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***Tetraria triangularis* (Boeck.) C. B. Clarke and the Cape Fold
Archipelago: An analysis of genetic and morphological variation in a
high-altitude species native to the mountains of the Cape Floristic
Region**

By

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Chapter 1 General Introduction

The Cape Floristic Region (CFR) is renowned for its high plant species richness and high floristic endemism (Marloth 1929, Takahatjan, 1986, Goldblatt and Manning 2000, Linder 2003). A small number of clades (33) account for 50% of the species diversity, indicating that much of the diversification has occurred *in situ*, rendering it reminiscent of island archipelago radiations (Marloth 1929, Linder 2003). At a higher taxonomic level, 16.2 % of genera and four families are endemic to the area (Goldblatt et al. 2005).

The montane flora of the CFR is ecologically isolated by its soils which, being derived from highly-leached quartzitic rocks, are critically deficient in many plant nutrients and so contrast sharply with the heavier, more-eutrophic Karoo Group soils that border the CFR to the north and east (Marloth, 1929; Takahatjan 1986, Goldblatt and Manning 2000, Linder 2003, Cowling et al. 2009). Within the CFR, vegetation types and soil boundaries coincide very closely (Marloth 1908), suggesting that soil substrate strongly controls vegetation distributions. The substrate isolation of the CFR is accentuated by the effects of a westerly storm system that dictates rainfall patterns, resulting in the western CFR receiving most of its rain in winter, but with a shift toward all year rainfall in the east (Chase and Meadows 2007). These frontal systems seldom affect southern Africa east of the CFR, creating a “seasonal rainfall island” that results in a Mediterranean-type climate which is not present in the rest of southern Africa.

The radiation of the Cape flora is thought to have been initiated at the end of the Miocene, by the onset of the winter-rainfall, summer-arid seasonality, that characterises

the climate of the CFR today (Levyns 1964). This aridification is seen as a consequence of the increase in Antarctic sea ice extent and reduced mean sea temperatures (Siesser 1980, Hendey 1983, Zachos et al. 2001, Linder 2003, Cowling et al. 2009). The existence of a subtropical woodland flora in what is currently the CFR implies a more aseasonal climate with cooler, moister conditions at the Miocene-Pliocene boundary (Coetzee 1978, Coetzee 1983, Deacon et al. 1992). Aridification is postulated to have precipitated the extinction of lineages present at the time, and driven subsequent radiation of surviving lineages into newly developed ecological niches (Linder et al. 1992). A recent review by Cowling et al. (2009), however, challenges the widely accepted view that landscapes in the CFR have been static since the late Miocene (Deacon et al. 1992, Linder 2003). Uplift of the southern African subcontinent, resulted in erosion of material from the Table Mountain Supergroup, exposing the underlying Malmesbury group shales and Granites of the Cape Granite Suite (Partridge and Maud 1987, Partridge and Maud 2000). This raises the possibility that increased speciation rates since the late Miocene could be related to increased niche diversity associated with erosion of the overlying continuous sandstone landscapes, creating the modern mosaic of interlinked sandstone highlands and shale lowlands.

While there is some indication that adaptive radiations of lowland taxa may be associated with Late Miocene climate change (Verboom et al. 2003, Klak et al. 2004), the evidence is less clear for highland groups (Verboom et al. 2009). This discrepancy may be attributable to the greater impact of aridification on lowland environments since, under modern climates, these areas have no buffer from summer drought, such as the input of water from southeast wind clouds experienced in the highlands (Marloth 1904, Cowling

and Lombard 2002). Lowland areas in the CFR also show higher substrate diversity, with shales, limestones and granites represented (Fuggle and Ashton 1979, Cowling et al. 2009). In contrast, the highlands are uniformly composed of sandstone or quartzite. Climatic conditions in highland habitats are thought to more closely resemble the less seasonal, mid-Miocene climate, suggesting that these highland habitats may represent the ancestral state for Cape lineages and long term refugia for species reliant on ample moisture (Verboom et al. 2009). Highland lineages have the potential to migrate up and down mountain slopes during Quaternary cycles (Dynesius and Jansson 2000, Jansson and Dynesius 2002) mitigating the need for plants to migrate over large geographical distance to track a shifting climatic niche, as would be required for lowland lineages (Linder and Vlok 1991, Midgely et al. 2002). Arguably, being able to track their climatic niche means that highland lineages will experience less selection for major ecological shifts through time, than lowland lineages (Thuiller et al. 2004).

Previous work on diversification of the Cape Flora has tended to focus on understanding the role of ecological gradients (Linder 1985, Linder 1991, Linder and Vlok 1991, Verboom et al. 2003, Verboom et al. 2004, Ellis et al. 2006, Ellis and Weis 2006) or pollination specialisation (Johnson 1996, Johnson and Steiner 2003) in stimulating adaptive speciation. Adaptive radiation can be recognised by the accumulation of substantial morphological and ecological diversity within rapidly speciating lineages (Schluter 1996, Schluter 2000). Thus, where this mechanism is prevalent, species should be differentiated on the basis of functional morphology and/or physiology as dictated by adaptation to the environment (Schluter 2000). Although rapid speciation has been demonstrated by various authors (Bakker et al. 1999a, Bakker et al.

1999b, Reeves et al. 2001, Richardson et al 2001, Goldblatt et al. 2002, Verboom et al. 2003, Linder et al. 2003, Klak et al. 2004, Mckenzie and Barker 2008), direct evidence for adaptive radiation has only been demonstrated for *Ehrharta* (Verboom et al. 2004) and *Agyroderma* (Ellis et al. 2006, Ellis and Weis 2006).

Geographical isolation through bioclimatic range fragmentation provides an alternative explanation for speciation in high-altitude taxa in the Cape (Goldblatt 1978, Linder 2003). Numerous studies document allopatry of sister species in a diversity of taxa (e.g. Rourke 1969, Williams 1972, Goldblatt 1981, Karis 1989). Aridification since the Miocene would have reduced the extent and connectivity of mesic montane areas, enhancing population differentiation via fragmentation and potentially increasing speciation in montane lineages (Wiens 2004a). Subsequent climatic oscillations during the Quaternary (Petit et al. 1999) would have driven a similar process of mesic highland area contraction and expansion, causing isolation or extinctions on mountains peaks in dry periods and allowing range expansion during cooling periods (Jansson and Dynesius 2002, Wiens 2004a). There is evidence to suggest that the western half of the CFR was wetter during the Pleistocene glacials and the eastern half of the CFR drier than at present; as a consequence, we expect that larger numbers of mesic-adapted lineages would have been able to persist in the western CFR, with the eastern CFR showing either a signal of high extinction or of widespread immigration (Chase and Meadows 2007). The mountains of the CFR are comprised of erosion resistant quartzite and sandstones, arranged as two sets of ridges lying parallel to the south coast and a single broader range lying parallel to the west coast (Fig. 1.1). Large distances separate areas of high ground (Fig. 1.1) and poor long distance dispersal abilities of plants in the Cape flora (Linder

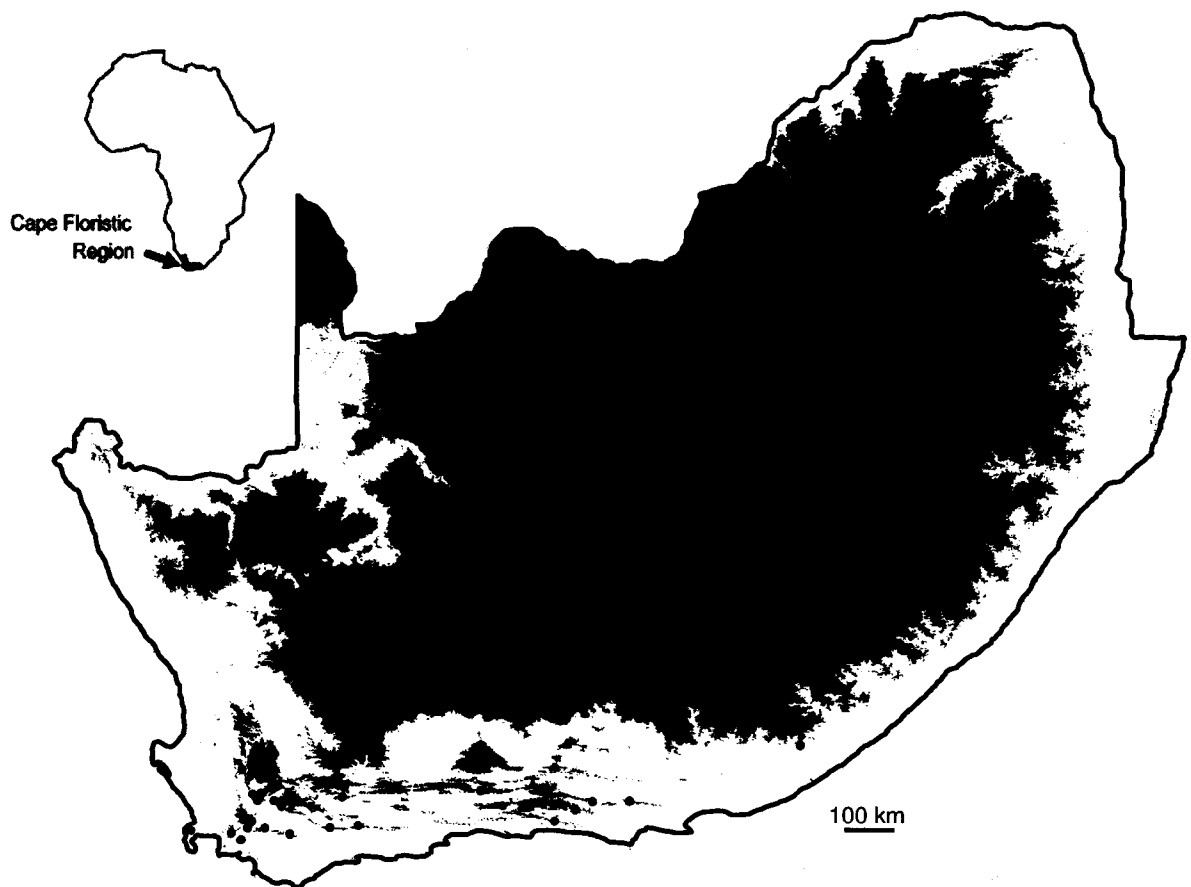


Figure 1.1: Distribution of known specimens of *Tetraria triangularis* derived from herbarium records and supplemented with new localities from this study. Most specimens are found in the CFR on the taller peaks of the Cape Fold Mountains. Outliers occur on the great escarpment at Somerset East and Hogsback, and the Natal Drakensberg at Cathedral Peak and Garden Castle

1985, Goldblatt 1997) mean that crossing of these “gaps” would be sufficiently rare to limit effective gene flow between populations (Linder 2003).

Fragmentation models have received little attention in the CFR, but the timing of first diversification in predominately highland CFR lineages tends to fall into a range consistent with a mid-late Miocene drying event (Richardson et al. 2001, Forest et al. 2007, Quint and Claßen-Bockhoff 2008). In most cases, however, taxon sampling is inadequate (e.g. 76% in van der Niet and Johnson [2009]) to permit meaningful inferences about the processes that have driven the most recent speciation events. In other areas characterised by complex topographies in which mountains are isolated by intervening lowland (termed Sky Islands by North American authors), there is considerable evidence for the influence of fragmentation process on speciation since the Last Glacial Maximum (LGM) (Masta 2000, Knowles 2001, DeChaine and Martin 2004, Galbreath et al. 2009). Some studies show evidence for the reconnection of populations on adjacent mountains during the LGM (Knowles 2001, Masta 2000), consistent with the requirements of the niche conservatism/niche tracking model of speciation proposed by Wiens (2004a). In addition several lineages contain species of a cryptic nature, characterised by low levels of morphological divergence, as one would expect if speciation is the result of niche conservatism and range fragmentation (Wiens 2004a). In the context of the CFR, low levels of allopatry amongst sister taxa are found when examining sister species pairs for evidence of recent speciation events (van der Niet and Johnson 2009). If speciation via niche conservatism is at work, this might be due to one or both of the “morphologically identifiable species” actually being composed of two or more cryptic species.

Within limits, climatically induced range fragmentation is expected to influence population differentiation and speciation in different lineages in a similar manner. This process offers a potential explanation for the existence of phytogeographical centres of endemism which have been identified across a number of clades (e.g. *Erica*, Proteaceae, and Restionaceae) that have radiated in the CFR (Weimarck 1941, Oliver et al. 1983, Goldblatt and Manning 2000). Oliver et al. (1983) found that endemic species richness is similarly distributed across phytogeographical areas in Restionaceae, *Erica*, Bruniaceae, *Aspalathus* and *Muraltia*. The boundaries between phytogeographical regions coincide with major barriers to dispersal, including deep river valleys and the east-west divide between winter and all-year rainfall zones, suggesting that these physical and environmental barriers have played a role in limiting gene flow. The probability of seeds or pollen traveling between peaks decreases with increasing distance between peaks. In the CFR the peaks that are separated by the greatest distances are also separated by the drainage systems of the major Cape Rivers. Thus, a species that is present on more than one peak is expected to occur on peaks on the same side of a major drainage line.

In this thesis, I use a combination of phylogenetic and phylogeographic tools to investigate genetic structure in *Tetraria triangularis* (Boeck.) C. B. Clarke, a widespread, high-altitude sedge species, native to the CFR (Fig. 1.1). *Tetraria triangularis* is a member of the reticulate-sheathed *Tetraria* clade, a group characterised by the possession of noded culms and distinctive fraying of the leaf sheathes to form a fine net around the base of the plant (Verboom 2006). The reticulate-sheathed *Tetraria* clade is in turn a member of the tribe Schoeneae (Verboom 2006). The focal species makes a suitable study system due its widespread distribution, with populations distributed throughout the

CFR, extending from the Cederberg and Cape Peninsula to the northern Drakensberg (Fig. 1.1). Its high altitude habitat (>900 m) accentuates the geographic separation between populations, and makes the designation of what constitutes population boundaries straightforward. Plants have an average height of 60 cm, with the leaves shorter than or equaling the height of the flowering culms (Levyns 1950, Archer 2000). Individuals are strong resprouters, such that populations should persist for long periods on peaks where they are able to establish, even if successful recruitment from seed is rare. The seeds of *T. triangularis* are hard nuts with no apparent modifications such as wings or eliasomes to facilitate long distance dispersal. Pollination is effected by wind, meaning that successful pollination over long distance would be entirely up to chance. *Tetraria triangularis* is common on open, dry, rocky slopes on both north and south aspects above 1000 m. This altitudinal minimum increases with distance from the coast (*pers obs*) and, on drier interior peaks and summits, plants are typically found in seasonally dry stream lines at altitudes greater than 1700 m.

In line with the hypothesis that historical range fragmentation has played an important role in shaping the accumulation of diversity within the CFR, I predict that:

- (i) Genetic diversity within *T. triangularis* is highly structured geographically. In addition, this structure should be correlated with topography and potentially congruent with previously recognised phytogeographical regions.
- (ii) *T. triangularis* potentially comprises a suite of species whose mode of speciation has, however, not stimulated the evolution of morphological divergence.

The first question is addressed in Chapter 2, in which I also attempt to infer the geographical centre of origin for *T. triangularis* as well as the route by which it has expanded its range. Chapter 3 deals with the second question and concludes with a reconsideration of the taxonomy of *T. triangularis*. Finally, Chapter 4 discusses the way in which the findings of this study inform our understanding of species richness and speciation process in the CFR.

Chapter 2: Genetic analyses of *T. triangularis* and its origins

Introduction

Overlaid on a complex topographical landscape, climatic oscillation may have powered allopatric speciation in montane lineages in the CFR (Linder 2003, Dynesius and Jansson 2000, Galbreath et al. 2009). Plants inhabit a particular altitudinal zone due to the prevalence of a particular set of precipitation and temperature conditions, required for growth and maintenance of stable population sizes (Hutchinson 1957, Wiens 2004a, Wiens and Graham 2005). When changes in climate cause a particular set of conditions to undergo altitudinal shifts, plants are predicted to “follow” their niche, through differential recruitment success in the direction in which the niche is moving (Wiens 2004a). During relatively cooler, wetter periods, mountain species are expected to occupy larger areas and populations on adjacent peaks will have smaller effective distances separating them, or even merge (Kozak and Wiens 2006). Warmer, drier periods tend to increase the geographical distances between populations, or isolate previously continuous populations, providing an opportunity for allopatric speciation to occur.

Linder (2003) invoked a similar mechanism of this type to explain the diversity of the high-altitude snow sugar bushes (*Protea cryophila* Bolus, *P. scabriuscula* E. Phillips, *P. pruinosa* Rourke, *P. scolopendrifolia* (Salisb. Ex Knight) Rourke) characterised by high-altitude distributions and non-overlapping ranges. Many altitudinally restricted *Protea* species, including *P. rupicola* Mund ex Meisn, *P. grandiceps* Tratt., *P. laevis* R. Br., *P. revoluta* R. Br. and *P. effusa* E. Mey. Ex Meisn, have distinctly allopatric ranges with confirmed gaps in distribution (Rebelo 1995). Although isolation between

populations of these species is not reflected by morphological divergence, it is possible that disjunct populations are in fact distinct evolutionary species as a result of restricted gene flow between populations, accentuated by trait or genetic fixation in small populations (Gittenberger 1991, Rundell and Price 2009). Masta (2000) showed that climate-induced contraction of woodlands fragmented populations of jumping spiders, resulting in monophyletic groups on isolated peaks. Owing to the restricted extent of the populations confined to mountain peaks, a pattern of strong gene tree structure with limited morphological divergence and deep genetic divergence is typical of such systems (Avice 2000). This pattern can be seen as a result of similar selective regimes being shared between mountain top habitats, providing little impetus for divergence from the ancestral morphology of sister populations (Wiens 2004a).

Above the species level, the interaction of climatic cycles and complex topography may explain the existence of “phytogeographic areas” or “centres of endemism” in the mountains of the CFR. The breaks between these areas often coincide with climatic, substrate and/or topographical boundaries, which could act as barriers to dispersal and gene flow. Weimarck (1941) attempted to designate centres of endemism in the CFR and recognised five areas (Fig. 2.1a). A later effort by Oliver et al. (1983), which described six areas of a distinctly different character (Fig. 2.1b), recognised lowland and highland centres as distinct. Subsequent authors (Cowling et al. 1992, Goldblatt and Manning 2000, Linder 2001, Linder 2003) have tended to use modified or combined versions of the Weimarck (1941) and Oliver et al. (1983) classifications (Fig. 2.1c-f).

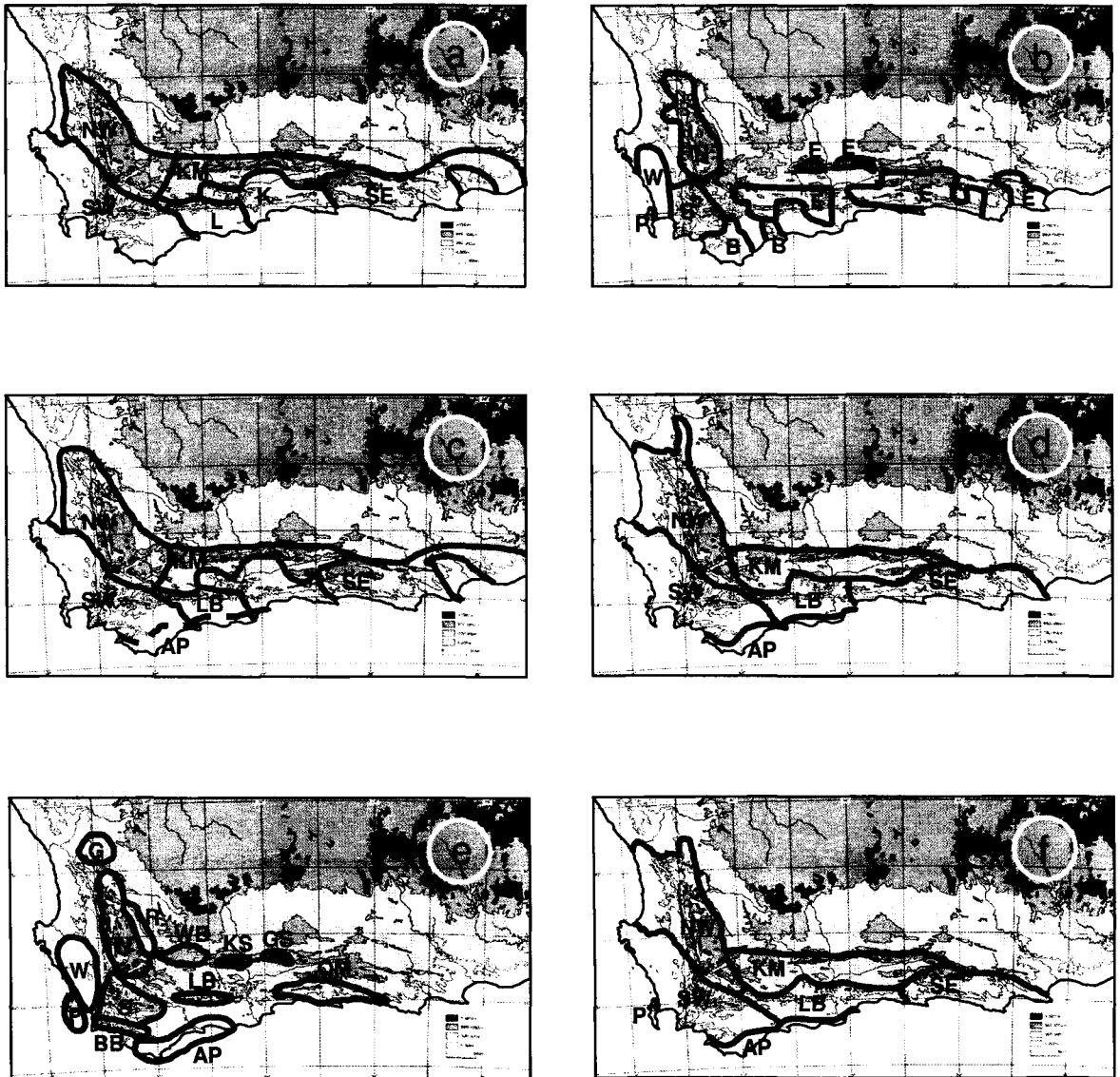


Figure 2.1: Representations of Phytogeographic Regions/Areas of endemism presented by (a) Weimarck (1941), (b) Oliver et al (1983), (c) Cowling et al (1992), (d) Goldblatt and Manning (2000), (e) Linder (2001) and (f) Linder (2003). Names have been altered to allow for ease of comparison, but boundaries are as near as possible to those laid out by the authors. .

Abbreviations: AP: Agulhas Plain, B: Bredasdorp, BB: Bettys Bay, E: Eastern Mountains, G: Gifberg, GS: Groot Swartberg, KS: Klein Swartberg, K: Knysna Interval, KM: Karoo Mountain, LB: Langeberg, N: Northern Mountains, NW: Northwest, OM: Outeniqua Mountains, P: Peninsula, R: Swart Ruggens, S: Southern mountains, SE: Southeast, SW: Southwest, W: West Coastal, WB: Witteberg

Linder (2001) examined endemism by assigning specimens of African Restionaceae to 0.0625 ° grid squares (Edwards and Leister 1971), thereafter clustering these grid squares to find areas of endemism (Fig. 2.1e). The resulting treatment resembles that of Oliver et al. (1983) in identifying Peninsula, West Coast and Agulhas Plain Centres, but differs in the recognition of individual mountain ranges as areas of endemism. Linder (2001) advanced the idea that larger areas of endemism may contain smaller areas of endemism and that it is important to use biologically meaningful areas to understand the relationships between patterns of endemism and speciation process. Since many lineages show congruent endemism patterns, it is logical to assume that these common barriers to dispersal have powered speciation at least in the montane lineages of the CFR.

Phylogeographic studies provide a powerful means for testing the role of topography and historical climate change in shaping the structure of genetic diversity within species and within complexes of closely related species (Knowles 2009). Population studies of *Melanoplus* grasshoppers (Knowles 2000) and *Habronattus* jumping spiders (Masta 2000) on sky islands in the Rocky Mountains and Southeastern Arizona, respectively, demonstrate that population-level sampling combined with estimation of divergence times allows examination of the effects of recent Pleistocene glaciation cycles in facilitating diversification and/or speciation in montane lineages. Ikeda and Setoguchi (2007) studied the phylogeography of the alpine plant *Phyllodoce nipponica* Makino, using the group to infer refugial areas for the Japanese alpine flora during Quaternary climate fluctuations. Kebede et al. (2007) explored genetic relationships using AFLPs between populations of *Lobelia giberroa* Hemsl. in the East

African highlands and found that well supported phylogeographic breaks within this species were congruent with interspecific boundaries as previously revealed by Knox and co-workers (Knox and Palmer 1998, Knox 1999).

Within the context of the CFR, phylogeographic studies are few and biased toward reptiles, mammals and insects, but work on dwarf chameleons (Tolley et al. 2009) and lizards (*Agama atra* Daudin; Swart and Matthee 2009) indicate the existence of abundant cryptic lineages in the CFR. The distributions of these lineages are not congruent with the recognised plant phylogeographical regions. Although these studies represent a great improvement on the resolution provided by morphological taxonomy alone, they would be improved by denser sampling across the CFR to allow the delimitation of distribution ranges of the cryptic lineages. While differences in the distribution patterns of different organisms are influenced by many factors, including organismal mobility and to the nature and location of dispersal barriers, it is worth noting that our ability to detect geographical structuring at the molecular level is determined by a number of other factors, including relative population sizes (Tajima 1983, Avise et al. 1988), the time since divergence (Avise and Ball 1990), the relative mutation rates of gene regions utilised (Hillis 1987, Avise 1994) and the availability of discordant gene histories within the lineage of interest (Knowles and Carstens 2007).

Modern phylogeography reflects a shift away from the descriptive techniques proposed by Templeton and co-workers (Templeton et al. 1992, Templeton and Sing 1993, Templeton 1995, Templeton 1998, Templeton 2004) toward approaches in which *a priori* hypotheses are statistically evaluated (Knowles 2004, Knowles 2008, Petit 2008). These approaches are, however, limited by their inability to deal with more complex

scenarios where *a priori* hypotheses are not readily formulated and do not allow for the inclusion of spatially explicit information to inform inferences (Lemmon and Lemmon 2008). They are also reliant on the use of multiple loci and the existence of polymorphisms within and between populations i.e. limited fixation of haplotypes between populations. Since populations in this study showed high levels of haplotype fixation with most pattern being based on information from a single marker (chloroplast DNA), the approach followed here is necessarily phylogenetic. In reconstructing historical range shifts in *T. triangularis*, I apply a likelihood-based method, which utilises a known gene tree in conjunction with the geographic co-ordinates of individuals at the tips, to estimate the geographic co-ordinates of ancestors at internal nodes in the tree (Lemmon and Lemmon 2008). The procedure is analogous to the reconstruction of continuous character states on a phylogeny (Schluter et al. 1997).

The aims of this chapter are: (i) to evaluate haplotype variation in *Tetraria triangularis* and to assess whether it is geographically structured; (ii) to determine whether haplotype boundaries coincide with major topographical breaks in the CFR, large distributional gaps and/or ecological boundaries; (iii) in the context of a dated tree, to determine the centre of origin of *T. triangularis* and the possible routes by which it expanded its range; and (iv) to determine whether haplotype divergence coincided with late Miocene aridification or whether it was more recent, perhaps being associated with Quaternary climate oscillations (Petit et al. 1999, Linder 2003, Verboom et al. 2009).

Methods

Field sampling, DNA isolation, amplification, sequencing and sequence alignments

Samples were collected from 37 localities representing as complete as possible a sampling of all *T. triangularis* populations shown to be geographically distinct as informed by the minimum altitude at which this species occurs (Table 2.1). Between 6 and 30 plants were collected per population, these being more than 100 m apart to ensure that individuals were a random sample, rather than a set of closely related individuals. One entire tiller was sampled from each plant and subsequently pressed to make a voucher specimen. Leaf samples were collected from neighbouring ramets of the same plant. The leaf samples were stored directly on silica gel in sealable plastic bags to ensure rapid dehydration and to prevent the growth of pathogens, allowing for DNA to be extracted successfully some time after collection (Chase and Hills 1991).

DNA was extracted from 5-24 individuals from each population using a standard cetyl trimethyl ammonium bromide (CTAB) protocol, with minor modifications (Doyle and Doyle 1987, Gawel and Jarret 1991). Leaf samples were ground in liquid nitrogen with a mortar and pestle to pulverise tough fibres, prior to the addition of CTAB.

Chloroplast haplotype variation across the range of *T. triangularis* was evaluated using the *trnH-psbA* intergenic spacer (Sang et al. 1997, Tate and Simpson 2003), owing to the high levels of variability shown by this marker. Thirty six populations were sampled for this marker, with two to 24 individuals being sequenced per population, these being sampled in such a way as to the maximize the spatial distances between sample plants (Table 2.1). Since the standard *trnH-psbA* primers (Tate and Simpson 2003, Sang et al. 1997) displayed non specific binding, it was necessary to design the

Table 2.1: Summary of samples used for and molecular analyses of *T. triangularis* complex. Rows show population localities and numbers of individuals sampled for each of the loci.

No.	Population name	Longitude °S	Latitude °E	Molecular locus					
				<i>trnH-psbA</i>	<i>rbcL</i>	<i>rps16</i>	<i>trnL-trnF</i>	ETS	<i>3'trnV-ndhC</i>
1	Sneeuberg	-32.48	19.14	8	0	0	0	0	0
2	Sneekop	-32.36	19.16	8	0	0	0	1	0
3	Koue Bokkeveld Sneeberg	-32.84	19.38	7	0	0	0	0	0
4	Groot Winterhoek	-33.12	19.17	6	0	0	0	0	0
5	Hanjesberg	-33.18	19.3	8	1	1	3	1	1
6	Wamboomsberg	-33.25	19.45	7	0	0	0	0	0
7	Vleiberg	-33.03	19.6	10	0	0	1	1	2
8	Baviaansberg	-33.21	19.59	6	0	0	0	0	0
9	Matroosberg	-33.38	19.67	9	0	0	3	0	1
10	Hogsback	-32.62	27	2	0	0	0	0	0
10	Milner Peak	-33.46	19.45	2	0	0	0	0	0
11	Western Hex	-33.5	19.37	8	0	0	1	1	0
12	Keeromsberg	-33.56	19.63	8	0	0	0	1	1
13	Naudesberg	-33.65	19.73	11	0	0	1	0	1
14	Du Toit's Peak	-33.75	19.2	17	0	1	2	1	1
15	Wemmershoek Peak	-33.8	19.17	24	1	2	3	2	3
16	Stettyns Peak	-33.86	19.3	8	0	0	2	0	0
17	Blokkop	-33.97	19.24	5	0	0	1	0	0
18	Mount Lebanon	-34.15	19.14	5	0	0	0	0	0
19	Landroskop	-34.04	18.98	9	0	0	0	0	0
20	Table Mountain	-33.96	18.43	8	1	1	1	1	1
21	Jonaskop	-33.97	19.5	18	0	1	4	2	2
22	Pilaarkop	-34.06	19.85	11	1	1	3	2	2
23	Misty Point	-33.96	20.44	5	0	0	0	2	1
24	Boosmansbos	-33.93	20.86	5	0	0	1	1	1
25	Kammanasie Mountain	-33.64	22.95	8	0	1	3	4	3
26	Hoopsberg	-33.66	23.28	8	0	0	0	0	0
27	Formosa Peak	-33.86	23.7	6	0	0	3	3	3
28	Kouga Kop	-33.72	23.99	9	0	0	3	2	2
29	Scholtzberg	-33.58	24.25	7	0	0	0	0	0
30	Cockscomb	-33.57	24.79	8	0	0	1	0	1
31	Bosberg	-32.7	25.53	6	0	1	2	2	2
33	Blesberg	-33.41	22.63	6	0	0	0	1	1
34	Swartberg	-33.35	22.04	8	0	0	1	0	0
35	Bloupunt	-33.4	21.44	7	0	0	0	0	0
36	Seweweekspoortberg	-33.38	21.36	7	1	1	1	1	1
37	Anysberg	-33.51	20.62	8	0	0	3	1	0
Total				303	5	10	43	30	30

species-specific primers; *psbA*^{TGC}, *trnH*^{ACT} and *psbAAAR*, to facilitate amplification (Table 2.3). Reactions were performed on ice in 30 µl volumes each containing 3 µl of 10 × DNA polymerase buffer, 0.6 µl of MgCl₂ (50 mM), 1.0 µl of each primer (10 µM), 1.2 µl of dNTP (10mM), 2.5 Units of Kapa *Taq* (Kapa Biosystems, Cape Town, South Africa) DNA polymerase and 2.0 µl of template DNA, and the balance made up with PCR water.

In order to obtain a robust estimate of the gene tree relating these haplotypes, a single carrier of each *trnH-psbA* haplotype (Table 2.2) was sequenced for an additional suite of plastid loci: *rbcL*, *rps16*, *trnL-trnF*, *3'trnV-ndhC* (Table 2.2, Table 2.3). Relationships among these individuals were also evaluated using an independently-assorting nuclear marker, a portion of the external transcribed spacer of the nuclear ribosomal DNA locus (ETS1f; Hershkovitz 1999, Starr et al. 2003). Amplification was carried out on an Applied Biosystems GeneAmp 2700 thermal cycler (Applied Biosystems, Foster City, CA, USA) using the following program; initial denaturation phase for 2 min at 94 °C; followed by 30-50 cycles of 60s at 94°C, 60 s at 52 °C and 2 min at 72 °C; and a final extension of 7 min at 72 °C. The PCR protocol was identical for all primers, the only adjustment made to the PCR protocol being to increase the number of cycles if amplification was insufficient. 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).

Table 2.2. Summary of unique *trnH-psbA* haplotypes included in phylogenetic analyses, indicating haplogroup membership of each haplotype. Haplogroups are sets of haplotypes with no base pair variation, but with differing lengths. X's mark samples if sequenced for a particular region

Haplogroup	Haplotype	Chloroplast					Nuclear
		<i>rbcL</i>	<i>rps16</i>	<i>trnL-trnF</i>	<i>trnH-psbA</i>	<i>3'trnV-ndhC</i>	ETS
Karoo Mountain	KM1	X	X	X	X	X	X
Karoo Mountain	KM2			X	X	X	X
Karoo Mountain	KM3			X	X	X	X
Karoo Mountain	KM4			X	X	X	X
Karoo Mountain	KM5		X	X	X	X	X
Karoo Mountain	KM6				X		
Karoo Mountain	KM7			X	X	X	X
Karoo Mountain	KM8				X		
Seweweekspoortberg	7W1		X	X	X	X	X
Anysberg	AB1			X	X		
Anysberg	AB2				X	X	X
Northwest	NW1		X	X	X		X
Northwest	NW2	X	X	X	X	X	X
Northwest	NW3			X	X		
Southwest	SW1			X	X	X	X
Southwest	SW2			X	X	X	X
Southwest	SW3	X	X	X	X	X	X
Hawequas	H1			X	X		
Hawequas	H2	X	X	X	X	X	X
Hawequas	H3	X	X	X	X	X	X

Table 2.3: Primers utilised for PCR amplification and sequencing of DNA

Locus	Primer name	Primer sequence	Amp	Seq	Reference
<i>rbcL</i>	rbc3	5' CTT TTA GTA AAA GAT GGG CCG AG 3'	Y	Y	Olmstead et al. 1992
<i>rbcL</i>	rbc5	5' ATG TCA CCA CAA ACA GAG AC 3'	Y	Y	Olmstead et al. 1992
<i>rps16</i>	rpsF	5' GTG GTA GAA AGC AAC GTG CGA CTT 3'	Y	Y	Oxelman et al. 1997
<i>rps16</i>	rpsR	5' TCG GGA TCG AAC ATC AAT TGC AAC 3'	Y	Y	Oxelman et al. 1997
<i>trnL-trnF</i>	trnC	5' CGA AAT CGG TAG ACG CTA CG 3'	Y	Y	Taberlet et al. 1991
<i>trnL-trnF</i>	trnF	5' TTT GAA CTG GTG ACA CGA G 3'	Y	Y	Taberlet et al. 1991
<i>trnL-trnF</i>	trnD	5' GGG GAT AGA GGG ACT TGA AC 3'	N	Y	Taberlet et al. 1991
<i>trnL-trnF</i>	trnE	5' GGT TCA AGT CCC TCT ATC CC 3'	N	Y	Taberlet et al. 1991
<i>ETS1-F</i>	ETS-1F	5'CTG TGG CGT CGC ATG AGT TG 3'	Y	Y	Starr et al. 2003
<i>ETS1-F</i>	18S-R	5' AGA CAA GCA TAT GAC TAC TGG CAG G 3'	Y	Y	Starr et al. 2003
<i>trnH^{GUG}-psbA</i>	psbA	5' GTT ATG CAT GAA CGT AAT GCT AAT GCT C 3'	Y*	N	Sang et al. 1997
<i>trnH^{GUG}-psbA</i>	psbA^{TGC}	5' TGC TGT TGA GTT CCT TCT ATC G 3'	Y	Y	This Study
<i>trnH^{GUG}-psbA</i>	trnH	5' CGC GCA TGG TGG ATT CAC AAA TC 3'	Y*	N	Tate and Simpson 2003
<i>trnH^{GUG}-psbA</i>	trnH^{ACT}	5' ACT GCC TTG ATC CAC TTG GC 3'	Y	Y	This Study
<i>trnH^{GUG}-psbA</i>	psbAAAR	5' AAA AAA AAA AGA GAA ATA CCC 3'	N	Y	This Study
<i>3'trnV-ndhC</i>	trnV^(UAC)	5' GTC TAC GGT TCG A(AG)T CCG TA 3'	Y	Y	Takahashi et al. 2005
<i>3'trnV-ndhC</i>	ndhC	5' TAT TAT TAG AAA TG(CT) CCA (AG)AA AAT ATC ATA 3'	Y	Y	Takahashi et al. 2005
<i>3'trnV-ndhC</i>	trnV_15	5' GCT TTT ATT TTC TCA TTT C 3'	N	Y	This Study
<i>3'trnV-ndhC</i>	ndhC_44F	5' TTT ATA GAT TGG TTA GTC G 3'	N	Y	This Study

Outgroups (Table 2.4) were selected such that a dating analysis could be calibrated using the dated node separating the Mapanioid sedges from the rest of Cyperaceae (Bremer 2002). Selection of outgroup taxa within the core Cyperaceae was informed by the topology of Verboom (2006), with all available sequences obtained from GenBank. Where sequences were not found on GenBank, they were generated using the methods described here.

Sequences were compiled using the program ChromasPro v 1.4.2 (www.technelysium.com.au/) and aligned using the program Bioedit v7.0.5 (Hall 2005). Stretches of sequence that could not be meaningfully aligned in some taxa (parts of the *rps16*, *trnL-trnF*, *trnH-psbA*, *3'trnV-ndhC* and ETS regions) were typically marked as unknown (?) for the unalignable taxa (typically outgroups). In cases where reliable alignment was impossible across all taxa (for example before and after homopolymers in the *rps16*, *trnL-trnF*, *trnH-psbA*, *3'trnV-ndhC* and ETS loci) the unalignable regions were excluded from the analyses entirely. The rationale in both cases was to minimise the number of false primary homologies.

Phylogenetic analysis

Phylogenetic relationships were inferred using both parsimony and Bayesian inference. In the absence of any supported conflict amongst the chloroplast loci, these were analysed in combination. In order to assess potential topological conflict between plastid and nuclear gene histories, the topologies produced by analysis of the combined chloroplast and nuclear data sets were compared by visual inspection. These included only those accessions common to both data sets. Owing to the presence of supported

Table 2.4. GenBank accession numbers (N/A = accession not available; sequence unpublished) and voucher information (collector, number, herbarium) relating to sequences used as outgroups.

Species	<i>rbcL</i>	<i>rps16</i>	<i>trnL - trnF</i>	<i>trnH-psbA</i>	3' <i>trnV-ndhC</i>	ETS
<i>Tetraria secans</i> C. B. Clarke	N/A (S. Africa: Verboom 653, BOL)	—	N/A (S. Africa: Verboom 653, BOL)	N/A (S. Africa: Verboom 653, BOL)	N/A (S. Africa: Verboom 653, BOL)	N/A (S. Africa: Verboom 653, BOL)
<i>Tetraria compressa</i> Turill	N/A (S. Africa: Verboom 658, BOL)	N/A (S. Africa: Verboom 658, BOL)	N/A (S. Africa: Verboom 658, BOL)	N/A (S. Africa: Verboom 658, BOL)	N/A (S. Africa: Verboom 658, BOL)	N/A (S. Africa: Verboom 658, BOL)
<i>Tetraria thermalis</i> (L.) C. B. Clarke	N/A (S. Africa: Verboom 643, BOL)	DQ058329 (S. Africa: Verboom 643, BOL)	N/A (S. Africa: Verboom 643, BOL)	DQ058308 (S. Africa: Verboom 643, BOL)	N/A (S. Africa: Verboom 643, BOL)	N/A (S. Africa: Verboom 643, BOL)
<i>Tetraria eximia</i> C. B. Clarke	N/A (S. Africa: Verboom 647, BOL)	—	—	N/A (S. Africa: Verboom 647, BOL)	—	—
<i>Tetraria sp. nov. aff. T. eximia</i>	N/A (S. Africa: Verboom 720, BOL)	—	—	—	N/A (S. Africa: Verboom 720, BOL) 3' portion	N/A (S. Africa: Verboom 720, BOL)
<i>Tetraria involucrata</i> (Rottb.) C. B. Clarke	N/A (S. Africa: Verboom 661, BOL)	N/A (S. Africa: Verboom 661, BOL)	N/A (S. Africa: Verboom 661, BOL)	N/A (S. Africa: Verboom 661, BOL)	—	—
<i>Tetraria bromoides</i> Lam.	N/A (S. Africa: Verboom 641, BOL)	—	—	N/A (S. Africa: Verboom 641, BOL)	N/A (S. Africa: Verboom 641, BOL)	—
<i>Tetraria flexuosa</i> Thunb.	N/A (S. Africa: Verboom 505, BOL)	N/A (S. Africa: Verboom 505, BOL)	N/A (S. Africa: Verboom 505, BOL)	N/A (S. Africa: Verboom 505, BOL)	N/A (S. Africa: Verboom 505, BOL)	N/A (S. Africa: Verboom 505, BOL)
<i>Tetraria microstachys</i> (Vahl) Pfeiffer	DQ058347 (S. Africa: Verboom 640, BOL)	DQ058328 (S. Africa: Verboom 640, BOL)	DQ058307 (S. Africa: Verboom 640, BOL)	N/A (S. Africa: Verboom 640, BOL)	N/A (S. Africa: Verboom 640, BOL)	N/A (S. Africa: Verboom 640, BOL)
<i>Tetraria nigrovaginata</i> (Nees) C. B. Clarke	N/A (S. Africa: Verboom 500, BOL)	N/A (S. Africa: Verboom 500, BOL)	N/A (S. Africa: Verboom 500, BOL)	—	N/A (S. Africa: Verboom 500, BOL)	N/A (S. Africa: Verboom 500, BOL)

Table 2.4. GenBank accession numbers (N/A = accession not available; sequence unpublished) and voucher information (collector, number, herbarium) relating to sequences used as outgroups.

Species	<i>rbcL</i>	<i>rps16</i>	<i>trnL - trnF</i>	<i>trnH-psbA</i>	3' <i>trnV-ndhC</i>	ETS
<i>Tricostularia pauciflora</i> (F. Muell.) Benth	AY725954 (Muasya et al., 2000)	—	AY230038 (Muasya et al., 2000)	—	—	—
<i>Schoenus nigricans</i> L.	Y12983 (Muasya et al., 1998)	DQ058331 (Saudi Arabia: Edmonson 3382, K)	DQ058310 (Saudi Arabia: Edmonson 3382, K)	—	—	—
<i>Epischoenus</i> <i>quadrangularis</i> (Boeck.) C. B. Clarke	DQ058349 (S. Africa: Verboom 636, BOL)	DQ 058332 (S. Africa: Verboom 636, BOL)	DQ058311 (S. Africa: Verboom 636, BOL)	—	—	—
<i>Tetraria crassa</i> Levyns	DQ058352 (S. Africa 507, BOL)	DQ058334 (S. Africa 507, BOL)	DQ058314 (S. Africa 507, BOL)	—	—	—
<i>Capeobolus brevicaulis</i> C. B. Clarke	DQ058343 (S. Africa: Verboom 646, BOL)	DQ058324 (S. Africa: Verboom 646, BOL)	DQ058303 (S. Africa: Verboom 646, BOL)	—	—	—
<i>Costularia natalensis</i> C. B. Clarke	DQ058345 (S. Africa: Verboom 773, BOL)	DQ058326 (S. Africa: Verboom 773, BOL)	DQ058305 (S. Africa: Verboom 773, BOL)	—	—	—
<i>Neesebeckia punctoria</i> (Vahl) Levyns	DQ058346 (S. Africa: Verboom 650, BOL)	DQ058327 (S. Africa: Verboom 650, BOL)	DQ058306 (S. Africa: Verboom 650, BOL)	—	—	—
<i>Machaerina</i> sp.	DQ058340 (New Guinea: Johns 9195, K)	DQ058321 (New Guinea: Johns 9195, K)	DQ 058300 (New Guinea: Johns 9195, K)	—	—	—

Table 2.4. GenBank accession numbers (N/A = accession not available; sequence unpublished) and voucher information (collector, number, herbarium) relating to sequences used as outgroups.

Species	<i>rbcL</i>	<i>rps16</i>	<i>trnL - trnF</i>	<i>trnH-psbA</i>	3' <i>trnV-ndhC</i>	ETS
<i>Gahnia baniensis</i> Benl.	DQ058342 (Malaysia, Simpson 2737)	DQ058323 (Malaysia, Simpson 2737)	DQ058302 (Malaysia, Simpson 2737)	—	—	—
<i>Mesomelaena pseudostygia</i> (Kuek.) K. L. Wilson	DQ058341 (Australia: Chase 2226, K)	DQ058322 (Australia: Chase 2226, K)	DQ058301 (Australia: Chase 2226, K)	—	—	—
<i>Evandra aristata</i> R. Br	AY725944 (Muasya et al., 2000)	—	—	—	—	—
<i>Caustis diocia</i> R. Br.	Y12976 (Muasya et al., 1998)	—	—	—	—	—
<i>Carpha alpina</i> R. Br.	AF307910 (Wardle et al., 2001)	—	AY230012 (Zhang et al., 2004)	—	—	—
<i>Cyperus rigidifolius</i> Steud.	Y13016 (Muasya et al., 1998)	AF449535 (Muasya et al., 2002)	AY040600 (Muasya et al., 2001)	—	—	—
<i>Ficinia paradoxa</i> (Schrud.) Nees	DQ058354 (S. Africa: Verboom 534, BOL)	—	DQ 058317 (S. Africa: Verboom 534, BOL)	—	—	—
<i>Rhynchospora brownii</i> Roem. et Schult.	DQ058353 (S. Africa: Verboom 616, BOL)	DQ058336 (S. Africa: Verboom 616, BOL)	DQ058316 (S. Africa: Verboom 616, BOL)	N/A (S. Africa: Verboom 616, BOL)	N/A (S. Africa: Verboom 616, BOL)	N/A (S. Africa: Verboom 616, BOL)
<i>Scleria distans</i> Poir. Schoeneae	DQ058339 (Kenya: Muasya 1023, EA, K)	DQ058320 (Kenya: Muasya 1023, EA, K)	DQ058299 (Kenya: Muasya 1023, EA, K)	—	—	—
<i>Gymnoschoenus. Shaeerocehalus</i> Nees	AY725945 (Muasya et al., 2000)	—	AY230033 (Zhang et al., 2004)	—	—	—

Table 2.4. GenBank accession numbers (N/A = accession not available; sequence unpublished) and voucher information (collector, number, herbarium) relating to sequences used as outgroups.

Species	<i>rbcL</i>	<i>rps16</i>	<i>trnL - trnF</i>	<i>trnH-psbA</i>	3' <i>trnV-ndhC</i>	ETS
<i>Cladium mariscus</i> (L.) R. Br	DQ058338 (Locality unknown: MJC 292, K)	DQ058319 (Locality unknown: MJC 292, K)	DQ058298 (Locality unknown: MJC 292, K)	N/A (Locality unknown: MJC 292, K)	N/A (Locality unknown: MJC 292, K)	N/A (Locality unknown: MJC 292, K)
<i>Hyplopytrum nemorum</i> (Vahl) Spreng.	Y12958 (Muasya et al., 1998)	AY344142 (Simpson et al., 2003)	AJ5777325 (Dhooge et al., 2003)	—	—	—
<i>Mapania cuspidata</i> (Miq.) Uittien	DQ058337 (Brunei: Marsh 4, K)	DQ058318 (Brunei: Marsh 4, K)	DQ058297 (Brunei: Marsh 4, K)	N/A (Brunei: Marsh 4, K)	—	—

conflict, no combined chloroplast-nuclear analysis was performed. However, a broader-sampled, chloroplast analysis was performed. The data used in each analysis are summarised in Table 2.2 (*T. triangularis*) and Table 2.4 (outgroups). All trees were rooted on the Hypolytreae (*Mapania cuspidata* and/or *Hypolytrum nemoralis*) as this group has been shown to be sister to the rest of the Cyperaceae (Muasya et al. 1998, Simpson et al. 2003). Parsimony analyses were performed in PAUP* version 4.01b10 (Swofford 2002), with 10 000 random addition replicates (Settings: TBR branch swapping, MULTREES in effect and retaining all of the shortest trees). Ten thousand bootstrap replicates (Felsenstein 1985) were performed to assess node support (Settings: simple addition sequence, TBR branch swapping and MULTREES in effect).

Bayesian analyses were performed using MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003), which allows different data partitions to take separate models of character evolution, their parameters being estimated from the data themselves. Separate GTR + I + Γ models were applied to each sequence region, for the analysis of the combined chloroplast (*rbcL*, *rps16*, *trnL-trnF*, *psbA* and 3'*trnV-ndhC*) and chloroplast and nuclear (ETS) datasets. This most complex available model was chosen in order to avoid model under-parameterisation, as recommended by Huelsenbeck and Rannala (2004). In each case parameters were treated as unknown, with uniform prior probabilities, and were estimated along with the tree topologies. Each analysis was performed with four Markov chains (three hot and one cold), each using a random starting tree. Each chain was run for 25×10^6 generations, sampled every 100th generation, yielding 25 000 samples for each run. Plots of the $-\log$ likelihoods against generation time and the comparison of posterior probabilities (PP) were used to

determine if and when stationary was achieved and to identify the burn-in period (Huelsenbeck et al. 2002). The trees from this initial burn-in were excluded from the summary tree and the calculation of Bayesian posterior probabilities for each node.

Association of clades with geographical area, elevation and climate

Several of the sampled *trnH-psbA* haplotypes were distinguished only by variation within a complex repeat sequence (Position 220-970 of 1100). Since length variation in this region could not be used to resolve relationships amongst haplotypes (due to ambiguous homology assessment), closely related haplotypes were amalgamated into haplogroups based on patterns of base pair variation on either side of the repeat sequence. Haplogroups were then treated as units of interest for the exploration of geographic structure in *trnH-psbA* variation.

The distributions of the major *trnH-psbA* haplogroups supported by the multi-locus phylogenetic analysis were plotted onto a map of the CFR, using ArcMap 9.2 (ESRI, 2008). To evaluate whether haplogroup turnover in the landscape was significantly associated with geographical isolation, topographical barriers (deep and /or more ancient river valleys) and/or contrasting bioclimatic conditions, metrics were derived to quantify separation between haplogroups for each of these. For example, the mean geographic distance between a pair of haplotypes, A and B, was calculated as the average of all geographical distances (straight line paths) separating populations containing haplogroup A individuals from those containing haplogroup B individuals. For this purpose, populations containing multiple haplogroups were treated as being comprised of multiple populations, each characterised by its own haplogroup. Thus,

where multiple haplogroups occurred at one site, this would yield a 'zero distance' between the co-occurring haplogroups. Similarly, bioclimatic distances were calculated as the mean straight line path lengths, in bioclimatic multivariate space, that connect populations of differing haplogroup. Finally, the topographical separation between haplogroups was calculated as the average of the minimum altitudes along the highest-elevation paths connecting populations of differing haplogroup.

The mean bioclimatic contrast between sites occupied by two haplogroups was calculated using data extracted from BIOCLIM (Hijmans 2005; <http://www.worldclim.org/bioclim.htm>). This 19-dimensional quantity for each population was then turned into a Euclidian distance matrix, to give a one-dimensional metric of "climate distance" between populations. Although it is common in niche modeling to reduce the number of variables by performing a correlation analysis of the variables and removing highly correlated variables through the use of a Pearson's coefficient (Phillips et al. 2006), this was not done here, since a different set of variables would need to be used for each haplogroup, making comparisons problematic. The aim of this exercise was to test whether haplogroups may have adapted to varying climates across the CFR, which may limit successful establishment on different mountain peaks. Topographical isolation was estimated by measuring the absolute maximum depth of valleys separating peaks (i.e. minimum altitude). This metric explores the impact of deep valleys on limiting gene flow and seed transport between groups of populations, and the potential effect of these larger barriers on the distribution of populations with the same haplogroup. It does not, however, accommodate the potential role of edaphic barriers in determining the probability of successful dispersal between peaks.

To test whether the mean geographical, topographical or bioclimatic separation between a particular pair of haplogroups was greater than would be expected by chance (i.e. observed association of haplogroup distribution with geographical distance is significant), the observed mean was compared against a null distribution of 1000 expected means derived by randomly shuffling the haplogroups amongst the populations, keeping the numbers of populations per haplogroup constant.

In the case of topographical isolation, I tested whether the mean lowest altitude connecting the populations with a common haplogroup was less than expected on the basis of chance, while in the case of bioclimatic contrasts, I evaluated whether the mean bioclimatic distance between haplogroups was greater than expected by chance. The metric for the lowest altitude between peaks is different, because we want to know whether the deeper valleys separate populations with different haplogroups, rather than whether one haplogroup has deeper valleys between populations than another. This was performed using a taxon shuffling procedure based on an R script (R version 2.8.1; R Development Core Team 2008) using the 'ade4' (Dray and Dufour 2007), 'ape' (Paradis et al. 2004) and 'picante' (Kembel et al. 2009) packages.

Knowing the geographical distance between populations with different haplogroups is of interest because successful gene flow by propagules is expected to decrease with increasing distance. Thus, more isolated peaks may be expected to have distinct haplogroups. The height difference between peaks is important, if we consider that successful propagule dispersal may be enhanced during periods when suitable *T. triangularis* habitat is found further downslope than at present, covering larger and more continuous areas. If this scenario is plausible, we may expect that the effect of a relatively

small geographic distance will be “amplified” by the depth of the valley separating populations. The use of a climatic variable accepts that rainfall regime is non-uniform across the CFR and that successful colonisation and competition among closely related populations may be limited by subtle differences in growth potential between various mountain peaks.

Molecular Dating

I performed a molecular dating analysis using the program BEAST version 1.4.7 (Drummond and Rambaut 2007), which accommodates variation in substitution rate amongst branches by allowing each branch to draw its rate from a discretized lognormal distribution estimated from the data (Drummond et al. 2006). This lognormal relaxed clock was preferred over non parametric rate smoothing (NPRS; Sanderson 1997), penalised likelihood (PL; Sanderson 2002) and the relaxed Bayesian approach implemented in Multidivtime (Thorne and Kishino 2002), because it does not assume rate autocorrelation, which may reduce the ratio of deep to shallow nodes through the systematic distortion of branch lengths (Hugall and Lee 2004, Martin et al. 2004). In addition, BEAST simultaneously estimates the topology along with the node ages, thus allowing sequence divergences to inform topology estimation (Drummond et al. 2006). Due to incongruence between the chloroplast and nuclear partitions of the data set, separate BEAST analyses were carried out on each partition. The data sets were the same as those used for parsimony and Bayesian inference of topology in each case.

Thirty outgroup taxa were added to facilitate calibration. Sequences were obtained from Genbank (NCBI: www.ncbi.nlm.nih.gov/), with additional loci sequenced using the

methods described earlier (Table 2.3, 2.4). In the absence of appropriately recent fossils, calibration was done indirectly, applying a date of 44.1 ± 8.8 Ma (Bremer et al. 2002) to the divergence of Hypolytreae from the rest of the Cyperaceae.

For the most part priors were set to be uniform, as defined by the default settings in BEAST. The only constraints imposed on the analyses were the monophyly of Hypolytreae as well as that of the remaining taxa, this defining the crown node of Cyperaceae (Muaysa et al. 1998). The age prior on the root node, was set to be normally distributed (Ho 2007) with a mean of 44.1 million years ago (Ma) and standard deviation of either (i) 1.0 Million years (Myr) (ii) 8.8 Myr. The application of two root age priors was done to evaluate the effect of variation in age of the root node on inferred ages of the nodes of interest.

The input files for analyses were prepared using the program BEAUti version 1.4.2 (Rambaut and Drummond 2007a). The nuclear ETS data was used as converted to xml by BEAUti, whereas the outputs for the five chloroplast partitions were manually compiled into a single mixed-model xml file. Posterior distributions for each parameter were obtained using a Markov Chain Monte Carlo (MCMC) procedure run for 20 million generations and sampled every 1000 generations. Results were inspected using Tracer version 1.4.1 (Rambaut and Drummond 2007b) to determine that stationarity was achieved in all cases and that the effective samples sizes were adequate. Trees were summarised using the maximum clade credibility tree function in TreeAnnotator, reporting median heights and 95% confidence intervals for nodes (Rambaut and Drummond 2007c), excluding the burn-in phase as determined by a visual inspection of

the log file in Tracer version 1.4.1 (Rambaut and Drummond 2007b). The summary tree was visualised using FigTree version 1.0 (Rambaut 2006).

Ancestral Area Inference

The inference of the ancestral geographical distributions was done using PhyloMapper (Lemmon and Lemmon 2008), which assumes a spatially explicit random walk model of migration to calculate ancestral node distributions in the context of a known gene tree. The random walk model has the property of inferring an ancestral range as being spatially intermediate between the two descendent ranges. This may be valid where the entire area between descendent populations constitutes potentially suitable habitat, but this is not true for range-restricted species. Despite these problems, the model may have utility in revealing range shifts at coarser (regional) spatial scales. Since PhyloMapper requires a single set of coordinates to be sampled for each terminal (haplogroup) included in the gene tree, it was not possible to accommodate the occurrence of most haplogroups at multiple localities.

To circumvent this problem, I used a six-terminal gene tree describing the relationships amongst the six principal haplogroup lineages identified by phylogenetic analysis of the *trnH-psbA* locus. The *trnH-psbA* locus was used, because it was the locus for which the most complete geographic sampling . The gene tree used was a reduced version of the time-calibrated BEAST tree used for dating analyses. To accommodate the fact that four of the six terminals were represented at multiple localities, the PhyloMapper analysis was repeated 10,000 times, randomly sampling a single locality from the set for each haplogroup.

Results

Sequence data

Except at the conserved ends, the *trnH-psbA* sequence data were difficult to align, owing to the presence of a region comprised of multiple repeats of varying length (ca. 10-100bp), situated between alignment positions 220 and 970. Ignoring length variation within this repeat region, the *T. triangularis* complex contained six unique *trnH-psbA* haplogroups, each comprising multiple haplotypes of variable length, which were differentiable on the basis of nucleotide variation within the conserved ends. The full alignment, including the indel zone, was 1364 bases pairs (bp) long with 16 polymorphic sites. Within the *T. triangularis* complex, the shortest sequence is 518 bp long and the longest 1100 bp (Table 2.5).

Four of the 37 sampling localities had more than one haplogroup present: Wemmershoek had three, Du Toits Peak two, Stettynsberg two and Jonaskop two (Fig. 2.2). In all other populations sampled, only one of the six haplogroups was recorded.

The *rbcL* alignment was straightforward, due to the absence of indel mutations. Alignment of the *rps16*, *trnL-trnF*, and 3' *trnV-ndhC* sequence regions was complicated by length variation and the need to insert numerous gaps into the alignment. The aligned *rbcL*, *rps16*, *trnL-trnF*, 3' *trnV-ndhC* sequences were 1376, 1135, 1636, 1366 bp long respectively. The nuclear ETS region was 633 bp long, with alignment not overly complicated by the need to insert gaps. A complete alignment, including all loci sampled is presented as a NEXUS file on the accompanying CD (Appendix 1).

Table 2.5. Summary of *trnH-psbA* length variation for each of the haplogroups. The total number of haplotypes is different from the total in molecular dating and phylogenetic analyses due to difficulties in getting successful amplification and sequencing for all loci for all of these length variants

Haplogroup	No. Length variants	List of Sequence lengths (bp)
Hawequas	3	862, 913, 945
South West	3	535, 594, 957
North West	2	780, 799
Karoo Mountain	7	518, 725, 947, 971, 993, 1054, 1100
Anysberg	2	851, 873
Seweweekspoortberg	1	971
Total	17	518-1100

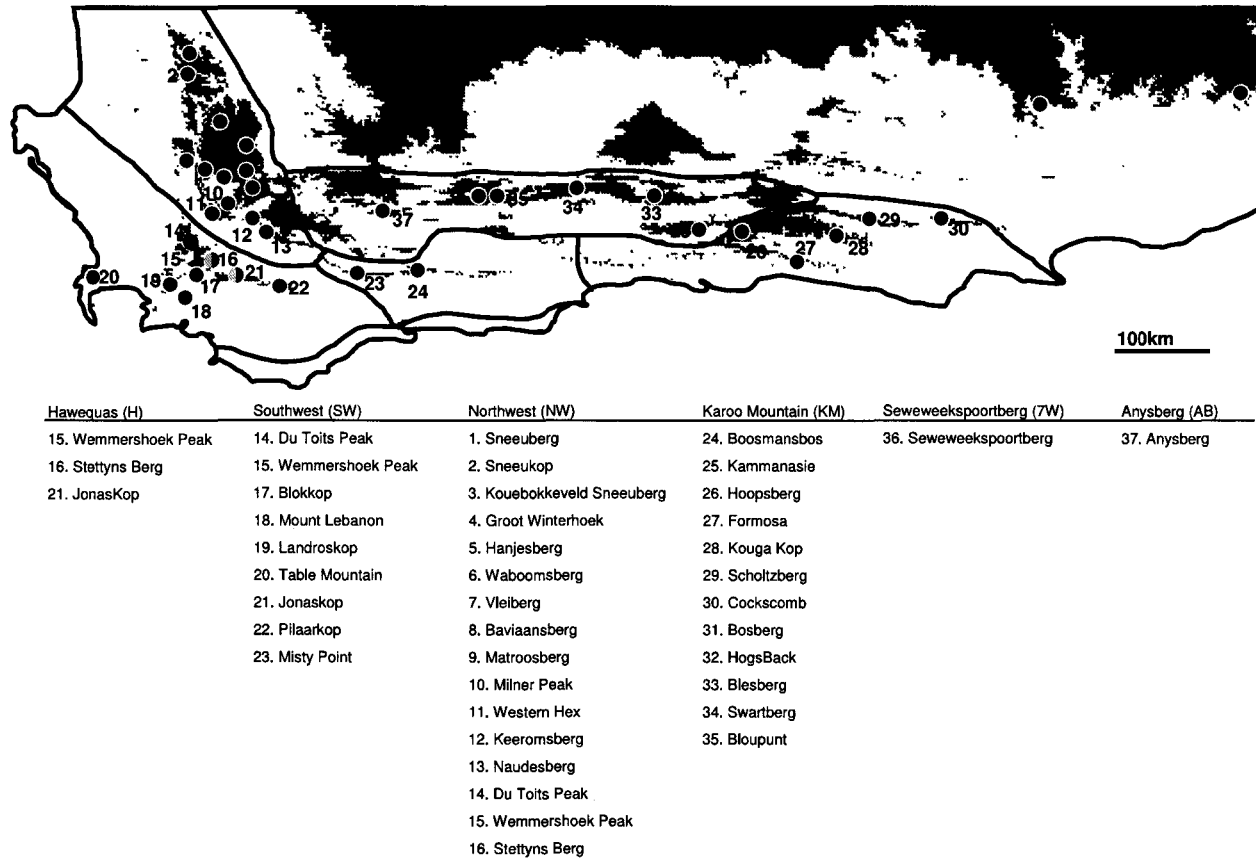


Figure 2.2: Map of *Tetraria triangularis* study area indicating locations and names of populations sampled. Population names are grouped according to *trnH-psbA* chloroplast genotyping and named for phytogeographical areas derived from Goldblatt and Manning (2000). Colours in circles represent the haplogroups yellow (Hawequas), red (Southwest), blue (Northwest), black (Karoo Mountain), pink (Anysberg) and purple (Seweweekspoortberg).

Phylogenetic analysis

Tetraria triangularis is identified as monophyletic by parsimony and Bayesian analyses of both the chloroplast and nuclear data (Fig. 2.3 and Fig. 2.4). Support for this pattern is generally high, although parsimony analysis of the nuclear data yields a low bootstrap value (51%). A high consistency index (Fig. 2.3 and Fig. 2.4) suggests that this is due to few character changes at the first *T. triangularis* branch, rather than contradictory signal in the data. For each data set, the parsimony and Bayesian results (Fig. 2.3) are largely consistent, except for some weak incongruence between the chloroplast topologies with regard to outgroup relationships. All analyses indicate that the other broad-leafed *Tetraria* species (*T. compressa*, *T. secans*, *T. thermalis*, *T. sp. nov.*) form a clade that is sister to *T. triangularis*.

Molecular sequence variation is strongly structured within *T. triangularis*, with five well-supported lineages identified for both the chloroplast and nuclear partitions (Fig. 2.3: Marked A-E). The chloroplast tree also provides strong support for the monophyly of AB1 + AB2 (Fig. 2.4), supporting subdivision of clade E (Fig. 2.3) into clades E and F as shown in Figure 2.4.

Whilst the haplogroups identified by chloroplast and nuclear partitions are identical, relationships between haplogroups differ slightly. In the chloroplast topologies, haplogroups A, B, and C are successively sister to the rest of the more recently diverged haplogroups in the complex. The nuclear topology, by contrast, places haplogroups A and B as sister to each other, with the remaining haplogroups forming a clade sister to them (Fig.2.3).

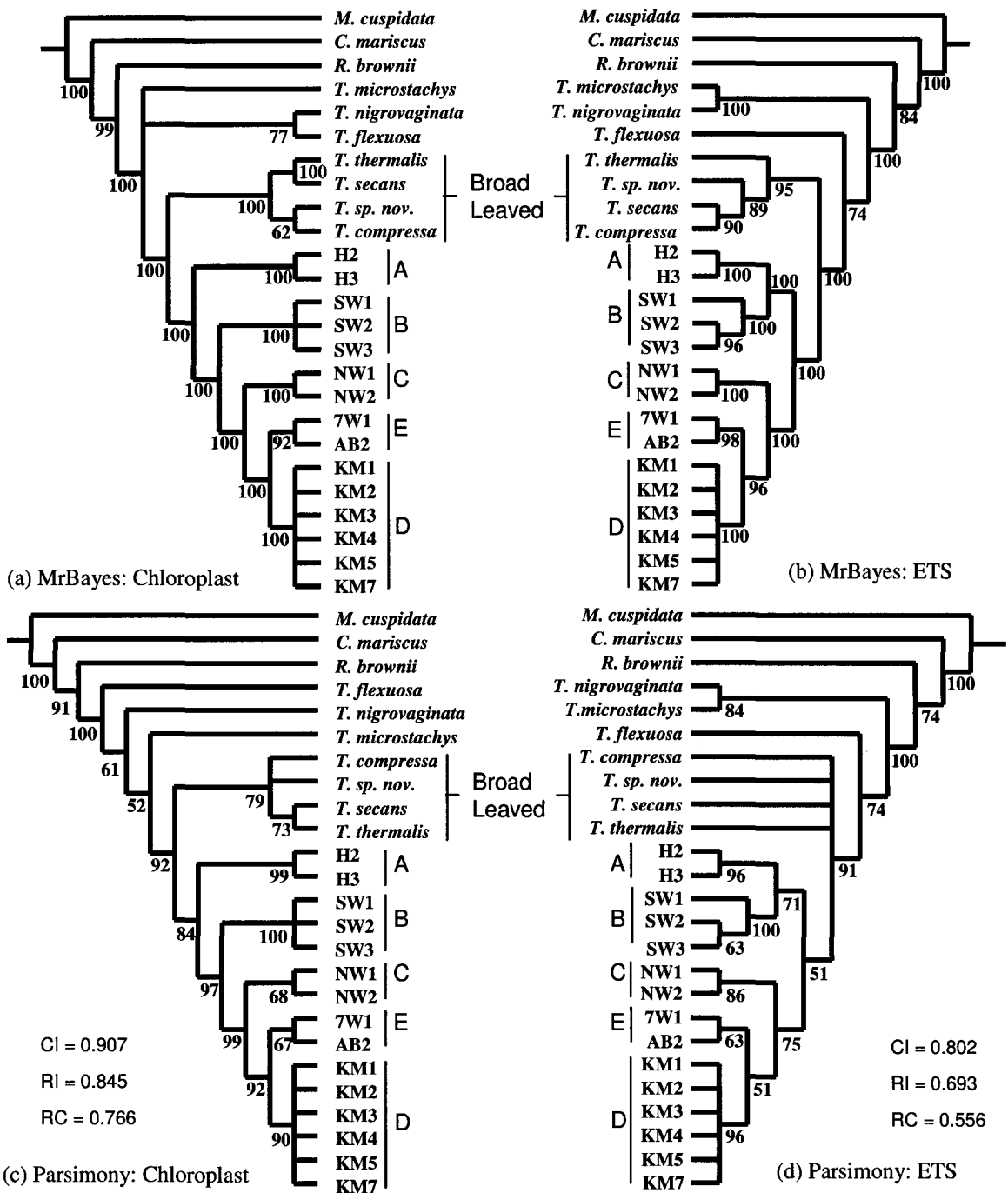


Figure 2.3: Results from parsimony and Bayesian analyses, examining conflict between (i) data partitions (a) vs. (b) & (c) vs. (d) and. Terminals marked with letter and number codes correspond to *tmH-psbA* haplotypes. The letters in bold show five well supported haplogroups within the *T. triangularis* complex. Numbers below the nodes are (i) Bayesian posterior probabilities in A & b and (ii) bootstrap values in c & d.

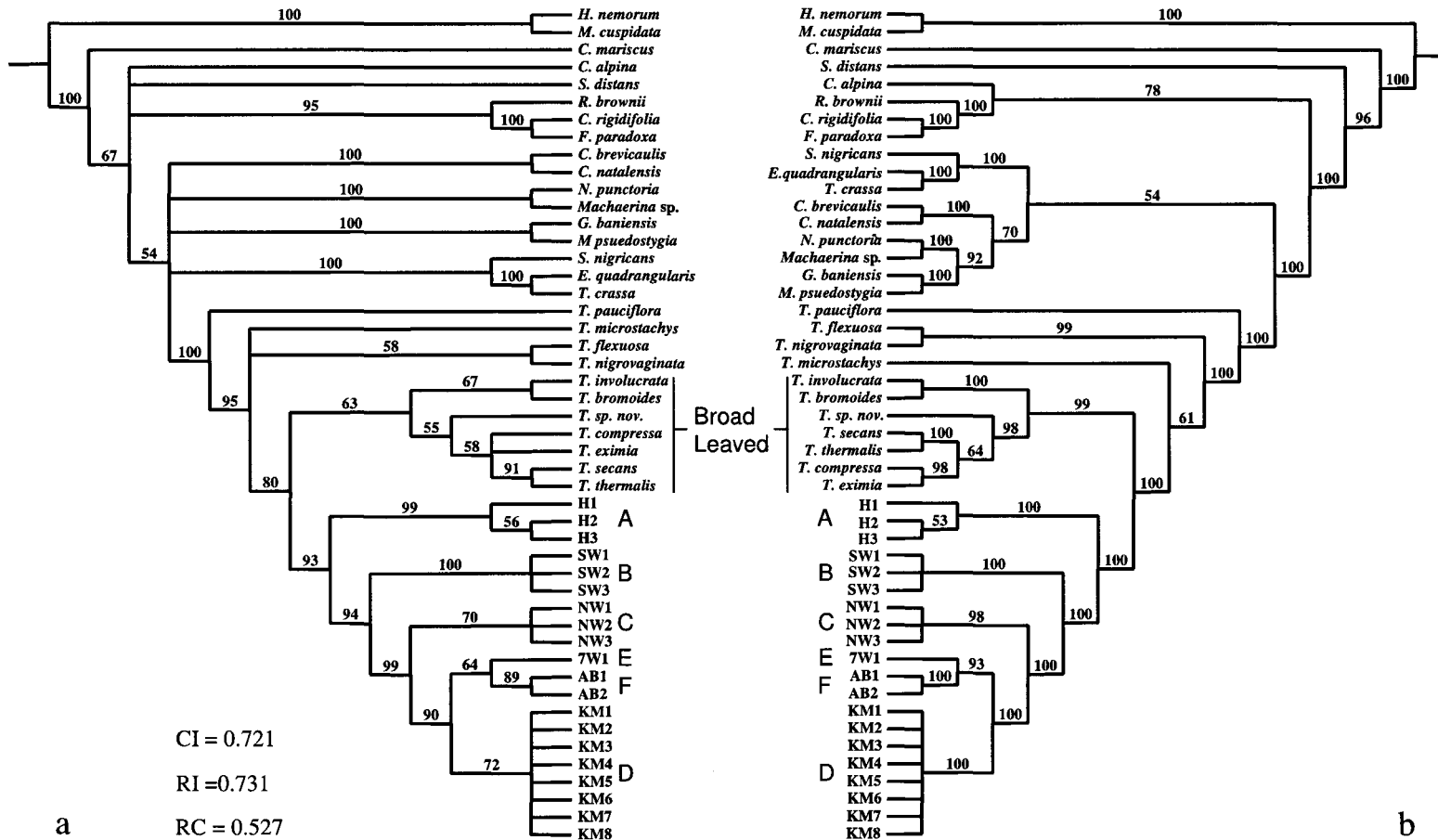


Figure 2.4: Plot of results for (a) Parsimony and (b) MrBayes analyses on the complete chloroplast data set. Scores for each node are (i) bootstrap and Bayesian posterior probabilities. Inset refers to the scores for the Parsimony Tree: CI= Consistency Index; RI = Retention Index; RC = Rescaled Consistency Index

Association of haplogroups with geographical area, elevation and climate

The distribution of the *trnH-psbA* haplogroups (Table 2.6) as well as the ETS haplotypes (data not presented), show strong geographical structuring, corresponding closely to the phytogeographic divisions set out by Goldblatt and Manning (2000). I follow their terminology in naming the *trnH-psbA* haplogroups (Table 2.5; Fig. 2.1d) where clade distributions are broadly consistent with phylogeographic areas and use my own terminology for those that aren't. Three haplogroups are widespread; one mostly confined to the area southwest of the Breede River valley (Southwest), another predominantly to the north of the Breede River valley and west of the Cogmanskloof (Northwest), and the last occurring on peaks to the east of the Cogmanskloof, excluding the low hills of the Outeniqua mountains (Karoo Mountain). The Hawequas haplogroup is confined to the mountains of the Hawequas range and Jonaskop. Two haplogroups are confined to single mountain populations: one to the Anysberg (Anysberg) and one to the Seweweekspoortberg (Seweweekspoortberg) (Fig. 2.2).

Although the Goldblatt and Manning (2000) phytogeographical scheme corresponds well to the haplogroup distributions reported in this study, there are exceptions (Table 2.6). Notably, the Southeast centre is not shown to be distinct from the Karoo Mountain centre, these areas being occupied by a single haplogroup, which I term the Karoo Mountain haplogroup. The Southwest haplogroup (marked in red) has an outlier population at Misty Point on the far side of the Breede river valley (Fig. 2.2; Population 23). Populations of the Northwest clade, marked in blue, are usually found

Table 2.6: Correspondence between clades and phytogeographic areas sensu Goldblatt and Manning (2000). Columns represent the phytogeographical areas from Goldblatt and Manning (2000) (Fig.2.1) and rows indicate the clades identified and named from this chapter from phylogenetic analysis and geographic distributions of haplogroups. The numbers reflect how many populations of each haplogroup occur in each phytogeographic area.

Haplogroup	Phytogeographic Area							Total
	Southwest	Northwest	Langeberg	Karoo Mountain	Southeast	Agulhas Plain	External	
Hawequas (H)	3	-	-	-	-	-	-	3
Southwest (SW)	8	-	1	-	-	-	-	9
Northwest (NW)	3	13	-	-	-	-	-	15
Karoo Mountain (KM)	-	-	1	4	5	-	2	12
Anysberg (AB)	-	-	-	1	-	-	-	1
Seweweekspoortberg (7W)	-	-	-	1	-	-	-	1
Total	14	13	2	6	5	0	2	42

north of the Breede River valley, barring three populations on peaks in the Hawequas range (Fig. 2.2; Populations 14, 15 & 16).

In general, geographical distances between populations with different haplogroups are significantly larger than expected by chance (Table 2.7). This is consistent with the observation of strong geographical structuring of haplogroups, in which peaks with the same haplogroups are rarely separated from each other by a population with a different haplogroup (Fig. 2.2). An exception to this pattern is the complete range overlap of the Hawequas haplogroup within the area occupied by the Southwest haplogroup, the geographic distance here being non-significant (Table 2.7). Geographic distance is non-significant for comparisons involving the Anysberg and Seweweekspoortberg haplogroups, because haplogroups are represented by just one locality each (Fig. 2.2, Table 2.7).

The minimum elevation connecting populations with contrasting haplogroups is often significantly lower than expected by chance, specifically in the western part of the range (Southwest-Northwest, Southwest-Karoo Mountain, Hawequas-Karoo Mountain, and Hawequas-Northwest). Most significant comparisons span the Breede River valley, identifying this as a potentially important topographical barrier. In the extreme Southwest, the Southwest and Hawequas haplogroups show no significant elevational separation, with the range of the latter entirely contained within that of the former. The elevational comparison between the Karoo Mountain and Northwest haplogroups is also not significant.

Significant climatic turnover between the Northwest and Southwest haplogroups may indicate a role for climatic factors in limiting dispersal across the Breede River

Table 2.7: Significance of the mean elevational minimum, the mean climatic distance, and the mean geographical distance amongst populations representing each haplogroup, as evaluated using a randomization procedure (see text). In the case of altitudinal minimum, a significant result implies that the observed value was lower than expected by chance, where for climatic and geographic distance it indicates a higher than expected value. Numbers in bold indicate comparisons where the p values are less than 0.05.

Southwest	Elevation	0.747				
	Climate	0.984				
	Distance	0.958				
Northwest	Elevation	0.013	P<0.001			
	Climate	0.121	P<0.001			
	Distance	0.258	P<0.001			
Karoo Mountain	Elevation	0.001	P<0.001	0.083		
	Climate	0.005	P<0.001	P<0.001		
	Distance	0.001	P<0.001	P<0.001		
Anysberg	Elevation	0.266	0.289	0.229	0.361	
	Climate	0.256	0.103	0.056	0.152	
	Distance	0.251	0.114	0.044	0.160	
Seweweeks poortberg	Elevation	0.253	0.295	0.248	0.325	1.000
	Climate	0.231	0.108	0.058	0.238	1.000
	Distance	0.254	0.114	0.055	0.291	1.000
		Hawequas	Southwest	Northwest	Karoo Mountain	Anysberg

valley. However, significance in one of these factors may be the result of autocorrelation, with significant climatic turnover often associating with significant geographical separation, presumably because climatic variation is spatially structured. Under this scenario, it may not be possible to use this method to distinguish which factor is causative. Nonetheless, the significant climate difference between Northwest and Karoo Mountain haplogroups, identifies climate as potentially more important than elevation in driving the separation of this, more recently-diverged, haplogroup pair.

Molecular Dating

Dating estimates obtained using the nuclear partition are generally two to three times older than those inferred from the chloroplast data set (Fig. 2.5; Table 2.8). The divergence of *T. triangularis* from its sister clade is estimated to be either 4-5 Mya (plastid) or 12 Mya old (nuclear) placing it within the mid Miocene-early Pliocene (Fig. 2.5; Table 2.8, Node B). Regardless of the calibration method, divergence of the clades within the complex is too old to be ascribed to recent Quaternary events. The first divergence within *T. triangularis* is dated at about 3 Mya (plastid) or 9 Mya (nuclear) (Fig. 2.5, Table 2.8: Node C), with most haplogroups diverging before the end of the Pliocene (Fig. 2.5: Nodes D, E, and F). The divergence between the Northwest and the eastern haplogroups (Karoo Mountain, Seweweekspoortberg, Anysberg) is estimated to have occurred during the early Quaternary by the chloroplast data, whereas the nuclear data shifts this split backward in time to ca. 5.5 Ma (Fig. 2.5: Node G). The more recent divergences between the clades in the eastern half of the CFR are estimated to have

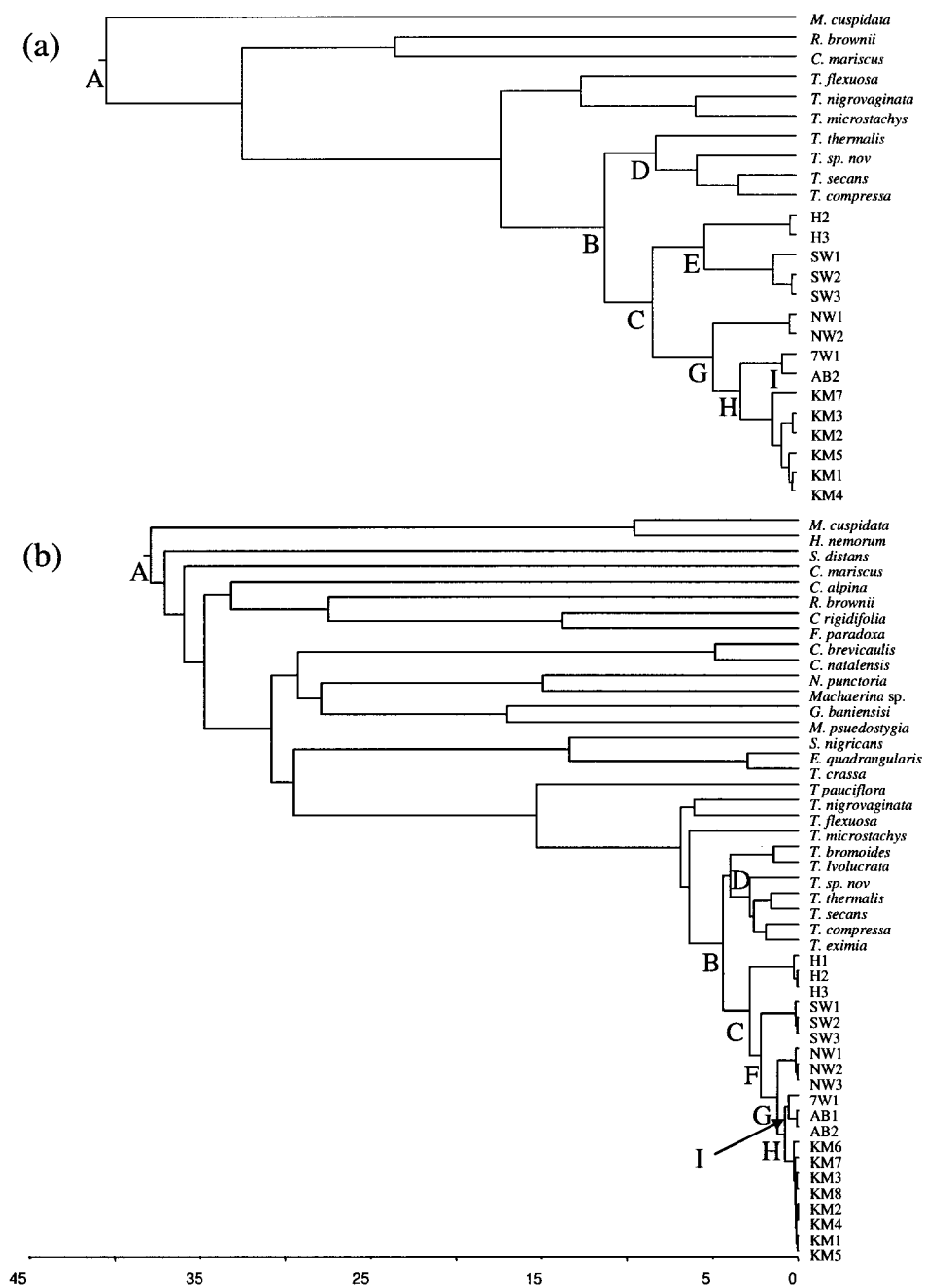


Figure 2.5: Plot of BEAST results for (a) Nuclear and (b) Chloroplast data sets. Illustrating the nodes of interest for *Tetraria* diversification. Table 8 (overleaf) shows the median ages of nodes and 95% confidence intervals. Time scale in millions of years.

Table 2.8: Results from BEAST Dating analysis. Columns show the result for each node for various reconstruction techniques. Rows show results for one of four methods. Within each cell the top row is the median age for that node, the middle row shows the upper and lower 95 % interval of the estimate and the bottom row is the Bayesian Posterior probability for that node.

Node	A	B	C	D	E	F	G	H	I
Chloroplast 44.1 ± 1.0 my	44.03	5.15	3.33	4.65	-	2.57	1.44	0.92	0.65
	44.08-46.00	4.28-6.09	2.57-4.16	3.82-5.55		1.95-3.28	1.01-1.97	0.61-1.29	0.32-1.01
	1.0	1.0	1.0	0.98		1.0	1.0	1.0	0.97
Chloroplast 44.1± 8.8 my	38.02	4.41	2.83	3.96	-	2.18	1.22	0.78	0.54
	19.51-57.72	2.21-6.83	1.28-4.42	2.01-6.21		1.01-3.48	0.52-2.04	0.31-1.33	0.18-1.02
	1.0	1.0	1.0	0.98		1.0	1.0	1.0	0.97
Nuclear 44.1 ± 1.0 my	44.02	11.08	8.31	8.11	5.23	-	4.74	3.11	0.81
	42.14-46.05	5.54-21.51	3.7-17.02	3.59-16.36	1.68-11.6		1.21-11.65	0.74-8.34	0.00-3.76
	1.0	0.98	1.0	0.95	0.99		1.0	0.89	0.99
Nuclear 44.1± 8.8 my	40.40	11.19	8.41	8.20	5.37	-	4.87	3.24	0.81
	28.14-54.9	5.27-21.11	3.81-17.04	3.82-16.69	1.78-11.74		1.34-11.89	0.80-8.57	0.00-3.84
	1.0	0.98	1.0	0.95	0.99		1.0	0.89	0.99

occurred within the Quaternary, according to the chloroplast data (Fig. 2.5: Nodes H and I).

Ancestral Area Inference

While a random walk model of dispersal is somewhat inappropriate in estimating the ancestral distributions of taxa whose potential ranges are discontinuously distributed, it may provide reliable insights into range shifts at broad spatial scales. In *T. triangularis*, for example, the ancestral area estimates for nodes F, G, H and I, suggest a western origin, with subsequent range expansion from west to east (Fig. 2.6). The limitations of the method become more apparent at fine spatial scales, with inferred ancestral areas not being restricted to mountain areas, and showing considerable variation for most nodes (depending on the specific set of localities used). By way of illustration, nodes F and G are commonly situated in low-lying areas where *T. triangularis* does not occur. On the other hand, the position of node I coincides with the Seweweekspoortberg population.

Discussion

As currently delimited (Archer 2000), the monophyly of *T. triangularis* is well supported, the complex comprising six well-supported, evolutionarily independent lineages demonstrated by both chloroplast and nuclear sequence data. While both chloroplast and nuclear data partitions recover all six clades, their inter-relationships vary. One possible cause of this gene-tree conflict is that gene flow with respect to pollen remained effective after the populations of the Hawequas and Southwest clades were spatially separated (Xiang et al. 2005). Alternatively it could reflect incomplete lineage

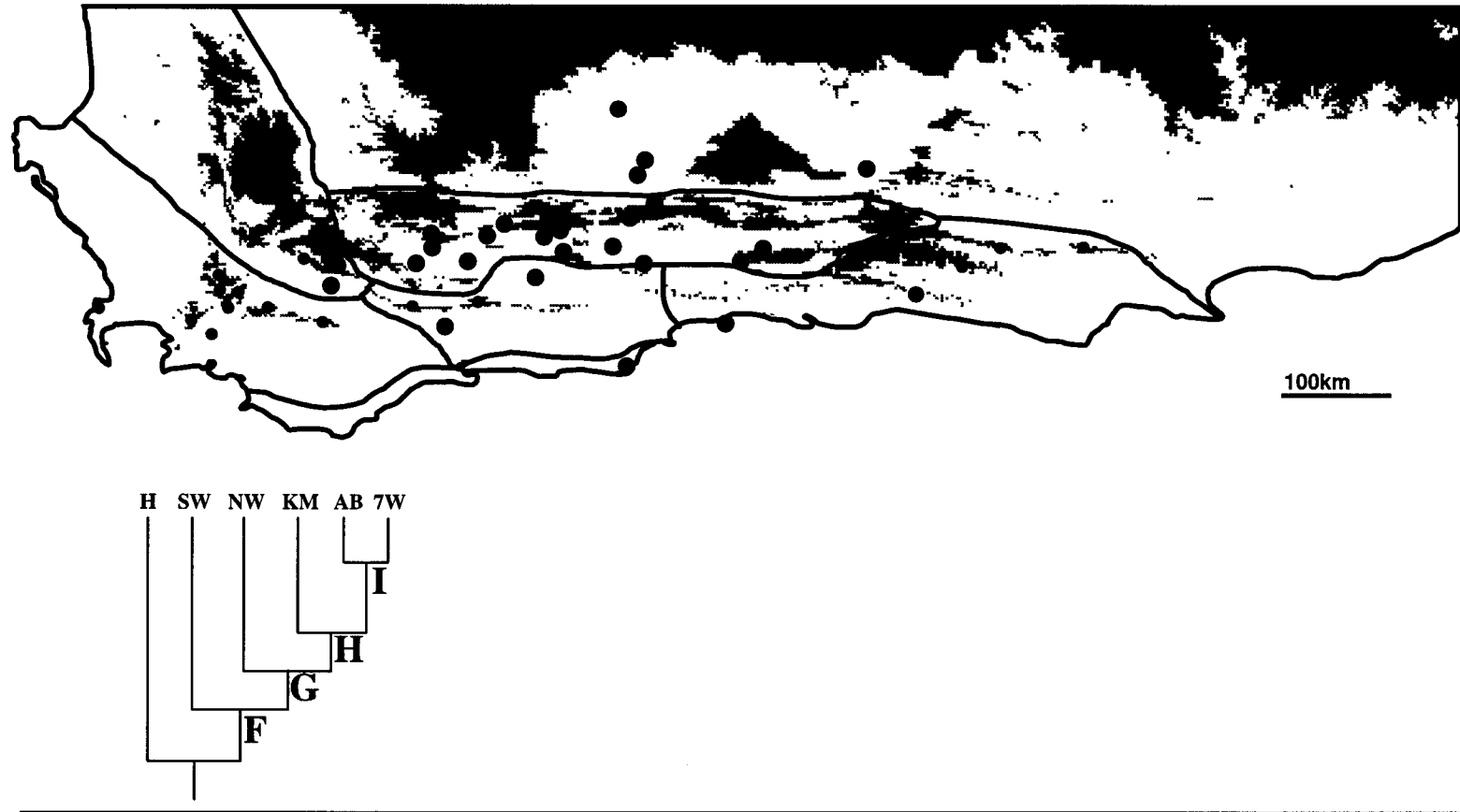


Figure 2.6: Map of *Tetraria triangularis* study area, indicating the locations for ancestral nodes inferred by PhyloMapper inference procedure, utilising the *trnH-psbA* dataset. Colours of the dots on the map match those of the letters on the tree. Points marked in black are the known occurrence localities of the *T. triangularis* complex. Letters on the tree are: H (Hawequas), SW (Southwest), NW (Northwest), KM (Karoo Mountain), AB (Anysberg), 7W (Seweweekspoortberg).

sorting, most likely in the nuclear locus, with an ancestral polymorphism being carried through to the stem lineage of the widely-distributed Southwest clade (Avice et al. 1983, Xiang et al. 2005, Carstens and Knowles 2007).

Each of the haplogroups recovered in the phylogenetic analyses has a coherent geographical distribution, with limited overlap in their distributions. For example, The Hawequas and Southwest haplogroups occur mostly south of the Berg and Breede river valleys, the Northwest haplogroup is restricted to mountains from Hawequas to the Cederberg (no further east than Naudesberg), and the Karoo Mountain haplogroup occurs nowhere west of Boosmansbos. Not all of the haplogroups are allopatric. The ranges of the Southwest, Hawequas and Northwest haplogroups overlap in the Hawequas Mountains (Fig. 2.2). The broad correspondence of the ranges of the Hawequas, Southwest and Northwest haplogroups to previously delimited phytogeographic centres suggests the existence of dispersal barriers that act to maintain their distributions. Despite *T. triangularis* being present in the Southeast Centre (Goldblatt and Manning 2000), there is no evidence for a haplogroup whose distribution corresponds to this area. In terms of barriers to dispersal it is difficult to see what differentiates the Karoo Mountain and Southeast Centres as defined by Goldblatt and Manning (2000), given that there is no clear break in the high ground across the boundary of these two centres. However, the existence of a boundary has been documented for rock Agamas and dwarf chameleons (Matthee and Flemming 2002, Tolley et al. 2006, Swart et al. 2009). It would be interesting to re-examine which species explain the definition of a Southeastern Centre and to see if these are lowland species as opposed to montane species. Perhaps an

ecological gradient is acting on the lowland flora, which does not act as dispersal barrier to montane species.

Geographic distances between populations with the same haplotype are often very large, indicating that long distance dispersal is possible if conditions are favourable. In the Karoo Mountain haplogroup, the distances separating the Cockscomb-Bosberg and Bosberg-Hogsback are approximately 150 km and 200 km respectively (Fig. 2.2). Presumably, the distance between populations serves to accentuate the topographic, substrate and climatic barriers that separate them. Topography has been implicated in being more strongly associated with divergence between populations of habitat restricted species than distance alone (Holland and Hadfield 2002, Moya et al. 2007, Wang 2009). Turnover between the Northwest and Karoo Mountain haplogroups is associated with the change in rainfall regime from west to east across the CFR (Schulze and McGee 1978, Campbell 1983, Chase and Meadows 2007). While this pattern has been reported previously, its driving mechanism is poorly understood (Swart et al. 2009, Tolley et al. 2009). Rainfall patterns differ between the western and eastern CFR, with the west having both higher total rainfall and a higher percentage of total rain occurring in winter (Cowling et al. 1997, Chase and Meadows 2007). This is a function of the lower incidence of the low pressure, cyclonic, frontal rainfall systems reaching the interior (Deacon et al. 1992, Cowling and Lombard 2002, Linder 2003) and the incidence of cyclonic summer rainfall in the eastern CFR. Difference in climate seasonality could induce shifts in the timing of flowering in populations on either side of this boundary, a factor which has been implicated in facilitating genetic isolation in *Rhodocoma* (Linder and Vlok 1991, Hardy and Linder 2007).

There is some evidence of a role for topography in promoting the isolation of haplogroups in *T. triangularis sensu lato*, the lowlands with their shale-derived soils acting as effective topographical barriers to dispersal. Most marked is the separation of the Northwest haplogroup from the Southwest and Hawequas haplogroups by the Berg and Breede River valleys. Swart et al. (2009) suggest the same barrier to dispersal for *Agama atra*. In contrast, the large Table Mountain gap (Fig. 2.5, Populations 19 & 20) seems to be less of a barrier, perhaps due to populations being found at lower elevation due to the cooling oceanic influence and the more favourable Quaternary sands of the Cape Flats. However, the existence of a Cape Peninsula clade within *Agama atra* (Swart et al. 2009), implies that this gap is a barrier to dispersal for some taxa.

Chloroplast DNA estimates of divergence times within the *T. triangularis* clade are 20-70% younger than the estimates provided by the nuclear ETS data. Potential explanations for this phenomenon are the differences in sampling stochasticity and the difference in effective population sizes between the chloroplast and the nuclear locus. Under neutrality, the expected time for a nuclear locus to attain reciprocal monophyly is four times greater than that for a plastid locus, due to a fourfold difference in effective population size (Moore 1995, Palumbi et al. 2001). This effect is sometimes termed the “3x rule” (Palumbi and Cipriano 1998). Although the history experienced by the chloroplast and the nuclear genomes are identical, the smaller effective population size of the former means that a chloroplast gene has is more likely to reflect the timing (and order) of population divergence than any individual nuclear gene (Moore 1995). Bearing this in mind, I favour the chloroplast date estimates as a better indicator of ‘true’ population divergence times within *T. triangularis sensu lato*.

The chloroplast molecular dating indicates that diversification of the broad-leaved *Tetralia* clade, sister to *T. triangularis*, started around 5 Ma, whereas *T. triangularis* started to diversify around 3 Ma. In both cases, the onset of diversification coincides with the intensification of summer aridity ca. 5 Ma (Levyns 1964, Cowling et al. 1992, Linder 2003) as well as the accelerated erosion of the Cape Folded Mountain landscape (Partridge and Maud 1987, Partridge and Maud 2000, Cowling et al. 2009). These two events are associated with the Pleistocene uplift event which tilted the western and eastern halves of the CFR up by about 150 and 200-300 m respectively (Partridge and Maud 2000). Both erosion of mountain landscapes and aridification would have increased fragmentation of the ancestral southwestern *T. triangularis* habitat through decreasing the size of the “Sky Islands”, forcing populations further up slopes to the wetter mountain tops. The net effect would be reduced interconnectivity and limitation of dispersal across the Cape Folded Mountain landscape.

Subsequent expansion and contraction of montane habitats in association with Quaternary climatic oscillations (Petit et al. 1999, Hewitt 2000) may have driven the ‘peak jumping’. The general trend of eastward range expansion of the *T. triangularis* complex, as inferred by ancestral range reconstruction, is therefore best seen as a stochastic process, associated with cooler, wetter conditions during glacial periods (Chase and Meadows 2007). Range expansion is not only limited to the CFR, with rapid eastward expansion of the *T. triangularis* complex taking place within the last 1 Ma. Outlying populations of the Karoo Mountain haplogroup occurring on the escarpment (Fig. 2.2; Populations 31 and 32) and potentially the Drakensberg (not sampled) support

the general pattern of members of the Cape flora dispersing to the Drakensberg (Galley et al. 2007, Valente 2009) as proposed by Weimarck (1941).

The data presented here suggest an important role for topography in splitting populations and facilitating the diversification of lineages in the CFR, driving the development of distinct phytogeographic areas of endemism. It is not clear what differentiates barriers that separate haplogroups from those that separate populations within haplogroups, and it may be that they are similar but of different ages.

Alternatively, haplogroup barriers may persist through wetter periods, perhaps because shale substrates dominate in valleys or because they are deeper (e.g. Breede River valley), whilst those that separate populations within haplogroups disappear.

The co-occurrence of three haplogroups in the *Hawequas* may represent a large, persistent population whose genetic diversity has been retained over time. Alternatively, these haplogroups represent distinct evolutionary entities, whose co-occurrence is attributable to either allopatric divergence with subsequent “re-colonisation”, or divergence in sympatry. The following chapter makes the case for the evolutionary independence of the haplogroups. Given that boundaries between the haplogroups involved coincide with a major topographical feature (the Breede River valley) and that sympatric speciation remains to be convincingly demonstrated (Coyne and Orr 2004, Bolnick and Fitzpatrick 2007), I suggest that allopatric differentiation with subsequent recolonization best accounts for the patterns observed. These processes may also account for the high endemism found in this area for other floristic groups (Oliver et al. 1983).

Chapter 3: *Tetraria triangularis*: One or more species?

Introduction

Steep ecological gradients paired with rugged topography are thought to promote local species differentiation in the Cape Floristic Region (CFR) (Linder 1985, Cowling et al. 1992, Goldblatt and Manning 2000, Linder 2003) and species with wide distributions are unusual. For example, as few as 5 % of Proteaceae species confined to the CFR (18 out of 338 species) have a range breadth greater than 400 km, representing the total distance over which dispersal occurs if a species is freely breeding over its entire range, (Appendix 2: Data ex Rebelo 1995). By this standard, the 1200 km range breadth of *T. triangularis* extending from the Cape Peninsula to Cathedral Peak in northern KwaZulu-Natal, is remarkable. This is especially so because the highly insular, high-altitude distribution is expected to impede gene flow and enhance population differentiation (Jansen and Dynesius 2002, DeChaine and Martin 2005, Galbreath et al. 2009).

In *T. triangularis*, phylogenetic evidence indicates the existence of multiple independent lineages within the complex (Chapter 2). Previous morphology-based taxonomic treatments have applied at least two names to the group, and these potentially represent distinct species. However, a comprehensive survey of morphological variation in this group remains wanting. In Cyperaceae, the reduction of morphological features limits the number of characters available for species delimitation (Muasya et al., 1998). Molecular evidence (Chapter 2) for the existence of multiple, deeply divergent, lineages within *Tetraria triangularis* demands a thorough examination of the morphological evidence for multiple species.

The current application of the name *T. triangularis* as a widespread entity ranging from the Cape Peninsula northwards to the Cederberg and eastwards to KwaZulu-Natal is attributable to Archer (2000). Her treatment simplified a complex taxonomic history characterised by the application of multiple names, vague taxon delimitation and confusing typification (Table 3.1).

The basionym of *T. triangularis*, *Lepidosperma triangulare*, was published by Boeckeler (1874) on the basis of an Ecklon and Zeyher specimen whose locality is imprecise being given as “Cap. bonae spei” (Cape of Good Hope, which can refer to the Cape Peninsula, Cape Town, or the Cape Colony as a whole). Unfortunately this specimen, housed at the Berlin museum, was destroyed by fire during the Second World War. Consequently, *T. triangularis* lacks a type and its identity relies on Boeckeler’s description. Boeckeler’s characterisation of the stem as triangular with sharp edges and the spikelets as rusty red to dark brown, places it firmly in *T. triangularis s.l.* Clarke (1895, 1897) transferred *L. triangulare* to *Tetraria*, citing both the original specimen as well as *Burchell 659*. While the latter specimen could not be traced, Clarke supplies additional information confirming the inclusion of his concept of *T. triangularis* within *T. triangularis s.l.*

Subsequently, Boeckeler (1878) described *Chaetospora hexandra*, based on *MacOwan 1864* from Boschberg in the Eastern Cape Province of South Africa. Boeckeler did not associate this specimen with *L. triangulare*, but rather placed it in the same section as *C. circinalis* (= *Tetraria microstachys* (Vahl) Pfeiffer). The morphology of *C. hexandra*, however, clearly identifies it with *T. triangularis s.l.* lending the latter a widespread distribution. Clarke (1897) subsequently transferred *C. hexandra* to *T. macowani* but,

Table 3.1: Summary of the nomenclatural history of the *T. triangularis* complex. Each row indicates the contributing authors, the year of publication; the columns denote the name cited by that author for that specimen. Followed by the nomenclatural assignment of specimens cited by that author. Specimens marked with a (*) were not examined by the author.

Year	Author	<i>Lepidosperma triangulare</i> (Boeckeler)	<i>Tetraria triangularis</i> (Clarke)	<i>Tetraria bromides</i> var. <i>triangularis</i> (Kükenthal)	<i>Chaetospora</i> <i>hexandra</i> (Boeckeler)	<i>Tetraria</i> <i>MacOwani</i> / <i>macowaniana</i> (Clarke)	<i>Tetraria robusta</i> var γ <i>pauperior</i> (Kükenthal)
1874	Boeckeler	<i>Ecklon et Zeyher</i> (*)					
1878	Boeckeler	<i>MacOwan 1864</i>					
1895	Clarke	<i>Ecklon et Zeyher</i> (*)				<i>MacOwan 1864</i>	
1897	Clarke	<i>Ecklon et Zeyher</i> (*) <i>Burchell 659</i> (*)				<i>MacOwan 1864</i>	
1931	Kükenthal	<i>Ecklon et Zeyher</i> (*)				<i>Pillans 4856</i> <i>Schlechter 10078</i>	
1940	Kükenthal	<i>Ecklon et Zeyher</i> (*) <i>Burchell 659</i> (*)				<i>Pillans 4856</i> <i>Schlechter 10078</i> <i>Macowan 1864</i> <i>Sim 2842</i> <i>Flanagan 2303</i> <i>Hepburn 345</i>	
1976	Burt					<i>Macowan 1864</i> <i>Schlechter 10078</i> <i>Stokoe 18933</i>	

confusingly, placed this and *T. triangularis* into different subsections of *Tetraria*. Clarke (1897) associated *Tetraria macowani* with the distantly related and morphologically dissimilar *T. punctoria* (now a monotypic genus *Neesenbeckia punctoria*; see Verboom 2006), while *T. triangularis* was grouped with the broad-leafed members of the reticulate-sheathed *Tetraria* clade.

To confuse matters further, Kükenthal (1931) described a new variety of *T. robusta* (var. γ *pauperior*) on the basis of two specimens (*Pillans 4856*; *Schlechter 10078*) which clearly fall within *T. triangularis s.l.* He subsequently sank this variety into *T. macowani* (Kükenthal 1940), citing the original specimen (*MacOwan 1864*) amongst others (mostly from the Eastern Cape). Simultaneously, he reduced *T. triangularis* to a variety of *T. bromoides*, a perplexing step since the round stem of *T. bromoides* is inconsistent with Boeckeler's description of *T. triangularis* as having a sharply triangular stem.

Perhaps influenced by Levyns' (1950) inclusion of *T. triangularis*, but not *T. macowani*, in her list of sedges of the Cape Peninsula, Hilliard and Burt (1976) chose not to cite *Pillans 4856* (Table Mountain) under *T. macowaniana*, a legitimised replacement for the name *T. macowani*. Instead they cited additional specimens from the Drakensberg (*Hilliard & Burt 7792*) and Hex River mountains (*Stokoe 18933*), effectively circumscribing *T. macowaniana* as a species ranging from the Cederberg to the Drakensberg.

Bond and Goldblatt (1984) maintained the distinction between the two species, defining *T. triangularis* as distributed from the Cape Peninsula to Worcester and the Swartberg, and *T. macowaniana* as ranging from Worcester to the Eastern Cape (their

treatment does not deal with KwaZulu-Natal specimens). Most recently, Archer (2000) consolidated *T. macowaniana* into *T. triangularis*. Since Archer's (2000) concept of *T. triangularis* is the most inclusive available, it is hereafter referred to as *T. triangularis sensu lato* (*T. triangularis s.l.*).

The proposal of any new species hypothesis pivots on the concept used to define species (Wiens 2004b). Whilst a plethora of species concepts has been proposed, there is still no universal agreement on what is meant by a species, with different criteria being applied to different types of organisms (Wiley 1978, Mayden 2002). In practice, for example, plant species are routinely defined on the basis of gross morphology (Cronquist 1978, Cronquist 1988, Rieseberg et al. 2006), whilst species in well studied animal groups may be delimited on the basis of behavioral, reproductive or genetic criteria (e.g. Paterson 1985). Indeed, it has been suggested that due to the vastly different types of organisms and breeding patterns in biology, it is unlikely that biologists will ever converge on a single species concept (Wheeler and Meier 2000, Hey 2006). Regardless of the particular species concept preferred, however, some authors have emphasised the need to be explicit about the concept being used, since the application of different species concepts to the same study group can yield different numbers of species (Laamanen et al. 2003, Denise et al. 2008).

In this chapter, I will use the evolutionary species concept (ESC) as defined by Wiley (1978): *A species is a lineage of ancestral descendent populations which maintains its identity from other such lineages and which has its own evolutionary tendencies and historical fate.* The ESC is a non-operational concept which assumes the objective reality of independent lineages that we may term species, and allows the hypothesis of

evolutionary independence to be tested on a variety of data types and analyses (Cotterill 2003, de Queiroz 2007). Although morphologically-delimited species are of most utility to end-users in conservation and management, these species should, as far as possible, be delimited to represent evolutionary entities. In this study I choose to use the ESC, because the recognition of evolutionary entities reflects the speciation history of lineages rather than the ability of a particular set of characters set to define groups. The use of single line of evidence i.e. morphological criteria alone will only recover one possible history, but many lines of evidence used in concert are more likely to recover the “true” history.

In many cases the definitional criteria associated with species concepts other than the ESC are useful for discovering evolutionary species. For example, evidence that two populations have lost the ability to interbreed (Mayr 1957: Biological Species Concept, Paterson 1985: Recognition Species Concept), confirms their evolutionary independence, since the appearance of intrinsic reproductive barriers imparts enforced genetic isolation. Conversely, however, the potential ability of two lineages to interbreed does not indicate non-independence, particularly where allopatric geographical distributions prevent gene exchange.

Genetic exclusivity (Simpson 1943, Dobzhansky 1950, Mayr 1969) and/or reciprocal monophyly (Mishler and Theirot 2000: Phylogenetic Species Concept) between two sets of populations also provide evidence for separate population histories (Sites and Marshall 2004). A lack of reciprocal monophyly however does not preclude evolutionary independence, since incomplete lineage sorting may lead lineages to share identical gene copies (Maddison 1997, Maddison and Knowles 2006).

Some species may inhabit divergent ecological niches that can be used with or without additional criteria to facilitate the identification of evolutionary independent lineages (van Valen 1976, The Ecological Species Concept). While this may be useful for the recognition of species in sympatry, it is less useful for diagnosing lineages in similar niches in different locations that are not interbreeding, because it is not possible to definitively prove that niche divergence rather than allopatry has been the primary cause of divergence (Wiens, 2004b, Orr and Smith 1998, Knox and Palmer 1998).

The existence of morphological characters with distinct breaks between them, such that lineages can be consistently and persistently distinguished, is the criterion most often used to define plant species (Cronquist, 1978; Morphological Species Concept). If no morphological adaptation or drift has occurred between species, however, we cannot rule out the existence of evolutionarily independent cryptic species which could be diagnosed via breeding incompatibilities, disjunctive distributions, divergent ecologies or exclusive gene lineages.

The various species concepts used by different authors may be viewed as diagnostic frameworks for the diagnosis of evolutionarily independent lineages (Wiley 1978, Mayden 2002, Sites and Marshall 2004). Some workers have noted that the effectiveness of a species concept or set of criteria in diagnosing species will depend on the stage of speciation at the time that diagnosis is performed (Cotterill 2003, de Queiroz 2005, 2007). The relationship between the successful discovery of a species and the time since speciation is a function of (i) the process of speciation itself and (ii) the nature of the criterion being used for diagnosis (i.e. the species concept), be they a mutation rate, the advent of discontinuity in a morphological character, or the evolution of intrinsic

reproductive isolation. Taxonomists should always consider that the tools or species concepts used to discover species are just that, tools in a toolbox, and that the species are there, whether we discover them or not (Simpson 1961, Wiley 1978, Wiley 1981).

The focus of this chapter is to test whether the six haplogroups identified by the cpDNA and nucDNA datasets in chapter two represent distinct species. By the genetic exclusivity criterion (Baum and Donoghue 1995, Baum 2009), we already have some evidence to suggest that these entities are evolutionarily independent. If the haplogroups are morphologically distinguishable and/or form distinct multivariate clusters which coincide with the cpDNA lineages discussed previously (Chapter 2), then this provides additional strong support for these lineages being distinct species. A failure to pass the test of morphological diagnosability does not, however, preclude evolutionary independence (Wiley 1978, Mayden 2002). If patterns of morphological and molecular variation are found to be congruent and this is maintained in sympatry, this provides an indication that the haplogroups concerned can no longer interbreed, making them good biological species. This is because morphological and cpDNA haplotype variation may be expected to assort independently of each other. In summary, this chapter explores the evidence for the hypothesis that these lineages represent good evolutionary species, using simple morphometric measures in combination with information regarding distributions, co-occurrence and genetic exclusivity.

Methods

Morphological Sampling

Morphological variation was assessed using voucher samples representing the same individuals and populations which had been surveyed for *trnH-psbA* sequence variation (Chapter 2). All samples from the cpDNA survey that were suitable for measurement were scored for morphology (Table 3.2). Herbarium specimens were not used, since these specimens had not been characterised with regard to chloroplast haplogroup. Specimens used for the morphological survey comprised entire tillers, allowing an estimation of plant height in addition to a range of other characters. As far as possible, sampled individuals had mature spikelets with evidence of exposed anthers or stigmas. Unavoidably, however, the specimen material of two populations (Table 3.2: Swartberg and Cederberg Sneeuberg) was reproductively immature and therefore unsuitable for measurement (Sokal and Sneath 1963). This is important because, although plant size is constant through the year, spikelets expand considerably up until flowering. Overall, 34 populations were sampled for morphology (1-28 per population), yielding a total of 276 individuals (Table 3.2).

Variables measured and equipment

Specimens were measured for each of 12 length and one count variable. Measurement details are provided in Figure 3.1 and Table 3.3. Coarse measurements, on the order of 10 mm to 1 m, were made with a metre ruler, with measurement precision as indicated in Table 3.3. Spikelets were measured using an eyepiece graticule installed in a

Table 3.2: Summary of study sites and 273 samples used for morphological analysis of the *T. triangularis* complex

No.	Population	Longitude °S	Latitude °E	N
1	Anysberg	-33.51	20.62	8
2	Baviaansberg	-33.21	19.59	8
3	Blesberg	-33.41	22.63	10
4	Blokkop	-33.97	19.24	3
5	Boosmansbos	-33.93	20.86	7
6	Bosberg	-32.70	25.53	1
7	Cockscomb	-33.57	24.79	8
8	Du Toit's Peak	-33.75	19.20	25
9	Formosa Peak	-33.86	23.70	4
10	Groot Winterhoek	-33.12	19.17	3
11	Hanjesberg	-33.18	19.30	6
12	Western Hex	-33.50	19.37	2
13	Hogsback	-32.62	27.00	1
14	Hoopsberg	-33.66	23.28	10
15	Jonaskop	-33.97	19.50	19
16	Kammanasie Mountain	-33.64	22.95	9
17	Keeromsberg	-33.56	19.63	6
18	Koue Bokkeveld Sneeuberg	-32.84	19.38	9
19	Kouga Kop	-33.72	23.99	13
20	Landroskop	-34.04	18.98	5
21	Matroosberg	-33.38	19.67	9
22	Milner Peak	-33.46	19.45	5
23	Misty Point	-33.96	20.44	5
24	Mount Lebanon	-34.15	19.14	1
25	Nameless1935	-33.40	21.44	9
26	Naudesberg	-33.65	19.73	10
27	Pilaarkop	-34.06	19.85	7
28	Scholtzberg	-33.58	24.25	8
29	Seweweekspoortberg	-33.38	21.36	7
30	Sneeuberg	-32.48	19.14	0
31	Sneeukop	-32.36	19.16	3
32	Stettyns Peak	-33.86	19.30	1
33	Swartberg	-33.35	22.04	0
34	Table Mountain	-33.96	18.43	7
35	Vleiberg	-33.03	19.60	13
36	Wamboomsberg	-33.25	19.45	6
37	Wemmershoek Peak	-33.80	19.17	28

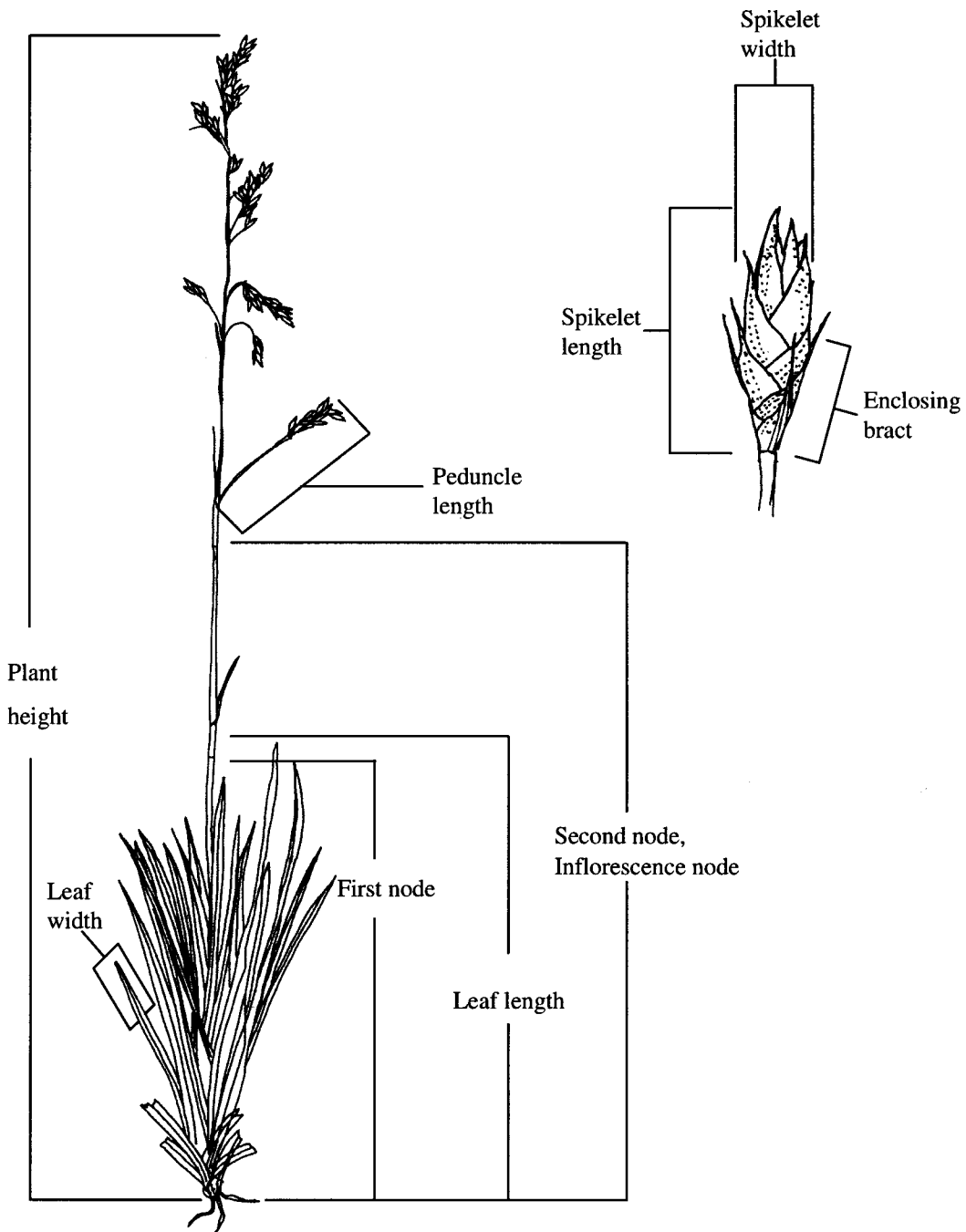


Figure 3.1: Illustration of the dimensions measured in the morphological analysis. Culm width not illustrated here, but measured as the widest dimension of the triangular culm at 10 cm from the base

Table 3.3: Description of characters measured and units used in morphological analyses

Variable name	Variable description	Measurement method	Units	Precision
Plant height	Length of culm from plant base to terminal spikelet	MR*	mm	10 mm
First node	Length from plant base to lowest culm node	MR	mm	5 mm
Second node	Length from plant base to second lowest culm node	MR	mm	5 mm
Inflorescence node	Length from plant base to lowest node subtending an inflorescence branch	MR	mm	5 mm
Culm width	Maximum of culm, measured 10cm above plant base	MR	mm	0.5 mm
Leaf length	Length from plant base to leaf-tip of an average leaf	MR	mm	10 mm
Leaf width	Width of leaf measured 10 cm from plant base	MR	mm	0.5 mm
Peduncle length	Length of first peduncle measured from the first inflorescence node to the terminal spikelet	MR	mm	5 mm
Spikelet length	Length of spikelet from base to tip	CMG**	mm	0.16 mm
Spikelet width	Width of spikelet at widest point	CMG	mm	0.16 mm
Spikelet count	Total number of spikelets in the inflorescence	Count	Count	True
Enclosing bract	Length of bract subtending spikelet	CMG	mm	0.16 mm

*MR: Metre ruler; **CMG : dissecting microscope graticule.

Leica MS 5 dissecting microscope at 63× magnification; these measurements were made precise to 0.16 mm.

Analysis

Testing significant discrimination between groups

To test the hypothesis that the haplogroups identified in Chapter 2 (Fig. 2.2, 2.4) are morphologically distinguishable, discriminant functions analysis (DFA) was performed in STATISTICA (StatSoft, Inc. 2007). Genotyping using the *trmH-psbA* marker (Fig. 2.2) was used to assign specimens to one of six groups, corresponding to the haplogroups identified in Chapter 2. Since the measured variables differed in their scales and units of measurement they were all \log_{10} transformed to ensure that they were similarly scaled (Sokal and Sneath 1963) and that their distribution more closely approximated the normal distribution required by DFA. DFA derives linear combinations of variables, termed canonical covariates (CV), in such a way that correlations between assigned group membership and CV scores are maximized (Krzanowski 1990, Sokal and Sneath 1963). The CVs represent a weighted combination of the morphological variables that provides the best overall discrimination between groups (Sokal and Sneath 1963). An F-statistic based on the Mahalanobis distance between groups allows for the evaluation of significant multivariate differences between group centroids (Krzanowski 1990, Sokal and Sneath 1963). For each CV it is possible to derive a vector of function coefficients which indicate the partial contribution of each variable to that CV. Also derived is a vector of factor structure (r), which reflects the correlations of the original variables in the model with each CV (Sokal and Sneath 1963).

Identification of distinguishing characters and construction of a dichotomous key

For each morphological variable, non- parametric Kruskal–Wallis tests were performed in STATISTICA (StatSoft, Inc. 2007) to test the null hypothesis of equality of means among groups. The Kruskal-Wallis test was used as a consequence of the data not being normally distributed (Kruskal and Wallis 1952, Siegel and Castellan 1988). Data were not transformed to approximate a normal distribution, in order to allow direct comparison of the variables being measured (Siegel and Castellan, 1988). This was used in conjunction with the standardised coefficient scores from the DFA, to identify which traits best separated the groups. Box and whisker plots were used to summarise the Kruskal-Wallis results and to allow visual inspection of the degree of overlap between groups for each variable. Post-hoc Newman-Keuls tests were performed to identify homogenous groups for each variable (Newman1939, Keuls 1952, Winer et al. 1991).

Owing to the degree of overlap in all characters amongst the species, preparation of a dichotomous key was difficult. A combination of the Kruskal–Wallis results and an assessment of inter-specific character overlap as described by the box and whisker plots, were used to decide the couplet order of the key. The Newman-Keuls-derived homogenous groups allow for the easy identification of species that are grouped or differentiated by a particular variable (Newman 1939, Keuls 1952, Winer et al. 1991). Sequential two-group DFA's were then employed to identify the two most discriminatory variables for each couplet. In each case, a zone of overlap was identified within which individuals were likely to be misidentified. This is recorded as a percentage of potentially incorrect classifications. Trait measurements for each species were entered into DELTA (Dallwitz et al. 2000) format, along with additional descriptive characters that permit

species within this complex to be discriminated from each other, and from other members of the broad-leafed reticulate-sheathed *Tetraria* clade. These data were then exported from DELTA, and used to generate natural language descriptions (TONAT, Dallwitz et al. 2000). The information was then converted into INTKEY format (Dallwitz et al. 2000) to produce a digital key, allowing for the identification of species using multiple variables.

Results

Discriminant functions analysis

Three significant canonical variates, accounting for 96.5% of the overall variation between the six haplogroups, were extracted in the DFA (Table 3.4). All six groups were shown to be significantly different from each other at the $P < 0.0001$ level (Table 3.5). Of the 273 samples, 91 % were classified correctly, with no misclassification of specimens assigned *a priori* to the Hawequas or Southwest groups (Table 3.6). A plot of CV1 vs. CV2 shows very little overlap between these two groups (Table 3.5, Fig. 3.2).

The level of correct prediction for the Northwest group was 91.8 %, with two samples misclassified as Southwest. Notably, these samples were not from sites where the Northwest haplogroup was sympatric with individuals from the Southwest and Hawequas haplogroups. Individuals in these populations were correctly assigned, and are embedded in the core cloud of points representing the Northwest haplogroup. Four of the five Northwest samples misclassified as Karoo Mountain were from the Naudesberg. Naudesberg is the easternmost population representing the Northwest haplogroup and is geographically closest to a Karoo Mountain population (Boosmansbos) (Fig. 2.2).

Table 3.4: Factor structure matrix for the first three canonical variates derived from the discriminant functions analysis across the six clades. Each value denotes the correlation (r) between one of the canonical variates and the original variable. The total variance associated with each canonical variate is indicated below.

Character	CV1	CV2	CV3
Plant height	0.32	-0.65	0.07
First node	0.06	-0.26	0.13
Second node	0.24	-0.45	0.20
Inflorescence node	0.51	-0.60	0.30
Culm width	0.49	-0.03	-0.14
Leaf length	-0.02	-0.23	0.05
Leaf width	0.37	0.02	-0.34
Peduncle length	0.07	-0.24	-0.04
Spikelet length	0.62	-0.04	-0.61
Spikelet width	0.34	0.09	-0.20
Spikelet count	-0.16	-0.51	-0.40
Enclosing bract	0.31	0.07	-0.11
Percentage of among-group variance	48.3	26.5	19.73

Table 3.5: Summary of comparisons between squared Mahalanobis distances for haplogroups (below diagonal) and F stats (above diagonal) between groups. Full model stats: Wilks' Lambda= 0.03012; approximate $F_{60,1202} = 22.301$; $P < 0.0001$. Asterisks indicate significant discrimination at $P < 0.0001$

	Hawequas	Southwest	Northwest	Karoo Mountain	Anysberg	Seweweeks-poortberg
Hawequas	-	35.2*	42.6*	29.8*	17.9*	5.1*
Southwest	25.2	-	63.7*	44.0*	12.8*	12.8*
Northwest	26.7	21.8	-	33.4*	6.7*	7.5*
Karoo Mountain	19.7	16.5	9.6	-	4.6*	4.0*
Anysberg	41.1	25.6	12.9	8.9	-	7.5*
Seweweeks-poortberg	11.7	25.8	14.5	7.8	27.0	-

Table 3.6: Results of the classification analysis. Numbers in bold in table indicate: the total number individuals from a haplogroup assigned to a haplogroup by the model, in parentheses the number of individuals assigned *a priori* to a haplogroup. The percent correct column is the result of the latter divided by the former (*100). All *F*-statistics for the classifications are significant at $P < 0.0001$. P-values in the column headers indicate significance of the Post-hoc assignment of individuals to haplogroups.

Haplogroup	Percent correct	Post-hoc assignment					
		Hawequas p=.092	Southwest p=.213	Northwest p=.359	Karoo Mountain p=.286	Anysberg p=.0256	Seweweeks-poortberg p=.0256
Hawequas	100.0	25(25)	0	0	0	0	0
Southwest	100.0	0	58(58)	0	0	0	0
Northwest	91.8	0	2	90(98)	5	1	0
Karoo Mountain	84.6	1	5	3	66(78)	1	2
Anysberg	85.7	0	0	0	1	6(7)	0
Seweweeks-Poortberg	57.1	0	0	1	2	0	4(7)

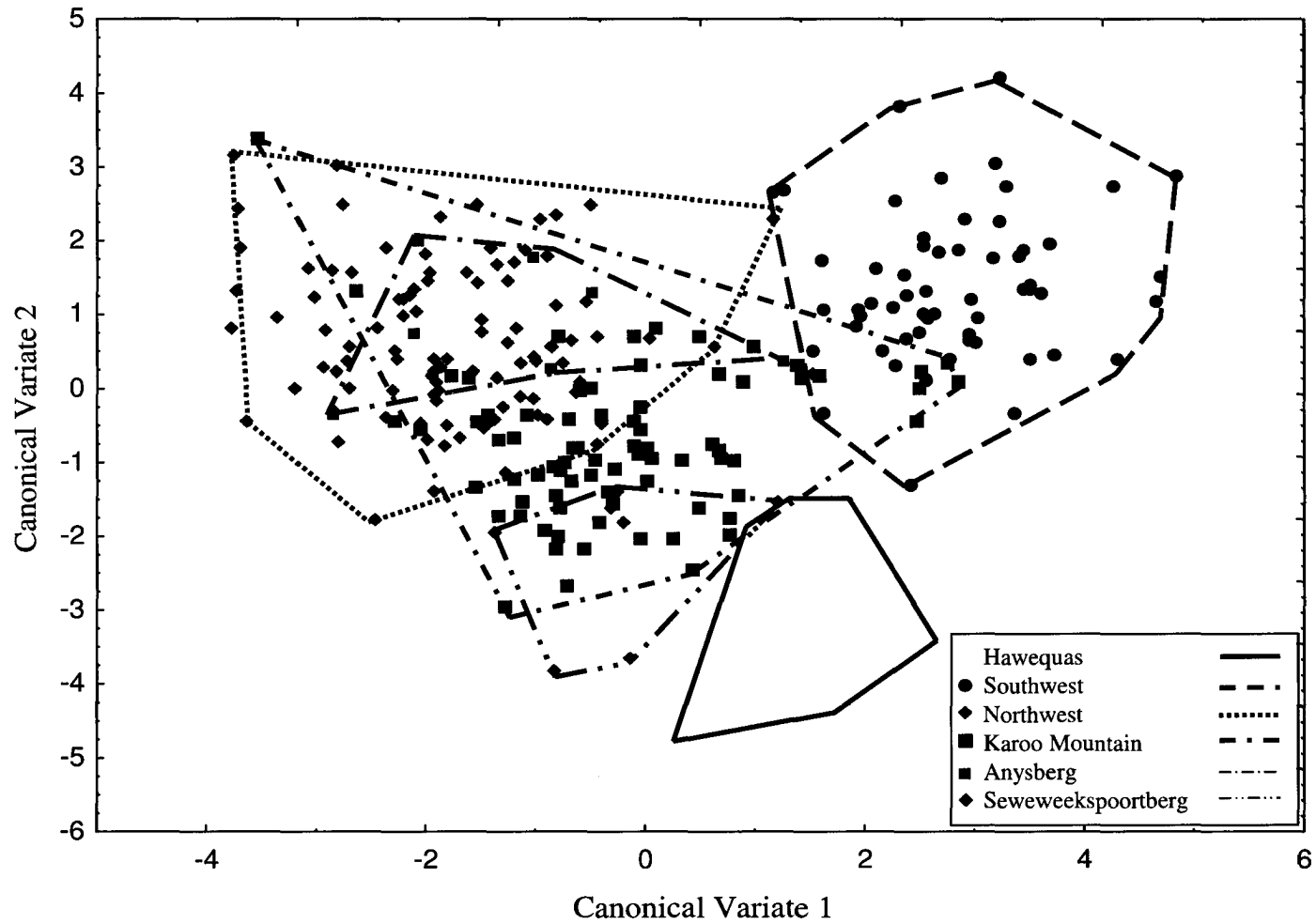


Figure 3.2: Ordination of the specimens on Canonical Variates 1 and 2 extracted from the discriminant functions analysis applied to 13 morphometric variables for the six groups. Symbols are included in the inset.

Similarly, the five Karoo Mountain specimens misclassified as Southwest occur at Boosmansbos, this being the Karoo Mountain population which is closest to a Southwest population (Misty Point) (Fig 2.2). The single-population haplogroups, Anysberg and Seweweekspoortberg, both showed high levels of incorrectly classified samples, mostly accounted for by their morphological similarity to the closely related Karoo Mountain haplogroup.

The first CV accounted for 48 % of the variation and discriminated the Hawequas and Southwest haplogroups from the remaining haplogroups (Fig. 3.2). The factor coefficient scores indicate that these groups have their first inflorescence nodes situated further up the culm, thicker culms and longer spikelets (Table 3.4, Fig. 3.2). The second CV discriminates (i) between the Hawequas and Southwest haplogroups, and (ii) between the Seweweekspoortberg and Anysberg haplogroups (Fig. 3.2). In both cases, the first group is taller, has its first inflorescence nodes situated further up the culm, and possesses more spikelets per inflorescence (Table 3.4, Fig 3.2). The third CV (not shown) discriminates only the Anysberg and Seweweekspoortberg haplogroups, with the latter group having longer and more numerous spikelets. The Northwest and Karoo Mountain groups show overlap along all five CVs (including non significant CVs), though they are somewhat separated along CV2 and CV3. This reflects the fact that the Karoo Mountain plants are taller, with the second culm node and first inflorescence node further up the culm. There is a strong correspondence between variables with high standardised coefficient values and those with high factor structure coefficients for a given CV. However, neither of these shows a perfect correspondence to variables identified as most discriminatory by the Newman-Keuls tests (Fig 3. 3).

Kruskal–Wallis

All Kruskal–Wallis comparisons were highly significant ($P < 0.0001$), but this may be due to the effect of one or more groups that are very different from the others (Table 3.7). The Newman-Keuls tests show which groups are significantly different for each variable (Fig. 3.3; Table 3.7). The Hawequas and Southwest groups are often part of the same homogenous group (Table 3.7). There is no single variable that perfectly distinguishes any group from every other group.

Key

A large number of specimens are incorrectly placed at most couplets within the key (Table 3.8, Fig. 3.4). Step 4, distinguishing the Northwest and Anysberg haplogroups, performs especially poorly, potentially misclassifying 85 % of the samples (Fig. 3.4). Comparatively, Steps 1 and 5 perform well with 28 % and 20 % ambiguously placed specimens respectively (Fig. 3.4). These two steps correspond with the deeper divergences within the complex, as illustrated by the DFA results (Fig. 3.2), where the samples can be most readily divided into three groups. Step 1 separates Hawequas and Southwest haplogroups from the remaining groups (Fig. 3.4), while step 5 separates the Hawequas and Southwest haplogroups (Fig. 3.4).

Discussion

Under the Evolutionary Species Concept, a *species is a lineage of ancestral descendent populations which maintains its identity from other such lineages and which has its own evolutionary tendencies and historical fate* (Wiley 1978). Thus evolutionary

Table 3.7: Summary of results from Analysis of variance; Population mean \pm standard deviations are given for each variable measured. Letters in bold indicate homogenous means based on a Newmann-Keuls test for homogeneity.

Variable	Hawequas	Southwest	Northwest	Karoo Mountain	Anysberg	Seweweeks poortberg
Plant height (mm)	1012 \pm 168 a	649 \pm 133 b	536 \pm 110 c	723 \pm 107 b	497 \pm 123 c	827 \pm 138 d
Leaf length (mm)	469 \pm 90 ab	394 \pm 101 ac	414 \pm 96 a	459 \pm 91 ab	330 \pm 79 c	511 \pm 111 b
Leaf width (mm)	9.35 \pm 1.91 a	9.67 \pm 2.15 a	6.86 \pm 1.54 b	6.53 \pm 1.48 b	5.64 \pm 1.35 b	7.71 \pm 1.95 ab
Spikelet length (mm)	15.30 \pm 0.71 a	14.98 \pm 1.17 a	12.25 \pm 0.79 b	11.93 \pm 1.30 b	10.65 \pm 1.28 c	13.24 \pm 0.80 d
Spike width (mm)	3.49 \pm 0.31 a	3.79 \pm 0.59 b	3.12 \pm 0.32 c	3.10 \pm 0.38 c	2.98 \pm 0.31 c	3.32 \pm 0.41 c
First node (mm)	241 \pm 59 a	190 \pm 63 b	173 \pm 60 b	221 \pm 56 ab	181 \pm 50 b	256 \pm 45 a
Second node (mm)	460 \pm 85 a	350 \pm 77 bc	288 \pm 66 d	390 \pm 64 b	304 \pm 79 cd	447 \pm 61 a
First inflorescence node	666 \pm 104 a	416 \pm 89 b	231 \pm 73 c	457 \pm 100 b	304 \pm 79 d	479 \pm 89 b
First peduncle	153 \pm 47 a	113 \pm 66 bc	99 \pm 26 bc	115 \pm 33 bc	85.71 \pm 22 c	129 \pm 47 ac
Number of spikelets (count)	61 \pm 21.24 a	15.98 \pm 7.06 b	28.48 \pm 13.16 c	23.90 \pm 9.61 bc	16.57 \pm 5.10 b	31.29 \pm 12.63 c
Culm width (mm)	4.82 \pm 0.96 a	4.84 \pm 0.87 a	3.22 \pm 0.69 b	3.55 \pm 0.65 b	3.14 \pm 0.48 b	3.00 \pm 0.50 b
Spikelet enclosing bract (mm)	6.79 \pm 1.21 a	8.34 \pm 1.69 bc	6.05 \pm 1.44 bc	6.37 \pm 1.66 bc	4.10 \pm 0.60 c	7.12 \pm 0.50 ab

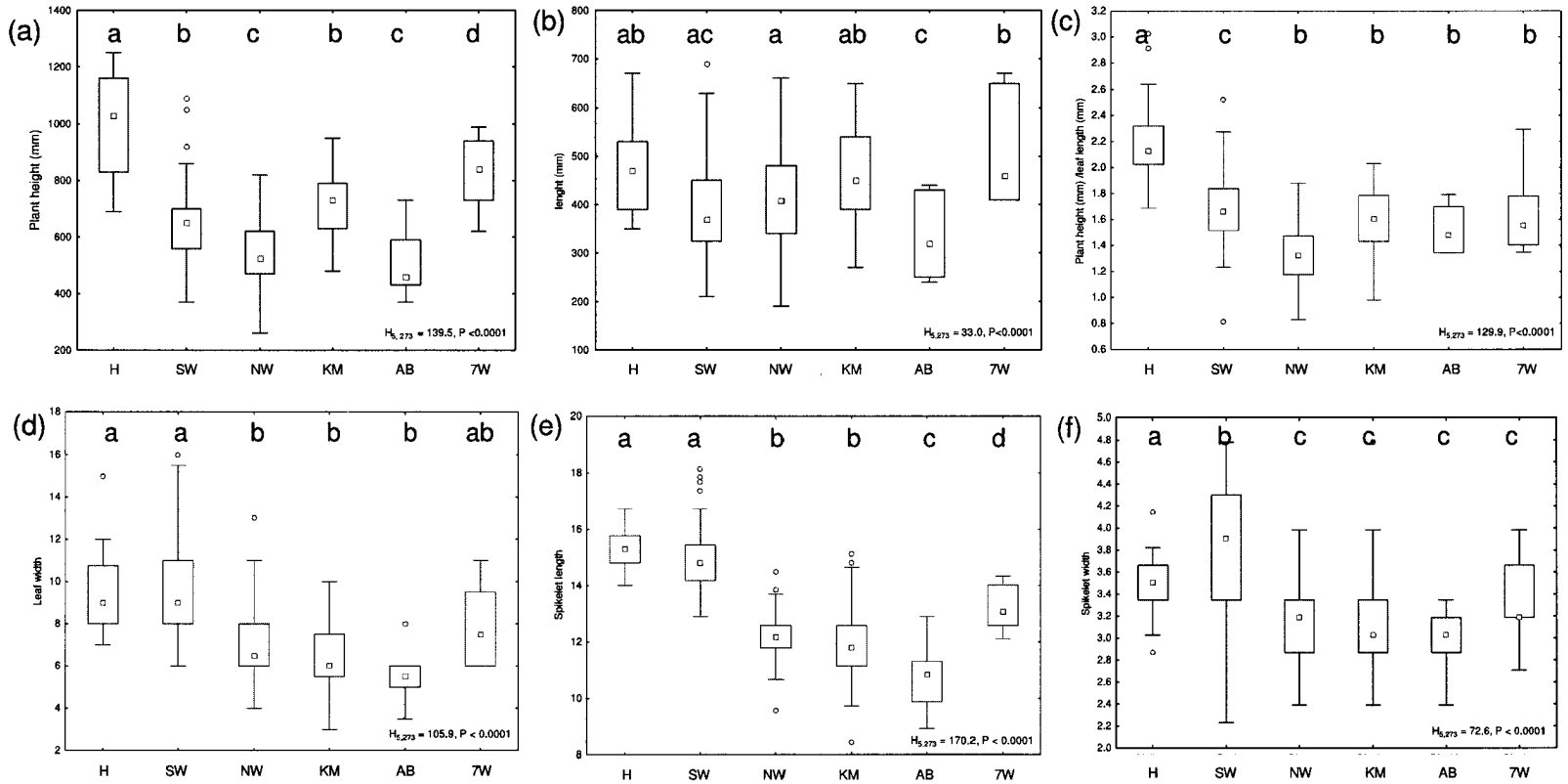


Figure 3.3: Results of Kruskal–Wallis analyses between haplogroups. Letters above graphs show Newman-Keuls test for homogeneity (a) Plant height (b) Leaf length (c) Plant height / Leaf length (d) Leaf width (e) Spikelet length (f) Spikelet width. Group Abbreviations: Hawequas (H), Southwest (SW), Northwest (NW), Karoo Mountain (KM), Anysberg (AB), Seweweekspoortberg (7W). Symbols: Boxes 25-75% = Confidence interval, whiskers Non-outlier range, circles = outliers and crosses = extreme values

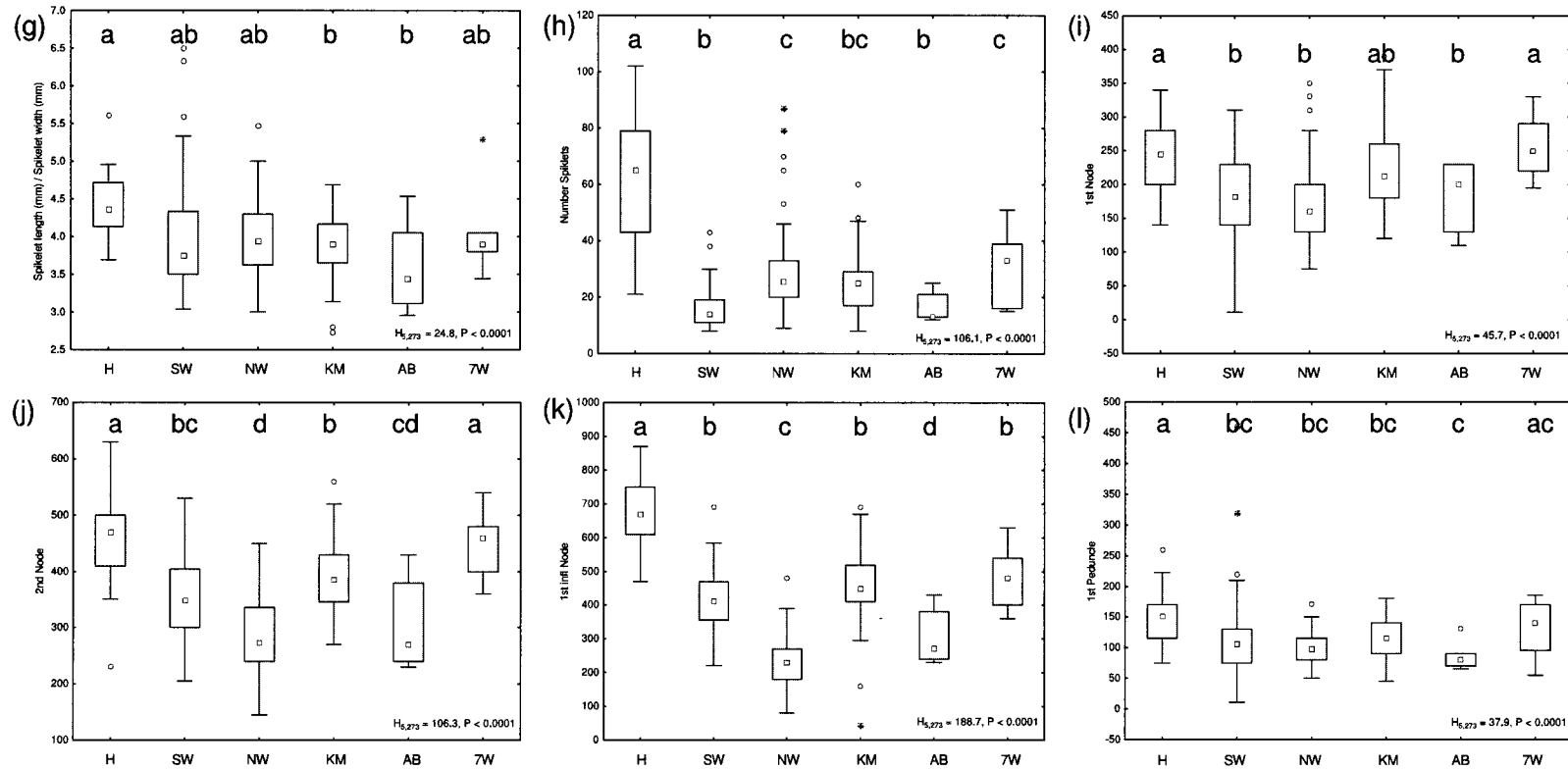


Figure 3.3: Results of Kruskal–Wallis analyses between haplogroups. Letters above graphs show Newman-Keuls test for homogeneity (g) Spikelet length (mm) / Spikelet width (mm) (h) Number of spikelets (i) First node (j) Second node (k) inflorescence node (l) Peduncle length. Group Abbreviations: Hawequas (H), Southwest (SW), Northwest (NW), Karoo Mountain (KM), Anysberg (AB), Seweweekspoortberg (7W). Symbols: Boxes 25-75% = Confidence interval, whiskers Non-outlier range, circles = outliers and crosses = extreme values

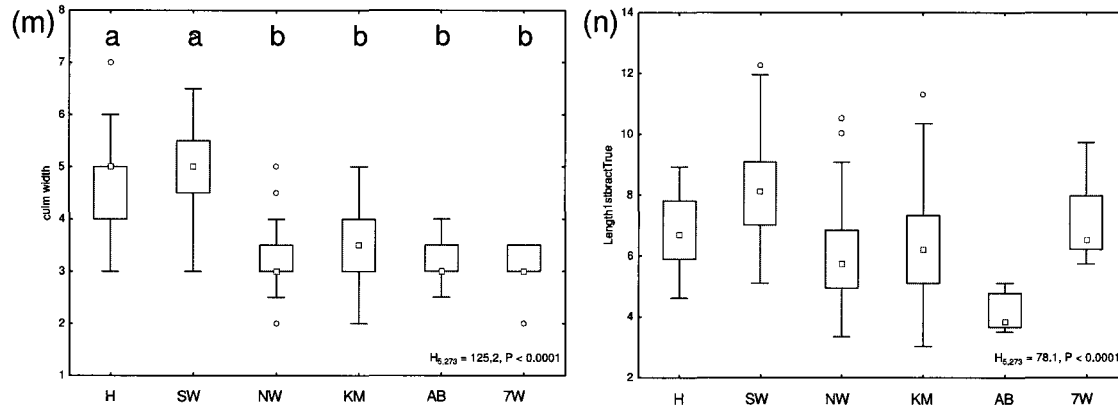


Figure 3.3: Results of Kruskal–Wallis analyses between haplogroups. Letters above graphs show Newman-Keuls test for homogeneity (m) Culm width (n) Enclosing bract (o). Group Abbreviations: Hawequas (H), Southwest (SW), Northwest (NW), Karoo Mountain (KM), Anysberg (AB), Seweweekspoortberg (7W). Symbols: Boxes 25-75% = Confidence interval, whiskers Non-outlier range, circles = outliers and crosses = extreme values

Table 3.8: Key to the six species with the *T. triangularis* complex. With percentage of samples ambiguously placed at each couplet presented below

1 Culms > 5mm wide, spikelets < 15.5mm Long.....	2
Culms < 3mm wide, spikelets > 13mm Long.....	5
Percentage potentially misidentified 28 %:	
2 Inflorescence Node < 295mm, Plant height < 480mm.....	3
Inflorescence Node > 480mm, Plant height > 820mm.....	4
Percentage potentially misidentified 34 %	
Northwest/Anysberg misidentified 44%	
Karoo Mountain/Seweweekspoort misidentified 22%	
3 No. of Spikelets > 25, Spikelet length >13mm.....	Northwest
No. of Spikelets < 25, Spikelet length < 9.5mm.....	Anysberg
Percentage potentially misidentified 85 %	
Anysberg misidentified 41%	
Northwest misidentified 44%	
4 Second Node < 360mm, Spikelet length < 12.25mm.....	Karoo Mountain
Second Node > 360mm, Spikelet length > 12.25mm.....	Seweweekspoortberg
Percentage potentially misidentified %	
Karoo Mountain misidentified 30%	
Seweweekspoort misidentified 100%	
5 Spikelet Number > 43 (rarely < 38); Plant Height > 1100.....	Hawequas
Spikelet Number < 21 (rarely > 38), Plant Height < 690.....	Southwest
Percentage potentially misidentified 20 %	
Hawequas misidentified 28 %	
Southwest misidentified 17 %	

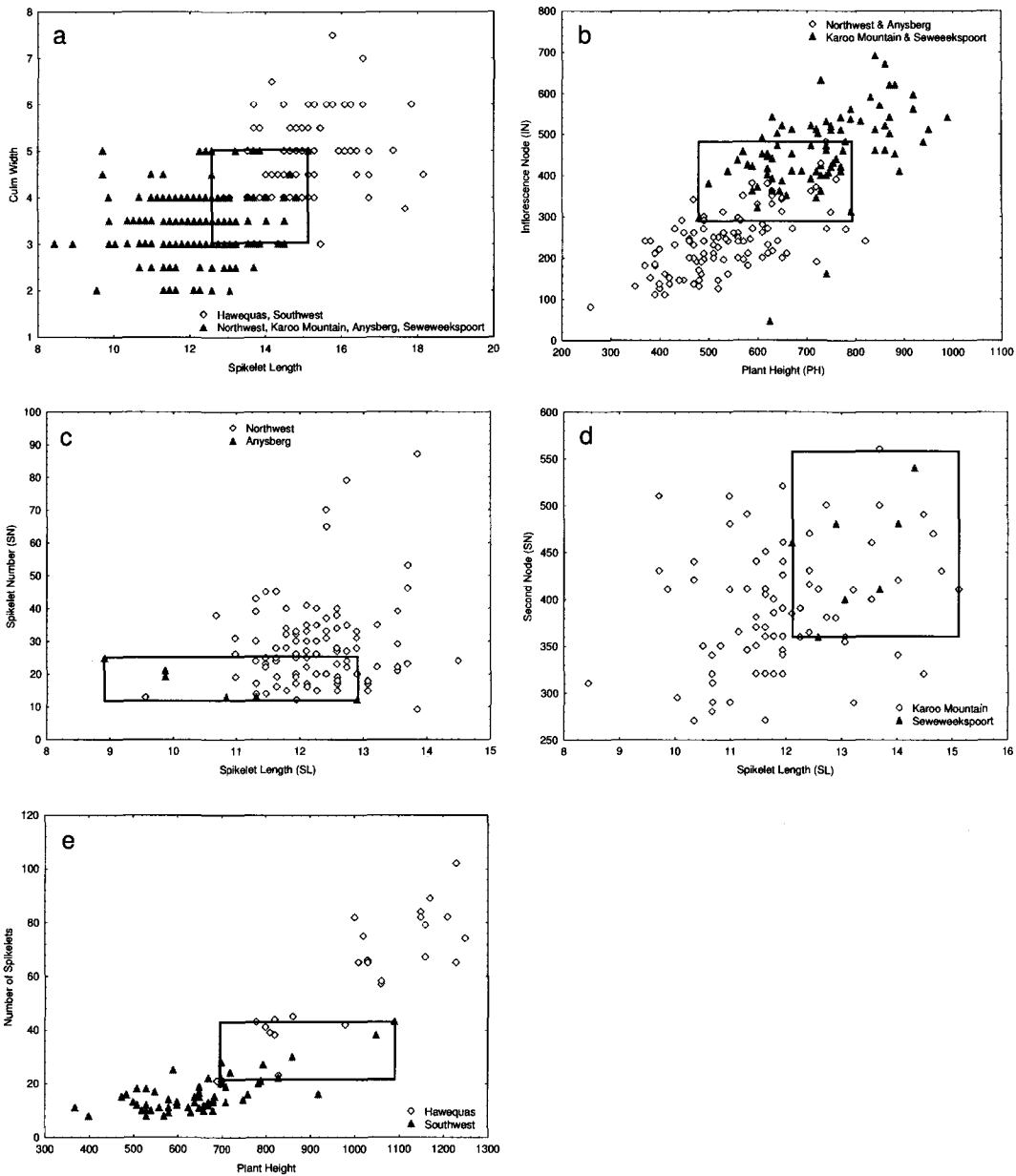


Figure 3.4: Bivariate plots showing the discrimination of taxa / taxonsets across each couplet of the key, using the two variables identified with the highest discriminating power. Couplets 1-5 are represented by (a) – (e). The squares in the diagrams indicate the areas of uncertainty associated with using the pair of measures in discriminating groups in the couplets.

species are defined purely in terms of their distinctness as evolutionary entities, their diagnosability being of secondary concern. In the *Tetraria triangularis* complex, I find that there are six identifiable evolutionarily independent lineages worthy of recognition as species, based on diverse lines of evidence.

The multi-locus DNA data set, including five chloroplast loci and one nuclear locus (Chapter 2), shows deep divergence between six haplogroups with morphology corresponding with genetic exclusivity. This is indicative of a period of isolation between these groups sufficient for unshared nucleotide changes (private alleles) to arise in each group (Templeton 2006). The phylogeographic pattern of the *trnH-psbA* chloroplast marker also shows that these genetic groups are geographically structured, with very little range overlap. Although comparable, geographically-coherent lineages have been reported in several studies (Kebede et al. 2007, Kuchta et al. 2009, Pyron and Burbrink 2009), these are rarely accorded formal recognition as species due to the absence of clear morphological divisions. For example, Kuchta et al. (2009) demonstrate genetically exclusive lineages with non-overlapping ranges within the salamander *Ensatina eschscholtzii*, but refrain from describing them as new species. This theme recurs in Kebede et al. (2007), Nicolas et al. (2008), Thum and Harrison (2009) and Venegas-Anaya et al (2008), all of whom recognise independent lineages with no geographical overlap, but do not term these species, referring to them only as independent lineages. Presumably, this situation is a result of there being insufficient morphological divergence in the characters measured upon which to base species divisions. The failure to elevate genetically distinct allopatric lineages to species status may be a function of taxonomic tradition, the particular species concept being applied, implicit taxonomic objectives

and/or neglect (Agapow et al. 2004). Following Wiley (1978), I take the view that geographical isolation paired with genetic exclusivity indicates that individuals within haplogroups in *T. triangularis* are not interacting as a single lineage, and that haplogroups constitute independent lineages and thus species.

In addition to their geographic isolation and genetic exclusivity, DFA confirms that haplogroups within *T. triangularis* s.l. are significantly different from each other on the basis of a combination of morphological variables. Strikingly, there is almost no overlap in the CV space between the more deeply divergent lineages. The use of bivariate morphological criteria to distinguish these haplogroups is not very successful, but this does not detract from the significant correspondence between the haplogroups and their significantly divergent morphologies. While these haplogroups lack clear uni- or bivariate breaks that are the hallmark of traditional morphological species (Cronquist 1978), this correspondence is evidence of their evolutionary independence. The morphological distinctiveness of some haplotype groups in sympatry is indicative of the emergence of reproductive isolation (Perrie et al. 2000). In the Hawequas Mountains (Fig. 2.2, Populations. 14, 15 & 16) and on Jonaskop (Fig. 2.2, Population 21), where haplogroups occur in sympatry, individuals can be reliably assigned to one of the three haplogroups known to be present by eye. This is confirmed by the DFA which groups all individuals from these sites with their correct haplogroup. By this measure, the Northwest, Southwest and Hawequas haplogroups are good biological species with respect to each other. If individuals from different haplogroups were interbreeding, we would expect to find evidence of morphological intermediates (Stebbins 1957) and/or a lack of correspondence

between morphological groups and genetic groups in areas of sympatry, because these trait sets are expected to segregate independently.

The tendency in some cases for populations near the edge of haplogroup ranges to resemble individuals of the geographically adjacent haplogroup may be a product of climate mediated selection. An example is the Naudesberg population (Fig.2.2; population 13) that genetically sorts with the Northwest haplogroup, but whose morphology is more similar to the Karoo Mountain haplogroup. Alternatively, this pattern could reflect interbreeding, perhaps by pollen movement between populations, given that this phenomenon is found in haplogroups that do not naturally occur in sympatry. Where haplogroup ranges do not overlap it is impossible, without targeted crossing experiments, to assess their potential to interbreed. This is, however, irrelevant in terms of my objective of recognising evolutionarily independent lineages, rather than biological species. Geographic isolation, lack of shared genotypes and correspondence of haplotypes and morphology where haplogroups co-occur, demonstrate that these haplogroups are on independent evolutionary trajectories with respect to each other, and thus constitute good evolutionary species. It would be desirable to test the patterns found in *trnH-psbA* against a nuclear locus to show conclusive evidence that there is no gene flow between lineages. There is some cross-corroboration from the ETS dataset for broad clade boundary congruence, but sampling is insufficient to be conclusive.

Although these haplogroup lineages qualify as evolutionary species (ESU: Ryder 1986, Agapow et al. 2004), they are not always readily diagnosable. There are large overlaps in multivariate space for several of the species despite the means being significantly different. An attempt to develop a dichotomous key using the two most

discriminatory characters available at each couplet was largely unsuccessful, with large percentages of individuals being ambiguously assigned. A multiple-access digital key was developed using DELTA (Dallwitz et al. 2007) and accompanies the thesis on a compact disc (Appendix 3). This is better able to assign individuals correctly to groups due to the simultaneous use of many variables.

Phylogeographers and taxonomists appear reluctant to describe cryptic species even where they show evidence of range disjunction and/or genetic exclusivity (Kebede et al. 2007, Kuchta et al. 2009, Pyron and Burbrink 2009). Although there is support equating independent evolutionary lineages to species (Venegas-Anaya et al. 2008, Funk et al. 2008.), taxonomists appear to feel some pressure to describe only species that are readily identifiable by ordinary means. There is a drive to discover evolutionary significant units (ESU), however, which can be defined as populations or groups of populations that merit separate management or priority for conservation because of high distinctness, in order to preserve evolutionary diversity (Moritz 1994, Allendorf and Luikart 2007). While the distinction between discovering an evolutionarily significant unit and naming a new species may seem trivial, it seems likely that unnamed lineages will fail to attract the attention of conservation managers and policy makers responsible for biodiversity conservation. Two of the species described here, *T. anysbergensis* and *T. seweweekspoortbergensis* are single-population species that are virtually impossible to identify reliably, using morphology alone, and it would be all too easy to subsume this diversity into a more inclusive encompassing group along with *T. macowaniana*. It would, however, constitute neglect to ignore this evolutionary diversity, which dates to 0.5-1.0 Ma (Fig. 2.5).

Wiens (2004a) has highlighted how niche conservatism, paired with range fragmentation, may promote speciation in montane taxa, resulting in species with low levels of morphological divergence. The *T. triangularis* complex is an example of this type of speciation. Prior to this phylogeographic study, *T. triangularis* was viewed as an “early-diverging” species, situated at the end of a relatively long branch and placed as sister to the morphologically diverse broad-leafed *Tetraria* clade. Re-evaluation of *T. triangularis*, however, reveals it to comprise a set of six morphologically indistinct species. This pattern recurs in *Lobelia giberroa* in the East African highlands (Kebede et al. 2007, Knox and Palmer 1998) and in *Ensatina eschscholtzii* salamanders in California (Kuchta et al. 2009). In each instance, divergent selection has created a clade of morphologically divergent species on the one hand, while selection for stasis accompanied by fragmentation has promoted a clade of morphologically indistinct species on the other.

Given the potential importance of fragmentation processes as a driver of speciation in the Cape (Chapter 2, Linder 2003), it is possible that several lineages may possess complexes of cryptic species. This is difficult to evaluate given the paucity of studies employing phylogeographic sampling of species in the CFR, particularly for montane species. The ranges of many other currently recognised species in the CFR share common barriers to gene flow, making it possible that they comprise many, as yet unrecognised, species (Weimarck 1941). In the Cape-endemic Proteaceae, for example, nine of the 18 species whose ranges extend beyond 400 km show documented coincidence of morphological variation with geographical distribution, potentially indicating the existence of multiple taxa (Appendix 2, Data from Rebelo, 1995). It seems

clear that additional phylogeographic studies and molecular biogeographies of this and other groups have the potential to elucidate the boundaries of many more evolutionarily independent lineages and, in so doing, better uncover the true process of speciation in the CFR. The striking coincidence of shared species distributions in many unrelated taxa, as indicated by the phylogeographic regions in the CFR, suggest a common allopatric mode of speciation at least in the montane zone. The utility of phylogeographic molecular sampling of organelles (mitochondrion and chloroplast), in discovering within species breaks congruent with known species distributions, has previously been demonstrated in northwestern North America (Funk et al. 2008, Knowles 2000, Knowles 2001) and the Japanese archipelago (Ikeda and Setoguchi 2007).

Here, I formally recognise six species within the *T. triangularis* complex and present the nomenclature for each. The name *T. triangularis* is identified with the Southwest group on the basis of Boeckeler's (1874) description of the type as having reddish spikelets, and Clarke's subsequent (1897) characterisation of the same material as possessing few (10-12), reddish-brown spikelets per inflorescence (Fig. 3.3h). *Tetraria macowaniana* is assigned to the Karoo Mountain haplogroup, because the type locality falls within an area occupied exclusively by that haplogroup. Although a specimen that identifies with the Northwest haplogroup is cited as a syntype of *T. robusta* var *γ pauperior* (Kükenthal 1931), the other syntype falls within *T. triangularis*. Kükenthal (1940) later subsumed this name into *T. macowani* (= *T. macowaniana*). Given that the rule of priority does not extend across ranks, there is no compulsion to use the name *pauperior*, I elect to apply a more descriptive name, *T. cederbergensis*, to the Northwest haplogroup (ICBN, Article 11). The Hawequas haplogroup is morphologically rather than

geographically distinct, prompting me to assign a name describing its tall and slender appearance and collection locality (*T. gracillioccultus*). Since there are no names available for the Anysberg and Seweweekspoort haplogroups these are assigned new names which reflect their native distributions (*T. anysbergensis* and *T. seweweekspoortbergensis*). The descriptions have not been provided in Latin here, in order to prevent the propagation of pseudo-descriptions, but will be provided in a publication.

Tetraria triangularis (Boeck.) C. B. Clarke, in Durand and Schinz, *Conspect. Fl. Afr.*: 663 (1895, nomen); *Fl. Cap.* 7: 286 (1897).

= *Lepidosperma triangulare* Boeck., in *Linnaea*, 38: 336 (1874).

= *T. bromoides* (Lam.) var *triangularis* (Boeck.) Kük., in *Fedde. Rep. Sp. Nov.* 29: 190 (1931).

Type: South Africa, Western Cape, Table Mountain; June 2005; *M.N. Britton* TM5 (BOL, neo!, designated here because holotype destroyed).

= *T. robusta* (Kunth) C.B. Clarke var γ *pauperior* Kük., in *Fedde, Rep. Sp. Nov.* 29: 190 (1931), in part.

Type: South Africa, Western Cape, Table Mountain; *Pillans 4856* (K, syn!), but excluding: South Africa, Western Cape, Koue Bokkeveld, Wagendrift, 5500 ft; 1897; *Schlechter 10078* (BOL, E, K, S, syn!).

Plants perennial. Tufted. *Tiller bases* barely enlarged, 40–60 mm in diameter. *Leaves* mostly basal, 21–63 cm tall, considerably shorter than the culms, or about equalling the

culms, about 0.4–0.8 times as high. *Basal leaf sheaths* yellow brown, dull, fraying at maturity, but not to form a distinctive net-like sheath. *Leaf blades* folded along the midrib, 6–15.5 mm broad, margins coarsely scabrid. *Cauline leaves* not overtopping the inflorescence, sheaths pale brown. *Culms* 37–85 cm high, 3.0–6.5 mm in diameter, trigonous, with sharp edges, having 1–2 nodes below the lowest inflorescence node. *Inflorescence* a contracted panicle, erect, 10–56 cm long, with the lowest node situated 22–69 cm above the plant base. *Spikelets* all bisexual, 8–40 in number, solitary, or in pairs or triplets, not or hardly enclosed by the inflorescence bracts, lanceolate, distinctly laterally compressed, red brown, 7.5–11.4 mm long, 1.4–3 mm wide. *Glumes* 8–9, distichous, chartaceous, rough, margins glabrous to minutely ciliate, lower and upper bract apices acuminate. *Flowers* 2, upper bisexual, lower male infertile. *Female-fertile flowers* 1 per spikelet. *Perianth bristles* present, 8 in number, 1–1.5 mm long, glabrous. *Stamens* 3. *Fruit* ovoid, terete, 4–5 mm long, 2–3 mm wide, smooth, shiny, yellowish brown, glabrous, gynophore absent, beak distinct, wrinkled, obtuse, 0.75–1.25 mm long. *Distribution range*, Table Mountain, Hottentots Holland, Hawequas, and Riviersonderend Mountains and an outlying population at Misty Point. *Altitude range* > 900 metres, or >1200 metres. *Vegetation* Mountain fynbos. *Substrate* Sandstone. *Flowering* February to April.

Tetraria gracilliocultus M.N. Britton, sp. nov., Similar to *T. triangularis* but taller with spikelets longer and blackish-brown. Spikelets more numerous, hanging free from the culm rather than closely adhering to the culm.

Type: South Africa, Western Cape, Wemmershoek Mountains, farm Elandspad, QDS 3319CC; April 2007; *M.N. Britton* W1 (BOL, holo.!).

Etymology: The name is based on the fact that this species is taller (700-1300mm) and more slender in appearance than *T. triangularis* and the fact that it had evaded discovery in a much collected area.

Plants perennial. Tufted. *Tiller bases* barely enlarged, 40–60 mm in diameter. *Leaves*, mostly basal, 35–67 cm tall, considerably shorter than the culms, about 0.3–0.6 times as high. *Basal leaf sheaths* yellow brown, dull, fraying at maturity, but not to form a distinctive net-like sheath. *Leaf blades* folded along the midrib, 7–12 mm broad, margins coarsely scabrid. *Cauline leaves* not overtopping the inflorescence, sheaths pale brown. *Culms* 69–125 cm high, 3–6 mm in diameter, trigonous, with sharp edges, with 1–2 nodes below the lowest inflorescence node. *Inflorescence* an open panicle, lax, 12–62 cm long, with the lowest node situated 47–87 cm above the plant base. *Spikelets* all bisexual, 21–102, solitary, or in pairs or triplets, not or hardly enclosed by the inflorescence bracts, narrowly-lanceolate, distinctly laterally compressed, dark brown, 8.5–10.5 mm long, 1.8–2.6 mm wide. *Glumes*, 8–10 bracts, distichous, chartaceous, rough, bract margins glabrous to minutely ciliate, lower and upper bract apices acuminate. *Flowers* 2, upper bisexual, lower male infertile. *Female-fertile flowers* 1 per spikelet. *Perianth bristles* 8, 1–1.5 mm long, glabrous. *Stamens* 3. *Fruit* ovoid, terete in cross section, 5–6 mm long, 2.5–3.5 mm wide, smooth, shiny, yellowish brown, glabrous, gynophore absent, *Beak* distinct, wrinkled, obtuse, 1–1.5 mm long.

Distribution range Hawequas Mountains, Jonaskop. *Altitude range* > 900 metres.

Vegetation Mountain fynbos. *Substrate* Sandstone. *Flowering* February to April.

Tetraria cederbergensis M.N. Britton, sp. nov., Differs from *T. triangularis* in that spikelets are narrower (2.4-4.0mm) and more numerous (10-85). Spikelets are chocolate brown rather than bright ferruginous brown.

Type: South Africa, Western Cape, Cederberg Mountains, western slopes of Cederberg Sneekop, QDS 3219AC; January 2005; *M.N. Britton* SK1 (BOL, holo!).

= *T. robusta* (Kunth) C.B. Clarke var. *γ pauperior* Kük., in Fedde, Rep. Sp. Nov. 29: 190 (1931), in part.

Type: South Africa, Western Cape, Wagensdrift, 5500ft; 1897; *Schlechter 10078* (BOL, E, K, S syn.); but excluding: South Africa, Western Cape, Table Mountain; *Pillans 4856* (K, syn.).

Etymology: The name refers to the Cederberg mountain range, whose vegetation typifies the natural habitat of this species, and which contains the type locality.

Plants perennial. Tufted. *Tiller bases* barely enlarged, 20–40 mm in diameter. *Leaves* mostly basal, 19–66 cm tall, considerably shorter than the culms, or about equalling the culms, about 0.5–1.2 times as high. *Basal leaf sheaths* yellow brown, dull, fraying at maturity, but not to form a distinctive net-like sheath. *Leaf blades* folded along the midrib 4–11 mm broad, not coiling at the tips, margins coarsely scabrid. *Cauline leaves* not overtopping the inflorescence, sheaths pale brown. *Culms* 26–82 cm high, 2.5–4 mm in diameter, trigonous, with sharp edges, with 1–2 nodes below the lowest inflorescence

node. *Inflorescence* a contracted panicle, erect, 13–58 cm long, with the lowest node situated 8–39 cm above the plant base. *Spikelets* all bisexual, 9–87 in number, solitary, or in pairs or triplets, not or hardly enclosed by the inflorescence bracts, narrowly-lanceolate, distinctly laterally compressed, dark brown, 6–9.1 mm long, 1.5–2.5 mm wide. *Glumes* 8–10, distichous, chartaceous, rough, margins glabrous to minutely ciliate, lower and upper bract apices acuminate. *Flowers* 2, upper bisexual, lower male infertile. *Female-fertile flowers* 1 per spikelet. *Perianth bristles* present, 8 in number, 1–1.5 mm long, glabrous. *Stamens* 3. *Fruit* ovoid, terete in cross section, 4.5–5 mm long, 2.5–3 mm wide, smooth, shiny, reddish brown, glabrous, gynophore absent. *Beak* distinct, wrinkled, obtuse, beak 0.75–1 mm long.

Distribution range Waboomsberg, Keeromsberg, Naudesberg, Vleiberg, Cederberg, KoueBokkeveld, Hex River and Hawequas Mountains. *Altitude range* >1200 metres. *Vegetation* Mountain fynbos. *Substrate* Sandstone. *Flowering* February to April.

Tetraria macowaniana B.L. Burt, in Notes from the Royal Botanic Garden Edinburgh 34: 282 (1976).

= *Chaetospora hexandra* Boeck., in Flora 61: 37 (1878); non *Tetraria hexandra* (Nees) Kük .

= *Tetraria macowanii* C.B. Clarke, in Durand and Schinz, Consp. Fl. Afr. 5: 661 (1895, nomen) in Fl. Cap. 7: 290 (1898); nomen illegit.

Type: South Africa, Eastern Cape, Somerset East, on the summit of the Boschberg, 4500 ft; *MacOwan* 1864 (Z, holo.!; BOL, K, iso.!)

Plants perennial. Tufted. *Tiller bases* barely enlarged, 20–40 mm in diameter. *Leaves* basal, 48–95 cm tall, considerably shorter than the culms, or about equalling the culms, about 0.5–1 times as high. *Basal leaf sheaths* yellow brown, dull, fraying at maturity, but not to form a distinctive net-like sheath. *Leaf blades* folded along the midrib, 3–10 mm broad, not coiling at the tips, margins coarsely scabrid. *Cauline leaves* not overtopping the inflorescence, sheathes pale brown. *Culms* 48–95 cm high, 2–5 mm in diameter, trigonous, with sharp edges, with 1–2 nodes below the lowest inflorescence node.

Inflorescence a contracted panicle, erect, 9–58 cm long, with the lowest node situated 30–68 cm above the plant base. Spikelets all bisexual, 8–60, solitary, or in pairs or triplets, not or hardly enclosed by the inflorescence bracts, narrowly-lanceolate, distinctly laterally compressed, brown, or dark brown, 5.3–9.5 mm long, 1.5–2.5 mm wide. *Glumes* 8, distichous, chartaceous, rough, margins glabrous to minutely ciliate lower and upper bract apices acuminate. *Flowers* 2, upper bisexual, lower male infertile. *Female-fertile flowers* 1 per spikelet. *Perianth bristles* present, 8 in number, 1–1.5 mm long, glabrous. *Stamens* 3. *Fruit* ovoid, terete, 4.5–5 mm long, 2.6–3 mm wide, smooth, shiny, brown, or yellowish brown, glabrous, gynophore absent. *Beak* distinct, wrinkled, obtuse; beak 0.75–1 mm long.

Distribution range Boosmansbos, Somerset Sneeukop, Hogsback, Baviaanskloof mountains and Groot Swartberg Mountains. *Altitude range* >1200 metres. *Vegetation* Mountain fynbos. *Substrate* Sandstone. *Flowering* March to May.

Tetraria anysbergensis M.N. Britton, sp. nov., Similar to *T. macowaniana* but with plants smaller in stature (350-710mm), spikelets shorter (9-13mm) and occurring on the Anysberg.

Type: South Africa, Western Cape, Klein Swartberg Mountains, south slopes of the Anysberg, QDS 3320DA; June 2006; *M.N. Britton* AB5 (BOL, holo.!).

Etymology: Named for the Anysberg, the only locality where the species occurs.

Plants perennial. Tufted. *Tiller bases* barely enlarged, 20–30 mm in diameter. *Leaves* mostly basal, 37–73 cm tall, considerably shorter than the culms, or about equalling the culms, about 0.5–0.8 times as high. *Basal leaf sheaths* yellow brown, dull, fraying at maturity, but not to form a distinctive net-like sheath. *Leaf blades* folded along the midrib, 3.5–6 mm broad, not coiling at the tips, margins coarsely scabrid. *Cauline leaves* not overtopping the inflorescence, sheaths pale brown. *Culms* 37–730 cm high, 2.5–4 mm in diameter, trigonous, with sharp edges, with 1–2 nodes below the lowest inflorescence node. *Inflorescence* a contracted panicle, erect, 13–30 cm long, with the lowest node situated 23–43 cm above the plant base. *Spikelets* all bisexual, 12–30, solitary, or in pairs or triplets, not or hardly enclosed by the inflorescence bracts, narrowly-lanceolate, distinctly laterally compressed, brown, 5.5–8.1 mm long, 1.5–2.1 mm wide. *Glumes* 8, distichous, chartaceous, rough, bract margins glabrous to minutely ciliate, lower bract apices acuminate, upper bract apices acuminate. *Flowers* 2, upper bisexual, lower male infertile. *Female-fertile flowers* 1 per spikelet. *Perianth bristles* present, 8 in number, 1–1.5 mm long, glabrous. *Stamens* 3. *Fruit* ovoid, terete, 4–4.5 mm long, 2.5 mm wide,

smooth, shiny, yellowish brown, or greenish grey, glabrous; gynophore absent. *Beak* distinct, wrinkled, obtuse, 0.5–0.75 mm long.

Distribution range Anysberg. *Altitude range* >1200 metres. *Vegetation* Mountain fynbos.

Substrate Sandstone. *Flowering* March to April.

Tetraria seweweekspoortbergensis M.N. Britton, sp. nov., Similar to *T. macowaniana*, but taller in stature, with larger spikelets, broader leaves and occurring on the Seweweekspoortberg.

Type: South Africa, Western Cape, Klein Swartberg Mountains, north slopes the Seweweekspoortberg, July 2007; *M.N. Britton* 7W7 (BOL, holo.!).

Etymology: Named for the Seweweekspoortberg, the only locality where the species occurs.

Plants perennial. Tufted. *Tiller bases* barely enlarged, 40–60 mm in diameter. *Leaves* basal, 62–99 cm tall, considerably shorter than the culms, about 0.4–0.7 times as high. *Basal leaf sheaths* yellow brown, dull, fraying at maturity, but not to form a distinctive net-like sheath. *Leaf blades* folded along the midrib, 6–11 mm broad, margins coarsely scabrid. *Cauline leaves* not overtopping the inflorescence, sheaths pale brown. *Culms* 62–99 cm high, 3–3.5 mm in diameter, trigonous, with sharp edges, with 1–2 nodes below the lowest inflorescence node. *Inflorescence* a contracted panicle, erect, 10–46 cm long, with the lowest node situated 36–63 cm above the plant base. *Spikelets* all bisexual, 15–51, solitary, or in pairs or triplets, not or hardly enclosed by the inflorescence bracts,

narrowly-lanceolate, distinctly laterally compressed, pale brown, 7.6–9 mm long, 1.7–2.5 mm wide. *Glumes* 8 in number, distichous, chartaceous, rough, bract margins glabrous to minutely ciliate, lower bract apices acuminate, upper bract apices acuminate. *Flowers* 2, upper bisexual, lower male infertile. *Female-fertile flowers* 1 per spikelet. *Perianth bristles* present, 8 in number, 1–1.5 mm long, glabrous. *Stamens* 3. *Fruit* ovoid, terete, 4.75–5 mm long, 2.25–2.5 mm wide, smooth, shiny, yellowish brown, or greenish grey, glabrous, gynophore absent. *Beak* distinct, wrinkled, obtuse, 1 mm long.

Distribution range Klein Swartberg Mountains, Seweweekspoortberg. *Altitude range* >1200 metres. *Vegetation* Mountain fynbos. *Substrate* Sandstone. *Flowering* March to April.

Chapter 4: Diversification in *T. triangularis*: an example of non – adaptive radiation in the CFR

The CFR is renowned for the phenomenal richness and high levels of endemism of plant species (Good 1953, Goldblatt 1978, Takhtajan 1986, Cowling et al. 1992, Goldblatt and Manning 2000), which have primarily been defined on the basis of morphological diagnosability. Molecular tools provide an opportunity to define species that have arisen via a non-adaptive mechanism, potentially enabling the identification of lineages that are not readily distinguished by morphology. This study represents one of the very few examples of population level genetic investigation within a widespread Cape plant species. An investigation of this nature simultaneously raises questions about the nature of species diversity and the process by which it originates. Here, I briefly review the main findings of this study and examine their implications for our understanding of the floristic diversification of the CFR.

Populations of *T. triangularis* show strong geographic structuring of chloroplast haplotypes that has been generated over a period of at least 3 million years, and possibly as much as 8 Ma. The dual impacts of continental uplift across the CFR and intensification of summer aridity in the western half of the CFR during the Miocene-Pliocene are implicated in the reduction and fragmentation of suitable montane habitats, thus increasing isolation between montane lineages, as a mechanism for increasing the isolation between populations of montane lineages, through the reduction of habitat area. Remarkably, despite the long divergence times involved, morphological divergence between populations is weak, resulting in deeply diverged clades displaying very similar

morphology. However, what little morphological differentiation is evident is highly correlated with molecular structure. Thus, the earliest diverging clades show the greatest morphological variation, whereas the most recently diverged clades are, for practical purposes, impossible to distinguish on the basis of morphology.

The correspondence of morphological and molecular divergence, and the long periods over which they have been generated, leads to rejection of the hypothesis of a single widespread species, *T. triangularis* (Leache 2009). Instead, using this evidence under the framework of the Evolutionary Species Concept (Wiley 1978), I have opted to delimit six species. I do this despite an overall reluctance by past workers to recognise formally well-supported, morphologically-cryptic lineages as species (Kebede et al. 2007, Pyron and Burbrink 2009). Two of the species I describe constitute single populations, while the others are somewhat more widespread. The spatial distribution of the species is strongly reminiscent of the phytogeographic regions that have been noted by previous workers (Weimarck 1941, Oliver et al. 1983, Cowling et al. 1992, Goldblatt and Manning 2000, Linder 2001, Linder 2003), and suggests that the geographical processes that have been at work in this clade may also be responsible for diversification in other Cape clades.

Most genera of Cape plants were last revised prior to the era of molecular systematics (e.g. *Phylica*; Pillans 1942, *Brunia*; Pillans 1947, *Agathosma*; Pillans 1950, *Leucodendron*; Williams 1972, *Leucospermum*; Rourke 1972); thus most molecular phylogenetic analyses rely heavily on morphology-based taxonomies to guide sampling, and rarely incorporate multiple accessions of widespread species. The implication is that many more taxa, which like those within the *T. triangularis* complex are morphologically

indistinguishable, may in fact represent multiple lineages. While further phylogeographic studies are required to test this hypothesis conclusively, Swart et al. (2009) corroborate this pattern in the southern rock agama (*Agama atra*). Cryptic species of this nature are probably concentrated within taxa such as Poaceae, Restionaceae and Cyperaceae whose reduced morphological features limit the availability of characters upon which to base species divisions.

The existence of more-or-less allopatrically-distributed, cryptic species indicates a potential role for non-adaptive processes (see Rundell and Price 2009) in driving speciation in the CFR. Paired with geographical isolation, rugged topography and spatial heterogeneity in climate underpin the insular, archipelagic distribution range of the *T. triangularis* complex. In the absence of explicit fine-scale paleoclimatic reconstruction scenarios for the Quaternary and earlier, it is very difficult to evaluate the exact effect of historical climate shifts on the distribution of the species included within the complex. In the western half of the CFR, however, species are significantly associated with topography on either side of the Berg and Breede river valleys, while on the boundary between the western and eastern CFR, the interface between *T. cederbergensis* and *T. macowaniana* is more readily characterised by difference in climate.

The speciation processes invoked in this study differ starkly from the predominantly ecologically-driven mechanism proposed by previous workers to explain the floristic richness of the CFR (Linder and Vlok 1991, Verboom et al. 2003, Ellis et al. 2006). Lineages in the *T. triangularis* complex satisfy the predictions of an allopatric speciation mechanism mediated by niche conservatism, as documented by Wiens and co-workers (Wiens 2004a, Kozak and Wiens 2006). Under this mechanism, species

differentiate precisely because they are unable to adapt to novel environments (phylogenetic niche conservatism: Ricklefs and Latham 1992, Peterson et al. 1999, Webb et al. 2002). Phylogenetic niche conservatism is apparent in the *T. triangularis* complex since it is restricted to montane habitats and has not adapted to the diversity of edaphic and climatic conditions that characterise the lowlands, over the ca. 3 Myr period over which diversification has occurred. This does not, however, preclude the existence of some adaptation to local conditions in isolation, since this seems inevitable wherever there is allopatry (Rundell and Price 2009). Allopatry mediated by phylogenetic niche conservatism is merely the initial mechanism that keeps populations separated during speciation.

Crucially, this complex does not fulfill the requirements of a speciation process driven by adaptive radiation, in that there is little evidence for the characteristic accumulation of substantial morphological and ecological diversity (Schluter 1996, Schluter 2000). Indeed, a comparison of morphological trait divergence amongst species within the *T. triangularis* complex with that shown by species in its low-altitude sister clade, reveals greater variation in the latter for all variables measured (Fig. 4.1). The implication is that ecological speciation mechanisms may play a greater role in lowland taxa, where range overlap may occur more readily and sooner after speciation, than in altitudinally restricted species.

Despite the potential importance of topography and climate boundaries as barriers to gene flow, and hence as drivers of allopatric speciation, there is evidence that some of the newly recognised species have traversed these boundaries subsequent to their initial

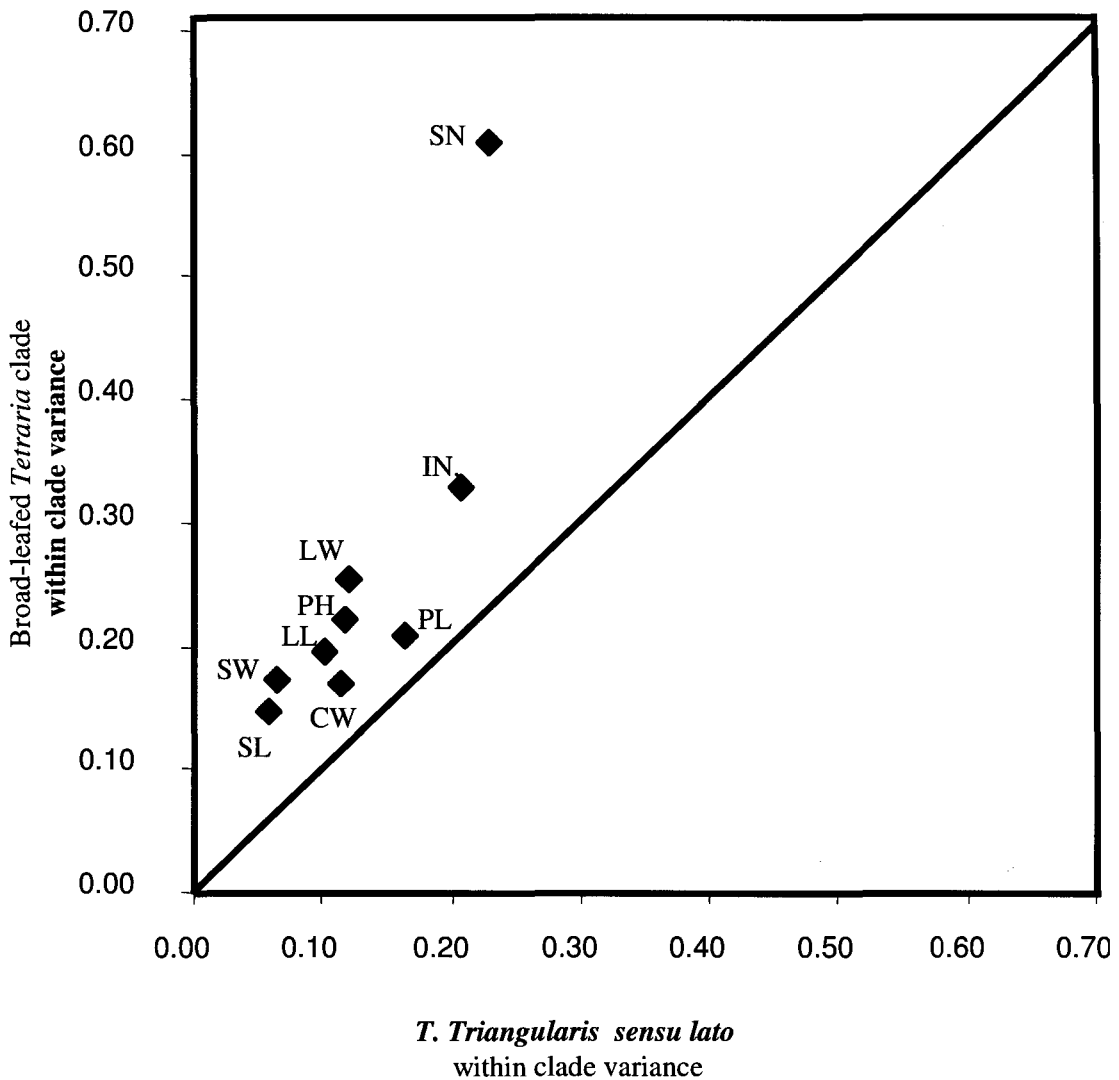


Figure 4.1: Plot comparing trait variance within the broad-leafed *Tetraria* (Fig 2.4) and *T. triangularis* clades. Traits from the data used in Chapter 3, which were obtainable for species in both the *T. triangularis* clade and the broad-leafed *Tetraria* clade, were used. All variables were transformed to log10 to give ensure a comparable order of magnitude for species of various sizes. Factorial ANOVA was performed on each of the clades and the within group variances plot here against a line with a slope = 1 (The expectation if trait variance is identical in both groups). Characters shown : Plant height (PH), Leaf length, (LL), Length of first peduncle (PL), Inflorescence node (IN), Leaf width (LW), Culm width (CW), Spikelet number (SN), Spikelet length (SL), Spikelet width (SW).

differentiation. For example, populations of *T. cederbergensis* occur on the tops of peaks in the Hawequas mountains, separated from the remaining populations by the Berg and Breede river valleys. The timing of haplotype divergence between these lineages (< 1 Ma) suggests that this “back-colonisation” was recent, and possibly associated with glacial cycles during the Quaternary. Interestingly, the fact that the association of morphology and cpDNA variation remains intact even when species are in contact suggests that the initial period in allopatry was sufficient to allow reproductive isolation to develop (cf. Moyle et al. 2004, Bolnick and Near 2005). This identifies the species occurring in the Hawequas Mountains as good biological species (Mayr 1957), despite their cryptic nature.

In the context of published molecular studies on the Cape Flora, this study is characterised by the sampling of many individuals from populations from virtually all of the known localities of the species. This enhances the scope to identify evolutionary species and to explore the speciation process. Most published molecular work in this system has been phylogenetic in nature, with single accessions used to represent entire species. One of the most completely sampled examinations of the spatial aspect of speciation in the Greater CFR is that Ellis and co-workers (Ellis and Weiss 2006, Ellis et al. 2006), dealing with *Argyrodema*, but even this does not adequately demonstrate monophyly of the species concerned, nor is the entire range sampled. The single accession approach limits the ability to discover deep divergences that have not given rise to distinct morphological characters. In the context of a phylogenetic sampling strategy, climate and edaphic gradients are far simpler to invoke as explanations for species divergence, thus much speciation is attributed to ecological process. In addition,

incomplete taxon sampling results in loss of signal at the tips of the tree. Thus sampling is crucial to uncovering the geographical (or not) nature of recent speciation events, because the signal from recent speciation events rapidly fades and is overwritten by subsequent adaptation and dispersal (Losos and Glor 2003).

To conclude, the landscapes of the Cape Folded Mountain system have powered the production of new species within the *T. triangularis* complex, some of which would not be recognised by a traditional morphologically-based taxonomic approach. Population level sampling of molecular and morphological data, for other montane lineages will be required to ascertain if cryptic species are indeed common. If cryptic species are found to be general phenomenon, then the already-remarkable species richness of the CFR would be far greater than currently estimated.

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