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Niche specificity influences gene flow across fine-scale habitat mosaics in Succulent Karoo plants

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Abstract

While the tempo of diversification in biodiversity hotspots has received much attention, the spatial scale of diversification has often been overlooked. Addressing this deficiency requires understanding the drivers of population divergence and the spatial scales at which they operate in species-rich clades and ecosystems. South Africa's Succulent Karoo (SK) hotspot provides an excellent system for such research, being both compact (ca. 110,000 km^2) and home to spectacular in-situ radiations, such as the ruschioid Aizoaceae. Here we use GBS to document genetic structure in two co-occurring ruschioid species, at both coarse (>10 km) and fine (<500 m) spatial scales. Where *Ruschia burtoniae* shows strong between-population genetic differentiation and no gene flow, *Conophytum calculus* shows weak differentiation, with high levels of admixture suggesting recent or ongoing gene flow. Community analysis and transplant experiments reveal that *R. burtoniae* occupies a narrow, low-pH edaphic niche, and at scales of a few hundred metres, areas of elevated genetic turnover correspond to patches of edaphically unsuitable habitat. In contrast, *C. calculus* occupies a broader niche and exhibits isolation-by-distance without a habitat effect. We suggest that edaphic specialisation, coupled with highly restricted seed and pollen dispersal in heterogeneous landscapes, has played a major role in driving rapid diversification at small spatial scales in this system. However, the contrasting patterns in our study species show that these factors do not influence all organisms uniformly, being strongly modulated by lineage-specific traits that influence both the spatial scale of gene flow and habitat specificity.

KEYWORDS
Aizoaceae, dispersal, edaphic specialisation, genotyping-by-sequencing, greater Cape floristic region, speciation

1 | INTRODUCTION

Biodiversity hotspots are areas characterized by high concentrations (per unit area) of species richness and taxonomic endemism (Guilhaumon et al., 2008; Mazel et al., 2014; Mittermeier et al., 2011; Myers et al., 2000; but see Orme et al., 2005). Therefore, notwithstanding the critical role of long-term climatic stability in facilitating the local accumulation of endemic species (Harrison & Noss, 2017), interrogating the processes that promote diversification at small spatial scales is central to understanding the richness of these hotspots. Speciation requires either strong divergent selection or a reduction in gene flow between populations occurs (Coyne, 1992; Coyne & Orr, 2004; Slatkin, 1985; cf. Nosil, 2008), and so the challenge of understanding what drives speciation in hotspots reduces to understanding the factors—both environmental and organismal— that promote ecological divergence and/or restrict gene movement at small spatial scales.

Environmental heterogeneity influences gene flow both by presenting barriers of unsuitable habitat that impede between-population dispersal and so result in population isolation, and by presenting contrasting selective environments which simultaneously stimulate adaptation to local conditions and select against gene flow between the locally-adapted forms (Rundle & Nosil, 2005; Sobel et al., 2010). The relative influence of these mechanisms varies from one system to the next, depending on the magnitude and spatial configuration of landscape heterogeneity. In oceanic archipelagos and high-elevation mountain systems, for example, population isolation by areas of unsuitable habitat (e.g., sea water or low-elevation habitat) commonly provides the initial impetus for differentiation leading to the formation of new species across broad spatial scales (Gillespie & Roderick, 2002; Grant, 1996; Steinbauer et al., 2016). In rainforest systems, by comparison, local adaptation across ecotones may be more important in powering speciation, the strength of selection being sufficient to counter gene movement (Brousseau et al., 2020; Fine et al., 2013; Kirschel et al., 2011; Misiewicz & Fine, 2014; Misiewicz et al., 2020; Moritz et al., 2000; Smith et al., 1997). The latter process can also operate at fine spatial scales, underpinning the "budding" speciation pattern observed in small, sympatric or parapatric populations of monkeyflower (Grossenbacher et al., 2014) and in twelve plant families in the California Floristic Province (Anacker & Strauss, 2014).Importantly, regardless of the mechanism involved, heterogeneity-driven divergence is not expected to yield a pattern of isolation by distance, the latter being more consistent with intrinsically limited short-range dispersal in a continuously-distributed population (Wright, 1943).

The spatial scale of gene flow is, of course, also influenced by intrinsic organismal features, particularly those influencing the spatial scale of gene transmission and the degree of niche specificity. Seed and pollen dispersal distances in plants are influenced primarily by floral and fruit/propagule traits that determine pollinator/disperser type, as well as by other traits, such as plant height and seed mass (Boucher et al., 2017; Dick et al., 2008; Givnish, 2010; Schurr et al., 2005; Thomson et al., 2011; Vittoz & Engler, 2007). As such,

traits affecting seed and pollen movement determine the scale of environmental heterogeneity that is relevant to gene flow and population genetic differentiation. This is further influenced by breeding system (outcrossing versus selfing), plant life span, plant habit (annual versus perennial; woody versus herbaceous), geographic range extent, and rare and unpredictable long-distance seed dispersal events (Hamrick & Godt, 1996; Hamrick et al., 1992; Nathan & Muller-Landau, 2000). Similarly, niche specificity is influenced by specific traits (e.g., plant habit, plant size, and environmental stress tolerance; Boucher et al., 2017; Boulangeat et al., 2012) which in turn determine the scale of environmental heterogeneity at which immigrant inviability limits gene flow. To sum up, the spatial scale of gene flow is influenced by organismal traits, and this undermines our expectation of a simple relationship between landscape heterogeneity and the spatial scale of speciation, which should instead be modulated by lineage-specific idiosyncrasies (Fine & Baraloto, 2016).

In the Cape biodiversity hotspot (i.e., Cape Floristic Region, CFR, sensu Goldblatt, 1978) of South Africa, complex topography, and the habitat heterogeneity that it entails, appears to underpin the exceptional species richness and local endemism of its flora (Goldblatt, 1978, 1997; Goldblatt & Manning, 2002; Linder, 1985, 2003; Verboom et al., 2015). However, this may be less true of the adjacent Succulent Karoo (SK) biodiversity hotspot (Desmet & Cowling, 1999). Characterized by a flat to gently-undulating topography which is a product of its easily-eroded, predominantly-shale rocks (Bradshaw & Cowling, 2014), the SK nevertheless accommodates ±5,000 plant species in an area of 111,212 km^2 (Hilton-Taylor, 1996). This makes it one of the most species-rich and endemic-rich arid floras globally (Cowling et al., 1998). Much of this diversity and endemism (860 species, ±17% of flora; ±540 species endemic; Snijman, 2013) resides in the ice-plant family Aizoaceae, particularly its subfamily Ruschioideae, which radiated in the Late Miocene (Klak et al., 2004; Valente et al., 2014). In the absence of rugged topography, some authors have identified fine-scale edaphic heterogeneity as an alternative driver of localized speciation in the SK (Ellis & Weis, 2006; Ellis et al., 2006), while others have invoked organismal traits, particularly the evolution (in Ruschioideae) of rain-triggered, hygrochastic seed capsules whose sophisticated seed retention structures restrict primary dispersal distances to <2 m (Ihlenfeldt, 1994; Klak et al., 2004; Parolin, 2001, 2006). Another possibility is that the generally small size of succulent plants promotes diversification either by limiting dispersibility or by enhancing sensitivity to environmental gradients (Boucher et al., 2017).

The richness of the SK flora is exemplified by the plants of the Knersvlakte (Figure 1), a ca. 60 km wide plain which, on the evidence of contemporary species distributions, has hosted the radiations of at least two Aizoaceae clades: *Argyroderma* (11 species, all Knersvlakte-endemic; Ellis et al., 2006) and *Monilaria-Dicrocaulon-Oophytum* (14 species, nine Knersvlakte-endemic; Klak, Bruyns, et al., 2013; Schmiedel, 2002). In total, the Knersvlakte accommodates >850 plant species of which >15% are Knersvlakte-endemic (Snijman, 2013), prompting Born et al. (2007) to identify the area as a local centre of endemism. A striking feature of the Knersvlakte
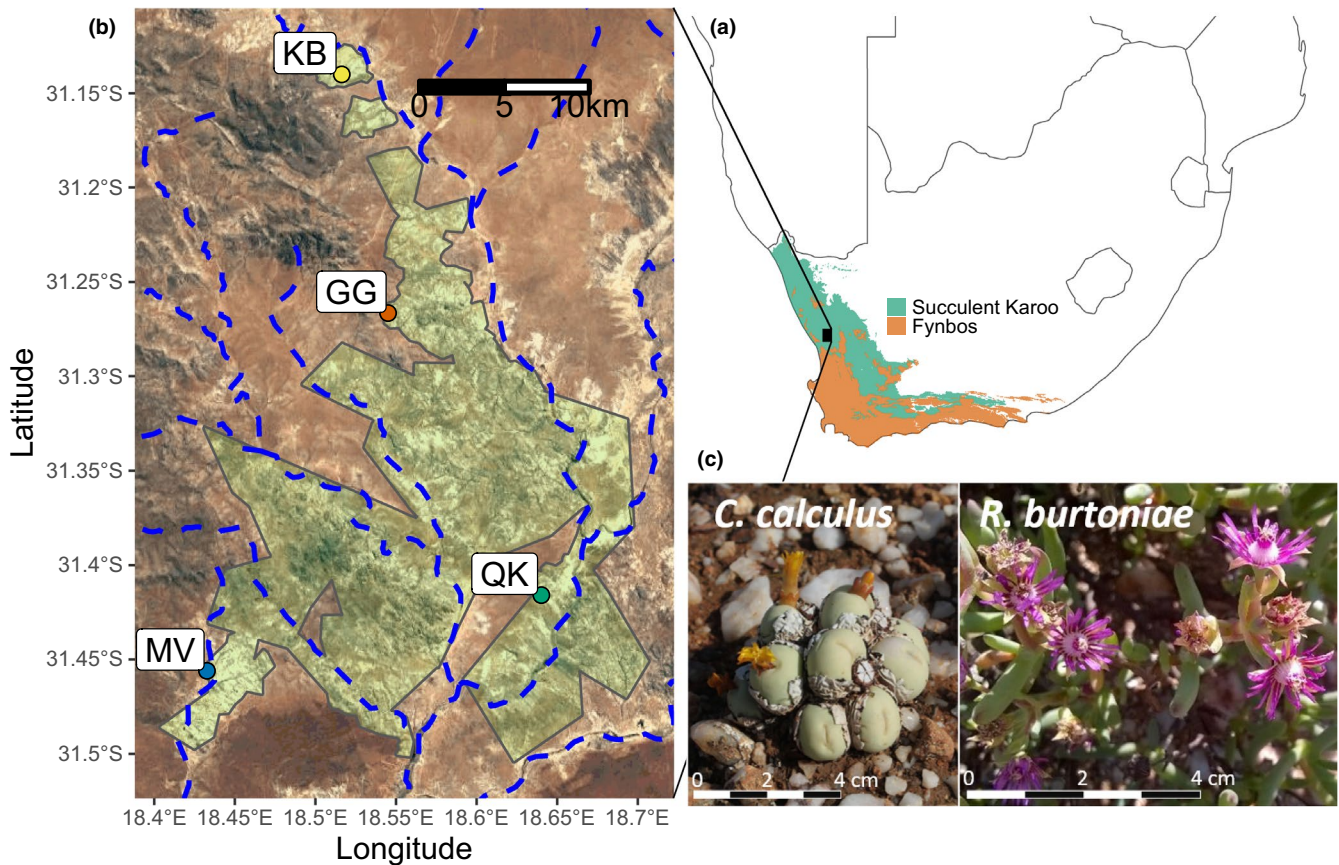
FIGURE 1 (a) Map of South Africa showing the distributions of the Fynbos and Succulent Karoo biomes, which make up the majority of the Greater Cape Floristic Region, and the location of the Knersvlakte (black box). (b) Google satellite image of the Knersvlakte showing the rough extent of the region containing quartz fields (shaded area), site localities (GG, Groot Graafwater; KB, Kareeberg; MV, Moedverloren; QK, Quaggaskop) and locations of rivers (dashed lines). (c) Images of this study's two focal species in flower: *Conophytum calculus* and *Ruschia burtoniae* [Colour figure can be viewed at wileyonlinelibrary.com]

landscape is the presence of a complex system of quartzitic gravel "islands", commonly termed quartz fields, which range from ca. 1–>100 m across (Schmiedel & Jürgens, 1999) and are separated from each other by a sharply-contrasting intervening matrix of red sands (Schmiedel, 2002; Schmiedel et al., 2015). Since the high albedo of the quartz pebbles ameliorates the desiccating effect of the hot, dry summers, these quartz fields provide refuge to many species, in particular a charismatic suite of drought-intolerant, dwarf-succulent plants (Musil, Schmiedel, & Midgley, 2005, 2009; Schmiedel & Jürgens, 2004), and the plant communities they support are distinct in composition and growth form from those of the intervening red sands (Schmiedel & Jürgens, 1999; Schmiedel et al., 2015).

Overall, the Knersvlakte quartz fields host 63 locally-endemic plant species, of which 37 belong to Aizoaceae (table 27 in Schmiedel, 2002). This richness is in part facilitated by the presence of environmental heterogeneity within quartz fields, particularly in edaphic properties. Particularly important is pH, which varies from neutral or slightly acidic to strongly acidic, with changes often being spatially abrupt (Ellis & Weis, 2006; Schmiedel & Jürgens, 1999; Schmiedel et al., 2015). This heterogeneity underlies striking changes in local species composition over tens of metres, producing a mosaic of distinct plant communities mostly dominated by Aizoaceae

(Schmiedel, 2002; Schmiedel et al., 2015). These features make the Knersvlakte an excellent system for studying both coarse and fine-scale genetic patterns in plants and how they relate to environmental heterogeneity (Jelinski, 1997).

In this paper, we examine spatial patterns of genetic variation in two quartz field-endemic Aizoaceae species, *Ruschia burtoniae* L. Bolus and *Conophytum calculus* (A. Berger) N.E.Br., in order to test the hypothesis that fine-scale spatial edaphic heterogeneity promotes fine-scale genetic differentiation in quartz field succulent species, and to elucidate the mechanism through which heterogeneity limits gene flow. For this purpose, we quantify patterns of genetic diversity at two spatial scales using single nucleotide polymorphism (SNP) variation obtained via genotyping-by-sequencing (GBS; Elshire et al., 2011). Using plant community and soil pH data, plus a common-garden comparison of each species' growth on different soils, we also characterize each species' edaphic preferences and, using this information, assess the extent to which genetic differentiation in each is a consequence of gene flow limitation associated with (a) barriers of edaphically-unsuitable habitat; or (b) divergent adaptation to soils of contrasting pH, or both. In the absence of adaptive divergence, strong genetic structure would point to a primary role for edaphic barriers. We also test

for isolation by distance effects expected if genetic structuring is influenced primarily by intrinsic, and not extrinsic, limitations to gene flow.

2 | MATERIALS AND METHODS

2.1 | Study system

The study was conducted in the Knersvlakte region of the Succulent Karoo biodiversity hotspot (Figure 1). To broaden the scope of our findings, we selected species with contrasting morphological and ecological traits, which represent the broader variability that resides within Ruschioideae. Where *R. burtoniae* is an erect, succulent-leafed shrub growing to ca. 60 cm tall, having small, open, pink flowers borne on a many-branching cyme, and fruit capsules with complex seed-retention structures, *C. calculus* is a miniature succulent growing to no more than 10 cm tall with tubular, yellow, night-scented flowers borne singly in the axil of each leaf pair, and fruit capsules lacking complex seed-retention structures. Both species are largely confined to the Knersvlakte: *R. burtoniae* extends marginally into the hills to the north and east (Klak & Buys, 2012), while *C. calculus* is endemic excluding an outlying population located some 200 km to the north-east (Hammer, 2002) which is recognized as taxonomically distinct (subspecies *vanzylii*). Klak, Bruyns, et al. (2013) found *Ruschia* to be polyphyletic and that *R. burtoniae* falls outside the core *Ruschia* clade, being placed instead within a four-species clade alongside *Ruschia phylicoides* and the two species of *Scopelogena*. This clade is typified by a lack of closing bodies in the capsules and an unusual preference (in Aizoaceae) for relatively low-nutrient soils (Klak, 2000; Klak, Buys, et al., 2013; Klak & Buys, 2012) but is otherwise typical of the other woody ruschioid shrubs that dominate the SK flora. *Conophytum*, on the other hand, is a large, monophyletic genus with 108 species and 165 taxa (Hammer & Young, 2017; Klak, Bruyns, et al., 2013; Powell, 2016), of which 60% are endemic to the SK region and 28% have a total range size <10 km² (Young & Desmet, 2016). All are miniature leaf succulents and lack complex seed retention structures, but floral traits vary greatly in terms of morphology (from short and open to long and tubular), colour (white, yellow, pink and magenta) and circadian phenology (70% day-flowering, 30% night-flowering; Hammer & Young, 2017; Liede & Hammer, 1990). We selected four quartz field sites situated across the Knersvlakte at which both study species occurred (Figure 1). These were: a site in the Groot Graafwater area of the central Knersvlakte ("GG"); a northern site in the Kareeberg area ("KB"); a southern site on the farm Quaggaskop ("QK"); and a south-western site in the Moedverloren area ("MV"). All sites except QK were within the Knersvlakte Nature Reserve. The exact locations of each site are withheld to combat poaching and can be obtained on request. The distances between GG, the central site, and the other sites ranged from 17 to 28 km, with the peripheral sites being 24–42 km apart. Each site spanned an area of less than 1 km² and had largely unbroken quartz cover.

2.2 | Sampling design

At each site, variation in community composition and associated soil chemistry was sampled in October 2014 using a series of circular plots, each of 5 m² area. The number of plots varied between sites, with 37, 35, 51 and 29 plots at GG, KB, MV and QK, respectively. Plot locations were nonrandom, and were chosen to represent the diversity of quartz field plant communities present at each site, with *C. calculus* occurring in 13, 11, 13 and 7 plots, and *R. burtoniae* occurring in 26, 19, 19 and eight plots, at GG, KB, MV and QK, respectively. For each plot, a list of all Aizoaceae taxa (rank dependent on taxonomic uncertainty; see Schmiedel, 2002) present was noted and a soil sample was taken, combining soil from two to three points within the plot, at a depth of ≤10 cm. These samples were submitted to the Analytical Services facility at Elsenburg (Western Cape Department of Agriculture) for pH determination. This was done by sifting the soil to remove grains >1 mm and suspending in KCl, followed by measurement of pH using a standard pH meter.

For assessment of genetic patterns, 23–24 individuals of *C. calculus* and *R. burtoniae* were sampled from each site, giving a total of 95 samples per species across all four sites. Individual plants were sampled from across the species' distribution at each site, taking care wherever possible to sample individuals >5 m apart. Samples comprised leafy cuttings with leaves in good condition.

2.3 | Analysis of community and soil data

In order to characterize the ecological niches of the two focal species in a community context, we explored the relationship of community composition to soil pH across the four sites. Unless otherwise stated, community analysis was conducted in R v. 3.5.3 (R Core Team, 2018) using VEGAN v. 2.5-4 (Oksanen et al., 2019). Using only species present in ≥10 plots across all four sites in order to avoid biases introduced by rare species, between-plot Jaccard distances, based on species presence/absence data, were calculated using "vegdist". Thereafter, "varpart" was used to determine the proportion of variance in the matrix of Jaccard distances explained uniquely by pH (irrespective of site) and by site (irrespective of pH) using adjusted R^2 values. To visualise and assess the significance of the effect of pH on community composition, irrespective of site, we implemented the constrained ordination procedure introduced by Legendre and Anderson (1999) known as distance-based redundancy analysis (dbRDA) using the function "capscale". We specified site as a conditioning variable to ensure that the constrained axis (CAP1) corresponded to variance in community composition explained by pH after "partialling out" the effect of site, and that the unconstrained axes (denoted MDS1, MDS2, etc. and corresponding to orthogonal axes of variance in community composition not explained by pH) were also independent of site-specific variance. Significance was assessed using the randomisation procedure implemented in the function "ANOVA. cca", with 999 randomisations. The niche characteristics of *C. calculus* and *R. burtoniae* were assessed visually using biplots of CAP1 and

MDS1 showing both plot and species mean values. As a complementary analysis, we also assessed species co-occurrence in a pairwise manner and ignoring site and pH, using the method of Veech (2013) implemented in COOCCUR v. 1.3 (Griffith et al., 2016). This provided a probabilistic method to test whether pairs of species co-occur significantly more or less frequently than expected by chance.

2.4 | DNA extraction and sequencing

DNAs were extracted from freshly field-sampled leaf material. For *C. calculus*, we used the Qiagen DNEasy Plant Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's recommendations, with minor modifications. 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. Approximately 0.1 g of PVP was also added to reduce the polyphenol content. 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 modifies the widely used CTAB method of Doyle & Doyle (1987). Where necessary, samples for both species were cleaned and concentrated using the Zymo Genomic Clean and Concentrator kit (Zymo Research).

Samples were sent to the Cornell Institute of Genomic Diversity for GBS (Ithaca, New York) to be processed using a workflow which generates reduced representation libraries for Illumina sequencing following the laboratory protocol outlined in Elshire et al. (2011). EcoT22I (restriction site ATGCAT) was used as the restriction enzyme for digestion as it produced fragments of mostly <500 bp with no evidence of repetitive DNA amplified by PCR. The enzyme PstI (CTGCAG) was also tested but was deemed inferior as it produced fragments ranging from 160 to 1,000 bp. The 95 samples of each species were run, alongside a blank, on separate lanes of a 100 bp single-end Illumina HiSeq 2000 instrument at the Cornell Core Laboratories Centre.

2.5 | Read alignment, SNP calling and SNP filtering

Read parsing, alignment, SNP calling and SNP filtering were all conducted separately for the two species. Raw reads were parsed, quality-filtered and trimmed using Trimmomatic (Bolger et al., 2014), and demultiplexed using the GBS-SNP-CROP pipeline v. 1.1 (Melo et al., 2016). Read alignment and variant calling were conducted following the DDOCENT pipeline v. 2.2.19 (Puritz et al., 2014). Reads were aligned to a draft genome of *Schlechteranthus steenbokensis* (H.E.K. Hartmann) Klak (S. A. Schlebusch, unpublished data), another species within the core Ruschioideae, using the BWA-MEM algorithm in BWA v. 0.7.15-r1140 (Li, 2013) with clustering similarity = 90% and all other parameters set to the (conservative) defaults. The draft genome is ca. 1 Gb in size with scaffold N50 = 4.8 Kbp. We implemented three variant calling algorithms: FREEBAYES v. 1.3.2 (Garrison

& Marth, 2012), NGSEP3 v. 3.3.2 (Tello et al., 2019) and PLATYPUS v. 0.8.1.1 (Rimmer et al., 2014), all with the default parameters. For each of the resultant variant call format (VCF) files a first round of filtering removed individuals with >60% missing data (five individuals of *R. burtoniae* and one of *C. calculus*), flagged variants, indels, nonbiallelic SNPs, SNPs with a minor allele count of one, genotypes with read depth <5, and SNPs with >10% missing data. For these steps we used VCFtools (Danecek et al., 2011) and VCFLIB v. 1.0.0 (Garrison, 2012). Of the remaining SNPs, we kept only those called by all three variant callers using BCFTOOLS v. 1.9 (http://samtools.github.io/bcftools), using the FREEBAYES VCF as the template for downstream analyses. Next, SNPs in putative linkage disequilibrium (LD) were removed using the sliding-window "indep-pairwise" function in PLINK v. 1.90b4 (Purcell et al., 2007), with pairwise LD R^2 calculated for SNPs within 50 bp windows, SNPs pruned until no pairs with $R^2 > .2$ remained, and the window shifted five SNPs after each step. Finally, loci present in putative repeat regions of the *S. steenbokensis* genome (SA Schlebusch, unpublished data) were removed using BEDTOOLS v. 2.28 (Quinlan, 2014).

2.6 | Analysis of genetic data

All analyses, unless otherwise indicated, were conducted in R v. 3.5.3 (R Core Team, 2018). VCF files were read into R using VCFR v. 1.9.0 (Knaus & Grünwald, 2017) and converted to genlight and genind objects native to ADEGENET v. 2.1.2 (Jombart & Ahmed, 2011) for analysis. Global between-site genetic differentiation and bootstrapped 95% confidence intervals were estimated using the F_{ST} estimator of Weir and Cockerham (1984), which is appropriate for large SNP data sets (Willing et al., 2012), using the functions "wc" and "boot.vc" in HIERFSTAT v. 0.4.22 (Goudet, 2005).

We implemented two methods to assess genetic structure at the between-site scale. Firstly, a principal component analysis was done using the "glPCA" function in ADEGENET. Secondly, individual assignment analysis was conducted using the sparse non-negative matrix factorization (sNMF) algorithms developed by Frichot et al. (2014), which are analogous to those used in other genetic clustering (i.e., "STRUCTURE-like"; Pritchard et al., 2000) programs, to produce individual ancestry coefficient estimates for the number of clusters (K) ranging from 2 to 8, with 100 runs for each K, using the R package LEA v. 3.0.0 (Frichot & François, 2015). VCF file conversion to STRUCTURE format was done using PGDSPIDER v. 2.1.1.5 (Lischer & Excoffier, 2011). The entropy-based cross-validation technique (implemented in LEA) and PCA eigenvector plots were used to aid the choice of most likely K for each species, as recommended by Frichot and François (2015). The resulting set of Q-matrices of ancestry proportion estimates were summarised using the CLUMPAK v. 1.1 online server (http://clumpak.tau.ac.il/; Kopelman et al., 2015), which identifies major and minor modes based on the degree of consensus between runs within each K. The resulting estimated ancestry coefficients were plotted using the R package POPHELPER v. 2.3.0 (Francis, 2017).

Mantel tests (Diniz-Filho et al., 2013; Mantel, 1967) were used to test for isolation-by-distance (IBD) between the four study sites. For this purpose, Weir and Cockerham's F_{ST} was used to estimate pairwise genetic differentiation between all site pairs (with 95% confidence intervals), using the package STAMPP v. 1.5.1 (Pembleton et al., 2013). Between-site Euclidean (i.e., straight-line) geographic distances were calculated using the "dist" function after coordinates had been transformed to Mercator projection using GEOSPHERE v. 1.5-7 (Hijmans, 2017). In addition, the function "genleastcost" in POPGENREPORT v. 3.0.4 (Adamack & Gruber, 2014) was used to estimate least-cost on-quartz pairwise distances between sites. This was done using a polygon which roughly outlined the distribution of quartz on the Knersvlakte, determined by visual inspection of Google satellite imagery and generated in QGIS (QGIS Development Team, 2016), and which imposed 100-fold lower "cost" values for connectivity on quartz relative to non-quartz, thereby serving to minimize the off-quartz component of the estimated paths. Correlations between linearized F_{ST} ($F_{ST}/(1-F_{ST})$) and log-transformed geographic distances were tested using Mantel tests implemented in VEGAN v. 2.5-4 (Oksanen et al., 2019). Since six pairwise comparisons yield 24 Mantel permutations of the data (including the observed permutation), these tests had limited statistical power, with a minimum possible p-value of .042.

Two approaches were used to investigate spatial genetic structure between individuals within sites. Firstly, the correlation between log-transformed Euclidean geographic and genetic distance for each species at each site was assessed using Mantel tests calculated in vegan, with 9,999 permutations. Pairwise genetic distance matrices were estimated using "gd.kosman" in POPGENREPORT v. 3.0.4 (Adamack & Gruber, 2014), which determines Kosman's genetic distance (Kosman & Leonard, 2005). Secondly, we implemented the "Mapping Averaged Pairwise Information" (MAPI) approach in MAPI v. 1.0.1 (Piry et al., 2016) to visualise and test two-dimensional genetic divergence patterns for each species at each site, again using Kosman's genetic distance. Briefly, the MAPI approach entails the following: (a) a hexagonal grid is drawn so as to encompass the full geographic extent of all sampled individuals; (b) each pairwise genetic distance has its value assigned to an ellipsis connecting the pair; and (c) each grid cell is assigned the mean value of all ellipses that intersect it (this is denoted m_w). Then, to test the null hypothesis that m_w values are randomly distributed in space, a null distribution of m_w values is generated by randomly permuting the sampling localities 5,000 times, each time repeating the MAPI procedure. Using this null, the m_w of each cell is assigned a probability of being either higher or lower than expected, and each p-value is subjected to the false discovery rate procedure of Benjamini and Yekutieli (2001) to account for positive spatial autocorrelation. Since the eccentricity of the ellipses may affect the outcome of the analysis, the above procedure was repeated for eccentricities of 0.8, 0.9, 0.975 and 0.99. The results were qualitatively similar for different eccentricities (see Supporting Information) so we continued with the default value of 0.975.

To assess whether areas of unsuitable edaphic habitat, as determined on the basis of pH, corresponded to areas of high genetic

turnover, genetic distance values were extracted from the MAPI plots for each point with community and pH data using RASTER v. 3.0.12 (Hijmans, 2020), and the relationship between pH and genetic distance was assessed using separate linear models for each species at each site.

2.7 | Common garden experiment

We conducted a common garden experiment to assess: (a) the potential influence of edaphic heterogeneity in limiting the dispersal and establishment of *C. calculus* and *R. burtoniae* at both coarse (between sites) and fine (within sites) scales; and (b) the extent to which adaptation to local soils might explain genetic differentiation between the four sites. For this purpose, seeds of both species were collected at the central GG site from at least 40 individuals, mixed thoroughly, and then counted under a dissecting microscope. Seeds of each species were sown into four soils: (a) "home soil", being soil sampled from a single plot at GG within which the focal species was abundant; (b) "alternative soil", being soil sampled from a single plot at GG from which the focal species was absent; (c) soils sampled from single plots at each of the other three sites within which the focal species was abundant ("KB soil", "MV soil", "QK soil"); and (d) "matrix soil", being soil sampled from the sandy matrix separating quartz patches, at a point close to GG (at 31°17′42.5″S, 18°29′08.2″E). To ensure that the alternative soils contrasted with those favoured by *C. calculus* and *R. burtoniae* soils, these were sampled from plots dominated by species (*Argyroderma framesii* and *Knersia diversifolia*, respectively) which show strong separation from the focal species in the community ordination (Figure 2). In all instances, soils were first sifted to remove particles >2 mm, mixed with 50% (by mass) sterile river sand to improve drainage and avoid fungal infection in the more humid Cape Town climate and under greenhouse growth conditions (S. Hammer, personal communication), and dispensed into 5 cm × 5 cm × 6 cm seedling pots.

For each species-soil type combination, 200 seeds were sown into two pots and ultimately thinned to eight individuals (per pot) situated >0.5 cm apart to minimize competitive interactions. Where germination rates were too low to yield eight seedlings, additional seedlings were added from reserve pots in which 50 seeds had been sown. Growth monitoring was started within a week of germination, with measurements taken on a monthly basis and continuing for up to 4 months (125 days). Both species exhibited low growth rates, with *R. burtoniae* plants growing vertically and producing on average three to four leaf pairs over the course of the experiment, and *C. calculus* plants growing by expansion of a single spheroidal leaf pair generally with a diameter «1 cm. In *R. burtoniae*, therefore, growth was measured in terms of plant height, using a 0.5 mm-resolution steel ruler, while in *C. calculus*, growth was measured in terms of diameter, using a dissecting microscope at 10X magnification equipped with a 0.1 mm-resolution eyepiece graticule. Pots and focal individuals were marked uniquely, the latter being achieved by sinking pins with heads of different colours into the soil next to sample individuals. If
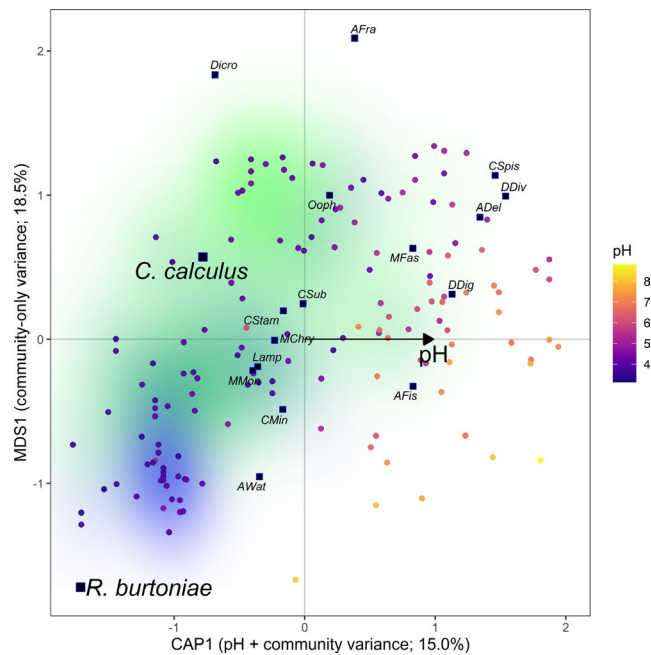
FIGURE 2 Ordination biplot of the distance-based redundancy analysis (dbRDA) of Aizoaceae community composition across the four study sites, with variation between sites conditioned out. The plot is scaled relative to species using Hill scaling. The proportion of variance in the first constrained axis (CAP1) explained by pH, and its direction, are indicated by the arrow. Colouring of points indicates plot pH. The first unconstrained axis (MDS1) represents variation in community composition not associated with site or pH. The percentage of variance explained by each axis is indicated in the axis titles. The two focal species are highlighted in larger text, and density kernels are drawn based on plots inhabited by *Conophytum calculus* (green shading) and *Ruschia burtoniae* (blue shading). See Table S1 for the key to abbreviated species names [Colour figure can be viewed at wileyonlinelibrary.com]

a new seedling was discovered to have germinated within 0.5 cm of a focal individual within a nongrowth pot, monitoring was ceased to ensure that competition between neighbouring plants during the period between measurements did not affect the results.

The pots were arrayed in a grid with pots randomly arranged relative to soil type but grouped by species in 7 × 4 pot trays arrayed in two rows of two 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 Upper Campus, and the grid was placed in a sunny, airy part of the facility to avoid detrimental effects of excessive humidity (S. Hammer, personal communication).

We followed the approach of Paine et al. (2012) in using (mostly) nonlinear models to estimate separate growth curves for each group of interest, with 95% confidence intervals used to determine statistical significance. For each species, we fitted independent models for each soil type, relating size (i.e., height or diameter) measurement day, with pot and individual included as random effects to account

for block effects and repeated measurements, respectively. Models were fitted using NLME v. 3.1-143 (Pinheiro et al., 2019), with logistic, Gompertz and linear link functions. The best-fitting models were chosen using pseudo-R^2, calculated following Paine et al. (2012) for the logistic and Gompertz fits, and following Nakagawa et al. (2017) for the linear fits, in the package MUMIN v. 1.43.15 (Barton, 2019). In most cases, growth curves were asymptotic, for which logistic and Gompertz fits performed almost identically; therefore, logistic fits were preferred arbitrarily. In these cases, asymptotes were allowed to vary randomly by individual and pot. Where linear fits worked best, intercepts were allowed to vary randomly. We followed Paine et al. (2012) in calculating absolute (AGR) and relative growth rates (RGR) based on estimated model coefficients, with confidence intervals estimated using hierarchical bootstrapping (R code modified from code written by B. Bolker available in Supporting Information), which accounts for uncertainty in the random effect parameter estimates by sampling both within and between individuals. We recalculated AGR and RGR for each bootstrap iteration in which a new model was estimated in order to ensure equivalent confidence intervals across metrics. Parallelised bootstrapping was performed with up to 1,100 sampling iterations using the package FUTURE.APPLY v. 1.4.0 (Bengtsson, 2020). Some iterations resulted in models that did not converge, and these were excluded.

3 | RESULTS

3.1 | Edaphic variation and community composition

Variance partitioning showed that both pH and site independently explained a substantial proportion of the total variance in Knersvlakte succulent community composition, though the effect of pH was much greater (pH|site: adj. R-squared = 0.151; site|pH: adj. R-squared = .0632). A nonparametric ANOVA of the dbRDA model suggested that pH was highly significant as a main effect after conditioning for the effect of site ($F_{1,147} = 25.9$, $p = .001$) implying a constant effect of pH on community composition at all sites. Figure 2 shows the results of the dbRDA (see Table S1 for abbreviation key). The constrained axis (CAP1), corresponding to variation in community composition that correlated with pH, explained 14.96% of the total variance in community composition after conditioning out the effect of site, while the first unconstrained axis (MDS1), representing variation not explained by site or pH, explained an additional 18.54%.

Ruschia burtoniae displayed the strongest association with low pH soils of any species, with the lowest CAP1 score (−1.72), a mean pH of 3.98 ± 0.50 *SD* and a pH range of 3.1 to 6.0 across plots in which it occurred ($n = 72$). In contrast, *C. calculus* displayed a weaker affinity for low pH soils, with a CAP1 score of −0.78, a mean pH of 4.22 ± 0.67 *SD*, and a pH range of 3.4 to 7.0 ($n = 44$). A two-sided *t* test revealed a significant pH difference between plots occupied by *R. burtoniae* and *C. calculus* ($t = −2.01$, $df = 72.3$, $p = .0483$). *Ruschia burtoniae* also had more unusual and
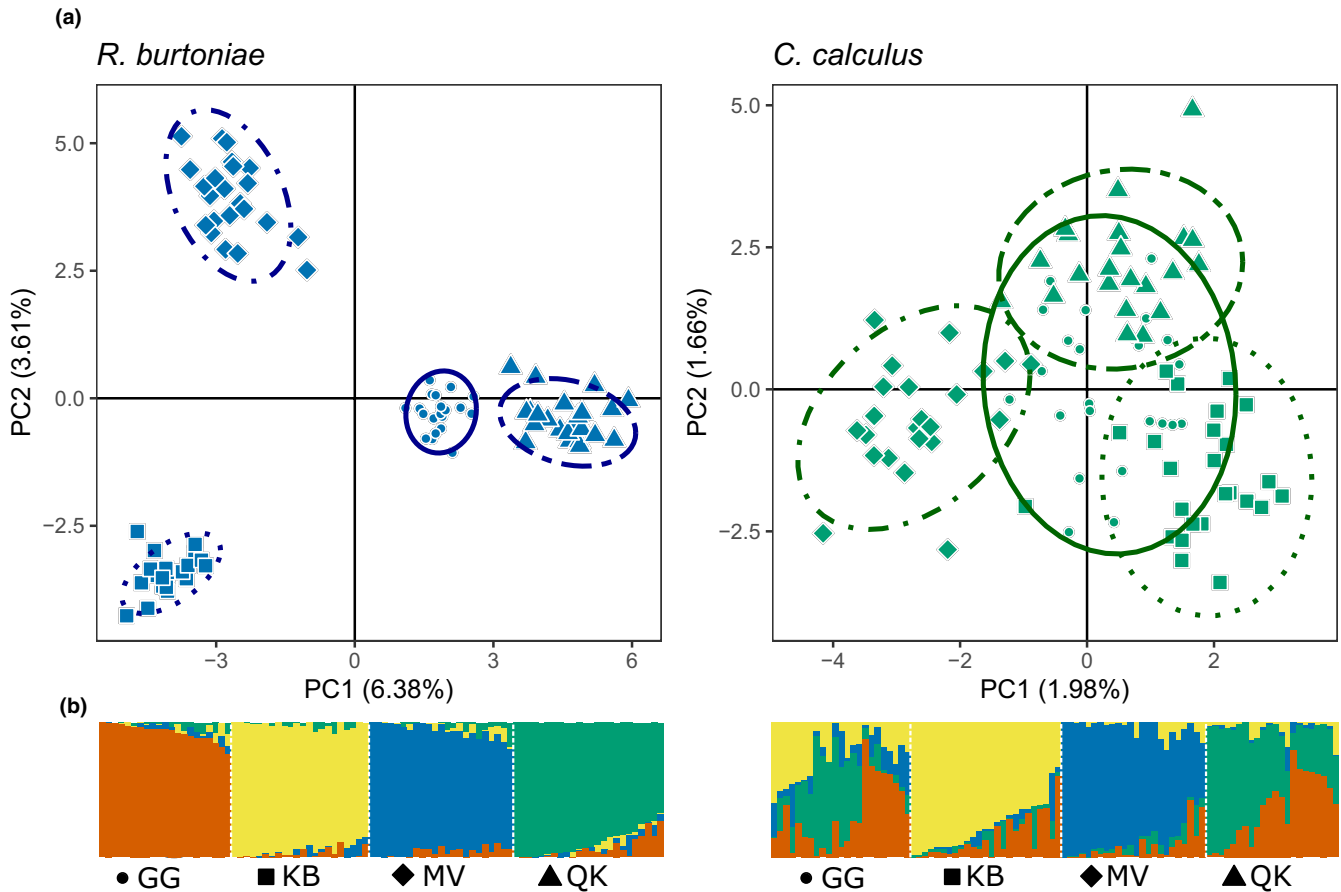
FIGURE 3 (a) Principal component analysis biplots and (b) sNMF individual assignment results showing estimated ancestry coefficients for the major mode identified by CLUMPAK (summarised over 100 sNMF runs) at $K = 4$ for *Ruschia burtoniae* and *Conophytum calculus*. In the PCA plots (a), the proportion of variance explained by each axis is shown in parentheses, points represent individuals, shapes represent sites (as in b) and 95% confidence ellipses are drawn for each site. In the assignment plots (b), colours represent different genetic clusters, bars represent individuals (grouped by site), and the proportion of each colour in each bar represents the estimated ancestry coefficient for that cluster for that individual. For clarity, individuals are sorted by their estimated ancestry coefficient within each site. Note that CLUMPAK placed all 100 runs at $K = 4$ under the major mode for *R. burtoniae*, whereas 65/100 runs at $K = 4$ were placed under the major mode for *C. calculus*. Note also that $K = 4$ was the best-supported K for *R. burtoniae* but $K = 1$ was best for *C. calculus*; we show $K = 4$ only for comparison [Colour figure can be viewed at wileyonlinelibrary.com]

extreme community-level associations than *C. calculus*, as shown by its more extreme position on MDS1 (MDS1 = −1.72 and 0.57, respectively).

Consistent with this result, field observations suggest that *R. burtoniae* often occurs as the only plant species in large swathes of quartz field habitat. Specifically, where *R. burtoniae* occurred as the only Aizoaceae species in nine out of 72 plots, *C. calculus* never occurred alone in a total of 44 plots. *Ruschia burtoniae* stood out in the co-occurrence analysis for showing the strongest and largest number of negative co-occurrence coefficients with other species (Figure S1), whereas for *C. calculus* most pairwise associations were random (Table S2). Nonetheless, *C. calculus* did occur with *R. burtoniae* more frequently than expected by chance (observed = 28 plots, expected = 20.8, $p = .008$). Altogether these results indicate that habitat suitability decreases with increasing pH for both focal species, but that *R. burtoniae* is limited to a narrower and more extreme (low) pH range.

3.2 | Genetic data

Sequencing produced 273,628,335 raw reads for *R. burtoniae* and 260,757,274 for *C. calculus*, of which ca. 90% contained a barcode and cut site. Respectively, reads from *R. burtoniae* and *C. calculus* aligned to 152,702 and 185,828 unique sites on 76,682 and 84,753 unique scaffolds of the *S. steenbokensis* draft reference genome, with coverage means of 364.2 and 265.6 and read mapping rates (reads aligned per reads sequenced) of 20.3% and 18.9%. Following variant calling and filtering, we retained 91 individuals and 7,560 SNPs on 2,298 scaffolds for *R. burtoniae*, and 94 individuals and 15,354 SNPs on 2,470 scaffolds for *C. calculus*.

All analyses consistently recovered greater genetic structure in *R. burtoniae* than in *C. calculus*. For *R. burtoniae*, global F_{ST} was 0.0676 (0.0636–0.0717 95% CI; $p = .001$), with both the assignment analysis and PCA (Figure 3 and Figure S2) indicating that the four sites represent genetically distinct populations. The cross-validation

by entropy criterion analysis showed the lowest entropy at $K = 4$ (Figure S4), and PCA eigenvectors showed a drop in explained variance from the first to fourth component axes, followed by a plateau (Figure S5), also supporting four distinct clusters. In contrast, global F_{ST} was 0.0090 (0.0078–0.0102 95% CI; $p = .001$) for *C. calculus*, which showed very limited spatial genetic structure, with both the assignment analysis and PCA recovering MV as somewhat distinct from the other three sites, which were not reliably distinguishable from each other (Figure 3 and Figure S3). For *C. calculus*, entropy showed a steady increase from $K = 1$ to $K = 8$, while PCA eigenvectors showed a steep drop in explained variance from the first to second axes (Figures S4 and S5), reflecting the distinctness of MV and a lack of further structure in the data.

Evidence of between-site isolation-by-distance (IBD) also differed markedly between the species (Figure 4). Although differentiation was low in *C. calculus*, it showed a strong and significant pattern of IBD between sites. The stronger IBD effect for on-quartz distance was caused primarily by the MV-QK pair, which has an on-quartz distance almost twice that of its straight-line distance (see Figure 1). In contrast, *R. burtoniae* showed no IBD pattern.

At the fine-scale (i.e., within sites, with between-individual distances <500 m) spatial genetic structure was remarkably strong in both species but considerably more pronounced in *R. burtoniae*. Mantel tests (Table 1) showed strong correlations between straight-line distance and Kosman's genetic distance at all sites for *R. burtoniae*, with *C. calculus* also showing significant but weaker correlations at all sites except GG. The MAPI analysis (Figure 5) revealed a strong pattern of spatially 'clustered' genetic variation in *R. burtoniae* at all sites except GG, with large, contiguous groups of grid cells having

higher genetic turnover than expected by chance separating small, relatively close (<50 m apart) groups of genetically similar individuals. In contrast, the MAPI analysis for *C. calculus* showed a much more even distribution of genetic turnover at all sites, with no notable regions of significantly high or low turnover, reflecting pure isolation-by-distance rather than spatial genetic clustering.

For *R. burtoniae*, plots with high pH had greater inferred genetic distances at all sites except GG (Table 2, Figure S6), with the MAPI analysis (Figure 5) demonstrating that genetically diverged clusters of genetically similar individuals are separated by unsuitable, high pH habitat. In contrast, this pattern was never observed for *C. calculus*.

3.3 | Common garden experiment

The number of individual plants with repeated growth measurements in each soil type ranged from 10 to 19 for *R. burtoniae* (all with four repeated measurements), and from 10 to 16 for *C. calculus* (three to four repeated measurements). Growth profiles (Figure 6) were explained well by logistic fits for all soil types for *R. burtoniae* (pseudo-R^2 range .82–.94), whereas they were generally less effective for *C. calculus*, with linear fits being favoured for growth profiles in home, matrix and QK soil (pseudo-R^2 .77, .90 and .78, respectively) and logistic fits being favoured for alternative, KB and MV soil (pseudo-R^2 .84, .92 and .85, respectively). Nonoverlapping 95% confidence intervals in growth curves as well as in changes in AGR and RGR over time for *R. burtoniae* indicated significantly reduced growth in soil taken from high-pH communities on quartz (alternative soil) as well as in soil from the surrounding nonquartz matrix
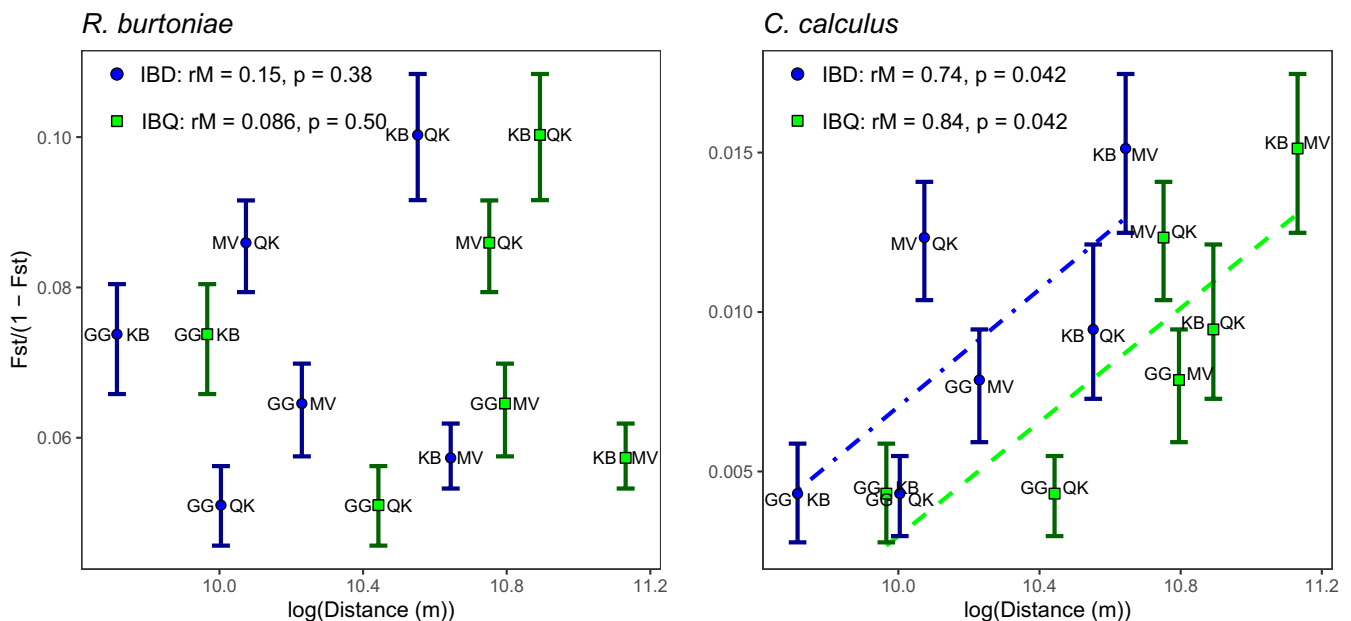

FIGURE 4 Plots to show isolation-by-distance (IBD; blue circles) and isolation-by-quartz (IBQ, i.e., on-quartz distance; green squares), indicating the correlation between linearised pairwise F_{ST} and log-transformed geographic distances. Error bars are bootstrapped 95% confidence intervals. The identity of each pairwise comparison is indicated at each point. Lines show predictions of linear models, although significance was tested using Mantel tests, the results of which are displayed. rM = Mantel's r [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Results of the fine-scale Mantel tests for each species at each site

Species	Site	Mantel's r	p-Value
Ruschia burtoniae	GG	0.248	<.001***
	KB	0.270	<.001***
	MV	0.661	<.001***
	QK	0.364	<.001***
Conophytum calculus	GG	−0.084	.771
	KB	0.149	.006**
	MV	0.171	.027*
	QK	0.118	.042*

Note: p-values are derived from 9,999 permutations of the data.
*p < .05;
**p < .01;
***p < .001.

(matrix soil) relative to that in home soil (Figure 6 and Figure S7). In addition, there was some evidence of a contracted growth period in QK soil relative to home soil. However, no differences in growth profile were observed in MV and KB soil. By contrast, *C. calculus* showed no evidence of reduced growth rate in any soil relative to home soil, with seedlings unexpectedly performing worse in home soil than in alternative soil (Figure 6).

4 | DISCUSSION

Consistent with our expectation of fine-scale differentiation in SK plants, population-level genetic differentiation in *R. burtoniae* was strong (global $F_{ST} = 0.0676$) given the proximity of our sampling localities (17–42 km apart). This pattern is comparable with that observed in another Knersvlakte-endemic succulent, *Argyroderma pearsonii* (Aizoaceae), which showed a global $F_{ST} = 0.07$ across six populations located <10 km apart (Ellis et al., 2007). Although comparable levels of population differentiation have also been observed in several CFR plants, the spatial scale of sampling in these species was typically much broader. For example, *Protea repens* (Proteaceae) had a global $F_{ST} = 0.063$, but this was based on sampling of sites spanning a range of >800 km (Prunier et al., 2017). Similarly, *Restio capensis* (Restionaceae) had a global $F_{ST} = 0.030$, based on sampling of sites spanning a range of >500 km (Lexer et al., 2014). Finally, within the white *Protea* clade (Proteaceae) mean pairwise population differentiation within genetically defined species clusters was $F_{ST} = 0.081$ (Prunier & Holsinger, 2010), while species within the *Seriphium plumosum* complex (Asteraceae) had F_{ST} in the range 0.004–0.061 (Shaik, 2019). In both these examples, most species have ranges spanning several hundred kilometres. Although the fine spatial scale of genetic differentiation observed in both *R. burtoniae* and *A. pearsonii* is suggestive of generally finer-scale differentiation in SK plants than in fynbos plants, such generalization is premature, pending corroborative evidence from additional species. Indeed, the other species sampled in the present study, *C. calculus*, showed much

weaker differentiation (global $F_{ST} = 0.009$), a pattern which we ascribe to differences in the species' biology (see below).

The hypothesis that fine-scale genetic differentiation in the SK flora is linked to fine-scale patchiness of edaphic habitats appears to hold for *R. burtoniae*. Our results support previous work showing that pH strongly affects plant community composition in the Knersvlakte (Schmiedel & Jürgens, 1999), and the transplant experiments show that *R. burtoniae* seedlings experience reduced growth in unsuitable soils. Reduced seedling growth during the winter rains is likely to affect survival probability over the hot, dry summer as competition with adult plants intensifies (Milton, 1995; Musil et al., 2005, 2009), increasing the likelihood that unsuitable soils present a barrier to movement. Furthermore, because our common garden experimental approach controls for other potential soil-specific factors (e.g., herbivory and droughting) that may further reduce performance in unsuitable soils, the results probably more closely reflect the effect of edaphic variation alone.

Community and common garden transplant data indicate that *R. burtoniae* prefers strongly-leached, low-pH soils, a preference shared by the closely-related *Scopelogena bruynsii* and *R. phylicoides* (Klak, Bruyns, et al., 2013), both of which associate with fynbos vegetation growing on low-nutrient, acidic sands (Klak, Bruyns, et al., 2013; Klak & Buys, 2012). Since soils of this type, on the Knersvlakte, are typically associated with higher-lying areas, which are inselberg remnants of fluvial erosion processes that have dissected the Knersvlakte landscape since the Early Cretaceous (Kounov et al., 2008), their contemporary distribution is patchy. The strong edaphic specificity of *R. burtoniae* explains both why genetic variation in this species is clustered at the fine spatial scale (i.e., within-site, between individuals <500 m apart), with genetic turnover at this scale being greatest across areas of unsuitable edaphic habitat, and why genetic differentiation shows no relationship to geographic distance at the coarse spatial scale (i.e., across sites, 17–42 km). The spatial scale of gene flow is not, however, a function of edaphic barriers alone, with seed and pollen dispersal distances influencing whether gene movement across such barriers is possible (Wiens, 1976). Our results support the hypothesis that the complex seed retention structures of *R. burtoniae* (and most Ruschioideae) restrict long-distance seed dispersal (Ihlenfeldt, 1994; Parolin, 2001, 2006) and, in line with expectations for spring-flowering SK Aizoaceae that employ a generalist pollination syndrome (Ihlenfeldt, 1994; Scodanibbio, 2002), they suggest that pollination in *R. burtoniae* is localised.

In contrast to *R. burtoniae*, the edaphic heterogeneity hypothesis does not explain genetic structure in *C. calculus*. Instead, geographic distance appears to be the primary determinant of genetic divergence in this species. Whether the isolation-by-distance pattern observed in *C. calculus* is a product of ongoing gene flow or recent range expansion (Peter & Slatkin, 2013), both scenarios imply greater dispersal ability in *C. calculus* than in *R. burtoniae*. In part, this may be due to the weaker edaphic specificity of *C. calculus* compared with *R. burtoniae*, which may facilitate this species' movement both within and between quartz islands. Although our common
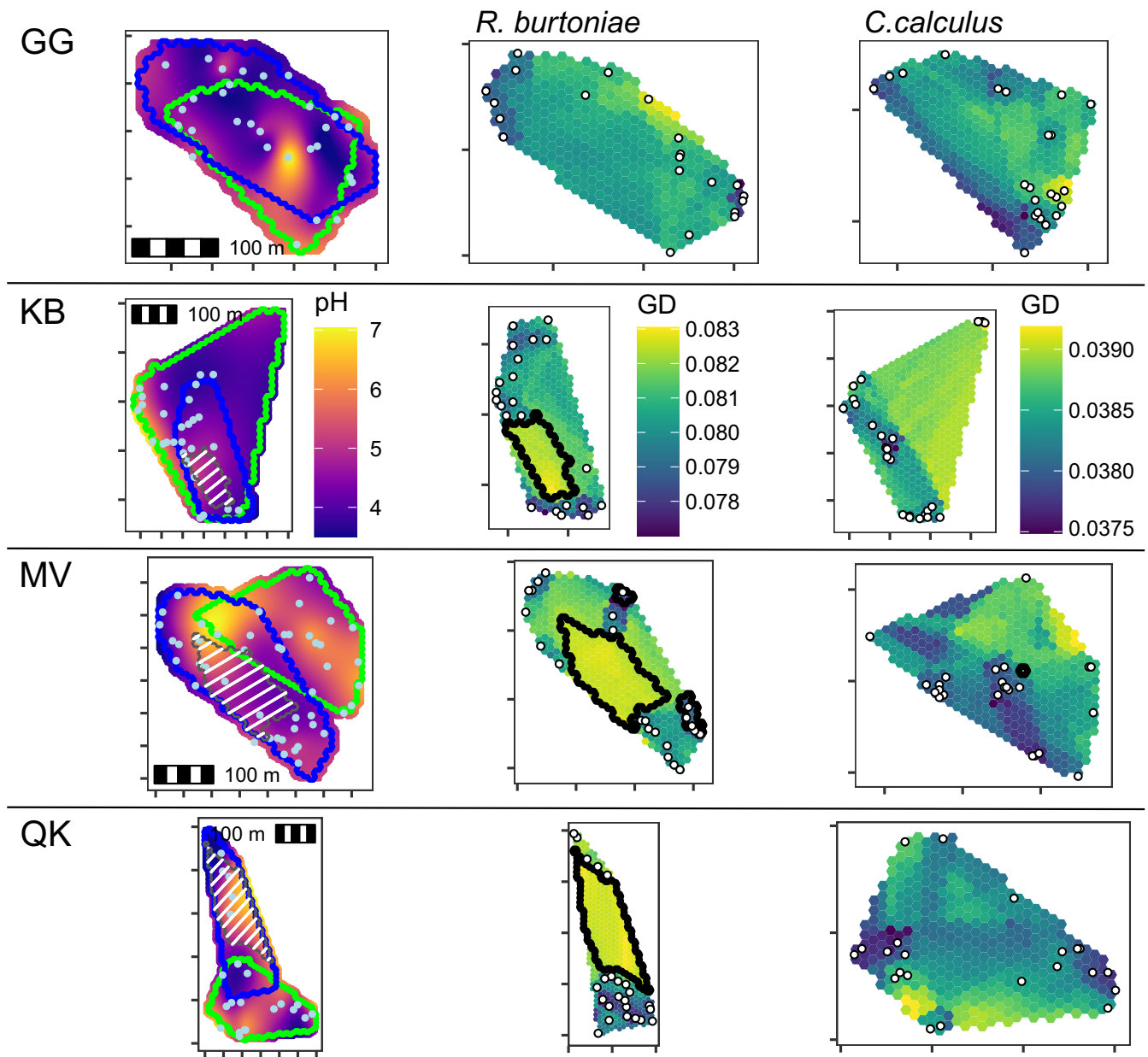
FIGURE 5 Maps to show spatial variation in pH and genetic distance. First column: maps of each site showing outlines of the MAPI grids for *Ruschia burtoniae* (blue) and *Conophytum calculus* (green), pH sampling points (pale blue points), interpolated pH values (colour shading), and areas of higher-than-expected genetic distance for *R. burtoniae* (hatched regions). Second and third columns: results of the MAPI procedure showing inferred mean pairwise genetic distances, genetic sampling locations (points), and areas of higher or lower than expected genetic turnover (black). GD, Kosman's genetic distance. Note the different scales for genetic distance for the two species [Colour figure can be viewed at wileyonlinelibrary.com]

garden results suggest that *C. calculus* is capable of growing in matrix soils, however, it is unlikely to do so in the wild as the wind-blown nature of these sands would result in seedling burial, and the lack of quartz would make them susceptible to overheating (Musil et al., 2005, 2009; Schmiedel & Jürgens, 2004). Instead, it seems likely that *C. calculus* is able bridge the gap between quartz islands by persisting in marginal quartz habitats which have isolated pebbles that provide shelter to seedlings. In addition, a lack of complex seed retention structures and large numbers of seeds per fruit may facilitate colonisation of isolated quartz patches. Moreover, finding low

to moderate overall genetic differentiation in several *Conophytum* species elsewhere in the SK, Powell et al. (2019) have suggested that wind-mediated dispersal of whole capsules enables gene flow between isolated *Conophytum* populations separated by >60 km of unsuitable habitat. Finally, long-distance pollen movement may also contribute to genetic connectivity in *C. calculus*, as its moth pollinators (Jürgens, 2004; Jürgens & Witt, 2014) are likely to be strong flyers and may be forced to search farther afield for nectar sources in late summer when little else is flowering (Hammer, 2002; Liede & Hammer, 1990). The flowers of this species are also scented

Species	Site	Slope (SD)	Adjusted R^2	df	p-Value
Ruschia burtoniae	GG	−1.55e−4 (1.22e−4)	0.01	44	.21
	KB	5.73e−4 (2.28e−4)	0.15	28	.018[*]
	MV	5.06e−4 (1.84e−4)	0.1	56	.008[**]
	QK	8.12e−4 (3.43e−4)	0.26	12	.035[*]
Conophytum calculus	GG	−0.44e−5 (6.71e−5)	−0.05	16	.72
	KB	2.89e−5 (6.70e−5)	−0.03	26	.67
	MV	1.01e−4 (6.98e−5)	0.06	16	.168
	QK	3.10e−5 (6.74e−5)	−0.06	14	.653

Abbreviations: *df*, degrees of freedom; *SD*, standard deviation.

[*]$p < .05$;

[**]$p < .01$;

[***]$p < .001$.

(Jürgens, 2004) which is likely to enhance their attractiveness to pollinators (Jürgens & Witt, 2014; Parachnowitsch et al., 2012). While a comparison of maternally versus biparentally inherited markers would provide insight into the importance of seed versus pollen-mediated gene flow, we were unable to confidently recover adequate chloroplast markers from our data set to do this.

In addition to its effect as a barrier to seed and pollen dispersal, edaphic heterogeneity may promote genetic divergence via local adaptation, and the associated genetic hitchhiking/pleiotropy and selection against immigrants that this entails (Kawecki & Ebert, 2004; Rice & Hostert, 1993). Whereas *C. calculus* showed no evidence of reduced growth in soils from other sites, *R. burtoniae* exhibited reduced growth in QK relative to home soil which might be interpreted as evidence of local adaptation, though confirmation of this idea would require reciprocal transplants showing that QK plants perform poorly on GG soil. Importantly, however, no reduction in growth was observed in soil from the other two sites (KB and MV), suggesting that genetic differentiation in *R. burtoniae* may be driven more by landscape barriers to gene flow than by local edaphic adaptation. This inference is corroborated by the relative genetic similarity of GG to QK (compared to that of GG to KB and MV; Figures 3 and 4), the only site to show any potential signal of local adaptation. While we acknowledge that soil variation represents just one axis of selective heterogeneity between sites, with local climate differences potentially being a stimulus for local adaptation, we consider the potential influence of climate to be limited given the relative uniformity of the landscape and the proximity of our sampling sites. Nevertheless, the contrast between *C. calculus* and *R. burtoniae* suggests that reduced gene flow goes hand in hand with local adaptation. This accords well with the work of Ellis et al. (2007) showing that in the Knersvlakte-endemic dwarf succulent *A. pearsonii*, landscape barriers to gene flow are a prerequisite for genetic differentiation even in the context of strong divergent selection between sites.

Although *R. burtoniae* and *Argyroderma* show highly localized genetic differentiation, *C. calculus* reveals that these patterns do not automatically generalize to the entire SK flora, nor even its Aizoaceae component. This is because the genetic mobility of individual species is idiosyncratic, being dependent on a suite of species- and lineage-specific traits that dictate habitat specificity, seed dispersibility and pollen mobility. Given its minute stature, the relatively weak genetic differentiation shown by *C. calculus*, compared to that shown by the taller *R. burtoniae*, is somewhat surprising given that plant size has been identified as a potential driver of diversification rate/density (Boucher et al., 2017, 2020). However, the potential influence of other traits means that this relationship will often be noisy (Boucher et al., 2017) and certainly it does not hold here. Given that minute stature has evolved several times independently in Aizoaceae (Klak, Bruyns, et al., 2013) and is prevalent in other important SK families, this observation brings into focus the question of whether and how minutism might drive diversification.

Inferring speciation mechanism from patterns of population-level genetic differentiation is not straightforward (Wiens, 2004). Given their genetic and even ecological distinctness, the four populations of *R. burtoniae* sampled in this study might represent distinct evolutionary species (De Queiroz, 2007; Wiley, 1978). In our experience, however, these populations show no discernible morphological divergence. Since most plant species, including those that make up the remarkable SK flora, have been differentiated on the basis of morphological distinctiveness (Bickford et al., 2007; Ellis et al., 2014), it is unclear how our understanding of cryptic population differentiation in *R. burtoniae* advances our understanding of the floristic radiation of the SK generally. One possibility is that genetic differentiation represents an important step towards the reproductive isolation of populations (Coyne & Orr, 1989; Moyle et al., 2004; Scopece et al., 2010) which are then free to diverge morphologically, via genetic drift and/or selection (Rieseberg & Willis, 2007), to the point where taxonomists would recognise them as separate species (Treurnicht et al., 2017). Another is that the process of population differentiation described here for *R. burtoniae* is atypical for SK plants, with most differentiation being adaptive and accompanied by morphological divergence. For example, it may be that erosional fragmentation of the Knersvlakte landscape (Kounov et al., 2008) fosters a more neutral mode of differentiation than is typical elsewhere in the SK.
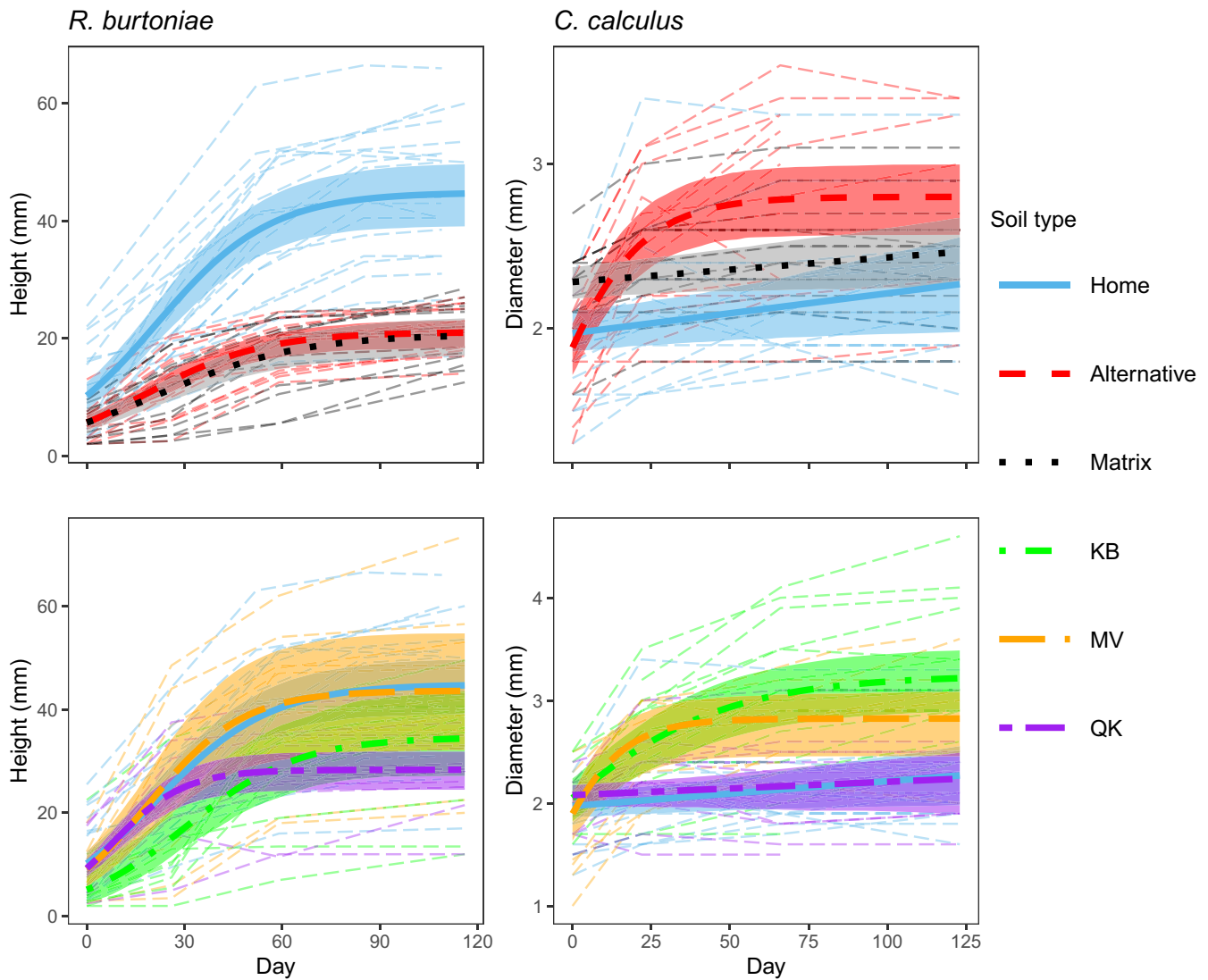
FIGURE 6 Plots to show change in size over time for *Ruschia burtoniae* (left column) and *Conophytum calculus* (right column) seedlings germinated and grown in different soils in a greenhouse, with plots separated into within- and between-site comparisons (top and bottom rows, respectively). Within-site comparisons contrast growth in home (i.e., occupied) quartz soils with that in alternative (i.e., unoccupied, high pH) quartz soil and matrix (i.e., nonquartz) soil. Between-site comparisons contrast growth in home soils with that in occupied quartz soils from other sampling sites. Raw values per individual are shown as narrow dashed lines (coloured by soil type). Predictions of (non-) linear mixed-effects models are shown as thick lines with shaded areas representing 95% confidence intervals determined by bootstrapping [Colour figure can be viewed at wileyonlinelibrary.com]

In another contrast to *R. burtoniae*, *C. calculus* is known to exhibit some variation across the Knersvlakte in vegetative and floral traits (Hammer & Young, 2017; S. Hammer, personal communication), which might reflect adaptive genetic divergence (despite gene flow) or phenotypic plasticity. The closest relatives of *C. calculus* are the sister species *Conophytum breve* and *Conophytum flavum* (Figure 6.3 in Powell, 2016). While the former resembles *C. calculus* in both floral and vegetative traits, the latter is distinct in having larger, day-opening flowers of a different type (Liede & Hammer, 1990) and in being tetraploid (de Vos, 1947) where the majority of *Conophytum*, including *C. calculus*, are diploid (Hammer & Liede, 1992). Both *C. breve* and *C. flavum* typically occur on quartzitic substrates to the north of the Knersvlakte where they are broadly sympatric (Hammer & Young, 2017; Young & Rodgerson, 2017). This pattern is consistent

with the idea that both allopatric/budding speciation (mediated by long-distance seed dispersal) and sympatric speciation (mediated by shifts in floral traits and/or ploidy) have contributed to diversification in *Conophytum* (Hammer & Liede, 1992; Powell, 2016; Powell et al., 2019). Given that there is floral divergence despite evidence of extensive gene flow between *C. calculus* populations, we encourage further research on speciation process in *Conophytum*, e.g., investigating the extent of gene flow between sister taxa with different pollination syndromes.

In conclusion, understanding the uneven global distribution of biodiversity requires that we understand the factors that promote differentiation at fine spatial scales. By focusing on patterns of genetic and ecological divergence in response to eco-spatial landscape features, we hope to gain insight into the early stages

of speciation at fine spatial scales in the SK biodiversity hotspot. Although our data corroborate existing studies (Ellis & Weis, 2006; Ellis et al., 2006, 2007) in identifying soil heterogeneity as a potentially critical driver of fine-scale differentiation, we find that the scale of differentiation is influenced by other factors, notably organismal traits influencing niche specificity and seed and pollen dispersal distances. Future work examining additional species, and integrating genetic, community and trait data, is needed to assess the generality of the processes and patterns described here. In addition, there is a need for comparative studies that relate processes taking place at the population level to diversification pattern at the phylogenetic scale.

AUTHOR CONTRIBUTIONS

G.A.V., S.D.M., and A.G.E. conceived and designed the study. S.D.M. carried out the experiments, analysed the data and produced the figures. S.D.M. wrote the manuscript with the assistance of G.A.V., and A.G.E. S.A.S. assisted with genetic data analysis and contributed unpublished data. All authors reviewed the manuscript.

DATA AVAILABILITY STATEMENT

Illumina reads have been deposited in the NCBI Sequence Read Archive (SRA) under BioProjects PRJNA670095 and PRJNA670851. Community and transplant experiment data and R scripts for their analysis, plus the key file for the GBS data and filtered VCF files for each species, are available for download from Figshare (https://doi.org/10.25375/uct.13129883).

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