as a proportion of the maximally dominant community.	functional evenness	Includes redundant variation.	This study
Pairwise phylogenetic evenness (*pPEve*)	The degree to which the relative abundance of entities represents the average proportion of phylogenetic distance between them and all other entities in the assemblage, expressed as a proportion of the maximally dominant community.	phylogenetic evenness	Includes redundant phylogenetic branch length.	This study

Table 2.1: (continued on next page)

Table 2.1: (continued)

Measure	Basis	Component	Weakness	Reference
Pairwise functional redundancy (*pFRed*)	One minus the sum of all near neighbour distances divided by the sum of all furthest neighbour distances.	functional redundancy	Includes redundant phylogenetic branch length.	This study
Pairwise phylogenetic redundancy (*pPRed*)	One minus the sum of all near neighbour distances divided by the sum of all furthest neighbour distances.	phylogenetic redundancy	Includes redundant phylogenetic branch length.	This study
Multidimensional				
Convex hull volume / functional richness (*FRic*)	The volume of the minimum convex polygon encompassing the set of species on n orthogonal niche dimensions.	functional richness	Does not capture all variation when the number of traits exceeds the number of entities in the community.	Cornwell *et al.* 2006, Villeger *et al.* 2008
Functional evenness (*FEve*)	The regularity of spacing between species in the volume of trait space calculated by down-weighting the branches in a minimum spanning tree by the abundances of the species they join. Maximized when all weighted branches are equal.	∼functional evenness	Only takes nearest neighbours into account.	Villeger *et al.* 2008
Functional divergence (*FDiv*)	The spread of abundance within the volume of trait space calculated by comparing the abundance weighted deviances of species from the centroid calculated independently of abundances.	∼functional dispersion + ∼functional evenness	Decreases in range with increasing numbers of species.	Villeger *et al.* 2008
Functional dispersion (*FDis*)	Average distance of species to the abundance weighted centroid of the community functional space.	∼functional richness ∼functional dispersion	Is not set monotone. Decreases in range with increasing numbers of species.	Laliberte & Legendre 2010

2.4 New measures of evenness and redundancy

2.4.1 Functional and phylogenetic evenness

Greater evenness among functional or phylogenetic entities in an assemblage is achieved as the relative abundance of each entity approaches the proportion of unique functional variation or evolutionary history it contributes to the assemblage. Cadotte *et al.* (2010) developed a series of measures of phylogenetic evenness based on this principal that could equally be used to measure functional evenness. Phylogenetic abundance evenness (*PAE*; see Table 2.1 for details) measures the degree to which the distribution of abundances among terminal branches in the phylogeny represents the relative length of each branch, ignoring internal (shared) branches. The phylogenetic measure of imbalance of abundance at higher clades (*IAC*) measures deviation in the distribution of abundance among branch tips from the expectation that abundance is split equally across each node in the tree, ignoring branch length. In other words, the expected abundance at each individual tip is $nj = N/2^{vj}$ where vj is the number of nodes between the tip j and the root (including the root) of a fully resolved sample tree. Lastly, Cadotte *et al.* (2010) derived H_{AED} and E_{AED} which are equivalent to Shannon's *H'* and Pielou's (1966) *J*, but substituting the abundance of each species and the total community abundance with abundance weighted evolutionary distinctness and Faith's (1992) *PD*, respectively (see Table 2.1).

I propose a new hierarchical measure of functional or phylogenetic evenness that is explicitly based on the length of both internal and terminal branches. If evenness is maximized when abundance is split across each node in the sample tree (including only the species in the assemblage) in proportion to the sum of the branch length on either side of the split, then the expected abundance n at each tip *j* of the tree is:

$$n_j = N * \prod\nolimits_{i=0}^{m} b_i \tag{2.1}$$

where *N* is the total number of individuals in the assemblage, *b* is the proportion of the branch length descended from the node (i.e. towards the tips) that lies on the same side of the node as the tip, 1 is the basal node, *i* is the node immediately subtending the tip and *m* is the number of nodes joining the tip to the root, including the root (see Figure 2.2). An hierarchical index of evenness that ranges between near zero (low evenness) and 1 (high evenness) can then be calculated as:

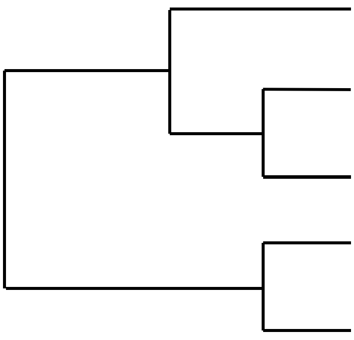
$$hEve = 1 - \frac{\sum_{j=1}^{s} \Delta T_j}{1 + Max(\sum_{j=1}^{s} \Delta T_j)} \tag{2.2}$$

where ΔT_j is the absolute difference between the observed and expected (Equation 2.1) abundance at tip *j*. Equation 2.2 expresses evenness in the assemblage as one minus the deviation of abundances from expected expressed as a proportion of the maximum possible deviation from the expected. One is added to the maximum sum of absolute differences in the denominator $Max(\sum_{j=1}^{s} \Delta T_j)$ so that the index can never be zero. The maximum sum of differences is calculated by comparing the expected abundances with the assemblage that is least even (i.e. maximally dominant). This is the assemblage where the observed abundances are distributed such that all species are allocated a single individual, and remaining individuals are allocated to the species with the lowest expected abundance. Where more than one species shares the minimum expected abundance, as is commonly the case for pairs of sister species, all individuals are randomly allocated to one or the other. Equation 2.2 can be used to calculate evenness based on trees without branch length by making the expected abundance at each tip $n_j = N/2^{vj}$ where vj is the number of nodes between the tip j and the root (including the root) of a fully resolved sample tree. It can also be used to calculate a pairwise distance matrix measure of functional or phylogenetic evenness (*pEve*) by making the expected optimum abundance of each entity $n_j = Sd_j/Gd * N$ where Sd_j is the sum of all pairwise distances from our focal species to all other species, Gd is the sum of all pairwise distances between all species pairs, and N is the number of individuals in the assemblage.

2.4.2 Functional and phylogenetic redundancy

There are currently no explicit measures of within-assemblage functional or phylogenetic redundancy. A hierarchical measure of within-assemblage redundancy can be calculated as the sum of each of the redundant branch lengths multiplied by the number of entities that share them, expressed as a proportion of itself plus the total length of unique branches:

$$hRed = \frac{\sum_{i=1}^{R}(n_i * r_i)}{\sum_{i=1}^{R}(n_i * r_i) + \sum_{j=1}^{U} u_j} \tag{2.3}$$



O	E	ΔT_j	Max O	Max ΔT_j
24	28	4	1	27
24	21	3	116	95
24	21	3	1	18
24	25	1	1	24
24	25	1	1	24
120	**120**	**12**	**120**	**188**

$\nwarrow \sum_j \Delta T_j$

$\nwarrow \mathrm{Max}[\sum_j \Delta T_j]$

$hEve = 1 - \sum_j \Delta T_j / (1 + \mathrm{Max}\,[\sum_j \Delta T_j])$

$hEve = 1 - 12/(1 + 188)$

$hEve = 0.9365$

Figure 2.2: Illustration of the calculation of hierarchical evenness. The observed (O) abundance values for the five species in the community have been set so as to maximize traditional species evenness (i.e. all species have equal abundance). The expected (E) values are calculated using the equation $n_j = N * \prod_{i=0}^{m} b_i$ (see text for details) and represent the expected values should the abundance of each species represent its unique functional variation or evolutionary history (branch length). The sum of the absolute values of the differences between the observed and expected abundances $(\sum_{j=1}^{s} \Delta T_j)$, and the sum of the absolute values of the differences between the hypothetical maximally dominant distribution of abundances $(MaxO)$ and the expected $(Max(\sum_{j=1}^{s} \Delta T_j))$, are used to calculate *hEve*.

Here r_i is the ith redundant branch length, n is the number of species sharing that branch, u_j is the jth unique branch length, and R and U are the number of redundant and unique branches respectively. This index ranges from zero (no redundancy) to near one (almost complete redundancy). *hRed* can never equal one because all species add at least some unique branch length.

The functional or phylogenetic redundancy of an entity in a pairwise distance matrix is best expressed as one minus the minimum distance $(Min[d_j])$ to any other entity in the assemblage divided by the maximum distance to any other entity in the assemblage $(Max[d_j])$, because two entities become more redundant as the functional difference or phylogenetic distance between them decreases. The redundancy of the assemblage calculated using pairwise distances (*pRed*) can then be expressed as one minus the sum of all minimum distances divided by the sum of all maximum distances $(1 - \sum_{j=1}^{S} Min(d_j) / \sum_{j=1}^{S} Max(d_j))$. Both *hRed* and *pRed* increase with an increase in the number of entities in an assemblage or a decrease in the dispersion among those entities.

2.5 Exploring relationships between measures

There is currently little structure to the plethora of measures used in studies of functional and phylogenetic diversity. To allow comparison between studies that measure different diversity components or use different methodological approaches we require an understanding of the relationships between measures. I investigated the relationships between components of functional and phylogenetic diversity and assessed the consistency between methodological approaches in measuring each component by comparing a large set of measures on a simulated dataset. Twenty thousand assemblages, 1000 of each of 20 species richness categories (5 to 100 in increments of 5), were drawn from a pool of 150 species with a lognormal metacommunity abundance distribution. A random ultrametric tree representing phylogenetic relationships between species was generated, and species values for each of 10 traits were sampled from normal distributions. All functional diversity measures were calculated using Euclidean distance and trait values standardized to a mean of zero and unit variance. All measures except the evenness measures and Shannon's *H'* were calculated from a presence/absence community data matrix (i.e. excluding relative abundances) such that they each measure only one component of diversity. Multidimensional functional diversity measures were based on the first four axes (one less than the minimum species richness) derived from a principal components analysis according to the method of Laliberte & Legendre (2010). There was no collinearity in the trait data because values were generated independently (maximum $R^2 = 0.09$). Species were assigned to 16 functional groups based on shared height on a dendrogram generated using UPGMA according to the method of Laliberte & Legendre (2010).

Functional and phylogenetic diversity measures were first compared across all 20

000 assemblages using Spearman's rank-order correlation to negate the need for data transformation. These results were summarized into dendrograms (Figures 2.3 & 2.4), using correlation coefficients (ρ) as similarity measures and clustering the diversity measures using UPGMA. Since many of these relationships are autocorrelated due to their co-dependence on species number, however, it was necessary to exclude the effect of species number by comparing each measure separately for each species richness category. Figures 2.3 & 2.4 illustrate a series of line plots comparing Spearman's ρ (ranging from -1 to 1) between each pair of measures across the range of species richness values. A horizontal line in mid Y-axis of each line plot represents no relationship ($\rho = 0$) between two measures across all species richness', while a horizontal line near the top (e.g. comparison of H' and J) or bottom of the frame indicates a strong positive or negative relationship respectively. P-values are not reported and correlation coefficients are not dependent on sample sizes because all species richness categories comprise 1000 assemblages. All analyses were performed in R 2.9.2 (R Development Core Team 2010) using the 'ape' (Paradis *et al.* 2004), 'picante' (Kembel *et al.* 2010), 'FD' (Laliberte & Legendre 2010) and 'ecoPD' packages (Cadotte *et al.* 2010).

Most measures of functional and phylogenetic diversity were highly correlated with species number (Figures 2.3 & 2.4). However, when comparisons were corrected for differences in species number, many of the relationships were no longer evident (Figures 2.3 & 2.4). This highlights the importance of considering autocorrelation between measures due to co-dependence on species number.

Following correction for species richness, three independent groups of inter-correlated measures were identified which correspond to measures of evenness, redundancy and richness + dispersion (Figures 2.3 & 2.4). The richness and dispersion measures are further segregated into measures that correlate with species richness (richness measures) and those that do not (dispersion measures). These results suggest that different methodological approaches exist for quantifying the same diversity components.

Faith's (1992) phylogenetic diversity (PD) correlated poorly with Shannon's H' weighted by evolutionary distinctness (H_{AED}) when corrected for species richness, implying that one is an inappropriate measure of phylogenetic richness. PD fits the predictions for a measure of richness in that it is positively correlated with measures of dispersion and negatively correlated with measures of redundancy when species richness is accounted for. H_{AED} on the other hand showed mostly positive relationships with measures of both

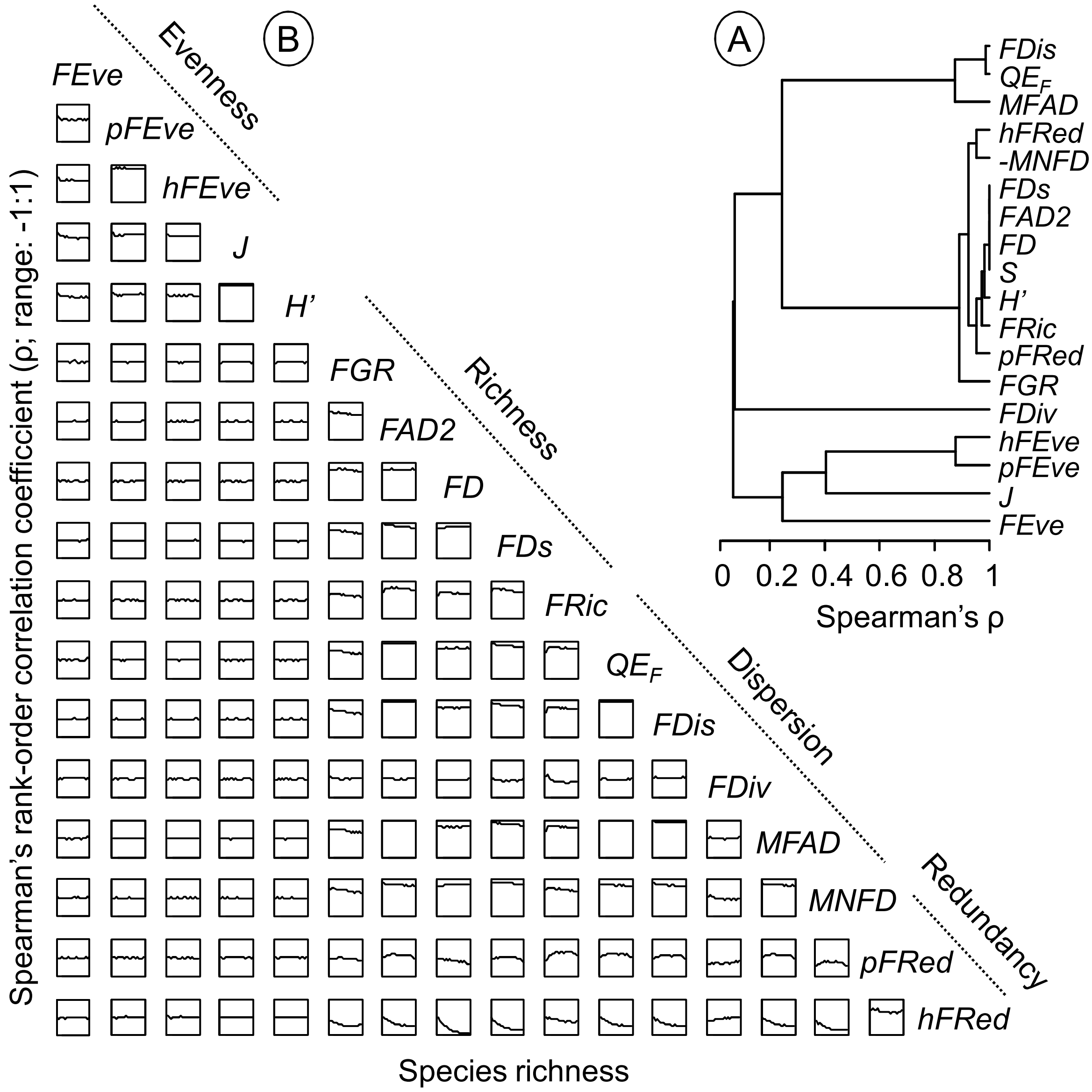


Figure 2.3: Comparison of functional diversity measures. Full names, formulas and sources are given in Table 2.1. A) Relationships between measures for all species richness' (5-100), clustered according to their Spearman's rank-order correlation coefficients. B) Line plots for each pair of metrics illustrating the Spearman's rank-order correlation coefficients (ranging from -1 to 1) for each of 20 species richness categories (5-100). Measures are grouped by the diversity component they are proposed to measure. Note that richness and dispersion measures are highly correlated because the effect of species richness has been removed. See text for details.

dispersion and redundancy when corrected for species number, implying that it is not an appropriate measure of richness. Results were qualitatively identical when H_{AED} included species abundances.

Most measures of dispersion where strongly correlated when corrected for species richness, although the mean nearest taxon distance (*MNTD*) and mean nearest functional entity distance (*MNFD*) were less strongly correlated with the rest. These measures were negatively related to species richness, because they average near-neighbour distances (phylogenetic or functional) which become smaller as more taxa are included in the phylogenetic tree or in multivariate functional space, and showed strong negative correlation with measures of redundancy. This increase with increasing richness and decrease with increasing dispersion make *MNTD* and *MNFD* good proxies for redundancy. The functional divergence (*FDiv*) metric of Villeger *et al.* (2008) showed weak negative correlations with *pFRed* and *hFRed* only and it is unclear what this metric measures when it is based on presence/absence data only.

Matching predictions, measures of redundancy were positively related to species richness (Figures 2.3 & 2.4) and negatively related to dispersion (Figures 2.3 & 2.4). The phylogenetic measures of pairwise and hierarchical redundancy are more highly correlated than their functional counterparts, likely due to distortion of the pairwise functional relationships between species when they are linked into a dendrogram to allow the calculation of hierarchical functional redundancy.

All measures of functional and phylogenetic evenness were correlated with each other but showed no relation to species number. Shannon's *H'* became equivalent to Pielou's measure of species evenness (*J*) when corrected for species number. Both measures showed weak positive relationships to most functional and phylogenetic evenness measures and strong negative relationship to the imbalance of abundances at the clade level (*IAC*), low values of which indicate high evenness. These results highlight the fact that measures of functional or phylogenetically weighted evenness are more or less similar to traditional measures of species evenness depending on whether they include branch lengths or distances between entities. Specifically, the *IAC* is most similar to *J* because its calculation is based on the number of nodes and balance in the phylogenetic tree and does not take branch lengths into account. In general, hierarchical and pairwise measures of evenness may be expected to become more similar to categorical measures when the relationships between entities become more equal, reflecting equivalent categories. For hierarchical measures this occurs

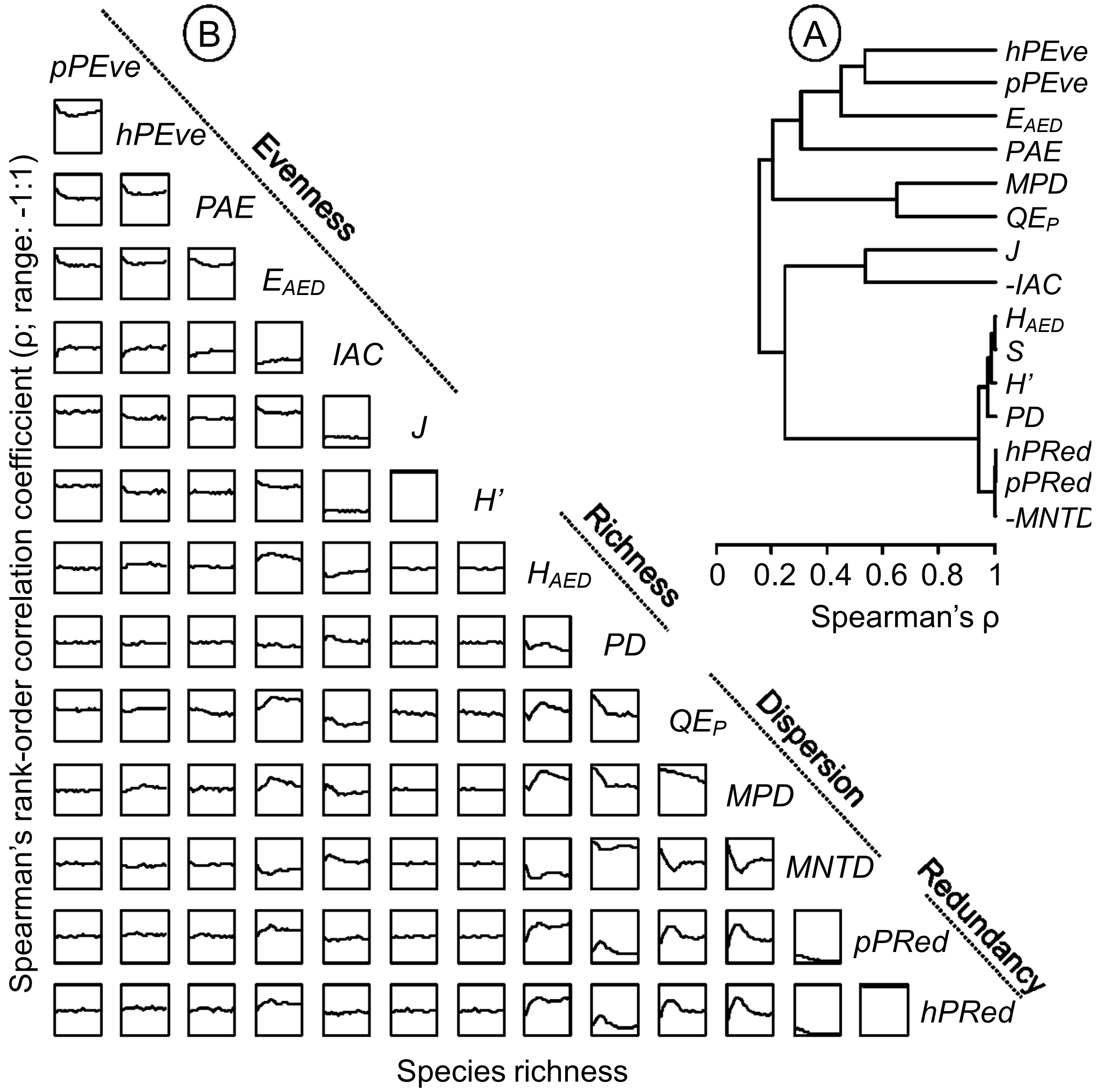


Figure 2.4: Comparison of phylogenetic diversity measures. Full names, formulas and sources are given in Table 2.1. A) Relationships between measures for all species richness' (5-100), clustered according to their Spearman's rank-order correlation coefficients. B) Line plots for each pair of metrics illustrating the Spearman's rank-order correlation coefficients (ranging from -1 to 1) for each of 20 species richness categories (5-100). Measures are grouped by the diversity component they are proposed to measure. See text for details.

when the tree topology becomes more balanced, or when branch nodes are clustered deep in the tree.

In all, the analysis confirms that there is much autocorrelation between measures due to their co-dependence on species richness, and that richness, dispersion, evenness and redundancy are unique components of functional and phylogenetic diversity which may be quantified using various methods. Measures of evenness scale on a continuum from categorical species evenness to evenness weighted by contribution to unique function or evolutionary history depending on how the metric is calculated. The relationships between measures within each component are likely to be dependent on the relationships between species (i.e. tree shape and branch lengths or the distribution of pairwise distances).

Although each diversity component may be quantified using different methodological approaches, there are a number of pros and cons associated with each approach. Table 2.1 indicates the full list of measures, which component of diversity they quantify or attempt to quantify, and potential weaknesses of each. Some general issues are apparent. Firstly, pairwise measures do not represent unique variation within sets of species because there is often much redundancy in the distances between species pairs in terms of shared branch length or variation in multivariate space (Petchey & Gaston 2006). This 'double counting' gives greater weight to the set of larger pairwise distances, down-weighting the importance of more closely related or functionally similar entities. Pairwise measures of diversity are not necessarily erroneous, but should be treated with caution until the effects of 'double counting' have been more thoroughly explored. Secondly, the range of values that can be attained using most measures of dispersion is highly dependent on the number of species in the assemblage, the range decreasing as the number of species increases, making comparison between assemblages of differing species richness difficult. This dependence on species richness can be reduced by calculating the standardized effect size or 'z-transforming' the dispersion measures (e.g. *NRI* and *NTI*, Webb *et al.* 2002), though some dependence on species richness may remain.

2.6 Conclusions

The vast number of measures that are used to quantify functional and phylogenetic diversity can be categorized by the diversity component or set of components they quantify and the statistical approach upon which they are based. Richness, dispersion, evenness and

Table 2.2: The components of diversity, their definition, application and example metrics

Component	Definition	Information required	Application	Metrics
Richness	The total amount of unique functional variation or evolutionary history in an assemblage.	Relationships among species or entities. Community samples.	Any application that traditionally used species richness, including for setting conservation priorities (Faith 1992) and testing biodiversity-ecosystem function hypotheses (Petchey & Gaston 2002, 2006).	Functional: *Functional group richness, FD, FDs FAD2, Fric*: Phylogenetic: *Species richness, PD*
Dispersion	The unique functional or phylogenetic differences between species in an assemblage, or that component of richness unexplained by species number.	Relationships among species or entities. Community samples.	Testing for the influence of local or regional processes on the assembly of communities by testing for phylogenetic or functional clustering or overdispersion (Webb *et al.* 2002). Testing for the influence of relatedness or functional similarity among species on ecosystem function (Heemsbergen *et al.* 2004).	Functional: *MFAD, FDis*; Phylogenetic: *MPD (NRI)*
Evenness	The parity in the distribution of the abundances among species with different trait values or phylogenetic position.	Relationships among species or entities and their relative abundance. Community samples.	Can be used to test for biodiversity effects on ecosystem function (Vile *et al.* 2006, Hillebrand *et al.* 2008). Allows investigation of local and regional community assembly processes (Hardy 2008). Allows investigation of subtler changes in assemblages (Hillebrand *et al.* 2008).	Functional: *hFEve, pFEve, Feve*; Phylogenetic: *PAE, IAC*, E_{AED}, *hPEve, pPEve*
Redundancy	The overlap in function or shared evolutionary history of a group of species in an assemblage.	Relationships among species or entities. Community samples.	Provides a measure of the potential reliability and stability of an ecosystem through its influence on ecosystem resistance and resilience (Diaz & Cabido 2001). Provides a measure of the vulnerability of an assemblage to losing unique functional or phylogenetic diversity (Faith 1992).	Functional: *hFRed, pFRed*; Phylogenetic: *hPRed, pPRed*

redundancy make up the components of the functional or phylogenetic diversity of an assemblage, and can be measured using categorical, hierarchical, pairwise or multidimensional approaches. Each of these components has proven useful for investigating the distribution, origin and maintenance of various forms of life, deciphering how species and assemblages influence and respond to ecosystem processes and functions, and helping to set priorities for the conservation of threatened species and ecosystems. The use of a common set of diversity measures and an understanding of how the various measures relate to each other will hopefully improve communication and collaboration between researchers and practitioners in the fields of evolutionary biology, community ecology, functional ecology and conservation biology, and lead to a holistic understanding of biological landscapes that will aid in their conservation and management.

Tillers of *Tetraria involucrata* resprouting after fire, Tierkop, Outeniqua mountains.

3 Measuring phylogenetic dispersion and testing for phylogenetic structure

3.1 Abstract

In the context of community ecology, phylogenetic information provides an easy-to-measure proxy for the integrated functional differences between species, and explicitly links local ecological processes with trait evolution and biogeography. Recognition of this point, along with technological advances and increasing availability of phylogenetic and community data, has led to a rapid proliferation of community phylogenetic studies (phyloecology) and associated methodological approaches. I review and test a range of methods that compare dispersion between communities of organisms and test for significant phylogenetic structuring, highlighting pitfalls and important considerations. Careful sampling design and choice of statistical method and null model are required to determine which factors influence phylogenetic structure within and between communities. All metrics that measure phylogenetic dispersion are sensitive to community species number, necessitating the use of a nonparametric rank-order standardization to compare dispersion between communities of different species richness, and rendering the use of null models which do not hold community species richness constant inappropriate. The power of methods to detect significant phylogenetic structuring depends on species pool size, whether abundance or presence/absence data are used, the branching architecture of the phylogenetic tree relating the species concerned, and constraints on the null model. Metric approaches are poor at detecting phylogenetic evenness, and perform worse when phylogenetic trees are well balanced. By contrast, linear regression approaches detect both evenness and clustering well when used in combination with a suitable measure of species co-occurrence, except when trees are highly skewed. A trade-off between power to detect pattern and the ability to tease apart the various influences that affect the pattern, and dependence of metrics and co-occurrence coefficients on tree shape, suggest the need for improved statistical methods. I provide a guide to assist the choice of appropriate methods to compare levels of phylogenetic dispersion amongst communities or to test for the existence of significant phylogenetic

structure.

3.2 Introduction

The study of community assembly examines the processes that determine the distribution and co-occurrence of individuals and species in communities, with reference to their functional traits and phylogenetic relationships (Diamond 1975, Weiher & Keddy 1999, Webb *et al.* 2002).

Due to the effects of competition and habitat filtering (including effects of mutualists, facilitation, predators, pathogens and prey availability; Weiher & Keddy 1999), co-occurring species may be more or less similar than expected by chance, in terms of their functional attributes. Moreover, where close relatives share similar functional traits, this may lead to significant phylogenetic structuring of communities (Webb *et al.* 2002). By extension, trait conservatism may validate the use of phylogenetic relatedness as a proxy for the functional differences between species, bypassing the need for exhaustive measurement of all functional traits (Faith 1992). This is useful because it is seldom known with certainty which traits are most important in determining community structure. Paired with its utility as a link between ecological and biogeographical-evolutionary scales, this has led to the recognition of phylogenetic information as a valuable tool in community ecology (Webb *et al.* 2002, Cavender-Bares *et al.* 2009). The result is the emergence of a new field termed ‘community phylogenetics’ or ‘phyloecology’ (Webb *et al.* 2002, Vamosi *et al.* 2009, Cavender-Bares *et al.* 2009) which incorporates phylogenetic information in the study of community assembly. Despite its short history, phyloecology has contributed significantly to our understanding of ecological interactions, trait evolution, and the influence of adaptation, speciation and biogeography on the assembly and structure of contemporary communities (see Losos 1996, Webb *et al.* 2002, Emerson & Gillespie 2008, Vamosi *et al.* 2009, Cavender-Bares *et al.* 2009 for review).

The rapid expansion of phyloecology has led to a proliferation of methodological approaches (Webb *et al.* 2002, Cavender-Bares *et al.* 2004, Lovette & Hochachka 2006, Slingsby & Verboom 2006, Helmus *et al.* 2007*a*,*b*, Hardy & Senterre 2007, Hardy 2008), these being geared either towards comparing the degree of phylogenetic dispersion shown by different communities (e.g. Snyder *et al.* 2007, Bryant *et al.* 2008), or testing whether the degree of dispersion within communities differs significantly from chance expectation (phylogenetic structure; Hardy 2008). In a similar vein, these methods have also been used to evaluate dispersion in functional trait values within and amongst communities (Slingsby & Verboom 2006, Kraft *et al.* 2008, Cornwell & Ackerly 2009, Ingram & Shurin 2009, Swenson & Enquist 2009).

In this chapter, I review and evaluate the principal methods that are used in phyloecology. Firstly, I survey the range of statistical approaches used to quantify phylogenetic dispersion and test for phylogenetic structure, exploring their advantages and limitations by testing their performance on simulated datasets. I then discuss issues of sampling design and the use of null models and the manner in which they can affect statistical power and impact on analytical outcomes.

3.3 Evaluating phylogenetic dispersion and testing for structure

Phylogenetic dispersion is currently evaluated in three ways which I term the ‘metric’, ‘correlative’ and ‘turnover’ approaches. The metric approach quantifies dispersion among species or individuals within single communities using a metric of dispersion, based on either the average phylogenetic distance between species (Webb *et al.* 2002, Hardy & Senterre 2007) or the sum of the branch length connecting species on the phylogenetic tree (Faith 1992). Where a community is to be tested for significant phylogenetic structure, this is typically achieved by comparing its metric value against a distribution in which each value reflects the level of structure within a ‘random’ community, generated under an appropriate null model (Figure 3.1). In contrast to the metric approach, correlative methods evaluate dispersion across multiple communities, testing whether pairwise species’ co-occurrence is a function of their phylogenetic relatedness (Cavender-Bares *et al.* 2004). This is often achieved using a randomized null, similar to that used for metric approaches. Here, however, the use of randomization procedures is also necessary because non-independence of species pairs (Mantel 1967) and expectation of triangular, rather than linear, relationships (Slingsby & Verboom 2006) violate the assumptions of ordinary least squares regression. While correlative methods have the benefit of being able to incorporate species abundances, as well as any spatial, temporal or environmental correlations among sites/communities (Helmus *et al.* 2007*b*), they do not permit an evaluation of structure within single communities. They also ignore the potential effects of positive or negative interactions with more than one species within each community because co-occurrence is treated in a pairwise manner, and co-occurrence estimates involving rare species are often poor, potentially biasing results

(Slingsby & Verboom 2006). Finally, the turnover approaches use an index, such as *UniFrac* (Lozupone & Knight 2005) or *PhyloSor* (Bryant *et al.* 2008), to quantify turnover, from one community to another, in the total phylogenetic branch length connecting species within each community (i.e. turnover in the phylogenetic diversity metric of Faith (1992)). Phylogenetic turnover may also be quantified by some metric approaches, through additive partitioning of within-community versus between-community phylogenetic diversity (e.g. Hardy & Senterre 2007, Chave *et al.* 2007).

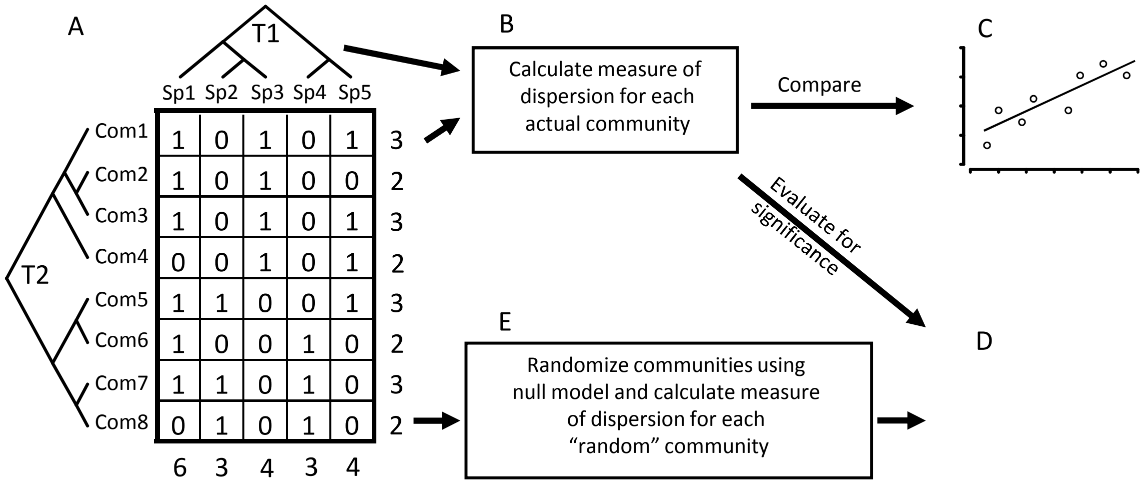


Figure 3.1: The steps involved in measuring phylogenetic dispersion amongst communities, and testing for significant phylogenetic structuring within a community or set of communities. The set of communities must be sampled resulting in a data set (A) comprising a species by site (community) data matrix (presence/absence matrix shown here) and the various community properties that may influence phylogenetic structure. Row sums represent species richness per community; column sums represent species prevalence (occupancy) across sites. Tree T1 represents the phylogenetic or functional relationships between species and tree T2 represents the spatial or temporal separation or environmental similarity between sites. The degree of phylogenetic dispersion between co-occurring species is quantified using an appropriate measure (B). One can then compare the measure of dispersion between communities (C), or test for significant phylogenetic clustering or evenness by comparing the observed measure against a distribution of expected values (D) generated using a null model (E). Where a correlative approach is used the relationship between co-occurrence and phylogenetic distance between species pairs is established at step B and compared against a distribution of relationships derived from sets of null communities at step D. Null models operate by shuffling cells in the matrix or shuffling species or communities on the tips of the trees with various constraints (see Table 3.1).

Table 3.1: Some null models and the properties of the community dataset that they retain

Null model	Operation	Species richness within sites	Species prevalence across sites	Total abundance within sites ϕ	Total abundance within species ϕ	Spatial co-occurrence between species	Spatio-temporal autocor-relation between sites	Environmental correlation between sites	Functional or phylogenetic relationships between species
Sites and species	This is when the matrix is shuffled by species (within columns) and by sites (within rows) equiprobably. It can be done with or without replacement.	✗	✗	✗	✗	✗	✗	✗	✓
Sites within species	Cells within columns (species) in the matrix are shuffled equiprobably.	✗	✓	✗	✓	✗	✗	✗	✓
Species within sites	Cells within rows (sites) in the matrix are shuffled equiprobably.	✓	✗	✓	✗	✗	✗	✗	✓
Independent swap (within sites)	Two pairs of cells are swapped between two species (columns).	✓	✓	✓	✗	∼	✗	✗	✓
Independent swap (within species)	Two pairs of cells are swapped between two sites (rows).	✓	✓	✗	✓	✗	✗	✗	✓
Independent swap (weighted by spatial relationship - within species or sites)	Two pairs of cells are swapped between two species or sites but the probability of swapping is down-weighted by increasing spatial distance between them.	✓	✓	✓$*$	✓$^{\psi}$	∼$*$	∼	✗$^{\triangle}$	✓

Table 3.1: (continued on next page)

Table 3.1: (continued)

Independent swap (weighted by environmental similarity - within species or sites)	Two pairs of cells are swapped between two species or sites but the probability of swapping is down-weighted by decreasing environmental similarity between them.	✓	✓	✓*	✓$^{\psi}$	∼*	✗	∼	✓
Independent swap (weighted by spatial and environmental relationships - within species or sites)	Two pairs of cells are swapped between two species or sites but the probability of swapping is down-weighted by increasing distance and decreasing environmental similarity between them.	✓	✓	✓*	✓$^{\psi}$	∼*	∼	∼	✓
Phylogeny shuffle	Taxa are shuffled on the tips of the phylogeny.	✓	✗	✓	✗	✗	✓	✓	✗1
Phylogeny shuffle within prevalence classes	Taxa with similar prevalence across sites are shuffled on the tips of the phylogeny.	✓	∼	✓	✗∼	✗	✓	✓	✗2
Phylogeny shuffle within abundance classes	Taxa with similar abundances are shuffled on the tips of the phylogeny.	✓	✗∼	✓	∼	✗	✓	✓	✗3
Phylogeny shuffle within prevalence and abundance classes	Taxa with similar prevalence across sites and abundances are shuffled on the tips of the phylogeny.	✓	∼	✓	∼	✗	✓	✓	✗4
Phylogeny shuffle constrained to clades	Only taxa within a particular clade or clades are shuffled.	✓	✓	✓	✓	✗	✓	✓	∼5

Notes:

Table 3.1: (continued on next page)

Table 3.1: (continued)

✓ retained

✗randomized

ϕ excluded when using P/A data (and when P/A data are independent of metacommunity abundance see text)

$*$ when shuffled within sites

ψ when shuffled within species

$\sim$ partially maintained

Δ environmental correlation is likely to be retained if environmental conditions are spatially correlated

1 randomizes the relationships between species, breaking down all association between relatedness and the 7 other properties of the community dataset

2 randomizes relationships between species, but retains some association between relatedness and species prevalence (and abundance if correlated with prevalence)

3 randomizes relationships between species, but retains some association between relatedness and species abundance (and prevalence if correlated with abundance)

4 randomizes relationships between species, but retains some association between relatedness and species prevalence and abundance

5 randomizes relationships between a select group of species - can be adapted to include any of the other phylogeny shuffle options

Measures of phylogenetic dispersion within a 'local' community may depend on the number, identity and relative abundance of each species. These properties are determined by 'local' processes, such as competition and habitat filtering, and 'regional' processes, such as migration of species from the broader meta-community. For example, species that are more abundant in the broader meta-community are more likely to occur within a local community and to show greater relative abundances. Similarly, they are likely to occur in a larger number of local communities across the landscape (greater prevalence or occupancy). Thus, if the abundance of species in the meta-community is phylogenetically structured (e.g. due to biogeographic history), this can lead to phylogenetic structure in species co-occurrence and relative abundance within local communities, or their prevalence across a set of communities, even in the absence of local structuring processes (Hardy 2008, Kembel 2009). In addition, through their effect on local ecological processes and migration, the spatial and temporal proximity of two or more communities, as well as their environmental similarity, will influence how similar they are in terms of species composition, abundance and, consequently, phylogenetic dispersion (Helmus *et al.* 2007*b*, Bryant *et al.* 2008). Evaluating the relative influence of local and regional processes thus requires knowledge of a number of 'community properties,' including the number and relative abundance of species within and across communities, species' prevalence across communities, species' co-occurrence within communities, and any spatio-temporal and environmental associations between communities (Figure 3.1, Table 3.1, Hardy 2008).

Some of these community properties are expected to affect some community-structuring processes more than others, and determining which processes have been most influential in shaping community structure is typically achieved by evaluating the effect, on phylogenetic dispersion, of varying one or more of these properties, while keeping the remainder constant. This requires three things: i) sampling communities in such a way that all community properties are either quantified or held constant; ii) using a measure of dispersion that incorporates the property or properties of interest, while controlling for those that are not; and, iii) where one is testing for phylogenetic structure, using a null model that randomizes the properties of interest while maintaining any pattern in the remainder. Unfortunately, there are some impediments to fulfilling these requirements. Firstly, effective sampling is constrained by the large quantities of data required. In practice, ecologists are often forced to collate data that have been sampled in different ways, to utilize existing data with inappropriate or partially known sampling designs, or to limit their sampling strategy according to financial or logistical constraints. Secondly, correct use of dispersion measures and null models is limited by poor understanding of their behavior and their sensitivity to community properties (but see Kraft *et al.* 2007, Hardy 2008, Schweiger *et al.* 2008, Kembel 2009). Finally, we probably still do not appreciate the full range of factors that affect phylogenetic dispersion, limiting our ability to take them into account (Hardy 2008, Kembel 2009).

3.3.1 Dependence of dispersion measures on tree shape and species number

A large array of metrics has been developed to measure within-community phylogenetic dispersion (see Table 3.2 for a list of metrics and their calculation). Some of these metrics are highly dependent on community species richness and phylogenetic tree shape (Schweiger *et al.* 2008), with consequences for tests of phylogenetic structure (Kraft *et al.* 2007). Figure 3.2 displays the distributions of six commonly used metrics for a sample of all possible presence/absence communities derived from the three 16-species trees of different shape shown in Figure 3.3.

The means and/or variances of all metrics evaluated vary with community species richness and tree shape. Thus, if communities are compared using one of these metrics, differences in species richness will influence the comparison. This is particularly a problem where phylogenetic structure is evaluated using a null model that does not preserve species richness within communities (see Table 3.1). For example, comparison of the MPD metric for a two species community against random communities of varying species number is likely to reveal significant clustering or evenness (Figure 3.2). Conversely, a species rich community will rarely reveal significant phylogenetic structure when compared against communities of only two species. Use of null models which do not retain species number should be avoided unless explicitly justified.

The net relatedness index (NRI) and nearest taxon index (NTI) metrics were developed to permit comparison of dispersion between communities of different species richness (Webb *et al.* 2002). These metrics appear to control for the effect of species richness since they are, respectively, the standardized effect size of MPD and MNND. Unfortunately, despite homogeneity of their means and variances, the margins of their distributions still vary with community species richness, often positively (Figure 3.2), with the result that comparisons may be systematically biased by species richness.

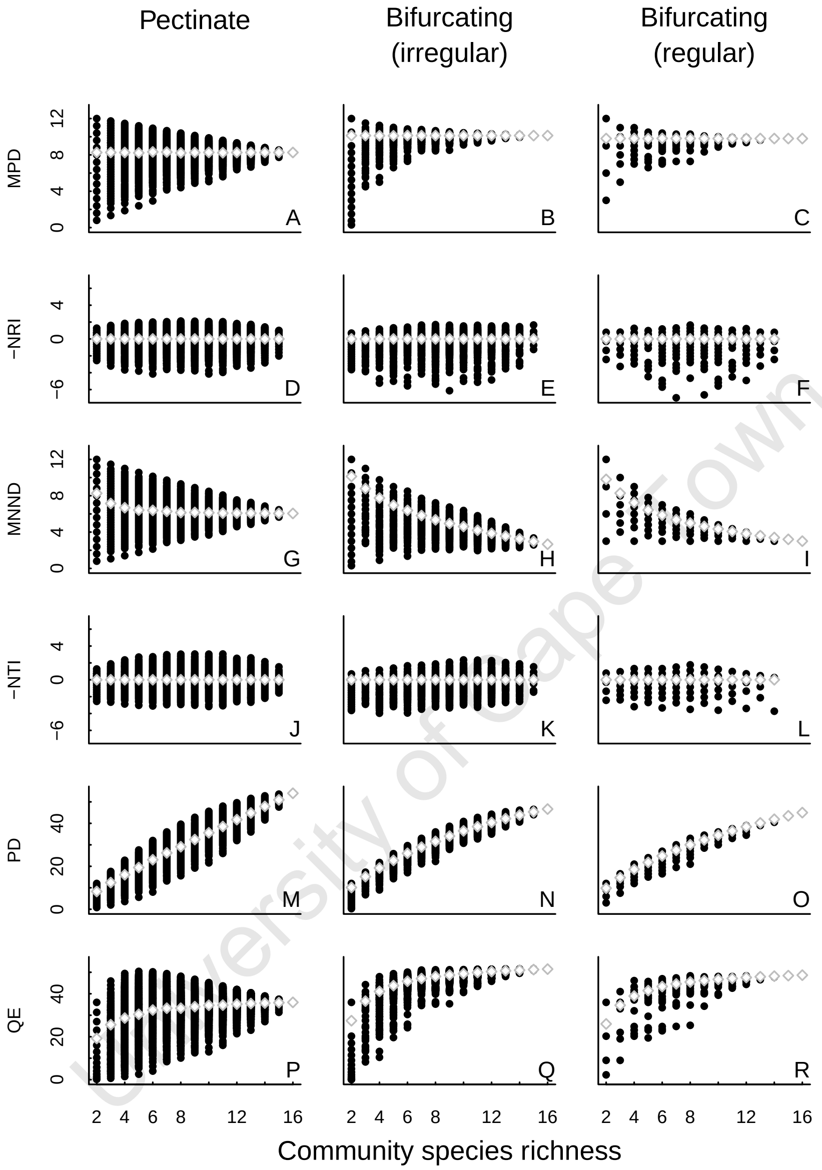


Figure 3.2: The distribution of values of six phylogenetic relatedness metrics for all possible communities of 2 to 15 species derived from the three phylogenetic trees in Figure 3.3, see text and Appendix A for details. MPD: mean phylogenetic distance, NRI: net relatedness index, MNND = mean nearest neighbor (taxon) distance, NTI: nearest taxon index, PD: phylogenetic diversity, QE: Raos quadratic entropy using phylogenetic distances.

Table 3.2: Metrics that measure phylogenetic relatedness and their properties

	Basis	Formula	Mean independent of species number?	Range independent of species number?	Can include abundance?	Includes frequency across sites?	Reference
Metric approaches							
Average taxonomic distinctness (AvTD)	Average pairwise taxonomic distance between all taxa in a community	$\sum d_{ij}/np$	Yes	No	Yes	No	(Clarke & Warwick 1998)
Mean pairwise distance (MPD)	Average pairwise phylogenetic distance between all taxa in a community	$\sum d_{ij}/np$	Yes	No	Yes	No	(Webb *et al.* 2002)
Phylogenetic species variability (PSV)	Expected variability in trait values between taxa in a community based on a Brownian motion model of evolution along branches	1-c	Yes	No	Yes (as PSE: phylogenetic species evenness)	No	(Helmus *et al.* 2007*a*)
Mean nearest neighbour distance (MNND)	Average of the shortest distance to any other taxon on the community phylogenetic tree for each species in the community	$\sum md_i/n$	No	No	Yes	No	(Webb *et al.* 2002)
Net relatedness index (NRI)	The standardised effect size of MPD x -1	$-(MPD - \overline{MPD})/sd(MPD)$	Yes (but slope of upper and lower bounds of variance change with increasing species number)	Yes (but slope of upper and lower bounds of variance change with increasing species)	Yes	No	(Webb *et al.* 2002)
Nearest Taxon Index (NTI)	The standardised effect size of MNND x -1	$-(MNND - \overline{MNND})/sd(MNND)$	Yes (but slope of upper and lower bounds of variance change with increasing species)	Yes (but slope of upper and lower bounds of variance change with increasing species)	Yes	No	(Webb *et al.* 2002)
Phylogenetic diversity (PD)	Sum of the minimum branch length required to join all taxa in the community on the phylogenetic tree		No	Yes	No	No	(Faith 1992)

Table 3.2: (continued on next page)

Table 3.2: (continued)

	Basis	Formula	Mean non-independent?	Range non-independent?	Abundance?	Frequency?	Reference
Rao's Quadratic Entropy (QE)	Pairwise distance between all taxa in a community weighted by abundance	$QE = \sum\sum d_{ij}a_ia_j$	No	No	Yes	No	(Rao 1982, Hardy & Senterre 2007)
Correlative approaches							
	Regression of co-occurrence coefficient against pairwise distance using linear or quantile regression or a Mantel test.	There are various co-occurrence indices. Schoener's coefficient, deviation from Dice's expected co-occurrence, Jaccard's coefficient and checkerboard scores were used in this study.	N/A?	N/A?	Yes (if abundance is incorporated in the co-occurrence index)	Yes	(Mantel 1967, Cavender-Bares *et al.* 2004, 2006, Slingsby & Verboom 2006, Lovette & Hochachka 2006, Helmus *et al.* 2007*b*)

d_{ij} = distance on the tree (branch length) between entity i and j. Entities are typically species but become individuals when abundance data are included.

$np = (N^2 - N)/2$ the number of pairwise comparisons between entities in community.

c = the average of the off-diagonal elements of the covariance matrix summarizing the correlation structure of the community phylogeny.

md_i = minimum distance on the tree (branch length) from species i to any other.

n = number of species in community.

a_i = relative abundance of species i within a community.

Comparing dispersion between communities in a way that compensates for the effect of species richness is best done using a rank order approach. This evaluates the rank of each community being compared, in terms of some dispersion metric, within the full sets of potential communities of equivalent species richness generated using an appropriate null model (where matching communities are assigned the same rank). Since the number of ranks or possible communities (n) will vary with species richness (k), according to the binomial coefficient ($C(n,k) = n!/(k!(n-k)!)$), it is important that the number of ranks be equalized among the communities being compared. This can be achieved by setting the number of ranks to the smallest number of possible communities associated with any single species richness category included in the comparison (either the most species-poor or species-rich community), and binning the ranks of other communities to make them equivalent in number . This approach applied to MPD or MNND generates a nonparametric form of NRI or NTI respectively, and may be particularly useful for comparing multiple sets of communities of different kinds of organisms or from different localities.

The phylogenetic diversity (PD) metric of Faith (1992) and Rao's (1982) quadratic entropy (QE), as implemented by Hardy & Senterre (2007), are positively related to community species richness (Figure 3.2) and are thus poor measures of phylogenetic dispersion. PD is a measure of cumulative evolutionary history (Proches *et al.* 2006) and increases for a set community species richness as the phylogenetic distances between the species increases. As phylogenetic beta diversity metrics such as *UniFrac* (Lozupone & Knight 2005) and *PhyloSor* (Bryant *et al.* 2008) are based on the calculation of turnover in PD they are highly sensitive to changes in species number between communities. This emphasizes the need to identify which component of phylogenetic diversity (e.g. richness, evenness or dispersion) is under investigation in community phylogenetic studies (Chapter 2). PD can only be used as a measure of phylogenetic dispersion when it is corrected for species richness, in which case it closely resembles MNND, and its use for testing for phylogenetic structure is thus appropriate if the null model used keeps species richness constant. Similarly QE when corrected for species richness approximates MPD.

The QE, MPD, MNND and the phylogenetic species evenness (PSE; Helmus *et al.* 2007*a*) metrics can incorporate relative species abundances. The decision to include relative abundance in metrics requires careful consideration, however, as all metrics in current use arbitrarily weight the importance of conspecific individuals relative to that of individuals belonging to different species. This weighting is based on a measure of evenness in abundances (Magurran 2004), rather than the unique genetic variation contributed by each individual (phylogenetic distance) as the metrics imply (Hardy & Senterre 2007); but see (Helmus *et al.* 2007*a*). Intraspecific genetic variation has important effects on ecological processes and interactions within communities (Johnson & Stinchcombe 2007, Hughes *et al.* 2008) and, if genetic variation among individuals in a community is to be appropriately incorporated in phylogenetic dispersion metrics, this will require that the unique genetic variation contributed by each individual (or a sample of individuals) be estimated. Notwithstanding, the determinants and effects of species evenness or dominance is of great interest in studies of both community assembly and ecosystem function (Hillebrand *et al.* 2008), and merits explicit investigation. New metrics which measure evenness in the representation of unique evolutionary history within communities (Cadotte *et al.* 2010; Chapter 2) will be useful in this regard.

3.3.2 Detecting significant phylogenetic structure

A number of studies have compared community phylogenetic methods in terms of their power to detect significant phylogenetic structure (Kraft *et al.* 2007, Hardy 2008, Swenson 2009, Kembel 2009). Using simulated communities assembled under an ecological model, Kraft *et al.* (2007) explored the properties of MNND and MPD in some detail, demonstrating that they have greater power to detect significant phylogenetic evenness (overdispersion) and clustering respectively. This is attributable to the fact that MNND measures the phylogenetic distances between species at the tips of the tree whereas MPD measures the average phylogenetic distances between all species, thereby including deeper phylogenetic nodes (Webb *et al.* 2002). They also demonstrated that the power to detect evenness was poor for both metrics, and varied with tree shape, community size, and the size of the regional pool upon which the null model was based. Similarly, Hardy (2008) and Kembel (2009) used simulated communities to demonstrate that many methods can be overly liberal and are often sensitive to hidden structure in the dataset when tested using various null models.

To illustrate the limitations of the statistical methods, I explored the power of four metric (MPD, MNND, PD and QE) and three correlative methods (linear regression, quantile regression and a Mantel test) to detect significant phylogenetic structure in simulated clustered or even communities. The tests of the correlative methods were done using four co-occurrence coefficients (DO: deviation from Dice (1945) expected co-occurrence (Slingsby & Verboom 2006), Schoener's measure of proportional similarity (Schoener 1970), Jaccard's similarity coefficient (Jaccard 1901) and Checkerboard scores

(Stone & Roberts 1990)). Since the specific aim is to test the statistical methods used to detect phylogenetic structure, rather than the ecological theory behind the assembly of communities, communities were generated mechanistically, based on phylogenetic relatedness, rather than using realistic community assembly models such as those employed by Kraft *et al.* (2007), Hardy (2008), Kembel (2009). This approach has the advantages that all communities display strong structuring and any potential biases or weakly structured communities generated by an ecological assembly model are excluded.

To test the effect of tree shape, even and clustered communities were derived from each of the three, variably-balanced, 16-species trees shown in Figure 3.3. I tested recursively for structure in sets of the communities whose structure was degraded by randomizing 10% of the communities at a time until no structure remained (Figures 3.4 and 3.5). Randomization was performed by swapping species identity within communities, thus holding community species richness constant and avoiding confounding the results with changes in statistical power due to changes in species richness. This was done for clustered and even sets of 1000 communities for each phylogenetic tree.

Communities with even structure were generated by randomly selecting one species from the set of 16 with uniform probability, randomly selecting a community species richness from a log-normal distribution, and successively adding the most distantly related species (with random tie breaking) until the community species richness was reached. Clustered communities were generated in a similar manner, successively adding the most closely related species. Assuming phylogenetic relatedness reflects functional similarity, this approach is a simplified version of the ecological assembly models employed in previous tests of community phylogenetic methods (Kraft *et al.* 2007, Kembel 2009) where even communities are structured by competition between close relatives and clustered communities are structured by filtering of close relatives into the same habitats.

The approach adopted draws species from a uniform species abundance distribution in the broader metacommunity because sampling from a log-normal distribution commonly resulted in one or more species not being represented in the set of 1000 communities. In addition, drawing species from a log-normal metacommunity abundance distribution did not result in a log-normal species abundance distribution for the set of 1000 structured communities, demonstrating a strong influence of the structuring processes. Various ecological and statistical processes have been suggested as drivers of the log-normal metacommunity abundance distribution commonly observed in empirical studies (reviewed

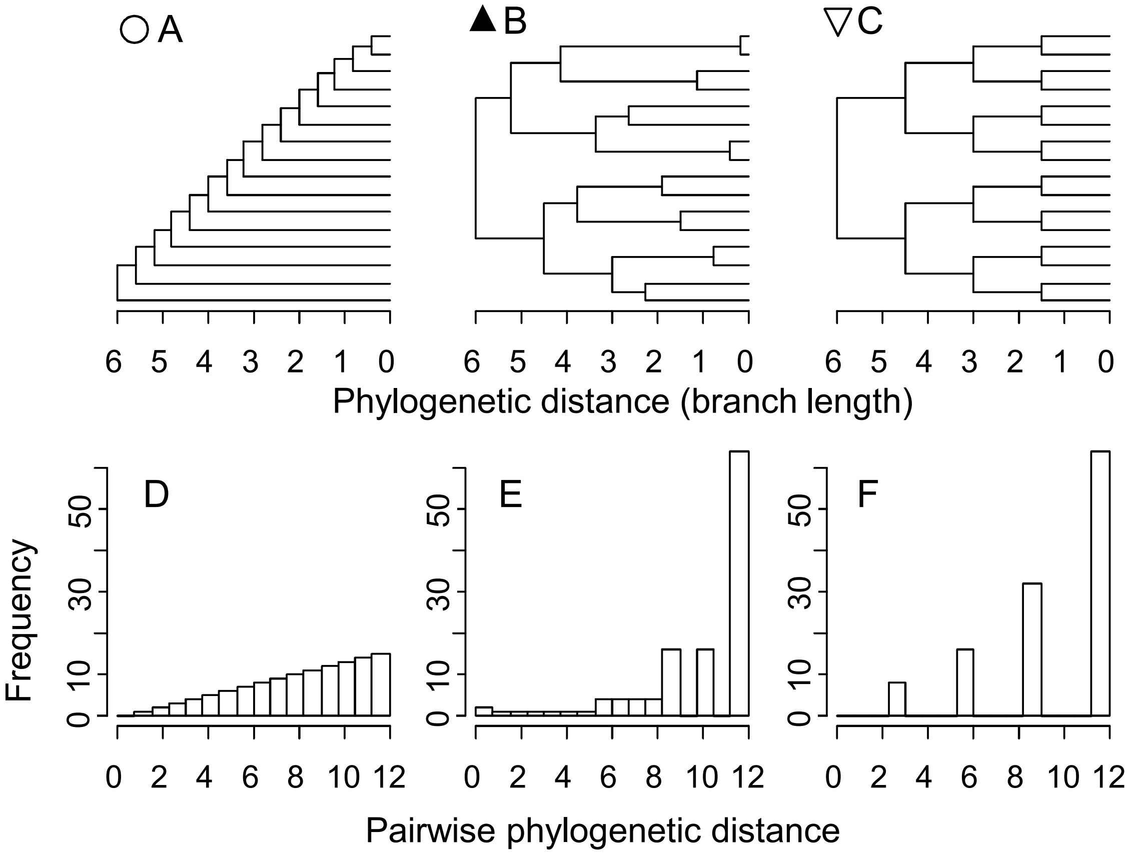


Figure 3.3: The pectinate (A), irregular balanced (B) and regular balanced (C) trees used in this study and the frequency distributions of the phylogenetic distances between species pairs derived from each tree.

in Hubbell 2001), but exploration of these drivers is beyond the scope of this chapter. I acknowledge that the species relative abundance distribution of the sets of 1000 structured communities may have an important influence on the performance of the statistical methods and merits more detailed exploration in future studies. The species abundance distribution of the simulated communities used here varied between log-normal, normal and multimodal depending on tree shape and phylogenetic structure.

Tests of significant structure using the MPD, MNND and PD metrics were performed for each community within each set by ranking the dispersion value for the ‘observed’ community against the values for 1000 null communities (Figure 3.1). The percentage of communities in which structure was detected was plotted against the percentage of communities which were known to be structured. Significant structure measured using the

QE metric approach was tested by comparing the phylogenetic differentiation coefficient between community samples (P_{ST}) for the 'real' community with the distribution of P_{ST} values from 1000 sets of null communities as described in Hardy & Senterre (2007). Statistical significance for the Mantel approach was evaluated using a standard Mantel test for correlation between two matrices (Mantel 1967). The linear and quantile regression methods plotted each co-occurrence coefficient against the pairwise phylogenetic distances between species. The quantile regression method used here differed from previous studies (Slingsby & Verboom 2006, Lovette & Hochachka 2006) in that it ranked the slope of the quantile with the greatest slope for the real set of communities against the slopes of the same quantile for 1000 sets of null communities, rather than always comparing a predefined quantile (e.g. 0.75). The linear regression method evaluated significance by ranking correlation coefficients against 1000 sets of null communities. All null communities for all methods were generated by shuffling species identity on the tips of the phylogenies. This null model preserves community species richness (Table 3.1), thus controlling for potential bias generated by the dependence of the metrics on species number, and has been shown to be the least biased null model when species presence/absence data are used (Hardy 2008).

Figures 3.4 and 3.5 display the power of each method to detect significant phylogenetic structure in sets of 1000 presence/absence communities that ranged from 0 to 100% clustering or evenness. The MPD, MNND and PD metrics detect the percentage of significantly clustered communities very well irrespective of tree shape, but are less capable of detecting evenness when communities are derived from balanced trees (Figure 3.4). This is due to a paucity of unique possible communities having very few or very many species, a larger proportion of which have identical metric values when the tree is balanced and/or regularly branched (Figure 3.3). For example, since the maximum number of unique pairwise distances derived from a tree is the number of unique internal node heights, there are only four possible dispersion values for any pair of species sampled from the regular balanced tree (Figure 3.3F), and six possible values for a set of three species. By contrast, the pectinate and irregular balanced trees offer 15 and 105 possible values for two- and three-species communities, respectively. Swenson (2009) supports this finding in showing that trees with more polytomies and, therefore, fewer unique node heights, offered less power to detect phylogenetic structure. Tree shape also influences statistical power because the number of species pairs sharing a particular pairwise distance is the product of the number of species on either side of the node (Figure 3.3 D-F). For example, 15 pairs pass through the basal node in the pectinate tree, while 64 pairs pass through the basal nodes of the balanced trees, all having a pairwise distance of 12. Trees that are well-balanced, especially at the deeper nodes, will thus have a more strongly skewed distribution, with greater numbers of communities sharing values of distant relatedness. Thus, the inability of the metric approaches to detect maximum evenness (α = 0.05) when trees are balanced, is a simple consequence of the fact that more than 5% of the total set of possible communities share this maximum value (Figure 3.3 and 3.4). This bias towards evenness results, conversely, in clustering being more easily detectable. This is consistent with the findings of Kraft *et al.* (2007) who showed that the NRI and NTI metrics had much greater statistical power to detect phylogenetic clustering than evenness, and that the power to detect clustering increased with greater tree balance, while the power to detect evenness decreased. Although Hardy & Senterre (2007) QE method successfully detected significant structure in clustered and even communities derived from the pectinate and irregular balanced trees with $>$20% structuring (Figure 3.4), it performed poorly where structuring was based on the regular balanced tree, reflecting behavior similar to other metric approaches.

Correlative approaches are based on the notion that a positive relationship between co-occurrence and phylogenetic distance (pairwise, between species) reflects phylogenetic evenness, while a negative relationship reflects clustering (Cavender-Bares *et al.* 2006). Three regression approaches are considered here because each has its own pros and cons. Linear regression allows calculation of a correlation coefficient, but its assumptions are violated by phylogenetic autocorrelation and heteroscedasticity (i.e. variance in the response variable changing as a function of the predictor variable). Autocorrelation between species pairs requires the use of a null model in combination with the linear regression analysis (e.g. Cavender-Bares *et al.* 2004) or the use of a Mantel test (Mantel 1967). A quantile regression approach circumvents violation of the assumption of variance homogeneity and is more realistic because, while phylogenetic relatedness is expected to set an upper bound on co-occurrence, it does not necessitate co-occurrence (Slingsby & Verboom 2006, Lovette & Hochachka 2006).

Performance of the correlative approaches was variable, depending on the co-occurrence coefficient and statistical method employed (Figure 3.5). Most combinations of statistical methods and co-occurrence coefficients detected significant clustering and evenness in communities derived from the balanced trees, but analyses based on the pectinate tree often performed poorly. Plots of the distributions of co-occurrence values against phylogenetic distance (Figures 3.6 and 3.7) imply that most co-occurrence coefficients suffer a pathology associated with pectinate trees, a number of them displaying slopes opposite to

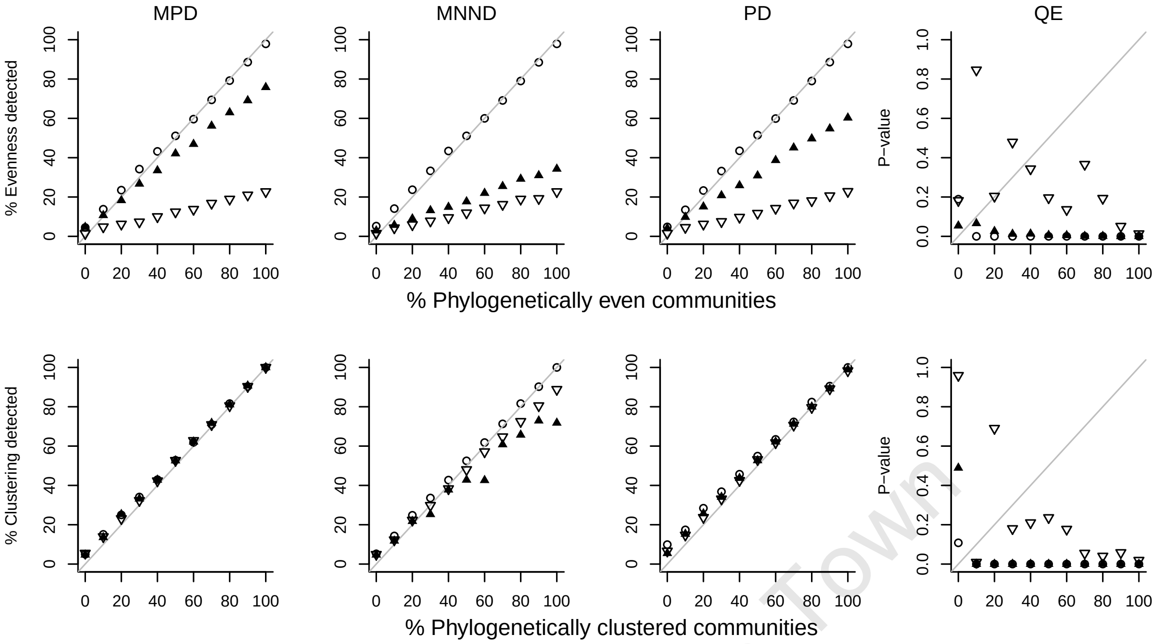


Figure 3.4: The performance of four metric approaches to detect significant phylogenetic structure in sets of even (overdispersed) and clustered communities derived from the three phylogenetic trees in Figure 3.3 (pectinate = open circles, irregular balanced = closed triangles, regular balanced = open triangles). The independent variables are the percentage of even (A) or clustered (B) communities. The dependent variables represent the percentage of communities detected as having significant structure, or the P-values attained by tests for structure across the whole set of communities. MPD = mean phylogenetic distance, MNND = mean nearest neighbor (taxon) distance, PD = phylogenetic diversity, QE = Raos quadratic entropy using phylogenetic distances.

those predicted for even or clustered communities. The quantile regression approach also displayed high Type I error in detecting evenness, though the approach implemented here differs from previous applications. Further investigation of the sensitivities of the quantile regression method is thus required. The DO index, in combination with the linear regression approach or a Mantel test, offered the best correlative test of significant evenness. On the other hand, tests of clustering worked best when performed with Schoener's coefficient, in combination with any of the correlative methods.

Most studies using correlative methods have detected at least some phylogenetic evenness (Cavender-Bares *et al.* 2004, 2006, Slingsby & Verboom 2006, Lovette & Hochachka 2006, Helmus *et al.* 2007*b*), and our results suggest that these methods may be more powerful than metric approaches in detecting phylogenetic evenness. They do, however,

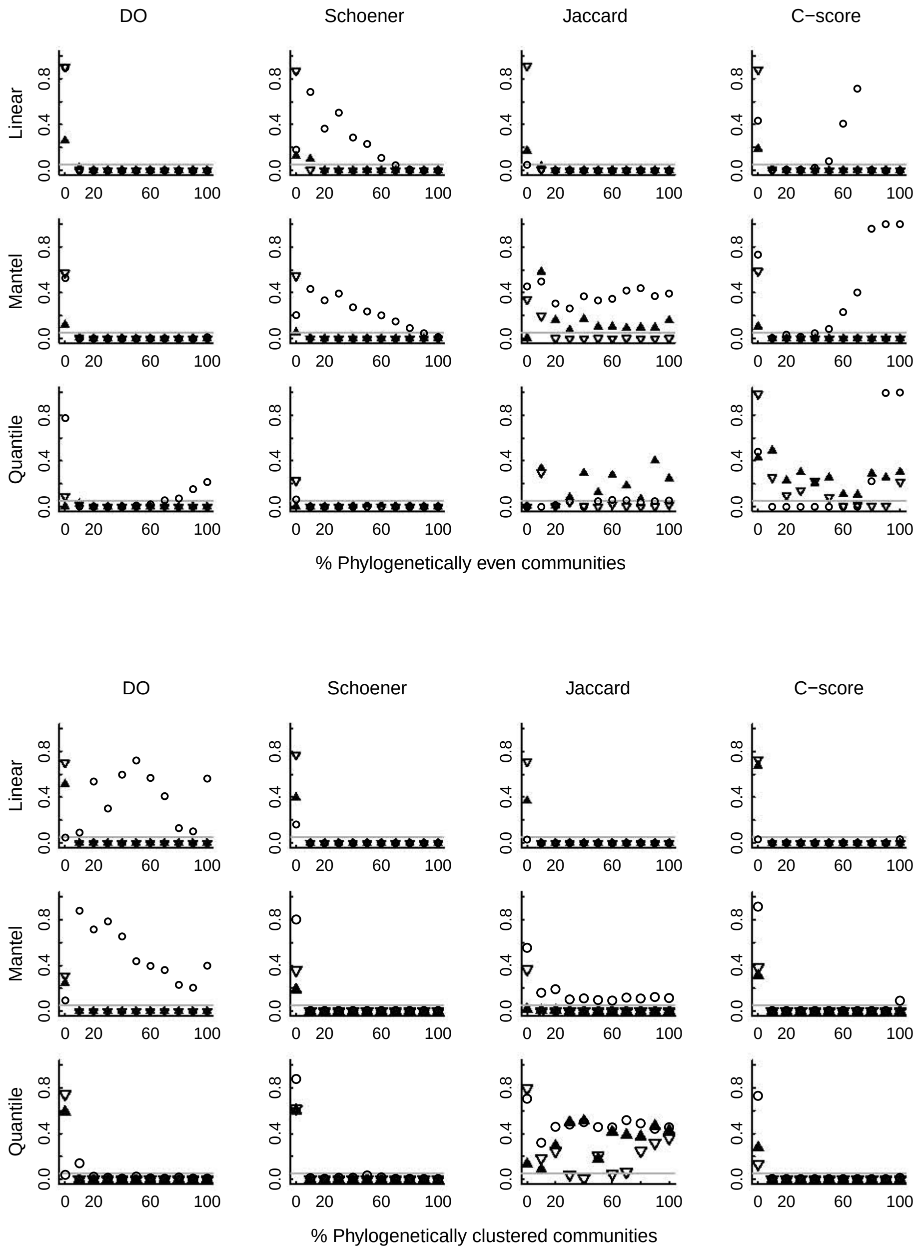


Figure 3.5: The performance of three correlative approaches, in combination with four co-occurrence coefficients, to detect significant phylogenetic structure in sets of even (overdispersed) and clustered communities derived from the three phylogenetic trees in Figure 3.3 (pectinate = open circles, irregular balanced = closed triangles, regular balanced = open triangles). The independent variables are the percentage of even (A) or clustered (B) communities. The dependent variables are the P-values attained by tests for structure across the whole set of communities. DO = deviation from expected co-occurrence, C-score = Checkerboard score.

have several disadvantages: their statistical power is highly dependent on the number of species pairs in the analysis; they are sensitive to large co-occurrence estimation errors associated with species of low prevalence; and co-occurrence coefficients appear sensitive to highly skewed trees. Further investigation of these and other co-occurrence coefficients or measures of spatial overlap (e.g. Diamond & Case 1986, Weiher & Keddy 1999, Lovette & Hochachka 2006, Helmus *et al.* 2007*b*) is required, including a consideration of their sensitivities to low species prevalence and tree balance. There is ample opportunity for the development of co-occurrence coefficients that incorporate species abundances or account for spatial, temporal or environmental relationships between sites, allowing for detailed examination of the determinants of phylogenetic structure (e.g. Cavender-Bares *et al.* 2006, Helmus *et al.* 2007*b*).

3.3.3 Sampling design

The manner in which communities are sampled and the data recorded determines which community properties or structuring processes can be examined or controlled for, and can constrain the statistical power of analyses. The most important components of sampling design are spatial, temporal, environmental and taxonomic scale, and whether species occurrence is recorded as presence-absence or as abundance.

Knowing the spatio-temporal relationships and environmental similarity among communities (sites or samples) is vital for comparing phylogenetic dispersion between communities, and for testing phylogenetic structure. This is because many communities are known to be structured by species' abiotic preferences, dispersal dynamics, successional stage and seasonal variation (Diamond & Case 1986, Weiher & Keddy 1999). While these factors can render a study biologically meaningless if ignored, they can either be controlled for at the sampling stage (e.g. sampling homogeneous communities only) or incorporated at the data analysis stage (e.g. Helmus *et al.* 2007*b*). The pattern of dispersion is expected to change with increasing spatial scale because the relative strength of density dependent interactions is expected to decrease while that of ecological sorting increases, especially where additional abiotic environments are sampled (Cavender-Bares *et al.* 2009). Similarly, sampling at spatial scales that transcend barriers to dispersal can create pattern in the data due to dispersal limitation or the nature of the speciation process (Slingsby & Verboom 2006), confounding biogeographic history and the signal of local ecological processes (e.g. competition, ecological sorting or mutualistic interactions). Temporal factors may also be

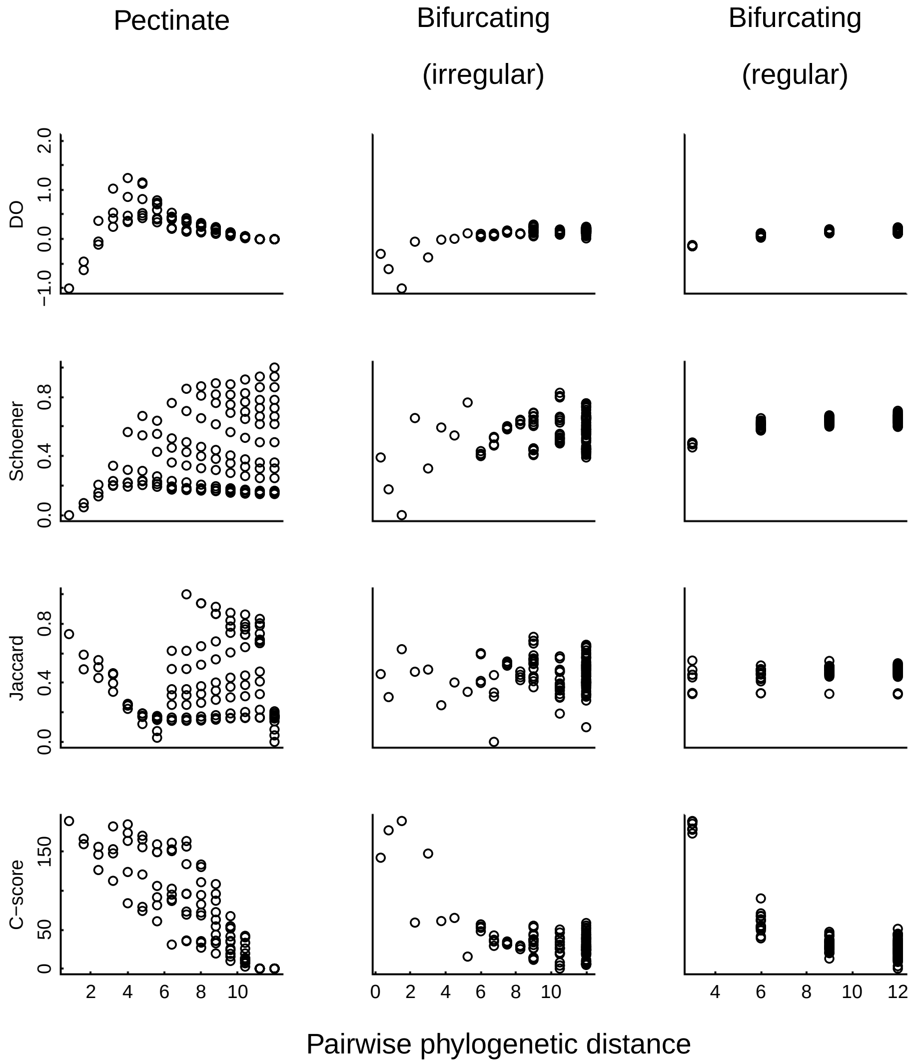


Figure 3.6: Plots of co-occurrence coefficient values against phylogenetic distance for pairs of species derived from the 100% evenly structured datasets presented in Figure 3.5 derived from each of the three trees presented in Figure 3.3. The expectation for even communities is a positive linear relationship between the co-occurrence coefficient and species richness. DO = deviation from expected co-occurrence, C-score = Checkerboard score.

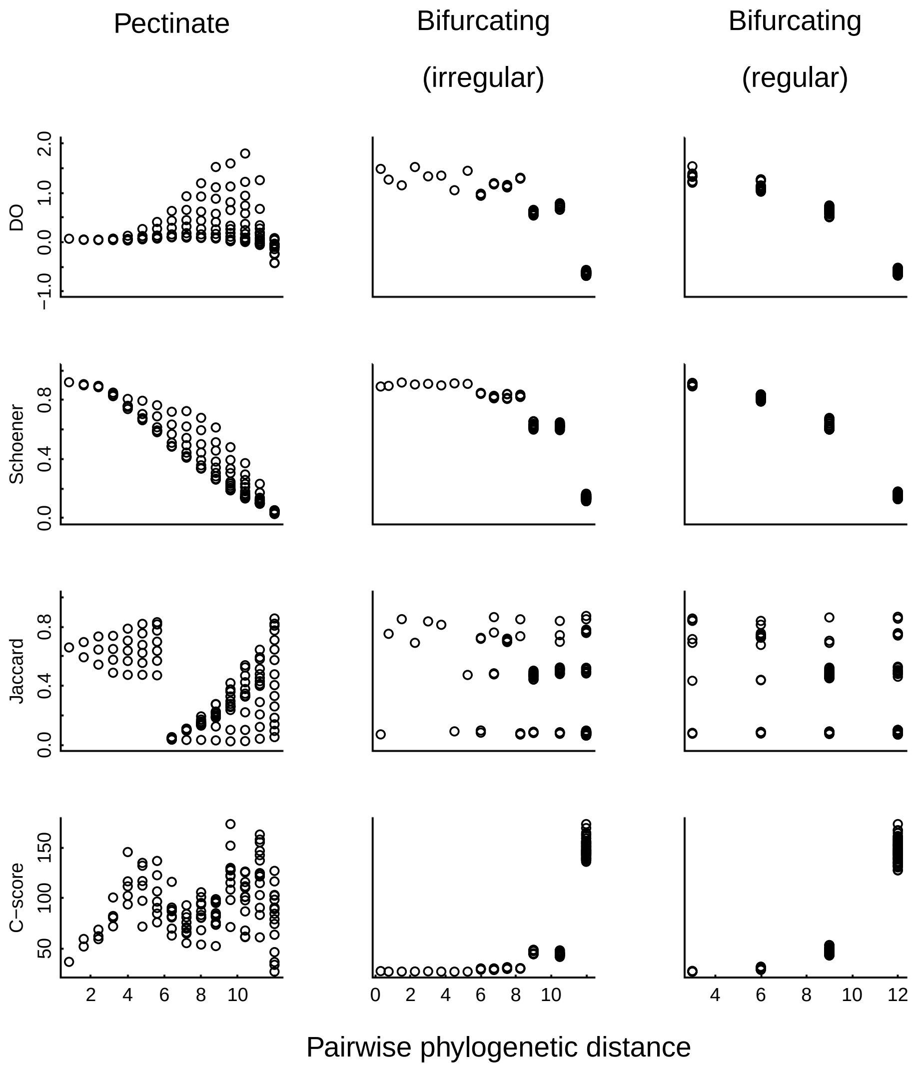


Figure 3.7: Plots of co-occurrence coefficient values against phylogenetic distance for pairs of species derived from the 100% clustered datasets presented in Figure 3.5 derived from each of the three trees presented in Figure 3.3. The expectation for even communities is a negative linear relationship between the co-occurrence coefficient and species richness. DO = deviation from expected co-occurrence, C-score = Checkerboard score.

influential since the signal of density dependent interactions may increase with succession, because time is required for interactions to run to their conclusion.

Taxonomic scope can influence the statistical power of analyses through its effect on species pool size (Slingsby & Verboom 2006, Kraft *et al.* 2007). It can also influence which interactions determine patterns of dispersion and the types of patterns that are likely to emerge. Studies performed at larger taxonomic scales typically detect phylogenetic clustering, whereas studies at finer taxonomic scales tend to detect evenness (Cavender-Bares *et al.* 2006, 2009, Emerson & Gillespie 2008, Vamosi *et al.* 2009). This probably reflects changes in the relative effects of competition and habitat filtering on co-occurrence as species become more distantly related, as well as differences in the lability of traits that determine α- versus β-niches (Ackerly *et al.* 2006, Silvertown *et al.* 2006).

Sampling multiple sites provides knowledge of species' prevalence throughout the set of communities and estimates of their relative abundances in the broader metacommunity, permitting investigation of the effects of dispersal dynamics on community dispersion. Levels of species co-occurrence within a local community may depend heavily on their abundances in the broader metacommunity. If the dispersal of a species into a local community is a function of its metacommunity abundance, and species' abundances in the metacommunity are phylogenetically structured, local co-occurrence may show significant phylogenetic structure due to purely neutral processes (Hardy 2008, Kembel 2009). It is important to exclude this influence if one wishes to invoke local deterministic processes such as habitat filtering and competition. This highlights the danger of ignoring any community properties, emphasizing the need to develop a better understanding of how various community properties affect phylogenetic dispersion. In particular, modeling approaches could be used to determine how well methods that test for significant phylogenetic structuring perform in spatially or environmentally structured landscapes.

Treating species occurrence as presence/absence, thereby ignoring the abundances of individuals, may permit the pattern of spatial occurrence to be distinguished from that associated with species' abundances within and across sites (Hardy 2008). Unfortunately, the ability to detect a species is rarely independent of its abundance, and the number of species recorded can depend on the number of individuals present and their body size (Gotelli & Colwell 2001). Thus, species richness is best assessed in a comparable manner as the number of species recorded for a set number of individuals of all species sampled at a site (Magurran 2004). If sampling area or effort is fixed, the numbers of individuals sampled often vary and the resulting data reflect species densities rather than species richness (Gotelli & Colwell 2001). Similarly, phylogenetic dispersion will be expressed per unit area rather than per set number of individuals. Density and richness may be correlated, but tend to diverge as the variance in the number of individuals sampled per site increases. This is affected by habitat differences or changes in individual body size, or when plots or samples of different size are compared. Measuring density rather than richness is of particular concern for studies investigating changes in phylogenetic community structure with spatial scale (plot size) or between different size cohorts, given that all metrics of dispersion are sensitive to species richness (Figure 3.2). Studies which sample communities per unit area or per sample rather than per number of individuals should make use of unbiased estimates of phylogenetic dispersion (see Kembel & Hubbell 2006).

Sampling strategy has an important effect on the statistical power of metric approaches since the number of communities with unique measures of dispersion that can be sampled by a null model increases when species abundance is included. The total number of unique presence/absence communities of particular species richness that can be drawn from a pool of n species is described by the binomial coefficient. When species abundances are included the total number of unique communities can be calculated by substituting the species pool size (n) with the number of unique species-abundance combinations, which drastically increases the number of possibilities. For null models that shuffle abundance only within species, n is the sum of the number of unique abundance values for each species in the dataset. Null models that allow abundance values to be shuffled between species allow an even greater number of possible communities as n is the total number of unique abundance values multiplied by the total number of species.

Despite its attractive features, including abundance in tests for phylogenetic structure using the metric approach can obscure the importance of properties such as species prevalence across sites, and requires that assumptions be made about the relative importance of species versus individuals and how each is weighted. The sensitivity of correlative approaches to the inclusion of abundances is unclear and merits further investigation. Inclusion of abundances could improve co-occurrence estimates without the need to assign a relative importance of species versus individuals (e.g. Cavender-Bares *et al.* 2006).

3.3.4 Null models

Null models are used to generate a distribution of the expected communities in the absence of the effects of the factors under study (Gotelli 2000). This is done by iteratively shuffling cells in the community data matrix or by swapping species attributes according to a set of defined criteria (Table 3.1, Figure 3.1). It is important to ensure that the null model used manipulates only the community properties of interest while controlling for the remainder. Since it is not always possible to exclude the influence of potentially confounding factors with a single null model (Table 3.1) a comparison using multiple null models can be useful. Helmus *et al.* (2007*a*), for example, demonstrated significant phylogenetic structure in sunfish prevalence across 890 lakes in Wisconsin, USA, by demonstrating that a null model which conserves species prevalence matched the observed data, while a null model that allows species prevalence to vary did not.

A central consideration when using null models to test for significant pattern is the number of possible combinations (null communities) and the number of unique phylogenetic dispersion or co-occurrence values that these represent. If these are limited in number, then the power of the model is greatly reduced, and running large numbers of iterations of the null model is meaningless. In addition to the effects of tree shape, species pool size and the use of presence/absence versus abundance data, the number of possible null communities and unique dispersion values can be severely reduced by the way in which the null model shuffles the community data. With a set of 16 species, for example, the total number of possible unique presence/absence communities having between one and 16 species is unimodal, peaking at eight species with 12 870 combinations, and totaling 65 535 altogether. If the null model employed holds species number per community constant, and the maximum species richness in the original community matrix is three, the possible set of unique communities is reduced to 696. Additional constraints such as those implemented in the independent swap algorithms (Table 3.1) will further reduce this number, and reduce the statistical power of the test. Similarly, shuffling n taxa on a phylogenetic tree results in $n!$ possible tree configurations, this number being drastically reduced as constraints on the shuffling procedure are introduced (e.g. Hardy 2008). There is thus a strong trade-off between power to detect statistically significant pattern and the resolution at which the pattern can be examined using null models.

3.4 Conclusions

Measuring phylogenetic dispersion and testing for structure in community properties requires careful sampling design and choice of statistical approach and null model. In Table 3.3, I provide a guide to the factors requiring consideration and the appropriate methods required to compare phylogenetic dispersion or test for phylogenetic structure of communities.

Phylogenetic structure may be apparent in many different properties of communities and researchers need to be explicit about which property they are measuring or testing and how their sampling design and choice of statistical approach and null model achieves this. Care must be taken to control for confounding variables which may not be of interest to the study, such as species richness or metacommunity abundance, and spatio-temporal autocorrelation or environmental similarity between communities. The structure of the landscape sampled also needs to be made explicit, as must the influence of ecological or biogeographic processes. Finally, sampling of species densities (number of species per sample unit) as opposed to species richness (number of species per set number of individuals) may confound comparisons of dispersion between size cohorts or spatial scales and necessitates the use of unbiased estimation procedures.

All metrics of phylogenetic dispersion depend on species richness. The least biased way to exclude the effect of species richness when comparing phylogenetic dispersion between communities is to compare their rank order from the set of potential communities of equivalent species richness, generated using a suitable null model. As all metrics are dependent on species richness, null models that do not hold species richness constant should not be used in tests of phylogenetic structure.

Metric approaches detect clustering more easily than evenness because the largest proportion of all possible unique communities that can be generated from any phylogenetic tree will share the maximum degree of dispersion. There is also greater power to detect clustering, and less power to detect evenness, as trees become more balanced. Consequently, I recommend using both metric-based and correlative methods when testing for phylogenetic evenness. Conversely, appropriate correlative approaches perform well at detecting both clustering and evenness, but the co-occurrence coefficients on which they are based may be sensitive to extreme skewness in the branching architecture of phylogenetic trees, favoring the use of metric approaches when trees are greatly skewed.

The statistical power of tests of structure depends on the number of possible unique communities that can be generated by the null model, as well as the number and frequency of dispersion measures which these can take. These are influenced by: i) the number of taxa (species pool size); ii) the balance of the phylogenetic tree; iii) the number of unique node heights in the tree; iv) the form of the data used (presence/absence versus species abundances); and v) constraints imposed on the null model. Consequently, there is a trade-off between the power to detect significant phylogenetic structuring and the resolution at which we can examine processes that structure communities using null models, highlighting a need for new and more flexible statistical methods in community phylogenetics. Co-occurrence coefficients used in correlative analyses allow inclusion of the properties of community data sets, such as the relative abundance of species and the spatial, temporal or environmental relationships between sites, permitting the use of less constrained null models and improving the resolution at which community assembly processes can be investigated. Further development of these methods and investigation of their sensitivities to tree balance, species prevalence and relative abundance, and null model construction is required.

While these findings are based on measures of phylogenetic dispersion and structuring they are equally applicable to studies investigating functional diversity and dispersion (Petchey & Gaston 2006) and testing for structure in the distribution of trait values (e.g. Slingsby & Verboom 2006, Kraft *et al.* 2008, Cornwell & Ackerly 2009, Ingram & Shurin 2009, Swenson & Enquist 2009).

Table 3.3: Considerations for phyloecological analyses.

A) Considerations for sampling design

Are species' abundances to be sampled or only presence/absence?
Abundance is preferable as it allows one to measure or estimate species richness as opposed to species density, and allows the use of rarefaction curves.

Are communities sampled per area or per number of individuals (species density vs species richness)?
Typically species richness is preferable, but if this means varying the size of sampling units then variation in environmental heterogeneity needs to be considered.

What size are the community samples?
Are the samples small enough (spatially and/or temporally) to assume that individuals are interacting within them? Are samples large enough that they are a fair reflection of true communities?

Table 3.3: (continued on next page)

Table 3.3: (continued)

What are the spatial, temporal or environmental relationships between the sampled communities?
These are often the factors of interest in community phylogenetic studies (see Helmus *et al.* 2007*b*), but should be considered a priori. In particular, if the goal is to determine whether the observed patterns are the result of ecological interactions or the history of speciation and dispersal limitation, one needs to know if there are biogeographic barriers within the study area. These factors can be considered a posteriori by comparing the spatial, temporal or environmental relationships between samples with a measure of phylogenetic turnover.

What are the relevant species pools?
This is determined by the spatial scale and taxonomic scope of the study. Are all species in the communities included in the analysis or only a set taxonomic, trophic or functional group? Does the species pool used for null model testing comprise only those species recorded in the sampled communities or all species within a defined region? It is important to have some idea of the prevalence of all species used for null modeling as this can influence results (Hardy 2008, Kembel 2009). While decisions regarding the species pool are largely driven by the question asked, it is important to consider that low species numbers can impede statistical power. It is also important to note that tests for significant structure are relative to the set of communities sampled or the regional species pool and vary depending on how the set of communities or species pool is delineated.

How many communities are sampled?
In addition to its influence on species pool, the number of samples also affects co-occurrence estimates for correlative tests for structure, and the ability to employ rarefaction curves in analyses. Greater numbers of samples are almost always preferable.

B) Considerations when comparing phylogenetic dispersion between communities

Are species richness or species density data being compared?
If the communities were sampled per unit area then phylogenetic dispersion based on species densities are being compared. As all measures of phylogenetic dispersion are sensitive to species number, comparing dispersion based on species densities rather than richness may create a bias. If species relative abundances are known then one can calculate unbiased estimates of phylogenetic dispersion or compare phylogenetic dispersion rarefaction curves. If relative abundances are not known then it is necessary to justify why species densities do not differ substantially from richness.

Do the numbers of species vary between communities?
If communities have uniform species richness then they can be compared directly using any metric. If species richness varies, comparisons should be made using the rank-order approach.

C) Considerations when testing for phylogenetic structure

Table 3.3: (continued on next page)

Table 3.3: (continued)

The results of this and other papers (Kraft *et al.* 2007, Hardy 2008, Kembel 2009) have revealed advantages and disadvantages of the different methods under different circumstances. Unfortunately, while the simulations performed by some of the studies were quite extensive, in all they represent only a tiny subset of the actual circumstances encountered in real datasets. There is thus no substitute for applying multiple methods to the data when testing for phylogenetic structure. The recommendations that follow are based on our current understanding from a limited number of simulation studies and should be treated with caution.

Testing for evenness
Correlative approaches appear to have greater statistical power to detect evenness than metric approaches. Linear regression or a Mantel test using the DO coefficient of co-occurrence appears to work best. Unfortunately, these methods perform poorly when trees are highly skewed. A metric approach using the MNND or PD metrics may also be useful because tests using these metrics perform well at detecting evenness, particularly where trees are skewed.

Testing for clustering
Both metric and correlative approaches detect clustering well, but the co-occurrence coefficients employed by correlative approaches perform poorly when trees are highly skewed. Conversely, metric approaches have poor statistical power when trees are well balanced. Linear or quantile regression using Schoener's measure of proportional similarity appear to be the best correlative methods for detecting clustering. MPD or PSV are best metrics in tests for clustering using the metric approach.

Including relative abundances in metric approaches
Inclusion of relative abundances of species in metric analyses converts the metric to a measure of phylogenetic dispersion and evenness and requires one to make a decision about the relative importance of an individual versus a species. Inclusion of relative abundances has the advantage of increasing statistical power. The results may, however, depend on the weighting of individuals versus species and the manner in which evenness is calculated. Where abundances are used it is recommended that presence/absence (equal abundances) analyses be performed for comparison of the relative effects of dispersion versus evenness. The MPD, MNND and QE metrics allow inclusion of abundances (Webb *et al.* 2008), as does PSV in the form of PSE (Helmus *et al.* 2007*a*).

Choosing a null model

Table 3.3: (continued on next page)

Table 3.3: (continued)

The choice of null model depends entirely on the goals of the study. Table 3.1 indicates the known properties of a community dataset that effect the dispersion of communities and some null models which retain or randomize them. Ideally one employs a null model which randomizes the property being investigated while retaining the observed structure in all others. Unfortunately, as more constraints are added to a null model the number of unique communities that the model can sample declines and the power to detect pattern is eroded. Should statistical power be a limitation, comparing multiple null models may allow the factor determining community structure to be inferred (e.g. Helmus *et al.* 2007*b*). As a general rule, null models which do not retain community species richness should not be used unless well justified.

Tetraria sp. nov. cf. triangularis A, Swartberg Pass.

4 Phylogenetics and diversification of *Tetraria*: Ecology limits the diversity of the Cape flora

4.1 Abstract

Understanding the ecology and evolution of the hyper-diverse Cape flora is dependent on developing an understanding of its component parts, best epitomized by the Cape floral clades which have diversified and are largely endemic to the region. Here I develop a dated phylogenetic hypothesis for the sedge genus *Tetraria*, one of the smaller Cape floral clades, to develop an understanding of the timing of diversification of the group, and to test if speciation rates have slowed subsequent to the initial diversification as the environment has stabilized and the available ecological niche space has become saturated. The radiation of *Tetraria* began approximately 16 million years ago, concordant with that of many other Cape clades and coincident with a period of climatic change which led to the current winter wet, summer dry regime. Diversification rates in the genus declined as lineage diversity accumulated, indicative of ecological limitation on speciation rates. This allows the development of heuristic predictions about the composition of *Tetraria* assemblages at various spatial scales, and suggests that closely related species should either be ecologically differentiated or have non-overlapping geographic distributions. The question of whether ecological limitation of diversity is a common phenomenon in other Cape lineages has important implications for our understanding of the evolution and ecology of the contemporary Cape flora as a whole.

4.2 Introduction

Diversification rates inferred from molecular phylogenies are commonly used to infer macro-evolutionary hypotheses such as those relating to adaptive radiation, key innovation or species selection (Pybus & Harvey 2000, Rabosky 2006*b*, McPeek 2008, Rabosky & McCune 2010). A topic that is garnering increasing interest is the potential role of ecology in limiting diversification rates (McPeek 2008, Phillimore & Price 2008, Rabosky & Lovette 2008*a*, Rabosky 2009*a*,*b*). Where the diversification rate of a lineage has declined as a function of the number of lineages present (i.e. is density-dependent) we can infer that ecological niche space has become increasingly saturated, with fewer empty niches for new species to evolve into (Foote 1997, Rabosky & Lovette 2008*a*). Decreasing rates of diversification through time (speciation minus extinction) have been reported for a diverse group of plant and animal lineages from different geographical areas based on both fossil (Sepkoski 1998) and phylogenetic evidence (Pybus & Harvey 2000, McPeek 2008, Phillimore & Price 2008, Rabosky & Lovette 2008*a*, Rabosky 2009*b*). This suggests that ecological saturation has played a crucial role in limiting the diversity of lineages and has constrained the composition and structure of biogeographic regions and communities (McPeek 2008).

Declining diversification rates may be the result of extrinsic or intrinsic constraints on speciation rates, but cannot result from increasing extinction because any signal in the phylogenetic data would be eroded (Rabosky & Lovette 2008*b*). There are a number of ways in which factors extrinsic to a lineage could affect speciation rates. For example, climatic change may increase genetic connectivity amongst populations distributed across a landscape, thereby reducing the probability of speciation in allopatry. Alternatively, a major geomorphological event may fragment a landscape triggering a brief burst of diversification via vicariant speciation. Declines in speciation rates may also have an intrinsic basis. The canalization of genetic diversity and developmental systems through successive divergence events may reduce the propensity for populations to diverge (Foote 1997). Similarly, if there is a limit to the available ecological niche space then speciation rates will decline as this space becomes saturated (Foote 1997, Rabosky & Lovette 2008*a*, Rabosky 2009*b*). One can discriminate between intrinsic and extrinsic explanations for decreasing speciation rates because that are expected to leave different signals in the diversification rates derived from phylogenies (Rabosky & Lovette 2008*a*). Where speciation rates depend on extrinsic factors diversification rates should decline as a function of time, whereas intrinsic factors should cause diversification rates to decrease as a function of the number of lineages present (i.e. indicate density-dependence).

The radiation of the highly diverse fynbos flora is largely thought to have been adaptive (Cowling *et al.* 1992, Linder 2003, 2005*a*, van der Niet & Johnson 2009), being driven by strong disruptive selection on traits relating to fire survival strategy (Schutte *et al.* 1995) and edaphic (Verboom *et al.* 2004), climatic (Linder & Vlok 1991, Verboom *et al.*

2003, 2009), pollinator (Johnson 1996, Johnson *et al.* 1998, Waterman *et al.* 2011) and microhabitat specialization. Phenological differences (Warren *et al.* 2011), low dispersal rates (Slingsby & Bond 1985, Schurr *et al.* 2005) and sterility barriers would greatly have aided cladogenesis across these divergent selective environments by limiting gene flow. Phylogenetic information suggests that a number of Cape lineages started to radiate during the late Miocene and early Pliocene (Linder & Hardy 2004, Linder 2005*a*, Verboom *et al.* 2009), potentially as a consequence of major environmental changes which would have modified the selective regime and created a novel adaptive zone (*sensu* Simpson 1953). Geomorphic evolution increased topographic heterogeneity by exposing new geologies (Cowling *et al.* 2009); cold water upwelling along the Atlantic coastline triggered climatic change and increased the extent of fynbos vegetation by creating a Mediterranean-type climate in the West and year-round rainfall in the East (Siesser 1980, Deacon *et al.* 1992, Zachos *et al.* 2001); and finally, the shift to a Mediterranean-type climate may have triggered the onset of modern day fire regimes (Hendey 1983, Deacon *et al.* 1992, Bytebier *et al.* 2011).

Phylogenetic studies have revealed evidence for declining diversification rates in two Cape clades, *Protea* (Valente *et al.* 2010) and Restionaceae (Linder & Hardy 2004), but they have not evaluated whether these declines are the result of extrinsic or intrinsic factors. The suggested model of adaptive diversification of the fynbos triggered by major environmental changes provides two competing hypotheses that could explain declining speciation rates. Firstly, assuming that the major environmental changes during the Pliocene were followed by a period of relative environmental stability (Cowling *et al.* 1996*a*, 2009, Linder 2003), we might expect Cape lineages to display declining rates of speciation subsequent to their initial radiation. Alternatively, if the diversification of these lineages was largely adaptive, with species radiating into vacant ecological niches, the speciation rate should have decreased as diversity accumulated because the available ecological space would have become increasingly saturated.

Almost half of the approximately 9000 plant species native to the hyper-diverse Cape Floristic Region belong to one of 33 ‘Cape floral clades’ that have diversified and are largely restricted to the region (Goldblatt & Manning 2000, Linder 2003). Understanding the origins of the Cape Flora is thus highly dependent on understanding the history of diversification in these clades (Linder 2003, 2005*a*, Linder & Hardy 2004, Verboom *et al.* 2009). *Tetraria*, a morphologically diverse genus of sedges (Cyperaceae), forms one of the ‘Cape floral clades’ and is a common, often dominant, component of most fynbos communities. Diversification rates in the genus are likely to have been greatly affected by both intrinsic and extrinsic factors. Miocene changes in climate and geomorphology are believed to have influenced the diversification of a number of Cape lineages (Linder & Vlok 1991, Verboom *et al.* 2003, 2009) and there is no reason to expect that these events were any less important for *Tetraria*. On the other hand, *Tetraria* species have broadly overlapping distribution ranges and closely related species show lower than expected levels of co-occurrence at fine spatial scales (Slingsby & Verboom 2006), suggesting that available ecological space is limiting. Speciation rates within the group may thus be expected to show a rapid increase through the Miocene and early Pliocene, followed by a density-dependent decline as environments have became more stable allowing interspecific interactions to run their course. In this chapter, I develop a phylogenetic hypothesis and estimate the timing of the initial radiation of the genus *Tetraria* using existing and new plastid and nuclear DNA sequence data. I then examine the rate of lineage accumulation through time and compare the fit of constant, time-varying and density-dependent models of diversification rate to cladogenetic events in the group as a whole and in each of its component clades. Key objectives are to establish whether the genus has undergone a Miocene-Pliocene radiation of the type reported for a number of other Cape groups, and to test whether diversification of the genus and each of its major clades has been constrained by limitations on available ecological space.

4.3 Methods

4.3.1 Taxon sampling

Fifty-three accessions, representing 37 of the 46 reticulate-sheathed *Tetraria* (ingroup) species native to the Cape and 16 outgroup species were included in the phylogenetic analysis. Nine ingroup species were not included (*Epischoenus cernuus*, *T. sp nov cf. burmannii*, *T. pillansii*, *T. robusta*, *T. sp nov cf. robusta*, *T. sp nov cf. capillacea*, *T. sp nov cf. pubescens B*, *T. sp nov cf. criniifolia* and *T. vaginata*) due to the difficulty of acquiring fresh leaf material or extracting DNA. Two purported ingroup species (*T. mlanjensis* and *T. usambarensis*) were not included because they do not occur within the Cape and their taxonomy is uncertain. The outgroup species include two representatives of Mapanioideae (*Mapania cuspidata* and *Hypolytrum nemorum*), two representatives of the Rhynchosporeae-Scirpeae-Cypereae clade (*Rhynchospora brownii* and *Ficinia paradoxa*) and 12 other representatives of the tribe Schoeneae (Appendix 4.1).

4.3.2 DNA isolation, amplification and sequencing

All new DNA sequences generated for this study were extracted from silica-dried field-collected leaf material and are represented by voucher specimens in the Bolus Herbarium, University of Cape Town (Appendix 4.1). Sequences obtained from other studies are indicated with the appropriate reference. Unfortunately it was not possible to obtain sequences for all markers for all species (Table 4.1, Appendix 4.1).

Four non-coding loci and two coding loci were sampled using PCR: the plastid *trnL-trnF* intron and spacer region (primers: 'c' and 'f'; Taberlet *et al.* 1991), the plastid *rps16* intron (primers: rpsF and rpsR2; Oxelman *et al.* 1997), the plastid *psbA-trnH* intergenic spacer (Sang *et al.* 1997, Tate & Simpson 2003, Britton 2010), the nuclear ribosomal *5S-rRNA* spacer (primers: P3 and P4; Cox *et al.* 1992), the plastid *rbcL* gene (rbc5' and rbc3'; Olmstead *et al.* 1992) and the nuclear ribosomal external transcribed spacer (*ETS*; primers: ETS-1F, 18S-R; Hershkovitz *et al.* 1999, Starr *et al.* 2003). Since the standard *trnH-psbA* primers (Tate & Simpson 2003, Sang *et al.* 1997) displayed non-specific binding for *Tetraria* species, the genus-specific primers; psbA^{TGC}, trnH^{ACT} and psbAAAR (Britton 2010) were used to amplify this region.

DNA was extracted using a standard cetyl trimethyl ammonium bromide (CTAB) protocol, with minor modifications (Doyle & Doyle 1987, Gawel & Jarret 1991). Leaf samples were ground in liquid nitrogen with a small amount of sterile sand using a mortar and pestle to pulverise tough fibres prior to the addition of CTAB. Reactions were performed on ice in 30μl volumes each containing 19.7 μl sterile water, 3 μl of 10 DNA polymerase buffer, 0.6 μl of $MgCl_2$ (50 mM), 1.0 μl of each primer (10 μM), 1.2 μl of dNTP (10 mM), 2.5 units of Taq DNA polymerase (Kapa Biosystems, Cape Town, South Africa) and 2 μl of template DNA.

Amplification was carried out on an Applied Biosystems GeneAmp 2700 thermal cycler (Applied Biosystems, Foster City, CA, USA) using the following program; initial denaturation of 2 min at 94°C; 30-50 cycles of 60 s at 52°C and 2 min at 72°C; and a final extension of 7 min at 72°C. Resultant PCR products were checked on 1% agarose and viewed under UV light with an UVIdoc gel viewing system (UVItec, Cambridge, England). Following purification by ethanol precipitation, products were cycle sequenced by Macrogen Inc. (Seoul, South Korea), with the BigDyeTM, using a 3730xl Automatic Sequencer (AB Biosystems; Foster City, USA). Forward and reverse sequences were assembled and edited in ChromasPro v 1.4.2 (www.technelysium.com.au/) before being exported to Bioedit v7.0.5 (Hall 2005) for manual alignment.

4.3.3 Phylogenetic analysis

Parsimony and Bayesian phylogenetic analyses were performed for each marker and for the combined plastid markers to allow for evaluation of conflict between gene regions and to assess the validity of combining all data to produce a total evidence tree.

Parsimony searches were conducted heuristically in PAUP∗ 4.0b10 (Swofford 2002) using 10,000 starting trees generated via random addition, with TBR branch swapping and MULTREES in effect. Branch support was estimated using 1,000 bootstrap replicates (Felsenstein 1985), with searches done heuristically as follows: 300 starting trees generated via random addition, with no more than 5 trees retained per replicate, TBR branch swapping and MULTREES in effect.

Bayesian inference was done using a mixed-model approach in MrBayes version 3.1.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003), as implemented in the high-performance computing facilities offered by the Computational Biology Service Unit at Cornell University (http://cbsu.tc.cornell.edu) and Bioportal at the University of Oslo, Norway (www.bioportal.uio.no). For each region and for the combined chloroplast and total combined analyses, the optimal data partitioning scheme was selected using the method of McGuire *et al.* (2007). This approach attempts to identify the minimum number of parameters required to capture the key features of the data, and was preferred over the universal application of the most parameter-rich model (as suggested by Huelsenbeck & Rannala 2004) because the dangers of over-parameterization are little known (McGuire *et al.* 2007). For *5S-rRNA* and *trnL-trnF* a single partition strategy was compared to one in which separate models were applied to coding versus non-coding sites. For *rbcL* a single partition was compared to one in which separate models were applied to first, second and third base-pair positions. For the combined chloroplast analysis, application of a single model to all loci was compared to a partitioning scheme which employed the optimal partitioning strategy previously identified for each loci. MrModeltest 2.3 (Nylander 2004) was used to determine the optimal model for each partition by comparing log-likelihoods. The preferred partitioning strategy for each analysis was then identified by comparing the harmonic mean likelihood (HML), Akaike information criteria for small samples (AICc) and Bayesian information criteria (BIC) for each partitioning strategy under comparison based on the optimal models of evolution (Table 4.2). For each partitioning scheme, the likelihoods were determined using six independent Metropolis-coupled Monte Carlo Markov Chain (MCMCMC) runs, each comprising one cold and three heated chains (temperature = 0.2). Each run lasted 10 million generations with a sample being drawn every 100th generation and, in all instances, only the last 45,000 samples were used to estimate posterior probabilities (PP), thus ensuring that only the stationary distribution was sampled. Plots of the log likelihoods against generation time, as well as comparisons of PP across independent runs, were used to confirm that the runs had converged and to determine whether and when stationarity was achieved. In addition, the adequacy of the effective sample sizes associated with each parameter estimate was evaluated using Tracer version 1.5.4 (Rambaut & Drummond 2007*b*). A combined set of the last 45,000 samples from each of the six runs was used to build the maximum clade credibility trees using TreeAnnotator (version 1.5.4; Rambaut & Drummond 2007*c*).

The presence of strong conflict between the nuclear *5S-rRNA* and plastid *trnH-psbA* regions for three species, *T. crinifolia*, *T. maculata* and *T. ferruginea*, meant that a combined analysis of plastid and nuclear sequences was unjustified. Here conflict is defined as contrasting topological arrangements reciprocally supported by PP>0.95 or parsimony bootstrap support values >50. Such topological incongruence reflects the independent histories of the different genes and can arise as the result of incomplete lineage sorting or introgressive hybridization (Doyle 1992, Maddison 1997).

Gene tree incongruence is common in many lineages and a number of approaches have been adopted to resolve such conflict (see Knowles 2009, Verboom et al. *In preparation b*). Coalescent-based methods could not be applied to the existing dataset because most species are represented by only a single accession and introgressive hybridization cannot be excluded as a cause of conflict (Knowles 2009). On the other hand, consensus and concordance methods are less desirable because they do not yield topologies with branch lengths (Adams 1972, Seelanan *et al.* 1997, Baum 2007). To accommodate and compare results using a range of different approaches, four separate analyses were performed. First, a total evidence approach was adopted, including all plastid and nuclear sequences (6,515 characters, 830 parsimony informative; Table 4.1) into a single combined analysis (Total Evidence). Second, a conflict decomposition analysis such as that employed by Pirie *et al.* (2008, 2009) was applied, splitting conflict taxa into separate accessions for the *5S-*

rRNA and plastid markers and analysing the combined dataset (Conflict Decomposition). Third, the *5S-rRNA* sequences for the conflict taxa were excluded from a combined analysis (Excluded). Lastly, the entire *5S-rRNA* marker was excluded and a combined analysis performed on the remaining sequence data (No5s). No analyses were performed excluding chloroplast data because a number of species are represented by chloroplast data only, including all the outgroup taxa. All analyses were performed in Mr Bayes as described for the analyses of separate markers above.

4.3.4 Molecular dating

Molecular dating was performed using a relaxed-clock Monte Carlo Markov Chain (MCMC) approach applying a log-normal model of rate variation among branches and Yule process prior for branching times implemented in BEAST (version 1.5.4; Drummond *et al.* 2006, Drummond & Rambaut 2007, Rambaut & Drummond 2007*a*). The analysis was performed on the Conflict Decomposition, Excluded and No5S combined datasets, applying separate models to each of the six markers. Additional partitioning within loci, favoured by the partitioning analysis (McGuire *et al.* 2007, Table 4.4), was not implemented in the BEAST runs because attempts to do so resulted in runs failing to reach stationarity and/or failing to converge. For the same reason, no BEAST results are reported for the Total Evidence data. Neighbour joining starting trees were used for all runs. The root node (divergence of Mapanioideae from the rest of Cyperaceae) age was constrained using a log-normal prior having a median of 44 million years (Myr) in accordance with the Middle Eocene fossil of *Volkeria messelensis* (Mapanioideae) identified by Smith *et al.* (2009) and corroborated by an age estimate from a dating analysis of Poales (Bremer 2002). Calibration was achieved by setting the mean root node age to 13.4 with an offset of 33.9, so as to set the Eocene/Oligocene boundary as solid lower bound, and setting the log(stdev) to 0.75175 such that the 95% confidence interval ranged from 36.2 to an upper bound of 78Mya, the age of the crown node of Cyperaceae identified by Janssen & Bremer (2004). A lognormal prior was used because it is most appropriate for calibrations based on fossil evidence (Ho & Phillips 2009), but while the fossil age is usually used as a solid lower bound here it is applied as the median for the lognormal prior to account for uncertainty in the fossil date (Smith *et al.* 2009).

Six independent MCMC runs of 20 million generations each, sampling every 2,000 generations, were performed for each analysis. The attainment of stationarity by each run as well as the convergence of independent runs was evaluated using Tracer (version 1.5; Rambaut & Drummond 2007*b*), the adequacy of sampling being assessed using the effective sample size (ESS) diagnostic. The individual runs converged quickly, identifying a burn-in of 2 million generations as adequate. The results of each analysis were summarised as maximum clade credibility (MCC) trees, built using TreeAnnotator (version 1.5.4; Rambaut & Drummond 2007*c*), and based on the combined posterior tree sets from all runs.

4.3.5 Rates and models of diversification

The timing of cladogenetic events was compared between the three MCC trees using cophenetic plots. Lineage-through-time (LTT) plots were generated for each of the dated MCC trees for the group as a whole and each of its major clades. Relative cladogenesis tests were then performed to identify rapid changes in the rates of species diversification through time (Purvis *et al.* 1995), and net diversification rates under a constant-rate lineage birth-death model were calculated for each tree and major clade using the method of Magallon & Sanderson (2001). This was done to allow comparison to rate estimates for Cape and other clades reported elsewhere (Magallon & Sanderson 2001, Warren & Hawkins 2006, Valente *et al.* 2010). Analyses were performed twice, with extinction rates set to proportions of 0 and 0.9 of the speciation rate. All analyses were performed using the *laser* 2.3 (Rabosky 2006*a*), *geiger* 1.3-1 (Harmon *et al.* 2008) and *ape* (Paradis *et al.* 2004) packages for R2.11.0 (R Development Core Team 2010).

For each of the three MCC trees, constant-rate and variable-rate diversification models were fitted to the branching times within the entire group and within each of its major clades and compared (Nee 2001, Rabosky 2006*b*, Rabosky & Lovette 2008*a*,*b*). This was done to test for a decrease in the rate of diversification and to test whether any such decline was density-dependent, as would be expected if there are ecological limits on diversification rate (Rabosky & Lovette 2008*a*,*b*, Rabosky 2009*a*, Figure 4.1). Likelihood based methods were preferred over the constant-rate test of Pybus & Harvey (2000) because they have been shown to perform better under a range of scenarios and provide greater insight into the diversification process (Rabosky 2006*b*). Constant-rate models applied here included both pure-birth and birth-death models which assume that diversification rates do not vary through time (Nee 2001). Four variable-rate models also were employed, each corresponding to a different evolutionary scenario. The two-rate Yule model is a pure-birth model which allows for a change in speciation rate at some point during the diversification of the clade, and should fit the data best if radiation was initially rapid and followed by a period of slow diversification at a constant rate (Rabosky 2006*b*). The time-varying speciation model (SPVAR, Rabosky & Lovette 2008*b*) fits a continuously declining diversification model by decreasing the speciation rate through time while keeping the extinction rate constant, and represents a scenario in which diversification rate is a function of time rather than the number of lineages present. One might expect this model to fit the data best if diversification was triggered by rapidly changing environmental conditions which then stabilized gradually with time. Finally, the linear and exponential density-dependent diversification rate models fit scenarios in which the rate of diversification is not directly dependent on time, but rather is a function of the number of lineages present at any point in time (Rabosky & Lovette 2008*a*). The density-dependent linear model is analogous to a logistic model of population growth but is termed the linear model because speciation rates decline linearly with increasing species number. The density-dependent exponential model fits a scenario in which the rate at which speciation decreases slows as the number of lineages present increases. This model should thus display stronger initial density-dependence than the linear model, but should never reach a point at which speciation is entirely inhibited. I do not report Rabosky & Lovette (2008*b*) model of time-varying extinction but constant speciation (EXVAR) or their model of time-varying speciation and extinction (BOTHVAR) because their study demonstrated that increasing rates of extinction cannot account for a pattern of explosive-early diversification. The SPVAR model outperformed the EXVAR and BOTHVAR models in all cases. Models were compared using Akaike's Information Criterion (AIC) because they were not nested, rendering the use of likelihood ratio tests unsuitable (Akaike 1974). All diversification rate models were fitted and compared using the *laser* 2.3 (Rabosky 2006*a*) package implemented in R2.11.0 (R Development Core Team 2010). The default starting parameters were used to fit the SPVAR model because results were insensitive to a broad range of starting parameters.

Diversification rate analyses are dependent on complete sampling of all extant taxa (Pybus & Harvey 2000, Rabosky & Lovette 2008*b*). To test for sensitivity of results to missing taxa, these analyses were performed, for each MCC tree, on a set of 1,000 trees which differed in having nine known missing taxa randomly added to the major clade to which, on the basis of morphology, they most probably belong. Their ages were sampled from an exponential distribution between 0 and the crown node age of the major clade. A more extreme scenario was also tested, in which double the numbers of missing taxa were added with ages sampled from an exponential distribution between 0 and half the age of the

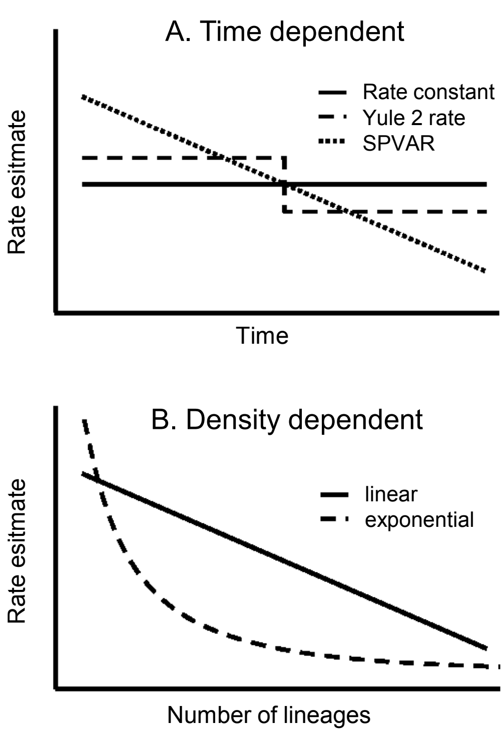


Figure 4.1: Diversification rate heterogeneity models illustrating the expected relationship between diversification rate and either time or the number of extant lineages. See text for details.

crown node for the major clade. This scenario concentrates the appearance of many taxa in the recent past, reducing the possibility of observing a decline in speciation rates. The number of random trees for which each model was preferred is reported, though the AIC values are not reported because they are not comparable when the underlying data are not identical.

4.4 Results

4.4.1 Phylogenetic relationships among species

More highly partitioned models were preferred for *5S-rRNA*, *rbcL* and the combined plastid analyses. The optimal partitioning scheme for *trnL-trnF* was ambiguous, however, with a comparison of harmonic mean likelihoods favouring the partitioned model and the

Akaike Information Criterion (AICc) and Bayesian Information Criterion (BIC) favouring the unpartitioned model (Table 4.2). Partitioned and unpartitioned models gave the same qualitative results, however, with near identical support values in all cases (partitioned model not shown).

Parsimony and Bayesian analyses of the independent molecular markers revealed variable levels of phylogenetic information with the plastid *trnL-trnF* and nuclear *5S-rRNA* and *ETS* regions resolving greater proportions of supported nodes (Figures 4.2 and 4.3). Sequence data for outgroup species were available or alignable only for the *rps16*, *trnL-trnF* and *rbcL* regions, with the latter two regions providing support for the monophyly of the reticulate-sheathed *Tetraria* species. Combined analysis of the plastid markers was justifiable given the absence of supported conflict between the *trnH-psbA*, *rps16*, *trnL-trnF* and *rbcL* regions and further supported the monophyly of the reticulate-sheathed Tetraria species (Figure 4.4).

Table 4.1: Summary statistics for each molecular marker included in this study

Region	Number of sequences	Total sites	Sites included	Variable sites included
trnL-trnF	43	1509	1370	375
trnH-psbA	26	1604	622	129
rps16	39	999	935	260
rbcL	29	1376	1376	236
5S-rRNA	26	460	254	99
ETS	32	567	567	217

Examination of the independently assorting markers revealed reciprocally supported conflict between the nuclear *5S-rRNA* analysis and the *trnL-trnF*, *rps16* and combined plastid analyses as indicated in Figure 4.5. Most of the markers provided some support for each of the three major clades (*microstachys-burmannii*, *fasciata-flexuosa* and *thermalis-bromoides*) identified by Slingsby and Verboom (2006), but while the plastid markers place *T. crinifolia* + *T. maculata* and *T. ferruginea* in two separate positions within the *fasciata-flexuosa* clade, the *5S-rRNA* analysis prefers these species to form a well supported outside the *fasciata-flexuosa* clade.

The Conflict decomposition and Total evidence combined analysis approaches gave near identical topologies for the ingroup species and split the ingroup into three well supported clades (Figure 4.6). The Conflict decomposition analysis placed the plastid

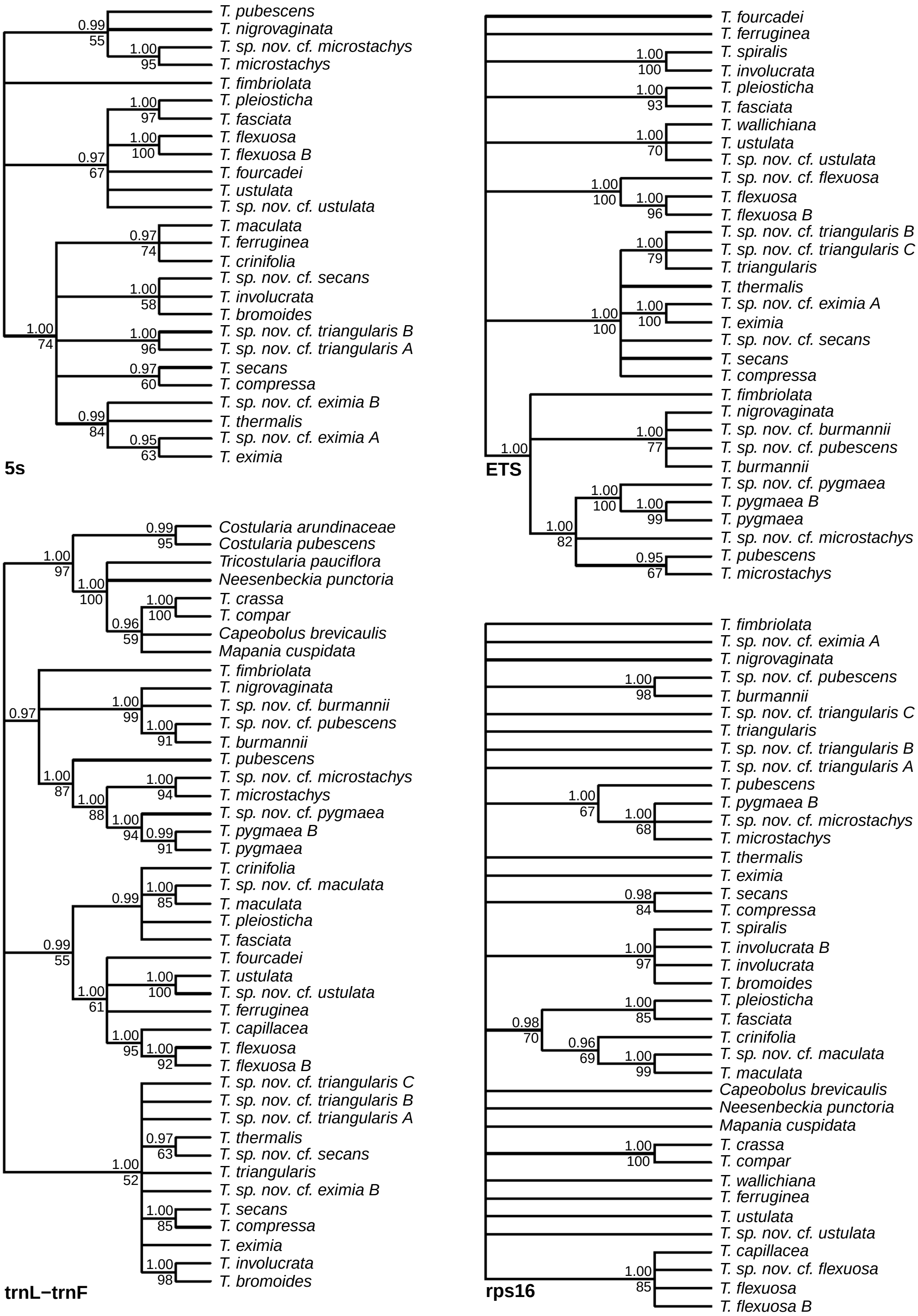


Figure 4.2: Bayesian maximum clade credibility (MCC) trees for the *5S-rRNA*, *ETS*, *trnL-trnF* and *rps16* molecular markers. Values above the nodes indicate Bayesian posterior probabilities >0.95, while values below the nodes indicate parsimony bootstrap support values >50.

Table 4.2: Results of the partitioning strategy analysis

	Pi	n	N	-HML	AICc	BIC
5S-rRNA Unpartitioned	11	254	26	1353.84	2730.77	3039.92
5S-rRNA Partitioned	16	254	26	1330.88	2696.06	3021.69
rbcL Unpartitioned	11	1376	29	4364.69	8751.58	9206.36
rbcL Partitioned	28	1376	29	4169.78	8396.77	8939.40
trnL-trnF Unpartitioned	11	1384	43	5213.73	10449.65	11107.34
trnL-trnF Partitioned	21	1384	43	5208.11	10458.90	11168.42
Combined plastid Unpartitioned	11	4318	56	15581.35	31184.76	32167.17
Combined plastid Partitioned	65	4318	56	15137.33	30406.68	31731.14

Notes: Partitioning strategies include splitting coding and non-coding regions for *5S-rRNA* and *trnL-trnF*, and separating first, second and third base pair positions for *rbcL*. The partitioned Combined plastid analysis employed separate models for *rps16*, *trnH-psbA*, *rbcL* and *trnL-trnF* regions, including the preferred partitioning strategy for *rbcL* and *trnL-trnF*. The number of parameters (Pi), number of sites (n), number of sequences (N), negative harmonic mean likelihood (-HML), Akaike information criterion for small sizes (AICc) and Bayesian information criterion (BIC) are reported for each model.

accessions of *T. crinifolia*, *T. maculata* and *T. ferruginea* in the *fasciata-flexuosa* clade, consistent with their placement in the other three combined analysis approaches (Figure 4.6 and 4.7), whereas the *5S-rRNA* accessions of *T. crinifolia*, *T. maculata* and *T. ferruginea* were placed amongst the outgroup species, for which *5S-rRNA* data were lacking. Other than the inclusion of the independent *5S-rRNA* and plastid accessions of the three conflict species, the Total evidence and Conflict decomposition analyses differed only in that the former did not provide significant support for the monophyly of *T. crinifolia* + *T. maculata* + *T. sp. nov. cf. maculata* + *T. fasciata* + *T. pleiosticha*. This is likely due to conflict between *5S-rRNA* and plastid sequences with respect to the placement of the conflict species. The Excluded and No 5S analyses did provide support for this clade and differed from the Total evidence and Conflict decomposition analyses only in that they did not find significant support for the *microstachys-burmannii* clade, while the No 5S analysis resolved fewer relationships among species within the *thermalis-bromoides* clade.

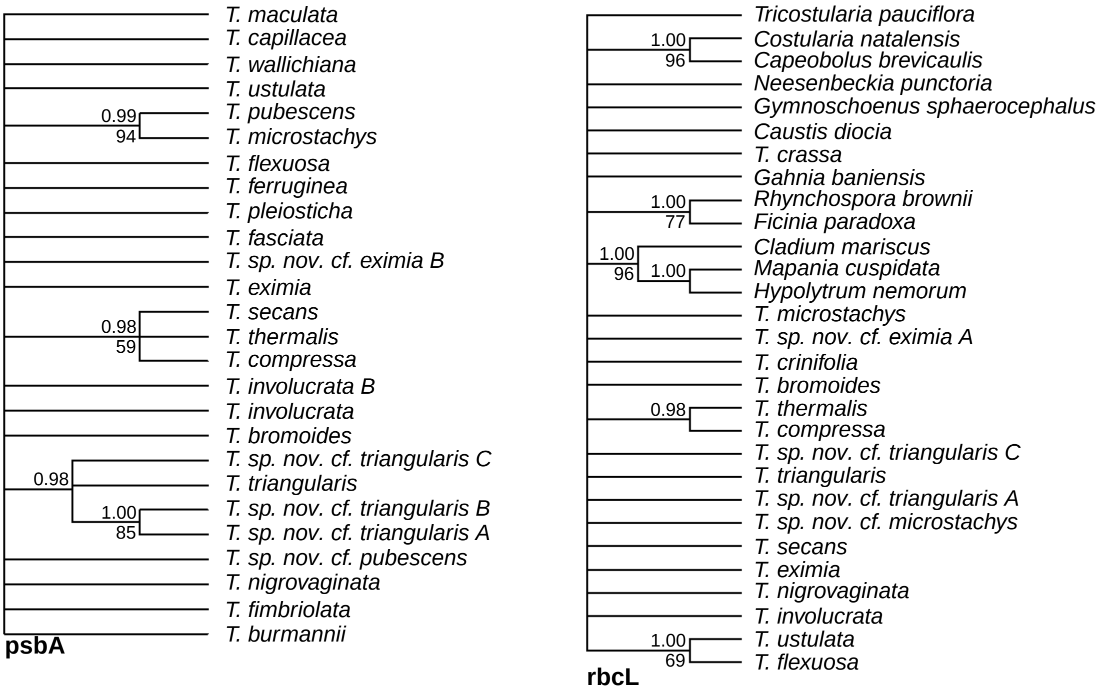


Figure 4.3: Bayesian maximum clade credibility (MCC) trees for the *trnH-psbA* and *rbcL* molecular markers. Values above the nodes indicate Bayesian posterior probabilities >0.95, while values below the nodes indicate parsimony bootstrap support values >50.

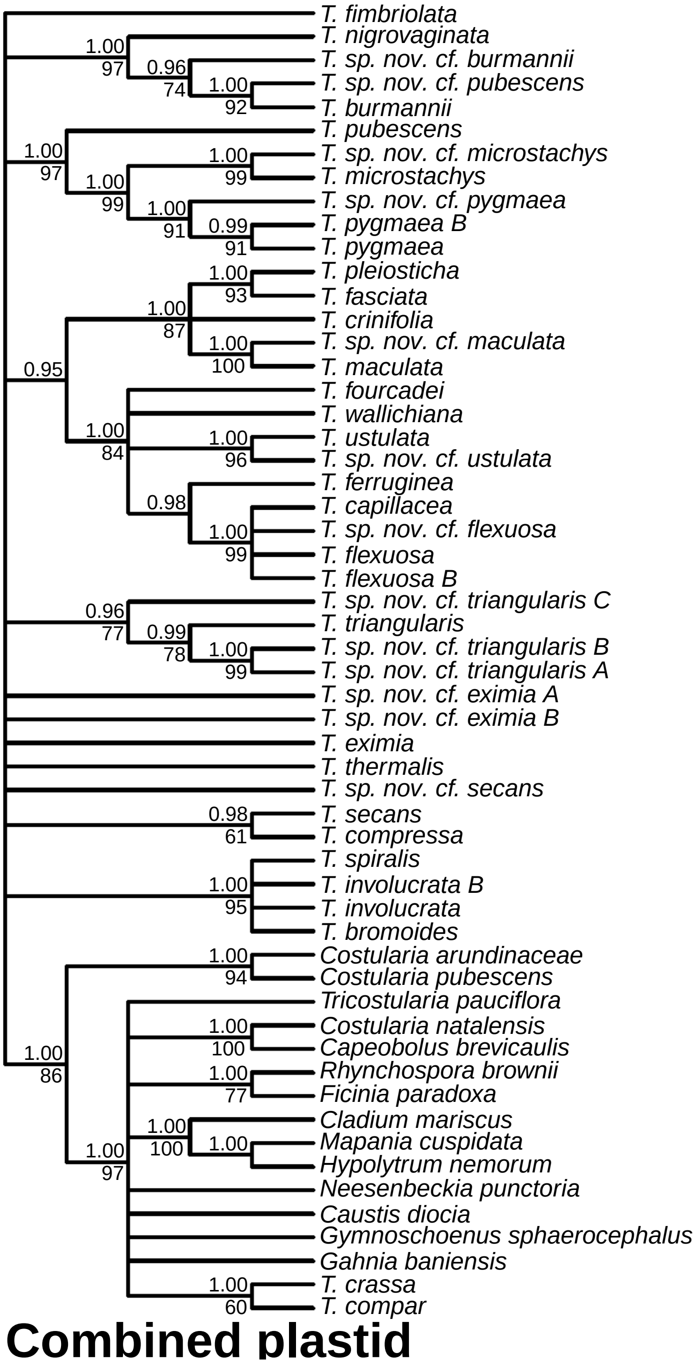


Figure 4.4: Bayesian maximum clade credibility (MCC) trees for the combined chloroplast markers. Values above the nodes indicate Bayesian posterior probabilities >0.95, while values below the nodes indicate parsimony bootstrap support values >50.

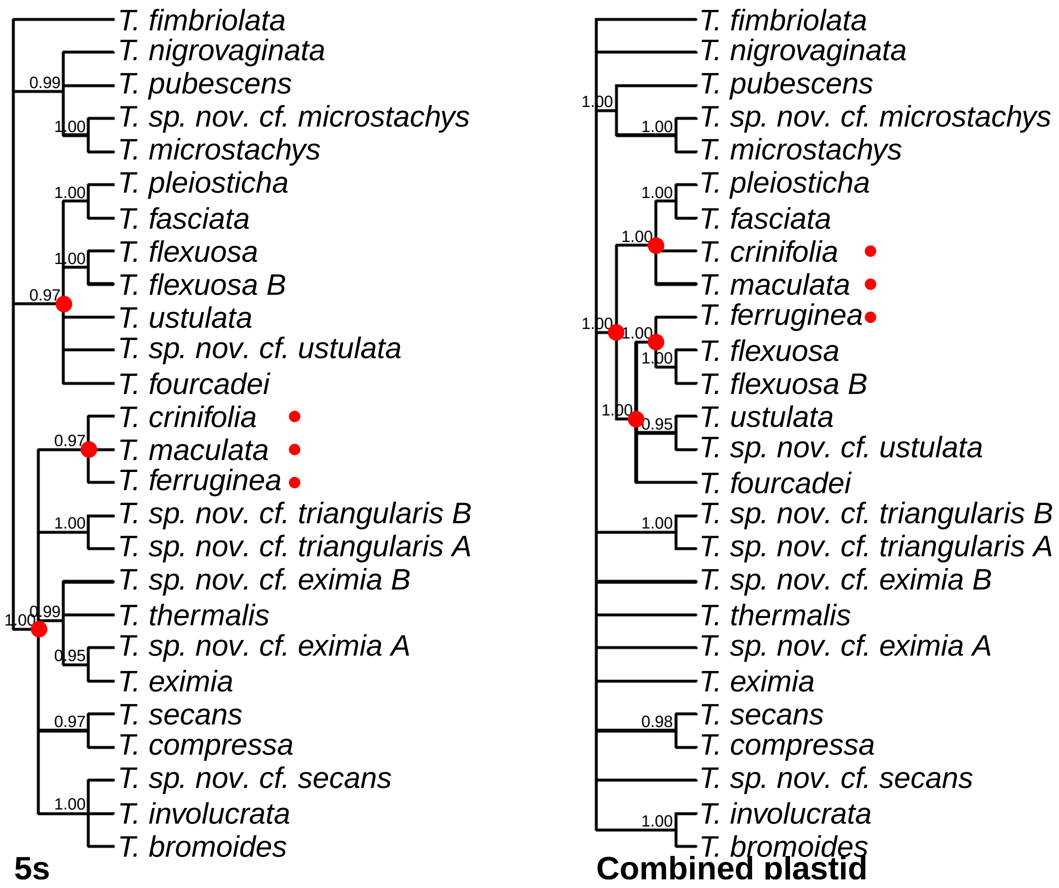
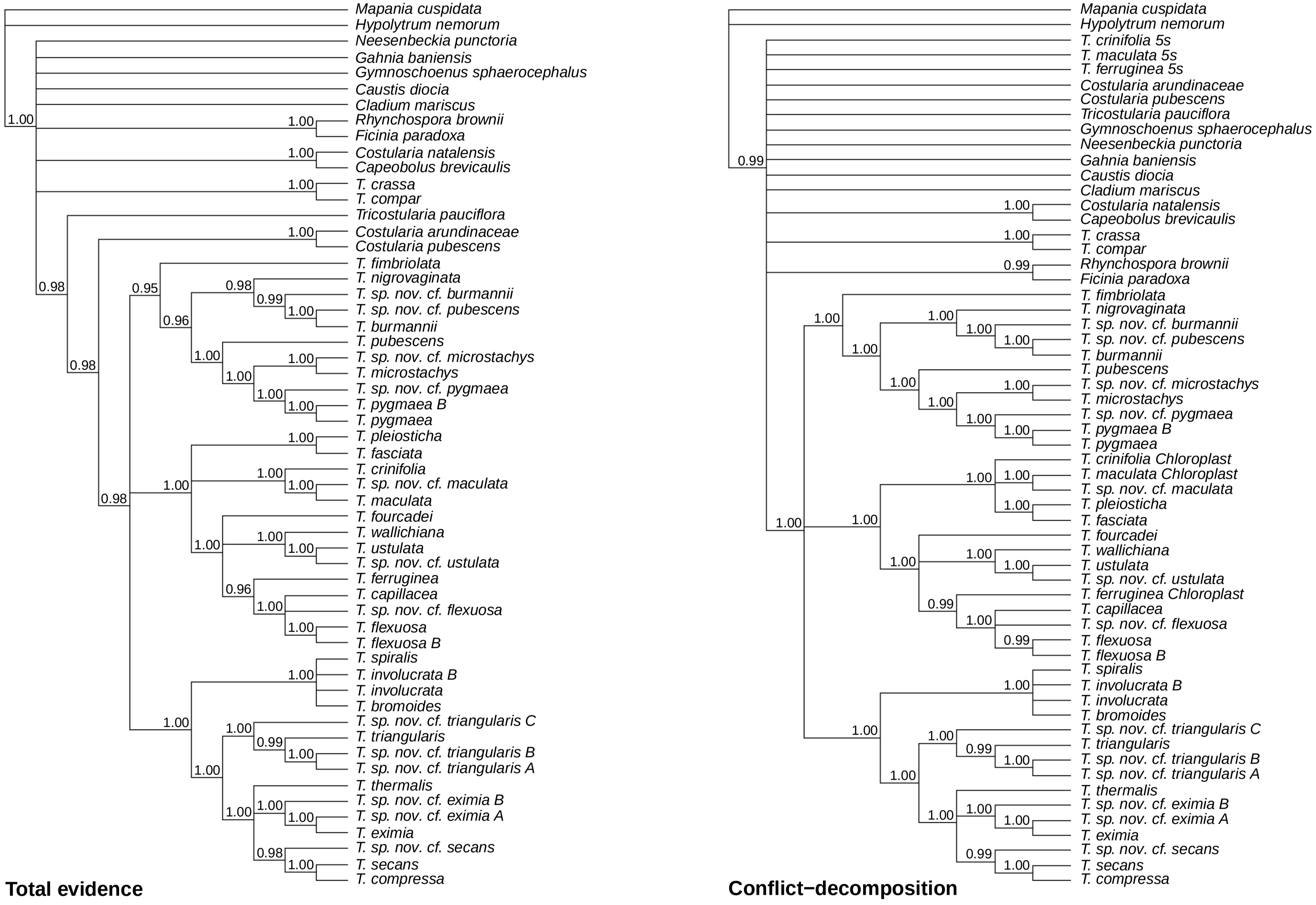


Figure 4.5: Trimmed combined chloroplast and *5S-rRNA* Bayesian MCC trees illustrating the conflicting positions for *T. maculata*, *T. crinifolia* and *T. ferruginea*. Red dots indicate conflict taxa and supported conflicting nodes. Values above the nodes indicate Bayesian posterior probabilities >0.95, while values below the nodes indicate parsimony bootstrap support values >50.



Total evidence

Conflict−decomposition

Figure 4.6: Bayesian MCC trees for the Total evidence and Conflict decomposition combined analysis approaches. Values above the nodes indicate Bayesian posterior probabilities >0.95, while values below the nodes indicate parsimony bootstrap support values >50.

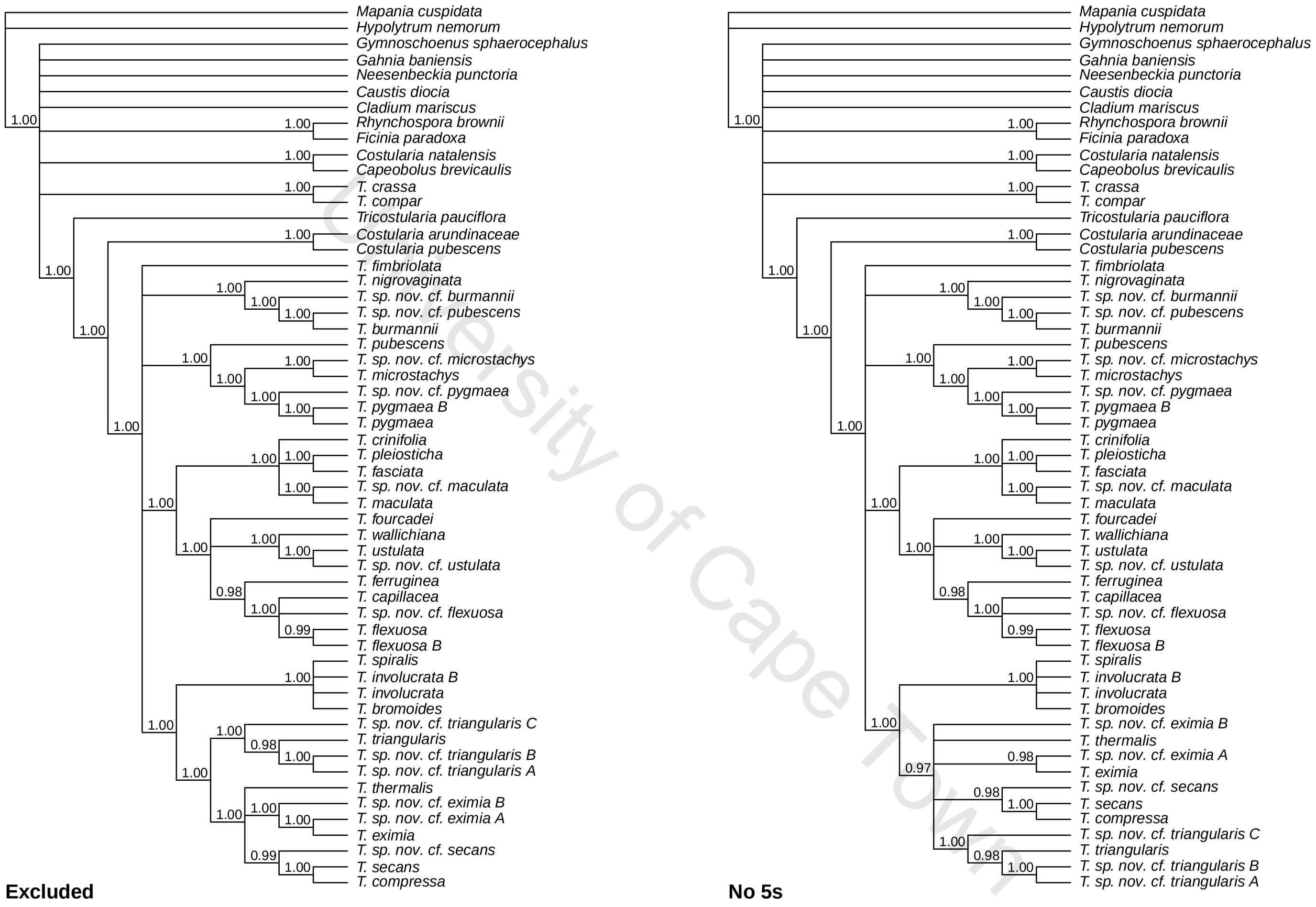


Figure 4.7: Bayesian MCC trees excluding *5S-rRNA* sequences from all taxa (Excluded) and from just the three conflict taxa (No 5S). Values above the nodes indicate Bayesian posterior probabilities >0.95, while values below the nodes indicate parsimony bootstrap support values >50.

4.4.2 The timing and rate of diversification

The molecular dating analyses for the three different combined analysis approaches provided slightly different estimates for the timing of cladogenesis within *Tetraria* (Figure 4.8). The Conflict decomposition analysis provided the oldest estimate of the crown node age at 16.56 Myr (95% confidence interval: 10.98 - 23.26), while the Excluded analysis pushed this date forward to 16.31 Myr (10.59 - 22.77) and the No 5S analysis provided the youngest estimate at 15.20 Myr (10.12 - 21.29). This provides a high degree of confidence that the genus has been diversifying since the early to mid Miocene.

Table 4.3: Net diversification rates for the genus *Tetraria* and each of the major subclades under two extreme extinction rates

Clade	Analysis	ε=0.0	ε=0.9
Tetraria			
	Conflict Decomposition	0.1822	0.0920
	Excluded	0.1846	0.0933
	No 5S	0.1982	0.1001
thermalis-bromoides			
	Conflict Decomposition	0.1612	0.0646
	Excluded	0.1739	0.0697
	No 5S	0.1645	0.0659
microstachys-burmannii			
	Conflict Decomposition	0.1176	0.0430
	Excluded	0.1174	0.0429
	No 5S	0.1240	0.0453
fasciata-flexuosa			
	Conflict Decomposition	0.1417	0.0556
	Excluded	0.1375	0.0540
	No 5S	0.1444	0.0567

Notes: Results reflect net diversification rates estimated using the method of Magallon & Sanderson (2001) implemented in geiger. All estimates were performed with no extinction (ε=0.0) and with the extinction rate set at 90% of the speciation rate (ε=0.9).

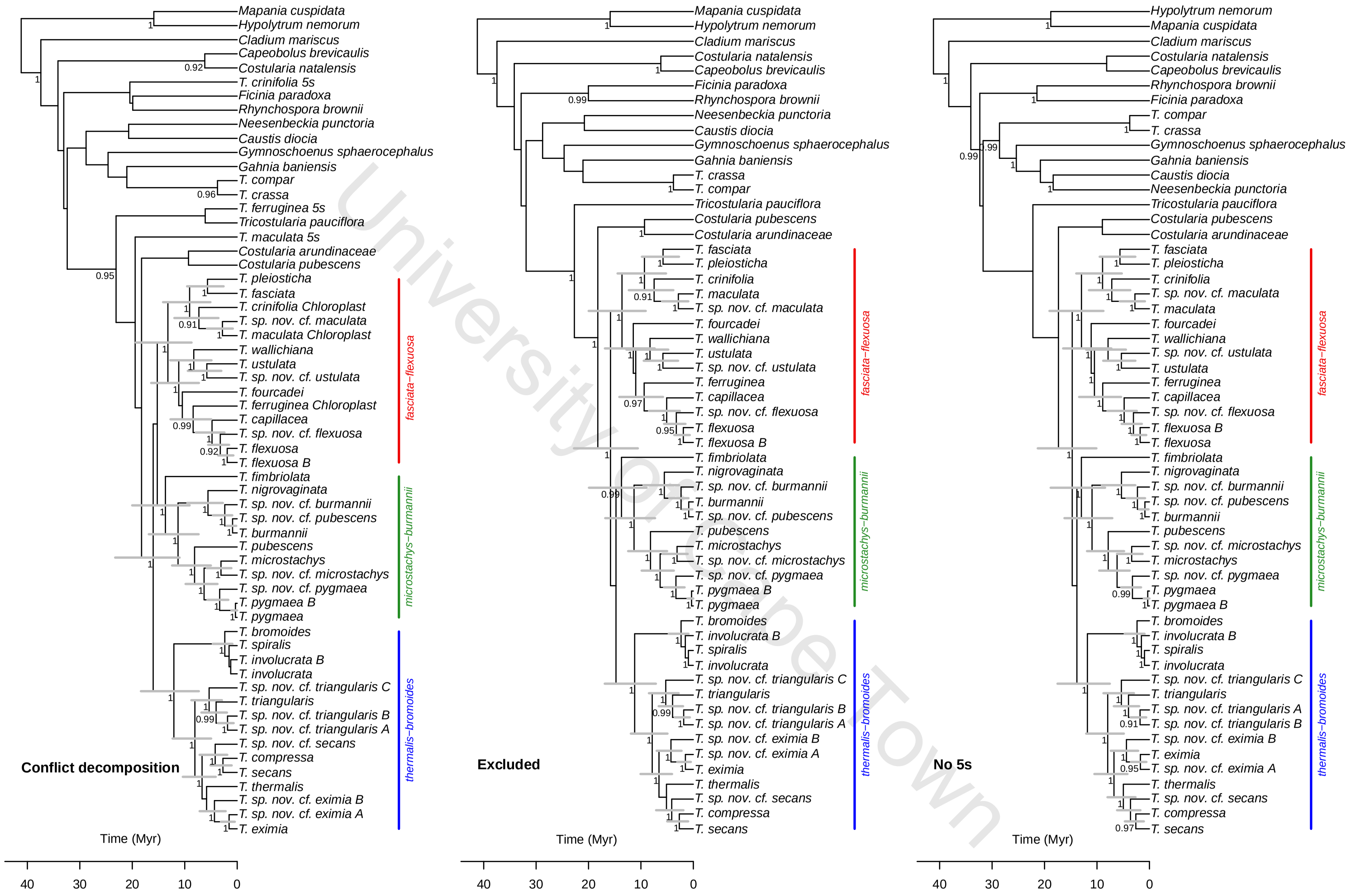


Figure 4.8: Dated Bayesian MCC trees for the Conflict decomposition, Excluded and No 5S combined analysis approaches indicating median node heights. Grey bars indicate 95% confidence intervals on node age estimates. Values above the nodes indicate Bayesian posterior probabilities >0.95, while values below the nodes indicate parsimony bootstrap support values >50. The three major clades within the ingroup are indicated by coloured vertical bars.

Estimates of node ages within the group are similar for all three dating analyses (Figure 4.8). Comparison of the pairwise distances between species for each of the three sets of trees revealed that the Conflict decomposition analysis provided slightly different estimates for some species pairs owing to slight differences in topology (Figure 4.9). The Conflict decomposition analysis placed *T. fourcadei* in a clade with *T. ferruginea*, *T.capillacea*, *T. flexuosa* and *T. sp. nov. cf. flexuosa*, this clade being sister to a clade comprising *T. ustulata*, *T. sp. nov. cf. ustulata* and *T. wallichiana*. In contrast, the Excluded and No 5S analyses placed T. fourcadei as sister to both these clades (Figure 4.8). The Conflict decomposition analysis also placed the *thermalis-bromoides* clade as sister to the other two major clades with PP = 0.93, while the Excluded and No 5S analyses identified the *fasciata-flexuosa* clade as sister with $PP < 0.9$.

Lineage-through-time plots were little affected by differences between the MCC trees, consistently indicating a slow decline in the rate of species accumulation for the three major clades and the genus as a whole since the early to mid Miocene (Figure 4.9). Relative cladogenesis tests revealed no significant rapid changes in the rates of diversification within any of the three MCC trees, or in the three major clades within each tree (results not shown). The net diversification rates of the three major clades, estimated under Magallon and Sanderson's (2001) birth-death model (Table 4.3), and the LTT plots indicate that the *thermalis-bromoides* clade has been diversifying more rapidly than the other two major clades.

Comparison of AICs between models of diversification rate identified variable-rate models, particularly the linear density-dependent model, as best describing diversification within the *thermalis-bromoides* and *fasciata-flexuosa* clades, and within the group as a whole (Table 4.4). This indicates that the rate of diversification of these clades has slowed through time, and that this decrease is better described as a function of lineage number than as a function of time. A pure-birth (constant rate) model was preferred for the *microstachys-burmannii* clade, indicating that radiation in this group has not slowed down and shows no signal of density dependence. Differences in the AIC between models were frequently below 2 units, however, suggesting that alternative models should not be rejected out of hand. In particular, within the *thermalis-bromoides* clade the linear density-dependent model was preferred over the pure-birth model by only 0.04 to 0.524 AIC units, while within the *microstachys-burmannii* clade the pure-birth model was preferred over the linear density-dependent model by between 0.689 and 0.945 AIC units. Differences between AICs were dependent on the MCC tree being used, with models based in the No 5S MCC tree

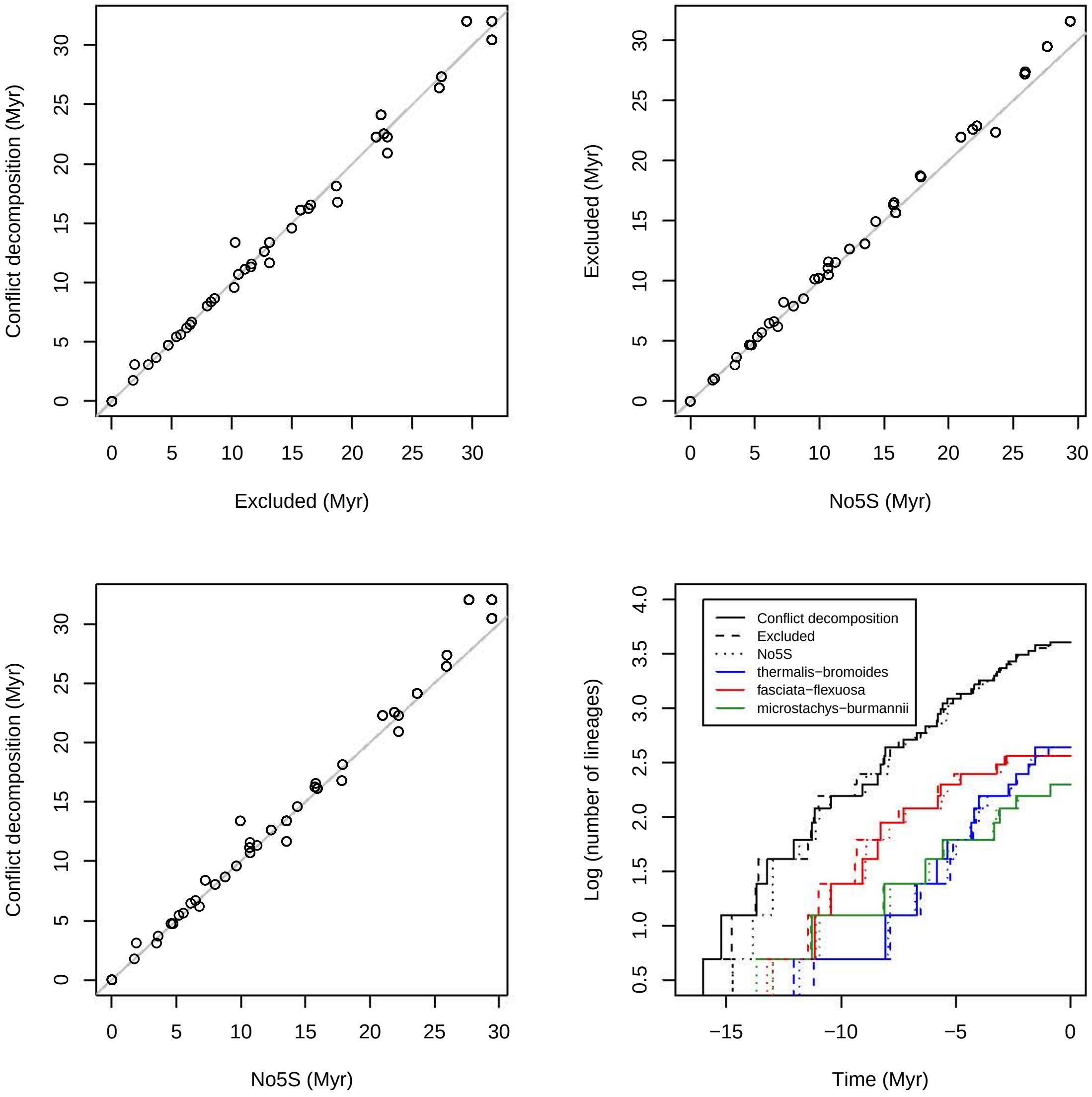


Figure 4.9: Cophenetic plots indicating similarity in node age estimates using each of the three combined analysis approaches and lineage-through-time plots for *Tetraria* and each of the three subclades generated for each of the combined analysis phylogenies.

showing the smallest differences.

The sensitivity tests used to account for incomplete taxon sampling generally favoured the same models as those identified above, implying that taxon sampling was generally not a problem. This was true even in the extreme case where twice the known number of missing taxa were included in half the age of the major clades. The only major difference was that the majority of the sensitivity analysis models for the full group based on the No 5S MCC trees preferred a density-dependent exponential model over the density-dependent linear model preferred by the original diversification rate analyses.

Table 4.4: Fit of constant and variable models of diversification rates.

Model	Model type	Rate type	np	Rate	LH	AIC	N_1	N_2
Tetraria								
Conflict decomposition								
pureBirth	yule	constant	1	0.139	-8.462	18.925	0	118
DDX	dd exponential	variable	2	0.709	-5.392	14.785	302	56
DDL	**dd linear**	**variable**	**2**	**0.310**	**-4.055**	**12.110**	**654**	**596**
yule2rate	yule	variable	3	0.181	-4.334	14.669	44	230
SPVAR	cont. decline	variable	3	0.386	-5.742	17.484	0	0
Excluded								
pureBirth	yule	constant	1	0.139	-8.373	18.746	0	0
DDX	dd exponential	variable	2	0.755	-5.172	14.344	415	339
DDL	**dd linear**	**variable**	**2**	**0.305**	**-4.194**	**12.389**	**439**	**429**
yule2rate	yule	variable	3	0.180	-4.454	14.908	146	232
SPVAR	cont. decline	variable	3	0.386	-5.624	17.248	0	0
No5S								
pureBirth	yule	constant	1	0.144	-7.213	16.426	0	21
DDX	dd exponential	variable	2	0.962	-3.372	10.743	777	476
DDL	**dd linear**	**variable**	**2**	**0.328**	**-2.552**	**9.104**	**155**	**360**
yule2rate	yule	variable	3	0.182	-2.412	10.824	68	143
SPVAR	cont. decline	variable	3	0.423	-3.935	13.871	0	0
thermalis-bromoides								
Conflict decomposition								
pureBirth	yule	constant	1	0.165	-11.057	24.115	231	407
DDX	dd exponential	variable	2	0.326	-10.717	25.434	0	0
DDL	**dd linear**	**variable**	**2**	**0.371**	**-9.795**	**23.591**	**748**	**582**
yule2rate	yule	variable	3	0.263	-9.901	25.802	21	11
SPVAR	cont. decline	variable	3	0.223	-10.985	27.971	0	0
Excluded								
pureBirth	yule	constant	1	0.174	-10.407	22.814	230	419
DDX	dd exponential	variable	2	0.372	-10.004	24.009	0	0
DDL	**dd linear**	**variable**	**2**	**0.392**	**-9.149**	**22.298**	**740**	**560**
yule2rate	yule	variable	3	0.290	-9.060	24.120	30	21
SPVAR	cont. decline	variable	3	0.250	-10.296	26.593	0	0

Table 4.4: (continued on next page)

Table 4.4: (continued)

Model	Model type	Rate type	np	Rate	LH	AIC	N_1	N_2
No5S								
pureBirth	yule	constant	1	0.171	-10.635	23.271	322	472
DDX	dd exponential	variable	2	0.314	-10.365	24.730	0	0
DDL	**dd linear**	**variable**	**2**	**0.358**	**-9.618**	**23.236**	**650**	**397**
yule2rate	yule	variable	3	0.218	-9.685	25.371	28	131
SPVAR	cont. decline	variable	3	0.212	-10.601	27.202	0	0
microstachys-burmannii								
Conflict decomposition								
pureBirth	**yule**	**constant**	**1**	**0.117**	**-12.357**	**26.715**	**523**	**852**
DDX	dd exponential	variable	2	0.287	-12.053	28.106	259	0
DDL	dd linear	variable	2	0.239	-11.817	27.634	161	30
yule2rate	yule	variable	3	0.149	-11.911	29.821	57	108
SPVAR	cont. decline	variable	3	0.138	-12.340	30.680	0	0
Excluded								
pureBirth	**yule**	**constant**	**1**	**0.117**	**-12.367**	**26.734**	**526**	**571**
DDX	dd exponential	variable	2	0.285	-12.067	28.134	264	158
DDL	dd linear	variable	2	0.237	-11.840	27.679	166	133
yule2rate	yule	variable	3	0.149	-11.916	29.832	44	137
SPVAR	cont. decline	variable	3	0.137	-12.351	30.702	0	0
No5S								
pureBirth	**yule**	**constant**	**1**	**0.121**	**-12.088**	**26.176**	**482**	**859**
DDX	dd exponential	variable	2	0.336	-11.702	27.404	302	0
DDL	dd linear	variable	2	0.263	-11.432	26.865	186	43
yule2rate	yule	variable	3	0.162	-11.463	28.927	30	97
SPVAR	cont. decline	variable	3	0.156	-12.044	30.087	0	0
fasciata-flexuosa								
Conflict decomposition								
pureBirth	yule	constant	1	0.106	-15.656	33.312	0	55
DDX	dd exponential	variable	2	1.040	-12.979	29.959	89	111
DDL	**dd linear**	**variable**	**2**	**0.507**	**-10.409**	**24.819**	**907**	**803**
yule2rate	yule	variable	3	0.202	-12.169	30.339	4	31
SPVAR	cont. decline	variable	3	0.463	-13.057	32.114	0	0
Excluded								
pureBirth	yule	constant	1	0.103	-16.027	34.054	0	31
DDX	dd exponential	variable	2	1.050	-13.295	30.590	136	162
DDL	**dd linear**	**variable**	**2**	**0.484**	**-10.877**	**25.755**	**857**	**737**
yule2rate	yule	variable	3	0.201	-12.341	30.681	7	70
SPVAR	cont. decline	variable	3	0.445	-13.423	32.846	0	0
No5S								
pureBirth	yule	constant	1	0.108	-15.518	33.036	0	44
DDX	dd exponential	variable	2	1.149	-12.741	29.482	140	190
DDL	**dd linear**	**variable**	**2**	**0.503**	**-10.420**	**24.840**	**852**	**739**

Table 4.4: (continued on next page)

Table 4.4: (continued)

Model	Model type	Rate type	np	Rate	LH	AIC	N_1	N_2
yule2rate	yule	variable	3	0.208	-11.897	29.794	8	27
SPVAR	cont. decline	variable	3	0.463	-12.909	31.818	0	0

Notes: Preferred models for each clade as inferred from AIC scores are highlighted in bold. Results of sensitivity analyses are indicated by subscripts 1=known missing taxa randomly added within the crown age of each major clade, 2=twice the number of missing taxa randomly added within half the ages of the major clades. Results of the birth-death model are not reported because parameter estimates and likelihoods were near identical to the pure-birth model, and AICs worse, in all cases. The extra parameter estimated by the birth-death models meant that these models were worse than the pure-birth models by 2 AIC units in all cases. dd=density-dependent, np= number of parameters, LH=likelihood, AIC=Akaike's information criterion, Rate=estimated initial diversification rate, N=number of trees with randomly added missing taxa from sensitivity analysis 1 and 2 that preferred the model.

4.5 Discussion

Improving our understanding of the timing and drivers of diversification of the Cape flora is dependent on thorough investigation of the lineages that best epitomise the flora (Linder 2003). Here I estimate the timing and rates of lineage diversification in the sedge genus *Tetraria*, one of the smaller Cape Floral Clades, in an attempt to test whether diversification was triggered by environmental change in the late Miocene/early Pliocene, and whether rates have slowed as environmental conditions have stabilized, possibly due to ecological limits to diversification.

Reciprocally supported conflict between the nuclear 5S and the chloroplast gene regions for three taxa rendered a combined total evidence analysis of the sequence data unsuitable. Four alternative approaches for dealing with this conflict provided similar topological arrangements, with no reciprocally supported conflict between them. All analyses split the genus into the three major clades (*thermalis-bromoides*, *fasciata-flexuosa* and *microstachys-burmannii*) identified in Slingsby & Verboom (2006), but failed to resolve the relationships between them with significant support (PP $\ll$0.95). The *thermalis-bromoides* clade consists of a set of 14 broad-leaved species that are typically large in stature and consist of few-to-many florets of leaves, or tillers (Plate 1A), the *microstachys-burmannii* clade consists of ten species that are both small in stature and consist of few tillers (Plate 1B), and the weakly supported *fasciata-flexuosa* clade consists of 13 small to medium sized species, individuals of which are often composed of large numbers of tillers

(Plate 1C). The chloroplast placement of the three conflict taxa, *T. crinifolia*, *T. maculata* and *T. ferruginea*, in the *fasciata-flexuosa* clade is most consistent with their morphology. The placement of the *5S-rRNA* accessions for these species among the outgroups in the Conflict Decomposition analysis raises scepticism about the utility of the *5S-rRNA* data for these species.

The fossil-calibrated molecular dating analyses placed the age of the crown node of the genus at approximately 16 Ma, with confidence intervals ranging from 24 to 10 Ma, indicating that the onset of diversification in the genus is concordant with an early- to mid-Miocene trigger as observed for a number of other Cape groups (Table 4.5). This is coincident with the opening of the Drake Passage around 28.5 Mya which is believed to have led to the establishment of the Benguela upwelling system and associated Mediterranean climate around 10 Mya (Siesser 1980, Linder 2003), suggesting that climatic change over this period triggered the radiation of the group.

When estimated under a constant-rate lineage birth-death model, net diversification rates for *Tetraria* are consistent with estimated rates for Cyperales as a whole and faster than those for angiosperms as a whole (Magallon & Sanderson 2001) (Tables 4.3 and 4.5). These rates are, however, lower than estimated rates within most Cape clades and most closely resemble those within the slowly diversifying Cape Restionaceae (Warren & Hawkins 2006). The low relative diversification rates of these two groups is surprising given that both Magallon & Sanderson (2001) and Davies *et al.* (2004) found Cyperales and Poales to be amongst the fastest diversifying lineages worldwide. A potential explanation for these lower rates relative to other lineages in the Cape is the absence of pollinator specialization, believed to be a major driver contributing to the diversification of the Cape Flora through disruptive selection or as a genetic isolating mechanism (Johnson 1996, Johnson *et al.* 1998, van der Niet & Johnson 2009, Waterman *et al.* 2011). The importance of pollinators in inflating speciation rates in the Cape flora could be tested by comparing diversification rates in biotic versus anemophilous clades within lineages having both pollination modes such as *Erica* or *Leucadendron*.

Table 4.5: Crown ages and net diversification rates for Cape and higher clades

Clade	Age (Myr)	Upper divergence		Lower divergence		Source
		ε = 0.0	ε = 0.9	ε = 0.0	ε = 0.9	
Heliophileae	10 - 7	0.6854	0.3973	1.0004	0.5799	Linder and Hardy 2004†
Indigofera∗	19 - 10	0.2001	0.1106	0.5631	0.3112	Linder and Hardy 2004†
Moraea	ca. 15	0.1993	0.1291	0.2939	0.1903	Linder and Hardy 2004†
Pelargonium∗	22 - 18	0.2070	0.1343	0.2876	0.1867	Linder and Hardy 2004†
Phylica	8 - 7	0.5397	0.3394	0.6168	0.3878	Linder and Hardy 2004†
Restionaceae∗	43 - 28	0.1161	0.0781	0.2487	0.1672	Linder and Hardy 2004†
Ehrharta	10 - 8	-	-	-	-	Verboom et al. 2003
Ixioideae	ca. 20	-	-	-	-	Linder and Hardy 2004
Muraltia	29 - 21	-	-	-	-	Forest et al. 2007
Melianthus	35 - 6	-	-	-	-	Linder et al. 2006
Protea∗	27 - 11	0.12 - 0.27	-	-	-	Valente et al. 2009
Cyperales	45	0.1908	0.1540	-	-	Magallon and Sanderson 2001
Angiosperms	132	0.0893	0.0767	-	-	Magallon and Sanderson 2001

Notes: Diversification rates for clades marked with a † were obtained from Warren and Hawkins 2006. Estimates were provided without extinction (ε = 0.0) and with the extinction rate set to 90% of the speciation rate (ε = 0.9). Diversification rates for Protea were estimated from a random sample of 1000 BEAST trees with extinction rates allowed to vary. Diversification rates for Cyperales and Angiosperms as a whole were based on mean data estimates.

Variable-rate diversification models provided a better fit to the timing of cladogenic events in *Tetraria* and two of the major clades than constant-rate models, implying that diversification rates have changed during the history of the group. The density-dependent linear model showed the best fit to the data, indicating that the decline in diversification rate is better explained by the number of lineages present than by time. This implies that the radiation of the genus has been constrained either by limitations on the ecological niche-space available for new species, or by canalization of genetic variation and developmental systems which reduces the propensity for subsequent divergence (Foote 1997, Rabosky & Lovette 2008*a*, Rabosky 2009*a*). Low levels of co-occurrence among closely-related, functionally similar species pairs at fine spatial scales (Slingsby & Verboom 2006) support the first explanation, suggesting that ecological niche-space is limiting and is a likely cause of the signal of density dependence evident in the diversification rates. The absence of a density dependent decline in the diversification rate for the *microstachys-burmannii* clade is consistent with this hypothesis because this clade contains fewer species that could potentially interact, and their smaller body size may allow them to partition niches at finer scales.

Estimates of diversification rates are highly sensitive to taxon sampling, with incomplete taxon sampling yielding an impression of a decrease in the rate of diversification through time (Pybus & Harvey 2000). Although nine species were missing from the analyses presented here, the randomized inclusion of placeholders for these missing species, within each of the major clades most consistent with their morphology indicates that the results are robust to the omission of these taxa. The results are also robust to an extreme scenario in which double the number of placeholders were added to each major clade, to branches that are younger than half the crown node age. Given that a current taxonomic treatment of the genus looks set to double the number of formally-recognised species (Verboom et al. *In preparation* a), it seems likely that a number of species remain to be discovered and described. Another potential problem in this regard is that the application of molecular techniques threatens to amplify the number of species further, the current species set being defined largely on the basis of morphological diagnosability. A recent phylogeographic study of *T. triangularis*, for example, revealed the existence of four cryptic species that are geographically distinct and have been genetically isolated for between 2 and 7 million years, but are diagnosable only by the subtlest differences in spikelet size, spikelet number and inflorescence architecture (Britton 2010). If other *Tetraria* species are also made up of multiple cryptic lineages there could be a large number of species not included in the analysis presented here.

Given evidence for density-dependent constraints on diversification rates in *Tetraria* it is tempting to propose that the radiation of the group was adaptive, dominated by ecological speciation driven by disruptive selection and slowing as the available ecological niches were filled, as suggested for North American wood warblers (Rabosky & Lovette 2008*a*). An equally plausible scenario, however, is that the radiation was non-adaptive, with non-ecological speciation happening in allopatry and ecological differentiation taking place subsequently due to neutral divergence, adaptation to divergent selective pressures or ecological character displacement in sympatry (Wiens 2004, Rundell & Price 2009). In this scenario diversification rates would have declined as the number of lineages increased because repeated allopatric speciation events could only have occurred across the same boundary once the initial pair of populations were genetically isolated and wouldn't introgress and species were sufficiently ecologically differentiated to allow co-occurrence. Consistent with this hypothesis, recent work on the *T. triangularis* complex of species found them to be largely allopatric with little morphological or ecological differentiation, suggesting non-ecological speciation in allopatry (Britton 2010). Where species in the complex do occur sympatrically they appear to assort along a moisture gradient and display accentuated morphological differences suggesting niche and character displacement (M.N. Britton *pers com.*).

Irrespective of the manner in which *Tetraria* has radiated, the density dependent decline in diversification rates in the group implies that there are ecological limits to the number of species that can be packed into the CFR and that sister species must either be geographically isolated or ecologically differentiated for diversity to be maintained. This provides strong links between the ecology of *Tetraria* species and their evolutionary history and provides a basis for developing predictions about the assembly of *Tetraria* communities at various spatial scales (Webb *et al.* 2002, Slingsby & Verboom 2006, McPeek 2008). Declining diversification rates have been reported in other Cape floral clades (Linder & Hardy 2004, Valente *et al.* 2010) and the extent to which density dependence, and ecological limitation in particular, have constrained the diversification of these clades could have major significance for our understanding of the diversity, distribution and dynamics of the contemporary Cape flora.

Appendix 4.1: Collection and GenBank accession numbers for accessions included in phylogeny reconstruction.

Species	*trnLF*	Source	*rps16*	Source	*rbcL*	Source	*trnH*	Source	*5S*	Source	*ETS*	Source
Mapania cuspidata	DQ058297	(Verboom 2006)	DQ058318	(Verboom 2006)	...	...	...	...	...	...	...	...
Capeobolus brevicaulis	DQ058303	(Verboom 2006)	DQ058324	(Verboom 2006)	...	...	...	...	...	...	...	...
Neesenbeckia punctoria	DQ058306	(Verboom 2006)	DQ058327	(Verboom 2006)	...	...	...	...	...	...	...	...
Caustis diocia	...	...	...	...	Y12976	(Verboom 2006)	...	...	...	...	...	...
Cladium mariscus	DQ058298	(Verboom 2006)	DQ058319	(Verboom 2006)	DQ058338	(Verboom 2006)	...	...	...	...	...	...
Costularia arundinaceae	AY230036	(Verboom 2006)	...	...	...	...	...	...	...	...	...	...
Costularia natalensis	DQ058305	(Verboom 2006)	DQ058326	(Verboom 2006)	DQ058345	(Verboom 2006)	...	...	...	...	...	...
Costularia pubescens	AY230037	(Verboom 2006)	...	...	...	...	...	...	...	...	...	...
Ficinia paradoxa	DQ058317	(Verboom 2006)	...	...	DQ058354	(Verboom 2006)	...	...	...	...	...	...
Gahnia baniensis	DQ058302	(Verboom 2006)	DQ058323	(Verboom 2006)	DQ058342	(Verboom 2006)	...	...	...	...	...	...
Gymnoschoenus sphaerocephalus	AY230033	(Verboom 2006)	...	...	AY725945	(Verboom 2006)	...	...	...	...	...	...
Hypolytrum nemorum	AJ5777325	(Verboom 2006)	AY344142	(Verboom 2006)	Y12958	(Verboom 2006)	...	...	...	...	...	...
Rhynchospora brownii	DQ058316	(Verboom 2006)	DQ058336	(Verboom 2006)	DQ058353	(Verboom 2006)	...	...	...	...	...	...
Tricostularia pauciflora	AY230038	(Verboom 2006)	...	...	AY725954	(Verboom 2006)	...	...	...	...	...	...
Tetraria compar	DQ058312	(Verboom 2006)	DQ058333	(Verboom 2006)	...	...	...	...	...	...	...	...
Tetraria crassa	DQ058314	(Verboom 2006)	DQ058334	(Verboom 2006)	...	...	...	...	...	...	...	...

Table 4.6: (continued on next page)

Table 4.6: (continued)

Species	*trnLF*	Source	*rps16*	Source	*rbcL*	Source	*trnH*	Source	*5S*	Source	*ETS*	Source
Tetraria bromoides	DQ419851	Verboom 641	DQ419883	Verboom 641	US	Verboom 641	US	Verboom 641	DQ419868	Verboom 641	...	...
Tetraria burmannii	US	Verboom 1219	US	Verboom 1219	...	...	US	Verboom 1219	...	...	US	Verboom 1219
Tetraria capillacea	US	Verboom 1313	US	Verboom 1313	...	...	US	Verboom 1313	...	...	...	...
Tetraria compressa	DQ419854	Verboom 653	DQ419886	Verboom 653	...	...	...	...	DQ419872	Verboom 653	...	...
Tetraria crinifolia	DQ058309	(Verboom 2006)	DQ058330	(Verboom 2006)	US	Verboom 638	...	...	DQ419877	Verboom 638	...	...
Tetraria eximia	US	Verboom 647	US	Verboom 647	US	Verboom 647	US	Verboom 647	US	Verboom 647	US	Verboom 647
Tetraria sp. nov. cf. eximia A	...	...	US	Verboom 720	US	Verboom 720	...	...	US	Verboom 720	US	Verboom 720
Tetraria sp. nov. cf. eximia B	US	Verboom 891	...	...	...	...	US	Verboom 891	US	Verboom 891	...	...
Tetraria fasciata	DQ419858	Verboom 644	DQ419890	Verboom 644	...	...	US	Verboom 644	DQ419878	Verboom 644	US	Verboom 644
Tetraria ferruginea	US	Verboom 527	US	Verboom 527	...	...	US	Verboom 527	US	Verboom 527	US	Verboom 527
Tetraria fimbriolata	DQ419862	Verboom 533	DQ419894	Verboom 533	...	...	US	Verboom 533	DQ419882	Verboom 533	US	Verboom 533
Tetraria flexuosa	DQ419859	Verboom 505	DQ419891	Verboom 505	...	...	...	...	DQ419879	Verboom 505	...	...
Tetraria flexuosa B	DQ419860	Verboom 733	DQ419892	Verboom 733	US	Verboom 733	US	Verboom 733	DQ419880	Verboom 733	US	Verboom 733
Tetraria sp. nov. cf. flexuosa	...	...	US	Kennedy A	...	...	...	...	...	...	US	Kennedy A
Tetraria fourcadei	US	Verboom 633	...	...	...	...	...	...	US	Verboom 633	US	Verboom 633
Tetraria sp. nov. cf. ustulata	US	Helme A	US	Helme A	...	...	...	...	US	Helme A	US	Helme A

Table 4.6: (continued on next page)

Table 4.6: (continued)

Species	*trnLF*	Source	*rps16*	Source	*rbcL*	Source	*trnH*	Source	*5S*	Source	*ETS*	Source
Tetraria involucrata	DQ419852	Verboom 661	DQ419884	Verboom 661	US	Verboom 661	US	Verboom 661	DQ419869	Verboom 661	US	Verboom 661
Tetraria involucrata B	...	...	US	Verboom 1283	...	...	US	Verboom 1283	...	...	...	...
Tetraria maculata	US	Verboom 528	US	Verboom 528	...	...	US	Verboom 528	US	Verboom 528	...	...
Tetraria microstachys	DQ058307	(Verboom 2006)	DQ058328	(Verboom 2006)	US	Verboom 640	US	Verboom 640	DQ419875	Verboom 640	US	Verboom 640
Tetraria sp. nov. cf. microstachys	DQ419856	Verboom 513	DQ419888	Verboom 513	US	Verboom 513	...	...	DQ419874	Verboom 513	US	Verboom 513
Tetraria nigrovaginata	DQ419857	Verboom 500	DQ419889	Verboom 500	US	Verboom 500	US	Verboom 500	DQ419876	Verboom 500	US	Verboom 500
Tetraria sp. nov. cf. maculata	US	Verboom 763	US	Verboom 763	...	...	...	...	...	...	...	...
Tetraria pleiosticha	US	Verboom 639	US	Verboom 639	...	...	US	Verboom 639	US	Verboom 639	US	Verboom 639
Tetraria pubescens	DQ419855	Verboom 651	DQ419887	Verboom 651	...	...	US	Verboom 651	DQ419873	Verboom 651	US	Verboom 651
Tetraria sp. nov. cf. pubescens	US	Verboom 1280	US	Verboom 1280	...	...	US	Verboom 1280	...	...	US	Verboom 1280
Tetraria pygmaea	US	Slingsby pygsil	...	...	...	...	...	...	...	...	US	Slingsby pygsil
Tetraria pygmaea B	US	Verboom 1276	US	Verboom 1276	...	...	...	...	...	...	US	Verboom 1276
Tetraria sp. nov. cf. pygmaea	US	Verboom 1304	...	...	...	...	...	...	...	...	US	Verboom 1304
Tetraria sp. nov. cf. burmannii	US	Slingsby 002	...	...	...	...	...	...	...	...	US	Slingsby 002
Tetraria secans	US	Verboom 658	US	Verboom 658	US	Verboom 658	US	Verboom 658	US	Verboom 658	US	Verboom 658
Tetraria spp nov cf. secans	US	Verboom 896	...	...	...	...	...	...	US	Verboom 896	US	Verboom 896

Table 4.6: (continued on next page)

Table 4.6: (continued)

Species	*trnLF*	Source	*rps16*	Source	*rbcL*	Source	*trnH*	Source	*5S*	Source	*ETS*	Source
Tetraria spiralis	...	...	US	Verboom 719	...	...	...	...	...	...	US	Verboom 719
Tetraria thermalis	DQ058308	(Verboom 2006)	DQ058329	(Verboom 2006)	...	...	US	Verboom 643	DQ419870	Verboom 643	US	Verboom 643
Tetraria sp. nov. cf. triangularis A	US	Britton BS3	US	Britton BS3	...	...	US	Britton BS3	US	Britton BS3	...	...
Tetraria sp. nov. cf. triangularis B	DQ419853	Verboom 518	DQ419885	Verboom 518	US	Verboom 518	US	Verboom 518	DQ419871	Verboom 518	US	Verboom 518
Tetraria triangularis	US	Britton SD1	US	Britton SD1	US	Britton SD1	US	Britton SD1	...	...	US	Britton SD1
Tetraria sp. nov. cf. triangularis C	US	Britton W28	US	Britton W28	US	Britton W28	US	Britton W28	...	...	US	Britton W28
Tetraria wallichiana	...	...	US	Kennedy B	...	...	US	Kennedy B	...	...	US	Kennedy B
Tetraria ustulata	DQ419861	Verboom 501	DQ419893	Verboom 501	US	Verboom 501	US	Verboom 501	DQ419881	Verboom 501	US	Verboom 501

Notes: Marker regions have been abbreviated; *trnLF: trnL-trnF, trnH: trnH-psbA, 5S: 5S-rRNA*. Where previously published sequences have been used the relevant reference is given in parentheses while new sequences are sourced to a voucher specimen (all vouchers housed at Bolus Herbarium (BOL), University of Cape Town). 'US' indicates sequences not yet submitted to GenBank.

Tetraria eximia, Silvermine, Table Mountain National Park.

5 Declining diversification rates, ecological differentiation and the role of geography

5.1 Abstract

A declining diversification rate is often taken to be an indication that the radiation of a lineage has occurred via adaptive divergence, with diversification slowing as the environment becomes increasingly ecologically saturated, and resulting in a set of ecologically differentiated species. Conversely, a stable or increasing diversification rate indicates radiation without adaptive divergence resulting in many ecologically undifferentiated species which co-occur via 'neutral-like' dynamics. Here I test these predictions by examining diversification rate heterogeneity and ecological differentiation among species in three subclades within *Tetraria*. I also test two modifications of these predictions based on the geographical arrangement of species. Firstly, sister species that occur in allopatry should display less ecological differentiation because speciation may not have been associated with adaptive divergence and there has been no opportunity for subsequent character displacement. Secondly, clades that display high levels of allopatry should display a lesser decline in diversification rate because ecological space becomes saturated more slowly. Generally the predictions are met, with little ecological differentiation among species in the *microstachys-burmannii* subclade, which shows no decline in diversification rate, while close relatives in the *thermalis-bromoides* and *fasciata-flexuosa* subclades, which had declining diversification rates, either display greater than expected morphological disparity or environmental niche partitioning in areas of geographical overlap. Of these, the *thermalis-bromoides* clade, which had greater levels of allopatry, showed less of a decline in diversification rate and allopatric species were more morphologically similar than expected by chance. I argue that geographical context and the mode of speciation are crucial factors affecting the rate of diversification and degree of ecological differentiation within a lineage. Ecological differentiation among close relatives can occur via non-ecological speciation in allopatry followed by niche or character displacement when they disperse back into sympatry. In this scenario diversification rates decline because founder populations which

disperse into allopatric regions are either eliminated by or introgress with close relatives and because colonization rates decline as founder populations have to disperse into regions that are increasingly ecologically saturated. Including geography in macroevolutionary models which explore the effects of ecological mechanisms on diversification dynamics provide exciting new avenues for synthesising our understanding of ecological dynamics, macroevolution and the assembly of species pools.

5.2 Introduction

Declining diversification rates are observed in almost half of all published phylogenies (McPeek 2008, Phillimore & Price 2008). Diversification rates which decline in relation to the number of extant lineages (i.e. are density dependent) suggest that diversification has been ecologically constrained (McPeek 2008, Rabosky & Lovette 2008*a*, Rabosky 2009*a*). In this scenario speciation is associated with ecological divergence, filling available ecological space rapidly, and diversification slows as the space becomes saturated (MacArthur 1965, Foote 1997, Schluter 2001, McPeek 2008, Rabosky & Lovette 2008*a*, Rabosky 2009*a*). This suggests that species in these lineages are competing, and provides the corollary that, for diversity to be maintained, species either need to be spatially isolated or partition their requirements on one or more niche-axes to be able co-exist (Hutchinson 1957, MacArthur & Levins 1967).

Using a simplistic metacommunity model of diversification McPeek (2008) provided theoretical support for the hypothesis that radiations which involve adaptive divergence exhibit density-dependent diversification rates. Species were allocated to communities positioned along a resource gradient and exhibited logistic population growth limited by the degree to which their position on the gradient deviated from their optimum resource preference. They shared the same dispersal rates and probability of speciation at each iteration and ‘new’ species were assigned a resource preference by adding a random deviate sampled from a normal distribution with zero mean to the resource preference of their progenitor. The degree of ecological differentiation allowed in the model was varied by altering the standard deviation of the distribution of random normal deviates. Thus, setting a large standard deviation is analogous to a scenario of ecological speciation because it allows species to fill the available niche space rapidly (Schluter 2001), while setting a low standard deviation represents a scenario where speciation is not accompanied by ecological differentiation, i.e. the radiation of the lineage is non-adaptive (Gittenberger 1991, Wiens

2004, Rundell & Price 2009). The ecological speciation model exhibited the expected high initial diversification rates, slowing as the niche space became saturated, with new species only able to invade communities by out-competing and replacing existing ones. Species were rapidly driven to extinction because fitness differences were typically large. By contrast, where ecological differentiation was low, diversification rates did not decline and even increased through time above that expected under a pure-birth model (Pybus & Harvey 2000). In this scenario high levels of diversity were maintained by 'neutral-like' dynamics where low fitness differences ($\approx$fitness equivalence; Hubbell 2001, 2005) meant that no species became dominant (Leibold & McPeek 2006). Species did not display stable co-existence in the theoretical and mathematical sense (Chesson 2000), but were transient, slowly being driven to extinction by weak species interactions or random drift.

The results of McPeek's (2008) metacommunity model yield testable predictions about the mode of speciation and degree of ecological differentiation among closely-related species given diversification rate heterogeneity. Speciation in lineages that exhibit declining diversification rates should be predominantly ecological, leading to a high degree of differentiation. Conversely, speciation in lineages with stable or increasing diversification rates should be associated with little ecological differentiation. The diversification of a lineage is not constrained to a single mode of speciation, however, and differences in diversification rate heterogeneity and ecological differentiation among clades may be the result of contrasting diversification histories involving combinations of multiple speciation processes (McPeek 2008). In particular, the geographical mode of speciation and the consequent spatial arrangement of species may have an important influence. Where speciation has been allopatric, and the distributions of sister species do not overlap, one might expect to see little or no ecological differentiation (Wiens 2004), no competition (Amarasekare 2003), and thus a weaker effect of density-dependence on diversification rates. In addition, where sister species are allopatric, they will not have experienced secondary contact and there will have been no character or niche displacement (Brown & Wilson 1956, Dayan & Simberloff 2005, Pfennig & Pfennig 2009).

High levels of endemism in lineages of Cape plants indicate that much of their diversity evolved in situ (Linder 2003) and make them ideal model systems for examining diversification processes (Barraclough 2006). Much of the diversification of the Cape flora is thought to have been adaptive (Cowling *et al.* 1992, Linder 2003, 2005*a*, van der Niet & Johnson 2009), suggesting that species are ecologically differentiated and that, according to the predictions of McPeek's model, the diversification of these lineages has been constrained by available ecological niche space. The Cape flora is characterized by a high number of narrow endemic species, however, with many species having non-overlapping distributions (Goldblatt & Manning 2000, 2002, Linder 2003). This may have lowered the rate at which the region has become ecologically saturated, reducing the effect of ecological constraint on diversification rates. The distinct and largely consistent geographical structuring exhibited by Cape clades with good distribution records (e.g. *Erica*, Proteaceae, Restionaceae, Bruniaceae, *Aspalathus* and *Muraltia*), has led to the identification of phytogeographical centres of endemism (Weimarck 1941, Oliver *et al.* 1983, Goldblatt & Manning 2000, 2002, Linder 2001, Moline & Linder 2006). These centres are typically separated by dispersal barriers, such as deep valleys or bioclimatic boundaries such as that differentiating the winter and year-round rainfall zones, and provides a valuable context for historical biogeography and the study of allopatric speciation in the Cape Floristic Region (CFR).

Here I aim to test the predictions of McPeek's (2008) model by examining diversification rates and ecological differentiation, in terms of differences in preferred environmental conditions and trait disparity, among species in the Cape genus *Tetraria* and each of its three major component subclades. I also aim to test two modifications of McPeek's model based on the geographical arrangement of species. Firstly, that clades containing close relatives with allopatric distributions should display lower declines in their diversification rates because regions should not become ecologically saturated and constrain diversification as quickly. Secondly, that these species may display little ecological differentiation because speciation likely occurred in allopatry and may not have been associated with adaptive divergence. Molecular phylogenetic evidence indicates that *Tetraria* is made up of three morphologically distinct subclades of similar age and diversity (Slingsby & Verboom 2006, Chapter 4). The *thermalis-bromoides* subclade consists of 14 broad-leaved species that are typically large in stature and consist of few-to-many rosettes of leaves, or tillers. The *microstachys-burmannii* clade comprises 10 species that are both small in stature and consist of few tillers. The *fasciata-flexuosa* clade contains 13 species of small to medium body size with individuals often composed of large numbers of tillers. Diversification within *Tetraria* has slowed as the number of extant species has increased (Chapter 4). Examination of diversification rates within the group revealed density-dependent declines in the *fasciata-flexuosa* and *thermalis-bromoides* subclades, while the *microstachys-burmannii* subclade indicated little or no decline, best fitting a pure-birth model. This leads to the prediction that diversification in the *fasciata-flexuosa* and *thermalis-bromoides* subclades has been largely ecological, resulting in a high degree of ecological differentiation among species, while diversification in the *microstachys-burmannii* subclade has not been ecological, resulting in species that are ecologically similar (McPeek 2008). In addition, breaks in the distribution of *Tetraria* species are largely coincident with the boundaries of the phytogeographical centres of endemism identified for other clades in the CFR (Goldblatt & Manning 2000, 2002), resulting in sister species with allopatric distributions and minimal ecological interaction (Britton 2010). Thus, subclades with higher levels of allopatry should exhibit smaller declines in their diversification rates and less ecological differentiation among close relatives.

To test McPeek's (2008) predictions and the two modifications based on the geographical arrangement of species I ask three questions: 1) Do closely-related species in clades with declining diversification rates display greater ecological differentiation in terms of their environmental preferences and morphological traits? 2) Are closely-related species that occur in allopatry more similar morphologically than would be expected on the basis of chance, while those in sympatry are more dissimilar? 3) Do clades with greater levels of allopatry among close relatives display shallower declines in their diversification rates? Answering these questions follows a five step process. First, I first measure the degree to which diversification rates have declined for each subclade. Second, I assess species range overlap by assessing co-occurrence in five phytogeographic centres of endemism. Third, where species pairs occur in sympatry, I test for significant differences in their environmental preferences within the region of overlap in terms of elevation and bioclimatic and solar radiation conditions. Fourth, I test for significantly greater morphological change among sympatric sister taxa and less change among allopatric sister taxa when compared to the expectations of a simple model of trait evolution. Finally, I relate the geographical, environmental and morphological differences among taxa within each subclade to the expectations based on heterogeneity in their diversification rates.

I examine both species' traits and the environmental conditions which characterize their distributions because each are useful for exploring different facets of ecological differentiation and specialization and provide information about species' ecologies that are applicable at different spatial scales. Species' relative performance and environmental preferences provide information about ecological differentiation in actual resource use (the realised 'Grinnellian' niche), while examination of species' traits gives an indication of their potential functional roles (the fundamental 'Eltonian' niche; Devictor *et al.* 2010). While examination of environmental conditions has the advantage that it provides a direct measure of species' niches without making assumptions about resource use mechanisms or the relative utility of a trait, it is disadvantaged by severe limitations on the collection of data of appropriate grain and extent. The availability of climatic data that can be extracted from Geographic Information Systems (GIS) has been a major advance in this regard (Hijmans *et al.* 2005), but is typically only available down to the scale of square kilometres and accuracy is highly dependent on interpolation procedures. In addition, comparing the environmental preferences of species from different geographical areas is problematic because the limiting environmental conditions for each species may not be known, and geographic constraint on environmental conditions and species distributions may create large differences in their realised niches (where they do occur) even when their fundamental niches (where they could occur) may be identical (Moen *et al.* 2009, Smith & Donoghue 2010). By contrast, detailed trait data are far easier to collect for large numbers of taxa (e.g. Wright *et al.* 2004, Moles *et al.* 2005, Chave *et al.* 2009) and examination of trait evolution and character displacement overcomes many of the shortcomings of examining environmental conditions. Examination of trait data also allow for investigation of fine-scale ecological processes that have less distinct geographical structure such as microhabitat or resource partitioning. A major limitation, however, is that the importance of the traits in determining habitat specialization or facilitating co-existence is difficult to demonstrate and must often be assumed (Leibold & McPeek 2006).

5.3 Methods

5.3.1 Diversification rate heterogeneity

Rate heterogeneity was measured for each of the three subclades by computing the gamma statistic of Pybus & Harvey (2000) on the branching times for the internal nodes of each of the subclades extracted from each of the three dated maximum clade credibility (MCC) phylogenetic trees produced in Chapter 4 (Figure 5.1). While likelihood based model fitting methods were employed to test for rate heterogeneity in Chapter 4, they do not provide a measure of the decrease in diversification rates that can be compared between the subclades (Rabosky 2006*a*). All analyses in this chapter are based on 37 of the 46 reticulate-sheathed *Tetraria* species for which phylogenetic data are available.

5.3.2 Assessing geographical overlap

Species' distributions and areas of distributional overlap were identified by dividing the CFR into five circumscribed areas and scoring species' presence or absence in each area (Figure 5.5). Species occurrence based on georeferenced locality data derived from specimens in the Bolus (BOL), Compton (NBG) and Pretoria (PRE) herbaria that were determined according to the most recent revision of the alpha taxonomy of the genus (Verboom *et al. In preparation*). Areas were based on the phytogeographical centres of endemism described by Goldblatt & Manning (2000) and Linder (2003) that were consistent with breaks in *Tetraria* species distribution. I followed Linder (2003) in recognising the Cape Peninsula as a separate phytogeographical centre because many species which occur in the Southwest centre do not extend to the Peninsula. The Agulhas Plain centre was included in the Southwest centre and the Karoo Mountain and Langeberg centres were combined into a single Central Mountain region because there was no evidence of disjunctions in *Tetraria* species' distributions between these centres. Localities occurring in the lowland area between the Peninsula and Southwest centres were considered to be from the Southwest because those species that had localities in this area and did not occur in both centres were only found in the Southwest. The Northwest and Southeast centres make up the last two areas included in this study.

5.3.3 Trait evolution

5.3.3.1 Trait selection

A set of traits which are potentially important for the ecology of the species and are easily measured from herbarium collections was identified (Figure 1.5, Appendix 5.1). This set included measures of leaf size and shape (basal leaf length and basal leaf width), inflorescence architecture (inflorescence height, length and culm diameter), spikelet characteristics (number, length and width) and seed characteristics (length, width and mass).

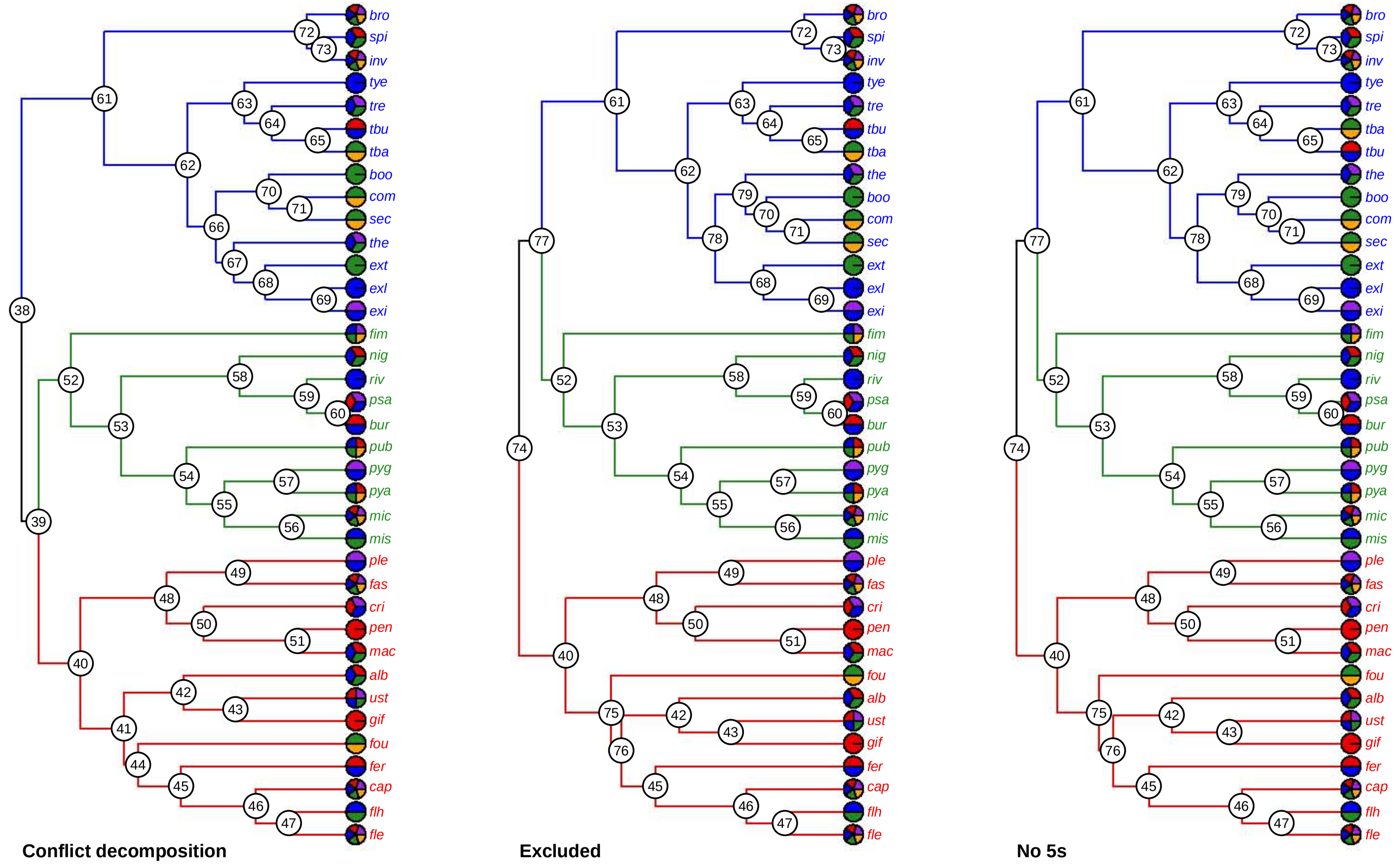


Figure 5.1: Three Bayesian maximum clade credibility (MCC) phylogenetic trees for each of the combined analysis strategies presented in Chapter 4. Pie colours indicate species occurrence in each of the five phytogeographic centres indicated in Figure 5.5. Branch colours indicate each of the three subclades: *thermalis-bromoides* (blue), *microstachys-burmannii* (green) and *fasciata-flexuosa* (red).

In *Tetraria*, leaf length determines leaf canopy height (R^2=0.99 for 37 species) because leaves emerge from basal corms (Figure 1.5). Leaf canopy height determines light interception properties and effects competitive ability and shade tolerance (Falster & Westoby 2003, 2005*a*). Leaf canopy height can also be linked to temperature and moisture regulation in fynbos environments through exposure to the cooling effects of wind (Yates *et al.* 2010), and through the interception of moisture from cloud condensation (Marloth 1904). Leaf width impacts on temperature regulation and transpiration through its influence on boundary layer thickness, with narrower leaves allowing higher sensible heat loss in low wind environments and facilitating higher transpiration rates (Yates *et al.* 2010).

Inflorescence architecture and spikelet morphology are important for the aerodynamics of wind pollination (Niklas 1985). While taller inflorescences benefit from greater wind interception, changes in size and aggregation of spikelets and inflorescences impact on boundary layer dynamics, influencing the efficiency of pollen dispersal and capture (Niklas 1985, Friedman & Barrett 2009). Inflorescence architecture can also manipulate airflow so as to selectively intercept conspecific pollen (Niklas 1985), an effect demonstrated for fynbos species by Linder & Midgley (1996). While *Tetraria* seeds show little differentiation for dispersal, taller inflorescences likely aid seed transport away from the parent plant, particularly in strong winds. Spikelet number is important for both pollen load and the potential number of seeds that can be produced, with seed size and seed number being important for seedling success, emergence depth (Bond *et al.* 1999) and competitor-colonizer trade-offs (Coomes & Grubb 2003).

5.3.3.2 Trait measurement

Traits were measured on pressed specimens housed in BOL and NBG. Trait means, standard deviations and number of individuals measured for each of 37 species are given in Appendix 5.1. Unfortunately, seed characteristics were measured for only 20 species owing to the difficulties of acquiring appropriate material. Seed length, seed width and seed mass (measured using a balance precise to 0.01 mg) were recorded from two to nine seeds for each species, depending on availability. All analyses were performed on ln-transformed and untransformed traits. Only results of analyses of ln-transformed traits are reported because these analyses do not violate the assumption of normality. Instances where analysis of untransformed traits contradicted results based on transformed traits are indicated.

5.3.3.3 Major axes of morphological variation

Variation in the trait dataset was explored by performing ahistorical and phylogenetically independent contrast (PIC) correlations (Felsenstein 1985) between all traits using species means and the three MCC trees. The major axes of trait variation were summarized using PCA of species means for the eight traits which had data for all 37 species. To justify the use of species means a PCA was initially done for all measured individuals to ensure that most of the trait variation was between rather than within species. Previous examination of a similar set of traits for species in the genus using a nested ANOVA indicated that typically more than 80% of the variation in traits is partitioned between species (Slingsby & Verboom 2006). A nested ANOVA could not be performed on this dataset, however, because unequal numbers of individuals were measured for each species, creating an unbalanced design. A second PCA was performed on the standardized PICs based on the species means. This analysis was performed to determine whether morphological divergence at cladogenetic events aligned with similar axes of variation as identified by the PCA of species means, and indicates the amount and direction of morphological change that occurred at each cladogenetic event.

5.3.3.4 Phylogenetic signal in traits

All traits and the first four axes from the PCA using species means were tested for significant phylogenetic signal using the method of Blomberg *et al.* (2003) on the three MCC trees. The method is based on the K statistic, which indicates the observed degree of similarity among relatives compared to a null expectation derived from a Brownian motion model of evolution based on the same topology and branch lengths. K varies from 0 (highly labile) through 1 (the expected amount of variability given the tree and branch lengths) to infinity (high phylogenetic signal). Significance is assessed by calculating K values for 999 simulated datasets in which the actual trait values are shuffled between the species in the phylogeny, thereafter ranking the observed test statistic against the distribution of null values. Traitgrams (Ackerly 2009) were generated for each of the first four axes from the PCA of the species means. Traitgrams depict the evolutionary history of a trait on a phylogeny by indicating the actual trait values for extant species and the reconstructed trait values at internal nodes on the X-axis, while indicating the relative age of the internal nodes on the Y-axis. Wide branching angles at internal nodes indicate large trait differences between descendent taxa, while narrow angles indicate small differences.

5.3.3.5 Testing trait disparity

If speciation is along ecological lines, or there has been character displacement to reduce competition amongst close relatives, the difference in trait values between species should be greater than expected if trait values were simply allowed to drift. Similarly, where speciation occurs in allopatry, species may be expected to retain their ancestral ecologies and display less trait divergence than expected by drift. Thus, when testing for significant differentiation or conservatism in traits between a pair of species it is not enough simply to demonstrate the presence or absence of a significant difference in trait values using conventional statistical tests. Indeed, it is necessary to demonstrate that the difference is greater or less than expected under a model of drifting trait evolution. I thus developed a test of trait disparity which compares the actual difference in a trait value across each node with a null distribution of 9999 trait differences for that node generated using a Brownian motion (drift) model of trait evolution. The null model generates trait values by allowing traits to evolve along the branches in the phylogeny at a constant rate such that the amount of change along each branch is proportional to the length of the branch and the variance set for the model. Model variance was set to the phylogenetic variance-covariance matrix estimated from the actual data for the trait being tested using the independent contrast method of Revell *et al.* (2007). Disparity tests were performed on each of the first four axes from the PCA of species means using each of the three MCC trees. All analyses were done using the MCC trees for the full species set and each subclade independently. This allowed estimation of independent phylogenetic variance-covariance matrices for each subclade because the variance in a trait is unlikely to be uniform across subclades.

5.3.4 Environmental niche differentiation

Differences in preferred environmental conditions in areas in which closely-related species have overlapping distributions were tested for using bioclimatic, incoming solar radiation (insolation) and elevational data extracted from GIS layers for the georeferenced herbarium specimen localities. Insolation and elevational differences between species were examined because turnover in species composition between adjacent slopes of different aspect are often as high as 100% (pers. obs.) and a number of *Tetraria* species are limited to high or low elevational zones (Britton 2010), reducing species co-occurrence despite geographical overlap. Elevational differences are likely to be correlated with climatic differences for the most part (Schulze 2007), but may also partition species which occur on different soil types because of the distinct stratification of most geological substrates in the CFR (see Cowling *et al.* 2009). Unfortunately appropriate soils data were not available.

5.3.4.1 Data extraction

All GIS layers were obtained from Hijmans *et al.* (2005; www.worldclim.org) and Schulze (2007), resampled to one minute grid cells to account for error in herbarium specimen georeferencing. Specimens that could not be reliably georeferenced to minute grid cells were excluded from the study and only one collection was included for each species in each minute grid cell, resulting in 1040 localities, ranging from 1 to 110 for each of the 37 species. Data were extracted for elevation and the 19 biologically meaningful climatic variables derived from monthly temperature and rainfall data by Hijmans *et al.* (2005), see Table 5.1 for descriptions. Insolation data were extracted from monthly mean insolation values for cloud free days provided by Schulze (2007) in MJ/m^2/day. To reduce collinearity among variables within each dataset and ensure that all variables were orthogonally related to allow the use of multivariate statistical tests, the bioclimatic and insolation datasets were summarized using principal components analyses (PCA).

Tetraria elevational zones often increase as one moves away from the sea due to the tempering effect of the ocean-driven weather systems and the effect of continentality (Britton 2010). As such, two *Tetraria* species that occur in the same phytogeographic region may appear to display elevational overlap even if they are segregated such that one occurs on high mountain peaks near the coast and does not occur inland while the other occurs at sea level near the coast and on peaks further inland. To account for this, elevational overlap was assessed by examining both elevation and the distance to the coastline for each locality. Both these variables were square-root transformed prior to statistical testing to ensure normal distributions.

5.3.4.2 Niche differentiation tests

Evidence for significant differentiation in preferred bioclimatic or insolation conditions or elevational zone among pairs of closely-related species was evaluated using multiple-response permutation procedure (MRPP) tests (Mielke *et al.* 1976, Wong *et al.* 1982). MRPP is a distribution free inferential technique for detecting differences amongst classified groups of objects in multi-dimensional space. The test statistic, δ, is based on the Euclidean

Table 5.1: Description of the environmental variables considered in this study

Variable	Description
bio1	Annual Mean Temperature
bio2	Mean Diurnal Range (Mean of monthly (max temp - min temp))
bio3	Isothermality (bio2/bio7)*100
bio4	Temperature Seasonality (standard deviation*100)
bio5	Max Temperature of Warmest Month
bio6	Min Temperature of Coldest Month
bio7	Temperature Annual Range (bio5-bio6)
bio8	Mean Temperature of Wettest Quarter
bio9	Mean Temperature of Driest Quarter
bio10	Mean Temperature of Warmest Quarter
bio11	Mean Temperature of Coldest Quarter
bio12	Annual Precipitation
bio13	Precipitation of Wettest Month
bio14	Precipitation of Driest Month
bio15	Precipitation Seasonality (Coefficient of Variation)
bio16	Precipitation of Wettest Quarter
bio17	Precipitation of Driest Quarter
bio18	Precipitation of Warmest Quarter
bio19	Precipitation of Coldest Quarter
sr1-12	Mean daily incoming solar radiation by month

distances between data points, and the permutation procedure evaluates whether data points show significantly greater clustering within as opposed to between the *a priori* classified groups than would be expected by chance. Tests were performed among all species pairs within each of the subclades of two to four species indicated in Figure 5.6. Three species, *fou, pub* and *fim*, were not included in this analysis because they are sister to large clades which would result in many comparisons between distantly-related species pairs.

5.3.5 Multiple comparisons

Given that each node or species pair need only differ significantly for one of the three environmental or four trait disparity tests to provide evidence of ecological differentiation there is a need to correct for multiple comparisons. The Bonferroni correction, which would result in a corrected α cut-off of 0.007 for the 7 different tests (α/m), only reduces the chances of detecting false positives, however, and does this at the cost of greatly increasing the chances of obtaining false negatives. This has led to the development of a large number of alternative procedures for correcting for multiple comparisons such as the false discovery rate (Benjamini & Hochberg 1995). Unfortunately most of these procedures require the comparisons to be made using the same statistical tests and sample sizes, limiting their use here. Here I use the mean α for m tests that can be derived from the Simes (1986) procedure, an adaptation of the Bonferroni correction, which is calculated as α(m+1)/2m, resulting in an approximate corrected α cut-off of 0.029 that can be applied to all tests. All analyses were performed using the *ade4* 1.4-14 (Dray & Dufour 2007), *adehabitat* 1.8.3 (Calenge 2006), *maptools* 0.7-38 (Lewin-Koh & Bivand 2010), *picante* 1.1.0 (Kembel *et al.* 2010) and *laser* 2.3 (Rabosky 2006*a*) packages in R2.11.0 (R Development Core Team 2010).

5.4 Results

5.4.1 Diversification rate heterogeneity

Tetraria and each of its three subclades displayed decreasing rates of diversification through time, as indicated by the negative gamma statistics in Table 5.2 (Pybus & Harvey 2000). The declines were only significant for *Tetraria* as a whole and for the *fasciata-flexuosa* subclade, however, with the *thermalis-bromoides* and *microstachys-burmannii* subclades showing less evidence of declining diversification rates.

Table 5.2: Gamma statistics for each subclade for each of the 3 MCC trees

	Conflict decomposition	Excluded	No5s
Reticulate-sheathed	-2.637*	-2.562*	-2.718*
fasciata-flexuosa	-2.515*	-2.491*	-2.478*
thermalis-bromoides	-1.065	-1.062	-0.908
microstachys-burmannii	-0.411	-0.399	-0.512

Notes: Values marked with a * are significant at $P<0.01$

5.4.2 Geographical overlap

Tetraria species tend to have large distribution ranges and geographical overlap between sister species is common throughout (Figure 5.1, Figure 5.5, Figure 5.7). Sister species often have either partially overlapping distributions or else one species' distribution is nested within that of the other. Only the *thermalis-bromoides* subclade has close relatives with distinct geographical distributions. The ranges of *tba* and *tbu* do not overlap, but do overlap with their next closest relatives, *tre* and *tye*. While *exi* and *exl* have overlapping distributions, neither overlap with their next closest relative, *ext*. The lower overlap in this subclade reflects the fact that these species tend to have far more restricted ranges with four of the 14 species being endemic to single phytogeographical centres and a further five restricted to just two centres.

Overall the species distributions imply that the different subclades diversified in different geographic centres. Ten of the 13 *fasciata-flexuosa* species occur in the Northwest centre, two of which are endemic. Of the 14 *thermalis-bromoides* species, ten occur in the Central mountain centre, two of which are endemic to this centre, and a further three which are endemic to the Central mountain and Southeast centres. Nine *thermalis-bromoides* species occur in the Southwest centre, two of which are endemic, and only four species occur in the Northwest. All ten of the *microstachys-burmannii* species occur in the Southwest centre, with six species extending into each of the Northwest and Central mountain centres and four to the Cape Peninsula. Maximum likelihood inference of range evolution using the dispersal-extinction-cladogenesis model of (Ree & Smith 2008) implemented using the software *lagrange* (http://www.reelab.net/lagrange) generally supported these findings, but support for the internal node states was very weak due to the large distribution ranges of most species (data not shown).

5.4.3 Trait evolution

5.4.3.1 Major axes of morphological variation

There was strong collinearity among the traits sampled, reflected in the large number of significant ahistorical correlations between traits (Table 5.3). A number of these correlations, notably between spikelet characteristics and other traits, were no longer significant when phylogenetic autocorrelation was accounted for. Correlations involving seed characteristics were generally weaker and less significant than other comparisons, possibly due to the reduced sample size (and thus degrees of freedom) associated with these analyses. Seed mass revealed strong ahistorical correlations with spikelet length and width, however, suggesting that spikelet measurements could be used as a proxy for seed mass within the genus. Correlations between seed mass and spikelet measurements were not significant when the PIC method was employed, however, implying that these traits did not evolve in a consistently concerted manner.

Principal components analysis of all measured individuals and of species mean trait

Table 5.3: Trait correlations

Ahistorical correlations

	Sd_l	Sd_w	Sd_m	Infl_ht	Cu_d	Bs_lf_l	Bs_lf_w	Spk_no	Spk_l	Spk_w	Infl_l
Sd_l		0.756	0.886	0.449	0.616	0.486	0.476	0.275	0.804	0.539	0.339
Sd_w	***		0.844	0.423	0.591	0.584	0.554	0.301	0.632	0.729	0.469
Sd_m	***	***		0.370	0.468	0.409	0.403	0.092	0.769	0.714	0.276
Infl_ht	*	0.1	NS		0.826	0.851	0.625	0.812	0.585	0.516	0.761
Cu_d	**	**	*	***		0.801	0.918	0.778	0.742	0.680	0.725
Bs_lf_l	*	**	0.1	***	***		0.632	0.686	0.585	0.572	0.631
Bs_lf_w	*	*	0.1	***	***	***		0.621	0.705	0.733	0.686
Spk_no	NS	NS	NS	***	***	***	***		0.391	0.281	0.779
Spk_l	***	**	***	***	***	***	***	*		0.774	0.540
Spk_w	*	***	***	***	***	***	***	0.1	***		0.382
Infl_l	NS	*	NS	***	***	***	***	***	***	*	

Phylogenetically independent contrast correlations

	Sd_l	Sd_w	Sd_m	Infl_ht	Cu_d	Bs_lf_l	Bs_lf_w	Spk_no	Spk_l	Spk_w	Infl_l
Sd_l		0.497	0.716	-0.413	0.083	-0.175	0.137	-0.400	0.278	0.062	-0.467
Sd_w	*		0.720	-0.065	0.261	0.199	0.424	-0.073	0.327	0.461	0.093
Sd_m	***	***		-0.071	0.143	0.075	0.207	-0.245	0.619	0.527	-0.080
Infl_ht	0.1	NS	NS		0.587	0.753	0.351	0.741	0.347	0.283	0.863
Cu_d	NS	NS	NS	***		0.496	0.862	0.710	0.495	0.339	0.487
Bs_lf_l	NS	NS	NS	***	**		0.287	0.614	0.185	0.084	0.717
Bs_lf_w	NS	0.1	NS	*	***	0.1		0.588	0.379	0.298	0.378
Spk_no	0.1	NS	NS	***	***	***	***		0.103	-0.067	0.779
Spk_l	NS	NS	**	*	**	NS	*	NS		0.776	0.201
Spk_w	NS	*	*	0.1	*	NS	0.1	NS	***		0.094
Infl_l	*	NS	NS	***	**	***	*	***	NS	NS	

Notes: All traits were ln-transformed. Trait codes are Sd_l: seed length, Sd_w: seed width, Sd_m: seed mass, Infl_ht: inflorescence height, Cu_d: culm diameter, Bs_lf_l: basal leaf length, Bs_lf_w: basal leaf width, Spk_no: spikelet number, Spk_l: spikelet length, Spk_w: spikelet width, Infl_l: inflorescence length. N for correlations including seed traits = 20. N = 37 for all other correlations. Correlations which are no longer significant when phylogenetically corrected are marked in italics. Correlations which become significant when phylogenetically corrected are marked in bold.

*** $P<0.001$
** $P<0.01$
* $P<0.05$
0.1 $P<0.1$
NS not significant

values revealed four major axes, each of which explained approximately 5% of the variance or more. The axes from the two analyses were qualitatively the same and only the results of the analysis using species means are reported (Figure 5.2). The first axis revealed positive loadings for all traits, reflecting variation in body size, and explained approximately 70% of the variance. Species from the three subclades were segregated along this axis with the *thermalis-bromoides* clade containing the largest-bodied species,

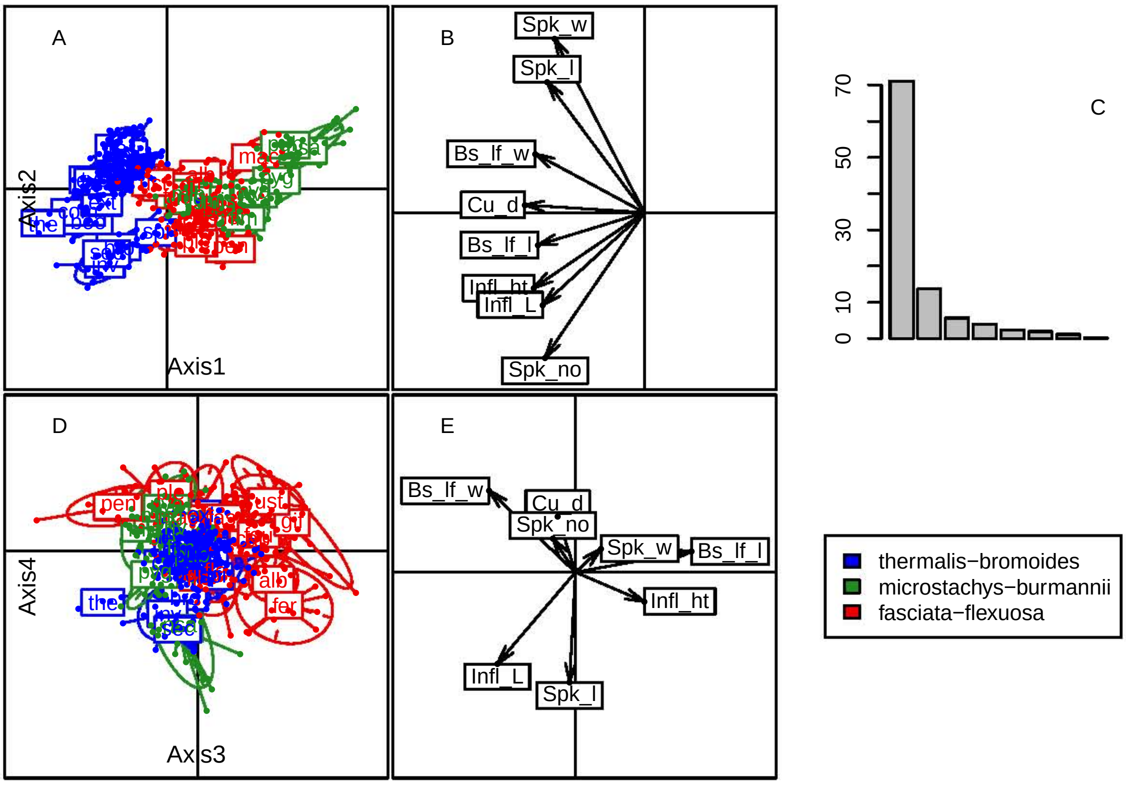


Figure 5.2: Principal components analysis of species morphological traits. Panels A and D indicate variation within species across the first four axes, whereas panels B, C and E report the results from analysis of species means only. Results based on individual measurements or species means were qualitatively the same. Translations of trait codes are given in Table 5.3.

the *microstachys-burmannii* clade containing species with the smallest body sizes, and the *fasciata-flexuosa* clade containing species with small to medium body size. The second major axis summarized variation in spikelet length, width and number, explained approximately 15% of the variance in the dataset, and segregated species within each of the subclades. The third major axis captured the residual variation in leaf characteristics (basal leaf width and basal leaf length), representing leaf shape, and explained approximately 6% of the total variance. This axis was most variable within the *fasciata-flexuosa* clade with the other two clades being far more constrained and showing much overlap. The fourth major axis explained approximately 4.5% of the variance, summarized most of the residual variation in inflorescence architecture and spikelet shape, and was most variable within the *fasciata-flexuosa* and *microstachys-burmannii* clades. These axes were qualitatively identical when trait values were not ln-transformed.

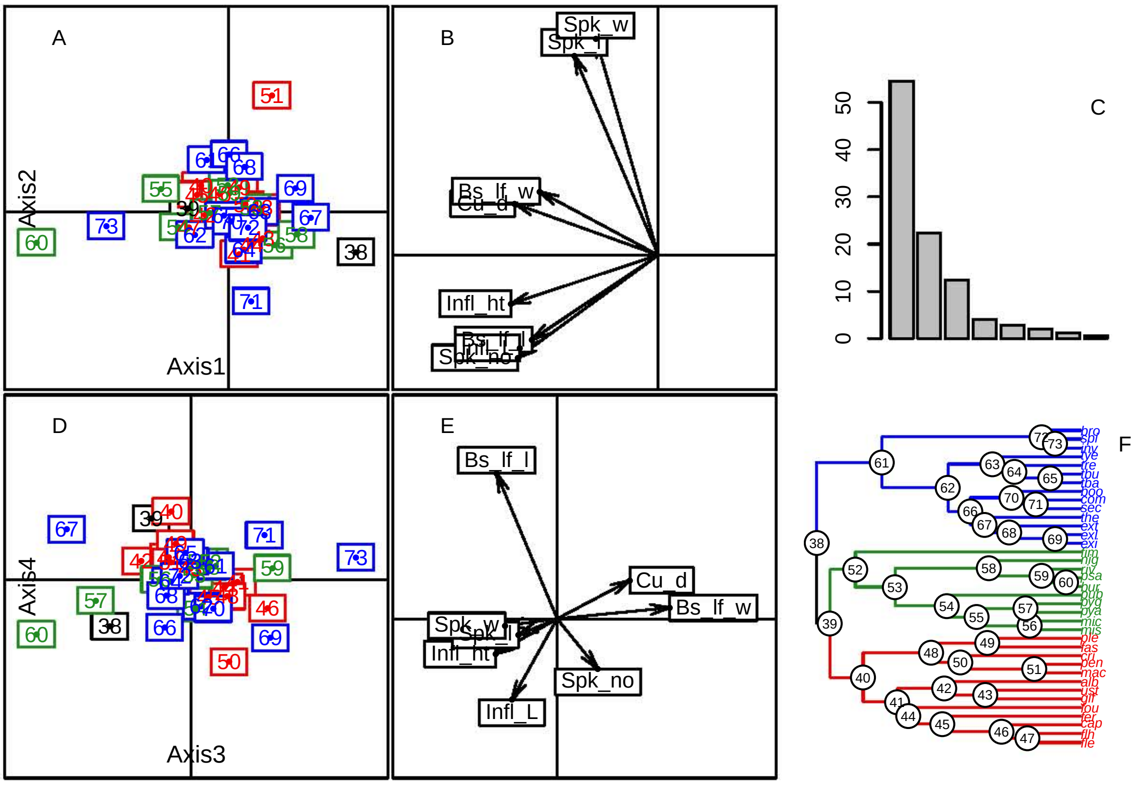


Figure 5.3: Principal components analysis of the phylogenetically independent contrasts of trait means indicating divergence along four trait axes at cladogenetic events. Numbers indicate node numbers on the phylogenetic tree.

Principal components analysis of the PICs revealed that most of the divergence in traits across cladogenetic events is aligned with four major axes of variation similar to those revealed by the PCA of the species means (Figure 5.3). The third and fourth axes differed slightly, the third becoming a dimension of leaf width and spikelet shape and the fourth an axis of leaf and inflorescence elongation. Except for the basal node (38), the more dramatic trait divergence events along each of the axes occurred among recent sister species pairs. The greatest divergence in body size (Axis 1) was between *bur* - *psa* (60), *spi* - *inv* (73) and *exi* - *exl* (69), while *com* - *sec* (71) and *pen* - *mac* (51) showed the greatest divergence in spikelet size, shape and number (Axis 2). The greatest divergence in leaf shape (Axis 3) was between *bur* - *psa* (60) and *spi* - *inv* (73). *Tetraria thermalis* (*the*) showed strong divergence from its sister clade (67) both in size and leaf shape. Although this species varied in its phylogenetic position depending on the MCC tree used (Figure 5.1), it remained distinct from its sister clade in terms of leaf shape (79).

5.4.3.2 Phylogenetic signal in traits

Most traits and axes of trait variation showed significant phylogenetic signal when compared to a random distribution of traits among species in the tree (Table 5.4). The level of phylogenetic signal was weak however, with *K* ranging from 0.373 to 2.446, and often falling below 1, the expectation under a Brownian motion model of trait evolution. Inflorescence length and the associated fourth axis of variation, which reflected inflorescence architecture and spikelet morphology, did not display significantly more phylogenetic signal than would be expected on the basis of chance. Species' mean positions on the first and second climatic PCA axes and on the second insolation PCA axis displayed significant phylogenetic signal, likely due to geographic structure in the distribution of the subclades and climatic and insolation conditions.

Table 5.4: Tests for phylogenetic signal

	K	observed variance	mean random variance	*P*	z	N
Sd_l	0.742	0.016	0.030	*	-1.128	20
Sd_w	0.854	0.011	0.023	*	-1.199	20
Sd_m	1.005	0.093	0.229	**	-1.428	20
Infl_ht	0.536	0.089	0.149	*	-1.570	37
Cu_d	1.831	0.025	0.139	***	-3.795	37
Bs_lf_l	1.136	0.025	0.089	***	-3.265	37
Bs_lf_w	2.446	0.033	0.241	***	-4.039	37
Spk_no	0.718	0.169	0.378	***	-2.267	37
Spk_l	1.018	0.014	0.043	***	-3.026	37
Spk_w	1.346	0.009	0.037	***	-3.551	37
Infl_L	0.373	0.136	0.156	NS	-0.562	37
Axis1	1.189	0.367	1.341	***	-3.544	37
Axis2	0.472	0.177	0.258	0.1	-1.429	37
Axis3	1.020	0.034	0.107	***	-2.862	37
Axis4	0.370	0.063	0.073	NS	-0.602	37
Clim1	0.596	0.444	0.833	**	-2.066	37
Clim2	0.666	0.368	0.757	**	-2.122	37
Clim3	0.449	0.398	0.549	0.1	-1.195	37
Clim4	0.371	0.061	0.069	NS	-0.539	37
Srad1	0.247	0.916	0.689	NS	1.390	37
Srad2	0.525	0.147	0.238	*	-1.680	37
Srad3	0.372	0.022	0.026	NS	-0.470	37
Srad4	0.297	0.003	0.003	NS	0.365	37

Notes: Tests are based on the method of Blomberg et al. (2003). See Appendix 1 for explanation of trait codes. All tests were performed using the Conflict decomposition tree with 999 null simulations. Results were qualitatively the same for the Excluded and No5S trees except for inflorescence height (Infl_ht) which became marginally significant ($P<0.07$). Results were qualitatively the same when tested on untransformed trait values except for spikelet number (Spk_no) which became not significant.

Traitgrams indicate that the three subclades experienced pronounced divergence in plant size early in their evolutionary history (PCA Axis 1; Figure 5.4), with these differences being retained through time such that the extant species from the different clades show little overlap. By contrast, each of the subclades has explored most of the variation in spikelet characteristics encompassed by the group (Axis 2), showing little structure among species within each subclade. Both leaf shape (Axis 3) and inflorescence architecture (Axis 4) are partially structured among subclades, with considerably more variation among the *fasciata-flexuosa* species than in the other two subclades.

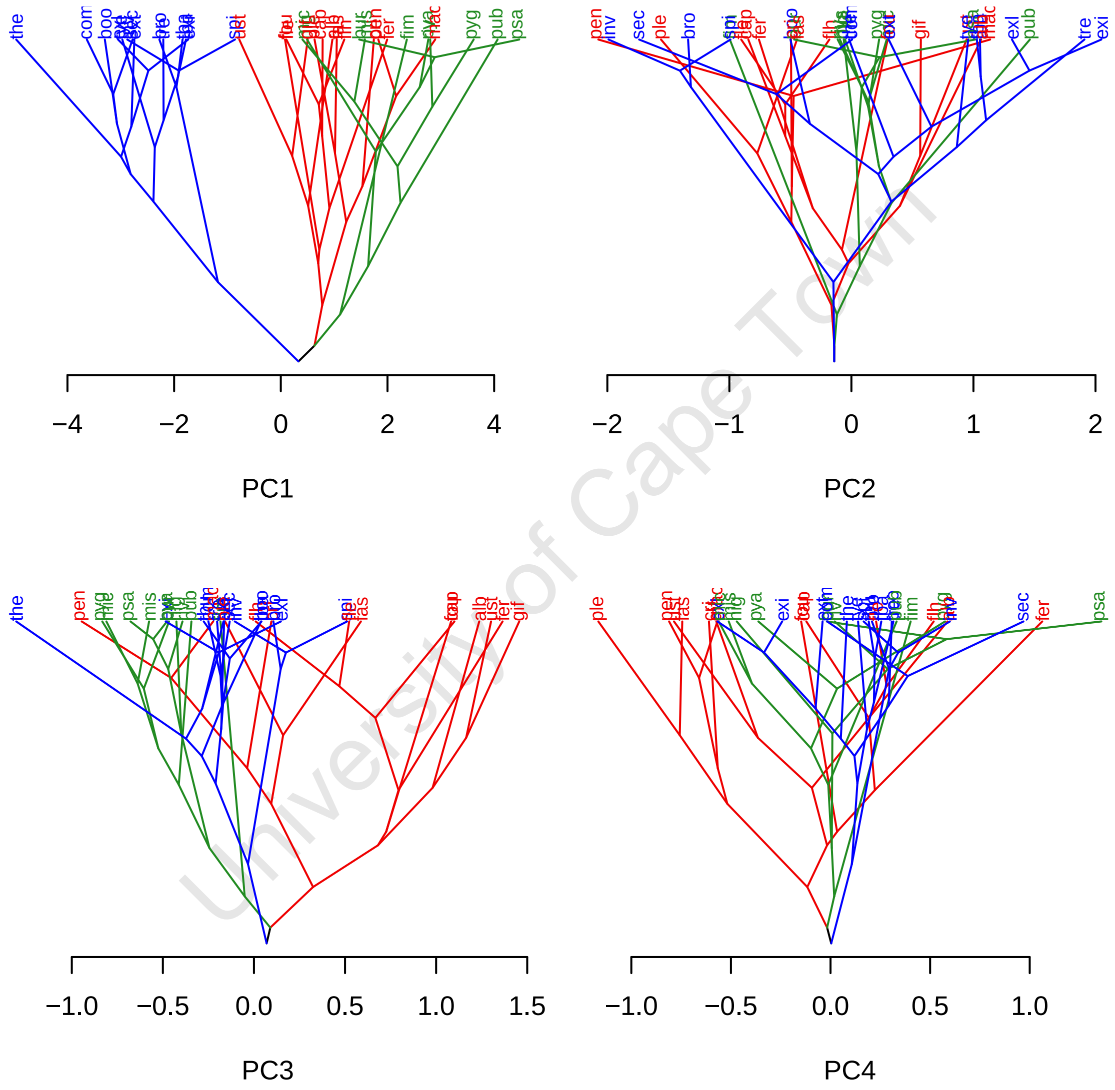


Figure 5.4: Traitgrams of the four major axes of trait variation. Trait values for extant taxa and reconstructed values for internal nodes are indicated on the x-axis. Node heights (y-axis) indicated estimated divergence times (see 4).

Table 5.5: Results of the trait disparity tests using each of the three MCC trees.

	Axis 1			Axis 2			Axis 3			Axis 4		
Node	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
38	H*	-	-	L***	-	-	NS	-	-	NS	-	-
39	NS	-	-	L	-	-	H	-	-	NS	-	-
40	NS	NS	NS	NS	NS	NS	H	H	H	NS	NS	NS
41	NS	-	-	NS	-	-	NS	-	-	NS	-	-
42	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
43	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
44	NS	-	-	NS	-	-	NS	-	-	NS	-	-
45	NS	NS	NS	L†	L*	L*	NS	NS	NS	NS	NS	NS
46	L	L	L	NS	NS	NS	H	NS	NS	NS	NS	NS
47	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
48	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
49	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
50	NS	NS	NS	L***	L***	L**	NS	NS	NS	L	L	L
51	NS	NS	NS	H***	H***	H***	H	NS	NS	NS	NS	NS
52	L***	L***	L***	NS	NS	NS	NS	NS	NS	NS	NS	NS
53	NS	NS	NS	NS	NS	NS	NS	NS	NS	L*	L*	L*†
54	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
55	NS	NS	NS	L***	L***	L***	L	L	L	NS	NS	NS
56	NS	NS	NS	NS	NS	NS	NS	NS	NS	L†	L†	L†
57	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
58	NS	NS	NS	L	NS	NS	NS	L	L	NS	NS	NS
59	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
60	H****	H****	H****	H***	H***	H***	NS	NS	NS	H****	H****	H****
61	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
62	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
63	NS	NS	NS	NS	NS	NS	L**	L***	L**	NS	NS	NS
64	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
65	L*	L*	L*	L*	L*	L*†	NS	NS	NS	NS	NS	NS
66	L	-	-	NS	-	-	NS	-	-	NS	-	-
67	NS	-	-	NS	-	-	H*	-	-	NS	-	-
68	NS	NS	NS	NS	NS	NS	L***	L***	L***	NS	NS	NS
69	NS	NS	NS	NS	NS	NS	H*	H	H	NS	NS	NS
70	L*	L*	L*	NS	NS	NS	NS	NS	NS	NS	NS	NS
71	NS	NS	NS	H	H	H	NS	NS	NS	H†	H†	H†
72	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
73	H	H**	H**	NS	H	H	H	H**	H**	NS	NS	NS
74	-	NS	NS	-	L	L	-	H	H	-	NS	NS
75	-	NS	NS	-	NS	NS	-	L*†	L*†	-	NS	NS
76	-	NS	NS	-	NS	NS	-	NS	NS	-	NS	NS
77	-	H**	H***	-	L***	L**	-	NS	NS	-	L	L
78	-	NS	NS	-	NS	NS	-	NS	NS	-	NS	NS
79	-	NS	NS	-	NS	NS	-	H**	H**	-	NS	NS

Notes: T1-3 indicate the three MCC trees (Conflict decomposition, Excluded, No 5S). Instances where change in a trait was greater (H) or lower (L) than expected at α=0.1 are indicated. Nodes marked with a * are significant at $P<0.05$, **$P<0.029$, ***$P<0.01$, ****$P<0.005$. Nodes which shifted a significance level when the tests were performed on the independent subclades (more different in all cases) are marked with a †. Differences in topolgy between the MCC trees mean that not all nodes are represented in each tree. Missing nodes are indicated with a '-'

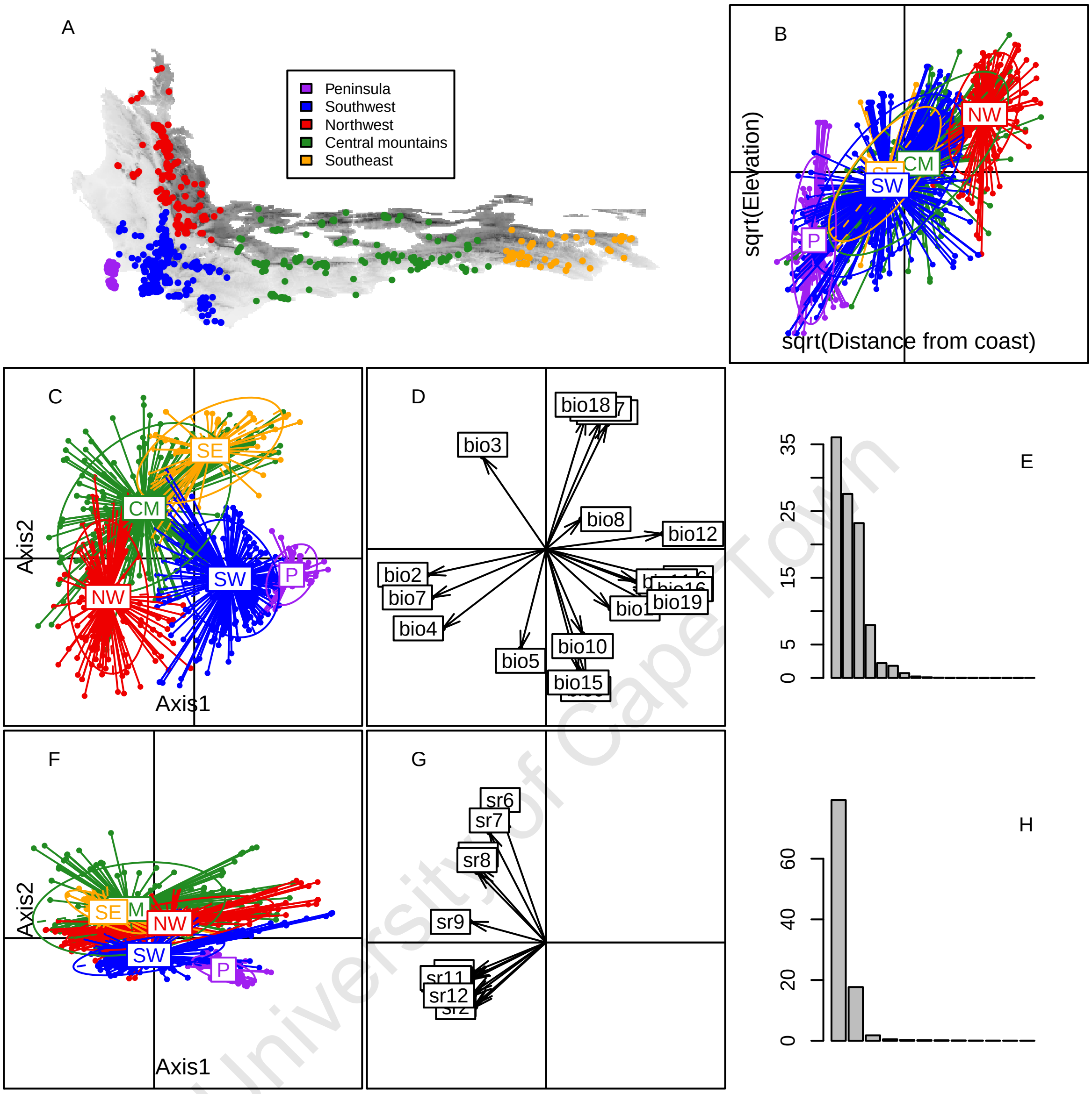


Figure 5.5: Map of the fynbos regions of the Cape Floristic Region indicating *Tetraria* species localities in each of five phytogeographic centres (A), scatterplot indicating the relationship between elevation and distance to the sea among localities in each phytogeographic centre (B), and results of principal components analysis of bioclimatic variation (C-E) and insolation (F-H) across localities in the phytogeographic centres. P: Peninsula, SW: Southwest, NW: Northwest, CM: Central Mountains, SE: Southeast, bio1-19: bioclim variables 1-19, sr1-12: monthly daily average solar radiation, January to December.

5.4.3.3 Disparity tests

The results of the disparity tests were largely consistent across the different MCC trees and when the tests were done on each subclade independently (Figure 5.6, Table 5.5).

Tests based on subclades tended to reveal more significant differences. Most differences in disparity test results between the MCC trees were due to topological differences. The species pairs *pen* - *mac* (node 51) and *psa* - *bur* (60) displayed significant trait divergence, similar to their contrasting niche preferences. However, some species pairs that had contrasting niche preferences, such as *gif* - *ust* (43) and *fle* - *flh* (47), did not display significant trait divergence. Two species pairs from the *thermalis-bromoides* subclade that did not show significantly different niche preferences, *exi* - *exl* (69) and *inv* - *spi* (73), did show significant trait divergence, the level of significance varying depending on the MCC tree employed and whether the tests were performed on the entire trees or independently on each subclade. A third pair, *com* - *sec* (71), differed at $\alpha = 0.1$.

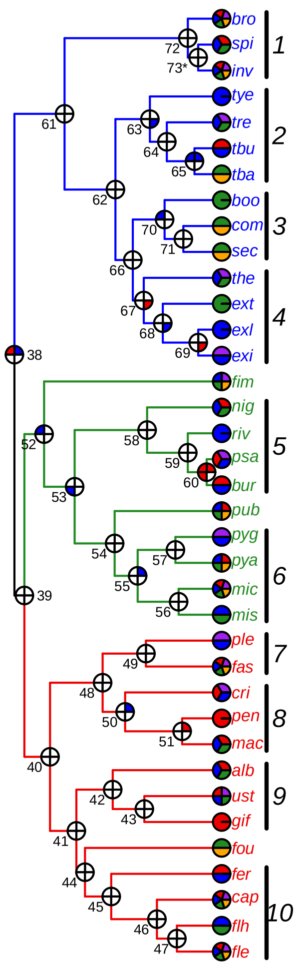
Trait divergence between taxa was commonly lower than expected on the basis of chance, notably between close relatives with allopatric distributions such as *tba* - *tbu* (65) and between *ext* and the pair *exi* - *exl* (68), and among a number of the internal nodes in the *microstachys-burmannii* subclade (52, 53, 55). Neither of the two species pairs from the *microstachys-burmannii* subclade that did not reveal significant niche differences, *mis* - *mic* (56) and *pya* - *pyg* (57), displayed significant trait divergence.

5.4.4 Environmental niche differentiation

The first two axes of the PCA based on bioclimatic variables explained almost 65% of the variance and revealed distinct climatic differences between the phytogeographical centres (Figure 5.5A, C - E). The first axis described mostly winter climatic conditions and temperature variation with positive loadings for rainfall and winter temperatures and negative loadings for temperature range and seasonality. This axis separated the wetter, more moderate Peninsula, Southwest and Southeast centres from the drier Central Mountain and Northwest centres, which are subject to more extreme temperatures, likely due to differences in elevation and proximity to the moderating influence of the sea (Figure 5.5B). The second axis described summer climatic conditions and rainfall variation with positive loadings for summer rainfall and negative loadings for summer temperatures and rainfall seasonality, and separated the western Peninsula, Northwest and Southwest centres from the eastern Central Mountain and Southeast centres. The PCA based on insolation data revealed two axes that explained 95% of the variance (Figure 5.5F-H). The first axis had positive loadings for all variables, mostly accounting for the mean daily amount of insolation each month, and revealed a slight East-West gradient among the phytogeographical centres. The second axis had positive loadings with winter and negative loadings with summer insolation and also revealed a slight East-West gradient. As all centres are at similar latitudes, differences in insolation between centres are likely to exist because mountain ranges lie parallel to the coast, running mostly North to South in the Western centres and East to West in the Eastern centres.

The environmental niche partitioning tests revealed that many sister species or close-relatives that co-occur within geographical centres showed significant shifts in one or more of their preferred bioclimatic, insolation or elevational preferences (Figure 5.6). Most of the non-significant comparisons are likely due to low numbers of localities of one or both species in the regions of overlap. Low numbers of localities limit the power of the permutation procedure because the total number of possible combinations is low. For example, the *alb* and *gif* comparison involved only one and three localities respectively, limiting the null model to four possible combinations and thus a minimum P-value of approximately 0.25. That the P value for differences in insolation and continentality are both 0.248 indicates that the *alb* locality is potentially very different from the *gif* localities for these two variables. Notable comparisons between sister species pairs which had reasonable sample sizes but did not reveal significant differences are *sec* - *com* and *inv* - *spi*, from the *thermalis-bromoides* subclade, and *mis* - *mic* and *pya* - *pyg*, from the *microstachys-burmannii* subclade.

Figure 5.6: Summary of morphological disparity tests and results of environmental differentiation tests. Pie slices at the tips of the phylogeny indicate species distribution ranges (see Figure 5.1) while pie slices on the nodes indicate the degree of change for each of the four trait axes (see Table 5.5) at that node (1 - 4 clockwise from top left). Red indicates significantly greater than expected change (α=0.05), blue indicates significantly lower than expected change, while white indicates no deviation from that expected by chance. Vertical bars indicate the subclades used for environmental differentiation comparisons in the adjacent table (labelled 1-10, column 1). The three major clades are separated in the table by solid horizontal lines while the subclades within them are separated by dashed horizontal lines. The number of localities used for each species in each pairwise comparison is indicates in brackets next to the species names. Regions of overlap (or allopatry) and MRPP test statistics and P values for each test are indicated. Significant differences at α <0.05 are indicated in italics while differences at α <0.027 are indicated in bold. The final two columns indicate whether each pair differs significantly for one or more environmental differentiation test (ΔE) or morphological disparity test on the four trait axes (ΔT). ✓indicates difference, ✗indicates no significant difference, $\sim$ indicates weakly significant difference and † indicates less change in morphological trait axes than expected.



				Bioclimatic			Insolation			Elevation				
C	Sp 1	Sp 2	Overlap	δO	δE	P	δO	δE	P	δO	δE	P	ΔE	ΔM
1	inv (52)	spi (22)	NW, SW, CM	5.53	5.58	0.091	4.40	4.42	0.208	1.28	1.27	0.791	✗	~
	inv (64)	bro (87)	P, NW, SW, CM, SE	5.58	5.70	**0.000**	4.04	4.10	**0.011**	1.46	1.55	**0.000**	✓	✗
	spi (22)	bro (66)	NW, SW, CM	5.01	5.14	**0.003**	3.47	3.46	0.623	1.34	1.47	**0.000**	✓	✗
2	tba	tbu	*Allopatric*	-	-	-	-	-	-	-	-	-	-	†
	tba (14)	tre (1)	CM	3.55	3.64	0.204	5.12	5.11	0.471	0.72	0.76	0.062	✗	✗
	tbu (6)	tre (17)	SW	2.05	2.31	**0.006**	4.17	5.42	**0.003**	0.62	0.70	**0.022**	✓	✗
	tba	tye	*Allopatric*	-	-	-	-	-	-	-	-	-	-	†
	tbu (6)	tye (10)	SW	1.45	1.61	*0.037*	4.49	4.94	0.081	0.44	0.43	0.647	~	†
	tre (17)	tye (10)	SW	1.99	2.14	**0.010**	4.53	4.73	0.114	0.62	0.70	**0.006**	✓	✗
3	sec (18)	com (8)	CM, SE	4.30	4.33	0.288	4.25	4.29	0.242	0.87	0.91	0.081	✗	~
	sec (10)	boo (1)	CM	4.16	4.06	0.638	4.45	4.19	0.818	0.83	0.89	0.277	✗	†
	com (3)	boo (1)	CM	5.30	4.31	1.000	3.36	3.00	1.000	1.62	1.46	1.000	✗	†
4	ext (2)	the (6)	CM	3.37	3.05	0.925	1.94	2.14	0.179	0.94	0.97	0.320	✗	✓
	exi (1)	exl (6)	SW	2.35	2.66	0.142	1.59	1.47	1.000	0.88	1.28	0.142	✗	✓
	ext	exi	*Allopatric*	-	-	-	-	-	-	-	-	-	-	†
	ext	exl	*Allopatric*	-	-	-	-	-	-	-	-	-	-	†
	exi (9)	the (22)	P, SW	3.10	3.31	**0.006**	2.36	2.96	**0.001**	1.21	1.42	**0.001**	✓	✓
	exl (6)	the (17)	SW	3.08	3.12	0.228	1.75	1.73	0.567	1.12	1.12	0.447	✗	✓
5	nig (33)	bur (11)	NW, SW	4.27	4.62	**0.001**	2.86	2.86	0.382	1.49	1.50	0.291	✓	✗
	nig (33)	psa (29)	NW, SW	4.08	4.91	**0.000**	3.59	4.40	**0.000**	1.37	1.50	**0.001**	✓	✗
	nig (15)	riv (1)	SW	3.45	3.62	0.188	1.93	2.17	0.060	1.46	1.53	0.129	✗	✗
	bur (11)	psa (29)	NW, SW	3.68	4.10	**0.000**	3.95	4.46	**0.003**	1.10	1.24	**0.001**	✓	✓
	bur (10)	riv (1)	SW	3.43	3.59	0.278	2.68	2.90	0.272	1.03	1.10	0.094	✗	✗
	psa (14)	riv (1)	SW	2.54	2.65	0.198	4.51	4.49	0.532	1.07	1.03	0.669	✗	✗
6	mis (21)	mic (17)	SW, CM	4.24	4.23	0.473	3.00	2.99	0.458	1.40	1.40	0.578	✗	✗
	mis (21)	pya (5)	SW, CM	4.25	4.21	0.598	2.33	2.33	0.354	1.64	1.63	0.556	✗	†
	mis (17)	pyg (6)	SW	2.96	2.93	0.572	1.94	1.90	0.890	1.33	1.35	0.229	✗	†
	mic (19)	pya (7)	NW, SW, CM, SE	4.88	4.88	0.451	3.50	3.59	0.164	1.44	1.52	*0.048*	~	†
	mic (25)	pyg (10)	P, SW	3.27	3.22	0.967	3.15	3.09	0.848	1.11	1.10	0.457	✗	†
	pya (4)	pyg (6)	SW	3.48	3.27	0.934	1.80	1.78	0.421	1.35	1.34	0.370	✗	✗
7	fas (37)	ple (26)	P, SW	3.45	3.49	0.093	3.15	3.13	0.540	1.46	1.51	**0.020**	✓	✗
8	cri (26)	mac (22)	NW, SW	3.84	4.53	**0.000**	3.61	3.98	**0.003**	1.12	1.44	**0.000**	✓	†
	cri (11)	pen (5)	NW	3.00	3.17	0.114	3.80	3.83	0.318	0.66	0.66	0.397	✗	†
	mac (20)	pen (5)	NW	2.63	3.12	**0.002**	4.16	4.22	0.233	0.58	0.68	**0.001**	✓	✓
9	alb (1)	gif (3)	NW	3.23	4.20	0.506	1.25	3.49	0.252	0.92	1.16	0.247	✗	✗
	alb (5)	ust (102)	NW, SW, CM	4.84	4.85	0.266	3.42	3.47	*0.038*	1.28	1.29	*0.035*	~	✗
	gif (3)	ust (45)	NW	3.54	3.70	**0.011**	3.85	4.05	*0.034*	0.89	0.90	0.146	✓	✗
10	fer (17)	fle (42)	NW, SW	3.87	4.54	**0.000**	2.54	2.83	**0.000**	1.34	1.74	**0.000**	✓	✗
	fer (6)	flh (7)	SW	2.72	3.11	*0.043*	4.29	4.09	0.681	0.75	0.86	*0.034*	~	✗
	fer (17)	cap (22)	NW, SW	4.06	4.71	**0.000**	2.81	3.26	**0.001**	1.14	1.56	**0.000**	✓	✗
	fle (48)	flh (8)	SW, CM	4.28	4.42	**0.006**	2.94	3.01	*0.045*	1.32	1.51	**0.000**	✓	✗
	fle (62)	cap (59)	P, NW, SW, CM, SE	5.24	5.40	**0.000**	3.62	3.69	**0.013**	1.45	1.46	0.288	✓	✗
	flh (8)	cap (38)	SW, CM	5.00	5.32	**0.001**	4.09	4.11	0.271	1.13	1.33	**0.000**	✓	✗

Figure 5.6: See over page for caption

5.4.5 Summary of ecological differentiation results

Most comparisons among close-relatives within the *fasciata-flexuosa* and *thermalis-bromoides* clades showed significant morphological divergence and/or differentiation in environmental preferences (Figure 5.6). Four of the 18 species pairs in the *thermalis-bromoides* clade were allopatrically distributed while ten of the remaining 14 differed significantly in morphology and/or environmental preference. Of the four which showed no significant difference, three involved comparisons with too few localities to attain statistically significant differences in environmental preferences while the fourth showed weak evidence of morphological divergence. All four comparisons of allopatric species pairs revealed lower divergence on one or more morphological axes than expected by chance.

Eleven of the 13 *fasciata-flexuosa* species pair comparisons differed significantly in morphology and/or environmental preference. One of the remaining pairs involved too few localities to attain significant differences in environmental preferences.

By contrast, only four of the 12 *microstachys-burmannii* species pair comparisons differed significantly in morphology and/or environmental preference. Low numbers of species localities would have resulted in low statistical power for three of the non-significant comparisons.

5.5 Discussion

Tetraria and two of its three major subclades exhibit density-dependent declines in diversification rate which, according to the predictions of McPeek (2008), suggests that speciation was associated with ecological divergence, slowing as the available ecological niche space became saturated. Consistent with this hypothesis, most closely-related species within the group display distinct ecological differentiation in terms of contrasting habitat preferences in areas of geographic overlap (ecological niche partitioning) or disparity in morphological traits that may be important for determining resource acquisition or facilitating species co-occurrence. In addition, the decline in diversification rates among clades varied depending on the degree of ecological differentiation among species, the geography of speciation and the spatial arrangement of species distribution ranges.

The degree of ecological differentiation within subclades and differences in the rates at which their diversification slowed fitted with the predictions of McPeek's (2008) metacommunity model of diversification. Most close relatives in the *fasciata-flexuosa* and *thermalis-bromoides* subclades display significant ecological differentiation in at least one environmental or morphological dimension. The density-dependent decline in their diversification rates are thus likely to be the result of asymmetrical competition between close relatives which dictates that 'new' species are rapidly driven to extinction or replace existing species (Chesson 2000, McPeek 2008). By contrast, the low degree of ecological differentiation among close relatives in the *microstachys-burmannii* subclade, apparent only in the *psa - bur* species pair, and the associated negligible decline in diversification rate, most likely arises because species are ecologically very similar and competition between close relatives is highly symmetrical. Here fitness equivalence and neutral-like dynamics allow species to be present in the system for a long time, slowly being driven to extinction by weak species interactions or random drift (Chesson 2000, Hubbell 2001, 2005, Leibold & McPeek 2006, McPeek 2008). An alternative coexistence mechanism that could produce a similar pattern is one in which competitive exclusion among species in the *microstachys-burmannii* subclade is greatly delayed because dispersal and recruitment limitation prevent the best competitors from establishing and dominating all available sites (Hurtt & Pacala 1995). This is plausible because all species in this subclade produce low numbers of small seeds and their smaller body size means that there are far more sites available to them than to species in the other two subclades.

The geography of speciation and spatial distribution of *Tetraria* species also appear to have had an important influence on diversification rates. Firstly, the lower decline in diversification rate in the *thermalis-bromoides* subclade when compared to the *fasciata-flexuosa* subclade is potentially attributable to the fact that closely-related species in the group show less geographical overlap, reducing competition through spatial niche partitioning (Amarasekare 2003). As a consequence, individual regions within the CFR (centres of endemism in this case) may have become ecologically saturated less quickly, imposing less constraint on diversification in the *thermalis-bromoides* subclade. Secondly, poor dispersal and speciation in allopatry may explain the higher overall rate of diversification observed in the *thermalis-bromoides* subclade (Chapter 4). On average, species in the *thermalis-bromoides* subclade occur in fewer phytogeographic centres than do species in the other two subclades, implying that species in this group do not disperse easily across the biogeographical barriers which isolate the centres. Poor dispersal ability means that founder events will be rare, but when they do occur, the founder and parent populations are likely to remain genetically isolated and result in speciation (Mayr 1963, Jablonski & Roy 2003). Conversely, high dispersal ability would allow many founder events, but gene transfer between populations would be common, resulting in few, broadly-distributed species (Mayr 1963, Jablonski & Roy 2003). This pattern is evident among the two subclades within the *thermalis-bromoides* group. *Tetraria bromoides* (*bro*), *inv* and *spi* form a clade of three broadly distributed species while its sister clade, including *the*, has radiated into 11 narrowly distributed species, despite having had the same time and ecological opportunity to disperse or diversify. The dispersal abilities of the 11 species in the *the* subclade may be lower because they have much larger seeds than other *Tetraria* species, including *bro, inv* and *spi* (Coomes & Grubb 2003).

The geography of speciation and the subsequent expansion of species ranges are also likely to have affected the degree to which *Tetraria* species are ecologically differentiated. The four species pairs which have allopatric distributions display lower than expected trait disparity suggesting that speciation was not associated with adaptive divergence, instead occurring via ‘non-ecological’ means (Wiens 2004, Kozak *et al.* 2006, Rundell & Price 2009). Here speciation involves a founder population colonizing the same ecological niche in a new geographic region, with the two populations diverging due to drift or weak divergent selection (Wiens 2004, Stephens & Wiens 2004, Moen *et al.* 2009). Morphological similarity may be maintained in the absence of gene flow if similar stabilizing selective pressures are present in the two regions. The extent to which non-ecological speciation is a more general phenomenon in *Tetraria* is unclear because non-adaptive radiations are difficult to detect in old radiations (Rundell & Price 2009). Ecological disparity among close relatives may accumulate with time due to drift and the subsequent dispersal of populations back into sympatry may affect the degree of ecological differentiation among close relatives in a number of ways. Firstly, gene flow can be re-established and any morphological differences between the populations become homogenized resulting in a single broadly distributed species. Secondly, if the two populations have diverged enough, but are not genetically isolated, trait divergence can occur through disruptive selection or lower hybrid fitness driving reinforcement via ecological or reproductive character displacement (Schluter 2001, Bolnick & Fitzpatrick 2007, Pfennig & Pfennig 2009). Lastly, if some isolating mechanism has evolved via genetic drift and fixation in allopatry, trait or niche divergence can occur via character displacement to reduce competition in sympatry (Brown & Wilson 1956, Pfennig & Pfennig 2009) or, as may be the case for the *microstachys-burmannii* species, there may be selection to maintain similar trait values to reduce differences in competitive ability and so to avoid competitive exclusion (Chesson 2000, Leibold & McPeek 2006, Mayfield & Levine 2010). Populations of *tre*, *tbu* and *tye* exhibit distinctive microhabitat and trait shifts in areas where they occur sympatrically when compared to populations where they occur alone (M. N. Britton *pers. comm.*). This suggests that character displacement may be an important driver of ecological differentiation among *Tetraria* species.

While the metacommunity model proposed by McPeek (2008) included spatial dynamics by allowing species to disperse between interconnected patches, it did not include geography and its effect on speciation and ecological differentiation in an explicit manner. Five percent of each species was allowed to disperse to all patches per iteration and there were no defined mechanisms of speciation, with all lineages sharing a constant instantaneous speciation rate. This excludes limited dispersal across biogeographic barriers and allopatric speciation as factors that may impact on diversification rates and may explain why McPeek (2008) found no effect of varying dispersal rates on diversification rates. Future, individual-based diversification models could explore the effect of dispersal limitation and allopatric speciation by including strong dispersal limitation between patches, or sets of patches nested within regions of varying connectivity.

A model that includes geography and non-adaptive speciation can still result in density dependent declines in diversification rates in lineages that undergo subsequent ecological differentiation in two ways. Firstly, multiple dispersal events back into sympatry may be required before a population can establish and undergo character or niche displacement without being competitively excluded. Secondly, under the expectation that species undergoing niche displacement are likely to occupy the biggest empty niche available, such as in the ‘broken stick’ model of niche apportionment (MacArthur 1957), successive speciation and niche shift events will result in species occupying smaller and smaller niches (ecological saturation). This will cause the number of founder events between regions and thus the speciation rate to decline, because, if dispersal is ecologically conserved, there will be smaller ‘islands’ of preferred niche available to disperse into. In addition, the spatial arrangement and connectivity between regions may have important effects on speciation rates and the degree to which regions become ecologically saturated. If one considers three regions in a linear arrangement such that the outer regions are connect to the central region but not to each other, it is clear that species range expansion and/or allopatric speciation across boundaries between regions will result in more rapid accumulation of diversity, and thus ecological saturation, in the central region. This will constrain founder events from the outer regions into the central region, but will still allow founder events from the central into the outer regions. Lineages which have their centres of diversity in the outer regions will thus suffer greater constraint on their diversification rates than lineages whose diversity is concentrated in the central region. The subclades of *Tetraria* are largely consistent with this model. Figure 5.7 provides a schematic of the five centres of endemism for *Tetraria* in the CFR indicating connectivity between regions and the diversity of each subclade represented within each region. The total diversity of each region clearly reflects their connectivity to other regions with the Southwest and Central Mountain centres connected to three regions each and containing the greatest diversity, followed by the Northwest centre with two connections and then the Peninsula and Southeast centres with one connection each. The concentration of diversity and the distribution of endemics within each subclade indicates their centre of diversity. The diversity of the *microstachys-burmannii* group, which showed no decline in diversification rate, is centred in the in the highly connected Southwestern region, while the centres of diversity for the *thermalis-bromoides* and the *fasciata-flexuosa* subclades, which do show declining diversification rates, occur in the peripheral Eastern and Northwest regions respectively.

Potential shortcomings of the analyses presented here relate to the scale of the environmental data, the limited set of traits examined and the sensitivity of the statistical tests employed. Environmental data used in this study were derived from GIS layers with 1 minute grid cell resolution, equivalent to an absolute area of 4 km^2 at the equator, masking much environmental variation along which species niches may be differentiated. Within the CFR an area of this size can be highly heterogeneous in terms of soil types, soil moisture and rainfall, depending on slope, aspect, geology and the influence of the sea (Campbell 1983, Cowling *et al.* 1996*a*,*b*, Cowling & Lombard 2002, Araya *et al.* 2010*b*). Summarizing these data into single averaged point values reduces the chances of detecting niche differentiation between species because each species will be recorded as occurring in a much broader range of conditions than is true of its actual habitat. Similarly, the lack of data pertaining to soil properties has limited this analysis to examination of climatic niches only. In the absence of these data, the interpretation that close relatives in the *microstachys-burmannii* subclade show little environmental niche partitioning may be premature. Similarly, the morphological data presented here were limited to traits that could be measured from dried, pressed herbarium material and represent only a small subset of traits that may be important for determining resource acquisition or facilitating species co-occurrence (see Cornelissen *et al.* 2003). Finally, the test for morphological disparity is dependent on maximum likelihood reconstruction of internal node states based on previously estimated phylogenies and does not estimate uncertainty in phylogenetic and ancestral state reconstructions simultaneously as do more advanced Bayesian techniques (Ronquist 2004, Pagel & Meade 2006). Similarly, the test is based on the assumption that constant rate Brownian motion provides an accurate model of trait evolution under drift, which is rarely true (Mooers *et al.* 2007). However, assuming a constant rate is desirable in this case because the aim is to detect instances where the rate of trait change has significantly increased or decreased relative to the net rate across the tree. In addition, basing the null on the variance-covariance matrix estimated from the actual values of the trait under scrutiny means that the test is conservative because it is based on the 'ghost of trait evolution past' which includes rapid trait shifts and thus greater variance than one would expect under actual drift.

Notwithstanding the analytical limitations, the analysis presented here provides support for the hypothesis that lineages which display declining diversification rates should be made up of ecologically differentiated species. It also highlights the importance of considering geographic context and speciation mode and the potential role for non-ecological speciation and character displacement in determining the diversification dynamics and ecological differentiation of lineages. Allopatrically distributed species need not be ecologically differentiated, while restricted distribution ranges result in regions becoming ecologically saturated more slowly, reducing the degree of ecological constraint on diversification rates. Finally, connectivity between regions can affect the rate at which they become ecologically saturated with important implications for lineage diversification rates and ecological differentiation. This highlights that, while incorporating ecological mechanisms that influence diversification into macroevolutionary models is invaluable, the geographical context cannot be ignored, and further tightens the links between ecological dynamics, macroevolution and the assembly of communities (Webb *et al.* 2002, McPeek 2008).

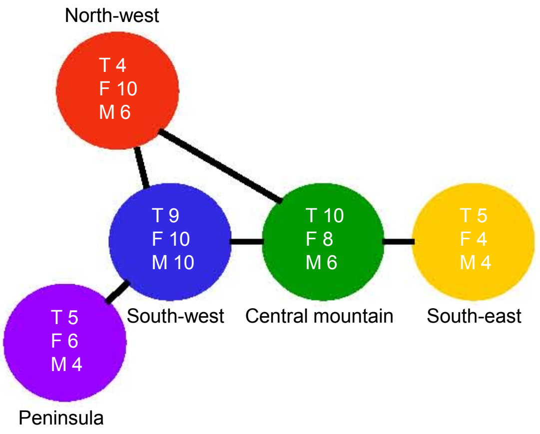


Figure 5.7: Schematic indicating the number of species from each of the *Tetraria* subclades in each of the centres of endemism across the CFR. T: *thermalis-bromoides*, F: *fasciata-flexuosa*, M: *microstachys-burmannii*. Connectivity between regions (bubbles) is indicated by black lines.

Appendix 5.1: Trait means, standard deviations and number of individuals measured for 37 *Tetraria* species.

Species	Code	Infl_ht (cm)	Infl_l (cm)	Cu_d (mm)	Bs_lf_l (cm)	Bs_lf_w (mm)	Spk_no	Spk_l (mm)	Spk_w (mm)	Sd_l (mm)	Sd_w (mm)	Sd_m (g)
Means												
T. wallichiana	alb	48.25	4.63	1.30	26.50	1.01	21.00	8.13	2.86	NA	NA	NA
T. sp. nov. cf. secans	boo	137.17	52.33	5.70	68.67	7.90	144.17	11.50	2.52	NA	NA	NA
T. bromoides	bro	101.92	35.00	3.67	52.46	3.22	433.85	10.38	1.90	3.48	1.82	0.003
T. burmannii	bur	32.42	21.88	0.95	15.67	1.13	29.08	5.54	1.84	1.25	1.10	0.001
T. capillacea	cap	61.53	13.95	1.17	37.67	0.67	39.83	6.73	1.64	2.60	1.50	0.003
T. compressa	com	149.60	57.00	5.70	49.00	7.66	200.00	14.40	3.32	6.66	3.57	0.018
T. crinifolia	cri	22.36	19.82	0.91	28.00	0.76	15.21	7.51	1.26	3.35	2.04	0.006
T. eximia	exi	45.00	26.00	2.31	26.67	5.50	37.00	16.67	4.79	4.88	3.56	0.028
T. sp. nov. cf. eximia A	exl	95.00	52.00	3.10	51.00	5.00	60.00	17.00	5.00	NA	NA	NA
T. sp. nov. cf. eximia B	ext	83.33	56.67	2.93	52.67	6.27	108.00	12.00	3.63	NA	NA	NA
T. fasciata	fas	45.53	14.66	1.15	26.47	0.59	31.42	9.37	1.48	2.75	1.66	0.002
T. ferruginea	fer	47.09	5.59	0.79	32.18	0.75	30.73	3.99	1.70	2.05	1.53	0.001
T. fimbriolata	fim	39.21	18.00	0.77	13.57	0.78	18.79	3.34	1.68	1.72	1.32	0.001
T. flexuosa	fle	72.13	17.92	1.70	29.70	1.09	77.33	6.42	1.94	2.07	1.37	0.001
T. sp. nov. cf. flexuosa	flh	33.88	13.88	1.43	20.13	1.18	29.13	5.51	2.05	2.16	2.58	0.002
T. fourcadei	fou	61.50	14.29	1.51	39.21	1.00	24.71	8.73	2.50	3.61	2.26	0.005
T. sp. nov. cf. ustulata	gif	54.50	11.75	1.05	42.50	0.68	17.25	10.13	2.45	NA	NA	NA
T. involucrata	inv	140.14	55.07	4.87	57.50	4.57	697.86	7.94	1.93	3.10	1.65	0.001
T. maculata	mac	30.43	4.57	0.91	6.93	0.65	6.86	10.18	1.57	3.57	1.07	0.004
T. microstachys	mic	30.32	24.64	1.28	16.73	1.83	41.73	10.64	2.05	1.94	1.56	0.001
T. sp. nov. cf. microstachys	mis	26.81	21.00	0.84	14.94	1.08	22.75	7.46	1.73	NA	NA	NA
T. nigrovaginata	nig	50.29	25.96	1.24	17.93	1.65	27.07	8.31	1.99	2.71	1.69	0.003
T. sp. nov. cf. maculata	pen	43.25	38.69	0.89	12.63	0.74	47.75	5.44	0.91	NA	NA	NA
T. pleiosticha	ple	51.22	40.33	1.10	26.00	0.76	58.11	8.49	1.13	3.10	1.74	0.002
T. sp. nov. cf. pubescens	psa	5.30	2.81	0.70	8.57	0.97	6.57	4.38	1.52	2.05	1.18	0.001
T. pubescens	pub	6.59	3.88	0.59	10.72	0.78	3.69	6.69	1.59	2.40	1.50	0.001
T. sp. nov. cf. pygmaea	pya	21.35	16.27	0.55	11.19	0.65	11.23	5.28	1.74	NA	NA	NA
T. pygmaea	pyg	9.50	6.79	0.70	9.43	0.91	11.71	4.94	1.39	NA	NA	NA
T. sp. nov. cf. burmannii	riv	18.50	14.63	0.58	14.50	0.93	10.25	4.88	1.60	NA	NA	NA
T. secans	sec	138.25	45.50	5.15	60.00	7.15	400.00	7.33	1.88	NA	NA	NA
T. spiralis	spi	79.31	23.92	2.32	42.08	1.55	128.85	7.88	1.97	NA	NA	NA
T. sp. nov. cf. triangularis A	tba	71.09	26.69	3.47	45.15	6.56	23.87	11.89	3.10	NA	NA	NA
T. sp. nov. cf. triangularis B	tbu	53.74	30.63	3.23	41.66	6.87	28.80	12.25	3.12	NA	NA	NA
T. thermalis	the	146.50	90.75	8.86	37.00	16.13	1048.75	18.86	4.08	NA	NA	NA
T. triangularis	tre	64.93	23.38	4.84	39.38	9.67	15.98	14.98	3.79	NA	NA	NA
T. sp. nov. cf. triangularis C	tye	101.24	34.64	4.82	46.88	9.35	61.12	15.31	3.49	NA	NA	NA
T. ustulata	ust	78.06	17.88	1.82	44.65	0.91	23.82	13.28	3.28	5.11	2.95	0.019
Number of individuals												
T. wallichiana	alb	8	8	8	8	8	8	8	8	NA	NA	NA
T. sp. nov. cf. secans	boo	6	6	6	6	6	6	6	6	NA	NA	NA
T. bromoides	bro	13	13	13	13	13	13	13	13	3	3	3
T. burmannii	bur	12	12	12	12	12	12	12	12	2	2	2
T. capillacea	cap	30	30	30	30	30	30	30	30	1	1	1

Appendix 5.1: (continued on next page)

Appendix 5.1: (continued)

Species	Code	Infl_ht (cm)	Infl_l (cm)	Cu_d (mm)	Bs_lf_l (cm)	Bs_lf_w (mm)	Spk_no	Spk_l (mm)	Spk_w (mm)	Sd_l (mm)	Sd_w (mm)	Sd_m (g)
T. compressa	com	5	5	5	5	5	5	5	5	4	4	4
T. crinifolia	cri	14	14	14	14	14	14	14	14	3	3	3
T. eximia	exi	9	9	9	9	9	9	9	9	2	2	2
T. sp. nov. cf. eximia A	exl	1	1	1	1	1	1	1	1	NA	NA	NA
T. sp. nov. cf. eximia B	ext	3	3	3	3	3	3	3	3	NA	NA	NA
T. fasciata	fas	19	19	19	19	19	19	19	19	2	2	2
T. ferruginea	fer	11	11	11	11	11	11	11	11	3	3	3
T. fimbriolata	fim	14	14	14	14	14	14	14	14	3	3	3
T. flexuosa	fle	30	30	30	30	30	30	30	30	4	4	4
T. sp. nov. cf. flexuosa	flh	8	8	8	8	8	8	8	8	1	1	1
T. fourcadei	fou	14	14	14	14	14	14	14	14	3	3	3
T. sp. nov. cf. ustulata	gif	4	4	4	4	4	4	4	4	NA	NA	NA
T. involucrata	inv	14	14	14	14	14	14	14	14	3	3	3
T. maculata	mac	14	14	14	14	14	14	14	14	3	3	3
T. microstachys	mic	11	11	11	11	11	11	11	11	1	1	1
T. sp. nov. cf. microstachys	mis	8	8	8	8	8	8	8	8	NA	NA	NA
T. nigrovaginata	nig	14	14	14	14	14	14	14	14	3	3	3
T. sp. nov. cf. maculata	pen	8	8	8	8	8	8	8	8	NA	NA	NA
T. pleiosticha	ple	9	9	9	9	9	9	9	9	1	1	1
T. sp. nov. cf. pubescens	psa	23	23	23	23	23	23	23	23	2	2	2
T. pubescens	pub	16	16	16	16	16	16	16	16	1	1	1
T. sp. nov. cf. pygmaea	pya	13	13	13	13	13	13	13	13	NA	NA	NA
T. pygmaea	pyg	7	7	7	7	7	7	7	7	NA	NA	NA
T. sp. nov. cf. burmannii	riv	4	4	4	4	4	4	4	4	NA	NA	NA
T. secans	sec	4	4	4	4	4	4	4	4	NA	NA	NA
T. spiralis	spi	13	13	13	13	13	13	13	13	NA	NA	NA
T. sp. nov. cf. triangularis A	tba	93	93	93	93	93	93	93	93	3	3	3
T. sp. nov. cf. triangularis B	tbu	99	99	99	99	99	99	99	99	NA	NA	NA
T. thermalis	the	4	4	4	4	4	4	4	4	NA	NA	NA
T. triangularis	tre	58	58	58	58	58	58	58	58	NA	NA	NA
T. sp. nov. cf. triangularis C	tye	25	25	25	25	25	25	25	25	NA	NA	NA
T. ustulata	ust	17	17	17	17	17	17	17	17	3	3	3
Standard deviation												
T. wallichiana	alb	12.80	1.19	0.29	7.13	0.12	6.82	0.88	0.18	NA	NA	NA
T. sp. nov. cf. secans	boo	23.58	9.24	0.59	13.14	2.34	80.21	1.05	0.44	NA	NA	NA
T. bromoides	bro	24.36	9.57	0.92	17.06	0.89	213.09	0.74	0.14	0.28	0.19	0.002
T. burmannii	bur	5.48	5.26	0.26	3.08	0.21	12.28	0.53	0.14	0.07	0.00	0.000
T. capillacea	cap	14.15	4.81	0.30	12.75	0.21	15.68	0.72	0.16	NA	NA	NA
T. compressa	com	24.67	10.86	0.85	7.78	1.56	116.62	1.08	0.51	0.15	0.20	0.007
T. crinifolia	cri	10.10	10.12	0.19	10.11	0.13	3.33	1.09	0.21	0.30	0.14	0.002
T. eximia	exi	7.00	10.49	0.36	6.65	1.16	14.47	1.03	0.40	0.11	0.08	0.018
T. sp. nov. cf. eximia A	exl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
T. sp. nov. cf. eximia B	ext	26.03	11.59	1.01	6.43	1.21	45.30	0.87	0.15	NA	NA	NA
T. fasciata	fas	13.26	4.50	0.15	6.68	0.12	9.96	0.70	0.13	0.30	0.06	0.001
T. ferruginea	fer	10.30	4.97	0.14	5.81	0.19	14.33	0.73	0.21	0.32	0.17	0.001

Appendix 5.1: (continued on next page)

Appendix 5.1: (continued)

Species	Code	Infl_ht (cm)	Infl_l (cm)	Cu_d (mm)	Bs_lf_l (cm)	Bs_lf_w (mm)	Spk_no	Spk_l (mm)	Spk_w (mm)	Sd_l (mm)	Sd_w (mm)	Sd_m (g)
T. fimbriolata	fim	9.13	8.33	0.14	3.84	0.15	7.27	0.50	0.24	0.24	0.17	0.000
T. flexuosa	fle	23.78	7.46	0.34	11.39	0.30	40.67	0.99	0.24	0.10	0.48	0.000
T. sp. nov. cf. flexuosa	flh	6.49	8.04	0.12	4.26	0.15	6.77	0.41	0.08	NA	NA	NA
T. fourcadei	fou	10.11	6.02	0.21	9.32	0.25	6.07	0.53	0.26	0.53	0.14	0.003
T. sp. nov. cf. ustulata	gif	14.06	9.22	0.10	8.66	0.10	2.63	1.11	0.10	NA	NA	NA
T. involucrata	inv	42.96	14.53	1.05	20.14	1.45	373.43	1.02	0.33	0.07	0.15	0.000
T. maculata	mac	10.88	2.59	0.15	4.73	0.19	4.26	1.01	0.30	0.67	0.50	0.002
T. microstachys	mic	9.63	9.12	0.24	3.72	0.39	14.72	1.14	0.20	NA	NA	NA
T. sp. nov. cf. microstachys	mis	2.83	1.83	0.11	4.14	0.29	4.89	0.50	0.17	NA	NA	NA
T. nigrovaginata	nig	13.80	9.53	0.16	2.95	0.39	11.49	0.89	0.14	0.10	0.10	0.001
T. sp. nov. cf. maculata	pen	9.77	9.46	0.18	4.47	0.21	15.23	0.50	0.11	NA	NA	NA
T. pleiosticha	ple	8.57	9.62	0.22	6.89	0.29	20.39	1.44	0.25	NA	NA	NA
T. sp. nov. cf. pubescens	psa	2.55	2.12	0.18	2.89	0.25	3.29	0.57	0.13	0.21	0.08	0.000
T. pubescens	pub	1.82	1.15	0.08	2.56	0.11	0.79	0.85	0.20	NA	NA	NA
T. sp. nov. cf. pygmaea	pya	6.56	5.24	0.13	4.06	0.31	2.39	0.53	0.20	NA	NA	NA
T. pygmaea	pyg	2.35	2.02	0.22	2.07	0.34	4.89	0.75	0.22	NA	NA	NA
T. sp. nov. cf. burmannii	riv	3.34	3.71	0.10	3.11	0.15	2.87	0.25	0.00	NA	NA	NA
T. secans	sec	44.21	25.65	1.45	8.16	1.05	177.95	1.14	0.22	NA	NA	NA
T. spiralis	spi	19.77	8.84	0.53	14.56	0.27	43.60	0.77	0.21	NA	NA	NA
T. sp. nov. cf. triangularis A	tba	13.12	10.44	0.64	9.93	1.55	9.89	1.37	0.38	0.50	0.12	0.003
T. sp. nov. cf. triangularis B	tbu	11.06	8.47	0.69	9.82	1.54	13.47	0.79	0.32	NA	NA	NA
T. thermalis	the	5.00	24.51	3.29	3.56	2.72	190.80	1.42	0.62	NA	NA	NA
T. triangularis	tre	13.33	8.68	0.87	10.06	2.15	7.06	1.17	0.59	NA	NA	NA
T. sp. nov. cf. triangularis C	tye	16.80	10.96	0.96	8.98	1.91	21.24	0.72	0.32	NA	NA	NA
T. ustulata	ust	16.08	7.07	0.32	15.33	0.17	8.95	1.15	0.66	0.97	0.42	0.009

Notes: See Figure 1.5 for translation of trait codes and indication of what was measured.

Plot 26, Silvermine, Table Mountain National Park.

6 Microhabitat segregation mediates the coexistence of *Tetraria* species within local communities

6.1 Abstract

The coexistence of large numbers of closely-related, morphologically-similar species in local fynbos plant communities is generally believed to be maintained by non-equilibrium mechanisms mediated by differences in species' regeneration strategies and variability in the fire regime. These mechanisms seem inadequate to explain the coexistence of species with similar regeneration strategies, however, and specialization to diverse microhabitats provided by the heterogeneous Cape environments provides a plausible alternative mechanism. Here I examine the morphological traits, gas exchange rates and distributions of 11 *Tetraria* species across a set of sixty 50 m^2 plots on a single mountainside, representing a gradient in soil properties. I test and provide evidence for the four criteria required to demonstrate that stable coexistence is maintained by classical niche differences in soil microhabitat preference. Species showed specialized niche preferences along a gradient of summer soil moisture and nutrient concentration and a gradient of winter soil moisture and soil depth (criterion 1). Species were segregated both in space and niche preferences (criterion 2). Species' positions along the two soil gradients were determined by a trade-off in traits and seasonal gas exchange strategies which influence their tolerance of summer drought and ability to optimize nutrient uptake when soils are wet and nutrients are available (criterion 3). Finally, species displayed reduced body size in the presence of stronger competitors (criterion 4), as inferred from their phylogenetic relatedness. Reduced body size in the presence of close relatives and greater spatial segregation among species within subclades validates the assuption that close relatives will be stronger competitors and provides support for Darwin's 'competition-relatedness hypothesis'. I propose that microhabitat specialization may be a common coexistence mechanism among closely-related fynbos species with conserved regeneration strategies, while coexistence in lineages with diverse regeneration strategies may be maintained by non-equilibrium mechanisms, allowing species to be less specialized in their habitat preferences.

6.2 Introduction

How large numbers of species coexist at fine spatial scales is a long-standing and contentious question in community ecology (Grinnell 1917, Hutchinson 1957, Silvertown 2004). The question becomes increasingly fraught when one considers the coexistence of close relatives, which are typically similar ecologically and morphologically due to phylogenetic constraint, phylogenetic inertia or niche conservatism (Darwin 1859, McKitrick 1993, Wiens *et al.* 2010). Classical niche theory requires that species exhibit distinct differences in resource requirements or resource utilization strategies in order to coexist, and predicts that species that are very similar should compete and exclude each other from the same environments (MacArthur & Levins 1967, Chase & Leibold 2003, Silvertown 2004). By contrast, more recent advances in coexistence theory such as lottery models or the ‘storage effect’ suggest that coexistence among sessile organisms (e.g. plants) is best maintained if species have similar reproductive output and competitive abilities and environmental fluctuations favour the differential recruitment success of species at different times (Chesson & Warner 1981, Shmida & Ellner 1984, Chesson 2000). In this scenario, coexistence is maintained by neutral-like dynamics with weak interspecific interactions slowly driving species to extinction (Leibold & McPeek 2006). These alternative mechanisms thus provide contrasting predictions about the coexistence of close relatives and should be readily testable in communities with many closely-related species (Mayfield & Levine 2010). Classical niche theory predicts niche-segregation, competition and rare co-occurrence of closely-related, functionally similar species, while non-equilibrium models predict broad niche-overlap and common co-occurrence of close relatives.

The Cape fynbos is a hyperdiverse flora composed of many closely-related, morphologically similar species, with over half the approximately 9000 species accounted for by just 33 lineages (Linder 2003). The diversification of the flora is generally believed to have been adaptive, driven by specialization to steep edaphic and climatic gradients, fire regimes and pollinators (Linder 2003). Maintenance of this diversity, on the other hand, and the coexistence of large numbers of species within local communities (high α-diversity), is believed to be facilitated by lottery models or a spatio-temporal storage effect whereby variability in the fire regime favours the differential recruitment of species at different times, preventing any species becoming dominant and competitively excluding others (Cowling 1987, Yeaton & Bond 1991, Bond *et al.* 1992, Kilian & Cowling 1992, Laurie & Cowling 1995, Laurie *et al.* 1997, Cowling *et al.* 1997*a*, Higgins *et al.* 2000*a*, Thuiller *et al.* 2007). There are several reasons to believe, however, that fire-regime driven non-equilibrium models are not the sole mechanism by which the coexistence of close relatives and the rich local communities of fynbos are maintained. Firstly, these models require species to have different post-fire regeneration strategies (Grubb 1977, Chesson & Warner 1981, Angert *et al.* 2009), but the regeneration strategies of close relatives are often similar due to shared ancestry (McKitrick 1993, Wiens *et al.* 2010). Secondly, these models should facilitate the coexistence of any number of species, preventing ecological limitation of lineage diversification rates as observed in Chapters 4 and 5. Finally, they should mask any pattern of trait-environment matching (*sensu* Schimper 1903, Keddy 1992, McGill *et al.* 2006) because most species should occur and overlap over broad areas.

An alternative or complementary coexistence mechanism that may be operating in fynbos communities is segregation of species into different microhabitat niches or subtle differences in the ways in which species use the resources within microhabitats (Linder 2003, Silvertown 2004). The steep rocky mountains in the Cape provide very high environmental diversity in terms of soil depth and texture, drainage, nutrient concentrations and availabilities, shade, slope and aspect; providing ample opportunity for microhabitat segregation among species which co-occur within local communities at the scale of just a few metres (Campbell 1983, Linder 2003, 2005*b*, Araya *et al.* 2010*b*). While microhabitat diversity is commonly cited as important for the evolution and maintenance of fynbos species diversity (Cowling *et al.* 1997*a*, Linder 2003), studies of microhabitat specialization in fynbos are scarce. Recent studies suggest that species may be specialized to subtly different hydrological niches (Araya *et al.* 2010*a*,*b*) or mediate the use of the same microhabitat by partitioning rooting depth or phenology (Richards *et al.* 1995).

The three major components of fynbos environments that are believed to have had a pervasive influence shaping the flora are fire, seasonal drought and soils with low nutrient status (Cowling 1992, Cowling *et al.* 1997*a*, Linder 2003, Mucina & Rutherford 2006). In fynbos, as with many other ecosystems, soil water and the availability of nutrients, particularly nitrogen (N), are often linked and are modified by soil properties such as texture and depth (Campbell 1983, Richards *et al.* 1997, Brady & Weil 2002). This, combined with evidence for links between nutrient uptake and water use efficiency (Patterson *et al.* 1997, Cramer *et al.* 2008, 2009), and for the co-limiting influence of water availability and N on plant growth and community composition (Hooper & Johnson 1999, Harpole *et al.* 2007), suggests that fynbos species may face a trade-off in their drought tolerance and nutrient acquisition and retention strategies. Coexistence among fynbos species may thus be facilitated by differentiated strategies for coping with this drought tolerance - nutrient acquisition trade-off (Chase & Leibold 2003, Everard *et al.* 2010).

Stomatal control of gas exchange allows plants to control their tissue water status (Tardieu & Simonneau 1998). Plant responses to declining soil water availability range in a continuum from keeping their stomata open irrespective of declining soil moisture availability and allowing their tissue moisture status to fluctuate (anisohydry) to reducing stomatal conductance to retain relatively constant tissue water status (isohydry). The trade-off between these two strategies is that anisohydry is more likely to result in mortality due to hydraulic failure or loss of cell turgor pressure while isohydry risks mortality due to carbon starvation if stomata are closed for long periods of time (McDowell *et al.* 2008) or damage to photosynthetic tissues due to reduced transpirational leaf cooling. Isohydric species thus require the ability to maximize nutrient uptake and carbon sequestration when soil water is not limiting and a mechanism to avoid excessive leaf temperatures during times of drought. Reduced leaf size or increased leaf dissection can achieve both these objectives by manipulating boundary layer dynamics to increase sensible heat loss in summer and increase transpiration rates despite low evaporative demand in winter (Nicotra *et al.* 2008, Yates *et al.* 2010). Nicotra *et al.* (2008) found that *Pelargonium* species with highly dissected leaves had higher optimal growth temperatures and higher carbon gain and water loss rates than species with less dissected leaves when grown under the same conditions, while Yates *et al.* (2010) found that Proteaceae leaf size was positively correlated with leaf temperature in low wind environments and negatively correlated with transpiration rates. Yates *et al.* (2010) further suggested a link between stomatal conductance and nutrient uptake in that higher transpiration abilities in the wet season allow enhanced nutrient uptake via mass-flow during the period when nutrients are more readily available. The prediction is thus that as one moves from seasonal environments with short periods of high soil moisture and nutrient availability (e.g. well-drained soils) to more constant soil moisture there should be a grade in preferred strategy from anisohydry to isohydry, with overlap in strategies in intermediate environments. The range of strategies between the two extremes should be reflected by a trade-off in species leaf traits, with large leaves designed to minimize transpiration rates in the absence of stomatal control favouring anisohydry, while leaves designed to maximize transpiration rates and sensible heat loss favour isohydry. Differentiated gas exchange strategies thus provide a potential mechanism mediating species coexistence in fynbos via a drought tolerance - nutrient acquisition trade-off (Chase & Leibold 2003, Everard *et al.* 2010).

Tetraria species are a common component of most fynbos communities with as many as six species co-occurring within an area of only 50m^2 (Slingsby & Verboom 2006). A non-equilibrium coexistence mechanism mediated by variation in the fire regime is unlikely to explain the co-occurrence of multiple *Tetraria* species because they show little differentiation in post-fire regeneration strategy, with all species resprouting rapidly after fire and flowering and setting seed in the first year or two. That cladogenetic events within the group are associated with distinct divergence in morphological traits or shifts in climatic or altitudinal preferences implies that *Tetraria* species are ecologically specialized (Chapter 5), and suggests a role for niche segregation as a mechanism maintaining their coexistence.

Silvertown (2004) derived four criteria from classical competition theory which are critical for demonstrating that niche differences maintain the stable coexistence of species. The tests are based on the notion that for stable coexistence to be maintained a species that has become rare should increase in abundance (the 'invasibility criterion'; Chesson 2000) and require: 1) that intraspecific competition is stronger than interspecific competition; 2) that species are segregated along a resource or environmental niche axis; 3) that there are trade-offs between traits or in performance on niche axes; and 4) that species display niche shifts in response to experimental manipulation of competitors. Here I test these criteria on *Tetraria* communities sampled at two spatial scales on a single mountainside: 50 m^2 plots along a 2 km transect, and 5 m^2 quadrats within 50 m^2 plots. In particular I ask: are *Tetraria* species specialized to particular microhabitats? [Criterion 1]; Are species spatially and environmentally segregated and is this segregation mostly between close relatives? [Criterion2]; Are there trade-offs in species leaf traits relating to soil water availability, seasonal water use and nutrient uptake strategies? [Criterion 3]; Is species' performance reduced in the presence of close relatives? [Criterion 4]

6.3 Methods

6.3.1 Survey and environmental data

The study was performed on the slopes of Wolfkop and Higher Steenberg Peak in the eastern portion of the Silvermine section of Table Mountain National Park on the Cape Peninsula (34.09 S, 18.43 E). The entire study falls within 'mesic oligotrophic proteoid fynbos', one of fifteen plant communities described for the Cape Peninsula in Cowling *et al.* (1996*a*), and occurs on Clovelly soils derived from Peninsula Formation sandstone with an annual rainfall of approximately 900 mm. Sixty 5 by 10 m plots, each divided into ten 2 x 2.5 m quadrats, were placed at ± 70 m intervals along a transect (Appendix 6.1). Vegetation that had burnt within the previous year or had signs of past disturbance by human impacts or alien species was avoided, but otherwise there were no selection criteria for the placement of plots, including the presence or absence of *Tetraria* species. Plots were placed such that their long edge followed the horizontal contour, and the corners were marked with steel fencing droppers.

For each individual of all *Tetraria* species in all plots the species specific size class (Appendix 6.2) and exact geographic location, using the NW corner as the origin, was recorded. Vegetation age was extracted from a fire history GIS of the Cape Peninsula provided by South African National Parks and verified using node counts of live *Leucadendron laureolum* individuals (number of nodes passed from root to apical meristem - 1) for each plot. Node counts are a reliable method of estimating vegetation age to within three years as many Proteaceae form a new node each year after flowering (Rebelo 2001). For each of the 2 x 2.5 m quadrats within each plot, the proportions of rock cover and bare ground (% surface area), and soil depth (measured by sinking a metal spike in the corners of each quadrat) were recorded. Seasonal soil moisture availability was monitored four times throughout the year by measuring gravimetric soil moisture content from 0.03 m diameter soil cores collected from each of the four corners and centre of each plot and taken to the maximum depth of up to 0.3 m. One 0.3 m deep, 0.05 m diameter soil core was collected from each of the four corners and the from centre of each plot for nutrient and texture analysis. Each soil core was analysed for pH, Bray II P (plant available P), percentage nitrogen (%N) and soil texture. Phosphorus was extracted using the Bray II method described in Benton-Jones (2001) using the malachite green colorometric reagent because P levels in the soils were very low. Concentrations were measured with a Multiskan spectrum V1.2 spectrophotometer using a 24-well plate and a calibration curve with standards ranging from 0 to 10 μM phosphate. Soils were suspended in 1 M $CaCl_2$ solution and pH measured using a hand held probe (Hanna instruments The Non-Affiliated Soil Analysis Work Committee 1990). Percentage N, %C and δ^{13}C were measured using a mass spectrometry (See 6.3.4 for details). Soil texture was measured by sifting each air-dried soil sample into six fractions after removing all particles greater than 2 mm, weighing each fraction, and expressing each fraction as a proportion of the total sample. The fractions were <20 μm, 20-53 μm, 53-106 μm, 106-250 μm, 250-500 μm and 500-2000 μm, following the standard protocol of the Soil Science Society of South Africa (The Non-Affiliated Soil Analysis Work Committee

1990). Silt and clay fractions were not determined because the fractions $<53\ \mu$m typically made up less than 0.5% of the sample. Only the combined medium and course sand fraction (250-2000 μm) was used for further analysis because it was negatively proportional to all finer fractions.

6.3.2 Trait selection and measurement

A set of quantitative leaf traits which have been reported to convey utility for temperature regulation, water use and nutrient retention and are easily measured were selected, including maximum leaf length, maximum leaf width, specific leaf area (SLA), leaf dry matter content, stomatal density and percentage leaf nitrogen. Leaf length determines leaf canopy height in *Tetraria* (R^2=0.99 for 37 species, Chapter 5), because leaves emerge from basal corms. Leaf canopy height determines light interception properties and affects competitive ability and shade tolerance (Falster & Westoby 2005*b*), and can be linked to temperature and moisture regulation in fynbos environments through exposure to the cooling effects of wind (Yates *et al.* 2010) and the possible interception of moisture from cloud condensation (Marloth 1904). Leaf width impacts on temperature regulation and transpiration through its influence on boundary layer thickness, with narrower leaves allowing higher sensible heat loss in low wind environments and facilitating higher transpiration rates (Yates *et al.* 2010). Specific leaf area, leaf dry matter content and percentage leaf nitrogen form part of a suite of leaf traits that are considered important for water use strategy, photosynthetic rates, leaf structural integrity and orientation, phenology, leaf turnover and decomposition rates (Westoby *et al.* 2002, Wright *et al.* 2004, Duru *et al.* 2009, Marino *et al.* 2010). Stomata and stomatal densities are responsible for gas exchange and leaf temperature regulation and thus control water use efficiency, the balance between water loss and carbon gain (Tardieu & Simonneau 1998, Xu & Zhou 2008, Franks & Beerling 2009). Stomatal densities have also been found to scale allometrically with SLA and other leaf traits (Loranger & Shipley 2010).

Leaf traits were measured from three fully expanded fresh leaves from each of 15 individuals per species. Five individuals were sampled from each of three sites for each species. Sites were selected to encompass as much of the variation in soil moisture and soil depth as the species encounters across the set of plots as possible. Nine leaves were collected for each individual, three for determining each of % leaf N and stomatal density and three for determining leaf length, leaf width, SLA and leaf dry matter content, and placed in zip-locked bags with damp tissue paper. To quantify SLA, the single-sided surface area of each leaf was measured with an LI-3000 Area Meter (LI-COR). These leaves were then weighed fresh (saturated), dried at 70°C for 48 h and re-weighed. Specific leaf area was calculated as the surface area of a single side of a leaf divided by the dry weight of the leaf and expressed in m^2 kg^{-1} (Cornelissen *et al.* 2003). Leaf dry matter content was calculated as the oven-dry mass divided by the water-saturated fresh mass and expressed in mg g^{-1} (Garnier *et al.* 2001). Stomatal densities were determined by examining impressions of the epidermis of the leaf, made by painting and peeling clear nail varnish on the upper surface of the middle of the leaf, counting the number of stomata visible in the field of view of a compound microscope set at 400x magnification and expressed per m^2. None of the species examined had stomata on the undersides of their leaves and there was no consistent variation in stomatal density along the length or width of leaves. Percentage leaf N was determined from mass spectrometry performed on one sample of three leaves for each individual (see 6.3.4 below) and expressed as the %N per dry mass.

6.3.3 Gas analysis

Rates of photosynthesis and transpiration were measured using a LI-6400 portable infra-red gas analysis system (LI-COR, Lincoln, NE, USA) on the same five individuals of each of the 11 species on a clear, cloudless day once in the dry summer and once in the wet winter. These measures were used to calculate an estimate of water use efficiency (WUE) for each season. Gas exchange of the youngest fully expanded leaves of each plant was measured with the blue-red light-emitting diodebank of the LI-COR LI-6400-02B light source emitting 1500 μmol quanta m^{-2} s^{-1}. Measurements were made with cuvette conditions set to match the air temperature of the day of measurement (20°C in winter and 25°C in summer) and 400 ppm CO_2. Leaves were then detached after each measurement and the leaf area enclosed in the cuvette measured with an LI-3000 Area Meter (LI-COR) so that measurements could be adjusted for the correct leaf area. Leaf samples collected from each individual in summer and in winter were analysed for $\delta^{13}C$ using mass spectrometry.

6.3.4 Mass spectrometer isotope analyses

All leaf samples were dried in an oven at 70°C for 48 h and milled in a ball mill and all soil samples were air dried and passed through a 2 mm sieve prior to mass spectrometer analysis. Mass spectrometer analysis was performed by weighing c.40 mg of sample into tin capsules (Elemental Microanalysis Ltd., Devon, UK), combusting them in a Thermo Flash EA 1112 series elemental analyser and feeding the gases into a Delta Plus XP isotope ratio mass spectrometer (Thermo Electron Corporation, Milan, Italy). Carbon isotope ratios of each sample were expressed relative to the Pee Dee Belemnite standard (Ehleringer & Rundel 1989).

6.3.5 Testing for spatial segregation

Species segregation was assessed using a null model analysis on the presence-absence and abundance data matrices for the plots using the program *CoOccurrence* (Ulrich 2006). The use of abundance data allows detection of subtle effects of competition such as consistent dominance hierarchies (Ulrich & Gotelli 2010). Presence-absence analysis was performed by evaluating the C-score (Stone & Roberts 1990) for the observed data matrix against a distribution of 999 null values generated using an independent swap algorithm (Gotelli 2000). The C-score metric counts the number of occasions in the data matrix that a pair of species in two plots is segregated such that they do not co-occur ('checkerboard units'), while the independent swap null model retains as much structure in the data matrix as possible by maintaining both the frequencies with which species occur across plots and the species richness within each plot (Gotelli 2000). Gotelli (2000) found that of a set of four co-occurrence indices and nine null models the C-score combined with the independent swap algorithm provided the lowest Type-I error rates and good power to detect community structure.

Segregation was evaluated for the abundance data matrix using the *CA* metric and *IT* null model of Ulrich & Gotelli (2010). The *CA* metric is an expansion of the C-score which counts 'abundance checkerboard units where two species are numerically dominant in each of a pair of plots in turn. The *IT* null model employs a 'floating zero' individual-based algorithm which randomly assigns individuals to plots and species with probabilities proportional to the row and column abundance totals in the original data matrix, stopping when the original row and column totals are reached. It thus retains the original row, column and matrix-wide total abundances but does not preserve species occurrences and species richness. Ulrich & Gotelli (2010) found that of a set of six co-occurrence metrics and 14 null models the *CA* metric and *IT* null model had the lowest Type-I error rates and good power to detect community structure.

To test the sensitivity of the results of both analyses to the presence or absence of any particular species in the dataset, each analysis was rerun, dropping each species in turn. To test whether spatial segregation was predominantly between species within each of the three major clades (Chapter 4), as opposed to between clades, additional analyses were done in which null model randomization was only permitted among species within each clade. Finally, within-plot segregation among species was tested by comparing the average within-plot near-neighbour distances to conspecific versus heterospecific individuals across all plots using a Wilcoxon matched pairs test (Crawley 2007). Edge effects were not excluded because the majority of individuals were closer to the edge of the plot than to another individual. Including edge effects typically biases near-neighbour distances to be greater than if edge effects were excluded (Crawley 2007), but there is no reason to believe that this bias would be any different between conspecific and heterospecific comparisons.

6.3.6 Testing for microhabitat specialization and niche segregation

Microhabitat specialization was tested for using the Outlying Mean Index (OMI) multivariate niche analysis method of Doledec *et al.* (2000). This method uses a principal component analysis (PCA) ordination of plots based on their environmental variables and infers each species' preferred habitat conditions by estimating its position in ordination space based on presence in plots and weighted by relative abundance. The method assesses habitat specialization by calculating the distances (OMI) between the mean habitat conditions for each species (species centroid) and a hypothetical generalist species that occurs in all habitat conditions with uniform abundance, represented by the origin of the ordination. Advantages of the OMI method are that it makes no assumptions about the shape of the species response curves (they can be linear or unimodal), all sites are weighted equally, and it describes the mean position of species relative to mean habitat conditions (i.e. indicating the degree of habitat specialization). Significance was assessed by ranking the observed OMI for each species relative to a distribution of 999 null OMI values generated by randomizing species presence in plots, retaining their frequency of occurrence and abundance, and recalculating the OMI at each permutation. The analysis was performed on the full set of eight measured environmental variables and on a subset of four soil variables which are of primary interest in relation to seasonal water use and nutrient uptake strategies: soil depth, %N and summer and winter soil moisture. The analysis was performed in R 2.11.0 (R Development Core Team 2010) using the *ade4* package (Dray & Dufour 2007).

Segregation of microhabitats between species was further tested using multiple-response permutation procedure (MRPP) tests (Mielke *et al.* 1976, Wong *et al.* 1982). MRPP is a distribution-free inferential technique for detecting differences amongst classified groups of objects in multi-dimensional space. The test statistic, δ, is based on the distances between data points, and the permutation procedure evaluates whether data points show significantly greater clustering within as opposed to between the a priori classified groups (species) than is expected by chance. Tests were based on the PCA axes using the full set of environmental data and were performed at two scales, firstly grouping plots by species occurrence, and secondly grouping quadrats by species occurrence. Tests were performed for all species together and by species pair using the R package *vegan* (Oksanen *et al.* 2010).

Microhabitat segregation among species was also assessed at the quadrat level for each of the four soil variables of primary interest (soil depth, %N and summer and winter soil moisture) using a multiple comparison test between treatments in a Kruskal-Wallis test (Siegel & Castellan 1988) implemented in the R package *pgirmess* (Giraudoux 2010). A Bonferroni correction was applied resulting in an α cut-off of 0.0009 for the 55 pairwise comparisons for each soil variable. Additional Wilcoxon tests were performed between individuals from species pairs that co-occurred within three or more plots using quadrat data from those plots. This was done to circumvent any spatial autocorrelation issues associated with the other niche segregation tests and represent very conservative tests because they test for significant differences in the areas in which species' niches will be most similar.

6.3.7 Testing for trade-offs in traits or performance

Trait utility was investigated using correlative approaches to compare species traits to habitat variables, gas exchange variables and carbon isotopes. Two approaches were adopted. Firstly, community weighted mean trait values (CWM; Garnier *et al.* 2004) for the 53 plots with two or more species were compared with the mean soil variables for each of the plots using a generalized least squares (GLS) and a correlative approach. Secondly, the relationships between species' trait means, mean habitat conditions, seasonal gas exchange measurements and isotope values were investigated using ahistorical correlations and a phylogenetically independent contrast approach based on maximum likelihood (Felsenstein 1985). All analyses were performed in R 2.11.0 (R Development Core Team 2010) using the *ape* (Paradis *et al.* 2004), *car* (Fox & Weisberg 2010), *nlme* (Pinheiro *et al.* 2009) and *picante* (Kembel *et al.* 2010) packages.

The GLS analysis was preferred over more conventional regression techniques because it allows one to account for non-constant variance and spatial autocorrelation between sites. The analysis was performed using an eight step approach: 1) The plot-level CWM values for each trait were fitted with a complete model including all environmental variables without interacting effects. 2) A stepwise selection process based on Akaike's Information Criterion (AIC; Akaike 1974) was used to reduce the number of variables in the model. 3) A power variance function was fit to estimate and account for non-constant variance in the model (Pinheiro & Bates 2000). 4) A first order autoregressive correlation structure was applied to account for spatial autocorrelation between plots. This correlation structure was preferred over others by assessing autocorrelation in the model residuals (Pinheiro & Bates 2000). 5) A second stepwise selection process using AIC was used to eliminate variables whose contribution was negligible once non-constant variance and spatial autocorrelation were accounted for. 6) The significance of the contribution of each variable was tested by performing an ANOVA on the model. The ANOVA was applied to the marginal contributions of the model so that the P-value for each variable does not depend on the order of the variables in the model. 7) The overall fit of the model was assessed by regressing the observed CWM values against those predicted by the model. This approach was preferred over calculating an R^2 value for the model directly because R^2 values from models that include covariance among residuals are not comparable to R^2 values from ordinary least squares (OLS) models and can be easily misinterpreted (Lavin *et al.* 2008). 8) Finally, the assumption of normality in the model residuals was tested using Shapiro's test. Only leaf width required transformation (square-root) to satisfy the assumption of normality in model residuals. Interacting effects were not investigated because the analysis would become unstable, likely due to low sample size.

6.3.8 Testing for reduced performance in the presence of competitors

To test for evidence of reduced performance in the presence of competitors (criterion four) from natural variation in the occurrence of competing species I examined variation in the average size of individuals in species mixtures of varying phylogenetic relatedness across the plots. Unfortunately this criterion could not be tested experimentally because *Tetraria* individuals are long-lived and community dynamics are slow, with generation times in the order of decades or even centuries, making it unlikely that niche shifts would be observed within the time-frame of the research project. The approach adopted here is contingent on the assumption that close relatives are stronger competitors because they will be more similar ecologically due to shared ancestry (Darwin 1859, Webb *et al.* 2002). An average size-class across all individuals of all species in each plot was calculated by converting the species-specific size-class (small, medium, large) of each individual (Appendix 6.2) into a continuous variable (1, 2, 3) and taking the mean across all individuals. The use of species-specific size classes standardizes each species and excludes any influence of differences in species' actual plant sizes. These plot size-class averages were regressed against two measures of the phylogenetic relatedness among the species in each plot; the standardized effect size of the mean nearest taxon distance (sesMNTD) and the standardized effect size of the mean phylogenetic distance (sesMPD), as calculated by shuffling species identity on the phylogeny 999 times (Webb *et al.* 2002). Using the standardized effect size of the phylogenetic relatedness measures accounts for most, but not all, of their dependence on species number (Webb *et al.* 2002; Chapter 3). Results were qualitatively unchanged when based on a non-parametric rank-order standardization of MNTD and MPD.

6.4 Results

6.4.1 Spatial segregation

Eleven *Tetraria* species were recorded in the 60 plots surveyed. Three plots contained no *Tetraria* species and four contained only one species. Most of the remaining 53 plots contained two or three species, with a maximum of six occurring in two plots. The null model tests on the presence-absence and abundance community data matrices indicated that there was far greater spatial segregation among species than would be expected by chance (Table 6.1). These results were unchanged when each of the individual species was removed from the analysis in turn indicating that they are not dependent on segregation among particular species pairs. Similarly, constraining null model randomization to among species within each of the three major clades had little effect on the results, indicating that much of the spatial segregation within the dataset is between closely related species. This result was unchanged if *T. flexuosa* (fle) and *T. ustulata* (ust) were treated as a fourth clade, separate from the rest of the species in the *fasciata-flexuosa* clade (data not shown). Heterospecific near neighbour distances within plots were greater than between conspecifics, this difference being near-significant (W=255, P=0.058).

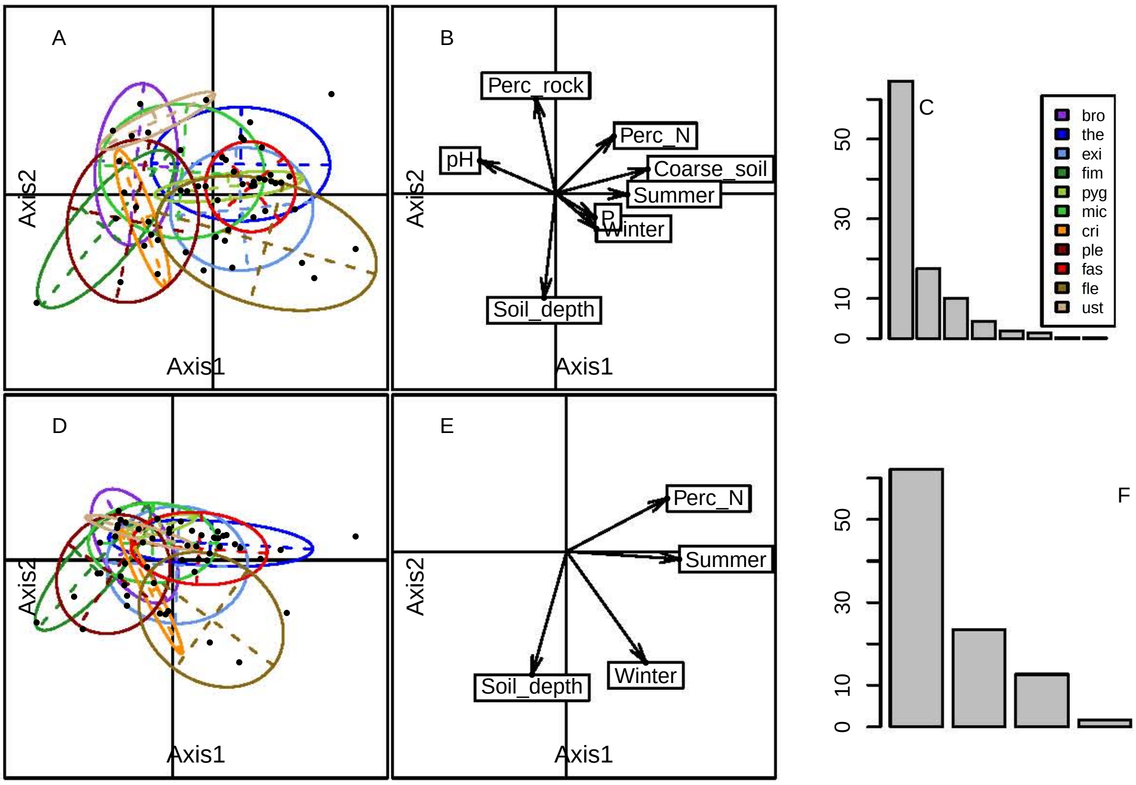


Figure 6.1: Results of the Outlying Mean Index niche specialization analysis. Coloured elipses indicate the distribution of species in niche space (A, D). Bar plots indicate the % of the variance in soil variables summarized in each axis. Arrows indicate the relative influence of each soil variable on each axis (B, E). Species three letter codes are indicated. Full species names are given in Appendix 5.1. Perc_rock: % rock cover, Perc_N: % soil N, Summer: summer soil moisture, Winter: winter soil moisture, Coarse_soil: fraction of soil 250-2000 μm.

6.4.2 Microhabitat specialization and niche segregation

The niche specialization analysis (OMI; Doledec *et al.* 2000) segregated species along two major niche axes (Figure 6.1). The first axis explained 66% of the variance and reflected predominantly a gradient of soil texture, summer soil moisture and pH with a weaker influence of soil %N, [P] and winter soil moisture. The second axis explained 18% of the variance and reflected variation in soil depth and rockiness with a weak influence of soil %N, [P] and winter soil moisture. A third axis, representing 10% of the variation, had weak loadings for most variables and was not included in any significance tests due to poor interpretability. Most species pairs (sisters in the context of the local species sample) within each major clade displayed distinct separation in habitat preference along one or both axes.

Tetraria thermalis (the) and *T. eximia* (exi) were separated along an axis of soil depth, both preferring higher nutrient, coarser soils with higher summer soil moisture than the related *T. bromoides* (bro). *Tetraria pleiosticha* (ple) and *T. fasciata* (fas), on the other hand, were segregated along the first axis, but *T. pleiosticha* (ple) displayed substantial overlap with the related *T. crinifolia* (cri). *Tetraria flexuosa* (fle) and *T. ustulata* (ust) were segregated along both axes while *T. fimbriolata* (fim), *T. microstachys* (mic) and *T. pygmaea* (pyg) displayed subtle differences in preferred summer soil moisture, soil texture and % soil N.

The OMI analysis based on all eight soil variables indicates that species are specialized to specific environmental conditions, with nine of the 11 species occurring in conditions significantly different to the average (Figure 6.1, Table 6.2). The two species that did not indicate significant habitat specialization (*T. pygmaea* and *T. ustulata*) were rare in the dataset, occurring in only three plots each, suggesting that the power of the statistical test may be an issue in this regard. An OMI analysis based on the reduced set of soil variables produced similar results with six of the 11 species displaying significant specialization along two axes, the first representing summer soil moisture and % soil N while the second represented soil depth and winter soil moisture.

The MRPP analysis revealed highly significant differences between species' positions on the first two OMI niche axes at both the plot and quadrat scales ($P<0.001$ for 999 null model iterations). Thirty-seven of the 55 pairwise species comparisons at the plot scale revealed significantly different environmental niche preferences based on the OMI analysis of eight soil variables (Table 6.3). This number increased to 46 of the 55 when comparisons were done at the quadrat scale. Eight of the non-significant plot level comparisons contained one of the two rare species, once again suggesting that poor statistical power is an issue.

Comparison of soil depth, % soil N and winter and summer soil moisture between the quadrats in which species occurred revealed significant differences along one or more of these gradients for 40 of the 55 species pairs at the α=0.0009 level (Figure 6.2), eight of the 15 non-significant comparisons involving one or both of the rare species. Comparisons between the 25 pairs of species that co-occurred in more than two plots based on quadrat data from those plots revealed significant differences between 18 pairs at the α=0.05 level and 21 pairs at the α=0.1 level on one or more gradients (Table 6.3).

In summary, all species pairs except *T. bromoides - T. ustulata* (bro-ust) showed significant differences in preferred niche space or position along gradients of soil depth, % soil N or summer or winter soil moisture either between plots or between quadrats within

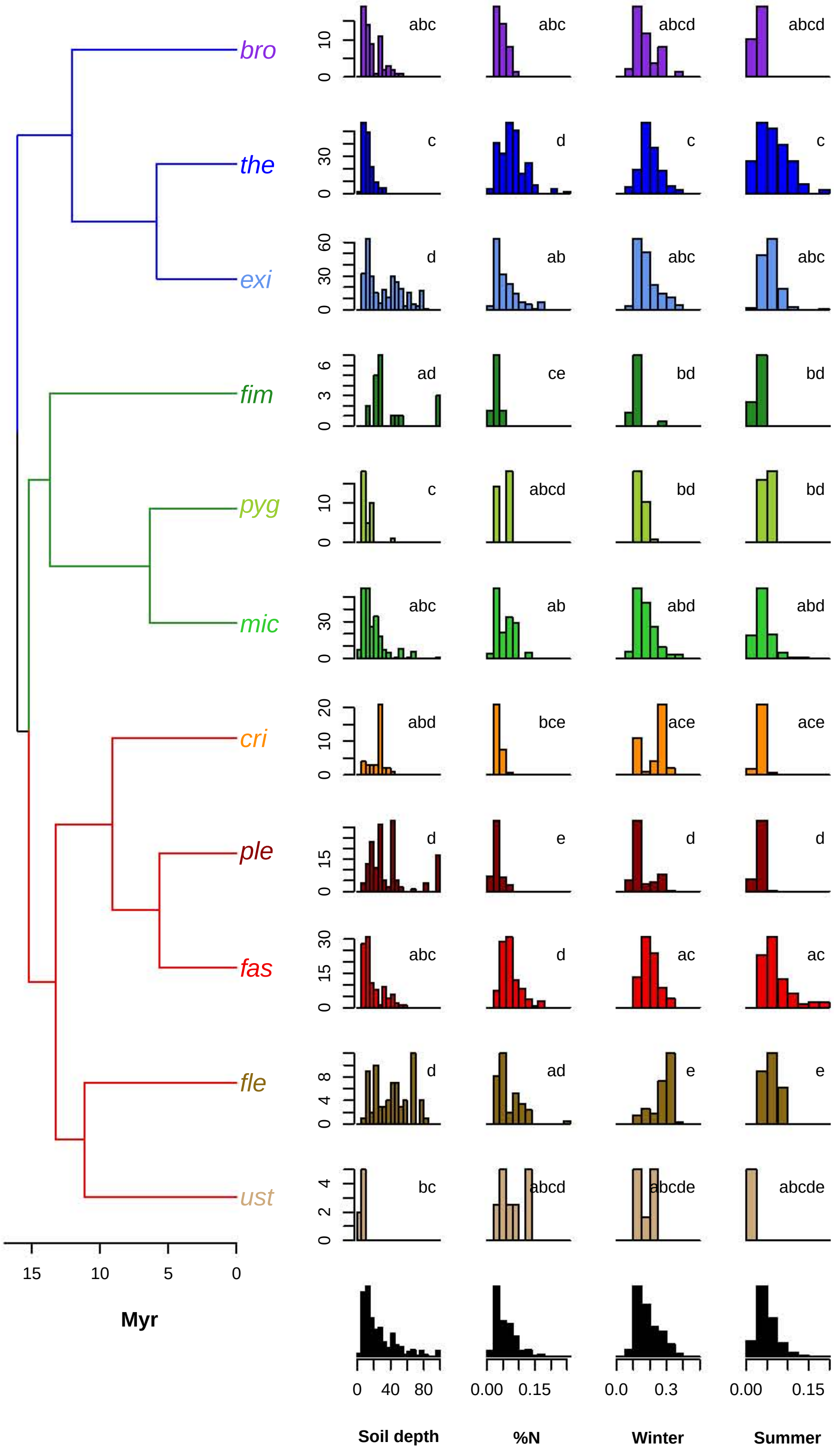


Figure 6.2: Phylogeny (see Chapter 4) indicating the relationships between species and microhabitat segregation among species in soil depth (cm), % soil N and summer and winter soil moisture (grams water / grams dry soil) preferences (histograms in columns). Tests were performed using a multiple comparisons test between treatments in a Kruskal-Wallis test on all quadrat data for each species. α=0.0009.

plots in which both species occur.

6.4.3 Trade-offs in traits and performance

Most of the variation in leaf traits was between, as opposed to within, species (Figure 6.3). Species with longer leaves had lower SLA values while species with wider leaves had lower leaf dry matter contents. The three major clades were segregated predominantly along an axis dominated by these four leaf traits which can be viewed as variation in leaf size while species within clades were separated by variation in % leaf N, stomatal densities and SLA.

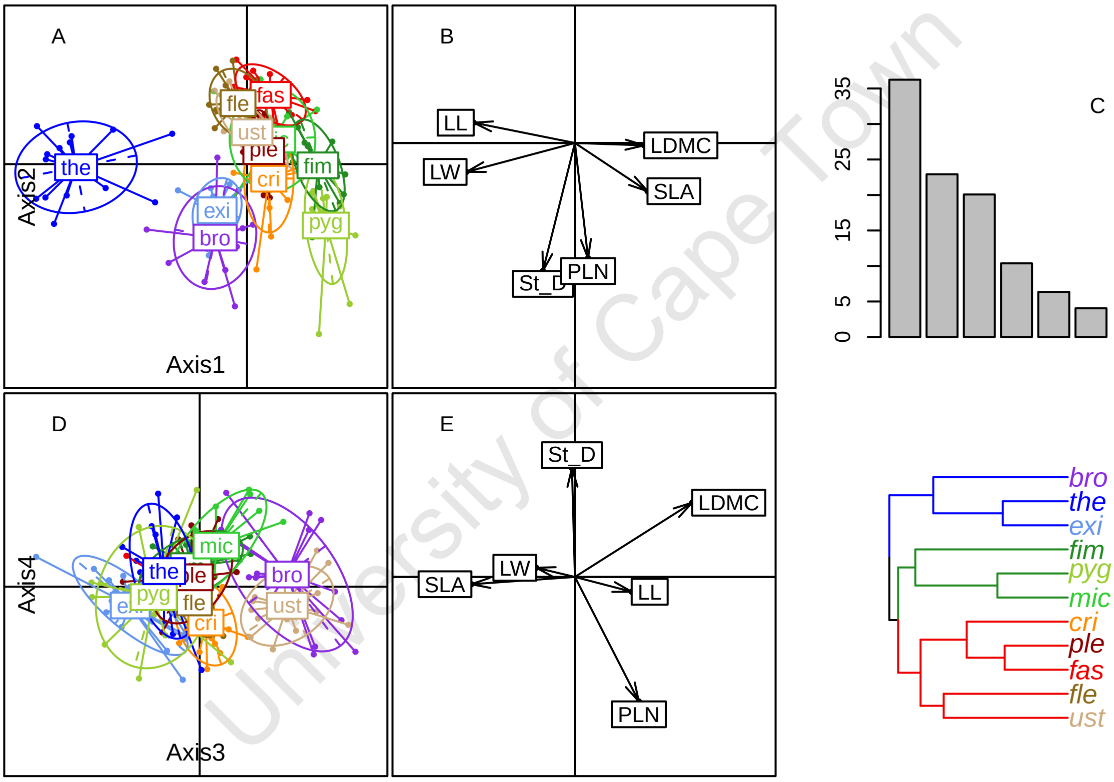


Figure 6.3: Principal components analysis indicating intraspecific variation in leaf trait measurements (A, D) and covariation between traits (B, D). Traits include maximum leaf length (LL), maximum leaf width (LW), specific leaf area (SLA), leaf dry matter content (LDMC), stomatal density (St_D) and percentage leaf nitrogen (PLN).

The GLS analysis identified models which provided reasonable predictions of CWM values for leaf length and width, SLA and leaf dry matter content across the plots once spatial autocorrelation and non-constant variance had been accounted for (Table 6.4). Leaf length, leaf width and SLA all showed strong association with soil depth and one of either

% soil N or summer soil moisture content, implying that these soil variables are important in determining species occurrence. Leaf dry matter content was further dependent on soil texture, which is related to % soil N, while stomatal density was weakly predicted by pH.

Ahistorical and PIC correlations based on species mean habitat conditions and trait values revealed only weak correlations, likely due to low sample size (n=11), and accounting for phylogenetic non-independence made little qualitative difference to the results (Table 6.5). Leaf length was significantly correlated with higher % rock cover and OMI axis 2 and weakly correlated with decreasing soil depth. SLA showed the inverse trend, being negatively correlated with % rock cover and OMI axis 2, but showed stronger negative correlation with winter soil moisture. Winter soil moisture was only weakly related to summer soil moisture and negatively correlated with increasing soil depth and reduced rock cover (data not shown) and is likely an indication of well drained soils subject to shorter periods of high soil moisture. Leaf dry matter content was negatively correlated with OMI axis 1, summer soil moisture and P, and weakly correlated with %N and pH. Percentage leaf N was weakly negatively correlated with soil %N, summer soil moisture and P. The lack of significant correlations for leaf width is likely because of an order of magnitude difference in values between *T. thermalis*, *T. eximia* and *T. bromoides* and the other eight species. Transformation of the data had little effect in reducing this difference.

Investigation of seasonal gas analysis revealed that species occurring on soils with high summer soil moisture had higher winter water use efficiency and a lower ratio of summer to winter water use efficiency (Table 6.6). This implies that plants that have access to higher soil moisture and can maintain gas exchange in summer need not transpire faster in winter. Species occurring on soils with higher %N also maintained higher summer water use efficiency, high winter photosynthesis and transpiration rates and a lower ratio of summer to winter photosynthesis rates.

Species with longer, wider leaves and lower SLA maintained higher water use efficiencies in winter (Table 6.6). SLA was also negatively correlated with summer photosynthesis and transpiration rates and positively correlated with winter transpiration rates and the ratio of summer to winter water use efficiency, implying that high SLA species mostly shut down in summer and operate at much higher rates in winter. Species with higher % leaf N also had higher transpiration rates and lower water use efficiencies in winter.

Summer and winter δ^{13}C values were highly correlated with each other and showed no correlation with any of the instantaneous seasonal gas exchange measurements (data not shown). Carbon isotope ratios are an integrated measure of WUE at the time of tissue expansion (Farquhar *et al.* 1989, Dawson *et al.* 2002) and should provide a good indication of species' water use strategies. As such isotope values showed no correlation with soil variables but were positively and negatively associated with leaf length and SLA respectively (Table 6.7).

6.4.4 Reduced performance in the presence of competitors

There was a weak but significant increase in the average size class of individuals of all species in plots occupied by more distant relatives, as measured using sesMNTD (Figure 6.4). This result remained significant when plots with the maximum or minimum average size class, or with the minimum sesMNTD were removed from the analysis, implying that it is not an artefact of extreme outlying values. The relationship was near-significant when sesMPD was used to measure phylogenetic relatedness (P=0.06).

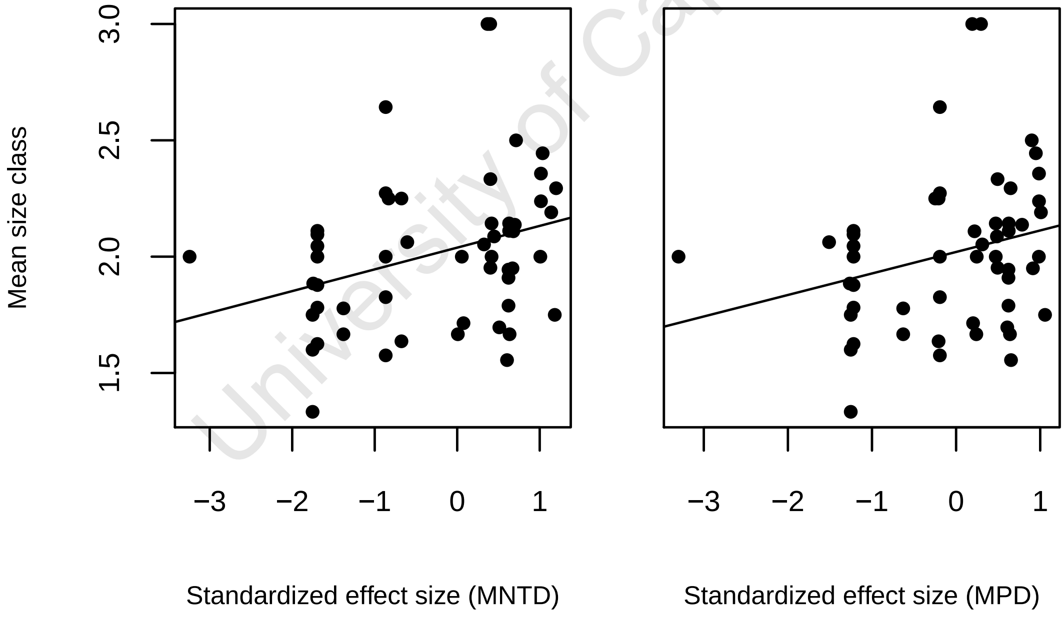


Figure 6.4: Reduced body size in the presence of close relatives indicating reduced performance in the presence of stronger competitors. The standardized effect size of the mean nearest taxon distance (sesMNTD): R^2=0.097, P<0.05, df=51, the standardized effect size of the mean pairwise distance (sesMPD): R^2=0.066, P=0.06, df=51.

6.5 Discussion

While the study of habitat specialization has a long tradition in fynbos ecology and systematics (Rourke 1972, Williams 1972, Linder & Ellis 1990, Linder & Vlok 1991, Richards *et al.* 1997, Shane *et al.* 2008), these studies typically explore pattern at large spatial scales and describe broad habitat differences relating to soil type or climate. Few studies have explored habitat specialization in fynbos at fine spatial scales (but see Richards *et al.* 1995, Araya *et al.* 2010*b*), and none have demonstrated stable fine-scale coexistence in local communities. Here I present evidence to show that the four criteria required to demonstrate stable coexistence maintained via classical niche segregation (niche specialization, niche or spatial segregation, trade-off in traits or in performance on niche axes, and niche shifts or reduced performance in the presence of competitors) hold for a set of 11 *Tetraria* species occurring on a single fynbos mountainside.

Species' habitat preferences differed significantly from the average soil conditions across the set of plots and species showed significant segregation in space and along soil resource axes (criteria 1 and 2). Species are thus specialized to a different niches in which they are competitively dominant. This implies that the population of each species is regulated by density-dependent negative intraspecific interactions within their preferred habitats, rather than by interspecific competition, and provides a mechanism whereby species will be able to increase from low abundance even in the presence of interspecific competitors (the 'invasibility criterion'; Chesson 2000). Niche specialization and segregation has been demonstrated for plant communities from across the globe (see Silvertown 2004 for review) and for species in the Proteaceae (Richards *et al.* 1995) and Restionaceae (Araya *et al.* 2010*b*) within fynbos.

Trade-offs in species' traits and seasonal gas exchange strategies determine their positions on two soil niche axes (criterion 3). Firstly, species were segregated along an axis of nutrient and summer soil water availability with wetter, higher nutrient sites dominated by species whose leaves are broader and have a low leaf dry matter content (Figure 6.5). Larger, wider leaves are likely to convey a competitive advantage because *Tetraria* species with larger leaves have larger canopies (Chapter 5) and are larger in stature (Falster & Westoby 2005*b*). The trade-off of larger, wider leaves, however, is that it restricts these species to soils with high summer moisture because wider leaves have thicker boundary layers, reducing sensible heat loss and increasing the need for transpirational cooling to prevent damage to photosynthetic tissues (Yates *et al.* 2010). These species thus need to adopt an anisohydric gas exchange strategy, maintaining their transpiration rates throughout the dry season. By contrast, narrow leaves allow for improved sensible heat loss and is advantageous in environments in which low summer soil moisture restricts transpirational cooling, favoring isohydric species that can reduce their gas exchange rates (Yates *et al.* 2010). Narrow leaves also tend to have high leaf dry matter content which is associated with nutrient retention and longer leaf lifespan and should be advantageous in these low nutrient environments (Garnier *et al.* 2004). The relationship between large leaf size and anisohydry provides a potential explanation for the dominance of large-leaved *Tetraria* species from the *thermalis-bromoides* clade in the less seasonal eastern CFR. Note that I adopt the use of the terms ‘isohydry’ and ‘anisohydry’ in the absence of leaf water potential data under the assumption that the gas exchange strategies described here have implications for tissue water status.

Variation in species’ position on the isohydry-anisohydry continuum further depends on soil depth and moisture availability in winter (the second major soil niche axis). This axis is largely independent of the first because summer and winter soil moisture readings were only weakly correlated. Nutrients in fynbos environments are most available in winter when soils are saturated (Richards *et al.* 1997). Deeper soils with lower moisture content in winter are likely to retain moisture for a shorter period of time and require plants to be capable of maximizing their transpiration rates and uptake of water and nutrients over the short period of time that they are readily available. Deeper soils which had low winter soil moisture content supported species with short, high-SLA leaves, traits which are typically associated with wet conditions, rapid transpiration and low water use efficiency (Geller & Smith 1982, Wright *et al.* 1993, Monclus *et al.* 2006). This implies that these plants take up water and nutrients rapidly for the short period that water is available, likely coming at a cost of increased risk of loss of turgor pressure and drought mortality during the dry season, favoring isohydric species which can reduce their gas exchange rates. Together these trade-offs interact to result in species that occur on soils which are wetter in summer and/or have wider leaves to have higher winter water use efficiencies and a lower ratio of summer to winter water use efficiency. Accordingly, species which occur on dryer winter soils and have higher SLA have poor winter water use efficiencies and a higher summer to winter ratio.

Araya *et al.* (2010*b*) demonstrated fine-scale niche segregation among Restionaceae species in multiple fynbos communities along similar environmental axes. They explored two components of the soil moisture gradient that are important for plant growth, an axis

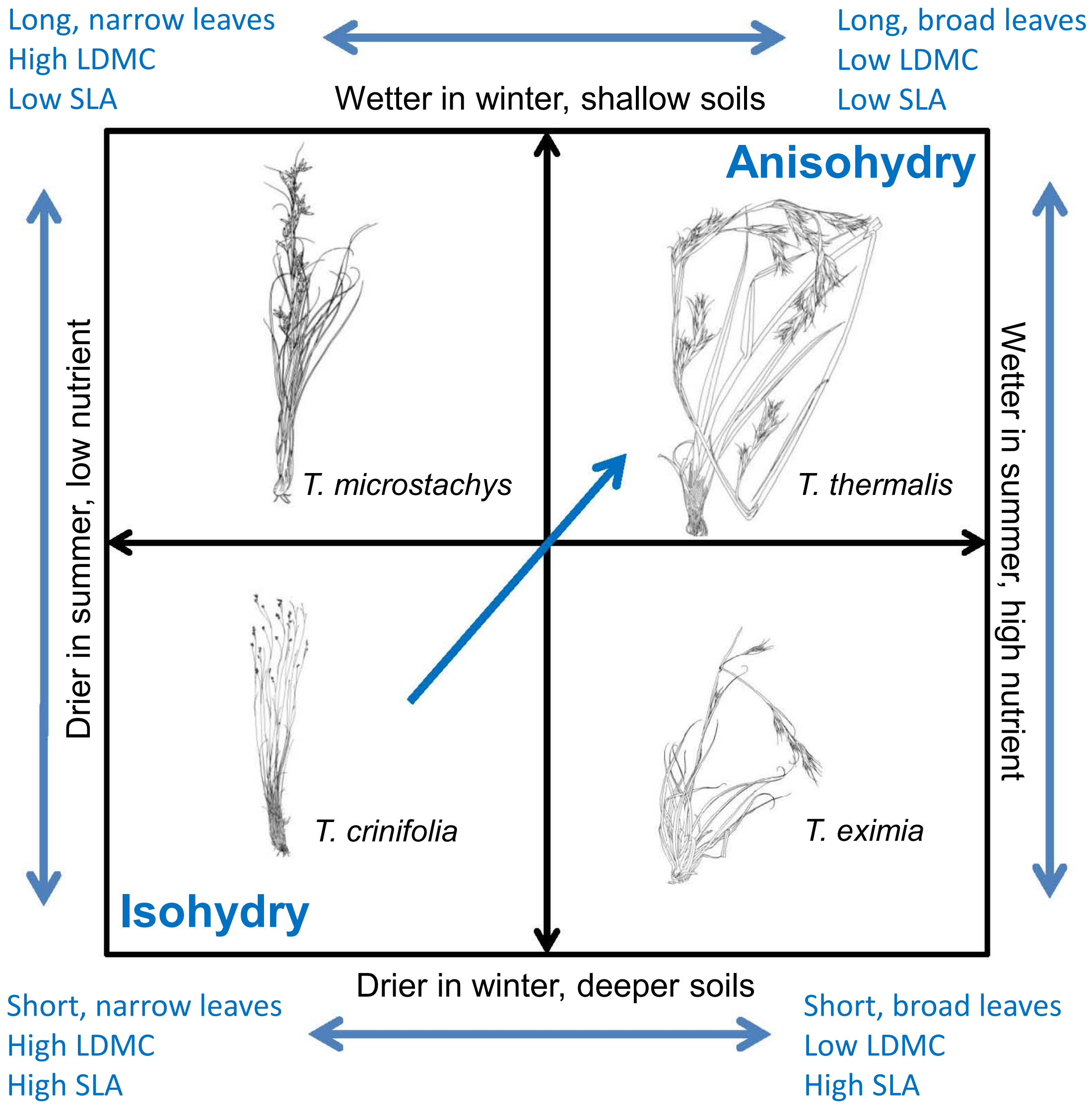


Figure 6.5: Summary diagram indicating the shift from isohydric to anisohydric gas exchange strategies and associated traits along the two major niche axes indicated in Figure 6.1. Habitat information is indicated in black while trait information is indicated in blue. Habit diagrams indicate species representative of each trait combination. LDMC: leaf dry matter content. Artwork: G. A. Verboom.

of summer drying stress and an axis of winter water-logging stress, and demonstrated that there is a trade-off in species' tolerance along each axis. Araya *et al.* (2010*a*) attempted to identify a mechanism behind this trade-off, but found that while plant WUE, as measured using δ^{13}C isotopes, differed between species it did not correlate with soil moisture status and suggested that plant phenology may be responsible for breaking down this expected relationship. While my method of soil moisture measurement differs from their studies, I have reproduced these results by showing that *Tetraria* species segregate along comparable soil moisture axes and that species' δ^{13}C values do not reflect their hydrological niches. In addition, I have demonstrated that species' water use efficiencies (δ^{13}C) are dependent on their seasonal gas exchange strategies which are in turn influenced by species' traits.

Two components considered in this study that were ignored by Araya *et al.* (2010*a*,*b*) and require further investigation are the role of nutrients and soil depth in determining species traits and habitat preference. Given the role of nutrients in regulating leaf gas exchange (Cramer *et al.* 2008, 2009) and the significant interaction between % soil N and gas exchange (Table 6.6), the apparently 'wasteful' use of water by species which increase their gas echange rates in winter is likely to be a mechanism to increase uptake of nutrients via mass-flow during the period when they are readily available. The role of nutrients in determining species' seasonal gas exchange strategies could be investigated by manipulating nutrient availability and observing changes in species' water use (e.g. Cramer *et al.* 2008).

Soil depth and rockiness impact on plant growth and survival by modifying soil nutrient and moisture environments. Other than the impact on drainage, shallow, rocky soils impact on root growth and resource acquisition through physical obstruction and reduced space (Semchenko *et al.* 2008, Kambatuku *et al.* 2011). Such obstructions can hamper root access to resources or cause root crowding and reduced root growth, reducing resource acquisition efficiency and requiring changes in root physiology or morphology to negotiate obstructions or acquire sufficient nutrients from limited volumes of soil (McConnaughay & Bazzaz 1991, Semchenko *et al.* 2007, 2008, Poot & Lambers 2008). Failure to quantify root traits such as specific root length and root profiles because of the logistical difficulties of excavating and removing stones from the roots of species that grow in shallow soils or in rock cracks is a testament to the potential importance of obstructions and reduced space for root growth and resource acquisition in *Tetraria* species. A number of root traits likely influence species' microhabitat niches through their influence on tolerance of shallow rocky soils (e.g. root placement; Poot & Lambers 2008, Semchenko *et al.* 2008), tolerance of soil drying (e.g. suberization; Moll & Sommerville 1985) and nutrient and water uptake (e.g. specific root length, cluster roots; Shane *et al.* 2008).

Finally, the average size of individuals of all species was significantly reduced in communities of closely-related species, suggesting that species respond negatively to the presence of stronger competitors (criterion 4). The test of this criterion is dependent on the assumption that close relatives should compete more strongly than distant relatives as suggested by Darwin (1859; the 'competition-relatedness hypothesis'). While there is some contention about the validity of this hypothesis and its predictions (Cahill *et al.* 2008, Mayfield & Levine 2010), the reduction of plant size in the presence of close-relatives and the existence of significant spatial segregation among species within each of the major clades suggests that the competition-relatedness hypothesis holds for *Tetraria*.

While previous studies have identified morphological or microhabitat segregation among fynbos species co-occurring at fine spatial scales (Cody 1986, Araya *et al.* 2010*a*), this is the first study to provide evidence for all four criteria required to demonstrate that classical niche differences maintain the stable coexistence of fynbos species at fine spatial scales. The extent to which microhabitat specialization is a general phenomenon responsible for maintaining fynbos diversity is of great interest for understanding the structure, composition and function of fynbos communities and their vulnerability to natural and unnatural perturbation. Improving our understanding of the heterogeneity of soils and other components of the microhabitat may greatly improve our understanding of fynbos plant population and community dynamics (Hutchings *et al.* 2003). There is little reason to believe that microhabitat differences are the sole mechanism mediating the coexistence of fynbos species, however, and non-equilibrium mechanisms such as lottery models or storage effects are likely to be important for maintaining the coexistence of species with differing regeneration strategies such as sprouting or reseeding after fire (Bond *et al.* 1992, Cowling *et al.* 1997*a*, Bond & Midgley 2001, 2003, Thuiller *et al.* 2007). The coexistence of close relatives in fynbos communities could thus be maintained via non-equilibrium mechanisms if species diverge in regeneration strategy or via classical niche segregation if species diverge in abiotic preferences. Which of these mechanisms mediates the coexistence of closely-related species is likely determined by the relative lability of regeneration strategies and traits which affect habitat preference. Comparison of co-occurrence patterns in lineages with diverse versus conservative regeneration strategies would be of great interest in this regard.

Table 6.1: Results of the spatial segregation analysis.

Species	Index	Sim	Z	Skewness	L95%	U95%
Presence-absence						
None	107.0	93.6	12.70	0.37	91.7	95.6
bro	109.0	100.0	8.84	0.24	98.1	102.0
the	97.7	82.6	11.80	0.57	80.3	85.4
exi	85.6	77.2	7.32	0.40	75.3	79.7
fim	113.0	99.6	11.60	0.45	97.6	102.0
pyg	124.0	107.0	14.30	0.37	105.0	110.0
mic	103.0	87.0	13.70	0.34	85.0	89.5
cri	115.0	105.0	9.23	0.35	103.0	108.0
ple	104.0	95.1	8.72	0.44	93.3	97.4
fas	94.1	80.3	10.50	0.49	78.0	83.2
fle	105.0	88.2	14.50	0.43	86.3	90.7
ust	123.0	108.0	13.30	0.54	106.0	111.0
By clade	106.7	44.9	8.50	0.11	31.3	59.4
Abundance						
None	0.0833	0.0446	7.04	0.22	0.0343	0.0559
bro	0.0867	0.0416	8.06	0.21	0.0306	0.0533
the	0.0740	0.0430	5.23	0.14	0.0322	0.0550
exi	0.0648	0.0426	4.13	0.12	0.0323	0.0538
fim	0.0904	0.0420	8.51	0.42	0.0317	0.0543
pyg	0.0974	0.0433	9.98	0.20	0.0332	0.0543
mic	0.0756	0.0433	5.58	0.20	0.0333	0.0550
cri	0.0913	0.0417	9.31	0.15	0.0318	0.0526
ple	0.0832	0.0451	6.68	0.12	0.0345	0.0564
fas	0.0740	0.0447	4.96	0.08	0.0334	0.0561
fle	0.0821	0.0423	6.91	0.22	0.0320	0.0543
ust	0.0970	0.0471	9.09	0.07	0.0362	0.0586
By clade	0.0833	0.0448	7.37	0.08	0.0352	0.0556

Note: Spatial segregation in the presence-absence matrix was tested using the C-score co-occurrence index and independent swap null model algorithm while segregation in the abundance matrix was tested using the CA index and the IT null model algorithm. All tests were run for 999 simulations. The species omitted, observed Index, mean simulated index (Sim), Z-score, null distribution skewness and lower and upper 95% confidence intervals are reported. Index scores greater than the upper or less than the lower 95% confidence intervals indicate significant segregation or aggregation at α=0.05 respectively. Rows labeled ‘By clade’ indicate analyses where null model randomization was constrained to among species within each of the three major clades.

Table 6.2: Outlying Mean Index analysis.

Species	OMI(8 vars)	P (8 vars)	OMI (4vars)	P (4 vars)	# plots
bro	4.609	<0.005	1.424	<0.05	10
the	1.449	<0.005	1.102	<0.005	28
exi	0.927	<0.05	0.085	0.653	36
fim	9.34	<0.05	4.343	0.057	7
pyg	2.187	0.695	0.858	0.668	3
mic	1.147	<0.01	0.546	<0.05	30
cri	5.97	<0.05	2.187	0.117	7
ple	4.649	<0.005	2.299	<0.005	13
fas	1.046	<0.05	0.544	0.065	27
fle	4.672	<0.01	3.313	<0.005	14
ust	7.715	0.077	2.739	0.164	3
OMI.mean	3.974	<0.005	1.767	<0.05	178

Note: Results reported for the full (8 vars) and reduced set (4 vars) of soil properties (see Figure 6.1). The OMI and P values, and number of plots in which each species occurs are reported.

Table 6.3: Niche segregation analysis.

Species	MRPP		Soil depth	%N	Summer	Winter	#Sp1/Sp2	#Plots
	Plot	Quad						
bro/cri	0.960	0.147	0.001	0.002	0.003	0.002	48/38	6
bro/exi	0.001	0.001	-	-	-	-	-	0
bro/fas	0.001	0.004	-	-	-	-	-	1
bro/fim	0.033	0.002	0.471	0.210	0.057	0.075	23/15	3
bro/fle	0.001	0.005	-	-	-	-	-	2
bro/mic	0.031	0.006	0.381	0.170	0.863	0.832	63/105	10
bro/pyg	0.023	0.005	-	-	-	-	-	0
bro/ple	0.464	0.006	0.001	0.000	0.000	0.613	53/81	7
bro/the	0.001	0.001	0.011	0.012	0.397	0.308	26/14	4
bro/ust	0.150	0.059	-	-	-	-	-	2
cri/exi	0.001	0.022	-	-	-	-	-	0
cri/fas	0.001	0.173	-	-	-	-	-	1
cri/fim	0.072	0.004	-	-	-	-	-	2
cri/fle	0.001	0.022	-	-	-	-	-	2
cri/mic	0.024	0.119	0.000	0.904	0.202	0.001	39/74	7
cri/pyg	0.016	0.030	-	-	-	-	-	0
cri/ple	0.634	0.357	0.027	0.237	0.084	0.003	39/83	7
cri/the	0.001	0.174	-	-	-	-	-	2
cri/ust	0.039	0.008	-	-	-	-	-	1
exi/fas	0.919	0.278	0.000	0.000	0.011	0.004	211/99	24
exi/fim	0.001	0.001	-	-	-	-	-	2
exi/fle	0.371	0.001	0.408	0.270	0.399	0.279	59/57	9
exi/mic	0.001	0.125	0.090	0.130	0.165	0.040	74/111	16
exi/pyg	0.851	0.001	0.040	0.626	0.019	0.586	24/34	3
exi/ple	0.001	0.001	0.043	0.995	0.970	0.004	33/50	3
exi/the	0.029	0.001	0.056	0.019	0.001	0.000	137/102	20
exi/ust	0.001	0.014	-	-	-	-	-	0
fas/fim	0.001	0.001	-	-	-	-	-	0
fas/fle	0.252	0.001	0.011	0.040	0.008	0.053	14/37	7
fas/mic	0.001	0.272	0.131	0.796	0.801	0.066	36/83	11
fas/pyg	0.728	0.009	-	-	-	-	-	2
fas/ple	0.001	0.002	-	-	-	-	-	1

Table 6.3: (continued on next page)

Table 6.3: (continued)

Species	MRPP		Soil depth	%N	Summer	Winter	#Sp1/Sp2	#Plots
	Plot	Quad						
fas/the	0.083	0.001	0.127	0.074	0.537	0.034	71/105	16
fas/ust	0.001	0.004	-	-	-	-	-	0
fim/fle	0.001	0.036	-	-	-	-	-	1
fim/mic	0.001	0.001	0.821	0.446	0.715	0.16	17/44	5
fim/pyg	0.012	0.001	-	-	-	-	-	0
fim/ple	0.512	0.003	0.067	0.534	0.312	0.639	19/85	6
fim/the	0.001	0.003	-	-	-	-	-	0
fim/ust	0.013	0.001	-	-	-	-	-	0
fle/mic	0.004	0.001	0.373	0.940	0.152	0.126	18/42	5
fle/pyg	0.523	0.002	-	-	-	-	-	0
fle/ple	0.001	0.006	0.512	0.038	0.296	0.151	12/43	3
fle/the	0.011	0.020	0.021	0.403	0.198	0.291	7/36	5
fle/ust	0.004	0.009	-	-	-	-	-	0
mic/pyg	0.497	0.006	-	-	-	-	-	2
mic/ple	0.002	0.001	0.000	0.003	0.006	0.706	98/119	10
mic/the	0.019	0.001	0.287	0.259	0.935	0.246	114/42	15
mic/ust	0.053	0.025	0.392	0.123	0.181	0.964	40/7	3
pyg/ple	0.019	0.008	-	-	-	-	-	0
pyg/the	0.443	0.002	-	-	-	-	-	1
pyg/ust	0.082	0.004	-	-	-	-	-	0
ple/the	0.001	0.006	0.171	0.474	0.153	0.278	5/11	3
ple/ust	0.010	0.006	-	-	-	-	-	1
the/ust	0.022	0.003	0.019	0.012	0.448	0.247	13/7	3

Note: Results of the MRPP tests for differences between species in niche space based on plot and quadrat data and Wilcoxon tests for differences between species among quadrats within plots in which they co-occur. Wilcoxon tests were not performed for species pairs which co-occurred in fewer than three plots.

Table 6.4: GLS analysis between community weighted means (CWMs) and soil properties.

Trait	Coarse soil	Soil depth	Summer	%N	pH	R^2	Shapiro (P)
LL	-	0.000	-	0.004	-	0.462	0.345
sqrt(LW)	-	0.000	0.007	-	-	0.335	0.942
SLA	-	0.015	-	0.000	-	0.474	0.570
LDMC	0.010	-	0.001	-	-	0.470	0.278
St_D	-	-	-	-	0.034	0.083	0.486
PLN	-	-	-	0.000	-	0.282	0.478

Note: Generalised Least Squares analysis of the relationship between plot level community weighted means (CWMs) for each trait and the set of soil variables. Variables retained in each model and the significance of their contribution are indicated. % Rock cover, winter soil moisture and [P] are not indicated because they were not retained for any of the models. The regression coefficient (R^2) indicates the power of the fitted values from the model for predicting the observed values. Sh(P) indicates the significance of the Shapiro - Wilk's W test of normality.

Table 6.5: Correlations between species trait means and mean habitat conditions.

	LL	LW	SLA	LDMC	St_D	PLN
Ahistorical correlations						
%Rock	**0.682**	0.095	*-0.588*	0.266	-0.168	0.187
Coarse soil	0.121	0.299	-0.029	-0.350	-0.201	-0.350
Soil depth	*-0.595*	-0.290	0.395	0.103	-0.044	-0.188
Winter	0.479	0.173	**-0.673**	-0.459	-0.240	-0.251
Summer	-0.113	0.314	0.096	**-0.732**	-0.148	*-0.518*
%N	0.435	0.459	-0.423	*-0.542*	-0.232	-0.478
pH	0.196	-0.160	-0.302	0.506	0.060	0.231
P	-0.223	0.252	0.148	**-0.716**	-0.087	*-0.530*
Axis1	0.026	0.317	0.004	**-0.619**	-0.165	-0.435
Axis2	**0.687**	0.242	*-0.527*	0.123	-0.083	0.142
Phylogenetically independent contrast correlations						
%Rock	**0.696**	0.230	*-0.569*	0.417	-0.108	0.082
Coarse soil	-0.030	0.136	0.048	-0.317	*-0.517*	-0.389
Soil depth	-0.508	-0.292	0.318	0.002	0.156	-0.044
Winter	0.346	0.140	*-0.591*	-0.385	-0.353	-0.384
Summer	-0.217	0.162	0.164	**-0.697**	-0.405	-0.476
%N	0.292	0.348	-0.345	-0.424	*-0.558*	**-0.604**
pH	0.337	-0.013	-0.424	*0.568*	0.244	0.139
P	-0.270	0.142	0.160	**-0.745**	-0.289	-0.498
Axis1	-0.127	0.151	0.105	*-0.588*	-0.449	-0.428
Axis2	**0.657**	0.303	-0.501	0.272	-0.202	-0.018

Note: Ahistorical and phylogenetically independent contrast correlations between species mean trait values and mean plot level habitat conditions. Only correlation co-efficients (R) are reported, values $>$0.6 or $<$-0.6 are significant at the α = 0.05 level and highlighted in bold, values significant at the α = 0.1 level are highlighted in italics, n=11 species.

Table 6.6: Infra-red Gas Analysis results.

	Summer			Winter			Summer/Winter ratio		
	A	*E*	WUE	*A*	*E*	WUE	*A*	*E*	WUE
Soil variables									
Coarse soil	0.22	0.38	-0.36	-0.47	-0.3	-0.42	0.47	0.24	0.03
Winter	-0.21	-0.37	0.50	0.50	0.47	0.14	-0.48	-0.33	0.30
Summer	0.11	0.07	-0.18	-0.08	-0.35	**0.66**	0.04	0.28	**-0.64**
%N	-0.28	-0.49	**0.61**	**0.69**	**0.63**	0.14	**-0.64**	-0.49	0.38
pH	0.32	0.42	-0.37	-0.49	-0.51	0.02	0.56	0.44	-0.32
P	0.04	-0.16	0.52	0.29	0.17	0.3	-0.18	0.03	0.14
Traits									
LL	*0.51*	0.32	0.12	0.06	-0.32	**0.83**	0.25	0.46	*-0.58*
LW	0.32	0.01	0.41	-0.08	-0.32	**0.77**	0.23	0.38	-0.29
SLA	**-0.65**	**-0.61**	0.30	0.21	**0.61**	**-0.77**	-0.48	**-0.69**	**0.87**
LDMC	-0.36	-0.18	-0.29	0.18	0.16	-0.20	-0.32	-0.32	-0.06
St_D	-0.00	-0.13	0.37	0.05	0.13	-0.13	0.00	-0.14	0.38
PLN	-0.16	-0.09	0.14	0.46	**0.66**	*-0.59*	-0.34	*-0.53*	**0.60**

Notes: Phylogenetically independent contrast correlations between gas exchange variables, soil variables and species mean trait values. *A*: photosynthesis (μmol CO$_2$ m^{-2}s^{-1}), *E*: transpiration (mmol m^{-2}s^{-1}), and WUE: water use efficiency) measured using an infra-red gas analyser for each season. Only correlation co-efficients (R) are reported, values $>$0.6 or $<$-0.6 are significant at the α = 0.05 level and highlighted in bold, values significant at the α = 0.1 level are highlighted in italics, n=11.

Table 6.7: PIC results for δ_{13}C isotope data.

Soil variables	Coarse soil	Winter	Summer	%N	pH	P
Summer	0.08	-0.17	-0.12	-0.07	0.33	-0.09
Winter	0.21	-0.36	-0.02	-0.34	*0.55*	-0.23

Traits	LL	LW	SLA	LDMC	St_D	PLN
Summer	**0.64**	0.25	*-0.54*	0.22	0.43	0.32
Winter	**0.68**	0.33	**-0.66**	0.20	0.35	0.09

Note: Correlation co-efficients (R) are reported for PIC correlations between isotope data, soil variables and species mean trait values.Values $>$0.6 or $<$-0.6 are significant at the α = 0.05 level and highlighted in bold, values significant at the α = 0.1 level are highlighted in italics, n=11.

Appendix 6.1: Plot GPS coordinates.

Plot	Lattitude	Longitude	Plot	Lattitude	Longitude
1	-34.0926209	18.4235937	31	-34.1029697	18.4459363
2	-34.0932968	18.4237367	32	-34.1022738	18.4464142
3	-34.0939914	18.4240213	33	-34.1018755	18.4471212
4	-34.0947315	18.4243729	34	-34.1014552	18.4476436
5	-34.0954084	18.4248002	35	-34.1006992	18.4476915
6	-34.0961506	18.4254186	36	-34.1002898	18.4472468
7	-34.0967629	18.4258938	37	-34.1001238	18.4464829
8	-34.0973927	18.4263093	38	-34.0997906	18.4452961
9	-34.0979654	18.4268977	39	-34.0995698	18.4445385
10	-34.0983541	18.4276622	40	-34.0993509	18.4434676
11	-34.0989137	18.4284288	41	-34.0983963	18.4425411
12	-34.0993669	18.4316669	42	-34.0979338	18.4419784
13	-34.0995337	18.4325072	43	-34.0971956	18.4412530
14	-34.0998402	18.4333467	44	-34.0963010	18.4415500
15	-34.1004819	18.4340507	45	-34.0959244	18.4404829
16	-34.1010322	18.4346517	46	-34.0955070	18.4399937
17	-34.1013768	18.4354116	47	-34.0952664	18.4391559
18	-34.1019137	18.4362889	48	-34.0946650	18.4383014
19	-34.1024094	18.4368597	49	-34.0909941	18.4295500
20	-34.1026651	18.4375807	50	-34.0903079	18.4293830
21	-34.1028594	18.4383487	51	-34.0897154	18.4298451
22	-34.1029467	18.4391413	52	-34.0897528	18.4306460
23	-34.1031424	18.4399680	53	-34.0896283	18.4314973
24	-34.1033095	18.4407856	54	-34.0893649	18.4321963
25	-34.1034303	18.4415238	55	-34.0894990	18.4330850
26	-34.1037104	18.4426788	56	-34.0896006	18.4338704
27	-34.1038026	18.4435895	57	-34.0870518	18.4296726
28	-34.1038187	18.4442970	58	-34.0867652	18.4289178
29	-34.1036557	18.4450728	59	-34.0865910	18.4279811
30	-34.1034841	18.4459937	60	-34.0871989	18.4277334

Note: The GPS coordinates (WGS84) for the 60 plots surveyed on the Wolfkop - Higher Steenberg Peak transect, Table Mountain National Park. All plots were marked with 0.9m metal fence droppers in 2007/2008.

Appendix 6.2: Basal diameter (cm) size classes for each species.

Species	Species code	Small	Medium	Large
T. bromoides	bro	<10	10 - 15	>15
T. thermalis	the	<25	25 - 50	>50
T. eximia	exi	<3	3 - 5	>5
T. fimbriolata	fim	<5	5 - 7.5	>7.5
T. pygmaea	pyg	<2	2 - 3	>3
T. microstachys	mic	<5	5 - 8	>8
T. crinifolia	cri	<10	10 - 12	>15
T. pleiostcha	ple	<5	5 - 10	>10
T. fasciata	fas	<10	10 - 15	>15
T. flexuosa	fle	<7	7 - 15	>15
T. ustulata	ust	<15	15 - 30	>30

Hex River Mountains, Western Cape.

7 Synthesis

Fynbos vegetation is comprised of moderately species-rich local communities (α-diversity) with very high turnover in composition between habitats (β-diversity) and between similar habitats in different regions (γ- or δ-diversity; Bond 1983, Cowling & Campbell 1984, Cowling 1990, Cowling *et al.* 1992). Turnover in growth form is very low, however, with most communities containing representatives of each of the major growth forms (Cowling & Campbell 1984, Cowling 1990). Taxonomic diversity displays similar low turnover because most communities contain members of the same 33 Cape Floral Clades that make up almost 50% of fynbos diversity (Linder 2003). This pattern may have been produced at the regional (γ or δ) scale via allopatric speciation, either combined with little ecological divergence among closely-related species or convergence to similar functional forms across species pools from different regions (Table 1.1, Chapter 1). The pattern would be reflected among communities within the same landscape (α and β scales) either through neutral processes, with high dispersal limitation explaining the high turnover in species composition between habitats or, if local coexistence is mediated by niche partitioning, with close relatives specialized to subtly different niches (Table 1.2, Chapter 1; Webb *et al.* 2002).

It is my thesis that ecological specialization is a key factor shaping the patterns of functional and phylogenetic diversity observed in fynbos through its affect on speciation, trait evolution and the occurrence and co-existence of species. As such I proposed the hypotheses that 1) the radiation of fynbos lineages should be constrained by available ecological space, resulting in declining diversification rates as species accumulate and fill that space; 2) close relatives with overlapping ranges should be ecologically differentiated while close relatives that occur in allopatry need not be ecologically differentiated; and 3) close relatives should be specialized to subtly different microhabitats, mediating species coexistence within local communities through resource partitioning in accordance with the principles of classical niche theory. These hypotheses were tested on a clade of schoenoid sedges in the genus *Tetraria* P. Beauv., one of the 33 Cape Floral Clades (Linder 2003).

The predictions were met. Diversification rates in the group declined as lineage diversity accumulated, indicating ecological limitation on speciation rates (Chapter 4).

Closely-related species with overlapping distribution ranges display greater morphological disparity than expected under a Brownian motion model of evolution and/or occur in significantly different elevational zones and bioclimatic or insolation conditions in their regions of geographical overlap (Chapter 5). By contrast, close-relatives with non-overlapping distributions were less disparate morphologically than expected under a Brownian motion model. Finally, a set of 11 *Tetraria* species occurring in local communities (5 x 10m plots) fulfilled the four criteria required to demonstrate that stable coexistence is maintained by classical niche differences in microhabitat preference (Chapter 6). Species were spatially segregated, displayed specialized preferences along soil nutrient-moisture and soil depth niche axes, and showed evidence of trade-offs in performance along these axes that was reflected in their morphological traits. Finally, they showed size-reduction in the presence of stronger competitors. In combination, these findings indicate that ecological specialization and ecological limitation, potentially through negative interspecific interactions, are key factors shaping patterns of functional and phylogenetic diversity observed in *Tetraria* communities, and possibly in fynbos as a whole.

There was also much evidence that 'non-ecological' biogeographic processes played an important role for the generation of diversity and the observed patterns of functional and phylogenetic diversity in *Tetraria* assemblages. Non-random distributions of species, clustering in areas of endemism, imply an important role for allopatry in facilitating speciation (Cracraft 1992). Congruent distributional boundaries among *Tetraria* species (Chapter 5) and species in a number of other Cape clades (Weimarck 1941, Oliver *et al.* 1983, Goldblatt & Manning 2000, 2002, Linder 2001, Moline & Linder 2006) can be taken as evidence of the predominance of allopatric speciation in shaping the Cape flora as a whole. A key point of interest is thus to establish the relative importance of ecological versus non-ecological processes in generating and structuring the observed patterns of extant diversity and the scale at which these processes operate.

Table 7.1: Similarity in species, functional and phylogenetic composition between *Tetraria* assemblages in different habitat types within each of the different areas of endemism.

		Species			Phylogenetic			Functional		
		Mean	F	P	Mean	F	P	Mean	F	P
Region	Within	0.204	11.162	<0.001	514.14	6.232	<0.05	506.24	0.041	0.839
	Between	0.153	-	-	457.541	-	-	511.048	-	-
Habitat type	Within	0.282	21.359	<0.000	437.64	0.227	0.634	604.547	4.608	<0.05
	Between	0.158	-	-	471.715	-	-	505.249	-	-

Notes: Species assemblages were identified by combining georeferenced species localities (Chapter 5) within ‘Vegetation Group’ GIS polygons provided in Mucina & Rutherford (2006). Thirteen vegetation groups (habitat types) were used. These are predominantly based on different soil types but identify wetlands and forests as different groups (Mucina & Rutherford 2006). Species assemblages were classed within regional areas of endemism identified in Chapter 5, Figure 5.5. Similarity in functional and phylogenetic composition was calculated using a non-parametric rank order standardization of similarity coefficients. Similarity in species composition was calculated using Sorenson’s coefficient, while unstandardized measures of similarity in phylogenetic and functional composition were calculated using the equation for Sorenson’s coefficient, substituting difference in species with difference in branch length derived from the dated MCC phylogenies generated in Chapter 4 (see Bryant *et al.* 2008) and from a dendrogram summarizing the morphological differences between species. The functional dendrogram was generated using UPGMA clustering of Euclidean distances between species based on a PCA using standardized data for the eight morphological traits measured for all species in Chapter 5 (see Chapter 2, Petchey & Gaston 2002). Similarity in functional and phylogenetic composition between sites was standardized by ranking each measure relative to a set of 999 null measures generated by shuffling species names on the tips of the phylogeny or dendrogram. This accounts for differences in the number of species between sites by holding similarity in species composition constant for the set of null measures and thus expresses functional or phylogenetic similarity between each pair of assemblages relative to 999 comparisons with the same degree of similarity in species composition. Tests were initially performed within versus between regions and within versus between habitat types using MRPP tests (Mielke *et al.* 1976) because the use of parametric statistics is inappropriate due to non-independence of data points. The results of a two-way ANOVA are presented because they were qualitatively identical to the MRPP tests. A two-way factorial ANOVA revealed no significant interacting effects. All analyses were performed in R2.11.1 (R Development Core Team 2010) using the packages ‘vegan’ (Oksanen *et al.* 2010) and ‘picante’ (Kembel *et al.* 2010).

Exploring patterns of functional and phylogenetic diversity within and between communities at different spatial scales provides a valuable starting point for identifying the relative influence of the different processes and will greatly aid in the development of integrated models of diversification and community assembly (Cavender-Bares *et al.* 2009, Chapters 2 and 3). Comparisons of similarity in species, functional and phylogenetic composition between *Tetraria* assemblages in different habitat types within each of the different areas of endemism are displayed in Table 7.1 (see caption for details of the analysis). Both region and habitat type were significant predictors of similarity in species composition, with assemblages being more similar within regions or habitat types than between, indicating the dual importance of biogeographical constraint and environmental filtering. Assemblages within regions were, however, more similar phylogenetically than assemblages between regions but showed no difference in functional similarity, this indicating an effect of geography on phylogenetic but not functional composition. By contrast, habitat type was not a significant predictor of phylogenetic similarity between assemblages but did have a significant effect on functional similarity, implying convergence in functional form between species in the same habitat type in different regions. Thus 'non-ecological' biogeographic processes seem to be structuring patterns of phylogenetic diversity while ecological pressures are structuring functional diversity by causing species' to converge in morphology and/or sorting them into the same habitat types in different regions (Scenario D, Figure 1.4, Chapter 1).

In addition to its effects on similarity in phylogenetic composition between regions, biogeography can affect phylogenetic dispersion within regions depending on their connectivity and the history of speciation. Investigating the effects of geography on phylogenetic dispersion within areas of endemism reveals that the outlying Peninsula and Southeast areas, each of which is connected to one region only, are comprised of distant relatives (Figure 7.1). This signal is likely to be the result of speciation in allopatry because sister pairs will be split between regions (Figure 1.3, Chapter 1). In contrast, the floras of regions with greater connectivity are less likely to be comprised predominantly of distant relatives because they are more likely to have been colonised by the expansion of species' ranges from multiple regions, masking any signal of overdispersion. These results were stronger for the analysis based on the 'independent swap' algorithm because this null model randomizes species distributions, breaking down the effect of geography. The analysis based on pairwise distances indicates that the Central Mountain and Northwest regions are phylogenetically clustered relative to the other regions (Figure 7.1), possibly because their

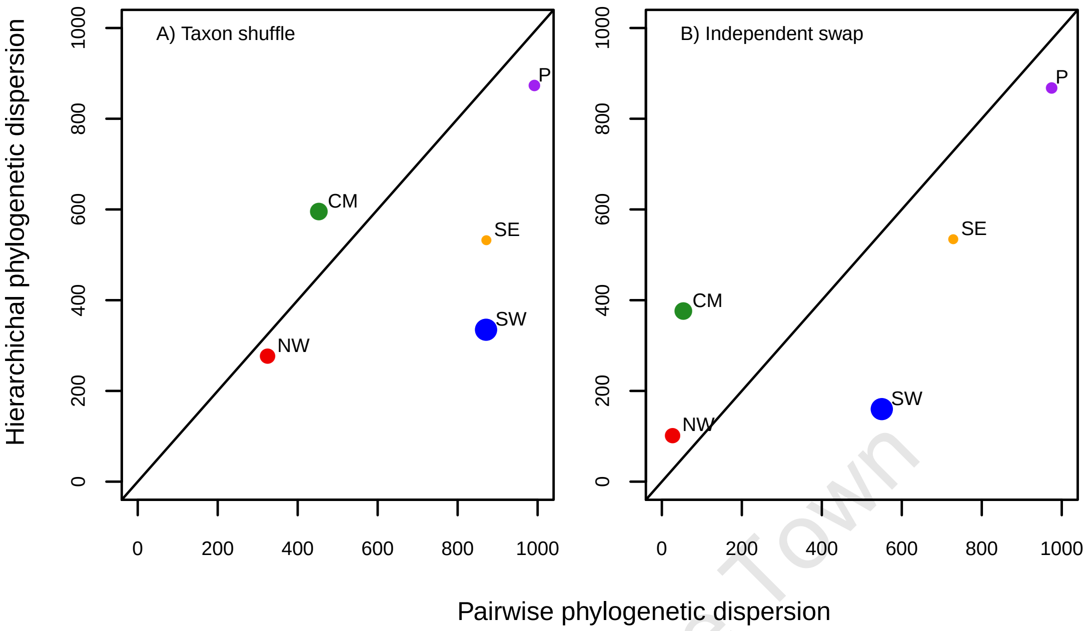


Figure 7.1: Comparison of rank-order standardized measures of hierarchical and pairwise measures of phylogenetic dispersion between areas of endemism using two null model approaches (see Chapter 2 for details of the standardization procedure). The 'Taxon shuffle' null model swaps species names on the phylogenetic tree, randomizing phylogenetic relationships but retaining species distributions, while the 'Independent swap' algorithm (Gotelli 2000) retains phylogenetic relationships but randomizes species distributions. The hierarchical measure of dispersion is based on the summed branch length joining the set of species in the community on the phylogeny (Faith 1992) and is most sensitive to the influence of close relatives while the pairwise measure is based on the mean of the distances between each pair of species (Webb *et al.* 2002) and is more sensitive to the influence of distant relatives. Colours represent the regions identified in Chapter 5, Figure 5.5, while the diameter of circles is proportional to the number of species within each region (see Figure 5.7, Chapter 5). Purple = Peninsula (P), Blue = Southwest (SW), Red = Northwest (NW), Green = Central Mountain (CM), Yellow = Southeast (SE).

diversity is dominated by the *fasciata-flexuosa* and *thermalis-bromoides* clades respectively. This pattern is not evident in the Central Mountain region when a hierarchical measure of phylogenetic dispersion is used because this metric is most sensitive to co-occurrence of close relatives (Chapter 3) and a number of sisters pairs in the *thermalis-bromoides* clade occur in allopatry (Chapter 5). The predominance of species in the *thermalis-bromoides* clade in the eastern regions also accounts for the Southeast region being comprised of more closely-related species than the Peninsula because the Southeast region is made up of distant relatives from within the clustered set of eastern species. The Peninsula flora, by contrast, contains more distantly-related species from the South West region, which contains similar numbers of species from all clades (Figure 5.7, Chapter 5).

The differential effects of biogeographic and ecological processes and the scale at which they impact on patterns of functional and phylogenetic diversity are best explored by examining a nested set of assemblages at different spatial scales (Chapter 3). Functional and phylogenetic dispersion from nested sets of communities from two localities in the CFR are presented in Figure 7.2. The degree of functional and phylogenetic dispersion at each spatial scale is assessed relative to the degree of dispersion of the broader species pools. Generally, functional dispersion reflected phylogenetic dispersion and assemblages became more even (i.e. more distantly related or functionally dissimilar) relative to the regional CFR species pool as the spatial scale decreased. This implies that similar processes, and density-dependent processes in particular, are acting at both localities.

Figure 7.2: Rank-order standardized hierarchical phylogenetic and functional dispersion at different spatial scales within two sites in the Cape Floristic Region. A) Phylogenetic dispersion on the Cape Peninsula and within the Silvermine plots; B) Functional dispersion on the Cape Peninsula and within the Silvermine plots; C) Phylogenetic dispersion in the Central Mountains and within the Langeberg plots; D) Functional dispersion on the Central Mountains and within the Langeberg plots. Dashed lines represent ranks the 95% critical bounds as defined by rank values of -475 and 475 respectively. CFR = Cape Floristic Region, Pen = Peninsula, CM = Central Mountains, Silv = Silvermine, Lang = Langeberg. Labels (e.g. CFR-Pen) indicate first the broader species pool (CFR) used to test dispersion in the finer species pool (Pen). Labels that are not hyphenated (e.g. Silv(T)) indicate mean dispersion values for a set of 5 x 10 m plots tested relative to the set of species occurring in all plots using the taxon shuffling (T) or independent swap (I) null model. All comparisons used the taxon shuffling null model based on the phylogeny or functional dendrogram (see Table 7.1) from the broader pool of species unless indicated with an 'I' (independent swap). Dispersion is expressed as the rank-order standardized dispersion minus 500 such that the null expected value is 0, <0 indicates clustering and >0 indicates evenness. The Silvermine dataset contains the 57 plots containing 11 species from Chapter 6, while the Langeberg dataset contains 239 plots containing 10 species from the Langeberg mountains surveyed by McDonald (1993*a*,*b*,*c*). *Tetraria* species composition in the Langeberg plots was updated to the new taxonomy (Verboom et al. *In preparation*) by re-determining the voucher specimens cited by McDonald (1993*a*,*b*,*c*).

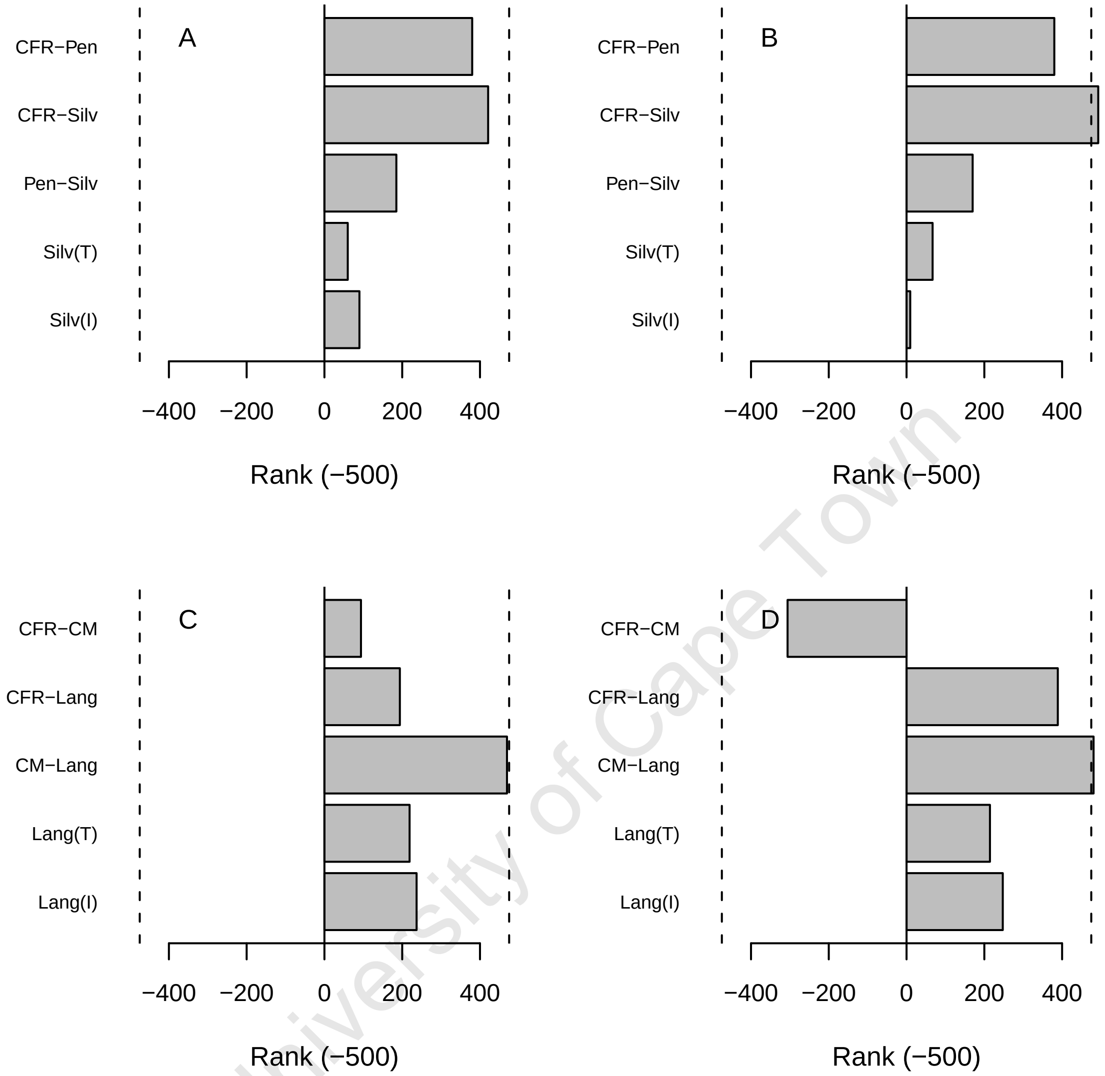


Figure 7.2: See over page for caption

Differences between the localities highlight the importance of regional context and spatial scale (Figure 7.2). Firstly, in accordance with Figure 7.1, the Peninsula represents a functionally and phylogenetically even set of all *Tetraria* species in the CFR while the Central Mountain assemblage shows a weak signal of phylogenetic evenness and is functionally clustered, likely due to the dominance of species from the *thermalis-bromoides* clade in this region (Figure 5.7, Chapter 5). The set of species occurring within the Langeberg plots is thus not as even functionally and phylogenetically as the set of species which occur in the Silvermine plots, when compared to the CFR species pool. Secondly, the greatest shift in degree of dispersion between spatial scales occurs between the CFR and Peninsula species pools for the Silvermine locality, while it occurs between the Central Mountain region and the plot scale in the Langeberg. Two factors may contribute to this pattern. Firstly, the Peninsula covers a much smaller area than the Central Mountain region and density-dependent processes may be important in limiting the Peninsula to a pool of functionally and phylogenetically even species. Secondly, comparing the Langeberg plots to the Central Mountain species pool reduces the influence of biogeographic processes on the pattern and excludes the clustering affect of the predominance of species from the *thermalis-bromoides* clade. The higher degree of functional and phylogenetic evenness within the set of Langeberg plots relative to the Silvermine plots (Figure 7.1) may be because the Langeberg plots were sampled over a much larger area, or because competition between close-relatives is stronger within the Langeberg because species are generally more closely-related than at Silvermine.

These results highlight how ecological and biogeographical processes both structure species assemblages and how their observable effects change with spatial scale as suggested in Figure 1.1, Chapter 1. In addition, the results allude to the interplay between ecology and biogeography, each affecting the other, emphasizing that any distinction between local and regional processes and species pools is artificial (Weiher & Keddy 1999, Hubbell 2001, Ricklefs 2004, 2006, 2008). Overcoming the need to make this distinction requires improving our understanding of how the various processes interact to produce the patterns we observe and how this varies with spatial and temporal scale. In the context of this study, a key step in this direction is deciphering the mechanism whereby density-dependent processes within assemblages interact with biogeographical processes to produce the observed patterns of functional and phylogenetic diversity and constrain species diversification rates as a whole. Adopting a traditional view one might argue that ecology limits diversification rates via an explicit conceptual analogue of density-dependent population regulation models (e.g. MacArthur 1957, 1965, MacArthur & Levins 1967) scaled up to the level of species and clades at broader spatial and temporal scales (Cracraft 1992). This view ignores biogeographic history, however, and would require the entire universe within which the lineage has diversified to be ecologically saturated. A more realistic model which includes biogeography is one in which the lineage has diversified among a set of spatially explicit regions with varying degrees of connectivity and ecological saturation. In Chapter 5 I argued that the geographical context and mode of speciation are crucial factors affecting the rate of diversification and degree of ecological differentiation within a lineage. If speciation occurs predominantly in allopatry and some regions are more ecologically saturated than others, e.g. because they have greater levels of connectivity to other regions (MacArthur & Wilson 1967) or occur more centrally (the 'Mid Domain Effect', Colwell & Lees 2000), then diversification rates may decline because founder populations which disperse into allopatric regions are either eliminated by or introgress with close relatives. In other words colonization rates, and thus diversification rates, decline as founder populations have to disperse into regions that are increasingly ecologically saturated.

Deciphering the mechanism whereby ecological interactions limit diversification rates is of great importance for understanding the ecology and diversity of fynbos communities and landscapes, and their consequences for ecosystem function. If the entire CFR is ecologically saturated, the implication is that assemblages are maintained under a fine balance of competitive hierarchies. In this scenario, any anthropogenic manipulation of environmental conditions would cause major shifts in community composition and potentially lead to cascading extinctions. For all we know this may already be happening but we fail to observe it because of insufficient long-term monitoring efforts (e.g. Slingsby 2009), or because insufficient time has passed for the effects to be readily detected. Alternatively, should the CFR be comprised of spatially segregated regions with varying levels of ecological saturation, the impacts of negative anthropogenic factors may be buffered because species may not experience the same pressures in all regions and will be allowed to respond in different ways.

Two testable corollaries of the spatially explicit model of ecologically constrained diversification are that outlying regions with lower connectivity should exhibit lower levels of ecological saturation, and that range expansion and allopatric speciation events are more likely to occur from more saturated into less saturated regions. Lower diversity in a region is not an indication of a lower level of ecological saturation because there are many factors which could potentially influence the number of species a region could support including area, climate, energy and environmental heterogeneity among others (Pianka 1966, MacArthur & Wilson 1967, Ricklefs 1977, 2006, Currie *et al.* 2004). If a region were ecologically saturated, one would expect its diversity to be related to one or more of these factors. A number of studies have explored these relationships within the CFR with mixed results. Linder (1991) divided the CFR into 55 quarter degree squares (QDS) and showed that species richness within five separate lineages was best predicted by rainfall and weakly correlated with altitude, number of substrates and vegetation type. Richardson *et al.* (2005) used a similar QDS approach to show that indigenous, alien and invasive alien species all increased in species richness in areas of greater climatic and topographic heterogeneity. The correlative approach based on QDS's used by these studies ignores biogeography, however, because all grid cells are considered equal and cells in ecologically saturated areas could dictate the pattern with cells from unsaturated areas simply adding noise to the relationship. A test for ecological saturation needs to consider differences in diversity and its environmental determinants between biogeographically meaningful areas. Cowling *et al.* (1992, 1997*b*) and Cowling & Lombard (2002) adopted such an approach by exploring environmental determinants as a potential explanation for the east-west gradient in regional species diversity in the CFR (SE+CM versus P+SW+NW regions in Figures 5.5 and 5.7, Chapter 5). They found that topographic heterogeneity, rainfall, temperature range and biological heterogeneity (number of community types) were not significant predictors of the difference in diversity and proposed that speciation-extinction history due to differences in climatic stability may be responsible for the observed pattern. The relationships between environmental variables and floristic diversity, and thus degree of ecological saturation, of areas of endemism in the CFR are yet to be explored.

The direction of range expansion and allopatric speciation events and whether or not they occur more frequently from more to less saturated regions can be inferred using models of geographic range evolution and overlap (Fitzpatrick & Turelli 2006, Ree & Smith 2008) or phylogeographic approaches (Knowles 2009, Lemey *et al.* 2009). Assuming that higher diversity in the Southwest region (Goldblatt & Manning 2000, 2002) implies greater ecological saturation, the few studies of this nature in the CFR generally support the spatially explicit model of ecologically constrained diversification. The majority of *Leucadendron* and *Muraltia* species occur in the Southwest region of the CFR and range reconstructions for both genera suggest that most instances of recent range expansion and allopatric speciation events involved shifts from the Southwest into neighbouring regions (Barker *et al.* 2004, Forest *et al.* 2007*b*). Inference of range evolution for *Tetraria* found a similar pattern for the *thermalis-bromoides* and *microstachys-burmannii* clades (data not shown, Chapter 5), while phylogeographic analysis of the *Tetraria triangularis* complex of species suggests that the lineage originated in the Southwest, spreading eastward (Britton 2010).

There is thus much evidence that range expansion and speciation among regions with varying connectivity and degrees of ecological saturation provides a plausible model for the diversification of the Cape flora. There are of course many unanswered questions. For example, it is unclear to what extent ecological constraint on diversification rates is a general phenomenon affecting Cape clades other than *Tetraria*, and thus the diversity of the flora as a whole. While there is evidence of habitat specialization (Linder & Vlok 1991, Richards *et al.* 1995, Latimer *et al.* 2009, Araya *et al.* 2010*b*, Carlson *et al.* 2011), morphological segregation (Cody 1986), disjunction between local and regional species diversity (Cowling *et al.* 1992, Richardson *et al.* 1995) and declining diversification rates (Linder & Hardy 2004, Valente *et al.* 2010) for some Cape lineages, these studies cover only a limited set of taxonomic groups and life history strategies. The elegance of a model of diversification based on ecological theory is that it allows us to predict the exceptions which can in turn be used to prove the rule.

A number of aspects of the biology of lineages should affect the degree to which their diversification rates and distributions are constrained by the interplay between ecology and biogeography. For example, the *microstachys-burmannii* clade showed no density-dependent decline in diversification rates. This may be because interactions among these species are less common or severe because, in terms of plant size, they are an order of magnitude smaller than other *Tetraria* species and because they have poor colonizing abilities, producing few, small seeds. Alternatively, these species could coexist via neutral-like dynamics because they show little niche or morphological differentiation and have similar competitive abilities (Chesson 2000, Leibold & McPeek 2006). Other potential exceptions which may show little or no decline in diversification rate are lineages which display little habitat specificity and coexist via differential recruitment under variable fire regimes (Cowling 1987, Thuiller *et al.* 2007), or lineages with specialized mutualisms, the added 'mutualism niche' dimensions allowing more species to be 'packed' into the same area (Waterman *et al.* 2011) and so reducing the degree of ecological saturation and ecological limitation. This highlights the importance of considering the effects of the mechanism by which species coexist (Wilson 1990, Chesson 2000, Hubbell 2001, Amarasekare 2003), and the positive and negative interactions between trophic levels (Agrawal 2007, Sargent & Ackerly 2008) in diversification and community assembly models. Other exceptions which merit attention are lineages which may have undergone ecological speciation in sympatry (van der Niet & Johnson 2009), or those which have good dispersal abilities and are less likely to be constrained by biogeography (e.g. Orchidaceae). Finally, a key component of this model which requires further investigation are interactions with the broader community of species not contained within the focal clade.

In conclusion, I have provided evidence that ecological specialization and niche differentiation have played a critical role affecting the genesis, maintenance and distribution of functional and phylogenetic diversity in the genus *Tetraria*, one of the Cape Floral Clades, and have proposed a model for the diversification of the Cape flora which integrates the interacting affects of ecology, biogeography and phylogeny across spatial and temporal scales. The high functional and phylogenetic diversity in regional *Tetraria* assemblages is likely the result of a predominance of allopatric speciation combined with convergence in form within habitats between regions and character displacement between close relatives within regions. Local *Tetraria* assemblages display similar patterns of diversity because close relatives have contrasting specialized microhabitat preferences but are generally conserved in functional form. Developing a better understanding of the degree to which the diversity of the Cape flora is limited by ecology, and the mechanism which determines this constraint, is of great importance for predicting the affects of anthropogenic impacts on the assembly of fynbos communities and landscapes and the consequences for ecosystem function.

Thirty-three plant and 70+ invertebrate species from a cubic foot of Silvermine, Table Mountain National Park. Courtesy of National Geographic, Copyright: David Liittschwager.

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