

Dispersal, gene flow, niche divergence and local adaptation in the hyper-diverse ruschioid Aizoaceae: Testing alternative modes of speciation in the Knersvlakte quartz field flora of the Succulent Karoo, South Africa

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Abstract

What are the roles of alternative modes of speciation in the generation of biological diversity? This question is fundamental to the debate surrounding the origins of extraordinarily diverse regions and clades. Diversification in the Cape flora of South Africa has been intensively studied owing to its remarkable diversity, for which significant roles for both ecological and non-ecological speciation have been invoked as drivers. However, much of this research has focused on the mesic fynbos vegetation, with far less attention paid to its neighboring biodiversity hotspot, the arid Succulent Karoo (SK), which has hosted the spectacular radiation of the ecologically dominant ruschioid Aizoaceae, a succulent group which exhibits extreme morphological diversity and convergence.

This thesis focused on ruschioid Aizoaceae in the Knersvlakte, a small region of the SK which holds a diverse and endemic-rich flora specially adapted to the ecologically unusual quartz fields – whose patchy distribution in the landscape suggests that diversification may have been facilitated by divergence of populations isolated on these ‘environmental islands’ in a similar fashion to serpentine systems such as those in California – and continues from previous work on the Knersvlakte-endemic ruschioid genus *Argyroderma* which supported an adaptive radiation in allopatry hypothesis. A population genomic approach was used to investigate the scale of seed dispersal (which is thought to be very limited due to the group’s highly specialized ballistic dispersal mechanism) as well as correlates of population divergence in two ruschioid quartz-field specialists with very different growth forms (the shrubby *Ruschia burtoniae* versus the dwarf *Conophytum calculus*) and which co-occurred at four sites distributed throughout the Knersvlakte. This, in combination with ecological and experimental transplant data to test the adaptive underpinning of edaphically-driven community structure, ecological isolation and niche divergence in these and other quartz field species, made it possible to tease apart the roles of local adaptation and limited dispersal in driving gene flow and speciation in the system.

Quartz fields were found to be a highly insular habitat with strong internal edaphic community structure, suggesting that they represent an environmental island system. In addition, intrinsic dispersal ability was very poor in the specialist shrub, which showed complete genetic isolation between the four populations separated by just 17-42 km. This species showed strong local adaptation between the populations as well as some evidence that this inhibited gene flow, though it is more likely that dispersal limitation allowed for fundamental niche divergence. In contrast, the dwarf showed surprisingly good dispersal ability and consequent weak genetic structure, which accounted for the lack of edaphic local adaptation between the populations. The study showed that, contrary to expectation, not all ruschioid Aizoaceae are poor dispersers, and also suggested that the likelihood of ecological speciation in response to edaphic heterogeneity is contingent on dispersal ability. Strongly limited dispersal may thus have contributed to the group's diversification either through non-adaptive radiation or edaphically driven adaptive radiation, but other factors are more likely to have driven diversification in sections of ruschioid Aizoaceae that possess mechanisms of long-distance dispersal. Future work might focus on inferring the scale of dispersal (e.g. based on morphological traits) and whether it predicts diversification rates. Finally, the lack of morphological variation in *R. burtoniae* belies its strong ecological and genetic divergence; in light of this, systematists are encouraged to investigate cryptic speciation in the group.

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Chapter 1 – Introduction: Current understanding of the drivers of the extraordinary floristic diversity of the Succulent Karoo, with emphasis on the Knersvlakte quartz fields

Speciation

What is speciation and how should we study it? This is the title of a classic article by John Wiens (Wiens 2004b) which argues that the focus on intrinsic reproductive isolation mechanisms promoted by the widely adopted Biological Species Concept (BSC; Coyne & Orr 2004) has diverted the study of speciation away from its real underlying causes because intrinsic isolation typically only evolves after speciation has actually occurred. The debate surrounding species concepts is central to Wiens's argument, which advocates for the Evolutionary Species Concept (ESC) in defining species as the largest lineage integrated by gene flow (Simpson 1951; Wiley 1978). De Queiroz (2007) outlined a 'Unified' Species Concept (USC) which highlights the distinction between the conceptualisation of species and the practical challenge of delimiting them, noting that many species concepts – including the BSC – are in fact practical in nature because they implicitly accept the ESC while simply proposing alternative methods to identify and delimit species. The recognition of this fact is crucial to the study of speciation because it shifts the focus away from finding the causes of any particular intrinsic property of species (e.g. evolved pre- or post-zygotic isolating mechanisms) and towards an examination of the factors which promote population divergence and incipient speciation (e.g. the cessation of gene flow or adaptation to different environments). This provides the basis of a research program which addresses the most important current questions about speciation (Wiens 2004b; The Marie Curie SPECIATION Network 2012).

One question that is fundamental to the debate surrounding the origins of extraordinarily diverse regions and clades is: What are the roles of alternative modes of speciation in generating biological diversity? A mode of speciation is a hypothesis about the microevolutionary processes that cause lineages to split and maintain their divergence, and two such hypotheses are particularly prominent. ‘Ecological speciation’, as defined by Rundle & Nosil (2005), is caused by adaptive divergence of populations in response to different environments (i.e. local adaptation) and is independent of geographic context. In contrast, ‘non-ecological speciation’ *sensu* Rundle & Nosil (2005) and Rundle & Price (2009) requires allopatry but is independent of divergent selection, and includes ‘mutation-order’ speciation under uniform selection as well as speciation resulting from polyploidy or genetic drift (Sobel et al. 2010). In recent times, ecological speciation has become the dominant hypothesis in the literature, at the expense of non-ecological speciation (Rundle & Nosil 2005; Schluter 2009; Nosil 2012).

There is, however, reason to question the *status quo*. Recent proponents of ecological speciation have generally adopted the BSC when concluding that non-ecological speciation (especially by genetic drift) is rare or unlikely (Turelli et al. 2001; Coyne & Orr 2004; Rundle & Nosil 2005; Sobel et al. 2010). But adopting the USC, in viewing speciation as a gradual process that may include but is not exclusively associated with intrinsic reproductive isolation, could lead to a different understanding of the issue. Rather than viewing speciation as binary it is more appropriate to account for a degree of uncertainty, and this includes acknowledging that speciation might be ‘incomplete’ (with respect to intrinsic isolation) when investigating its underlying causes (Nosil et al. 2009), as well as acknowledging the reality of cryptic speciation, a particularly thorny issue for plant taxonomists (Bickford et al. 2007). The latter encourages the taxonomic community to shift away from using morphology to delimit species, because genetic and ecological divergence – which may reflect true evolutionary divergence – are not necessarily reflected in morphological traits (Bickford et al. 2007). Finally, this approach promotes a shift in perspective: rather than focusing on geographic context by, for example, distinguishing allopatric and sympatric speciation, it emphasises

gene flow, the underlying process of interest which is affected by many factors apart from geography (Templeton 1981; The Marie Curie SPECIATION Network 2012).

In another shift away from traditional thinking, it has been convincingly argued that the terminology of the ecological/non-ecological speciation debate presents a false dichotomy because speciation invariably involves ecology (Wiens 2004a; Sobel et al. 2010). In particular, although allopatry is generally crucial to non-ecological speciation, it is often intimately tied to ecological forces (Wiens 2004b) and usually relates to niche conservatism, the common tendency of species to remain within their native environment rather than adapting to new ones (Donoghue 2008; Petitpierre et al. 2012). For any species, if environmental change results in a discontinuous distribution of its fundamental niche, the degree of subsequent range fragmentation (i.e. vicariance) will be influenced by its ability to adapt to new intervening environments (i.e. niche conservatism; Wiens et al. 1985). Given that niche conservatism is extremely common in nature (Donoghue 2008) and that range fragmentation usually reduces the spatial scale of gene flow, this means that the geographic pattern of environmental change over evolutionary time has a profound effect on the likelihood of speciation (Kozak & Wiens 2006). This applies to all modes of speciation because of the fundamental role of gene flow limitation in population divergence (Slatkin 1985).

The role of gene flow is not limited to non-ecological speciation because although ecological speciation is thought to be possible even in the presence of gene flow, it is nevertheless dependent on the strength of divergent selection being sufficient to prevent 'gene swamping', the ecological and evolutionary homogenising effect of gene flow (Lenormand 2002; Nosil 2008). Importantly, though, the relationship between selection and gene flow is reciprocal: strong environmental differences can enforce local adaptation which in turn restricts gene flow through immigrant inviability, a process called isolation by adaptation (IBA), and this results in a pattern of isolation by environment (IBE) in which there is a positive correlation between environmental and genetic divergence for both neutral and adaptive regions of the genome (Nosil et al. 2008). Conversely,

strong uniform selection can promote gene flow and prevent divergence even when gene flow is intrinsically very limited by countering genetic drift and favouring the establishment of infrequently dispersed vectors carrying advantageous alleles (Morjan & Rieseberg 2004). Finally, the relationship between gene flow and local adaptation is thought to be non-linear: for instance, extremely restricted gene flow may prevent local adaptation through the loss of genetic variation due to inbreeding depression; as such, local adaptation is perhaps most likely under an intermediate level of gene flow (Garant et al. 2007).

Isolation by adaptation is often cited as the primary mechanism of ecological speciation because it can profoundly affect phenotypic and genetic divergence (Shafer & Wolf 2013); however, factors apart from environmental variation that might promote divergence and local adaptation are not always investigated or acknowledged as contributing factors in speciation (Räsänen & Hendry 2008). As Wiens (2004b, p. 917) put it, for any taxon the 'ecological and evolutionary factors that cause allopatry and that determine how long geographic isolation will last', such as its ecological setting and intrinsic dispersal ability, are crucial in determining the likelihood of *any* mode of speciation. In conclusion, studying incipient speciation requires a holistic approach which addresses the ecology of allopatry in addition to how the spatial scale of gene flow is affected by local adaptation and intrinsic factors like dispersal limitation, and which acknowledges the interdependence of these factors and attempts to tease them apart by investigating each independently. Studies that focus on specialised species occupying naturally fragmented habitats, or 'environmental islands' (Ackerly 2003), are particularly well suited to this approach. Perhaps the best-studied systems of this kind are the serpentine floras of the world, particularly those in California, which typically comprise a number of radiations occupying edaphically unusual, patchily distributed geologies. The great potential of these systems for promoting ecological allopatry and both neutral and adaptive radiation has long been acknowledged (Kruckeberg 1986; Harrison et al. 2006; Kay et al. 2011), and genetic tools are now being used to tease apart the mechanisms of speciation that act within them (Turner et al. 2010; Anacker et al. 2011). The purpose of the remainder of this chapter is to introduce the Succulent

Karoo (SK) of South Africa as a highly diverse floristic region and to address the potential role that serpentine-like systems comprising environmental islands may have played in the evolution of its diversity.

The Succulent Karoo

The SK is an arid region that forms a major part of the Greater Cape Floristic Region (GCFR; *sensu* Born et al. 2007) and hosts the world's most diverse arid flora with over 5000 species, of which roughly 40% are endemic (Hilton-Taylor 1996), making it a global biodiversity hotspot (Myers et al. 2000). This diversity rivals that of the neighbouring Cape Floristic Region (CFR) which is primarily comprised of the Fynbos biome, a mesic shrubland that dominates the mountains of the Cape and hosts over 9000 species with ca. 70% endemism (Manning & Goldblatt 2012). Interest in the evolutionary factors responsible for the diversity of the GCFR has a long history (summarised by Ellis et al. 2014) and remains a focus of current research (e.g. Britton et al. 2014; Linder 2005; Schnitzler et al. 2011; Verboom et al. 2015). However, much of this research has focused on the CFR rather than the SK (but see Verboom et al. 2004; Ellis et al. 2006) and has also almost exclusively adopted a correlative approach, with phylogenetic methods employed to investigate associations between past trait or environmental changes and shifts in diversification rate (e.g. Schnitzler et al. 2011), or to use differences between sister taxa to infer the factors that caused their divergence (e.g. Van der Niet & Johnson 2009). In contrast, studies which investigate the drivers of population divergence (e.g. Ellis & Weis 2006; Ellis et al. 2007; Prunier & Holsinger 2010; Prunier et al. 2017) have been rare, despite the fact that they can be used to address causative hypotheses of speciation as a contemporary process (Ellis et al. 2014). Given the SK's global significance and the relative paucity of speciation research on its flora, there is a clear need for a greater understanding of the processes that gave rise to its extraordinary diversity. Furthermore, there are multiple reasons to suspect that different drivers of diversification acted in the SK and the CFR.

Diversification in the Succulent Karoo versus the Fynbos

Topography and geology. For the most part the SK lacks the great topographic heterogeneity of the CFR, which in that region has been linked to diversification through the promotion of strong environmental gradients and population isolation (Linder 2003; Linder 2005; Verboom et al. 2015), and evidence of cryptic speciation suggests that the importance of the latter in promoting non-ecological speciation may be under-appreciated (Britton et al. 2014). Rather, the much more easily eroded, predominantly shale substrates of the SK have produced a relatively flat to gently undulating topography (Figure 1; Bradshaw & Cowling 2014), whose ability to promote population isolation and consequent diversification remains an open question. In addition, the different underlying geologies of the two regions have resulted in very different patterns of edaphic variation (Bradshaw & Cowling 2014). The CFR is dominated by extremely low-nutrient soils, adaptation to which prevents lineages from occupying richer soils (Verboom et al. 2017) and may therefore promote non-ecological speciation by limiting dispersal. However, the origin of these soils does vary in the lowlands where, for example, specialised granite and limestone fynbos floras exist, and this large-scale edaphic heterogeneity may have promoted ecological speciation (Verboom et al. 2015). In contrast, the SK is dominated by relatively nutrient-rich shale-derived soils which are often heterogeneous at fine scales, often presenting very sharp edaphic boundaries with concurrent species turnover (e.g. Schmiedel et al. 2015), which suggests that edaphic variation may have promoted ecological speciation in the SK flora.

Age and climatic history. The two regions have very different environmental and consequently diversification histories. The SK is a recently emerged environment, having likely arisen as a result of global cooling and aridification in the Neogene (Dupont et al. 2005; Linder 2008; Verboom et al. 2009; Hoffmann et al. 2015), which contrasts with the long-term climatic stability of the CFR (Hoffmann et al. 2015) and the dominant, relatively ancient quartzitic substrates which host most of its diversity in the form of fynbos vegetation (Goldblatt 1978; Cowling et al. 2009). This is reflected in the phylogenetic histories of the floras: SK-endemic clades are consistently younger than their CFR-

endemic sister clades, and their estimated divergence times almost always postdate the hypothesised emergence of the SK, characterised by aridification in the Middle Miocene (13-17 Ma) and the development of strong seasonality ca. 6.5-8 Ma (Verboom et al. 2009; Hoffmann et al. 2015). Hence in the CFR long-term climatic and edaphic stability, the buffering effect of elevational heterogeneity, and consequent low extinction rates are thought to have provided the background for species accumulation at generally unexceptional speciation rates (Verboom et al. 2014); in contrast, the novelty of the SK suggests that its diversity arose primarily from rapid *in situ* diversification. Linder (2008) found support for this hypothesis, showing that estimated speciation rates were generally higher in clades with most species in Namaqualand (a major region of the SK) compared to majority-CFR clades.

Floristic composition. Despite their geographic proximity and some degree of floristic affiliation to one another (Born et al. 2007), there are numerous clear compositional differences between the SK and CFR floras (described in Bergh et al. 2014). For instance, the fynbos is characterised by families typical of temperate regions such as Restionaceae, Proteaceae and Ericaceae, while prominent families in the SK are Aizoaceae, Poaceae and Crassulaceae (Goldblatt 1978). Asteraceae is the most diverse family in both regions (Born et al. 2007) but exhibits notable regional phylogenetic signal, with the tribe Gnaphalieae being almost exclusive to the fynbos while its relatives within the Asteroideae (e.g. tribes Heliantheae and Astereae) are predominantly confined to the SK (Panero & Funk 2008; Verboom et al. 2014). This pattern suggests that strong environmental filtering has influenced the assembly of these floras. Indeed, fynbos has strong links to geographically distant but edaphically and climatically similar areas, particularly the Australasian flora (Crisp et al. 2009; Verboom et al. 2014), whereas most of the SK's flora has a high degree of compositional overlap with subtropical thicket and renosterveld vegetation types (Bergh et al. 2014), both of which fall within the CFR but consist mostly of radiations of African origin, as does the SK (Verboom et al. 2014). These differences suggest that the SK and fynbos floras may have responded in different ways to various (and perhaps independent) drivers of diversification.

Plant traits. One of the notable features of the SK flora is the preponderance of leaf succulence in a number of its ecologically dominant and most diverse families, many of which, particularly Aizoaceae and Crassulaceae, host a high proportion of dwarf species, a trait that is thought to promote diversification in general (Boucher et al. 2017). Cowling et al. (1998) suggested that the GCFR's Mediterranean climate, with unusually reliable winter rainfall and dry summers, promoted diversification by selecting for small plants with short life cycles and consequent high speciation rates. These authors also noted that periodic droughts in the SK, which induce high mortality especially in dwarves (Musil et al. 2005; Musil et al. 2009), should result in high generation turnover, which they proposed to be analogous to how periodic fires in the fynbos power diversification (Cowling 1987). Others have pointed to a role of plant size because small plants generally have high population densities, which should facilitate local adaptation and allow for fine-scale niche partitioning, thus promoting both adaptive speciation and high local species richness (Ellis & Weis 2006; Ellis et al. 2006; Ellis et al. 2007). Finally, dwarves' small stature might suggest limited dispersal ability (Thomson et al. 2011) and thus higher rates of diversification, but apart from the indirect estimates of dispersal in *Argyroderma* there are no data to test this assumption.

The evolution of succulence has also been suggested to explain the discrepancy in diversification rates between the SK and the CFR. Arakaki et al. (2011) pointed to a role of historical climate change coupled with advantageous 'pre-adaptations' of the local flora, showing that there was a worldwide burst of radiation in multiple succulent lineages in the late Miocene-Pliocene which was broadly coincident with a similar radiation of C4 lineages (Edwards et al. 2010). This postdates the well-known mid-Miocene cooling period which is thought to have resulted in global aridification, but coincides with a period between 8 and 14 Mya during which atmospheric CO₂ levels appear to have dropped precipitously (Tripathi et al. 2009; Zhang et al. 2013). Arakaki et al. (2011) suggested that this resulted in ecological release and subsequent diversification of succulent lineages because, like C4 plants, their high photosynthetic water-use efficiency made them pre-adapted to arid, low-CO₂ conditions. In the parlance of Donoghue & Sanderson (2015) this represents a 'confluence': the key

innovation of crassulacean acid metabolism evolved in response to reduced CO₂ levels against a backdrop of continued aridification, which culminated in widespread ecological release and thus extraordinary opportunities for rapid diversification.

However, relatively little is known about how the above factors which distinguish the SK from the CFR influenced adaptive and/or non-adaptive radiation. Adaptive radiation in response to the emergence of the SK has often been invoked to explain its floristic diversity, but as the case of research into the Aizoaceae demonstrates, much of our current understanding is still based upon speculation.

Aizoaceae: a case study

The rapid radiation hypothesis has some support from phylogenetic evidence from the Aizoaceae, which indicates a dramatic burst of radiation in the 'core' Ruschioideae clade beginning 2-8 Mya and producing over 1500 species and 100 genera (Klak et al. 2004; Valente et al. 2014). Klak et al. (2004) linked the extremely rapid radiation of the core Ruschioideae to a set of traits [wide-band tracheids, hygrochastic capsules (ballistically water-dispersed; Parolin 2006) and flattened leaves] facilitating their survival in the harsh SK climate, which they identified as key innovations allowing for adaptive radiation. One of the earliest authors to suggest a role for adaptive radiation in the group was Ihlenfeldt (1994), who explained the impressive morphological diversity of the Aizoaceae as the result of an unusually flexible growth architecture and proposed that this allowed the group to diversify into a wide array of niches. Valente et al. (2014) also addressed the hyperdiversity of this group in a spatial analysis, finding that across the GCFR there is a positive correlation between topographic heterogeneity and generic richness in the core Ruschioideae, but not in the species-poor Mesembryanthemoideae (ca. 100 species in 11 genera) and early-diverging Ruschioideae (22 species in 10 genera). An important difference between these groups relates to the structure of their seed capsules. Though all have hygrochastic capsules which limit primary dispersal to < 2 m (Parolin 2001; Parolin 2006), with secondary dispersal also being strongly limited due to the coupling of

dispersal and germination in response to rainfall events (Milton 1995), those of the core Ruschioideae have a number of highly specialised and effective seed retention structures (Parolin 2001). Both Ihlenfeldt (1994) and Klak et al. (2004) hypothesised that this trait led to accelerated diversification by causing a reduction in the spatial scale of gene flow. However, very little is known about gene flow in any of these groups, and moreover, gene flow limitation may promote but is not crucial to ecological speciation, whereas it is generally crucial to non-ecological speciation (Rundell & Price 2009).

Although the results of Valente et al. (2014) also suggest an interaction between complex seed retention structures and topographic heterogeneity in promoting the group's radiation, these authors called into question the primacy of this confluence. They noted the high representation of core Ruschioideae in the species- and endemic-rich quartz fields of the SK, which are among its more topographically heterogeneous elements, but emphasised that this lies in stark contrast to the species-poor early-diverging Ruschioideae whose distribution centre is in the south-western Cape, a large region which possesses much greater topographic heterogeneity than the SK. They concluded that topographic heterogeneity alone cannot explain the diversity of the core Ruschioideae, instead hypothesising a role for increased UV radiation exposure caused by the quartz's high reflectance leading to higher mutation rates, a process that is thought to promote diversification (Davies et al. 2004). However, mutation rates in Aizoaceae have yet to be quantified, and other regions of the SK host diverse, high-endemism communities but lack high-albedo substrates, such as the Richtersveld (Hilton-Taylor 1996). They also did not elaborate on whether high mutation rates might facilitate ecological or non-ecological speciation. However, they did draw attention to the particularly diverse flora of the SK's quartz fields, which is the subject of this thesis.

The Knersvlakte

The one exception to the dearth of population-level speciation research in the SK flora is a series of studies that truly sheds light on the nature of the core Ruschioideae radiation and was conducted in the Knersvlakte (Ellis & Weis 2006; Ellis et al. 2006; Ellis et al. 2007), a small region covering roughly

70 km north to south and 40 km west to east which was identified by Born et al. (2007) as one of the four highest centres of endemism in the entire GCFR, which is owed largely to its specialised quartz field flora. Of the 67 obligate quartz field taxa, a remarkable 94% are endemic and 56% belong to (mostly ruschioid) Aizoaceae (Schmiedel 2002). Apart from its diversity, the flora is of very recent origin: while most Succulent Karoo endemic lineages originated less than 10 Mya (Verboom et al. 2009), the quartz fields are thought to have emerged in the Pliocene between 5.3 and 2.6 Mya in response to regional erosional processes stimulated by continental uplift (Cowling et al. 2009). Such explosive radiations are typically interpreted as signatures of adaptive radiation (Ihlenfeldt 1994) but non-adaptive radiation following rapid range expansion can equally produce such a pattern (Rundell & Price 2009). The emergence of the quartz fields could have sparked such an event, particularly given that the initial distribution of the quartz would have been more island-like than it is at present (Ellis et al. 2006), providing greater opportunity for geographic isolation. Regardless, this is a system in which rapid *in situ* diversification clearly took place, though the drivers of this diversification remain uncertain.

Ellis and co-workers shed light on this problem by focusing on the Knersvlakte-endemic genus *Argyroderma* and exploring the links between patterns of genetic structure and divergence in morphology, flowering phenology and edaphic niche to understand the recent diversification of the group. The eleven species in the genus separate into three distinct genetic clades: the dwarf, unbranched *A. delaetii* group; the dwarf, typically branched *A. framesii* group; and the sole non-dwarf *A. fissum* (Hartmann 1977; Ellis et al. 2006). The dwarf species are all strongly associated with the Knersvlakte's most prominent geographic feature, the quartz gravel plains; in contrast, *A. fissum* occurs both on and off the quartz (Hartmann 1977; Schmiedel 2002). The studies showed that the largely allopatric *A. framesii* group taxa are genetically distinct, with genetic structure being associated with both spatial isolation and edaphic specialisation and divergence. Ellis et al. (2006) thus proposed a model for this group of diversification driven by adaptive divergence in allopatry and facilitated by limited long-distance gene flow. In contrast the *A. delaetii* group exhibited weak

genetic structure between taxa which correlated with divergence in flowering time and edaphic niche, leading Ellis et al. (2006) to suggest that incomplete adaptive differentiation occurred in allopatry in response to different edaphic environments, which in turn led to phenological differentiation.

Ellis et al. (2006) suggested that the process of diversification in the *A. delaetii* group is gradually being reversed as the taxa come back into contact, citing the prevailing hypothesis of the geomorphological evolution of the Knersvlakte quartz fields (KQF) as supporting evidence. The hypothesis states that although the distribution of the KQF is fragmented and in part island-like, as the quartzite beneath overlying rocks has gradually been exposed due to erosion the quartz 'islands' have continued to grow in size and so the potential for population isolation in quartz specialists has decreased with time. This could explain the complex, reticulate divergence patterns observed in the *A. delaetii* group, assuming a later origin of this group relative to the *A. framesii* group, which would have allowed the latter to evolve stronger reproductive isolation. The question remains, however, as to whether other groups in the KQF have similar histories to those of the *Argyroderma* dwarves.

Conclusions

The Ellis studies created the foundation for further population-level speciation research with the KQF as a focal system, and this thesis aims to continue this research by investigating ecologically driven allopatry, local adaptation, and gene flow in other KQF taxa. In particular the focus is to assess the generality of the ecological speciation in allopatry model proposed by Ellis et al. (2006). This thesis adopts the research program advocated by Wiens (2004) by focusing on the biology of allopatry, in other words viewing allopatry as not only a geographical phenomenon but also a biological one. In this sense the central question is: To what extent are quartz patches an insular (i.e. non-permeable) habitat, and how does the insularity and patchiness of the quartz fields interact with divergent selection to affect the probability of speciation in quartz specialists? The question is addressed using measures of niche divergence, local adaptation and gene flow between populations

within various species and species complexes which differ in growth form and life-history strategy, focusing on four sites distributed throughout the region.

Because of its fundamental role in all modes of speciation, gene flow and its mechanisms are the focus of Chapter 2. Using next-generation sequencing and population genomic analysis, unexpected patterns of spatial genetic structure were revealed in two quartz field specialists, which were shown to correspond well with certain aspects of capsule morphology relating to the potential for dispersal, with important implications for dispersal in these and other species of ruschioid Aizoaceae. Chapter 3 then focuses on the insularity and island-like nature of the quartz fields as well as within-species niche divergence and local adaptation to assess the potential for these factors to promote diversification. The results fortified the environmental island hypothesis of the Knersvlakte quartz fields and suggested a strong but complex relationship between realised and fundamental niche divergence in two of the four taxa studied. Finally, Chapter 4 presents a synthetic analysis aimed at teasing apart the roles of dispersal and pollen transfer limitation *versus* niche divergence and local adaptation in driving neutral and adaptive genetic divergence. Mechanisms limiting the likelihood of long-distance seed dispersal, which can be predicted based on plant traits, were hypothesised to be the primary drivers of genetic divergence in the system, with poor dispersers being far more likely to undergo both neutral and adaptive divergence in allopatry. These results have important implications not only for our understanding of diversification processes in the Knersvlakte, SK and ruschioid Aizoaceae, but also for Cape taxonomy and the conservation of evolutionary potential.

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Figures

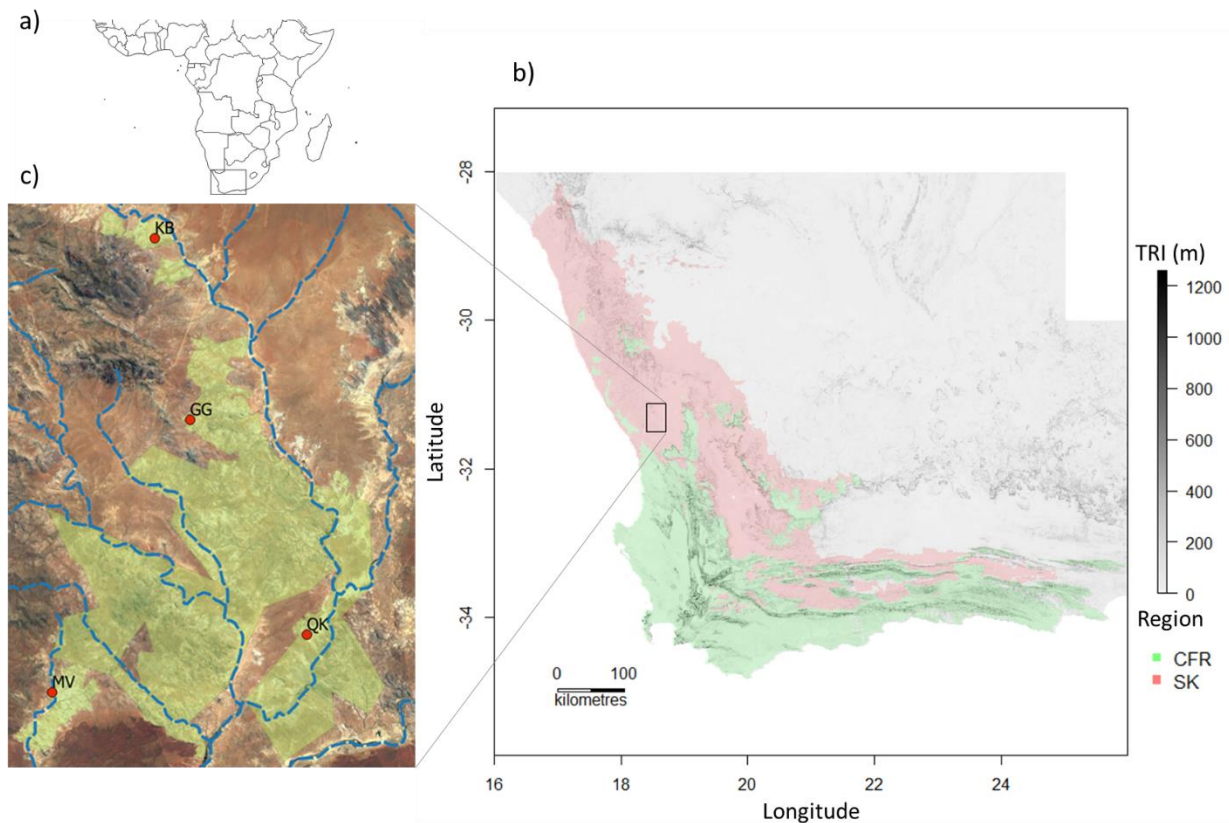


Figure 1: *a)* Map of sub-Saharan Africa showing the location of the GCFR at its southern tip; *b)* map of the GCFR showing the delineation of the SK and CFR and showing terrain ruggedness index (TRI), a measure of elevational heterogeneity which compares each pixel's elevation to that of its eight neighbours following Riley et al. (1999), computed in the R package 'raster' (Hijmans 2015) using a 450m resolution elevation layer modified from SRTM 90m resolution data (Jarvis et al. 2008), revealing far more extensive regions of high TRI in the CFR than in the SK; *c)* a Google satellite image of the Knersvlakte showing the rough distribution of the quartz fields (shaded green), river courses, and the locations of the four sites used in the thesis for genetic and ecological analysis (see chapter 2 for full site names and coordinates).

Chapter 2: Next-generation sequencing reveals unexpected patterns of spatial genetic structure in ruschioid Aizoaceae, with important implications for gene flow and dispersal limitation

Abstract

Genetic tools can be used to evaluate the limitations to mechanisms by which gene flow occurs between spatially isolated populations, which is fundamental to speciation because its likelihood decreases with increasing rate and scale of gene flow. This is particularly relevant in the Succulent Karoo because dispersal limitation has frequently been cited as a potential driver of diversification, especially in ruschioid Aizoaceae. Next-generation sequencing allows genetic divergence to be quantified with high resolution even at the individual level, which has spawned the field of population genomics. This approach was used to investigate long-distance dispersal and pollen transfer in two quartz field specialists with contrasting dispersal and pollination traits. The shrubby *Ruschia burtoniae* displayed remarkably strong genetic structure both between populations and between individuals within populations, with spatial patterns suggesting a complete lack of inter-population gene flow. In contrast, the dwarf *Conophytum calculus* showed a clear pattern of isolation by distance between populations and frequent panmixis within populations. It is hypothesised that long-distance dispersal of entire seed capsules containing hundreds of seeds occurs regularly in the dwarf, whereas this is extremely unlikely to occur in the shrub. These results have important implications for our understanding of diversification in this hyper-diverse family.

Introduction

In plants, gene flow between populations can take place via seed dispersal and pollen transfer, and the frequency and spatial scale of these processes are central to speciation because gene flow diminishes the effect of genetic drift on genetic divergence and can also lead to 'gene swamping,' making local adaptation (and therefore also adaptive speciation) less likely (Lenormand 2002). Consequently gene flow is generally accepted to be a homogenizing force which acts to diminish the likelihood of speciation (Slatkin 1985). Two predictions arise from this which are not mutually exclusive (Kisel & Barraclough 2010). Firstly, speciation should be more likely when gene flow between populations is reduced because of dispersal limitation resulting from intrinsic (i.e. biotic) and/or extrinsic (biotic and abiotic) factors. Following on from this, speciation should be more likely in larger regions partly because they should contain more landscape features that restrict gene flow, thus generating greater opportunity for divergence. However, species can retain genetic cohesion despite limited gene flow if there is strong stabilizing or spatially uniform directional selection on genes that are involved in speciation (Morjan & Rieseberg 2004) and, conversely, speciation can occur in the presence of high gene flow as a result of strong divergent selection (Nosil 2008; Niemiller et al. 2008). The body of evidence demonstrating speciation driven primarily by divergent selection ('ecological speciation') has grown in recent times, which has arguably shifted the focus away from speciation driven primarily by other factors such as genetic drift ('non-ecological speciation'; Schluter 2001; Coyne & Orr 2004; Schluter 2009). Nevertheless, Kisel & Barraclough (2010) found strong evidence to show that both geographic area and strength of gene flow are strong determinants of the probability of speciation in a broad range of taxa occupying oceanic islands, and Vamosi & Vamosi (2010; 2011) have demonstrated a strong effect of area on angiosperm diversity globally. While it is true that area may be associated with greater opportunity for not only non-ecological but also ecological speciation because larger areas typically contain more environmental variation, these studies demonstrate the importance of the spatial scale of gene flow in any mode of speciation.

The spatial scale of gene flow is particularly important in plant speciation because plants' sessile nature means that traits that influence the scale of seed dispersal and pollen transfer are subject to strong selective forces, many of which act to limit the spatial scale of gene flow (Rieseberg & Willis 2007). This is a particularly important subject in regions with high floristic diversity such as the Greater Cape Floristic Region (GCFR), where it has long received considerable attention in the literature on diversification (Goldblatt 1978; Ihlenfeldt 1994; Cowling et al. 1998; Linder 2003; Verboom et al. 2015) but has only very recently come under direct scrutiny (Ellis et al. 2014; see Ellis et al. 2006; Ellis et al. 2007; Lexer et al. 2013; Lexer et al. 2014; Britton et al. 2014; Prunier et al. 2017).

Gene flow: pattern and process

Typically, genetic differentiation is measured as F_{ST} , which is broadly defined as the proportion of total genetic variance that is explained by between-population variance, and can be estimated using a number of methods (Holsinger & Weir 2009). Orsini et al. (2013) provide an in-depth summary of the processes which underlie patterns of spatial genetic structure, and show that it is possible to infer the existence and strength of current gene flow between populations from a pattern of isolation by distance (IBD; Wright 1943), in which pairwise F_{ST} increases with pairwise geographic distance between populations. Mantel tests (Mantel 1967) can be used to estimate and test the strength and significance of IBD (Legendre & Fortin 2010; Diniz-Filho et al. 2013; Legendre et al. 2015). IBD might signify that there is ongoing gene flow and that its rate decreases with distance, a process termed isolation by dispersal limitation (IBDL *sensu* Orsini et al. 2013), which in the case of plants refers to both seed and pollen movement. However, IBD can also result from isolation by colonisation (IBC) if the sampled populations were colonised in a stepping-stone fashion (i.e. serially), but these processes can be teased apart through the use of dispersal route analysis to determine likely colonisation routes, such as along hypothesised least-cost paths (Storfer et al. 2007).

On the other hand, strong deviations from IBD can result from either a lack of IBDL due to panmixis or from IBC reflecting a complex colonisation history. These alternatives can be teased apart with assignment analysis, which provides a more nuanced view of genetic structure than F_{ST} because it evaluates genetic variation at the individual level, and also does not require any *a priori* population delineation (Davies et al. 1999; Pritchard et al. 2000). The degree of ambiguity in assigning individuals to populations based on their genetic make-up provides insight into gene flow [see Prunier & Holsinger (2010) for a summary and example]. For instance, unambiguous assignment of individuals to genetic clusters unique to each population suggests no or very limited contemporary gene flow between the sampled populations. Furthermore, immigrant individuals will cluster separately from others in their population, while hybrids resulting from between-population crosses will present ambiguous assignment. Finally, panmixis will result in a lack of genetic structure wherein individuals cannot be assigned to distinct clusters and so the optimal number of clusters is one.

When studying sessile organisms such as plants, another, more direct approach to studying dispersal limitation with genetic data is to measure IBD at much finer scales, where current gene flow and not historical factors should drive patterns, using measures of genetic distance between individuals within populations (Vekemans & Hardy 2004). At this level, in plants, seed dispersal and pollen transfer affect the strength of IBD at different scales (Hamrick & Trapnell 2011). It is useful to separate such fine-scale IBD into micro-scale (e.g. over tens of metres) and meso-scale (e.g. over hundreds of metres) IBD, and to compare these using Mantel correlograms which show how Mantel's correlation coefficient changes in size and significance at increasing distance classes (Vekemans & Hardy 2004). The effect of pollen transfer on fine-scale spatial genetic structure is contingent on the effect of seed dispersal because the latter solely determines the spatial distribution of individuals, such that strongly limited seed dispersal will produce local neighbourhoods comprising both siblings and mother-daughter pairs – resulting in a pattern of micro-scale IBD – irrespective of the source of the paternal genomes present in the neighbourhood (Dyer 2015). Therefore, fine-scale IBD will be strongest when both seed and pollen movement are

limited, while limited seed dispersal combined with widespread pollen transfer will result in reduced genetic differentiation only between pairs of individuals separated by larger distances, such that IBD will be strongest at the micro-scale and weaker or non-existent at the meso-scale. On the other hand, very limited pollen transfer will not produce micro-scale IBD if combined with widespread seed dispersal, and will essentially resemble panmixis resulting from unlimited pollen and seed movement: both scenarios will result in a lack of fine-scale IBD of any kind. Consequently, these patterns primarily reflect the scale of seed dispersal, and so can provide insight into how dispersal mechanisms influence gene flow at larger scales, which are relevant to speciation.

All of the methods described above can be undertaken using next-generation sequencing (NGS), which allows genetic variation in the form of single-nucleotide polymorphisms (SNPs) to be quantified with unprecedented precision, even for non-model organisms (Lexer et al. 2013; McCormack et al. 2013). However, translating genetic patterns into biological insights requires an understanding of the potential gene flow mechanisms of the organisms of interest.

Modes of gene flow in plants

In plants, long-distance seed dispersal (LDD) contributes to landscape level genetic structure and is thought to generally occur via 'non-standard' vectors such as extreme weather events which typically occur only rarely, making it challenging to determine the frequency of LDD events (Higgins et al. 2003; Nathan 2006; Nathan et al. 2008). However, plants might have genetic control over traits that influence LDD, making them potentially subject to selection. Extreme wind events are a likely candidate for causing LDD, and such events are expected to be more frequent in open environments (Nathan et al. 2008). In addition, wind-mediated secondary seed dispersal has been shown, using modelling approaches and experimental data, to vary unimodally with seed size, such that seeds of intermediate size disperse farthest (Schurr et al. 2005). The frequency of LDD can be inferred using genetic data at the landscape level with assignment analysis to identify potential immigrants, as well as at fine scales (i.e. within a population) provided the expected distance of dispersal via standard vectors is smaller than the spatial extent of the sampled individuals.

Gene flow via pollen dispersal has been demonstrated over large spatial scales (e.g. Ndiade-Bourobou et al. 2010; Craft & Ashley 2007; Sork et al. 2015) and in many instances is likely to influence landscape-scale gene flow more strongly than seed dispersal (Dyer 2015). However, long distance pollen transfer (LDP) is widely assumed to be most frequent in wind-pollinated plants (Whitehead 1969). In animal-pollinated plants, assuming there is overlap in flowering time among populations, LDP is influenced by i) pollinator flight patterns, abundance and population density, ii) the degree of pollen carry-over from one individual to the next visited by the pollinator, and iii) pollen longevity (Levin 1981). The movement patterns of pollinators are affected by plants at the individual, population and community levels. There is less incentive for pollinators to disperse when i) individual plants provide more abundant and reliable resources to pollinators, ii) plants occur in high population densities, and iii) communities have a high degree of flowering time overlap among species competing to attract the same pollinators. Species that attract generalist pollinators may be more likely to share pollinators with co-occurring species and so may be less prone to LDP, though on the other hand they might also have an increased chance of attracting wide-ranging pollinators (Waser et al. 1996). Plants with specialist pollinators typically evolve such a system in order to improve pollen transfer efficiency, and as such they may show more frequent LDP as less pollen will be lost to flowers of other species, resulting in greater carry-over (Levin 1981).

The Succulent Karoo and ruschioid Aizoaceae

The Succulent Karoo (SK) is a global hotspot of floral diversity and endemism which has played host to the exceptional radiation of the 'core' Ruschioideae clade within Aizoaceae, which rivals in both scale and tempo that of the cichlid fish of the African rift lakes (Klak et al. 2004; Valente et al. 2014). Dispersal limitation resulting in limited gene flow has long been cited as a putative cause of the group's high speciation rate (Ihlenfeldt 1994), though remarkably little work has been done to directly infer the spatial scale of gene flow within species in this or other important SK groups (Ellis et al. 2014). The only exception is the work of Ellis et al. (2006) on the Ruschioid genus *Argyroderma*, which is endemic to the Knersvlakte, a centre of floral endemism in the SK (Born et al. 2007). These

authors used data on genetic, edaphic niche and flowering time divergence and concluded that adaptive divergence in allopatry was facilitated by limited gene flow between isolated populations, making dispersal limitation a crucial factor in the group's radiation [see also Ellis et al. (2007)]. The most striking feature of the Knersvlakte is the extensive quartz gravel plains, and various lines of evidence suggest that these are an example of an archipelago-like continental system, including the fragmented distribution of the quartz fields in the landscape, the specialised nature of the plant species which inhabit them (most notably the diverse assemblage of dwarf succulents which have arisen from multiple independent radiations), and the high degree of endemism in the flora (Schmiedel 2002; Schmiedel & Jürgens 1999). Aizoaceae are notably dominant: they comprise 56% of the 67 quartz field flora and frequently occur in high population densities (Schmiedel 2002). These features, combined with the existence of previous work, make the Knersvlakte quartz fields (KQF) an excellent system for studying dispersal limitation in ruschioid Aizoaceae and its consequences for their diversification.

Seed dispersal in most ruschioid Aizoaceae is intimately tied to rainfall due to their hygrochastic capsules, which use the force of rain drops to release and disperse seeds over short (1-2 m) distances and usually have complex structures which protract the period of seed release (Parolin 2001; Parolin 2006; Ihlenfeldt 1994). Given this ballistic dispersal mechanism, plant height might be influential in determining seed dispersal distance, with dispersal distances being generally shorter in small stature species (Thomson et al. 2011). In addition, although there may be secondary seed dispersal via wind or water runoff, this is likely to be limited because germination is also stimulated by rainfall (Milton 1995). Consequently, seed dispersal via the standard mechanism is likely to be very limited. However, it is possible that entire seed capsules could be dislodged and dispersed long distances by wind, which would probably represent a non-standard dispersal mechanism. The larger size of the capsules (typically 5-10 mm in diameter) compared to the seeds (often well under 1 mm in diameter) favours wind-mediated long-distance dispersal of the former (Schurr et al. 2005), and furthermore, single capsules could generate multiple LDD events because they often contain

hundreds or even thousands of seeds. Two phenotypic constraints on this process that could be subject to selection are the ease with which i) the capsules are separated from the plant and ii) the seeds are released from the capsule. Ellis et al. (2006) cited these features of the group as potentially crucial in the diversification of *Argyroderma*, suggesting that colonisation of new sites via long-distance capsule dispersal occurred very rarely, while strongly limited dispersal in general facilitated between-population divergence and eventual speciation.

In contrast to LDD, relatively little attention has been paid to the potential for long-distance pollen transfer in ruschioid Aizoaceae, all of which employ insect pollinators – with Hymenoptera, Diptera, Coleoptera and Lepidoptera being the dominant groups (Struck 1994; Scodanibbio 2002) – and typically have large, dish-shaped, showy flowers which point to a generalised pollination system (Ihlenfeldt 1994). However, more specialised systems have evolved in some genera, notably *Conophytum* which has a high proportion of moth- and butterfly-pollinated species (Jürgens & Witt 2014). The strongly seasonal climate of the SK means that most flowering occurs in spring, and as such there is often a high degree of flowering time overlap in co-occurring species (e.g. Scodanibbio 2002), providing little incentive for pollinators to disperse large distances.

Study species and predictions

The aim of this chapter is to use NGS techniques to investigate spatial patterns of genetic structure in two species of ruschioid Aizoaceae (*Conophytum calculus*, a dwarf species, and *Ruschia burtoniae*, a non-dwarf) which occupy the same habitat within the KQF and have contrasting seed dispersal and pollination traits, with a view to improving our understanding of LDD and LDP in the group as a whole.

A number of inferences can be made about seed dispersal and pollen transfer in these two species based on observations of their biology. *Conophytum calculus* typically produces a few seed capsules each year, though these often contain hundreds of tiny seeds, whereas *R. burtoniae* produces several corymbose infructescences yearly, each with at most about a dozen capsules typically

containing few seeds. In *C. calculus* the capsules are situated between the leaves at a height of less than ten centimetres above the ground, whereas the infructescences of *R. burtoniae* extend above the plant and often exceed 50 cm in height. The internal structure of the capsules also differs markedly: *C. calculus*, like most other *Conophytum* species, has shallow cup-like capsules with highly reduced covering membranes and no closing bodies; *R. burtoniae* has deep, bell-shaped capsules with closing bodies and well-developed covering membranes, and these features have been shown experimentally to increase the distance of ballistic seed dispersal and reduce the number of seeds dispersed per raindrop impact (Parolin 2001). This also suggests that the upward-facing orientation of the capsule is important for effective ballistic dispersal of seeds by raindrops in *R. burtoniae* but not in *C. calculus*, in which the seeds are easily removed from the open capsule by water flow. Finally, the capsules of *C. calculus* are quite firmly attached to the plant and typically wedged between leaf pairs, whereas those of *R. burtoniae* are easily broken off, though the thicker basal stem of the infructescence is more difficult to break. These capsule structure and height differences indicate that standard (i.e. ballistic) seed dispersal is probably much more limited in *C. calculus* than in *R. burtoniae*. However, dispersal by whole-capsule removal seems more likely in *C. calculus* because, although its capsules are less easily removed, they contain many more seeds and are much more likely to release seeds after being removed from the plant. Because capsules are more likely to be dispersed long distances than individual seeds because of their size, LDD is expected to be more frequent in *C. calculus* than in *R. burtoniae*.

Conophytum calculus has night-scented tubular flowers and is likely to be moth-pollinated (Jürgens & Witt 2014), whereas *R. burtoniae* has typical *Ruschia* dish-shaped, small (1-2 cm in diameter) flowers with a central cone-shaped staminode and is therefore probably pollinated by small, highly territorial solitary bees or wasps (Struck 1994). In addition, the latter flowers during spring when many other co-occurring species are flowering, whereas the former flowers in late summer to autumn when other *Conophytum* species are in flower, but little else. The strong flight capabilities, limited territoriality, and greater pollen carry-over efficiency of moths compared to solitary bees and

wasps, together with greater incentive for long-range movements in pollinators that are active during autumn, suggest that LDP may be more frequent in *C. calculus*.

Overall these factors lead to the prediction that *R. burtoniae* will show stronger spatial genetic structure at the landscape level than *C. calculus* because LDD and LDP events are expected to be less frequent in the former. On the other hand, *R. burtoniae* is predicted to show weaker fine-scale genetic structure than *C. calculus* because seed dispersal via the standard ballistic mechanism is expected to be more limited in the latter.

Methods

Sampling, extraction and sequencing

Four quartz field sites spread across the Knersvlakte were selected. These were: a site on the farm Groot Graafwater in the central Knersvlakte ('GG'; 31°15'55.2"S 18°32'43.1"E); a northerly site on the farm Kareeberg ('KB'; 31°08'24.9"S 18°31'11.0"E); a southerly site on the farm Quaggaskop ('QK'; 31°24'51.6"S 18°38'29.0"E); and a south-westerly site on the farm Moedverloren ('MV'; 31°27'15.0"S 18°25'59.4"E). The distances between GG and the other sites ranged from 17 to 28 km, with the other sites being separated from each other by 24-42 km. Cuttings from 23-24 individuals of *C. calculus* and *R. burtoniae*, both of which are quartz field specialists confined primarily to acidic, low-nutrient soils (see Chapter 3), were taken from each site for DNA extraction. Cuttings were taken from individuals whose leaves were in good condition, and an attempt was made to sample widely at each site and to sample individuals > 5 m apart. For *C. calculus*, extractions were done using the Qiagen DNEasy Plant Mini Kit, following the manufacturer's recommendations. Fresh leaves weighing ca. 1 g had their cuticle removed using a scalpel blade and were crushed using pestle and mortar, with gradual addition of the ligation buffer (Buffer AP1) to the point where the mixture could be poured easily. A pinch of PVP was also added at this stage to reduce the polysaccharide content of the final extracts. For *R. burtoniae*, ca. 2 g of fresh material was ground into a fine powder using liquid nitrogen for use in the large-scale extraction protocol of Healey et al. (2014). This

protocol applies modifications to the widely used CTAB method of Doyle & Doyle (1987) to produce extracts with large quantities of high quality genomic DNA from plants with high amounts of phenolics and polysaccharides in their leaves. The modifications include using high sample quantities in 50 ml Falcon tubes; the addition of an RNase treatment step between the two chloroform: isoamyl alcohol solvent extractions; and DNA precipitation in a high salt buffer which increases the solubility of polysaccharides. All samples for both species were cleaned and concentrated using the Zymo Genomic Clean and Concentrator kit.

Samples were sent to the Cornell Institute of Genomic Diversity for genotyping-by-sequencing (GBS), a workflow which generates reduced representation libraries for Illumina sequencing following the laboratory protocol outlined in Elshire et al. (2011), using PstI (CTGCAG) as the restriction enzyme for digestion. The species' samples were run on separate lanes (one 95-sample lane each) of a 100-bp single-end Illumina HiSeq 2000 run at the Cornell Core Laboratories Centre. The Institute also conducted subsequent alignment and SNP calling using the combined reads from both species and the TASSEL reference pipeline (Glaubitz et al. 2014). For alignment using bwa version 0.7.8-r455 (Li & Durbin 2010) the Institute was furnished with highly fragmented draft genomes of *Polymita steenbokensis* and *Faucaria felina* [both members of the core Ruschioideae (Klak et al. 2013)] which are currently under construction at the Department of Molecular and Cell Biology at the University of Cape Town (S. Schlebusch unpublished data). A substantially greater proportion of the raw reads were uniquely aligned to the *P. steenbokensis* genome (21.4% versus 17.5%), so this alignment was used to generate the final SNP matrix which was provided in variant call format (VCF).

To generate a matrix of confidently identified neutral SNPs for each species, the VCF file was separated by species and each was filtered using 'vcftools' (Danecek et al. 2011) as follows. Firstly, genotypes were filtered according to read depth (DP): genotypes with DP greater than three times the mean DP across all genotypes were removed to account for SNPs that may have been called from repetitive regions of the genome, as were genotypes with DP < 3. Loci were then filtered to

include only biallelic loci having a minor allele frequency > 0.01, less than 10% missing data, and not deviating from Hardy-Weinberg equilibrium (p -value < 0.05). Finally, individuals with > 25% missing data were removed.

To detect loci potentially under selection ('outlier loci') that were not removed by the Hardy-Weinberg filtering step, the software BayeScan version 2.1 (Foll & Gaggiotti 2008) was used on the filtered data sets with a prior odds ratio of 10 and the recommended default settings for the Markov Chain runs. Outlier loci were detected in a liberal manner, as for SNP data with > 10 000 loci (see Results) this prior odds ratio has a high false discovery rate (FDR). The BayeScan R function 'plot_bayescan' was used to calculate the posterior odds (PO) threshold leading to an FDR below 10%, which was used to identify outlier loci to be removed from the final data set.

The resultant files were subjected to a round of filtering to remove loci that were potentially part of the chloroplast genome because its maternal inheritance means that spatial genetic structure will not reflect pollen transfer and so potentially bias the results. The genome used for alignment of the raw reads was chloroplast DNA-depauperate as it was constructed using DNA isolated using a protocol that sought to remove chloroplasts prior to extraction (S. Schlebusch pers. comm.). However, the protocol is not entirely effective, and so some chloroplast DNA may have been incorporated into the reference genome. To account for this, the reference genome was subjected to a BLAST search using blastn (Madden 2013) with the subject being a FASTA file including published whole chloroplast genomes downloaded from GenBank (Benson et al. 2005) of related species in the order Caryophyllales (*Mesembryanthemum crystallinum* in Aizoaceae [Yim et al. 2016]; *Colobanthus quitensis* [Kang et al. 2015] and *Silene latifolia* subsp. *alba* [Wu et al. 2015] in Caryophyllaceae; and *Haloxylon ammodendron* and *Haloxylon persicum* [Dong et al. 2016] in Amaranthaceae). Loci were removed if they were located on scaffolds which had sequences of any length aligning with an e-value less than 1×10^{-10} to any part of any of the chloroplast genomes.

Detection was done in a very liberal manner so as to maximize the likelihood of chloroplast locus removal.

Finally, loci with > 20% missing data were filtered out again as this was altered when individuals were removed. The resulting filtered VCF files were converted into the file formats necessary for further analyses using PGDSpider v.2.1.0.3 (Lischer & Excoffier 2012).

Analysis

Global population genetic differentiation was estimated using Weir and Cockerham's (1984) F_{ST} estimator, which is appropriate for large SNP data sets (Willing et al. 2012), using the R (R Core Team 2017) package 'HierFstat' (Goudet 2005). In addition, analyses of molecular variance (AMOVA) were conducted using the 'poppr.amova' function in package 'poppr' (Kamvar et al. 2014) and significance was assessed by randomisation using 'Ade4' (Dray & Dufour 2007), using 999 repetitions.

To assess IBD at the landscape level, F_{ST} (Weir & Cockerham 1984) was calculated for all pairwise population comparisons using 'HierFstat'. Then, the function 'genleastcost' in the R package 'PopGenReport' (Adamack & Gruber 2014) was used to calculate both Euclidean and least-cost on-quartz pairwise distances between populations. The latter used a raster file which roughly outlined the distribution of quartz in the Knersvlakte [determined using Google satellite imagery in QGIS (QGIS Development Team 2009)] and had lower 'cost' values for quartz relative to non-quartz, which served to limit the off-quartz length of the estimated paths to its minimum possible value while at the same time allowing the shortest on-quartz path to be determined. The correlations between F_{ST} and log-transformed geographic distances were then tested using Mantel tests (Mantel 1967; Diniz-Filho et al. 2013) implemented in the R package 'vegan' (Oksanen et al. 2013). The six pairwise comparisons yielded Mantel tests with 24 alternative permutations of the data, including the actual permutation, such that if all alternative permutations had lower correlation coefficient scores than the observed score, the p-value of the test would be 0.0417. Thus statistical power was somewhat limited.

Individual assignment analysis was conducted using the sparse nonnegative matrix factorization (sNMF) algorithms developed by Frichot et al. (2014), which are analogous to those used in other Bayesian clustering (i.e. 'STRUCTURE-like') programs, to produce individual ancestry proportion estimates of the number of clusters (K) ranging from 2 to 5, with 100 runs for each K, using the R package 'LEA' (Frichot & François 2015). The Q matrices containing ancestry proportion estimates were then summarised using the CLUMPAK software (Kopelman et al. 2015), which identifies major and minor modes based on the degree of consensus between the runs within each K and facilitates the plotting of these modes. In addition, the entropy-based cross-validation technique and scree plots from principal components analyses (PCA; both conducted in LEA) were used to aid in choosing the optimum K for each species, as recommended by Frichot & François (2015).

To assess IBD between individuals within sites, a pairwise genetic distance matrix was created using the 'bitwise.dist' function, which determines the proportion of allelic differences relative to the total number of possible differences between two individuals, using the package 'poppr' (Kamvar et al. 2015). The correlation between log-transformed geographic and genetic distance was again assessed using Mantel tests. To assess the spatial scale of IBD, Mantel correlograms which perform Mantel tests within a series of predetermined distance classes, were generated using 'vegan'. Correlograms are most interpretable when the number of pairs in each distance class is constant and four to five distance classes are used (Diniz-Filho et al. 2013), so the 20% quantiles of the full set of log-transformed pairwise distances for each species/population combination were used as the class breaks. All tests used 4999 randomisations, and p-values were adjusted for multiple testing using progressive Holm's correction as recommended by Legendre & Legendre (1998, p. 721).

Results

Final data set

Alignment and SNP calling by the Cornell Institute Genomic Diversity resulted in a combined matrix of 269 675 SNPs, which was reduced to 14 152 (93 individuals) and 10 384 (90 individuals) for *C.*

calculus and *R. burtoniae*, respectively, after filtering by genotype, locus and individual, and then removing potential chloroplast SNPs (254 and 147 SNPs, respectively) as well as potential outlier loci that were not detected by the Hardy-Weinberg filtering step (8 and 7 SNPs, respectively) and filtering again by locus-level missingness.

Patterns of genetic structure

All analyses consistently pointed towards greater genetic structure in *R. burtoniae* than in *C. calculus*. Global F_{ST} (according to Weir & Cockerham 1984) was 0.0598 for *R. burtoniae* and 0.00830 for *C. calculus*. AMOVA results showed significant structure and broadly agreed with F_{ST} , with between-population differentiation accounting for roughly 0.8% ($p = 0.001$) and 4.2% ($p = 0.001$) of total genetic variation for *C. calculus* and *R. burtoniae*, respectively. In both species all of the remaining variation was within individuals, with none being assigned to the 'between individual, within population' effect.

Evidence of IBD also differed markedly between the species (Figure 1). *C. calculus* showed a strong and significant pattern of IBD between populations and a stronger correlation of genetic distance with on-quartz compared to Euclidean distance (primarily caused by the MV-QK comparison, which deviated from Euclidean IBD because the on-quartz distance was almost twice as great as the Euclidean), which suggests that genetic divergence is influenced by the connectivity of the quartz fields between populations. In contrast, *R. burtoniae* showed no IBD pattern, instead showing relatively stronger divergence between pairs QK-MV and QK-KB, which have very different Euclidean distances but similar on-quartz distances, suggesting that quartz distribution influences genetic divergence to some extent. However, there was very low divergence between KB and MV, the most geographically distant pair (both in Euclidean and on-quartz distance), and therefore the distribution of quartz only partially explains genetic structure in this species.

The assignment analyses (Figure 2) suggested that the four sampled populations in *R. burtoniae* represent highly distinct genetic clusters; in contrast, *C. calculus* showed far less genetic structure.

The cross-validation by entropy criterion analysis for *R. burtoniae* showed the lowest entropy at K=4, and PCA eigenvector analysis showed a steep drop in explained variance followed by a plateau after the fourth component axis, thus also supporting K=4. In contrast, entropy showed a steady increase from K=1-8 for *C. calculus*, suggesting that all four populations might best be regarded as a single genetic cluster. However, the PCA eigenvector analysis for this species showed a sharp drop from 1 to 3 component axes and a subsequent plateau, suggesting K=3 to be most appropriate. When plotting the assignment scores, the fact that no greater resolution was apparent under K=4 or 5 provides further support for K=3 in this species and also suggests that there is very little genetic differentiation between individuals within populations. Taking K=3 for *C. calculus* showed that KB and GG individuals are scarcely differentiated and also that GG and QK are strongly connected. There was also evidence of a single potential immigrant (individual "V1") into GG via seed dispersal from QK or a population closely related to QK. In contrast, MV individuals clustered as unambiguously distinct from the other populations, and together these results suggest frequent ongoing seed dispersal between the three eastern populations, with less frequent seed dispersal to and from the south-west. In contrast, taking K=4 for *R. burtoniae* yielded almost completely unambiguous assignment of individuals to each population, suggesting a complete lack of inter-population gene flow, while taking K=5 indicated that individuals could be assigned to distinct clusters *within* populations, suggesting strongly limited gene flow between individuals even at fine scales.

In accordance with the above results, fine-scale IBD was considerably more pronounced in *R. burtoniae* than in *C. calculus*, with Mantel's *r* statistic across the full set of individual pairs being greater in the former in all populations (Figure 3). In a revealing comparison, *R. burtoniae* showed pronounced IBD at QK, whereas *C. calculus* showed only very weak IBD at KB. At both of these sites individuals were sampled from localities separated by unsuitable habitat occupied by different quartz field communities, giving rise to the distinct clouds of points visible in the plots for these populations in Figure 3, suggesting that even at small scales habitat barriers act to reduce gene flow

in *R. burtoniae* but not in *C. calculus*. In addition, Mantel correlograms (Figure 4) for *R. burtoniae* always showed IBD at both the micro-scale and the meso-scale at MV and QK, whereas in *C. calculus* there was only one instance of micro-scale IBD (at MV) and no evidence for meso-scale IBD.

Discussion

It is strikingly clear that the shrubby, widespread *R. burtoniae* faces much greater restrictions to gene flow on the Knersvlakte than the dwarf quartz-field specialist *C. calculus* at both the landscape level and at much finer scales. In fact *R. burtoniae* shows no evidence of gene flow between the sampled populations based on the assignment analysis and reflected in the lack of IBD. Furthermore, at the fine scale this species shows clear genetic structure as evidenced by the assignment analysis for $K=5$, which shows clear grouping of individuals within populations, and by the presence of strong fine-scale IBD which is influenced by local habitat barriers. This pattern suggests that despite its height and complex capsule morphology, primary and secondary dispersal of individual seeds is very limited in *R. burtoniae*, and that capsule dispersal events, which are more likely to cross habitat barriers because larger capsules should experience greater secondary dispersal distances than small individual seeds (Schurr et al. 2005), occur very infrequently if ever.

In stark contrast, the assignment analysis for *C. calculus* shows evidence of seed dispersal between populations, while the lack of micro-scale IBD (over tens of metres) cannot result from widespread pollen transfer alone because this does not determine the locations of individuals, and so it is best explained by widespread seed dispersal (Dyer 2015). The low stature and simple capsule morphology of *C. calculus* suggests that dispersal of seeds cannot account for the observation of panmixis over hundreds of metres in some populations, implying that widespread capsule dispersal accounts for the pattern. The strong pattern of landscape-level IBD, correlating best with on-quartz distance, suggests that inter-population capsule dispersal occurs frequently and typically in a step-wise fashion between neighbouring quartz patches.

In summary, these results suggest, firstly, that in line with predictions based on differences in the complexity of seed retention structures, long-distance capsule dispersal followed by seed release occurs extremely rarely in *R. burtoniae* but frequently in *C. calculus*, which accounts for landscape-level IBD in that species – therefore LDP does not need to be invoked to explain IBD, but because of the strong flight capabilities of its pollinators it also cannot be excluded as a gene flow mechanism. Secondly, ballistic and secondary seed dispersal is extremely limited in both species and therefore, contrary to expectation, capsule morphological complexity does not determine fine-scale genetic structure. Consequently, genetic differentiation in ruschioid Aizoaceae as a whole is hypothesised to depend primarily on i) the frequency and scale of dispersal of entire capsules and ii) the likelihood of seed release from capsules following capsule removal.

Mechanisms of long-distance capsule dispersal

The greater complexity of seed retention structures in *R. burtoniae* compared to *C. calculus* capsules suggests that seed release following capsule dispersal is less likely in the former, which could explain its lack of LDD if wind is the primary capsule dispersal vector. However, it is unclear whether the species differ in the frequency with which capsules are separated from the plant. It is possible that termites might target *C. calculus* capsules as easily accessed (due to their low height above ground) dry plant matter, as has been observed with *Argyroderma* capsules (AG Ellis personal obs.). Wind is also not the only potential vector: in the SK large numbers of seeds and seedlings were observed inside and germinating from the dung of various large mammals by Milton & Dean (2001), ca. 60% and 40% of which, respectively, belonged to Aizoaceae, leading these authors to suggest that endozoochory may be a mechanism of occasional LDD in this and other SK groups. Furthermore, these authors found that dung of the insectivorous Aardvark *Orycteropus afer* (which is common in the Knersvlakte) contained a disproportionately high number of Aizoaceae seedlings and suggested that the species ingests them along with its ant and termite prey, which implies a possible link between termite activity and endozoochory in LDD. However, Milton & Dean (2001) observed seeds of ruschioid species with complex seed retention structures germinating from Aardvark dung,

suggesting that factors other than capsule complexity prevent endozoochory in *R. burtoniae*. Clearly, a more thorough understanding of the dispersal biology of these species is needed in order to better explain the observed genetic patterns.

Comparison with other findings and implications for speciation

The F_{ST} of 0.06 for *R. burtoniae* is remarkably strong given the short inter-population distances, which range from 17 to 42 km. This is in line with previous work from the Knersvlakte by Ellis et al. (2006), who used AFLP loci and estimated a global F_{ST} of 0.07 among six *Argyroderma pearsonii* populations separated by on average ca. 5 km. This appears to be the only previously published intraspecific F_{ST} estimate from the Succulent Karoo. In contrast, a number of studies have investigated population differentiation in the neighbouring Cape Floristic Region. Tassone (2013), using 616 neutral nuclear SNPs in 51 populations of the wind-pollinated *Leucadendron salignum* distributed over the entire CFR (spanning > 600 km), found a global F_{ST} of 0.14; Lexer et al. (2014) used 14 278 neutral SNPs from *Restio capensis*, a wind-pollinated species endemic to the CFR, and estimated a global F_{ST} of 0.030 in ten populations separated by over 200 km on average; Prunier & Holsinger (2010) assessed differentiation within six *Protea* species in the CFR using 10 microsatellite loci and found an average F_{ST} of 0.08; and Prunier et al. (2017) assessed differentiation of 19 *Protea repens* populations distributed throughout the CFR using 1897 neutral SNPs and estimated a global F_{ST} of 0.06. *R. burtoniae* has a very similar capsule morphology to the hundreds of other shrubby species of ruschioid Aizoaceae, and these results show that gene flow in such species is limited to much finer scales than in many diverse groups in the CFR, while the data from Ellis et al. (2006) suggest that this also applies to at least some of the dwarf groups in the subfamily. Overall this suggests that limited gene flow has been a major diversification driver in ruschioid Aizoaceae by promoting population divergence, as hypothesised by various authors (e.g. Ihlenfeldt 1994; Klak et al. 2004).

This also supports the hypothesis that very infrequent long-distance seed dispersal has contributed to the exceptionally high species per unit area ratio and local endemism of the SK flora, which is

dominated by ruschioid Aizoaceae (Cowling et al. 1998; Desmet & Cowling 1999), by allowing ecologically equivalent taxa to evolve and diversify multiple times independently in different parts of the region (Givnish 2010), which is evidenced by the independent and remarkably similar radiations of *Argyroderma* and *Gibbaeum* in the Knersvlakte and Little Karoo, respectively (Schmiedel & Jürgens 1999). Furthermore, dispersal limitation has also been implicated in the broadly sympatric evolution of ecological equivalence using the unified neutral theory (Hubbell 2006), which could explain the SK's rapid diversification in a similar manner to that which has been shown for the fynbos (Latimer et al. 2005).

The results for *C. calculus*, however, seemingly contradict these hypotheses if one assumes that widespread seed dispersal is the norm in *Conophytum* because the genus is one of the largest in the family and the SK, with over 100 species (Hammer 2002). Nevertheless, landscape-level IBD shows that *C. calculus* is not panmictic at large spatial scales and that seed dispersal depends on the distribution of its habitat, suggesting that geographic isolation can occur over large enough distances or if there are large enough habitat breaks between populations separated by similar distances to those in this study. This is evident in the case of *C. calculus* itself, which comprises two subspecies (ssp. *calculus* in the Knersvlakte and ssp. *vanzijlii* in northern Namaqualand) confined to quartz field regions separated by > 200 km (Hammer 2002).

Conclusions

The first use of population genomics in the SK flora has revealed a complex picture of dispersal, gene flow and isolation in its most diverse and ecologically important family. Evidence of remarkably strong spatial genetic structure in *R. burtoniae* provides clear support for the long-held notion that dispersal limitation has been a key driver of diversity in ruschioid Aizoaceae (Ihlenfeldt 1994), but the high degree of genetic connectivity between populations of *C. calculus* opens the door for other factors that might limit or prevent gene flow such as isolation by adaptation (Nosil et al. 2008) or flowering phenology shifts (Young et al. 2015). Furthermore, demonstrating strong dispersal limitation and even weak intrinsic dispersal ability does not ultimately shed light on whether

speciation is driven by divergent selection or other, selectively neutral processes. The aim of the following chapter is to go some way towards addressing this issue.

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Figures

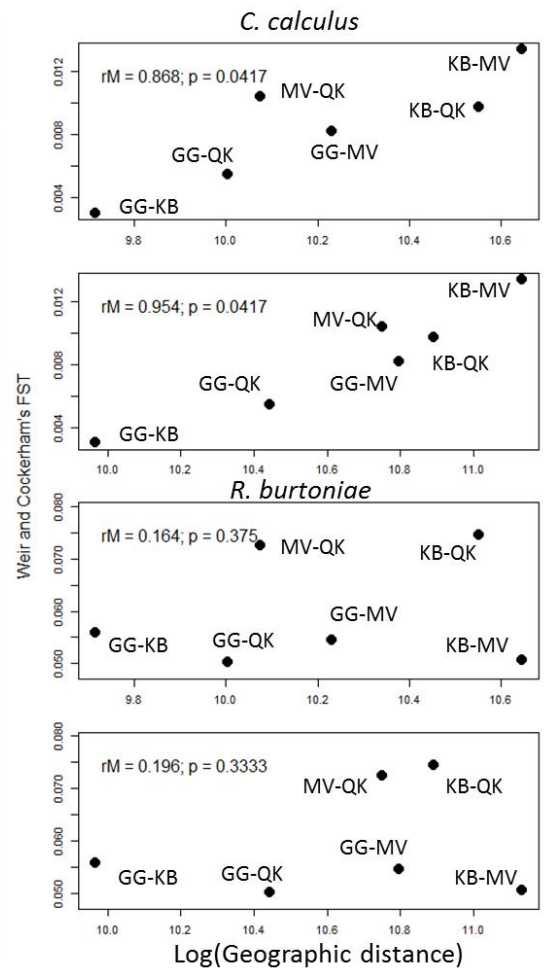
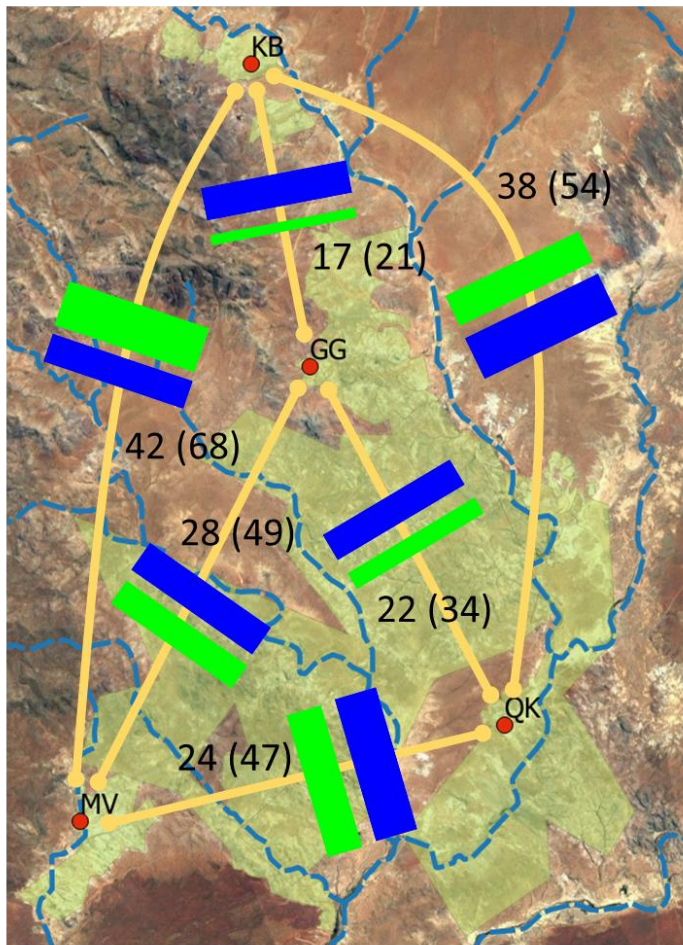


Figure 1: *Left:* satellite image of the Knersvlakte showing locations of populations; quartz distribution (shaded areas); inter-population Euclidean and on-quartz geographic distances (latter in brackets); and pairwise F_{ST} as a proportion of the species' maximum (green = *C. calculus*; blue = *R. burtoniae*). *Right:* Pairwise F_{ST} as a function of Euclidean (upper panel) and on-quartz (lower panel) distance for each species, with Mantel's r (rM) and p -values.

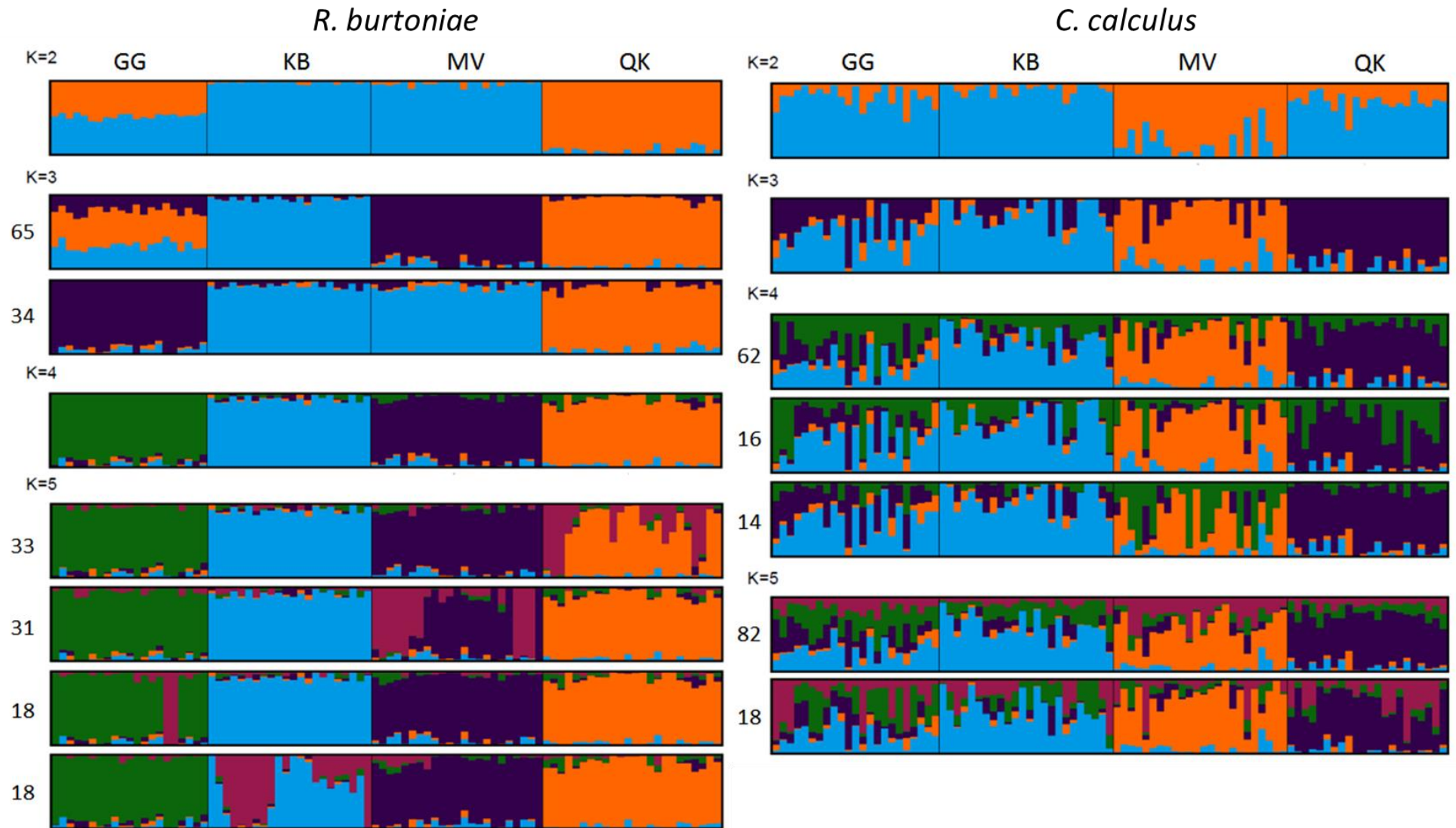


Figure 2: Assignment estimates for each individual, grouped by population, of the sNMF analysis summarised in CLUMPAK, showing major and minor modes for each K. Colours are consistent for each cluster across boxes. Numbers to the left of each box are the number of runs out of 100 included in the mode.

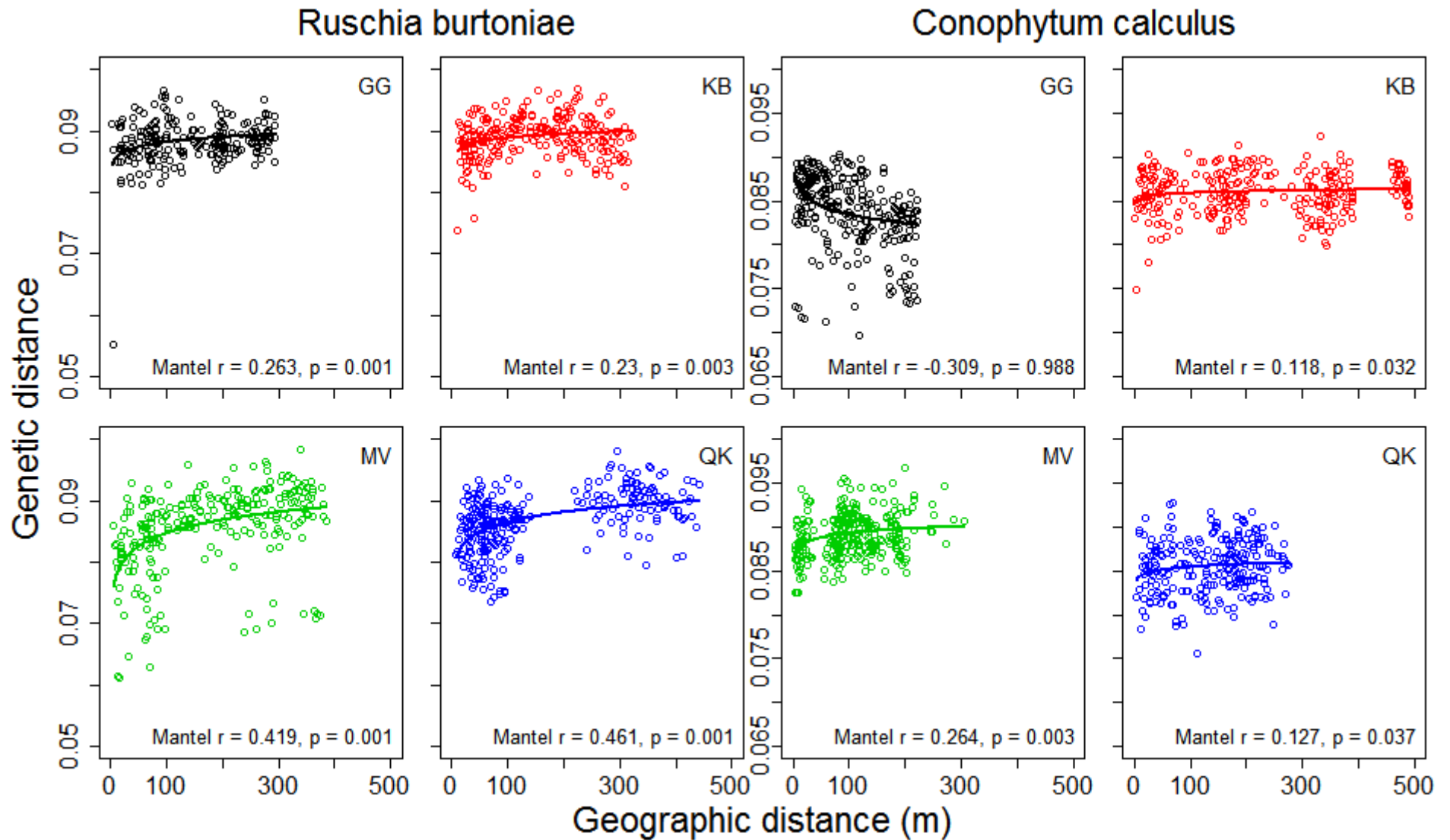


Figure 3: Isolation by distance plots on a pairwise individual basis, showing back-transformed linear model regression line of logged geographic distance versus untransformed genetic distance. Also shown are Mantel's r and p values. Note the different genetic distance scales between the species.⁶²

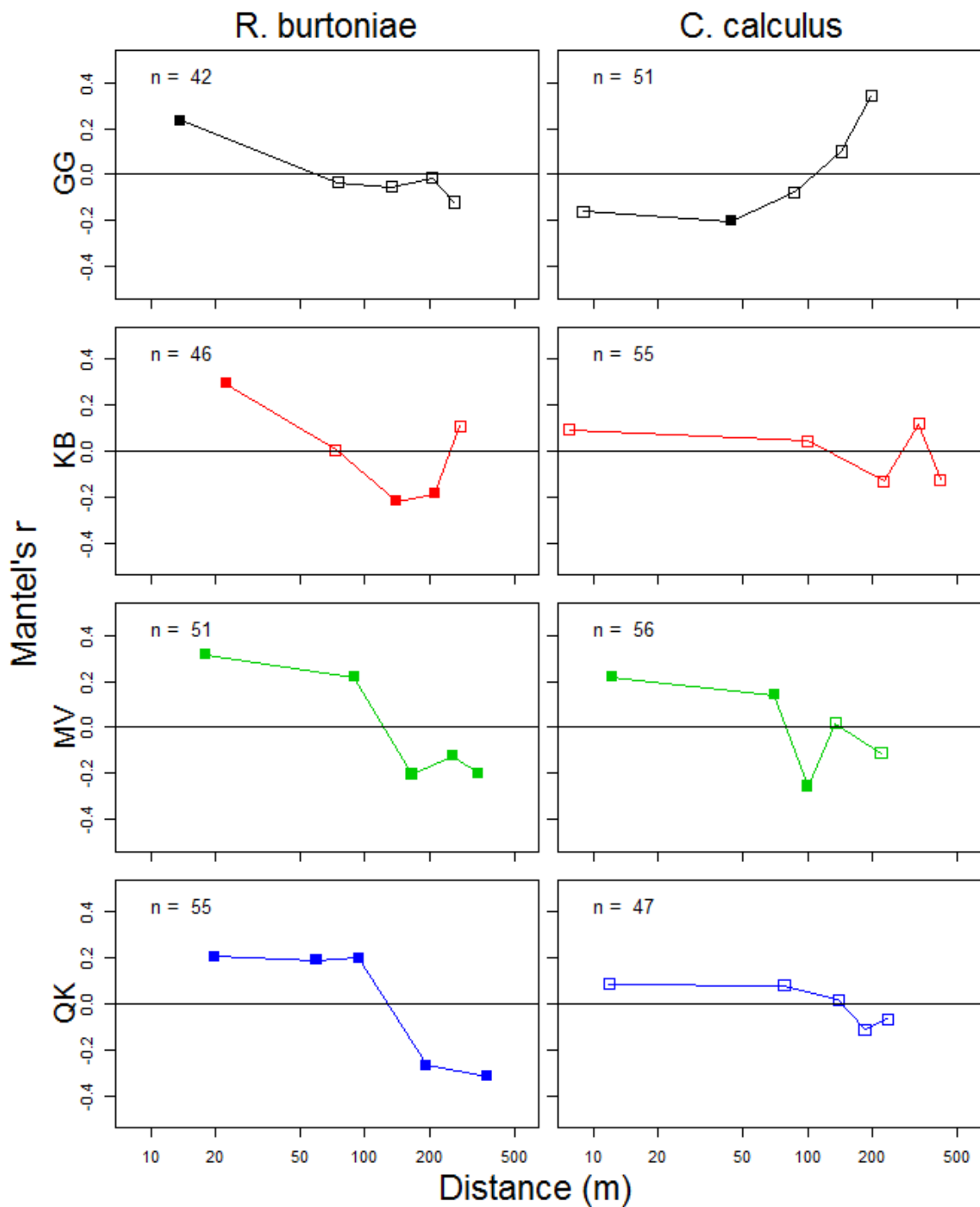


Figure 4: Mantel correlograms showing how isolation by distance within sites between individuals varies with spatial scale. Mantel's r of log-transformed geographic distance versus untransformed genetic distance at the mean of each geographic distance class (note the logarithmic scale) is shown. Filled squares indicate significant values ($p < 0.05$). Also shown is the number of pairs included in each distance class (n).

Chapter 3: Niche specialisation and divergence in the Knersvlakte quartz field flora: testing ecological versus non-ecological speciation models

Abstract

Ecology is likely to play a fundamental role in various modes of population divergence and speciation, highlighting the superficially misleading nature of the 'ecological' versus 'non-ecological' speciation divide in the literature. The rapid diversification in the Succulent Karoo flora has often been explained by niche divergence and local adaptation, with less attention paid to ecologically mediated geographic isolation between 'environmental islands'. However, population-level work directly investigating these factors is decidedly scarce. This chapter focused on the hyper-diverse ruschioid Aizoaceae in a well-studied system, the quartz fields of the Knersvlakte, to investigate the degree to which the hypothesis of adaptive divergence between environmental islands, which has been invoked by previous workers for the Knersvlakte-endemic genus *Argyroderma*, applies generally. This was done by characterising edaphic variation between quartz and non-quartz soils and between geographically isolated quartz patches, and studying the ecological and adaptive responses of communities and species to this variation. Consistent with previous findings, communities showed strong edaphic structure both within quartz fields and between them and the surrounding non-quartz soils, and transplant experiments suggested that these responses were adaptive, implying a strong environmental island effect of quartz edaphics. Within species and superspecies, niches were highly conserved between sites in some edaphic variables but frequently divergent in others. The link between niche divergence and local adaptation was idiosyncratic in the four taxa used in the transplant experiments, though three taxa showed a general link when

allowing multiple fitness proxies to be interpreted as evidence of local adaptation. The *Argyroderma framesii* group, whose members enjoy formal taxonomic recognition, showed a similar degree of divergence and local adaptation to *Ruschia burtoniae*, which has no taxonomic variation, highlighting the possibility of cryptic speciation in the latter. Together these results suggest that although local adaptation may have played an important role in some radiations, the insular nature and patchy distribution of the quartz fields themselves were crucial factors that promoted diversification in the KQF flora, and have the potential to continue to do so.

Introduction

The debate surrounding the drivers of biological diversification centres around two alternative modes of speciation. ‘Ecological speciation’ as defined by Rundle & Nosil (2005) is caused by adaptive divergence of populations in response to different environments (i.e. local adaptation) and, in principle, is independent of geographic context. In contrast, ‘non-ecological speciation’ (*sensu* Rundle & Price 2009) usually requires allopatry but is independent of divergent selection, and includes ‘mutation-order’ speciation under uniform selection and speciation caused by polyploidy or genetic drift (Sobel et al. 2010). These alternative hypotheses are frequently invoked when researchers frame questions about the drivers of diversification in any taxonomic or biogeographic unit. In recent times, ecological speciation has become the dominant paradigm in the literature primarily due to a growing body of evidence indicating ecological divergence between closely related species (Schluter 2009, Nosil 2012). As such, niche divergence and local adaptation need to be investigated when studying the initial stages of speciation.

However, a broader ecological understanding is crucial because ecological forces are central to both modes of speciation (Wiens 2004a; Sobel et al. 2010). Although non-adaptive divergence of populations generally requires allopatry, allopatry itself is often intimately tied to the ecology of species, and so studying this relationship is vital for understanding incipient speciation (Wiens 2004b). This has long been acknowledged in the literature on environmental islands such as those

occupied by the flora of serpentine soils in California, where specialisation to extreme edaphic conditions has resulted in highly fragmented ranges because areas with serpentine soils occur patchily in the landscape. Environmental islands are excellent systems for studying speciation at the population level, where the drivers of population divergence can be addressed directly.

Diversification in the Cape

In the Greater Cape Floristic Region (GCFR *sensu* Born et al. 2007) both ecological and non-ecological speciation have been cited as important processes contributing to the region's unparalleled floristic diversity (Linder 2008; Schnitzler et al. 2011; Verboom et al. 2009; Britton et al. 2014). Unfortunately most speciation research in the GCFR has focused on the fynbos biome rather than the Succulent Karoo (SK), despite the fact that the SK hosts one of the world's richest arid floras (Cowling et al. 1998), the diversity of which was likely driven by factors independent of those that acted in the fynbos (Verboom et al. 2009; Linder 2008; Ellis et al. 2014). Furthermore, speciation studies that investigate the causes of population differentiation are sorely lacking in the SK flora despite their widely acknowledged importance in understanding diversification (Ellis et al. 2014). The only exception to this is a series of papers which explored the links between patterns of genetic structure and divergence in morphology, flowering phenology and edaphic niche to understand the recent diversification of the genus *Argyroderma*, which is endemic to the Knersvlakte region of the SK (Ellis & Weis 2006; Ellis et al. 2006; Ellis et al. 2007). The ten dwarf species in the genus are strongly associated with the Knersvlakte's quartz gravel plains and separate into two distinct clades (Hartmann 1977; Schmiedel 2002), and the Ellis studies indicated a fundamental role for local adaptation facilitated by geographic isolation in the radiation of each.

The Knersvlakte quartz field flora

The KQF flora is very diverse and primarily of recent origin: most Succulent Karoo endemic lineages are less than 10 My old (Verboom et al. 2009), while the KQF are thought to have emerged in the Pliocene between 5.3 and 2.6 Ma in response to regional erosional processes stimulated by continental uplift (Cowling et al. 2009). Of its 67 obligate quartz field taxa, a remarkable 94% are

endemic and 56% belong to Aizoaceae (Schmiedel 2002), a family which has played host to one of the most remarkable plant radiations documented (Klak et al. 2004; Valente et al. 2014). Such explosive radiations are typically interpreted as signatures of adaptive radiation (Ihlenfeldt 1994) but non-adaptive radiation following rapid range expansion can equally produce such a pattern (Rundell & Price 2009). The emergence of the KQF could have sparked such an event, particularly given that the initial distribution of the quartz would have been more island-like than it is at present (Ellis et al. 2006), providing greater opportunity for geographic isolation.

The plant communities of the Knersvlakte quartz fields (KQF) exhibit strong fine-scale environmental structuring driven by edaphic variation (Schmiedel & Jürgens 1999; Schmiedel 2002) which suggests the potential for adaptive divergence in taxa other than *Argyroderma*. But they also show strong dependence on the quartz itself because of its cool microclimate (Schmiedel & Jürgens 2004; Musil et al. 2005; Musil et al. 2009), suggesting a role for niche conservatism in facilitating allopatric speciation. By addressing these alternatives the Ellis studies created the foundation for further population-level speciation research using the KQF as a focal system, and the focus of this chapter is to assess the generality of the ecological speciation model proposed by Ellis et al. (2006) by investigating edaphic niche patterns to understand the potential for ecologically driven isolation and testing for local adaptation in other KQF taxa.

Specifically, the aim was to address two alternative hypotheses relating to the radiation of the KQF flora. The *adaptive radiation hypothesis* posits that environmental differences between populations promote ecological speciation regardless of – but perhaps aided by – range fragmentation. The *non-adaptive radiation hypothesis* posits that range fragmentation underpinned by ecological factors promotes non-ecological speciation. These hypotheses lead to a number of contrasting, KQF-specific predictions about the nature of edaphic variation as well as inter- and intraspecific responses to that variation, including variation within quartz patches, between quartz patches, and between quartz and non-quartz soils.

Figure 1 and Table 1 provide an overview of the predictions presented below and the methods used to test them, which required data on plant community structure and edaphic variation in the KQF; the edaphics and ecology of the quartz/matrix divide; and edaphic niche divergence between populations within three species and – in the case of the *Argyroderma framesii*-group – between recognised taxa. Transplant experiments in the field and the greenhouse were also conducted to test the predictions about the underlying mechanisms responsible for the observed patterns (Hargreaves et al. 2014).

The non-adaptive radiation hypothesis yields the following predictions:

- i) soils from the non-quartz matrix are impermeable, and so differ edaphically from quartz soils and induce reduced fitness in individuals transplanted into them;
- ii) all quartz patches are edaphically similar, so patterns of edaphic variation within quartz patches and the community response to that variation do not differ between sites;
- iii) there is little realised edaphic niche divergence between populations within species; and
- iv) local adaptation is non-existent, so individuals transplanted into soils which their species occupies at other quartz patches ('extra-site soils') perform as well as individuals transplanted into their native soils.

In contrast, the adaptive radiation hypothesis predicts that

- i) 'matrix' soils may or may not be impermeable (but niche divergence between sites will be more pronounced if they are);
- ii) quartz patches differ in edaphic variation and edaphic community structure;
- iii) intraspecific realised niche divergence between populations is common; and
- iv) niche divergence reflects local adaptation, so that individuals transplanted into extra-site soils exhibit reduced performance.

Methods

Study sites and sampling strategy

Four study sites spread across the Knersvlakte were selected. These were: the 'core' site on the farm Groot Graafwater in the central Knersvlakte ('GG'; 31°15'55.2"S 18°32'43.1"E); a northerly site on the farm Kareeberg ('KB'; 31°08'24.9"S 18°31'11.0"E); a southerly site on the farm Quaggaskop ('QK'; 31°24'51.6"S 18°38'29.0"E); and a south-westerly site on the farm Moedverloren ('MV'; 31°27'15.0"S 18°25'59.4"E). The distances between GG and the other sites ranged from 17 to 28 km, with the other sites being separated from each other by 24-42 km. Twenty-three to twenty-seven 25 m² circular plots were used at each site to assess plant species associations and edaphic variation. For each plot the presence or absence of all Aizoaceae species occurring at the site was noted. Plots were not placed at random, but rather in order to maximise the representation of the ten most abundant Aizoaceae species or superspecies (Table 2) within the quartz field communities (see below). Soil samples were collected from every plot for chemical analysis. Roughly 1 kg of soil was collected from the top 10 cm of soil at three points distributed evenly within the plot. Care was taken to exclude the top layer of quartz pebbles, and large pebbles within the soil were excluded. Edaphic variables were measured by the Elsenburg Soils Lab. These variables were: pH; sand, silt and clay content (percentage by mass); resistance (Ohms); T value (i.e. cation exchange capacity), Ca and Mg (cmol/kg); and B, Cu, K, Mn, Na, P and Zn (mg/kg).

To compare quartz and non-quartz (i.e. 'matrix') soils, transects of varying length which spanned the quartz/matrix boundary at the GG and KB sites were conducted, with proportional quartz cover being estimated by eye and the presence or absence of Aizoaceae species known to occur on quartz being noted every 100 m in 25 m² plots. Soil samples were taken every 200 m. Soil chemistry was quantified as for the aforementioned set of samples.

Characterizing edaphic heterogeneity

All of the analyses described below were conducted in R version 3.4.1 (R Core Team 2017). Previous workers have found that the soils of these quartz patches can be separated into two broadly distinct

groups which differ markedly in pH (Schmiedel & Jürgens 1999; Schmiedel 2002). To assess bimodality and consistency across sites of patterns of edaphic heterogeneity including but not exclusive to pH, a principal components analysis (PCA) was conducted on all edaphic variables across all sites after log-transforming to improve normality and then standardizing to make their effects comparable in magnitude. Because the first three principal component (PC) axes explained a large proportion of the overall variance (see Results), density histograms of the first three PC axes as well as pH were plotted for visual assessment. As an analytical aid, model-based clustering was conducted on each variable both within each site and for the total variation across all sites, allowing variable variance, using the package 'mclust' (Fraley & Raftery 2002; Fraley et al. 2012).

Edaphic differences between quartz and non-quartz (i.e. 'matrix') soils were analysed by comparing the range of edaphic variation in important variables of quartz field plots occupied by the focal species used in the transplant experiments (Table 2), which represented the full range of quartz field edaphic communities identified in the study (see Results, especially Figure 3), to the edaphic variation in transect plots with quartz cover (QC) < 10% (the median) and QC > 10%, which were taken to represent matrix and quartz soils, respectively. Statistical tests were conducted on four variables which effectively characterised ecologically important axes of edaphic variation (see Figure 3): these were pH, [Na], T value and sand content. Variation in the latter was modelled using a generalised linear model (GLM) to predict percentage sand content based on plot type with errors fitted using the quasi-binomial distribution, which is similar to the binomial but accounts for overdispersion of variance by estimating a dispersion parameter. The other variables were log-transformed to improve normality of residuals and modelled using separate linear models. Differences between groups (four focal species, quartz and non-quartz transect plots) were assessed using Tukey's honest significant differences (HSD) in the package 'TukeyC' (Faria et al. 2017) for the linear models and 'multcomp' (Hothorn et al. 2008) for the GLM.

Edaphic heterogeneity and community composition

Because the goal was to assess the consistency of edaphic structuring of communities across sites, the ten most widespread and abundant species (occurring in at least five plots at each site) were chosen for analysis of the relationship between edaphic heterogeneity and community composition (Table 2). Specifically, the aim was to quantify and test the effect of edaphic variables on community composition, and to test whether the relationships were site-dependent. This was done using distance-based redundancy analysis (dbRDA; Legendre & Anderson 1999) implemented in the R package 'vegan' (Oksanen et al. 2016), which is a constrained ordination method similar to redundancy analysis (RDA) except that it allows for the use of non-Euclidean distance metrics. In this case, the Jaccard distance (Jaccard 1901) was used as it is appropriate for presence/absence data. The approach then uses the resulting distance matrix to conduct a principal coordinates analysis (PCoA), taking the resultant eigenvalues for use as the dependent variables in the RDA. As described by Legendre & Anderson (1999), two unique advantages of this method are that it can be used to test the significance of interaction terms and that, when doing so, it uses 'non-parametric permutation methods which do not rely on assumptions of multivariate normality' (p. 2). The explanatory variables used were edaphic PC1 (which correlated strongly positively with soil pH and general fertility); PC2 (which correlated strongly with soil texture variables); PC3 (which correlated positively with [Na] and [K] and negatively with [Zn]; see Results); and site. Initially all terms including interactions were included, and were removed from the model in a step-wise fashion using the Akaike's Information Criterion (AIC). Following the recommendations of Legendre et al. (2011) the final model was then subjected to a non-parametric ANOVA-like permutation test (9999 permutations) to evaluate the contribution (sum of squares) of each of the final marginal effects (analogous to "Type III" effects in ANOVA; i.e. all effects except main effects included in a significant [$p < 0.05$] interaction term), and to test their significance. Importantly, a significant interaction term between site and any of the edaphic variables would indicate that the community response to that variable differs between sites.

Edaphic niche divergence

The significance of the observed edaphic niche divergence between all population pairs of each of the ten focal species was tested using the method described by Broennimann et al. (2012) and the package 'ecospat' (Broenimann et al. 2016). This method generates kernel density functions on one or two environmental axes using occurrence records of pairs of populations, which are then used to estimate measures of niche overlap [D from Schoener (1968) and I from Warren et al. (2008)].

Two complementary permutation tests developed by Warren et al. (2008) and expanded on by Broenimann et al. (2012) were used to test whether the niches of the populations were more divergent than expected by chance. Firstly, the niche *equivalency* test simply determines a null overlap (D or I) distribution by randomly sampling from the full set of records the same number of occurrences in each group, each time calculating the overlap between groups. An observed overlap that is smaller than 95% of the randomly generated overlap values is considered to indicate non-equivalent niches. However, this test does not account for the possibility that even if the niches are divergent in the full niche space, the populations might occupy the most similar niche space that is locally available to them. Thus the niche *similarity* test randomly shifts the observed niche space (as opposed to randomly sampling actual occurrences) in one population, each time calculating its overlap with the observed niche in the other population. The test is directional and so is done for both comparisons. If there is unoccupied niche space in the first population which overlaps with the occupied niche space in the second, a high proportion of the random niches will have overlap values greater than the observed value, so the test will be non-significant. It follows that if the observed overlap is greater than 95% of the randomly generated values for both comparisons, the populations occupy more similar niches than expected by chance. Thus, evidence for niche divergence can be inferred from a *significant* result for the equivalency test coupled with *non-significant* results for either or both of the similarity tests (see Figure 1, contrasting niche shifts in species X and Y, for a simplified illustrative scenario). All null distributions were generated by 100 permutations of each procedure (the computationally intensive nature of the analysis precluded the use of a larger

number of permutations, but in trial runs of selected species-site combinations the resulting p-values were not greatly altered).

These tests can be conducted in a maximum of two dimensions, so niche divergence was assessed using edaphic PC1, PC2, PC3 and two-dimensional PC2:PC3 niche space. These variables were chosen based on their importance in determining community composition as shown in the dbRDA analysis (see Results). Tests were performed using both I and D for all pairwise population comparisons and for all ten species included in the above analyses. Results were summarised by assessing the strength of evidence for divergence as follows:

- Strong evidence was inferred from significant results for the equivalency test for both I and D, coupled with non-significant results for the respective similarity tests in both directions;
- some evidence if the above applied to only one direction of the similarity measure;
- weak evidence if only one measure showed any evidence of divergence;
- no evidence if the equivalency tests for I and D were both non-significant or if they were significant but the similarity tests showed that they occupied the most similar available habitat;
- inconclusive evidence if the tests based on I and D produced contrasting results.

Plant performance responses to edaphic heterogeneity

To test whether the relationship between community composition and edaphic variation is due to differential performance (i.e. it is adaptive), two sets of transplant experiments were performed, one in the field and one in the greenhouse. The field experiments were used as a means of assessing real-world fitness effects of transplants between quartz field sites and communities. The greenhouse experiments allowed for a comparison of the fitness costs of growing in different soils while controlling for the effects of climatic variation. Seeds were collected at the core GG site from at least 40 individuals of each species, mixed thoroughly, and then counted by eye under a dissecting microscope.

Both experiments involved the sowing of seeds in different soils and used the same set of 'focal' species, which were chosen to represent a range of communities and growth forms to allow for an interpretation of results informed by contrasting species traits (see Results and Figure 3). These were the dwarf (< 50 mm tall) *Argyroderma framesii* and *Conophytum calculus*, and the non-dwarf (typically > 200 mm tall) *Drosanthemum diversifolium* and *Ruschia burtoniae*. *C. calculus* and *R. burtoniae* exhibit a consistent preference for low pH, nutrient-poor soils, whereas *A. framesii* and *D. diversifolium* prefer nutrient-rich soils of moderate to high pH, and this dichotomy represents a broader distinction between communities which is consistent across the four sites (see Figure 3). For each species, seeds were transplanted into soils from GG in which the species occurred naturally (positive control), GG soils from the 'alternative' community (negative control), soils in which the species occurred naturally at the other three sites ('extra-site' transplants), and soils taken from the quartz-free matrix. Matrix transplants were only done for the greenhouse experiments due to the difficulty of assuring that seeds were not blown out of the arenas in matrix soils, whereas the quartz layer provides shelter from the wind.

Field transplant experimental design

The aim of the field experiments was to assess germination during spring (Aug-Sept) and growth and survival over the seedlings' first summer (Oct-Mar). This is a critical period for these plants as summers in the Knersvlakte are typically very hot, often exceeding 40 °C during the day, and, being a winter-rainfall area, rainfall is very infrequent. For the experiments, 200 seeds of each species were sown per treatment arena. The treatment arenas consisted of 25 cm-diameter, 5 cm tall circular metallic rings which were sunk into the ground so that seeds could not be washed away. All arenas were located within edaphically profiled plots (see above). For each species at the GG site, these consisted of seven plots within which the species occurred ('home community arenas'; denoted 'GG_H') and another seven within which the species was entirely absent ('alternative community arenas'; 'GG_A'). GG_A arenas were located in plots where one or both of the two focal species which characterised a broadly defined 'alternative community' were present. For example, for *A.*

framesii a GG_A arena was laid only in a plot where *C. calculus* and/or *R. burtoniae* were present. GG_H and GG_A transplants represented positive and negative controls, respectively. To test for evidence for local adaptation, seed from each species collected at the GG site was also sown in arenas at the MV, QK and KB sites ('extra-site' arenas). These arenas were always located within edaphically profiled plots in which the species occurred naturally.

The arenas were laid in December 2014. In early March 2015 they were watered thoroughly so as to promote the germination of any seeds that may have already been present; these seedlings were then removed prior to sowing the experimental seeds in mid-April 2015. After sowing, the arenas were briefly watered by mist sprayer so as to allow the seeds to settle between the pebbles. This was not sufficient to cause germination, which requires a succession of good rainfall events (Milton 1995). In August 2015, the arenas were observed to have very few seedlings, probably due to an unusually dry winter, and so to ensure sufficient sample sizes the arenas were watered thoroughly by mist sprayer twice over a period of four days, once in the evening and once in the early morning.

The first round of seedling counts and growth measurements was done in September 2015. For each arena the location of all seedlings was noted on a clear A4-size overhead projector slide placed on a Perspex tile, which was placed on the arena. The slides were oriented relative to a point on the arena, and the arena edge as well as selected pebbles were outlined to provide more precise orientation and to ensure that the same perspective was used for future monitoring. Up to 20 seedlings were chosen for growth monitoring, and if >20 seedlings were present they were chosen so as to cover as much of the arena as possible. For *D. diversifolium* and *R. burtoniae*, plant height was measured using a 0.5 mm resolution steel rule. For *A. framesii* and *C. calculus*, plant girth was measured using a digital calliper with a resolution of 0.1 mm. Counts and size measurements were taken again in April 2016.

Greenhouse transplant experimental design

For the greenhouse experiments, soil was collected from plots where each species occurred at all sites, sifted to remove particles larger than 2 mm, mixed with 50% (by mass) sterile river sand to

improve drainage in the more humid Cape Town climate, and dispensed into 5 cm x 5 cm x 6 cm seedling pots. The same procedure was done for soil collected from matrix soils close to GG (at 31°17'42.5"S, 18°29'08.2"E). As for the field experiments, seeds were sown into both home and alternative community soil from the GG site, and into home soil from the other sites. Alternative community (GG_A) soil was different for each species, being chosen to represent the soils occupied by a species from a different community but with a similar growth form. Thus, GG_A soil for *A. framesii* came from a site occupied by *C. calculus* and *vice versa*, while GG_A soil for *R. burtoniae* came from a site occupied by *D. diversifolium* and *vice versa*. In addition, seeds of all species were sown into the matrix soils.

For each species x treatment combination, 50 seeds were dispensed into seven pots intended for germination and survival monitoring, while 200 seeds were dispensed into two pots intended for growth monitoring. The growth monitoring pots were given more seeds as insurance against low germination rates, as the seedlings were thinned so that only eight which were more than 0.5 cm apart from each other remained in each pot. In order to increase sample size, especially where germination in growth pots was low for a particular species/treatment combination, individuals in the non-growth pots which germinated > 0.5 cm away from their nearest neighbour were also monitored for growth. Growth was monitored on a monthly basis. For *D. diversifolium* and *R. burtoniae*, plant height was measured using a 0.5 mm resolution steel rule. For *A. framesii* and *C. calculus*, plant girth was measured using a dissecting microscope at 10X magnification with the aid of an eye-piece graticule, which provided a resolution of 0.1 mm. Pots and focal individuals were uniquely marked. The latter was achieved by sinking pins with heads of different colours into the soil next to each individual. If a new seedling germinated within 0.5 cm of a focal individual within a non-growth pot, monitoring was ceased to ensure that competition between neighbouring plants did not confound the experiments. Newly germinated seedlings were incorporated into the growth study as needed, provided they were 0.5 cm from their nearest neighbour. Germination was monitored by

taking weekly counts of the number of individuals in each pot during the initial stages of the experiment.

The pots were arrayed in a tight grid with pots randomly arranged relative to treatment, but grouped by species in 7 x 4 pot trays arrayed in two columns of four trays each. This grid was rotated weekly to negate block effects, and was watered on a regular twice-weekly regime by mist-sprayers located 1 m above the pots. Watering sessions lasted for 1 min in winter (April-October) and 2 min in summer (November-March) and occurred in the early morning. The greenhouse was located at the University of Cape Town, and the grid placed in a sunny, airy part of the facility to avoid detrimental effects of humidity such as fungal infection (S. Hammer pers. comm.).

During the study it was noted that some pots retained water for longer periods than others, and that this appeared to be somewhat independent of treatment. To account for this, instead of watering the pots on a chosen day during the experiments 10 ml of water was poured into each pot and the time it took for all of the water to soak into the soil noted. This 'infiltration time' measurement was included as a covariate in the full models used in the greenhouse germination and growth analyses (see below). In addition, ANOVAs were used to determine the differences in infiltration time (log-transformed to improve normality) between treatments for each species, and pairwise contrasts were determined using Tukey's HSD tests.

Plant performance analysis

All analyses described below were conducted separately for the field and greenhouse experiments. Mixed-effects models were constructed using 'lme4' (Bates et al. 2015). The package 'car' (Fox and Weisberg 2011) was used for model simplification and to obtain p-values using 'Type II' tests which follow the principle of marginality, testing the effect of each term after removing all others except the term's higher-order relatives (e.g. main effects). Likelihood ratio tests were used for generalized linear models (GLMs), while χ^2 tests were used for generalized linear mixed-effects models (GLMMs) and linear mixed-effects models (LMMs). All descriptions below refer to the maximal model, i.e. the

model prior to simplification. Model validation was done by visual inspection of residual plots to determine the degree of normality and homoscedasticity.

Differences between species in germination and survival were analysed for both the greenhouse and field data in order to investigate their reproductive biology. Germination was compared between species using GLMs with a quasi-binomial error distribution with germination rate (proportion of germinated seeds per pot or arena) modelled in relation to species, treatment and, in the case of the greenhouse data, infiltration time, as well as all interaction terms. Differences in the probability of individual seedlings surviving their first summer were analysed using a GLMM of the binomial family with survival coded as binary and with fixed effects of species, size at first measurement (measured in September 2015 and standardised for each species to allow comparability), species x size interaction, treatment and species x treatment, and the random effect of arena.

The effect of treatment on **germination** was also analysed separately for each species for both experiments using GLMs with a quasi-binomial error distribution to predict germination rate per arena or pot based on treatment. For the greenhouse experiments the full model included infiltration time (log-transformed to improve normality) and infiltration time x treatment as additional predictors. For *A. framesii* only one seed germinated in QK soil in the first year of the experiment, but roughly ten germinated the following year. Including these in the germination modelling analysis did not alter the results and was deemed inappropriate as delayed germination was not monitored in the other species, but these individuals were monitored for growth and included in the growth analysis (detailed below).

Growth was analysed separately for each species using LMMs with random effects to account for unpredictable variation relating to arena and pot and, in the case of the greenhouse data, pseudo-replication due to repeated measurements of individuals. For the greenhouse data, plant size was modelled using the fixed effects treatment, time (the day the measurement was taken relative to

the first time the individual was measured), treatment x time interaction and infiltration time, and random effects individual within pot (i.e. main effect of pot and pot x individual interaction), allowing intercepts to vary and slopes to vary by time. Plant size and time were log-transformed to improve model fit. For the field experiments, relative growth rate (RGR) was calculated as $(\ln S_2 - \ln S_1)/(t_2 - t_1)$, where S_1 was the size at time t_1 (September 2015) and S_2 the size at time t_2 (April 2016), following Hunt (1982). RGR was modelled with treatment as a fixed effect, using only treatments with at least two survivors, and with arena as a random effect.

Survival in the field was also analysed separately for each species for treatment levels with survivors. The effect of treatment and initial size (measured in September 2015) on probability of survival was modelled at the individual level using GLMMs of the binomial family with arena as a random effect.

All of the analyses that were conducted separately for each species, with the exception of the greenhouse growth models, had final models lacking any interaction terms, and the significance of relevant contrasts in predicted values between treatment levels was assessed with Tukey's pairwise contrasts using the R package 'multcomp' (Hothorn et al. 2008). The contrasts of interest were: all pairwise contrasts involving GG_H, GG_A and, for greenhouse data, matrix treatments (to test the link between plant performance and edaphic variation between communities within quartz patches as well as between quartz and matrix soils); and GG_H versus all 'extra-site' treatments (to test for local adaptation). For the greenhouse growth models, which all included the time x treatment interaction term, differences in the effect of treatment were assessed by plotting the predicted values based on time and treatment using the R package 'visreg' (Breheny and Burchett 2016).

Relating niche divergence and local adaptation

Special attention regarding edaphic niche divergence was paid to the four species used in the transplant experiments because the two analyses were complementary. For example, if a species showed niche divergence between GG and KB, one would expect to find evidence for reduced fitness in individuals transplanted from GG into KB soils if edaphic niche divergence leads to local adaptation in the species. On the other hand, reduced fitness coupled with a lack of niche

divergence would suggest that factors other than edaphics caused local adaptation, while divergence without performance reductions would suggest non-adaptive divergence had taken place.

Results

Edaphic heterogeneity

The PCA effectively summarised edaphic heterogeneity, with the first three axes explaining 75.5% of total variation (PC1=46.2%, PC2=17.7%, PC3=11.6%). Loadings are summarised in Figure 4 to compliment the dbRDA analysis. PC1 correlated strongly and positively with pH and most of the micro-nutrients, suggesting that it could be viewed as a soil fertility axis. In contrast, PC2 corresponded to clay, silt and sand content, i.e. soil texture. PC3 was also associated with nutrient variation but correlated more strongly than PC1 with [Zn], [Na] and resistance, suggesting that it can be treated as a soil 'salinity' axis (*sensu* Jürgens & Schmiedel [1999]). Visual assessment of environmental axes (Figure 2) suggested that the distribution of PC1 and pH takes essentially the same shape and position at all sites, with strong bimodality, particularly in pH. In contrast, PC3 and, to a lesser degree, PC2 showed less conserved distributions across sites, though they too appeared to vary bimodally. Model-based clustering revealed bimodality in pH and PC2 at most sites as well as across all sites, but no bimodality in PC1 and PC3 probably because the peaks in the density histograms for these variables were close together.

Regarding edaphics of quartz and matrix soils, models indicated that the four edaphic variables of interest varied significantly between plots occupied by the focal species and transect plots identified as quartz and matrix ($df = 5$, $F > 4.5$ and $p < 0.001$ for all models). Matrix soils had higher pH and lower [Na] than quartz soils, sand content in matrix soils was similar to that in acid community soils, and the T values of matrix soils encompassed the full range of values in quartz soils (Figure 3).

Overall the results suggest that matrix soils are edaphically highly distinct from quartz field soils.

Edaphic heterogeneity and community composition

The final constrained dbRDA model explained 37.6% of the total community variation (inertia) between plots, with the first two constrained axes explaining 61.7% and 18.7% of this variation, respectively. The best model to predict community composition included: PC1 as a main effect with no interactions ($df = 1$, $F = 16.49$, $p = 0.001$); site and the interaction term between site and PC2 ($df = 3$, $F = 1.77$, $p = 0.0183$); and PC2, PC3 and their marginally significant interaction term ($df = 1$, $F = 1.94$, $p = 0.0498$). This indicates that PC1 structured communities similarly at all sites whereas the effect of PC2 varied, and that the effects of PC2 and PC3 were interdependent but, in combination, site-invariant. Figure 4 shows the broad distinction between communities occupying acidic, nutrient-poor soils (characterised by species such as *C. calculus* and *R. burtoniae*) and those occupying pH neutral, nutrient-rich soils (characterised by species such as *D. diversifolium* and those in the *A. framesii* group). Within these communities species separate out by variation along PC2 and PC3. For example, the *A. framesii* group species tend to occupy high PC2 and PC3 soils, suggesting a preference for saline soils with high silt and clay content; in contrast, *A. fissum* tends to occupy low PC2 and PC3 soils, suggesting a preference for non-saline soils with high sand content. Both of these taxa prefer high PC1 soils.

Community composition differed markedly between quartz and matrix habitats: the only species which occurred in both were *A. fissum*, *Cephalophyllum spissum* and *D. diversifolium*. Outside of the transect data, members of the *A. delaetii* group are known to occur on matrix soils marginal to the quartz fields (author, pers. obs.).(Anon n.d.)

Edaphic niche divergence

Overall the niche divergence analyses suggested a high degree of edaphic niche conservatism in the widespread quartz field plant species when comparing spatially separated sampling sites (Figure 5). When comparing populations across the study sites in a pairwise fashion for PC1, PC2 and PC3 the number of no divergence conclusions based on the equivalency and similarity tests outweighed the number of conclusions of any degree of divergence (55 to 3 for PC1, 38 to 16 for PC2, and 45 to 7 for

PC3). However, niche conservatism was not ubiquitous, and niche divergence was often strongly supported. In particular the 16 divergence conclusions for PC2 are non-trivial especially considering that there is strong evidence supporting most of them. In contrast, the tests for divergence in PC2:PC3 space indicated 15 no divergence and 36 divergence conclusions, most of which were strongly supported. This suggests the simultaneous involvement of PC2 and PC3 in divergence. In addition, the majority of divergence conclusions in PC2:PC3 space involved the MV site (23 out of 36), indicating that niches at this site were strongly divergent for many species.

When focusing on the site combinations used in the transplant experiments (i.e. all those involving GG), the taxa used in these experiments showed markedly different results for niche divergence (Table 3) and, furthermore, divergence was not always associated with the same variable(s).

Conophytum calculus showed no evidence for divergence along PC1 or PC3, a single case along PC2, and three cases in PC2:PC3 space (not shown), all involving the MV site; there was thus only one instance of divergence for the population pairs used in the transplant experiments, that being GG-MV in PC2:PC3 space, which was due to its occupation of unusually saline soils at MV. The other acid-loving species, *R. burtoniae*, showed no divergence along PC1 except for in the GG-MV comparison due to the MV population's relatively acidic and low-nutrient (i.e. low PC1) soils, while there were three cases of divergence along PC2 (two involving GG) and four cases in PC2:PC3 space (again two involving GG). In the contrasts involving GG divergence with MV and KB was due to the occupation of both low salinity and high sand content soils, while divergence at QK could not be attributed to variation in any individual variable.

For the *A. framesii*-group, four of the comparisons in PC2:PC3 space suggested niche divergence, including all three comparisons involving GG. Between GG and KB, divergence was also observed in PC1 and PC2:PC3 space, suggesting a strong difference in edaphic niche between *A. framesii* subsp. *framesii* (GG) and *A. theartii* (KB) related to the latter's occupation of more fertile, saline and higher pH soils. *A. pearsonii* (QK) also showed divergence from GG in PC2:PC3 space due to a similar but

slightly weaker pattern. Overall the data support grouping these taxa into those preferring infertile, low pH soil [*A. framesii* subsp. *framesii* and *A. framesii* subsp. *hallii* (at MV)] and those preferring fertile, pH neutral soil (*A. theartii* and *A. pearsonii*). This does not indicate clinal variation because the former group's geographic range bisects the range of the latter (Hartmann 1977; Ellis et al. 2006).

In *D. diversifolium* five comparisons showed divergence in PC2:PC3 space. Compared to GG, at MV it occupied relatively saline (high PC3) and sandy (low PC2) soils, at QK it occupied relatively sandy soils, and at KB the soils occupied were largely similar as indicated by divergence only in PC2:PC3 space.

To conclude, all focal taxa showed strong niche conservatism in PC1, but generally widespread niche divergence in other edaphic variables with the exception of *C. calculus* which showed little niche divergence overall. Notably, although the various taxa within the *A. framesii* group occupied divergent edaphic niches, the degree of divergence is unexceptional compared to that observed in *R. burtoniae* and *D. diversifolium* (Table 3).

Plant performance responses to edaphic heterogeneity

Because each experiment and its analysis included tests of multiple predictions, each prediction will be addressed in turn, drawing upon the relevant aspects of each experiment's results. First, though, some important aspects relating to the biology of the focal species which were revealed by the experiments will be described.

Comparison of species germination and survival rates

Table 4 provides results of marginal tests for significance of term for the models comparing germination and survival between species. Absolute germination varied dramatically between species in the field but not in the greenhouse experiments. In the latter, the number of germinated seeds for all species often reached or exceeded half of the number that was sown (Figure 6), and species was only marginally significant as a main effect. In contrast, species was a highly significant predictor of germination in the field, with extremely low germination in *C. calculus* relative to the

other species (Figure 6). Species was also a significant predictor of survival probability, which was considerably higher in *C. calculus* and *A. framesii* than in the other species (Figure 10).

The species survival model also indicated that the effect of starting size varied between species. Separate models for each species showed that starting size strongly affected survival in all species, with larger plants having greater survival probabilities, except for *C. calculus* which showed no size-dependence (Table 7). Together these results suggest that the dwarf species have low germination but high survival rates in the wild, while the reverse applies to the non-dwarf species, whose survival depends primarily on their initial size (i.e. size attained before summer heat stress, presumably, causes mortality).

Species-wise models: Overall results

Treatment affected germination rate significantly in all species in the field and greenhouse experiments with the exceptions of *R. burtoniae* and *C. calculus* in the greenhouse (Table 5).

Treatment also affected growth significantly in the greenhouse, with all species showing a significant main effect of treatment indicating that plants grew to different sizes, though no differences in growth rate could be detected in *R. burtoniae* and *D. diversifolium* as indicated by the non-significant time x treatment interaction (Table 6). In contrast, treatment did not affect RGR in the field for any species except *R. burtoniae* (Table 6), though this analysis was compromised by high mortality.

Finally, treatment did not affect field survival in any species except *A. framesii*, which showed a marginally non-significant effect (Table 7). Survival analyses were also compromised by high mortality but complete mortality was considered informative and was therefore interpreted qualitatively.

Responses to variation within the core site: Is edaphic community structure adaptive?

For all species, in the field germination in GG_A arenas was consistently lower than in GG_H arenas, though relatively low germination in GG_H arenas as well meant that none of the GG_H-GG_A pairwise contrasts were significant (Figure 6). In contrast, reduced germination in GG_A soils was not observed in the greenhouse. The growth data from the greenhouse revealed that i) unexpectedly, *C.*

calculus grew better in GG_A soil, ii) *R. burtoniae* and *A. framesii* showed reduced growth in GG_A soil, and iii) GG_A soil did not affect growth in *D. diversifolium* (Figures 7 and 8). For the field data the only species with survivors in GG_A arenas was *R. burtoniae*, for which neither growth nor survival probability differed between GG_A and GG_H (Figures 9 and 10). Together these results suggest that i) differential germination and mortality partly account for the edaphic positions of these taxa within the community; ii) differential germination is linked directly to edaphic variation only in *C. calculus*, whose germination rate is naturally low; and iii) edaphic variation affects growth rate in *R. burtoniae* and *A. framesii* but not in the other two species. To conclude, there is strong evidence to suggest that edaphic community structure is adaptive, though the mechanisms differ between species.

Responses to variation between quartz and matrix soils: Insularity

In the greenhouse, germination was consistently high in matrix soils for all species (Figure 6). For *D. diversifolium* and *A. framesii* germination in matrix soil was significantly higher than in GG_H, suggesting that the very fast infiltration time of matrix soils (Figure 11) stimulated germination in these species. For *C. calculus*, germination rate was high in both GG_H and matrix soils and did not differ between them, but infiltration time did not underlie this as it had no effect on germination overall (Table 5). In contrast to germination, the effect of matrix soils on growth varied greatly between species (Figures 7 and 8), with *R. burtoniae* and *A. framesii* showing reduced growth in matrix soils relative to GG_H, while *C. calculus* and *D. diversifolium* showed no response. Overall, these results are largely consistent with the observed distributions of these species, with *D. diversifolium* occurring commonly in matrix habitats and the others not, however edaphic variation cannot explain the absence of *C. calculus* in matrix habitats.

Responses to variation between quartz sites: Niche divergence and local adaptation

The link between niche divergence (Table 3) and local adaptation varied greatly in strength between species. Consistent with the observation of little realised edaphic niche divergence overall in *C. calculus*, this species showed no evidence of performance reductions in extra-site transplants. In

contrast, *R. burtoniae*, which showed widespread evidence of niche divergence, exhibited reduced growth in the greenhouse in KB and QK soils, though not in MV soils (Figures 7 and 8) despite the population at this site being the most edaphically distinct, occupying unusually low PC1 (acid) soils. However, in the field it displayed reduced growth at MV and KB (Figure 9), though neither differed significantly from GG_H. Evidence for local adaptation in *R. burtoniae* therefore primarily corresponded with niche shifts in PC2 and PC3. In contrast, *D. diversifolium* displayed no evidence for local adaptation despite much evidence of niche divergence. There was widespread niche divergence between the *A. framesii* taxa, and accordingly evidence for local adaptation in *A. framesii* ssp. *framesii* came from reduced growth in MV soils in the greenhouse (Figures 7 and 8), near-significant reduced growth in KB field arenas (post-hoc pairwise contrast: $z = 2.379$, $p = 0.0739$; Figure 9), and low germination and survival in QK soils, though these were non-significant relative to GG_H (Figures 6 and 10). Although the evidence comes from different experiments for each comparison, it does suggest a connection between niche divergence and local adaptation in the *A. framesii* group.

Treatment affected survival probability in *A. framesii* only and its survival in extra-site arenas did not differ significantly from GG_H arenas (Figure 10). This contrasts with the survival rate of zero in GG_A arenas in this species as well as in *C. calculus* and *D. diversifolium*, and therefore shows that there was no relationship between niche divergence and variation in first summer survival in these three species.

Discussion

The data presented here suggest roles for both ecological and non-ecological speciation in the diversification of the KQF flora, and in particular show that the link between ecology and divergence is likely to be strongly species-dependent. Evidence for reduced performance among quartz specialists in the edaphically distinct matrix soil suggests that the quartz fields are a highly insular environment, highlighting their patchy distribution as a potential driver of allopatric speciation.

Widespread niche conservatism along the fertility-acidity axis which strongly structures communities was countered by strong evidence of realised divergence in soil texture and salinity niches in a number of taxa (Figures 3 and 5; Table 3), but such divergence was not consistently associated with local adaptation in the taxa used in the transplant experiments (Figures 6-10). Even in the *A. framesii*-group taxa, whose members enjoy formal taxonomic status and whose diversification has been attributed to ecological speciation driven by edaphic divergence in allopatry (Ellis et al. 2006), the prevalence of niche divergence and local adaptation was comparable to that seen in *R. burtoniae* (Table 3), a non-dwarf species which shows remarkably little morphological variation across its range (author, pers. obs.) and has no taxonomically recognised variation – a situation which possibly warrants revision. Together these results suggest that although local adaptation may have played an important role in some radiations, the insular nature and patchy distribution of the quartz fields themselves were crucial factors that promoted diversification in the KQF flora, and have the potential to continue to do so.

The edaphic selective landscape and its floristic impacts

Understanding how KQF communities are edaphically structured is an important initial step in investigating the extent to which the edaphic landscape of the Knersvlakte as a whole influences population isolation. In keeping with previous findings in the KQF flora (Schmiedel & Jürgens 1999; Schmiedel 2002), the data presented here suggest that variation in fertility and pH strongly affect community structure, with distinct communities occupying acidic and pH neutral soils (Figure 3). However, while Schmiedel (2002) found that salinity (measured in this study by electrical resistance and [Na]) had a similar effect to pH, this study supported the finding of Jürgens & Schmiedel (1999) that these variables affect community structure somewhat independently. In accordance with these findings, all of the species used in the experiments showed marked reductions in performance when transplanted across the acid/neutral divide, and greenhouse data confirmed that this was caused by edaphic factors. Given this result it is likely that the salinity gradient observed within both communities also reflects fundamental niche differences between species, though further

experiments are required to confirm this. In conclusion, differences in individual fitness in response to edaphic heterogeneity – which manifest as differences in germination, growth and/or survival rates – are a major determinant of the community structure of the KQF flora. One implication of this is that edaphic niche variation between populations within species could reflect evolutionary change.

Insularity

In agreement with previous work (Schmiedel & Jürgens 1999; Schmiedel et al. 2015), the highly insular nature of the KQF relative to the surrounding soils was confirmed and was shown to be driven largely by edaphic variation. Matrix soils were significantly less saline and less acidic than the quartz soils occupied by the four focal species in the study (Figure 3), and the only species which were found to occur regularly on both quartz and matrix soils (*A. fissum*, *D. diversifolium* and *C. spissum*) showed a preference for the soils within the quartz fields which were edaphically most similar to matrix soils (Figure 4). The relationship between edaphic variation and the insularity of the KQF flora is better understood in the context of the quartz layer's thermal properties. It provides seedlings with shading and produces a relatively cool microclimate due to its high albedo (Schmiedel & Jürgens 2004), and several studies have shown that mild (3-4°C) experimental warming induces high mortality and performance reductions in adult plants in KQF Aizoaceae, especially in parts of experimental plots which had reduced ventilation or shading – suggesting an interaction with moisture availability (Musil et al. 2009; Musil et al. 2005; Schmiedel 2002). Dwarf species are particularly vulnerable to heat stress; for example, dwarf species such as *Argyroderma pearsonii* experienced much greater mortality increases than shrubs such as *R. burtoniae* and *D. diversifolium* in heated plots (Musil et al. 2005). Accordingly, dwarf seedlings (particularly of *C. calculus*) in the present study were frequently located underneath quartz pebbles (author, pers. obs.) and their chance of survival was independent of their size at first measurement, which supports the hypothesis that they depend on the cool microclimate of the quartz for their establishment and hence also their persistence. In contrast, the shrubs experienced relatively prolific germination rates

but also much greater (and often complete) first-summer seedling mortality than the dwarves under the same conditions (Figure 10), and furthermore the chance of an *R. burtoniae* or *D. diversifolium* seedling surviving its first summer was strongly dependent on its size at first measurement (Table 7). These results suggest that early germination and/or higher initial growth rate significantly increase individual fitness in non-dwarf seedlings, with thermal factors playing a minor role at most. The slow growth of *R. burtoniae* seedlings on matrix soils would therefore result in high mortality, which explains its natural absence from these soils in contrast to *D. diversifolium* whose seedlings grow equally well in quartz and matrix soils. Thus both edaphic and microclimatic variation are tied to the KQF's insularity. In terms of speciation, this implies that not only are dwarf plants likely to experience ecologically driven geographic isolation, but so too are shrubs that specialise to the KQF's distinct edaphic environment, and this is borne out by evidence of niche divergence and local adaptation in *R. burtoniae*.

Local adaptation

The results suggest that quartz field specialists are prone to range fragmentation, which should translate into a greater likelihood of speciation. However both ecological and non-ecological speciation are more likely under geographic isolation, so the next step is to investigate the role of local adaptation in divergence. While the data suggest that niche divergence is fairly common in widespread quartz field specialists, the link between niche divergence and performance reductions in the four species included in the transplant experiments was inconsistent, making it impossible to infer the prevalence of local adaptation in the other six species. The evidence of local adaptation is best interpreted on a case by case basis.

There was essentially no evidence of local adaptation in *C. calculus*. For the site comparisons which had transplant data, the species showed divergence only between GG and MV in PC2:PC3 space, with MV plants occupying unusually high salinity soils. However, evidence of reduced fitness in GG seedlings transplanted into MV soils was sparse, with reduced germination rates in the greenhouse but not in the field and no reduction in survival or growth, suggesting an absence of local adaptation

even when there is realised niche differentiation. These results contradict expectations given the strong dependence of *C. calculus* on quartz, which should promote geographic isolation and in turn facilitate local adaptation. One might conclude that the fundamental niche of the species is broader than its realised niche at any given site, which could reflect incomplete range filling caused, for example, by stochastically occurring patch mortality events (Leibold et al. 2004). However, under this scenario the general pattern of niche conservatism is unlikely, and a more parsimonious explanation is that local adaptation is limited perhaps by swamping due to gene flow or insufficient standing genetic variation (Lenormand 2002).

In contrast to *C. calculus*, *R. burtoniae* showed clear evidence of local adaptation. Although the effect of site on both germination and seedling survivorship was negligible, both greenhouse and field transplant experiments revealed a significant site effect on growth. Consistent with the observation of significant niche shifts between sites, especially along PC2 and PC3, GG plants grown in the greenhouse showed reduced growth in soils from KB (sandier soil) and QK (less saline), and in the field at MV (less fertile and sandier) and KB. Strong local adaptation in this species may be facilitated by geographic isolation arising from its dependence on quartz. This pattern contrasts with the apparent lack of morphological divergence in the species in the KQF (author, pers. obs.), suggesting that local adaptation has not led to morphological divergence, and points to potentially undiscovered cryptic diversity.

The data for *A. framesii* subsp. *framesii* (the GG taxon) revealed niche divergence in PC2:PC3 space in all comparisons involving GG, and along PC1 between GG and KB. The MV, QK and KB populations occupied, respectively, more saline, less saline, and less acidic soils than GG. Accordingly transplanted individuals showed reduced survival at the QK site and reduced growth in MV soils. Neither growth nor survival were reduced, however, there was some evidence of reduced germination in the neutral pH KB soils. It therefore seems reasonable to conclude that the *A.*

framesii group taxa studied here are locally adapted to distinct fundamental niches, supporting the hypothesis of adaptive divergence in the group (Ellis et al. 2006).

D. diversifolium showed considerable realised edaphic niche divergence between populations, but little to no evidence of concurrent fitness differences. It also showed limited evidence of reduced fitness in GG_A soils, with only a hint of slightly reduced germination in the field. Combined with its regular occurrence in the matrix, these results suggest that the fundamental edaphic niche of *D. diversifolium* is very broad and that its distribution in the KQF is primarily determined by non-edaphic factors. The lack of geographic isolation therefore explains the absence of local adaptation in this species despite its occupancy of different edaphic niches at each site.

Implications for modes of speciation

The findings presented in this chapter are in accordance with the view that the Knersvlakte is a rapidly evolving, recently emerged landscape which has provided ample opportunity for adaptive radiation in allopatry in its quartz field flora, and thus concurs with the model put forward by Ellis et al. (2006). It is likely that the highly insular nature of the KQF for both dwarf and non-dwarf plants, coupled with strong edaphic specialisation of communities and substantial edaphic variation between sites, has provided the foundation for diversification within the system. However, the model clearly does not apply generally: the case of *C. calculus* is a striking exception and highlights the importance of investigating dispersal and pollination biology in the context of ecologically-induced geographic isolation (see Chapter 2). Nevertheless there is a clear correlation between the degree of such isolation and adaptive divergence in the other species, and in particular the similarities between *R. burtoniae* and the *A. framesii* group point to possible cryptic species within the former. This highlights an important deficiency in Cape plant taxonomy, that of an over-reliance on morphological character variation in species delimitation (Bickford et al. 2007; Britton et al. 2014). In conclusion, these examples highlight the crucial role of geographic isolation in diversification and suggest that allopatric speciation, whether or not it is driven by adaptive divergence, may be the most prevalent mode of speciation in the SK, a system whose considerable

fine-scale environmental heterogeneity would have provided many opportunities for ecologically driven geographic isolation.

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Figures

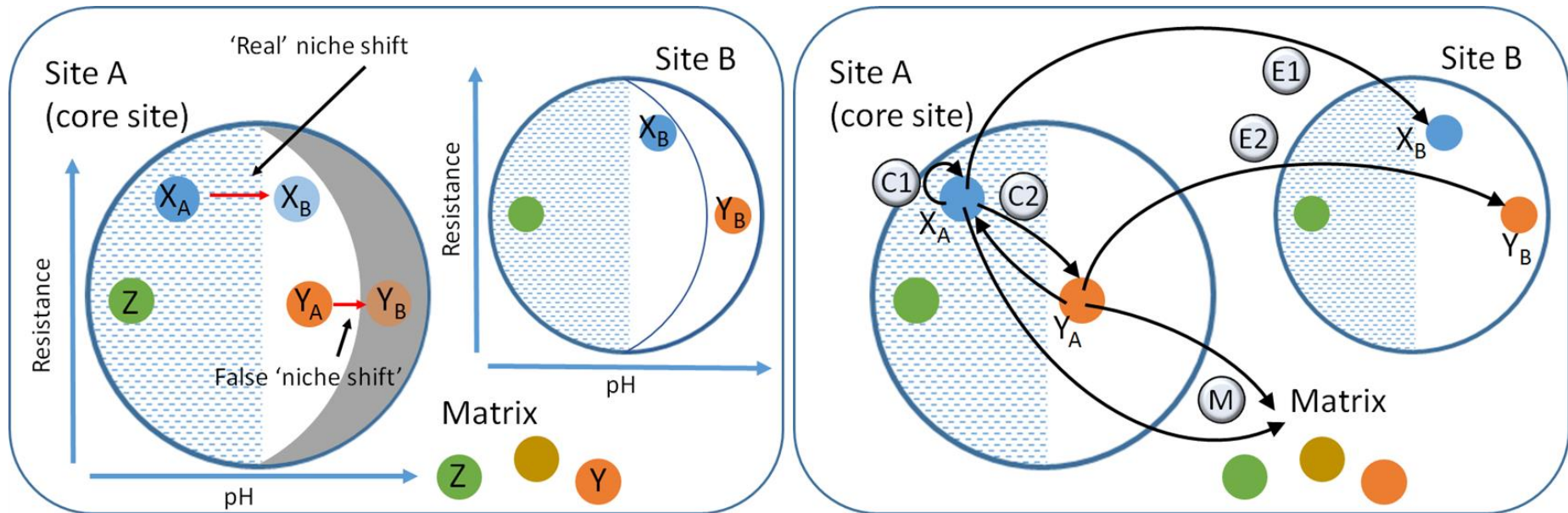


Figure 1: Schematic illustration of the framework, using edaphic data (*left*) and transplant experiments (*right*), for testing insularity, niche divergence/conservatism (accounting for habitat availability), and local adaptation. Axes indicate variation in pH and resistance; hatched and clear areas are low and high pH communities, respectively; crescent shaded grey or outlined represents niche space available at Site B but not Site A; coloured dots represent species and translucent dots show their occupied niche at the other site; red arrows show niche shifts; black arrows show transplants with directionality. Analyses can be summarised as follows: **Pure edaphics:** test whether edaphics differ between quartz and matrix, and whether edaphic variation is similar at all sites (compare Site A and B edaphic variation); **Is edaphic community structure conserved?:** determine which quartz species occur on matrix soils (e.g. species Y and Z) and test whether edaphic variation relates to community structure similarly at all sites; **Is edaphic community structure adaptive?:** test whether transplanting alone affects fitness (C1, negative control), and whether transplanting into chemically different quartz soils (C2, positive control) and into matrix soils (M) reduces performance ; **Niche divergence and local adaptation:** for species X test whether observed edaphic niche divergence (niche shift and full range of edaphic variation is available) translates into reduced fitness when transplanted into Site B (E1); for species Y test whether lack of observed niche divergence (niche 'shift' into chemically similar soil at Site B which is not available at Site A) is corroborated by no reduction in fitness (E2).

Table 1: Details of the predictions under each alternative hypothesis of population divergence drivers, and the methods used to test them.

	Adaptive divergence	Non-adaptive divergence	Methods
Importance of matrix soils	Matrix soils are not necessarily impermeable	Matrix soils are impermeable	Quantify edaphic and community differences between quartz and matrix; Transplant experiments (greenhouse) into matrix soils
Between-quartz site differences in edaphic variation pattern and community structure	Sites differ in edaphic variation and community structure	Sites are similar in edaphic variation and community structure	Visualize and quantify consistency of edaphic variation and community structure patterns between sites
Between-population <i>realised</i> ecological niche divergence	Niche differences are commonly observed	Niche differences are rarely observed	Quantify and test edaphic niche differences between spatially separated populations within species
Between-population <i>fundamental</i> ecological niche divergence	Niche differences are adaptive	Niche differences are non-adaptive	Transplant experiments (field and greenhouse) between sites and between soils from different sites

Table 2: Species and superspecies used in the community-edaphic analyses and transplant experiments (latter shaded). *Dicrocaulon* and *Oophytum* are genera whose taxonomy in the Knersvlakte is unresolved. Abbreviations used in the dbRDA and other analyses are also indicated, as are broad community characterisation and occurrence on matrix soils. Numbers in brackets refer to number of profiled plots at each site, and numbers below indicate how many plots were occupied by each species.

	dbRDA	Focal spp.	Community	Matrix occurrence	GG (24)	KB (23)	MV (23)	QK (27)	Total
<i>Conophytum calculus</i>	C. cal	CC	pH Acidic	No	8	8	7	7	30
<i>Dicrocaulon</i> spp.	Dicro		pH Acidic	No	9	5	14	6	34
<i>Ruschia burtonae</i>	R. burt	RB	pH Acidic	No	14	9	8	7	38
<i>Argyroderma delaetii</i> group	A. del		pH Neutral	Marginal	11	13	10	16	50
<i>Argyroderma fissum</i>	A. fis		pH Neutral	Yes	7	11	5	12	35
<i>Argyroderma framesii</i> group	A. fram	AF	pH Neutral	No	7	11	15	16	49
<i>Cephalophyllum spissum</i>	C. spis		pH Neutral	Yes	16	17	12	9	54
<i>Dactyloopsis digitata</i>	D. dig		pH Neutral	No	6	5	7	5	23
<i>Drosanthemum diversifolium</i>	D. div	DD	pH Neutral	Yes	14	17	10	10	51
<i>Oophytum</i> spp.	Oophy		pH Neutral	No	5	6	5	7	23

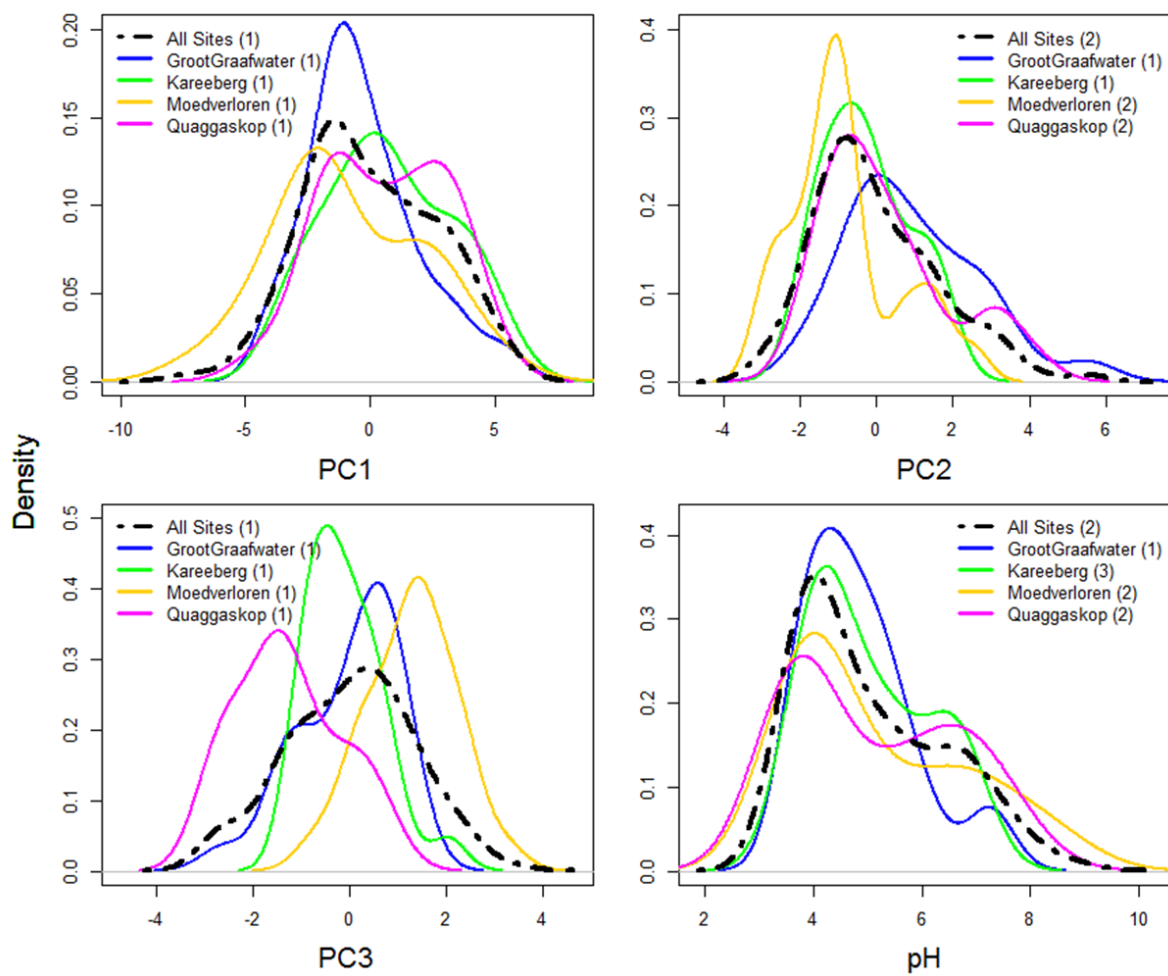


Figure 2: Probability density distributions of important axes of edaphic variation within the quartz fields. Dashed line shows the distribution of values across all sites; coloured lines show distributions by site.

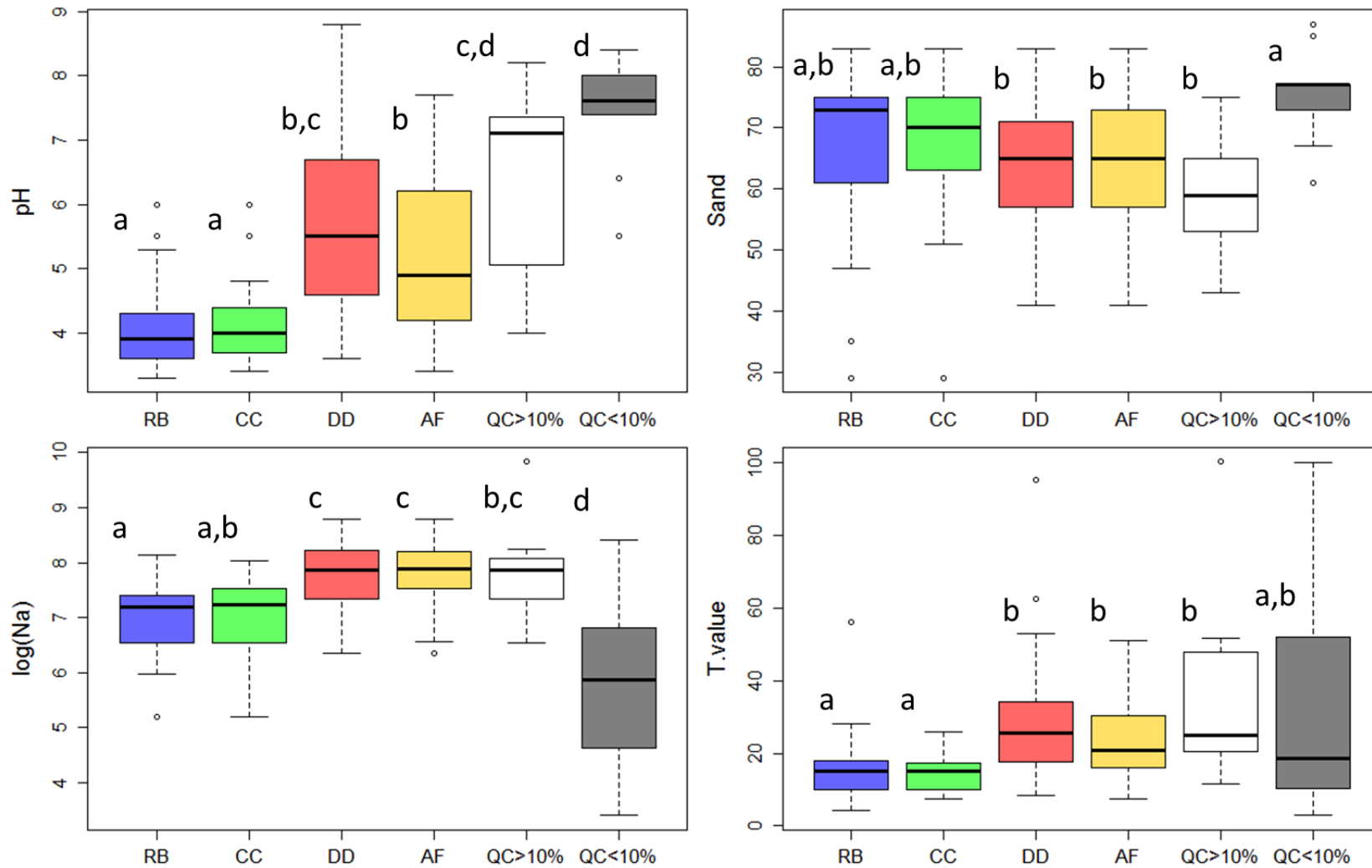


Figure 3: Boxplots showing variation in key edaphic variables pH, sand content (%), log-transformed [Na] and T value (cation exchange capacity) according to quartz plots occupied by the focal species, and plots along the quartz-matrix transects divided at the median 10% quartz cover (QC). Letters indicate group assignments based on post-hoc Tukey's HSD conducted on the ANOVAs.

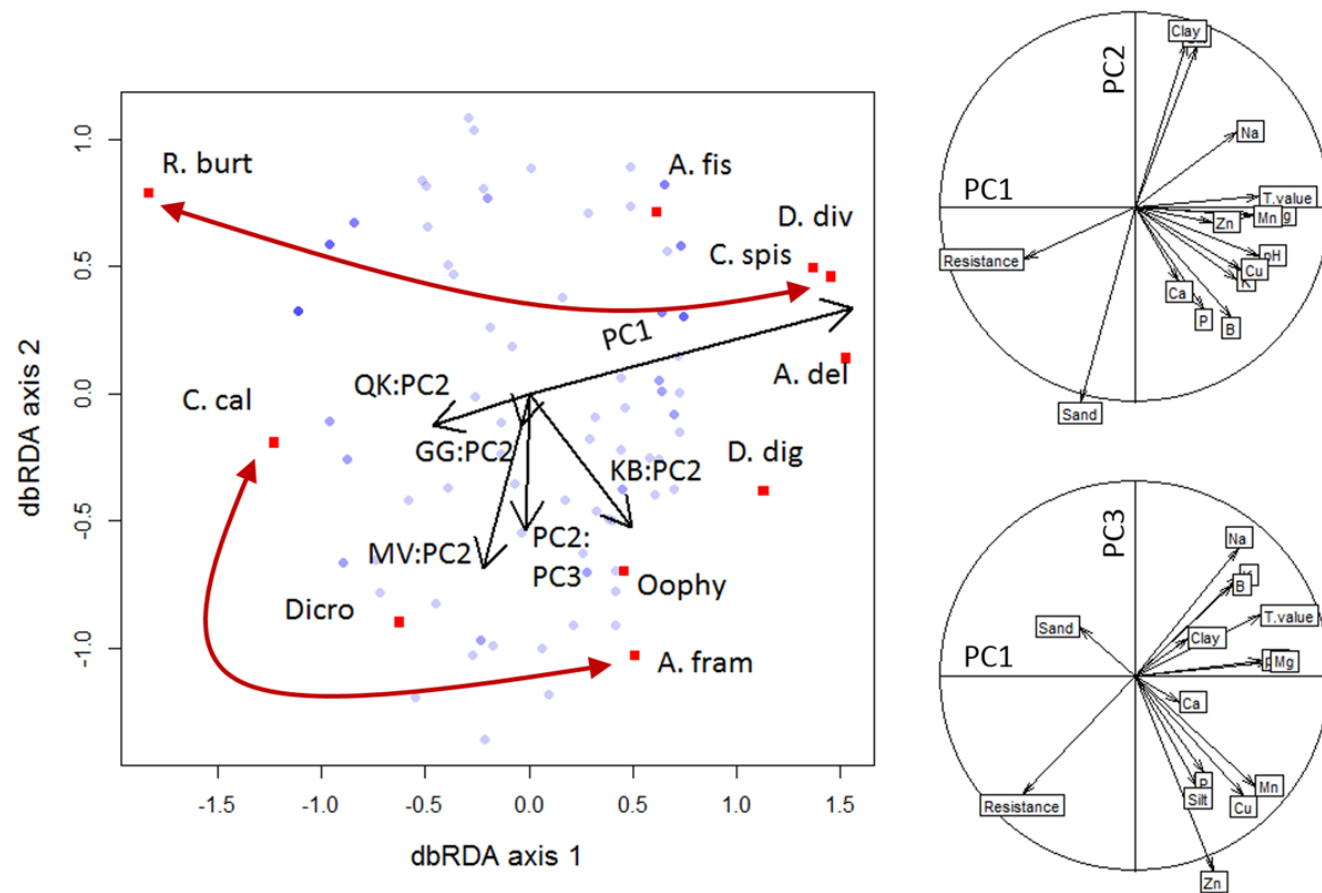


Figure 4: Results of the dbRDA analysis including plot locations in community space (blue dots; darker points have overlapping plots), relative weighted species locations (red squares), and contributions of each marginal term included in the final model (black arrows; length indicates relative contribution). Red arrows show the reciprocal transplants between species occupying different edaphic communities. The plots in the right-hand panel indicate the contribution of each variable to the principal component axes used in the dbRDA.

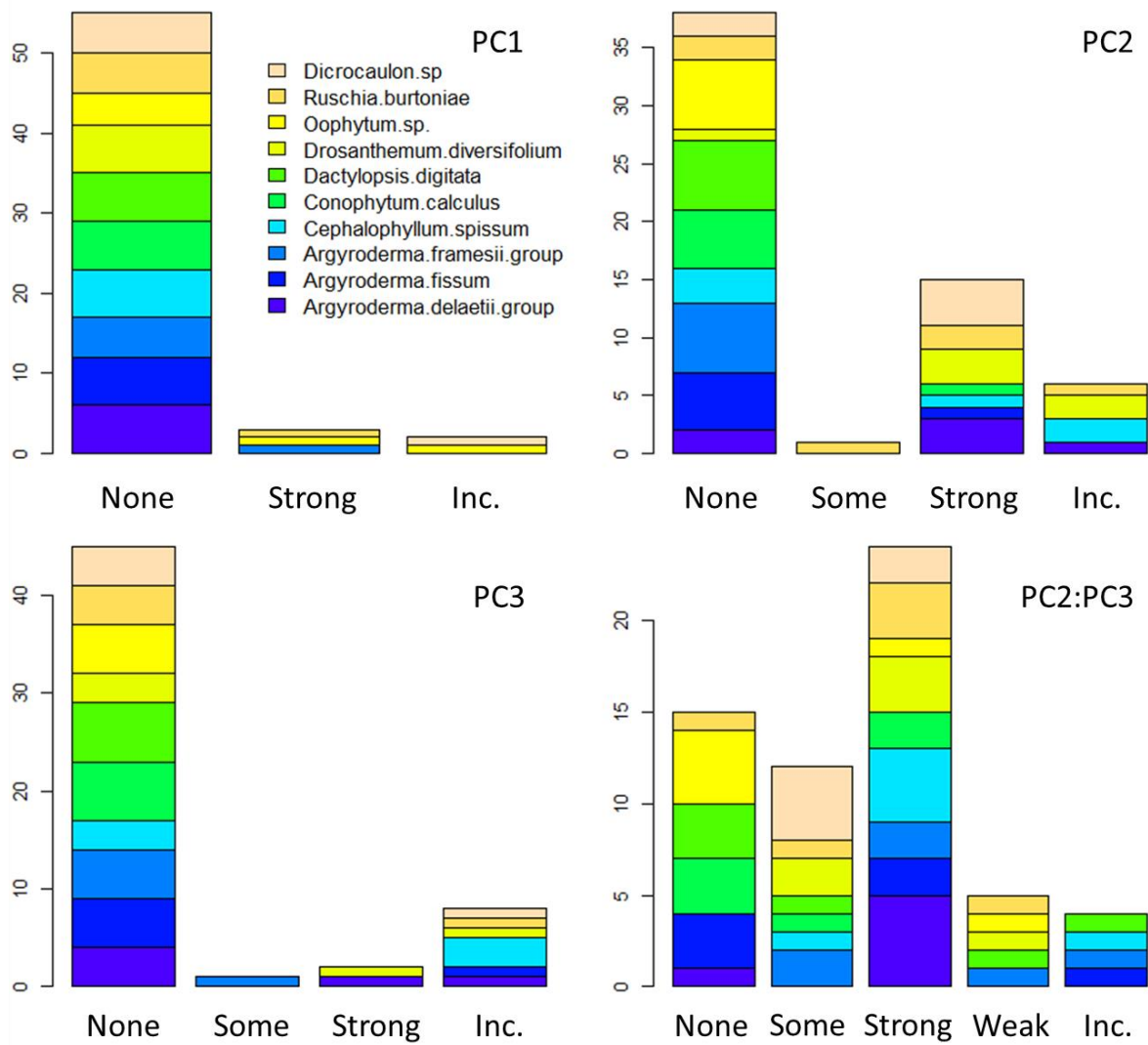


Figure 5: Barplots showing the frequencies of each conclusion reached after the niche divergence analyses using the equivalency and similarity tests (see text). "Inc." = inconclusive result due to disagreement between overlap measures D and I.

Table 3: Detailed results of the equivalency and similarity tests for the species and site combinations included in the transplant experiments and the conclusions about divergence based on the tests. Values of D (overlap) are shown for comparison between species. ** = $p < 0.05$ for both D and I; **^ = $p < 0.10$ for D and/or I; ns = not significant; '?' indicates that D and I presented contrasting results; 'x' indicates that the test is not applicable. A significant result for the equivalency test coupled with non-significant results for the similarity tests (forward [F] and reverse [R] comparisons) suggests strong evidence for niche divergence. Similarity tests are not applicable if the equivalency test is non-significant.

		GG-KB				GG-MV				GG-QK			
Species		PC1	PC2	PC3	PC2:PC3	PC1	PC2	PC3	PC2:PC3	PC1	PC2	PC3	PC2:PC3
<i>R. burtoniae</i>	Schoener's D	0.86	0.32	0.57	0.44	0.46	0.23	0.51	0.25	0.77	0.58	0.63	0.30
	Equivalency	ns	**	ns	?	**	**	ns	**	ns	ns	ns	**
	Similarity (F)		ns		?	ns	ns		ns				ns
	(R)		ns		**	ns	ns		ns				ns
	Divergence?	No	Yes	No	?	Yes	Yes	No	Yes	No	No	No	Yes
<i>C. calculus</i>	Schoener's D	0.75	0.38	0.43	0.45	0.49	0.31	0.48	0.31	0.46	0.39	0.64	0.51
	Equivalency	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	ns	ns
	Similarity (F)								ns				
	(R)								ns				
	Divergence?	No	No	No	No	No	No	No	Yes	No	No	No	No
<i>D. diversifolium</i>	Schoener's D	0.88	0.69	0.68	0.54	0.82	0.42	0.48	0.26	0.86	0.37	0.63	0.36
	Equivalency	ns	ns	ns	** ^	ns	**	**	**	ns	**	ns	**
	Similarity (F)				ns		ns	ns	ns		ns		ns
	(R)				ns		ns	ns	ns		ns		ns
	Divergence?	No	No	No	Yes	No	Yes	Yes	Yes	No	Yes	No	Yes
<i>A. framesii</i> group	Schoener's D	0.24	0.61	0.36	0.38	0.42	0.66	0.34	0.28	0.34	0.63	0.43	0.38
	Equivalency	** ^	ns	ns	** ^	ns	ns	ns	**	ns	ns	ns	**
	Similarity (F)	ns			ns				ns				ns
	(R)	ns			ns				ns				ns
	Divergence?	Yes	No	No	Yes	No	No	No	Yes	No	No	No	Yes

Table 4: Results of analyses of deviance of the GLMs and GLMM predicting germination and survival rate between species.

	LR χ^2	Df	P-value	Sig.
Greenhouse germination GLM				
Species	8.80	3	0.032	*
Treatment	30.24	5	< 0.001	***
Species:Treatment	48.72	15	< 0.001	***
Field germination GLM				
Species	24.61	3	< 0.001	***
Treatment	68.17	4	< 0.001	***
Species:Treatment	38.02	12	< 0.001	***
Field survival GLMM				
	χ^2			
Species	41.09	3	< 0.001	***
Size	29.47	1	< 0.001	***
Treatment	13.38	4	< 0.01	**
Species:Size	18.41	3	< 0.001	***
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1				

Table 5: Results of analyses of deviance of the GLMs predicting germination rate for each species in the greenhouse and field experiments

	LR χ^2	Df	P-value	Sig.
<i>R. burtoniae</i>				
Greenhouse				
Treatment	6.70	5	0.244	
Infiltration time	1.14	1	0.286	
Treatment: Inf. time	5.29	5	0.381	
Field				
Treatment	12.9	4	0.012 *	
<i>C. calculus</i>				
Greenhouse				
Treatment	8.44	5	0.133	
Infiltration time	0.02	1	0.893	
Treatment: Inf. time	7.15	5	0.210	
Field				
Treatment	11.3	4	0.024 *	
<i>D. diversifolium</i>				
Greenhouse				
Treatment	18.15	5	< 0.01 **	
Infiltration time	7.05	1	< 0.01 **	
Treatment: Inf. time	2.96	5	0.706	
Field				
Treatment	51.5	4	< 0.001 ***	
<i>A. framesii</i>				
Greenhouse				
Treatment	63.38	5	< 0.001 ***	
Infiltration time	3.66	1	0.056 .	
Treatment: Inf. time	5.32	5	0.378	
Field				
Treatment	24.9	4	< 0.001 ***	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

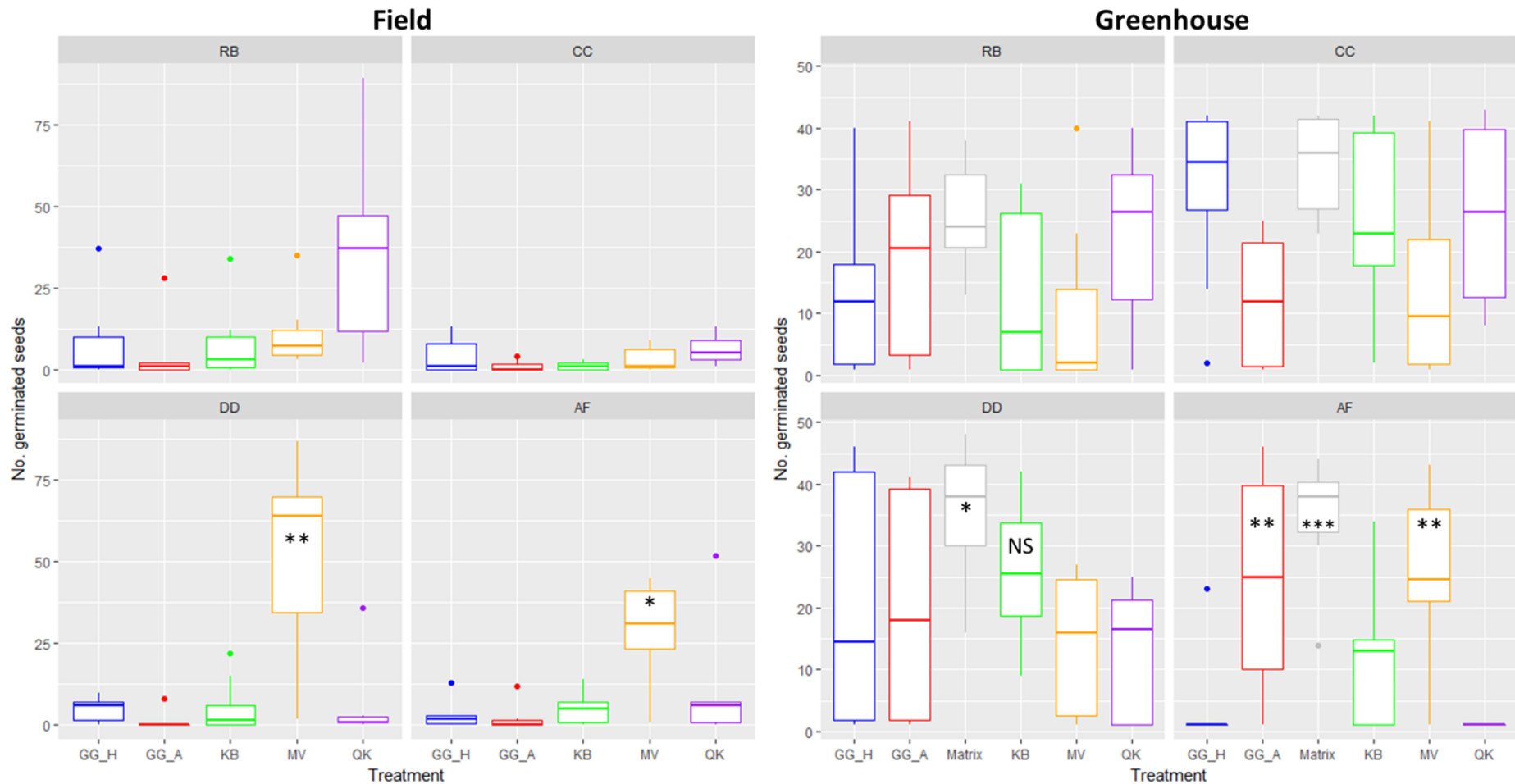


Figure 6: Number of germinated seeds (out of 200 and 50 sown, respectively) from the field and greenhouse experiments for each treatment. Results of likelihood ratio tests for the effect of removing treatment from the final model (which included infiltration time as a main effect for *D. diversifolium* and *A. framesii* in the greenhouse). Significance of post-hoc pairwise contrasts between each treatment and the control (GG_H) are shown for models excluding the non-significant infiltration time x treatment interaction term. Significance codes 0 '****' 0.001 '***' 0.01 '**' 0.05 '*'; 'NS' = near-significant.

Table GF Growth: Results of analyses of deviance of the GLMMs predicting growth for each species in the greenhouse and field experiments

	χ^2	Df	P-value	Sig.
<i>R. burtoniae</i>				
Greenhouse				
Treatment	69.83	5	< 0.001	***
Time	337.6	1	< 0.001	***
Treatment:Time	5.14	5	0.399	
Field				
Treatment	13.81	4	0.008	**
<i>C. calculus</i>				
Greenhouse				
Treatment	35.48	5	< 0.001	***
Time	262.9	1	< 0.001	***
Treatment:Time	71.81	5	< 0.001	***
Field				
Treatment	2.58	3	0.461	
<i>D. diversifolium</i>				
Greenhouse				
Treatment	12.09	5	0.034	*
Time	201.8	1	< 0.001	***
Treatment:Time	4.57	5	0.471	
Field				
Treatment	0.15	1	0.696	
<i>A. framesii</i>				
Greenhouse				
Treatment	59.33	5	< 0.001	***
Time	206.2	1	< 0.001	***
Treatment:Time	43.86	5	< 0.001	***
Field				
Treatment	5.82	3	0.121	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

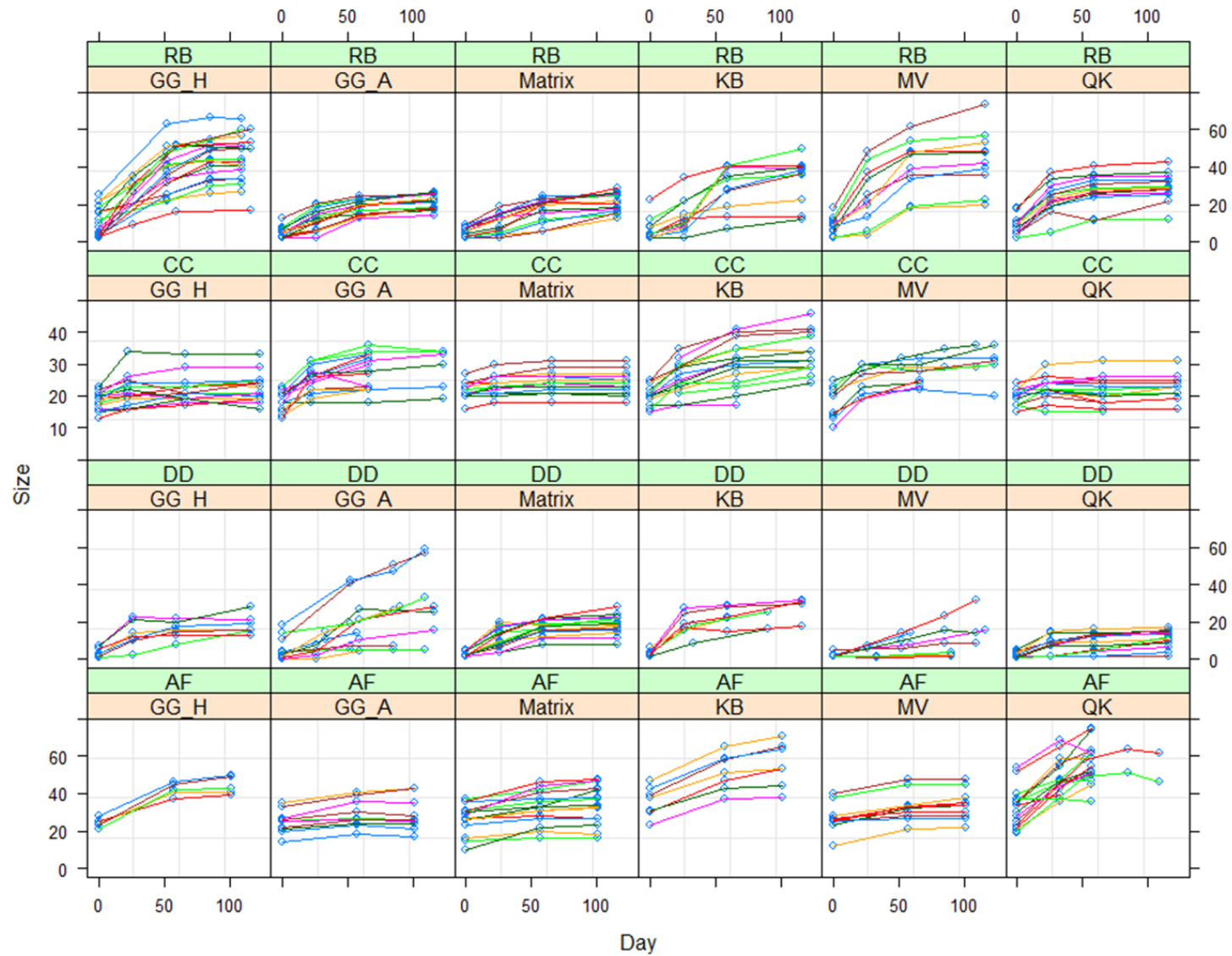


Figure 7: Plots to show change in size (measured as height in mm for RB and DD and width in tenths of millimetres for AF and CC) over time (in days after first measurement) of individual plants growing in the various treatments in the greenhouse. Colours are used to distinguish individuals and points represent measurements.

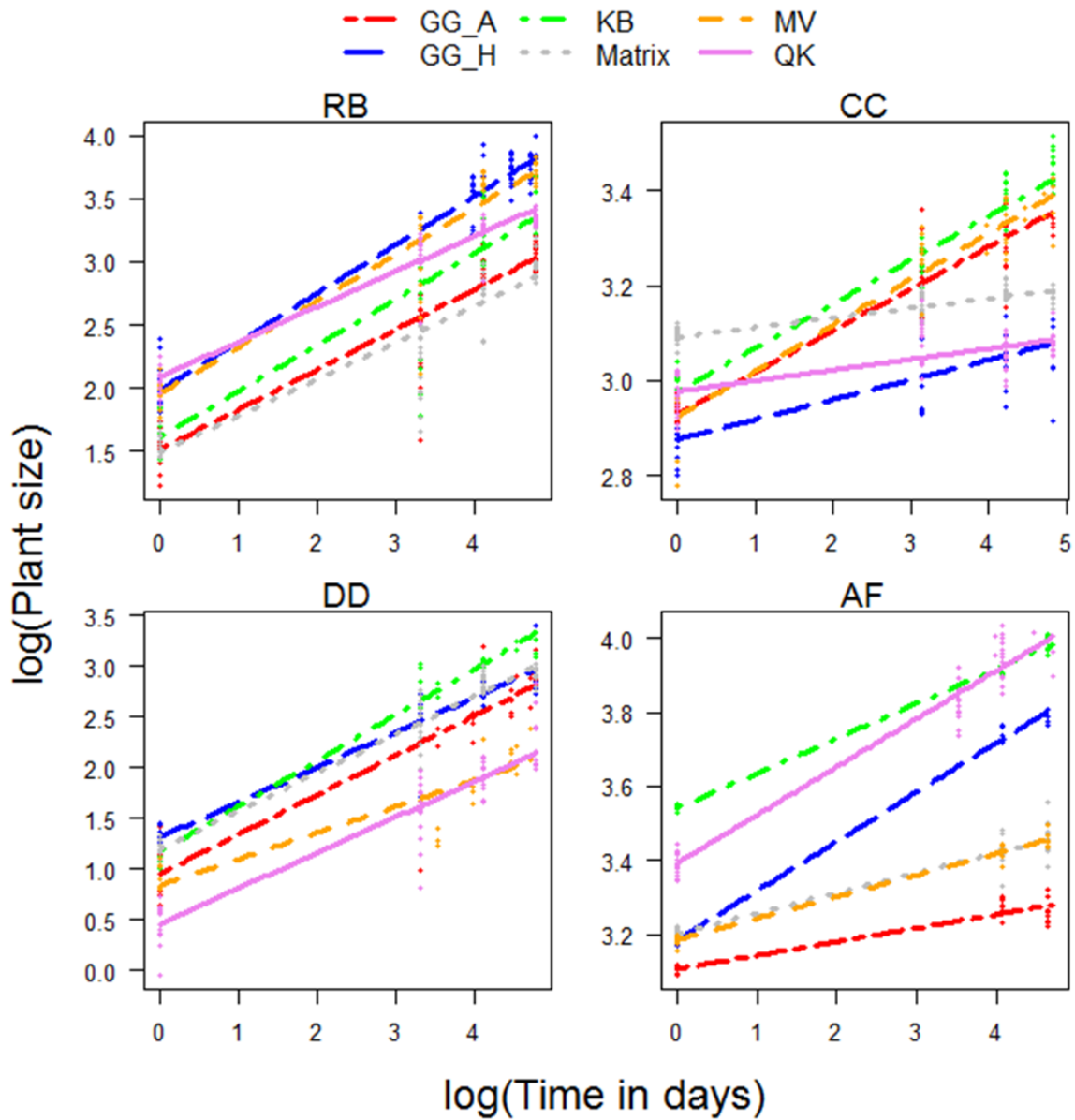


Figure 8: Visualisations of model predictions (lines) based on the maximal GLMM for each species, with observed values (dots), predicting growth (i.e. change in size over time) in relation to treatment in the greenhouse transplant experiments

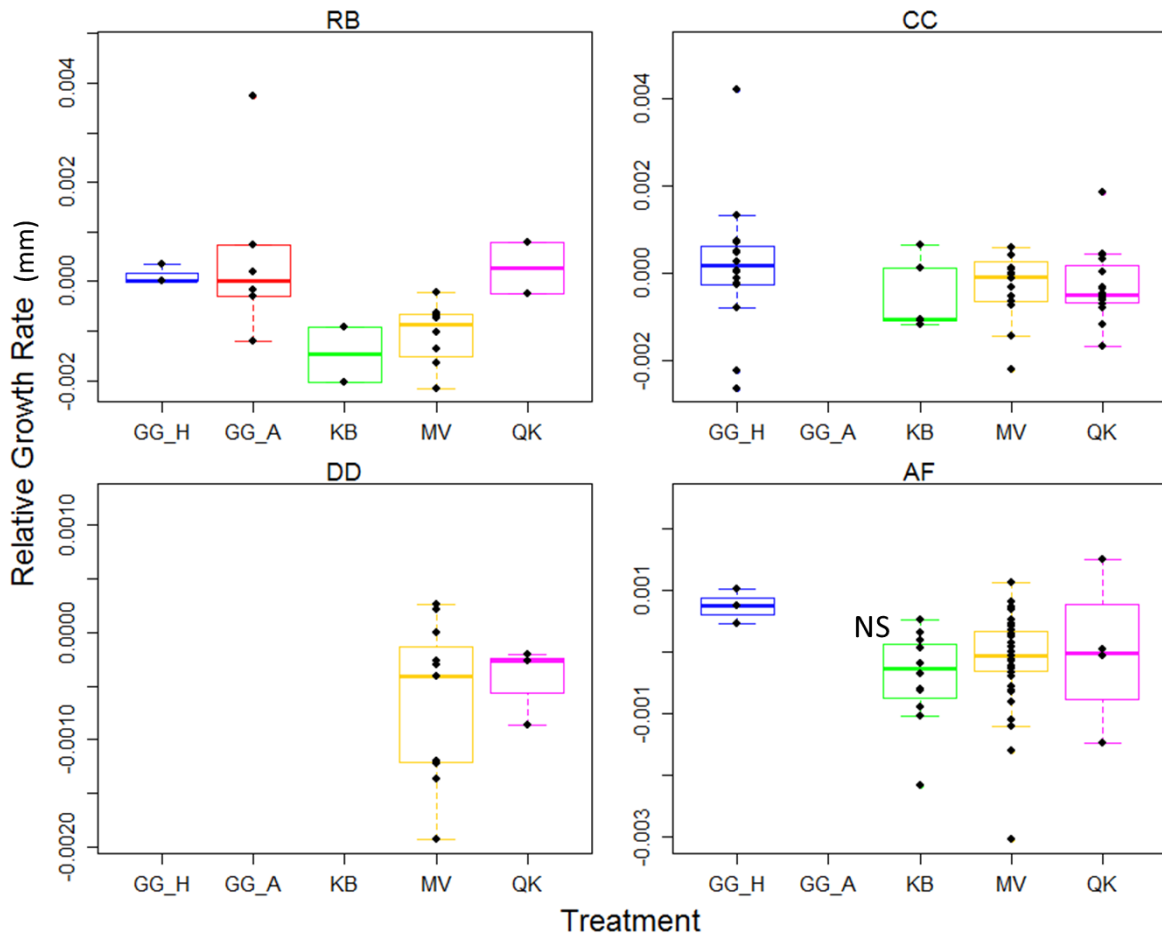


Figure 9: Boxplots to show the distribution of RGR of surviving individuals with observed values (dots) for each treatment for each species in the field. Treatments with no data showed complete mortality. None of the post-hoc pairwise contrasts between each treatment and the control (GG_H) were significant, though KB was near-significantly reduced in *A. framesii*. 'NS' = near-significant.

Table 7: Results of analyses of deviance of the GLMMs predicting survival at the individual level for each species in the field experiments

	χ^2	Df	P-value	Sig.
<i>R. burtoniae</i>				
Treatment	5.98	4	0.20	
Size	10.44	1	< 0.01	**
Treatment:Size	3.75	4	0.44	
<i>C. calculus</i>				
Treatment	5.64	3	0.13	
Size	0.49	1	0.48	
Treatment:Size	2.62	3	0.45	
<i>D. diversifolium</i>				
Treatment	0.44	1	0.51	
Size	17.55	1	< 0.001	***
Treatment:Size	0.01	1	0.94	
<i>A. framesii</i>				
Treatment	6.92	3	0.07	.
Size	7.33	1	< 0.01	**
Treatment:Size	1.24	3	0.74	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

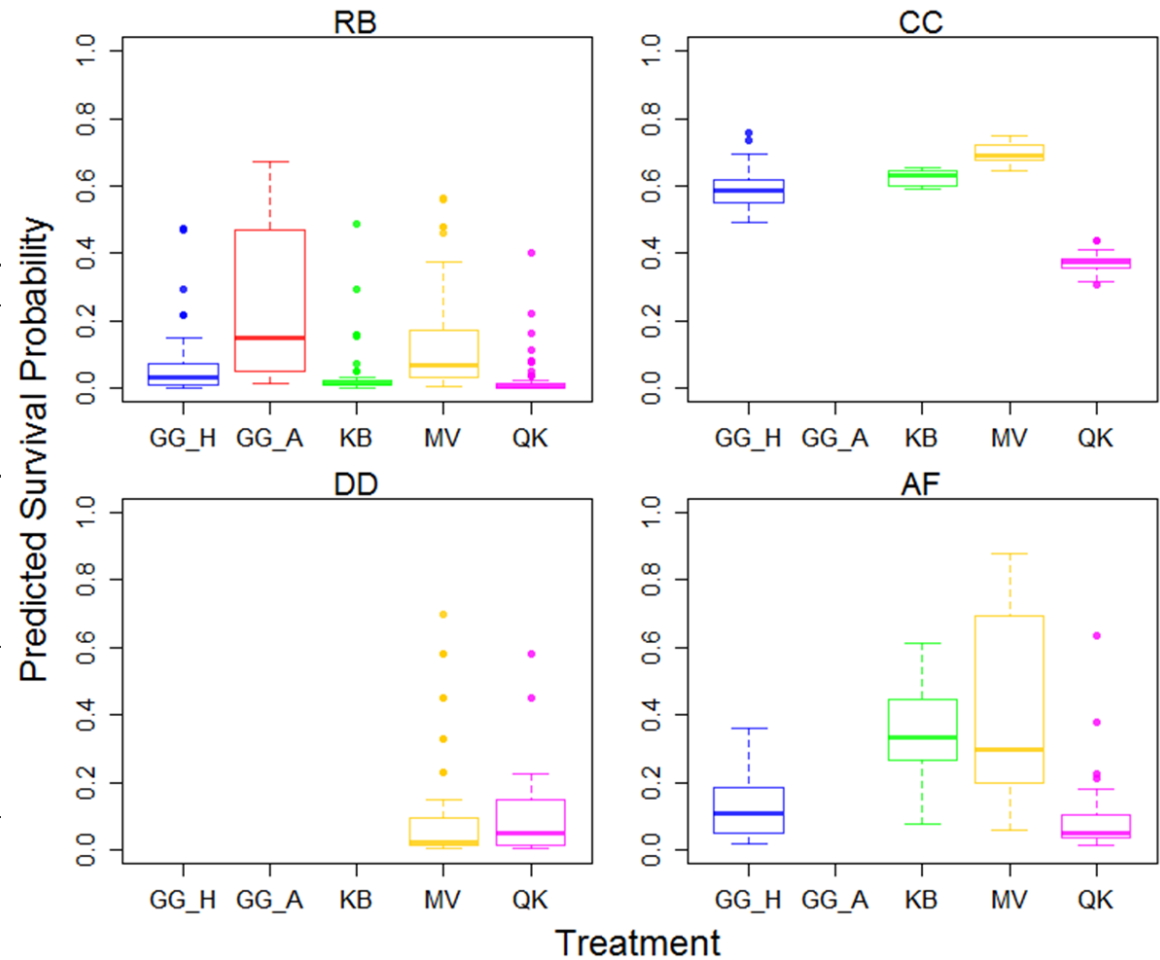


Figure 10: Boxplots showing the range of predicted survival probabilities per individual for each treatment based on GLMMs incorporating the effect of initial size where applicable but excluding the size x treatment interaction. Treatments with no data showed complete mortality. None of the post-hoc pairwise comparisons of interest were significant.

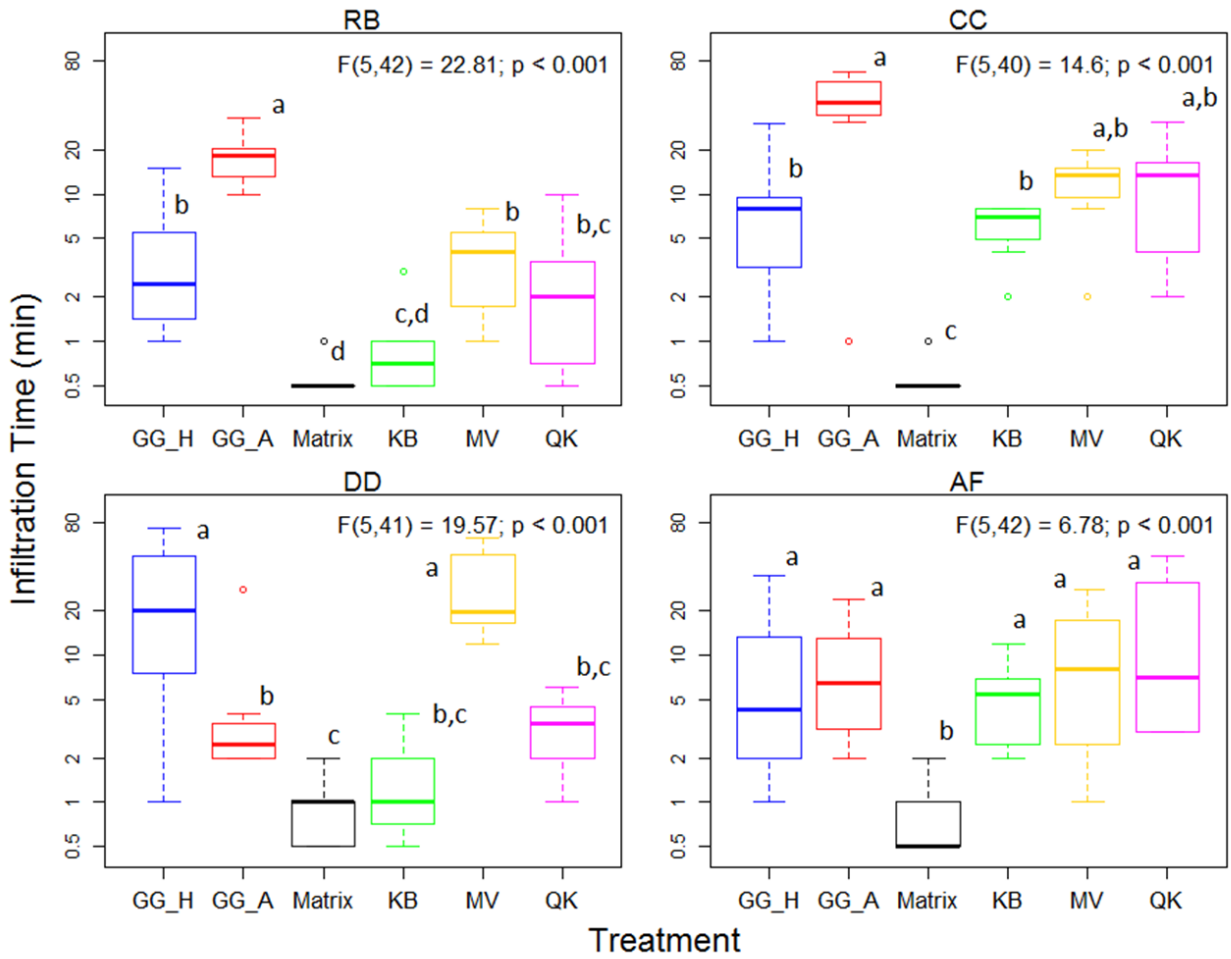


Figure 11: Boxplots showing the range of infiltration times (time taken for water to soak into soil) per pot for each treatment in the greenhouse experiments. ANOVA results are presented and groups identified by Tukey's HSD post-hoc tests are shown as letters next to each box. Note the logarithmic scale.

Chapter 4 – Synthesis: Linking genetic patterns to environmental isolation and local adaptation in the Knersvlakte

The Succulent Karoo (SK) is a region of particular interest to botanical systematists because of its extremely high floristic diversity which is largely the product of recent and rapid radiations (most notably that of the ruschioid Aizoaceae; Klak et al. 2004; Valente et al. 2014). Limited gene flow arising from dispersal limitation related to biological traits has frequently been cited as a cause of this diversity (Ihlenfeldt 1994; Klak et al. 2004), but a significant role for adaptive divergence has also been suggested, and is supported by population-level work on members of Aizoaceae, although this also cited gene flow limitation as a factor facilitating speciation (Ellis & Weis 2006; Ellis et al. 2006; Ellis et al. 2007). Although both factors are likely to contribute to diversification, the relative importance of each in the SK flora remains largely untested, as does the question of whether ecological or non-ecological speciation was more prevalent. However, novel genetic techniques which provide an unprecedented window into patterns of genetic isolation in non-model organisms (Lexer et al. 2013) can be coupled with ecological and geographic data to tease apart the processes which drive genetic divergence and ultimately speciation.

Linking genetic and ecological patterns

Within-species deviations from a genetic pattern of isolation by geographic distance (IBD) can reveal other processes apart from isolation by dispersal limitation (IBDL; i.e. gene flow decreasing with increasing distance) that affect genetic structure and ultimately speciation. This is a major focus of landscape genetics (Manel et al. 2003; Storfer et al. 2007), which aims to determine whether genetic structure relates to i) physical landscape features that block or enhance contemporary gene flow and dispersal, such as mountain ranges or habitat corridors; ii) historical factors such as colonisation

or vicariance patterns caused by environmental changes like geomorphological and climatic evolution; iii) ecological factors such as local adaptation stemming from environmental variation; or iv) interactions between these factors (Orsini et al. 2013). These are of interest because they relate directly to mechanisms of diversification, both past and present. However, teasing apart the influence of contemporary gene flow limitation, historical processes and ecological variation on patterns of genetic structure is challenging because these factors are often highly correlated and interdependent.

Population genomics makes it possible to investigate neutral and adaptive genetic variation separately by identifying loci that might be subject to divergent selection and investigating genetic structure in these loci separately from neutral genetic structure (Manel & Holderegger 2013; Orsini et al. 2013; Lexer et al. 2013). Selection can influence genetic structure through the process of isolation by adaptation (IBA) to generate a pattern of isolation by environment (IBE), in which genetic divergence increases with ecological divergence (i.e. fundamental niche divergence, aka local adaptation) between populations (Nosil et al. 2008). Under pure IBA, and assuming that geographic distance and ecological divergence are uncorrelated, no IBD is expected, but IBE is expected in neutral loci as well as loci under selection (Orsini et al. 2013). This is because gene flow under IBA will be determined by ecological similarity, with greater gene flow between more similar sites, rather than geographic distance; IBE may be stronger in loci under selection, however, because divergence in these loci is expected to be driven by both gene flow limitation and divergent selection.

In reality, multiple processes might act together to determine patterns of genetic structure (Orsini et al. 2013). Often genetic divergence shows a strong correlation with historical patterns of colonisation – the underlying process is termed isolation by colonisation (IBC). However, because of its historical nature, IBC can be eroded by gene flow, which itself may or may not be dependent on ecological divergence (IBDL and IBA, respectively). Under these scenarios IBE and IBD, respectively, are expected to develop over time, though the initial IBC phase might leave a legacy of elevated

overall genetic differentiation between populations. In addition, because gene flow can be limited by both distance and environmental divergence, it is important to first establish the scale of IBD and then control for its effect when testing for IBA. This can be achieved using causal modelling (Cushman & Landguth 2010) which makes use of partial Mantel tests to test the effect of ecological divergence on genetic divergence while controlling for the effect of geographic distance. However, such analyses must be interpreted in the context of the biological factors that are involved in inter-population gene flow limitation.

In plants, long-distance seed dispersal (LDD) and long-distance pollen transfer (LDP) are the primary means of inter-population gene flow and might be subject to selection provided that individuals have control over traits that influence them (Nathan et al. 2008). LDD might be selected for because it reduces kin competition, but the fate of the dispersed seed is the ultimate determinant of selection for or against LDD (Ronce 2007). After LDD, seeds have to germinate and eventually reproduce in order for their alleles to enter the new population, and this is strongly dependent on the strength of local adaptation. If it is weak, establishment of immigrants will be limited by the usual factors such as competition and environmental stochasticity, while strong local adaptation will interact with such factors due to reduced immigrant fitness, strongly limiting establishment and hence also gene flow. Local adaptation can affect LDP similarly provided that the offspring of inter-population crosses germinate locally, and might also generate a lasting barrier to gene flow via LDP if it causes populations to evolve non-overlapping flowering times or different pollinators, the first of which has been shown for the *Argyrodema delaeitii* group in the Knersvlakte (e.g. Ellis et al. 2006). However, the relationship between local adaptation and gene flow is reciprocal. The traits that affect the scale of dispersal and pollen transfer play a large role in how gene flow frequency relates to spatial scale – in other words, the shapes of the seed dispersal and pollen transfer kernels influence the shape of the gene flow kernel. If LDD or LDP occur frequently over large distances (i.e. seed and pollen kernels have ‘fat tails’) then gene swamping is likely to occur, which in turn reduces

the likelihood of local adaptation evolving (Lenormand 2002). On the other hand, if LDD and LDP events are rare and the distances involved vary stochastically, such as in environmental island systems in which plant species are strongly dependent on patchily distributed environments, then local adaptation is more likely to evolve (Ackerly 2003). This can result in IBA regardless of whether there is any change in the scale and frequency of LDD and LDP. Finally, local adaptation is irrelevant to neutral genetic divergence between populations if LDP and LDD never occur between them, in which case IBA is expected to be reflected only in regions of the genome that are experiencing divergent selection (Orsini et al. 2013). These considerations highlight the importance of understanding the dispersal and pollination biology of plants when investigating underlying drivers of population differentiation.

To conclude, insight into the potential for plant speciation driven by IBA can be gained by combining i) causal modelling of landscape scale genetic structure to determine the relative effects of dispersal limitation, historical colonisation and local adaptation on gene flow with ii) non-spatial assignment analysis and fine-scale analysis of IBD to determine the scale and means of inter-population gene flow (addressed in Chapter 2).

Gene flow and local adaptation in ruschioid Aizoaceae

The approach described above was implemented for *Ruschia burtoniae* and *Conophytum calculus*, for which both genetic and ecological data were gathered in this study, by causal modelling of genetic divergence in relation to geographic distance and environmental niche divergence between the four populations studied in the Knersvlakte in Chapters 2 and 3. To generate a matrix of loci putatively under selection ('outlier loci'), the procedure for filtering the SNP matrices was applied as in Chapter 2, with the exception of removing loci deviating from HWE *within* any of the four populations rather than over the entire set, because deviations from HWE can be caused by divergent selection among populations, whereas such deviations within populations are indicative of potentially spurious SNPs. This was done using custom perl scripts written by Chris Hollenbeck

(available at <http://ddocent.com/filtering/>) in concert with the program 'vcftools' (Danecek et al. 2011). BayeScan version 2.1 (Foll & Gaggiotti 2008) was then used to identify outlier loci as in Chapter 2, and F_{ST} was estimated for these loci following Weir & Cockerham (1984). IBE was assessed for the four populations in the study by testing the correlation between linearized pairwise genetic (using neutral and outlier SNPs) distance [$F_{ST} / (1 - F_{ST})$ following Rousset (1996)] and pairwise edaphic distance calculated as $1 - \text{Schoener's D}$ [i.e. similarity corrected for habitat availability (Broennimann et al. 2012)] for edaphic variables PC1, PC2, PC3 and two-dimensional PC2:PC3 edaphic space (see Chapter 3), using Mantel tests as well as partial Mantel tests accounting for the effect of log-transformed geographic distance, both implemented in the package 'vegan' (Oksanen et al. 2013).

This process identified 95 and 23 outlier loci for *R. burtoniae* and *C. calculus* which produced global F_{ST} estimates of 0.48 and 0.18, respectively, which, in line with these species' niche divergence and local adaptation patterns, suggests that adaptive genetic divergence is much greater in *R. burtoniae* than in *C. calculus*. Furthermore, there was no evidence of IBA in *C. calculus* along any edaphic axes but some evidence in *R. burtoniae* along PC3 (Figures 1 and 2), which could be broadly characterised as a salinity axis, suggesting that in *R. burtoniae* divergent selection and gene flow limitation both occur in response to local adaptation to soil salinity variation. The unpredictable nature of long-distance mechanisms of gene flow (Higgins et al. 2003) means that over long time scales local adaptation could have effected IBA and produced the pattern of IBE. However, the fact that only one of the PC3 pairwise comparisons was significant following equivalency and similarity testing (Chapter 3) casts some doubt on this conclusion, and the extremely poor dispersal ability of this species is further reason to question whether local adaptation can cause gene flow limitation at such large scales. In contrast, the strong dispersal ability of *C. calculus* supports the hypothesis that gene swamping is responsible for its strong niche conservatism. It therefore seems appropriate to conclude that local adaptation is highly contingent on strong dispersal limitation in this system.

LDD events in plants are thought to typically occur via non-standard vectors (Nathan et al. 2008), and this study supported this in ruschioid Aizoaceae by showing that the degree of spatial genetic structure correlated negatively with the likelihood of seed dispersal via the movement of entire capsules at both large and small scales (Chapter 2). However, there is also evidence that the frequency of LDD events can be predicted based on capsule morphology and, potentially, by gathering data on mechanisms of capsule dislodgement. Future work on incipient speciation in ruschioid Aizoaceae should therefore be guided by these factors.

Consequences for modes of speciation

What are the consequences of these results for the likelihood of different modes of speciation in ruschioid Aizoaceae and the SK? Ellis et al. (2006) proposed a model of diversification in the *A. framesii* group driven by adaptive speciation in allopatry and facilitated by limited long-distance dispersal and gene flow, and results from Chapter 3 showing that these taxa generally occupy divergent edaphic niches, and that divergence has resulted in local adaptation, lends support to this hypothesis. These results contrast with those for *C. calculus*, the other dwarf in this study, which showed limited niche divergence and little evidence for local adaptation between sites. The contrast between these taxa is revealing. The entire *Argyroderma* genus is endemic to the Knersvlakte (Hartmann 1977), suggesting strongly limited dispersal. In contrast, though *C. calculus* subsp. *calculus* is also endemic to the Knersvlakte quartz fields (KQF), *C. calculus* subsp. *vanzyliei* is found in similar habitat over 200 km to the north-east, with the region between these taxa predominantly comprising unsuitable non-quartz habitat (Hammer 2002). Vicariance may be responsible for the fragmented distribution, though this seems unlikely given the relatively recent emergence of the KQF (Cowling et al. 2009). Instead, surprisingly, the long-distance seed dispersal ability of the species appears to be the most likely explanation for this disjunction. This is certainly the best explanation for the lack of taxonomic variation and weak genetic structure within *C. calculus* subsp. *calculus*, and

the contrast with the *A. framesii* group highlights the importance of dispersal limitation in diversification.

The case of *R. burtoniae* provides further support for the primacy of dispersal limitation. The species has a fairly broad range extending from the KQF northwards into northern Namaqualand, where it occurs in non-quartz habitat (Manning & Goldblatt 2012), although it is confined to quartz fields in the Knersvlakte (Schmiedel 2002). This suggests that vicariance cannot be discounted as an explanation for its fragmented distribution in the KQF, and indeed, because it occurs primarily on eroding ridge-tops (Schmiedel 2002) it is possible that it once enjoyed a more contiguous distribution on the surface overlying the Knersvlakte and that the extent of its habitat has been shrinking due to erosion, resulting in an increasingly fragmented range. This, in combination with its very poor dispersal ability, is the likely cause of local adaptation in this species. Unlike the *A. framesii* group, the fragmentation of its habitat may have been too recent to have generated enough genetic and morphological divergence to warrant the recognition of distinct species, but the possibilities of incipient and cryptic speciation certainly cannot be discounted. This suggests that the Knersvlakte has the potential to continue to generate novel diversity. If not distinct species, these populations could certainly be deemed evolutionarily significant units *sensu* Moritz (1994), meaning that the habitat they occupy could be regarded as a priority in terms of conserving evolutionary processes in the SK (Desmet et al. 2002).

This raises the question of how lineages with strong dispersal ability might have diversified in the SK. It seems likely that dispersal ability will affect the way in which patterns of diversification vary across the full spectrum of scales available to SK-adapted lineages. For example, one might expect that lineages with traits promoting long-distance dispersal but which nevertheless show isolation by distance are more likely to diversify between biogeographic regions such as the Knersvlakte rather than within them (Gillespie et al. 2012). Consequently, if geographic isolation has been the dominant diversification driver in *Conophytum*, then assuming regular LDD its species should have anomalously

large range sizes compared to those in other ruschioid groups, particularly those with similar traits to *R. burtoniae*. However, Young & Desmet (2016) found that ca. 28% of the *Conophytum* species are point endemics (restricted to an area < 10 km²). Although this figure could reflect a tendency of *Conophytum* taxonomists to split taxa and, regardless, it is unclear how anomalous it is relative to other genera in the family, this nevertheless suggests the need to test diversification hypotheses in a phylogenetic context. For example, if point endemics occur in close proximity to their closest relatives this would suggest that other processes such as flowering phenology shifts [e.g. as documented by Young et al. (2015)] drove the genus's diversification. Testing the degree of phylogenetic structure in local assemblages of *Conophytum*, which sometimes contain up to ten co-occurring species, will also shed light on the prevalence of different modes of speciation in this genus (Powell et al. 2016).

Conclusions

The synthetic analysis presented in this chapter provides clear support for the importance of pre-zygotic isolation in determining likely modes of speciation. For example, the evidence suggests that ecological speciation is unlikely to occur without strong dispersal limitation in this group. This is perhaps less surprising than the finding that *C. calculus* – and potentially also a large proportion of the taxa in this diverse genus – is an excellent disperser, which has profound implications for the genus's diversification and warrants further research regarding the traits and mechanisms that determine the likelihood of long-distance dispersal in ruschioid Aizoaceae, and whether these factors correlate with diversification patterns. A starting point might be to test in a phylogenetic context whether capsule morphological complexity and seed count correlate with proxies of dispersal ability such as range size. Another important step in elucidating the mechanisms of speciation will be to test the degree to which local adaptation corresponds with reproductive isolation (Walter et al. 2016). More detailed genomic work to identify genes under divergent selection might compliment such analyses by revealing which environmental variables are the

strongest drivers of adaptive divergence (e.g. Prunier et al. 2017). To conclude, the ruschioid Aizoaceae of the Knersvlakte and the SK represents an excellent system for studying the patterns and drivers of diversification in species-rich, recently emerged environments, and will certainly reveal further surprises under the weight of continuing research.

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Figures

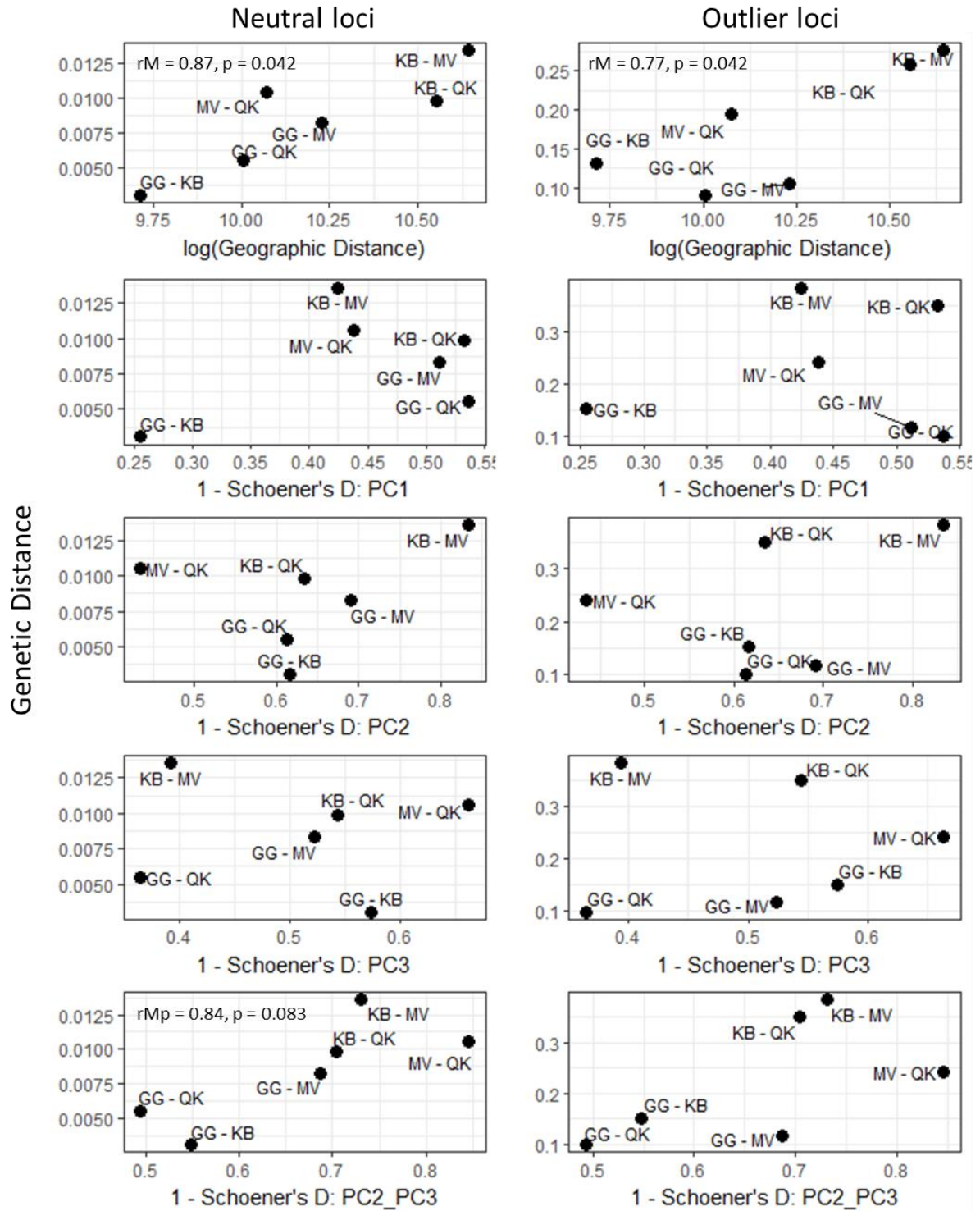


Figure 1: Scatterplots showing the relation between niche differentiation and linearised F_{ST} between population pairs of *C. calculus*. Population names are as in chapters 2 and 3. When $p < 0.1$, Mantel's r (rM) and/or partial r (rMp) with corresponding p -values are shown.

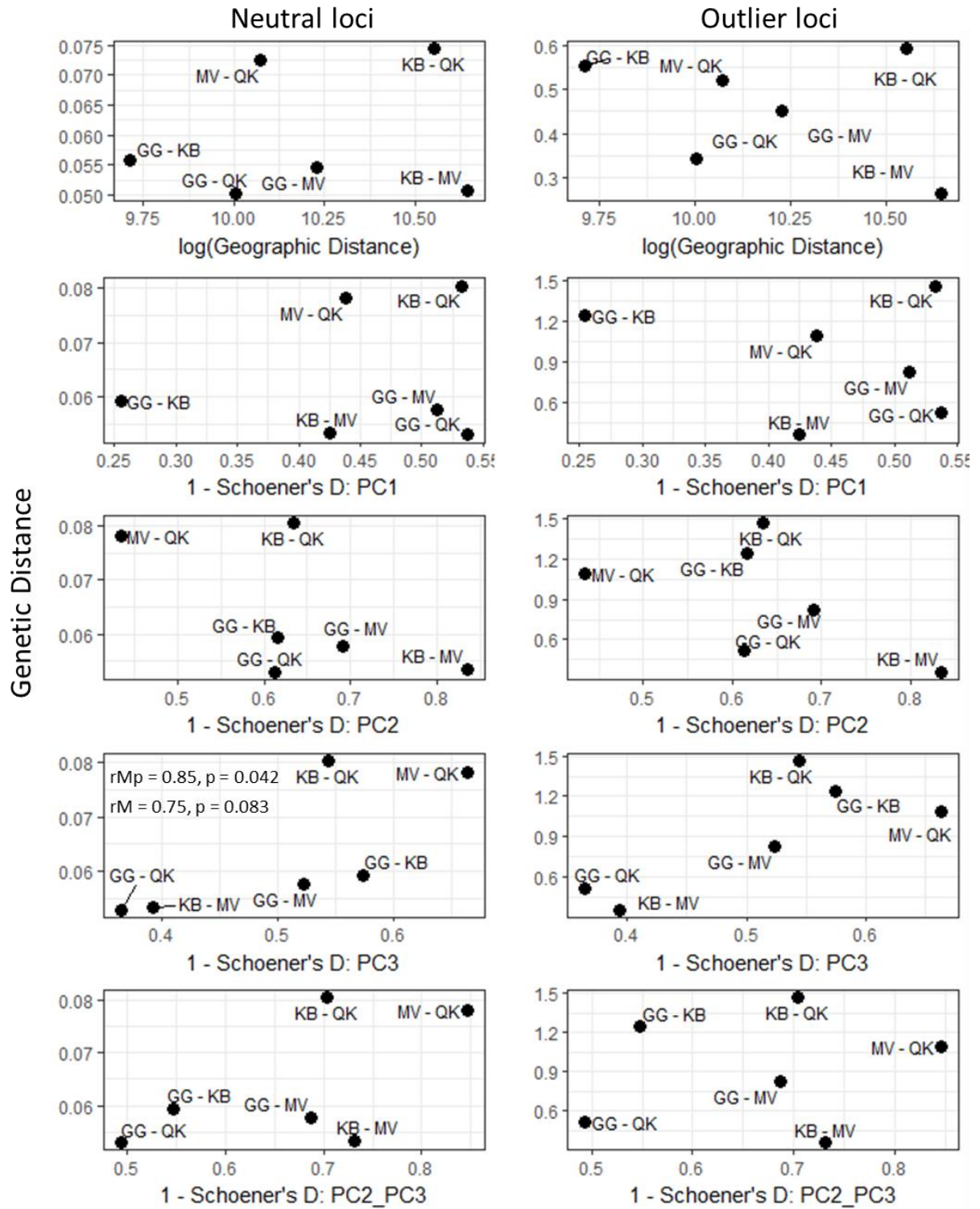


Figure 2: As for Figure 1 but showing results for *R. burtoniae*.