

# The shifting landscape of genes since the Pliocene: terrestrial phylogeography in the Greater Cape Floristic Region

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## 7.1 Introduction

The Greater Cape Floristic Region (GCFR) is considered megadiverse, and is recognized primarily for its extraordinary floristic richness and endemism (Chapter 4). Despite the deep origins of many clades in the Cenozoic (e.g. Chapter 5, Tolley et al. 2006; Forest, Grenyer, et al. 2007; Fjeldså and Bowie 2008), much of the present-day diversity within the GCFR is attributed to diversification during the Pliocene (5–2.5 Ma) and Pleistocene (2.5 Ma–20 000). This diversity is observable through the substantial phylogeographic structuring in many taxa examined to date, particularly for those that are specialists and/or lack vagility (e.g. Price et al. 2007; Smit et al. 2007; Price et al. 2010; Willows-Munro and Matthee 2011). However, because diversification on this timescale is relatively recent, the result is characteristically shallow genetic lineages, often within recently radiated species or species complexes (e.g. Mummenhoff et al. 2005; Tolley et al. 2006, 2009). Our understanding regarding patterns of floristic diversity in the GCFR have benefited greatly from molecular phylogenetic work, particularly for deeply divergent clades rooted in the Cenozoic (Chapter 5), with comparatively less work at the phylogeographic level. In contrast, a great deal of phylogeographic work has been produced on animal taxa. This floral–faunal imbalance probably reflects a bias due to the broad availability of suitable genetic markers for resolving phylogeographic patterns in animals. In particular,

the mitochondrial genome (mtDNA) has been successfully utilized across multiple animal taxa, despite the inherent bias of this marker due to its uniparental inheritance mode. The general focus on understanding the evolution of species-rich clades of plants (i.e. ‘Cape clades’; Linder 2003), and their use as proxies to unravel the causal factors responsible for the floral diversity of the GCFR, might also explain the limited number of phylogeographic studies for plant groups.

Typically, there are two time frames to which phylogeographic structure is attributed in the GCFR. The earlier diversification events are rooted in the Late Miocene and Pliocene, and these are often associated with recently diverged species, which are usually reciprocally monophyletic, but exhibit shallow divergences (e.g. Tolley et al. 2006; Price et al. 2010; Pereira-da-Conceicao et al. 2012). More recent diversification events are dated to the Pleistocene, but these appear to be either divergence events amongst populations within species (Smit et al. 2010) or in some cases between species that are not reciprocally monophyletic and share ancestral polymorphisms in the mtDNA (Tolley et al. 2006; Oatley et al. 2012). Often, discrete geographic boundaries amongst these clades are blurred, with alleles or haplotypes overlapping across geographic regions or habitat types (Swart et al. 2009; Russo et al. 2010). The lack of clear geographic pattern in the distribution of haplotypes could be due to shared ancestral polymorphisms in what are presently species (or populations) with restricted gene flow.

Alternatively, the geographic distribution of clades may not be adequately understood or characterized, making conclusions about how (and where) gene flow is restricted tentative at best.

Much of the phylogeographic work conducted in the GCFR has attempted to link patterns of geographic variation in genetic markers to causative factors, particularly with the intention of identifying barriers to gene flow that correspond to the geographic distribution of clades. In the GCFR, disruption of gene flow in the flora has been attributed to climate, edaphic factors, phenology, pollination systems, and fire-mediated life cycles, amongst others (e.g. Chapter 6, Warren et al. 2011). In terrestrial fauna, the presence of rivers, mountains, semi-arid plains, and rocky terrain appear loosely to delimit phylogeographic clades (e.g. Matthee and Flemming 2002; Sole et al. 2005; Price et al. 2007; Smit et al. 2007; Price et al. 2010; Daniels, Heideman et al. 2009; Daniels, Picker, et al. 2009; McDonald and Daniels 2012), whereas specialization for climate, habitat and niche have been implicated for a range of other taxa (e.g. reptiles (Tolley et al. 2008), small mammals (Smit et al. 2010), birds (Ribeiro et al. 2011; Oatley et al. 2012), and freshwater insects (Pereira-da-Conceicao et al. 2012)). While some physical components of the landscape seem to correspond to phylogeographic breaks, sparse sampling around these breaks is typically inadequate to distinguish whether the clades are truly bound by the identified geographic features because sampling is often not carried out to test such hypotheses explicitly (Schwartz and McKelvey 2009; Anderson et al. 2010). This can result in erroneous conclusions regarding the nature of geographic clustering, and can lead to a search for mechanisms to explain patterns that might be biologically meaningless. Furthermore, gene flow occurs only as a consequence of dispersal (and pollination in plants), which is in turn dictated by biological processes. Thus, phylogeographic patterns are not static in space and time but are influenced by a myriad of dynamic biotic and abiotic factors including ecological interactions, physiology, behaviour, adaptive potential, and dispersal ability (Chapter 6, Ricklefs and Bermingham 2002; Lomolino et al. 2010). Therefore, the correlations shown between palaeoenvironmental factors at the landscape level and phylogeographic patterns only allow for speculation of the actual mechanisms of diversification (Chapter 6). A genuine understanding of the drivers of phylogeographic patterns will emerge only through knowledge of organismal responses to environmental factors.

## 7.2 Phylogeography and the environment

Within the GCFR, phylogeographic structure of many lineages is typically shallow with most divergence estimates dated within the last few million years (i.e. Pliocene or Pleistocene). Thus, causative explanations regarding patterns, and direction or rate of gene flow within species or species complexes most likely relate to past environmental or biological conditions that correspond in timing with these divergence events. The GCFR environment during this period must therefore be thoroughly understood in order to have expectations towards understanding the drivers that generated the phylogeographic patterns observed today. Following a relatively warm and wet Miocene period, the Late Miocene–Early Pliocene climate was one of progressive dryness (Sepulchre et al. 2006; Senut et al. 2009; Bonnefille 2010). In addition, across Africa there was a major shift from biomes dominated by  $C_3$  plants (fynbos, forests, and thicket) to those dominated by  $C_4$  grasses (savanna and grasslands) 6–8 Ma (Cerling et al. 1997; Edwards et al. 2010, Cerling et al. 2011). In southern Africa, this shift resulted in the reduction of forest and thicket into relict fragments; fynbos, however, likely persisted throughout these climatic upheavals, especially on the infertile landscapes of the Cape mountains (Chapter 8). In the GCFR, this period also saw the establishment of the renosterveld and succulent karoo (Chapter 5, Verboom et al. 2009). Such a drastic change in vegetation structure would obviously have an impact on the distribution of other organisms (Cerling et al. 1997; Boissierie and Merceron 2011), and their resulting phylogeographic structure. For example, animal species specializing in the savanna/grassland niche (e.g. murid rodents, ungulates) have expanded their distributions since the Late Pleistocene (Boissierie and Merceron 2011; Edwards et al. 2011; Engelbrecht et al. 2011; du Toit et al. 2012; Lorenzen et al. 2012), although periodic wetter and warmer conditions throughout the Plio-Pleistocene (Dupont 2011) probably caused some savanna/grassland species to retreat into refuges (Lorenzen et al. 2012; Barlow et al. 2013). Conversely, forest specialists are currently confined to refugial patches across east and southern Africa, but were more widespread across the continent prior to the Oligocene reduction of pan-African forest cover (e.g. Couvreur et al. 2008; Tolley et al. 2008; Daniels, Picker, et al. 2009; Willows-Munro and Matthee 2009; Voelker et al. 2010). In the GCFR, afromontane forest is presently reduced to *c.* 900 patches totalling just

over 600 km<sup>2</sup> (Geldenhuys 1991). The largest fragment (c. 568 km<sup>2</sup>) is the Knysna–Tsitsikamma forest, which is home to a number of isolated endemic forest specialists (Carruthers and Robinson 1977; Branch and Hanekom 1987; Branch 1998; Mucina and Rutherford 2006; Tolley and Burger 2007; McDonald and Daniels 2012). Thus, phylogeographic patterns will be strongly influenced by habitat preferences of organisms (e.g. Potgieter, Herrel, Measey, Vanhooydonck, van Vuuren, and Tolley unpublished). In the GCFR, vagile species that inhabit expanding habitat types should show less phylogeographic structure than those with a narrow niche and/or those that inhabit dwindling habitats.

In addition to habitat changes, major geological changes influenced the Cenozoic environment of southern Africa, and are thought to have promoted diversification of some species through changes to the physical environment by promoting topographic heterogeneity (Chapter 5). In particular, uplift on a continental scale approximately 8 Ma created a topographic barrier that influenced atmospheric circulation, and resulted in continental-scale aridification of Africa (Sepulchre et al. 2006). In the GCFR, relatively mild uplift in the Early Miocene and Early Pliocene resulted in the dissection of the coastal forelands and large-scale exposure of clay-rich substrata, which is likely to have promoted diversification of lowland lineages (Chapter 5, Cowling et al. 2009). However, montane environments, owing to the resistant nature of Cape Supergroup quartzitic sandstones, have remained relatively stable throughout the Cenozoic.

A dynamic of key importance for the biogeography of lowland lineages were the fluctuations in sea level during the Plio-Pleistocene. During glacial maxima, a large amount of featureless, but relatively fertile plains-type habitat corresponding to the now submerged Agulhas Bank to the south of the current coastline was available (Chapter 8). It has been hypothesized that this allowed migrations of large mammals from the summer rainfall east to winter rainfall west while these same corridors were closed during interglacials (Chapter 8, Compton 2011). When sea level was lower, open corridors could have facilitated movements of many of the smaller faunal components (e.g. Schreiner et al. 2013). Conversely, elevated sea levels would have fragmented many lowland species. For plants, low sea levels would have made more limestone habitat available, resulting in a more varied version of diverse flora of the Agulhas plains (Cowling et al. 1988; Linder 2003). Another consequence is that the shifting coastline influenced the pattern of orographic rainfall, particularly to the inner Cape Fold Belt. Periods when

the Agulhas Bank was exposed led to a drier interior, leading to fragmentation of high moisture-dependent clades. Likewise, when the coastline was higher than it is today, these inner mountain ranges would have received increased orographic rainfall facilitating greater connectivity.

The environment of the GCFR, particularly the western GCFR, is thought to have been relatively stable since the start of the Pliocene (Linder 2003). This environmental stability has been invoked as an explanation for the elevated diversity and endemism in the GCFR (Cowling et al. 2009; Goldblatt 1997), because extinction rates are expected to be lower in stable refugia than in unstable environments, causing a gradual increase in species richness and genetic diversity. Indeed, genetic diversity and phylogeographic structure are greater in the west for many taxa (e.g. Tolley et al. 2006; Price et al. 2007; Smit et al. 2007; Daniels, Heideman, et al. 2009; McDonald and Daniels 2012; Barlow et al. 2013), lending support for the stability hypothesis. Conversely, many clades in the eastern GCFR are either extremely ancient (e.g. Cenozoic; Forest, Grenyer, et al. 2007) or extremely recent, having expanded into that region since the Late Pleistocene (Smit et al. 2007; Engelbrecht et al. 2011). Indeed, some rodents show essentially no phylogeographic structure within the GCFR and the region appears to have been recently colonized, particularly by clades that originated in savanna/grasslands and expanded southwards in the Late Pleistocene (see 7.9). These generalizations suggest that the stability hypothesis holds for the western GCFR for some taxa, but differences in life history and niche preference within and between taxonomic groups produce varying responses to environmental conditions (e.g. Smit et al. 2010; Edwards, Keogh, et al. 2012). Thus, there is a lack of phylogeographic congruence as a whole within the GCFR, not only between but also within major taxonomic groups (see also Chapter 4). A search for common processes that have generated the phylogeographic patterns in the GCFR is therefore not realistic, and phylogeographic patterns within the GCFR should rather be observed as dynamic, with multiple processes influencing the diversity through a myriad of circumstances.

### 7.3 Refugia in the GCFR

The concept of refugia (the location of an isolated or relict population of a species that was once more widespread) has been widely documented in the northern hemisphere, where glacial ice sheets formed during the Plio-Pleistocene and significantly altered the geographical distribution of many species

as they retreated into refugia (Hewitt 2000). The Plio-Pleistocene glacial cycles also had a pronounced influence on the fauna and flora of Africa, due to markedly cooler and more arid conditions globally during glacial phases, and warmer and more humid conditions during interglacial phases (deMenocal 1995; Zachos et al. 2001; deMenocal 2004; Trauth et al. 2009). Across Africa, these environmental changes provided periodic opportunities for the expansion of arid-adapted species, while forest habitats and their associated taxa contracted into one or more refugia (Bowie et al. 2006; Fjeldså and Bowie 2008; Tolley et al. 2009; Voelker et al. 2010; Barlow et al. 2013).

Phylogeography has proven to be a powerful method to infer the location of regional refugia and thereby provide a first pass at teasing apart the interactions between the abiotic and biotic forces which drive evolutionary change across species in regional biomes. More recently, researchers have started to validate genetic data with the use of species distribution models to develop predictive range distributions of species based on palaeoclimatic information for the region of interest (e.g. Richards et al. 2007; Barlow et al. 2013). This approach can be extended to modelling the spatial extent of biomes or other community level assemblages of taxa (e.g. Carnaval et al. 2009). In such models, when a species or habitat is present in a grid cell over multiple climate intervals (e.g. Last Glacial Maximum (LGM); Last Interglacial Maximum (LIM)), the cell is said to have high stability (Fjeldså et al. 2012) or low velocity (Sandel et al. 2011). The ability to determine putative areas of habitat or species persistence through time enables the development of spatially explicit hypotheses of the expected relationships amongst populations of the species of interest, and hypotheses to be erected about the demographic processes (population bottlenecks, population fragmentation, habitat persistence) that are likely to have shaped the pattern of genetic diversity in the species observed today (Chapter 8, Graham et al. 2010).

As a first step in the development of new predictive models of habitat dynamics encompassing both the LIM and the LGM, Bowie and colleagues (unpublished; see Fuchs et al. 2013 for methodology) have modelled 64 separate palaeoclimate surfaces spanning the past 120 kyr. From these layers, the stability (=habitat persistence through time) of major biomes in South Africa has been estimated under both a static (Plate 9a) and shifting refuge paradigm (not shown). The modelling approach is in good agreement with pollen core data which show, for example, that the Fynbos Biome gradually declined in extent from 30–22 ka,

then expanded considerably during the last LGM (22–12 kya). By 10 ka, the biome had reached its present extent, which it appears to have maintained since that time. Further, the model supports the inference of western and eastern refugia in the GCFR (see also Cowling and Lombard 2002), although the eastern refugium appears to have been larger and more stable over the course of the past 120 kyr.

The static model for the Fynbos and Succulent Karoo Biomes reveals that although these biomes became fragmented over the past 120 kyr, large patches of habitat persisted as refugia (green and blue areas in Plate 9a). In contrast, the Nama-Karoo Biome appears to have been much more dynamic, with relatively low stability (i.e. high velocity) apparent across the whole biome. The shifting refugia models (not shown) show a similar pattern, although areas of habitat persistence are broader. This is expected because the shifting refugia algorithm allows the modelled biome to track suitable habitat spatially through time. For instance, allowing for a biome dispersal rate of 10 km kyr<sup>-1</sup> further reinforces the pattern observed under the static model, suggesting that large parts of the present-day distribution of Fynbos and Succulent Karoo Biomes has remained intact over the past 120 kyr. This is in general agreement with the southern Africa palaeoclimate record (reviewed in Gasse et al. 2008) and the diversification of several plant genera (reviewed in Verboom et al. 2009).

These models also suggest that the point of contact between the Fynbos and Succulent Karoo Biomes has been in place almost continuously over the past 120 kyr, whereas the position of the Nama-Karoo Biome, relative to the above biomes, has repeatedly contracted over the past 120 kyr (at 10–22, 30–42, 58–70, 80–92 and 104–112 ka), expanding only recently, following the LGM (since c.10 ka). This expansion of the Nama-Karoo Biome has likely played an important role in the present placement of the contact zones for many taxa. For example, Cape white-eyes from the contact zone show admixture between alleles from the Nama-Karoo species (*Zosterops pallidus*) and the southern subspecies found in the Fynbos and Succulent Karoo Biomes (*Z. v. virens* and *Z. v. capensis*). Similarly, for puff adders (*Bitis arietans*) admixed individuals were found along biome contact zones, but also strong signatures of recent demographic expansions were identified for clades occurring in the Nama-Karoo and Savanna Biomes, with no such signatures being evident from clades found in the Fynbos and Succulent Karoo Biomes (Barlow et al. 2013). This pattern is consistent with a scenario encompassing GCFR (Fynbos/Succulent Karoo

Biomes) refugia, and a dynamic Nama-Karoo Biome in which populations expand and contract as the extent of the system fluctuates through time. Indeed, species distribution models suggest the presence of at least two refugia in the GCFR for puff adders, one in the Fynbos and one in the Succulent Karoo Biome, and these correspond surprisingly well with the habitat-based static model (Plate 9a). No refugia were predicted within the Nama-Karoo Biome, with puff adders currently in this area probably having expanded southwards from a refuge much further north (Barlow et al. 2013). This is consistent with a strong environmental barrier between the GCFR and Nama karoo region and, potentially, local adaptation to climatic conditions (e.g. Ribeiro et al. 2011; Ribeiro, Lopes, et al. 2012).

Very few phylogeographic studies of southern African taxa have made use of nuclear DNA (nDNA) markers, and therefore, it is unknown whether many other species also exhibit zones of contact or refugia within the GCFR. The use of new sequencing technologies will make it much easier and cost effective to obtain large numbers of independent nDNA markers, and this offers much promise for phylogeographic studies of species in the GCFR. As a future framework, we outline in Table 7.1 (modified from Werneck et al. 2012) how such multi-locus datasets could be utilized to estimate population metrics in order to validate the existence of putative refugia across the GCFR.

## 7.4 Plants

Despite its remarkable botanical diversity, relatively few studies have investigated phylogeographic patterns within the GCFR flora. The groups for which phylogeographic or population genetic patterns

have been investigated on a relatively large scale include the white sugarbushes, *Protea* section *Exsertae* (Proteaceae; Prunier and Holsinger 2010), the *Gladiolus carinatus* complex (Iridaceae; Rymer et al. 2010), *Elytropappus rhinocerotis* (renosterbos, Asteraceae; Bergh et al. 2007), *Erica coccinea* (Ericaceae; Segarra-Moragues and Ojeda 2010), *Schotia* (Fabaceae; Ramdhani et al. 2010; Potts, Hedderson, and Cowling 2013), *Aspalathus linearis* (rooibos, Fabaceae; Malgas et al. 2010), *Berkheya cuneata* (Asteraceae; Potts, Hedderson, Vlok, et al. 2013), *Nymanina capensis* (Meliaceae; Potts, Hedderson, and Cowling 2013), and *Pappea capensis* (Sapindaceae; Potts, Hedderson, and Cowling 2013). Studies confined to a relatively small scale, with associated constraints on phylogeographic inference, deal with *Argyroderma* (Aizoaceae; Ellis et al. 2006, 2007) and two aquatic species of *Oxalis* (Oxalidaceae; Oberlander et al. 2012).

A tendency towards greater variation amongst populations than within populations has been detected in some of these studies. In *Protea* section *Exsertae*, the results show that for most of the six species of this group, there is little gene flow between populations and that these have potentially diversified early in the evolutionary history of the group and subsequently remained relatively isolated from each other; this indicates that geographical isolation could be one of the main drivers of diversification in this group (Prunier and Holsinger 2010). Likewise, the two aquatic species of *Oxalis*, restricted to vernal pools near the Bokkeveld Mountains in the western part of the GCFR show high between-population variation but very low within-population diversity, with 31 of the 37 vernal pools investigated having only one haplotype (Oberlander et al. 2012). The opposite pattern has, however, been shown in the *Gladiolus*

**Table 7.1** Examples of population genetic metrics (modified from Werneck et al. 2012) that could be used to test the validity of putative areas of stability (= habitat persistence through time), identified from the palaeoclimate models.

Summary stat.	Description	Expectation (stable areas)	Expectation (unstable areas)
$\theta$	theta diversity parameter (no of segregating sites)	higher	lower
$h$	haplotype diversity	higher	lower
$\pi$	nucleotide diversity	higher	lower
Mean $D_a$	average net nucleotide diversity across localities	greater differences amongst localities (higher population structure within refugia)	fewer differences amongst localities (reduced population structure)
EBSF (Bayes Factor)	extended Bayesian skyline plots	no significant departure from a model of constant population size	significant departure (growth or decline) from a model of constant population size
Mantel test coefficient ( $P$ -value)	test for isolation-by-distance (IBD)	presence of IBD	lack of IBD

*carinatus* complex and in *Elytropappus rhinocerotis*, with most genetic variation being located within populations, rather than between populations (Bergh et al. 2007; Rymer et al. 2010). This contrasting pattern of low gene flow amongst populations versus high variation within populations due to high gene flow can be explained by differences in seed dispersal distances, for the few cases examined. Many plants have no particular active dispersal mechanisms and are disseminated locally (Goldblatt 1997) which would result in low gene flow. In the GCFR, this situation could be further exacerbated because about 15% of the flora is thought to have seeds dispersed by ants (myrmecochory; Bond and Slingsby 1983). Because of their relatively low dispersal capability (e.g. only a few metres), gene flow should be limited resulting in strong genetic structure between populations.

Two of the groups studied allow some inference about the directionality of population expansion, with a tendency for dispersion eastwards (and northwards) from the western GCFR. In the *Gladiolus carinatus* complex, two of the three species, *G. quadrangulus* and *G. griseus*, are restricted to the southwestern corner of the GCFR, from Saldanha to the Cape Flats, while *G. carinatus* is more widespread (i.e. from Namaqualand to Knysna). Rymer et al. (2010) showed that populations of *G. carinatus* are derived in relation to the other two species, thus indicating that populations are expanding or dispersing eastwards in this group. Likewise, in *Protea* section *Exsertae*, the only species found almost entirely outside the GCFR, *P. subvestita*, is nested in a clade comprising solely endemics or near endemics to the GCFR (Prunier and Holsinger 2010). It is worth noting that in *P. subvestita*, genetic diversity increases from east to west, which is consistent with the assumed west-east directionality of expansion of this species. This pattern is consistent with previous species-level studies that showed that many groups originated in the GCFR and dispersed northwards and most often eastwards (e.g. Forest, Nänni, et al. 2007; Galley et al. 2007).

Fire forms an integral part of ecosystem processes in the GCFR (Chapter 3) and plants have developed different ways to survive and exploit periodic fires. Most perennial plant species exposed to these fire regimes have been categorized as resprouters (i.e. adult plants survive fire) or reseeders (i.e. adult plants are killed, the next generation developing from a seed bank). One would expect that such divergent fire survival strategies will leave equally divergent signatures in the population structure of species. In *Erica coccinea*, reseedling populations have higher

within-population genetic diversity, but also twice as much amongst-population differentiation, than resprouting populations. This has been attributed to the shorter generation times and faster population turnover in reseedling populations (Segarra-Moragues and Ojeda 2010). Most reseedling and resprouting populations of *Erica coccinea* form genetically distinct and geographically structured clusters, with the reseedling populations found along the south coast and the resprouting population found inland and generally in mountainous areas. How this distribution pattern came into existence remains unclear, but the genetic differentiation between the resprouting and reseedling populations is unmistakable. Likewise, strong genetic differentiation between reseedling and resprouting populations has also been demonstrated in *Aspalathus linearis* (Malgas et al. 2010). This limited evidence suggests, unsurprisingly, that fire survival strategy has an important impact on the phylogeographic structure of a species.

Three studies allow the extrapolation of the observed phylogeographic patterns to the history of the vegetation type in which the species under study occur; a reasonable assumption as the taxa concerned are either endemic (or near endemic) to these assemblages or have their greatest density of individuals within them. A first study focusses on *Elytropappus rhinocerotis*, often a dominant component of renosterveld (Chapter 1). This work is the first to have examined the genetic variation of a widely distributed species in the GCFR (Bergh et al. 2007). Although the authors do not specifically extrapolate their results to explain the history of this vegetation type, the spatial genetic structure of *E. rhinocerotis* could be considered as a good surrogate to investigate the evolutionary history of renosterveld as a whole. The inter-simple sequence repeat (ISSR) markers used in this study uncovered a large amount of genetic variation, but this variation lacked strongly spatial structure. The authors attributed this to high level of recombination in ISSR, as a result of high levels of gene flow and outbreeding rates due to various biological characteristics of renosterbos itself (e.g. wind dispersed, high seed production). The authors further explored their results using two transects across the GCFR, on a north-south axis along the west coast (their 'western arm') and on a west-east axis along the south coast (their 'eastern arm'). On the north-south axis, genetic diversity decreases towards the north, which is suggestive of increased aridification in the Holocene (Chapter 8), taking place first in the north and progressing southwards. This is a rather more likely explanation for

this pattern than alternatives such as long-distance dispersal to the north from southern populations or the presence of smaller and more isolated populations in the north, resulting from present-day ecological conditions. Sites along the west–east axis, on the other hand, show high levels of genetic variability but with low between-site differentiation, potentially reflecting large population sizes and high gene flow (Bergh et al. 2007). This is indicative of fluctuating climatic conditions with limited or no directionality. These patterns are a consequence of the expansion and contraction of vegetation types, including renosterveld, in the region over the Pleistocene and Holocene, resulting from climatic oscillations (e.g. Chapter 8, Midgley et al. 2001; Jansson and Dynesius 2002). Together with the phylogeographic patterns uncovered in the *Gladiolus carinatus* complex and in *Protea* section *Exsertae* (see above), the patterns seen in *E. rhinocerotis* point to a potential refugial role for the southwestern part of the GCFR, with the rest of the region being more strongly affected by climatic oscillations (see also Forest, Grenyer, et al. 2007). Conversely, patterns of range contraction and fragmentation have been found using distribution modelling of *Berkheya cuneata* (Asteraceae), an endemic of the Little Karoo. Species distribution modelling shows a clear division between the western and eastern Little Karoo, although both regions show localized stability since the LGM (Potts, Hedderson, Vlok, et al. 2013).

Thicket vegetation (Albany Thicket Biome) is mostly composed of evergreen, often spiny shrubs and small trees. Located primarily in southeastern South Africa, it is also prevalent in some parts of the Little Karoo, and forms mosaics with other vegetation types across southern Africa, and within the GCFR (Chapter 1, Vlok et al. 2003; Cowling et al. 2005). It is one of the main components of the Maputaland–Pondoland biodiversity hotspot (Mittermeier et al. 2005). Thicket is thought to have been present in southern Africa for the past ca 40 Ma (see also Chapter 5), an estimate based on the presence of numerous major lineages making up this vegetation type (Cowling et al. 2005). The presence of thicket vegetation within neighbouring biomes such as the Fynbos and Grassland Biomes has led to interpretation of thicket as being ancient and having undergone significant contraction and expansion events in its recent history, having dominated the southern African landscape prior to the onset of Late Miocene aridification (Chapter 5, Cowling et al. 2005; Potts, Hedderson, Franklin, et al. 2013). One of the lineages associated with thicket is the genus *Schotia* from the legume family (Leguminosae), a small genus comprising four

species of trees with a unique association with thicket vegetation that provides insights into the evolution of this vegetation type. A study using a combination of plastid and nDNA sequence data examined the phylogenetic and phylogeographic patterns in this genus (Ramdhani et al. 2010). The results from this analysis showed that none of the four species were monophyletic. The patterns obtained from the plastid and nDNA are incongruent, which the authors attribute to hybridization and incomplete lineage sorting (Ramdhani et al. 2010). A long branch leading to the genus *Schotia* in a recent molecular analysis of the family (stem age of 48–49 Ma and crown group age of 5–6 Ma; Bruneau et al. 2008) is indicative of potentially important extinction events in the history of the genus and of recent diversification in the Pleistocene, triggered by climatic fluctuations. Some of the clades recovered in the study of Ramdhani et al. (2010) display disjunct distributions and limited spatial genetic structure, which supports the idea that the thicket is ancient and once covered a wider area than at present. Ramdhani et al. (2010) concluded that the eastern GCFR and part of the western GCFR served as thicket refugia. A second study used a comparative phylogeographic approach of three tree species found in thicket to assess the impact of primary drainage basins and mega-herbivore suitability on population dynamics within this biome (Potts, Hedderson, and Cowling 2013). For two of these species, *Pappea capensis* (Sapindaceae) and *Nymphaea capensis* (Meliaceae), the phylogeographic patterns show a significant correlation with the boundaries of primary drainage basins; haplotypes are tightly linked to each drainage basin as demonstrated by high genealogical sorting index values and network reconstructions (Potts, Hedderson, and Cowling 2013; see also Potts et al. 2014). The absence (or scarce occurrence) of long-distance dispersal has been identified as the causal factor responsible for the observed intraspecific isolation patterns in these species. Dispersal in both species is relatively limited, partly due to the complex topography of the Albany Thicket Biome (*N. capensis* is dispersed by wind and the fruits of *P. capensis* are disseminated by birds). The third species examined as part of this study, *Schotia afra*, presents a rather different phylogeographic pattern in which the drainage basins have little apparent influence on the population genetic structure of this species. This is attributed to the presence of long-distance dispersal facilitated by large herbivores (Potts, Hedderson, and Cowling 2013). The patterns uncovered within *S. afra* are probably indicative of the situation in the genus as a whole, as shown by the non-monophyly of its four

species (Ramdhani et al. 2010). Other studies focussing on species closely associated to the thicket vegetation will be needed to understand further the evolution of this biome and its potential role in the establishment of the current GCFR flora.

## 7.5 Invertebrates

Southern Africa in general, and the GCFR in particular, houses an abundance of invertebrate diversity, much of which is unknown, thus preventing accurate estimation of invertebrate richness and endemism (Chapter 4). The paucity of basic knowledge of invertebrate diversity and distributions is, in part, due to the small average size of invertebrates, the difficulty of their identification, their overwhelming diversity, and a lack of capacity to tackle baseline studies and taxonomy (Samways et al. 2012). Along these same lines, few invertebrate studies have sufficient taxonomic and/or geographic coverage of the GCFR to make adequate comparisons of phylogeographic patterns across the region, as many studies are limited by the high local endemism of the study taxa. Of these studies the majority focus on either freshwater crustaceans with limited terrestrial mobility (Daniels et al. 2001; Daniels 2003; Daniels et al. 2006) or insects which possess an aquatic life stage (Wishart and Hughes 2001; Pereira-da-Conceicao et al. 2012).

Of the studies that focus on terrestrial invertebrates, variation in vagility, from poor dispersers such as heelwalkers (Damgaard et al. 2008), flightless beetles (Sole et al. 2005), and velvet worms (Daniels, Picker, et al. 2009; McDonald and Daniels 2012) to highly vagile flying insects (beetles: Pitzalis and Bologna 2010; cicadas: Price et al. 2007, 2010; flies: de Jager and Ellis 2013), reduces the opportunity for direct comparison of phylogeographic patterns. Indeed even within the flying insects there is marked variation in dispersal capability, with some species showing high levels of habitat philopatry and a concomitant reduction in dispersal potential (cicadas: Price et al. 2007, 2010), and others being shown to be more vagile than initially assumed (mayflies: Pereira-da-Conceicao et al. 2012).

In all published studies of GCFR invertebrates, phylogeographic structure is prominent, indicating isolation of populations since the Early Pliocene (Daniels et al. 2001; Daniels 2003; Daniels et al. 2006; McDonald and Daniels 2012; Pereira-da-Conceicao et al. 2012; de Jager and Ellis 2013) and Pleistocene (Price et al. 2007; Pitzalis and Bologna 2010; Price et al. 2010) epochs. While most studies are restricted to the southwestern GCFR and focus on range-restricted

invertebrate species, all show significant population or phylogeographic structure. Within the GCFR, fynbos is postulated to house older lineages than the comparatively younger succulent karoo (Predel et al. 2012; de Jager and Ellis 2013).

Within the GCFR, invertebrate species that inhabit the Cape Peninsula, Hottentots–Holland and surrounding Cederberg Mountains have significant phylogeographic structure (Daniels et al. 2001; Wishart and Hughes 2001; Daniels 2003; Daniels et al. 2006; Daniels, Picker, et al. 2009; McDonald and Daniels 2012), implying the isolation of the Cape Peninsula invertebrate fauna from the remaining mountain chains in the GCFR. Furthermore the southwestern section of the Cape Fold Belt house invertebrate populations that are distinct from the eastern section of the Cape Fold Belt (Price et al. 2007; Damgaard et al. 2008; Daniels, Picker, et al. 2009; McDonald and Daniels 2012).

Comparison of terrestrial (Price et al. 2007, 2010) and semi-aquatic (Daniels et al. 2001; Wishart and Hughes 2001; Daniels 2003; Daniels et al. 2006) invertebrates highlights the importance of primary river catchments in structuring populations across the GCFR. These studies reveal west-flowing catchments, associated with the north–south running Cederberg Mountains, to be isolated from south-flowing catchments, associated with the east–west running mountains of the Cape Fold Belt (Daniels 2003; Daniels et al. 2006; Price et al. 2007). This catchment effect is especially apparent in the western portion of the GCFR where the elevated mountains and arid climate of the interior interact to further limit the opportunities for dispersal between primary catchments (Price et al. 2007, 2010). While the semi-aquatic invertebrates are restricted directly by their poor dispersal capabilities over land, volant terrestrial invertebrates such as cicadas are hypothesized to be restricted indirectly by the distribution of their host plants and a tendency towards habitat philopatry (Price et al. 2007, 2010).

Studies of terrestrial invertebrates restricted to the coastal regions of the GCFR are limited, but suggest population subdivision at the southern tip of Africa, Cape Agulhas (Price et al. 2007; Damgaard et al. 2008). This population structure is likely a result of Pleistocene eustatic sea-level changes which resulted in a southward extension of the coastline. This, in combination with lower glacial temperatures, may have resulted in unfavourable coastal habitat in the area of the Agulhas Plain during glacial cycles (Chapter 8, Price et al. 2007). However, this hypothesis would be strengthened with validation from additional studies of co-distributed coastal invertebrates.

Although timing of cladogenesis inevitably differs between invertebrate groups and across methods of calibration and analysis, much of the within-population demographic change is reported to have occurred in the Pleistocene, primarily in response to cyclical climatic fluctuations (Price et al. 2007; Pitzalis and Bologna 2010; Price et al. 2010; McDonald and Daniels 2012; Pereira-da-Conceicao et al. 2012). Within the invertebrates there is a trend towards long-term population isolation, with initial lineage divergences reported from the Middle Miocene (Daniels, Picker, et al. 2009; de Jager and Ellis 2013) and Early Pliocene (Daniels et al. 2001; Daniels, 2003; Daniels et al. 2006; Pereira-da-Conceicao et al. 2012). More recent population cladogenesis is reported from the Plio-Pleistocene and Middle to Late Pleistocene in some groups (Sole et al. 2005; Price et al. 2007, 2010; McDonald and Daniels 2012), suggesting that cladogenesis has been ongoing throughout the Plio-Pleistocene in the GCFR.

Most invertebrates studied within the GCFR show habitat specificity and highly restricted distributions. Suggested historical barriers to gene flow in invertebrates within the region include a combination of periglacial Pleistocene sea levels and glacial climates (Price et al. 2007) and the Plio-Pleistocene marine transgression of the low-lying Cape Flats region (Wishart and Hughes 2001; Daniels, Picker, et al. 2009; McDonald and Daniels 2012). Hypothesized barriers include major rivers (Sole et al. 2005), and the combination of the high elevation of the Cape Fold Belt and the associated structure of surrounding river catchment boundaries, resulting in little or no gene flow between catchments in both semi-aquatic (Daniels et al. 2001; Wishart and Hughes 2001; Daniels 2003; Daniels et al. 2006) and terrestrial (Price et al. 2007, 2010) invertebrates. Considering that the majority of studies target semi-aquatic groups, it is not surprising that most studies highlight river catchment boundaries as contemporary barriers to dispersal (Daniels et al. 2001; Wishart and Hughes 2001; Daniels 2003; Daniels et al. 2006). Unexpectedly, catchment boundaries have also been suggested as barriers to the dispersal of more volant terrestrial invertebrates, although this affect is likely indirect due to the restricted distribution of their host plants (Price et al. 2007, 2010). Studies of invertebrates with a greater dispersal potential (flies: de Jager and Ellis 2013; mayflies: Pereira-da-Conceicao et al. 2012) show little population structuring in response to this type of physical barrier, but rather are affected by environmental factors in the GCFR, including host plant distribution (de Jager and Ellis 2013) and changes in water pH mediated through streambed geology (Pereira-da-Conceicao et al. 2012).

## 7.6 Reptiles

Southern Africa is regarded as having one of the world's largest radiations of reptiles and the richest reptile diversity in Africa (Branch 2006). Within the GCFR, there are 16 families of reptiles in 60 genera. Of the 191 reptile species found in the GCFR, 45 are endemic to the region (Chapter 4). Changes in climate have been linked to the timing of major divisions in several taxa, with radiations extending back into the Middle to Late Miocene (e.g. Matthee and Flemming 2002; Bauer and Lamb 2005; Tolley et al. 2008; Daniels, Heideman, et al. 2009). These ancient diversifications notwithstanding, almost all taxa show distinctive phylogeographic structuring within the GCFR, with diversification during the Plio-Pleistocene period.

The primary phylogeographic pattern for multiple reptile taxa shows a particularly strong division between the eastern and western GCFR. This division corresponds with the change in direction of the Cape Fold Belt, from the east–west chain to the north–south chain (Daniels et al. 2007, 2009; Swart et al. 2009; Tolley et al. 2009; Barlow et al. 2013). The diversification associated with this geographical division for puff adders (*Bitis arietans*) has been dated at approximately 3.2 Ma (Barlow et al. 2013), but it is noteworthy that in this study (as in others: e.g. Swart et al. 2009) geographically adjoining clades are not necessarily sister clades. Further, it has been hypothesized that the division between some clades corresponds to the winter/seasonal rainfall boundary (see Tolley et al. 2009). This mountainous region, with its deep, often dry valleys, may not present a strong physical barrier to the taxa concerned, particularly because clades may have been formed in the Plio-Pleistocene under different environmental conditions to those observed today (Tolley et al. 2009). In addition, multiple clades are in allopatry in the montane regions of the western GCFR (c.20–21°E), suggesting gene flow is disrupted, potentially by climatic driven vegetation shifts (Tolley et al. 2009). To date, the identification of these patterns has relied on mtDNA and in the future more detailed investigations with fine scale nDNA markers (e.g. microsatellites) could be useful to examine the level of gene flow between the haplotype clades already identified.

Although phylogeographic studies have uncovered similar patterns between reptilian groups in the GCFR, no consistent geographical barriers have been identified, presumably confirming the diversity of dispersal mechanisms used by the reptilian fauna. One exception appears to be the Knersvlakte, an arid, topographically homogenous gap to the north of the

Cederberg Mountains, across which there are strong phylogeographic divisions for rupicolous reptiles, suggesting an absence of dispersal (Matthee and Flemming 2002; Swart et al. 2009; Daniels et al. 2010; Portik et al. 2011). However, such barriers are not necessarily relevant for all taxa. Lowland, particularly fossorial species, are thought to have barriers that correspond to current and past river barriers (Daniels, Heideman, et al. 2009; Heideman et al. 2011), although fine scale sampling to test this hypothesis is lacking.

In addition to these apparent geographical patterns, there are several examples of substrate specialization that are thought to have brought about strong phylogeographic structuring. For example, vegetation types are clearly associated with phylogeographic structuring in chameleons (*Bradypodion*), with most species showing spatial and substrate affinities that correspond to both morphology and genetics (Tolley et al. 2004, 2006, 2008; Potgieter 2013). Indeed, experimental work suggests that phenotypic adaptation to specific habitat structure in *Bradypodion* is a tangible, identifiable mechanism for limiting gene flow, and this is reflected in the phylogeographic patterns (Tolley et al. 2006; Herrel et al. 2013; Potgieter, Herrel, Measey, Vanhooydonck, van Vuuren, and Tolley unpublished). This combined body of work is formative for understanding the mechanisms that drive phylogeographic patterns, and provides a framework within which drivers can be identified at the organismal level, rather than simply a broad correlation with landscape features or climate. Regardless, recent shifts in vegetation structure obviously allow some permeability across habitats (e.g. *Bradypodion pumilum*, *B. ventrale*, *B. taeniabronchum*). In these instances, substantial morphological differences correspond with changes in vegetation structure, yet there are shared mtDNA haplotypes across the same gradient (Tolley et al. 2006; Measey et al. 2009; Hopkins and Tolley 2011). Other authors have reported strong phylogenetic patterns associated with bedrock type (in geckos: Bauer 1999; Bauer and Lamb 2005), and habitat structure (lacertid lizards: Edwards, Vanhooydonck, et al. 2012), although these divergences are much older than the Plio-Pleistocene.

Of particular importance may be the change in rainfall regimes associated with the change in position of the southern coastline given sea-level fluctuations (Daniels et al. 2007; Daniels, Heideman, et al. 2009; Tolley et al. 2009; Engelbrecht et al. 2013). Glacial fluctuations during the Pleistocene have likely resulted in the changes in distribution of many reptiles. During glacial periods, the considerable drying of the interior of southern Africa, including many of the GCFR

mountains (especially in the western GCFR corresponding to the discovery of divergent clades there), may have isolated many reptilian lineages, making the GCFR an important refuge for currently widespread reptiles. Barlow et al. (2013) demonstrated this scenario, as well as potential refugia in the south west for a large pan-African adder, *Bitis arietans*, which contains considerable structural diversity within the GCFR. This hypothesis receives additional support regarding the importance of precipitation for reptile fauna from species distribution models (e.g. Houniet et al. 2009; Tolley et al. 2009; Barlow et al. 2013). In particular, mountainous areas, such as the Cederberg, the Hottentots–Holland Mountains and several inselbergs in the Little Karoo, may have maintained appropriate climatic conditions and acted as refugia for some reptile taxa during the climatic fluctuations of the Pliocene (e.g. Daniels, Heideman, et al. 2009). Conversely, when the sea level was higher than today, interior regions likely received higher rainfall, and this presumably allowed for the passage of some reptile taxa between the eastern and western GCFR. This may explain some apparently odd sister relationships between clades in the far eastern and the southwestern corner of the GCFR (e.g. Daniels, Heideman, et al. 2009; Swart et al. 2009; Tolley et al. 2009; Barlow et al. 2013; Engelbrecht et al. 2013).

## 7.7 Amphibians

Sub-Saharan Africa has a notable diversity of anuran amphibians (996 species; 86 genera; 15 families), with few caecilians (28 species; seven genera; four families) and a total absence of salamanders (Stuart et al. 2008). The GCFR is relatively poor in terms of species richness (when compared with tropical Africa), with only 48 species of Anura, but it does contain a surprisingly high family-level diversity (ten families represented), especially considering the temperate climate, and has a large number of narrow-range endemic species within the subregion (Angulo et al. 2011). Amongst vertebrates, amphibians are frequently singled out as poor dispersers (e.g. Blaustein et al. 1994), with populations showing strong phylogenetic structuring (Avice 2000). However, the increase in molecular studies addressing phylogenetic questions has revealed that amphibians vary greatly in their dispersal potential, from species that have been shown to cross oceanic barriers (e.g. Vences et al. 2004; Measey et al. 2007) and move across large terrestrial areas (e.g. Pramuk et al. 2008; Van Bocxlaer et al. 2010), to those which are narrow endemics resulting from vicariant speciation (e.g. Tolley, de Villiers, et al. 2010; Turner

2011). These differences are frequently linked to the life-history traits of the species concerned, so that only a subset of species are capable of extending across different biomes, especially when these are particularly dry (e.g. Nama-Karoo). Typically, large species with cosmopolitan breeding habitats are widespread (Van Bocxlaer et al. 2010), while those which are smaller, with specialized breeding requirements tend to be narrow-range endemics (see Mokhatla et al. 2012). Some species appear to be linked to particular breeding habitat types, with fragmented ranges (Evans et al. 1997; Minter et al. 2004; Tolley, de Villiers, et al. 2010; Turner 2011; Mokhatla et al. 2012).

Existing phylogeographic studies on frogs in the GCFR suggest a range of dispersal abilities, resulting in a wide variety of phylogeographic patterns. For example, within the family Bufonidae, large toads such as *Amietophrynus rangeri* show no variation in 556 bp of the mitochondrial 16S marker throughout the GCFR (Cunningham and Cherry 2004), and maintain this same haplotype far beyond this area. On the other hand, small toads, such as *Capensibufo rosei* and *C. tradouwi* show a strong phylogenetic signal, with lineages that represent cryptic species and deep divergences rooted in the Miocene (Tolley, de Villiers, et al. 2010). This pattern fits with the proposed optimal range-expansion phenotype for toads (Van Bocxlaer et al. 2010), which contrasts large species with high fecundity and good dispersal attributes with small, low fecund, and range-restricted taxa, and it is possible that this model fits many of the GCFR amphibian taxa. There are, however, exceptions, such as the large toads that show a high level of endemism and phylogeographic structuring within and between the sister species (e.g. *Amietophrynus pardalis* and *A. pantherinus*) in the eastern and south west GCFR (Eick et al. 2001; Measey and Tolley 2011). A disjunction within the distribution of *A. pantherinus* of only 100 km showed no shared haplotypes for the mtDNA markers screened (Measey and Tolley 2011).

Even amongst wide-ranging southern African species investigated, there is some evidence of phylogeographic structuring between the winter (including aseasonal rainfall zones) and the summer rainfall areas. For example, in the clicking stream frog (*Strongylopus grayii*) there are two distinct clades within the GCFR, one located in the southwestern corner, and the other more widespread in the central and eastern GCFR extending into the Albany Thicket Biome (Tolley, Braae, et al. 2010). These two clades have moderate divergence (1.3% sequence divergence for mitochondrial ND2) given that they

represent a single species. Furthermore, the common platanna (*Xenopus laevis*) is one of the most widespread amphibian species in South Africa (Measey 2004), perhaps because of its propensity to invade (Measey and Davies 2011; Measey et al. 2012). Despite this, there is phylogeographic structure within the GCFR pointing to a division between summer and winter rainfall regimes (Grohovaz et al. 1996; Measey and Channing 2003; Evans et al. 2004), although samples from the all-year rainfall zone have not been examined. The southwestern lowland clade of *X. laevis* was found to extend as far as Cape Agulhas (Evans et al. 1997), and several hundred kilometres inland, where they co-occurred with individuals bearing haplotypes of the summer rainfall clade (du Preez et al. 2009).

The southwestern corner of the GCFR holds a particularly high diversity (from populations, through species, to genera and families) of amphibians (Chapter 4), and much of this diversity appears to be attributable to vicariance. For example, the two described species of *Capensibufo* (dwarf mountain toadlets), are each comprised of several cryptic species with strong affinities to isolated montane areas (Tolley, de Villiers, et al. 2010; Cressey et al. 2014). These two species contain multiple deep lineages, corresponding roughly to one species for each mountain range. A slightly more complex pattern is shown by the moss frogs of the genus *Arthroleptella*, which also display strong divergence patterns associated with individual mountains. Three species (*A. lightfooti*, *A. villiersi*, and *A. rugosa*) are found in the extreme southwest, while representatives of the other clade are mixed between the inner and outer mountains of the Cape Fold Belt (Turner 2011; Turner et al. 2004). Interestingly, the most widespread species, *A. villiersi*, contains little phylogeographic structure (Turner 2011). It has been hypothesized that this relates to the versatility of *A. villiersi* to withstand periodic drying of breeding habitats, as well as its dispersal capacity (individuals have proportionately longer legs compared to other species in the genus). Accordingly, the genetic differentiation between populations of this widespread taxon is low, which contrasts to the strong divergence between the narrow endemics (Turner 2011). Both *Arthroleptella* and *Capensibufo* are small frogs, largely reliant on montane seepages as habitats, and this may (in part) explain their similarity. However, current sampling has revealed unexpected phylogenetic structuring; but intraspecific phylogeographic studies at a finer scale are still required.

Strong phylogeographic signals are also present in lowland GCFR amphibian species where these are

associated with particular habitat types. The Cape platanna, *Xenopus gilli*, is confined to acidic blackwater pools in the southwestern GCFR, with a disjunct distribution similar to that of *A. pantherinus*. However, investigations into a range disjunction of around 100 km have suggested a deep divergence corresponding to 4–6 Ma (Evans et al. 2004; Fogell et al. 2013). The only remaining population in the west (in a small area on the Cape Peninsula) shows weak genetic diversity, while that in the east shows a large degree of variation within (Evans et al. 1997; Fogell et al. 2013).

While there are currently few phylogeographic studies on amphibians in the GCFR, those that exist suggest three groups of amphibians, each with different phylogeographic signals: (a) widespread species with cosmopolitan breeding habits that have minimal phylogeographic structuring in the GCFR; (b) species having strong structuring within the GCFR that appears to coincide with the occurrence of rainfall zones; and (c) narrow endemics, often with specific breeding requirements, that are specific to either individual mountains or particular breeding sites (e.g. lowland acidic fynbos pools or perennial streams).

## 7.8 Birds

Considering the decades of research conducted on southern African birds and their rich diversity, it is surprising that so few phylogeographic studies have focussed on birds from the subcontinent, and none have focussed on species solely within the GCFR. Despite the moderate proportion of endemic birds (c. 98 in southern Africa, 13 in the GCFR), the role of evolutionary processes in shaping avian population histories across the GCFR, and the entire southern African subcontinent has been sorely neglected. Regardless, the few studies on widespread species in southern Africa provide some insight into the evolutionary history of birds with distributions that include the GCFR. In general, there is an indication that many diverse taxa are geographically structured in association with particular biomes. Recent macroecological studies have suggested that turnover (beta diversity) in southern African avian communities occurs at biome boundaries (van Rensburg et al. 2004; McInnes et al. 2009; van Rensburg et al. 2009), with the transition between the Fynbos and Nama-Karoo Biomes being particularly 'impermeable' (but see Chapter 4). Thus, ecotones may play an important role in both driving and maintaining biogeographic boundaries amongst populations of southern African birds (see also Oatley et al. 2012).

Along these same lines, cladogenesis appears to be related to a combination of physical barriers (e.g. Clancey 1994) and biome-level vegetation boundaries (e.g. the boundary between the winter rainfall succulent karoo and summer rainfall Namib–Kalahari; Ryan et al. 1998; Ryan and Bloomer 1999). In contrast, a recent study investigating phylogeographic structure in the endemic southern ant-eating chat (*Myrmecocichla formicivora*) showed no phylogeographic structure across its large distributional range, which includes the GCFR, but also extends northwards across the Nama karoo and grassland regions (Voelker et al. 2012). Placing this species in phylogenetic context with other members of the same genus, suggests that, in stark contrast to more dispersal-limited vertebrate species whose genetic structure often shows strong intra-regional spatial patterns, inter-regional drivers of speciation may be more influential, possibly through contraction and expansion of habitat corridors between the southwestern and northeastern arid biomes (see also Outlaw et al. 2010; Cohen 2011).

More recently, researchers have started to combine the investigation of spatial variation in mtDNA with that from the biparentally inherited nDNA and have thereby revealed striking patterns of discordance. Ribeiro and colleagues (Ribeiro et al. 2011; Ribeiro, Lopes, et al. 2012) recovered strong patterns of mtDNA structuring in the karoo scrub-robin (*Erythropygia coryphoeus*) that were associated with the steep aridity gradient between the coastal scarp (i.e. fynbos and succulent karoo) and interior plateau of western South Africa (i.e. Nama karoo). In contrast, neutral nuclear loci (microsatellites and introns) revealed estimates of gene flow 50 times larger than the estimates for mtDNA genes, with pronounced nuclear gene flow throughout the species range. Given that females are the dispersive sex in the karoo scrub-robin (Ribeiro, Lloyd, et al. 2012), the authors suggest that selective pressures on physiology, possibly mediated by the mtDNA genome, may be a common mechanism for facilitating local adaptation to climatic conditions.

Recent work on the Cape white-eye species complex (*Zosterops virens* and *Z. pallidus*) identified a central/northern clade (Nama karoo, grassland, and northern savanna) and a southern/eastern clade (fynbos, succulent karoo, thicket, forest, lowland savanna of the north east) within South Africa (Plate 9b, Oatley et al. 2011, 2012). However, incongruent with the genetic data, the Cape white-eye species complex encompasses three taxa based on plumage characters that are strongly associated with different vegetation types (Plate 9b): (a) the peachy-flanked Orange River white-eye

(*Z. pallidus*) of the arid interior, primarily occupying Nama karoo, grassland, and savanna; (b) the grey-bellied form of the Cape white-eye (*Z. virens capensis*) within the GCFR, inhabiting fynbos and patches of succulent karoo and thicket; and (c) the green-bellied form of the Cape white-eye (*Z. virens virens*), occupying coastal forest in southern and eastern South Africa, as well as lowland wooded savanna in northeastern South Africa (Hockey et al. 2005). Analysis of nuclear intron data (Oatley, Voelker, and Bowie unpublished) separates *Z. pallidus* from the other two taxa (3.5–4.5% mtDNA divergence). The remaining two taxa (*Z. virens capensis* and *Z. virens virens*) are not distinguishable from each other using available nDNA or mtDNA data (0.3–1.5% divergence), and form a single genetic group. However, these taxa can be readily separated by plumage colouration (Oatley et al. 2011, 2012). Further, the nDNA and mtDNA, together with morphological analyses, identified several contact (introgression) zones between the three taxa (Plate 9b). These include points of contact where *Z. pallidus* and *Z. virens capensis* hybridize where succulent karoo/fynbos meets Nama karoo, as well as along a broad contact zone where the Nama-Karoo, Grassland, and Albany Thicket Biomes intersect (the two circles indicated for contact zone 4 on the map, Plate 9b). These two forms are genetically and phenotypically distinct, except at points of contact where they form hybrids that exhibit intermediate plumage characters.

Because most phylogeographic studies of vertebrate taxa within the GCFR have made use of mtDNA, we do not know whether multi-locus studies of divergent small mammal or reptile mtDNA clades would also identify zones of secondary admixture between previously separate arid and mesic lineages, as identified for the Cape white-eye species complex. The combination of nuclear and mitochondrial markers in future phylogeographic studies could provide interesting insight into the evolution of species within the GCFR. Regardless, the information to date for birds and other organisms does present a compelling argument that the transition between the Fynbos and the Succulent Karoo and Nama-Karoo Biomes is a phylogeographic barrier for many taxa.

## 7.9 Mammals

Southern Africa has a mammalian fauna comprising approximately 350 species in 190 genera, but only c.90 species, of which just four are endemic, are found in the GCFR (Bronner et al. 2003; Skinner and Chimimba 2005). The region was once home to several

large mammals that are now extinct in the wild due to human exploitation (Boshoff and Kerley 2001), extirpation or habitat loss (e.g. African elephant, black rhinoceros, eland, hippopotamus, lion, spotted and brown hyena, warthog), or are severely reduced in numbers (e.g. mountain zebra; Moodley and Harley 2005). At least one GCFR endemic mammal, the blue-buck (*Hippotragus leucophaeus*) is extinct due to human exploitation (Boshoff and Kerley 2001). In terms of species richness, small mammals (<2 kg body weight; c.50 species) seem to be more diverse than medium/large mammals ( $\geq 2$  kg body weight; c.40 species; Boshoff et al. 2001; Skinner and Chimimba 2005). Indeed, specialization to specific habitats, as well as a lack of vagility would promote diversification for small mammals, resulting in reduced gene flow particularly across biomes, and this signal is often detectable in phylogeographic studies. In contrast, large mammals are capable of dispersal and migration on large scales, crossing what might be formidable barriers for small mammals. Therefore, they might be considered less prone to isolation, with gene flow occurring across larger geographic scales than small mammals. At present, most large mammals are confined to nature reserves and/or have been reintroduced following human mediated declines, and this certainly impacts on their phylogenetic and population genetic patterns, although this issue is not considered further here.

Most phylogeographic work on mammals in the GCFR has been aimed at small mammals, particularly the rodent family Muridae (subfamily Murinae), Macroscelididae (elephant shrews), and Soricidae (shrews). Few studies, however, have concentrated on patterns within the GCFR, but rather on establishing the presence of phylogeographic patterns on a sub-continental scale, while including the GCFR. In general, emergent patterns show that species with large distributions across the subcontinent are divergent between biomes (Edwards et al. 2011; Engelbrecht et al. 2011; du Toit et al. 2012) most likely due to habitat specialization (e.g. Meynard et al. 2012). For example, the four-striped mouse (*Rhabdomys pumilio*) is thought to occur from the GCFR across the subcontinent to Tanzania (Skinner and Chimimba 2005). Phylogeographic sampling, however, shows the presence of multiple divergent clades suggestive of species-level divergence that corresponds with biomes (du Toit et al. 2012).

Of the small mammals investigated, genetic diversity and phylogeographic structure within the GCFR is, in most cases, low (e.g. Smit et al. 2010; Edwards et al. 2011; Engelbrecht et al. 2011). Typically, there is a single GCFR clade that extends both along the western

margin of the continent into succulent karoo and along the southern margin of the continent throughout the GCFR (Edwards et al. 2011; Engelbrecht et al. 2011; du Toit et al. 2012). Indeed, the Murinae show signatures of very recent radiations in the GCFR, mainly within the Late Pleistocene (Edwards et al. 2011; Engelbrecht et al. 2011). This suggests that the GCFR has not played a strong role as refugia for these taxa, but is an area into which clades have only recently expanded. In contrast, genetic diversity and phylogeographic structure appears to be higher outside the GCFR, within grassland/savanna environments (Engelbrecht et al. 2011; du Toit et al. 2012). The low diversity, coupled with the characteristic signatures of demographic expansion into the GCFR, suggests that some groups of small mammals recently radiated into this region, probably expanding southwards from savanna/grasslands. Most murid clades within the GCFR appear to be substantially younger than the Fynbos Biome itself suggesting that the radiation into the GCFR is not a result of the formation of the GCFR biomes, but rather colonization of the existing GCFR biomes by individuals from savanna clades. For example, the savanna/grassland and fynbos clades of *Rhabdomys*, *Micaelamys*, and *Otomys* diverged 1–2 Ma. Strong phylogeographic signal characterizes the savanna/grassland region with multiple clades present, whereas the GCFR primarily contains a single clade (Russo et al. 2010; Engelbrecht et al. 2011; du Toit et al. 2012). It is likely that some Murinae expanded southwards with the spread of savanna systems, colonizing the GCFR within the past 1–2 Myr (Rambau et al. 2003). What remains unclear is the extent of population-level structure within the GCFR and whether the western GCFR holds greater diversity than the east, as is observed for other taxa (Tolley et al. 2006; Smit et al. 2007; Swart et al. 2009; Tolley et al. 2009; Willows-Munro and Matthee 2011).

While most small mammal taxa lack phylogeographic structure within the GCFR, others show very different patterns. There is strong phylogeographic structure within the GCFR for shrews (*Myosorex varius*) and elephant shrews (*Elephantulus edwardii*). Notably, multiple clades form a contact area in the western GCFR for both taxa, whereas only one clade is present in the eastern GCFR (Smit et al. 2007; Willows-Munro and Matthee 2011). This highlights the western GCFR as an area in which phylogeographic diversity is high in contrast to the eastern GCFR. Despite the comparatively high diversity in the west, cladogenesis seems to have occurred only in the Late Pleistocene (<2 Ma).

Although the GCFR probably has not acted as a refugium for most small mammals investigated, an

exception is *Myosorex longicaudatus*, which is found within the GCFR, but is restricted to the fragments of afrotemperate forest in the Tsitsikamma/Outeniqua Mountains. Its closest relative is the afrotemperate forest-endemic *M. geata* from the Uluguru Mountains in Tanzania, whose divergence from *M. longicaudatus* has been dated to *c.*7 Ma (Willows-Munro and Matthee 2009). This divergence corresponds broadly to the fragmentation of afrotemperate forest in southern and eastern Africa (Jacobs 2004; Sepulchre et al. 2006; Couvreur et al. 2008) and suggests that *M. longicaudatus* is a surviving relict of a previously more widespread group. Thus, for the more ancient afrotemperate restricted taxa, such as *M. longicaudatus*, the surviving forest fragment in the GCFR does indeed seem to be a refuge.

Phylogeographic structure is not readily apparent within the GCFR for medium and large mammals, although very few studies have been conducted to date. Indeed some taxa show little structure across southern Africa (e.g. yellow mongoose), whereas others (e.g. Cape mountain zebra (*Equus zebra zebra*); chacma baboon (*Papio ursinus*); rock hyrax (*Procavia capensis*)) show some genetic structure over the subcontinent, but no structure within the GCFR (Prinsloo and Robinson 1992; van Vuuren and Robinson 1997; Moodley and Harley 2005; Sithaldeen et al. 2009). This pattern of weak phylogeographic structure for medium and large mammals suggests that size and vagility plays a role in promoting gene flow in comparison to smaller mammals. Where sampling is sufficient in the GCFR, however, some population-level structure emerges. Scrub hares from the western GCFR show a contact point between two populations, one of which forms a single, widespread population in the eastern GCFR extending northwards into the savanna and grassland regions (Kryger et al. 2004).

## 7.10 Next-generation phylogeography

The information provided by DNA-based tools and the development of associated analytical approaches, particularly in the last two decades, has considerably improved our understanding of species phylogeographic patterns and the processes responsible for these. Phylogeography, until recently, has relied mostly on the sequencing of a few DNA regions, predominantly from the plastid genome in plants and the mtDNA for animals, although nuclear regions have also been used on occasion (e.g. ribosomal internal transcribed spacer in plants, and microsatellite markers and introns in animals). In recent years, new

sequencing technologies have become available that provide numerous opportunities for research in the field of phylogeography. The field of phylogeography has not yet fully taken advantage of next-generation sequencing technologies (e.g. Metzker 2010; Glenn 2011; Lexer et al. 2013). Reasons for this include the fact that phylogeographic studies concentrate on non-model organisms, and often require data from hundreds of individuals per species (McCormack et al. 2013). Several options have potential for phylogeographic studies, such as amplicon sequencing (high-throughput sequencing of PCR products) better suited to relatively small projects since it uses a limited number of DNA regions, restriction-digest methods (a combination of restriction enzyme digestion and selection of a range of fragment sizes prior to sequencing; includes restriction-site associated DNA, or RAD, sequencing) more suitable for intraspecific studies, and target enrichment (selection of genomic regions using RNA probes before sequencing) for species-level and above studies (McCormack et al. 2013).

Phylogeographic studies using next-generation sequencing approaches are few but steadily increasing (e.g. McCormack et al. 2012; Puritz et al. 2012; Zellmer et al. 2012). Furthermore, the integration of phylogeography and ecological genomics (the discovery of the genetic basis of organisms' response to environmental factors) is a natural outcome of the increasing use of next-generation sequencing data in these disciplines. For the GCFR, these new approaches will provide means to understand the processes responsible for the speciation and persistence of species in this biodiversity hotspot (see Lexer et al. 2013 for a more detailed narrative). The uncovering of potential causal factors behind this diversity, such as adaptation to fire regimes, varied pollination syndromes, and topographic heterogeneity to name only a few, will soon be within grasp in many groups. The future holds promising opportunities for phylogeography in the age of high-throughput sequencing (e.g. Lexer et al. 2013; McCormack et al. 2013).

## 7.11 Conclusions

Phylogeographic patterns in the GCFR do not reveal a suite of congruent patterns across taxa investigated to date. Instead, patterns appear to be dependent upon species life history and dispersal capability. Despite this, a few broad generalizations can be made. In particular, the southwestern region is often considered more diverse than the east, and this is attributed to the southwest having acted as refuge during climatic changes, with species later expanding east and/or north. In

plants, there appear to be contrasting patterns, potentially due to differences in seed dispersal capability and fire survival strategies. In some species, limited dispersal results in highly localized genetic structure and little variation within a population, but conversely wide dispersers show high diversity across a large region and lack localized structure. For mammals, clades are usually widespread and lack phylogeographic structure within the GCFR, but do show structure across southern Africa. In contrast, invertebrates show multiple divergent clades across the GCFR and any generalization is difficult to draw. This is likely due to the very wide range of taxa investigated, but also because patterns may be related to associations with host plants, and this is not typically taken into account. Regardless, most taxa show some broad division between the western and eastern regions, and very often a distinct Cape Peninsula clade. Many amphibians show very localized patterns of phylogeographic structure, as well as much deeper phylogenetic structure on very small scales within the GCFR, which is attributed to low dispersal ability and habitat specificity. However, examples of amphibians with good dispersal ability lack strong phylogeographic structure. Reptiles typically show strong patterns of phylogeographic structure in the GCFR, with the eastern region usually consisting of one (or a few) widespread clade(s), while the western GCFR is a region that encompasses multiple clades that have potentially expanded out of multiple refugia. There is a lack of studies for avian taxa within the GCFR, but larger-scale studies of birds with southern African distributions (including the GCFR) show that phylogeographic patterns most probably follow biome boundaries, with hybridization at the biome transition zones. Overall, however, it appears that any search for common environmental drivers to explain phylogeographic patterns within the GCFR is untenable. Indeed, to better understand the factors that have produced the observed patterns, we must gain a comprehensive understanding of adaptive processes (Chapter 6). Much more useful in this respect would be studies that take into account mechanisms at the organismal level, rather than simply addressing broad correlations between landscape features and palaeoclimate shifts.

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