South African Journal of Botany 88 (2013) 401–413

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Pleistocene range dynamics in the eastern Greater Cape Floristic Region: A case study of the Little Karoo endemic *Berkheya cuneata* (Asteraceae)

Alastair J. Potts [a,*], Terry A. Hedderson [b], Jan H.J. Vlok [a], Richard M. Cowling [a]

[a] Department of Botany, Nelson Mandela Metropolitan University, P.O. Box 77000, Port Elizabeth 6031, South Africa
[b] Department of Biological Sciences, University of Cape Town, University Private Bag, Rondebosch 7700, South Africa

ARTICLE INFO

Article history:
Received 15 February 2013
Received in revised form 24 July 2013
Accepted 16 August 2013
Available online xxxx

Edited by JC Manning

Keywords:
Berkheya cuneata
Chloroplast DNA
Phylogeography
Nuclear DNA
South Africa
Species distribution modelling

ABSTRACT

The glacial–interglacial climate cycles of the Pleistocene played a significant role in dramatically altering species distributions across the globe. However, the climate of the Greater Cape Floristic Region is thought to have been decoupled from global fluctuations and the current Mediterranean climate remained relatively buffered during this period. Here we explore the roles of climate stability and the topographic complexity of the region on the range history of an endemic Little Karoo plant, *Berkheya cuneata*, using ensemble species distribution modelling and multi-locus phylogeography. The species distribution models projected onto downscaled climate simulation of the Last Glacial Maximum demonstrated a considerable range contraction and fragmentation into the western and eastern Little Karoo, separated by the Rooiberg inselberg. This population fragmentation is mirrored in the phylogeographic structuring of both chloroplast and nuclear DNA. These results suggest that sufficient climatic buffering coupled with regionally complex topography ensured the localised population persistence during Pleistocene climate cycles but these features have also promoted population vicariance in this, and likely other, Little Karoo lowland species.

1. Introduction

The Greater Cape Floristic Region (GCFR, Born et al., 2007) consists of the Fynbos and Succulent Karoo biomes, both of which are globally recognised for their unusually high levels of plant diversity and endemism (Goldblatt and Manning, 2002; Linder, 2003) and are considered biodiversity hotspots (Myers et al., 2000). This diversity is suggested to have arisen, in part, due to exceptional climatic stability during the Pleistocene (Cowling, 1992; Cowling and Lombard, 2002; Cowling et al., 2009; Dynesius and Jansson, 2000; Hopper, 2009). Pleistocene climate cycled between glacial and interglacial states and this had global impacts on the size, distribution, and local extinctions of species' ranges (Hewitt, 2000; Jansson and Dynesius, 2002). However, the climate of the south-western Cape was decoupled from the global Pleistocene climate fluctuations, and the current rainfall regime (winter-wet and summer-arid) is suggested to have remained relatively buffered during this period (Chase, 2010; Chase and Meadows, 2007; Dynesius and Jansson, 2000; Meadows and Sugden, 1993). This localised climatic stability would weaken the effects of orbital-forcing on species' range dynamics and thus reduced species and population extinction rates (Dynesius and Jansson, 2000).

In this study we investigate the role of Pleistocene climate on the range dynamics within the GCFR using species distribution modelling (SDM) and phylogeography (*sensu* Hugall et al., 2002; Svenning et al., 2011). Species distribution modelling can predict ranges under current and altered climate conditions (Franklin, 2010). We project the SDM onto modelled and downscaled climatic conditions of the Last Glacial Maximum; this period represents a glacial extreme of the Pleistocene cycles where global ice volumes were at their maximum. We then compare these predictions with phylogeographic patterns as range dynamics leave genetic imprints on species (Avise, 2000; Waltari et al., 2007). Species distribution modelling and phylogeographic analyses may therefore help to understand the role of climatic factors in promoting differentiation (*e.g.* Hugall et al., 2002) and, ultimately, speciation within the GCFR flora.

Our focus is on *Berkheya cuneata* (Thunb.) Willd. (Fabaceae), a plant species that is endemic to the Little Karoo sub-region of the eastern GCFR. The Little Karoo is a dry intermontane valley within the Cape Fold Belt of the Western Cape, South Africa (Fig. 1). The region is complex, both in terms of topography and climate (Vlok and Schutte-Vlok, 2010; further details below). Our results suggest that sufficient climatic buffering coupled with regionally complex topography ensured localised population persistence during Pleistocene climate cycles and that

* Corresponding author at: Botany Department, Nelson Mandela Metropolitan University, South Africa.
E-mail address: potts.a@gmail.com (A.J. Potts).

0254-6299/$ – see front matter © 2013 SAAB. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.sajb.2013.08.009

these have played key roles in promoting population vicariance in *B. cuneata*.

2. Materials and methods

2.1. Study area and species

The Little Karoo lies between the western winter and eastern summer rainfall regimes and this gives rise to an aseasonal regime that experiences year-round rainfall (Fig. 1A). This sub-region is separated from the more mesic winter-rainfall coastal region of the GCFR by the Langeberg and Outeniqua mountains to the south, and from the more arid and largely summer-rainfall Great Karoo by the Witteberg and Swartberg ranges to the north. These mountains have a large influence on the climate in the Little Karoo, especially the southern mountain ranges which block winter-rainfall frontal systems from the Atlantic and moist rain-bearing air from the Indian Ocean (Fig. 1B, Vlok et al., 2005). A number of inselbergs rise up from the valley floor, the most notable of which is the Rooiberg mountain range. The mountain ranges and inselbergs are geologically associated with the infertile Cape Folded Belt, whereas lowlands comprise more fertile soils derived from Bokkeveld shale and Enon mudstones and conglomerates.

The lowland vegetation within the Little Karoo is dominated by Succulent Karoo vegetation, comprising predominantly of leaf succulents, mainly from the Mesembryanthemaceae, as well as drought-hardy members of the Asteraceae (Vlok and Schutte-Vlok, 2010). Fynbos and renosterveld are vegetation types that require more soil moisture, and are found on the nutrient-poor summits and foothills (respectively) of Little Karoo mountains.

B. cuneata (Thunb.) Willd. belongs to a genus of 75 species endemic to southern and tropical Africa (Goldblatt and Manning, 2000). It is a relatively small perennial shrub with spiny, velvety-grey leaves. The species has typical yellow asteraceous flowers, and is insect-pollinated (Whitehead, 1984). The bracts harden to form a tumbling flowerhead that is dispersed by wind. See Appendix A for habit and habitat photos of this species (Figs. A.1 and A.2). This species is endemic to, but widespread and common in, the Little Karoo, and within the region has strong association with the Succulent Karoo vegetation (Vlok and Schutte-Vlok, 2010). It has experienced a population decline and range contraction in a number of localities due to overgrazing by livestock, and the extinction of at least three local populations has been observed (JHJ Vlok, unpublished observations). The ploidy level within this species and across populations is unknown.

2.2. Species distribution modelling

We used an ensemble modelling approach (Araújo and New, 2007) that addresses the uncertainties surrounding the choice of predictor variables, algorithms and localities. The predictor variables, global climate models, distribution models and thresholds were identical to those used by Potts et al. (2013b). In brief, an ensemble of models was generated using a combination of six sets of climate predictor variables, three locality datasets (obtained *via* k-folding), and six different distribution modelling algorithms (Bioclim, Domain, GLM, GAM, Maxent, Random Forest — settings are given in Table 1), producing a total of 108 models (see Potts et al., 2013b for details). Each of these models was projected onto current conditions and two downscaled global climate models (CCSM and MIROC); these climate layers are available at www.worldclim.org (Hijmans et al., 2005). To facilitate the comparison of outputs from different modelling algorithms (which are incompatible at times), the 'probability of occurrence' maps were reclassified to binary data ('present' or 'absent') using the maximum sensitivity plus specificity threshold criterion (Liu et al., 2005). A mask from 32.5° S to 34.5° S and 19.5° E to 23.0° E was used to sample background data from the climate layers; some of the modelling methods require data akin to absences (known as background or "pseudo-absences"). These background points were generated by sampling all cells (~3500), including cells that coincided with a presence point within the mask, in order to characterise the climate of the sub-region. Of note is that each set of climate predictor variables consists of seven bioclimatic variables randomly selected from a set of 19 (Table B.1); however, in order to avoid multicollinearity – which may affect the modelling of climate envelopes (Dormann, 2007) – a selected variable that had an $R^2 > 0.7$ with any previously selected variables in a given set was replaced by another variable selected at random but with a lower correlation (see Appendix B for details). A minor addition from the details given in Potts et al. (2013b) is that in order to minimise any sampling bias, localities were filtered to ensure that there was a 10 km buffer between stepwise randomly selected localities

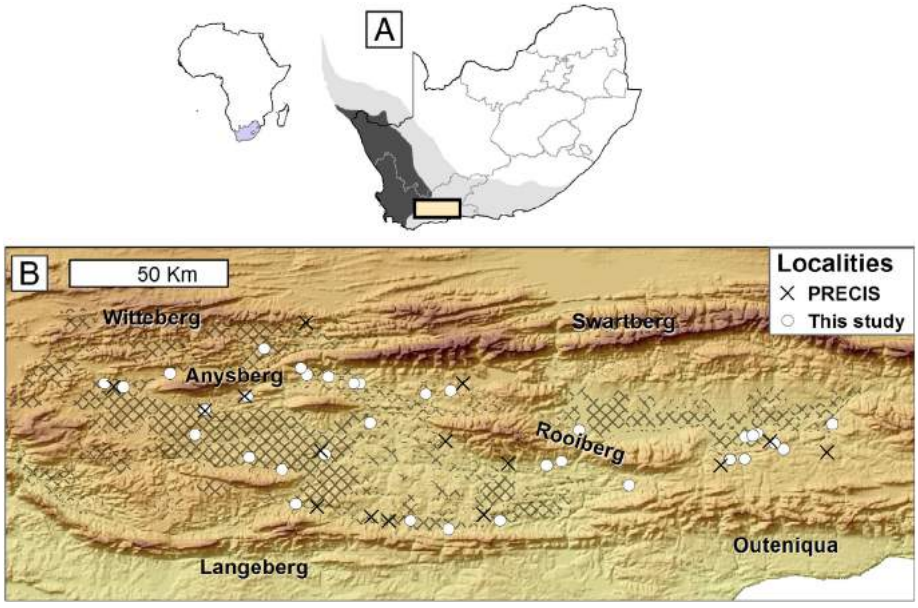

Fig. 1. A) The geographic position of the Little Karoo. The winter, year-round and summer rainfall zones are shown (dark grey, light grey and white shading, respectively). B) The Little Karoo is an intermontane basin that lies between the Witteberg and Swartberg mountain ranges to the north and the Langeberg and Outeniqua mountain ranges to the south. The distribution of the Succulent Karoo vegetation (hatched shading) and localities of *Berkheya cuneata* are shown. *Berkheya cuneata* is widespread in, and endemic to, the Succulent Karoo vegetation of the Little Karoo.

(*i.e.* removing all localities that fell within the buffer of a selected locality). Locality data were obtained from either GPS coordinates of populations visited for genetic sampling or by georeferencing locality descriptions (only localities that could be georeferenced to within an estimated 5 km of the actual sampling locality were used) from the PRECIS herbarium database which is maintained by the South African National Biodiversity Institute. Model performance was evaluated for each combination using a standard statistical measure of predictive ability, the area under the receiver operating characteristic curve (AUC). The AUC statistic ranges from 0.5 (model prediction is no better than random) to 1.0 (perfect model prediction of presence *versus* absence).

2.3. Sample collection, DNA extraction and PCR amplification

Potential sampling sites were identified from herbarium collections, a vegetation survey (Vlok et al., 2005) and from local expert knowledge. Eleven other *Berkheya* species occur in either parapatry or sympatry with *B. cuneata*. However, field identification was unambiguous as numerous leaf and fruit traits distinguish *B. cuneata* from congeners. Specimens from three outgroup taxa, *Berkheya fruticosa* (L., Ehrh.), *Berkheya spinosa* (L.f., Druce) and *Berkheya coriacea* (Harv.), were also sampled to determine the rooting of the chloroplast network (Table D.1). Leaf samples were collected from 34 sites across the species range (Fig. 1B). We used a two-stage sampling scheme for genetic analyses because we had no *a priori* information on how to define populations (Harwood, 2009). First, only a single individual was sequenced per sampling site (Städler et al., 2009). Second, multiple individuals (two to five) were sequenced from a subset of samplings site to establish the significance of observed phylogeographic breaks. Individuals within sites were sampled more than 50 m apart. This scheme was used so that we could first assess the regional genetic variability and then increase the sample size to confirm major regional genetic breaks as we had sampled all known populations east of the Rooiberg Mountain. All samples were desiccated with silica granules.

In plants, spatial variation is affected by the range and direction of seed and pollen dispersal (Ennos, 1994; Schaal et al., 1998). In angiosperms, seeds carry both cytoplasmic and nuclear genomes whereas pollen generally only contains the nuclear genome (Reboud and Zeyl, 1994). Since seeds and pollen may have strongly contrasting dispersal modes (*e.g.* wind *versus* insects) and provide different dispersal means for cytoplasmic and nuclear genomes, we selected regions from both of these genomes to provide information on seed and pollen dispersal.

Genomic DNA was extracted from silica-dried leaf material using a modified version of the method specified by Gawel and Jarret (1991). Polyvinylpyrrolidone-40 (PVP) was added when grinding the leaf material in liquid nitrogen using a mortar and pestle. Two cpDNA regions were sequenced for all samples: *trnQ*$^{(UUG)}$*-5 rps16* and *psbD-trnT*$^{(GGU)}$ (Shaw et al., 2007). Nuclear variation was sampled for the ITS region of the 18S-26S cistron using the primers ITS5m (Sang et al., 1995) and ITS4 (White et al., 1990). See Appendix C for PCR amplification and sequencing conditions. Of note is that high-fidelity Taq polymerase was used for all PCRs of nDNA.

2.4. Sequence assembly, alignment and phylogeographic analyses

Both cpDNA and nDNA sequences generated in this study were assembled with CODONCODE ALIGNER v2.0.1 (Codon Code Corp, http://www.codoncode.com) and automatically aligned using CLUSTALW (Thompson et al., 1994). Each base-call within every sequence was assigned a quality score using the automated base-calling program PHRED (Ewing et al., 1998) to improve the speed and accuracy of identifying DNA variations among assembled sequences. Polymorphic sites in the nDNA dataset were identified through the presence of dual peaks in electropherograms (Brumfield et al., 2003) using automated detection, through the 'find mutations' option in CODONCODE ALIGNER, and by visual inspection. Polymorphic sites were then coded using IUPAC polymorphism codes (International Union of Pure and Applied Chemistry, http://www.iupac.org). All scored variable characters were either nucleotide polymorphisms or indels that were not in simple repeats. A number of long mononucleotide repeats in the cpDNA alignment were observed to have length variation, but these were excluded because they were difficult to score with confidence. As cpDNA gene regions are maternally inherited in tandem without recombination (Reboud and Zeyl, 1994), the two gene regions were combined for all subsequent analyses.

Statistical parsimony and neighbour-net splits graphs were used to ascertain the genealogical relationships among cpDNA haplotypes and nDNA sequences, respectively (Bryant and Moulton, 2004; Templeton et al., 1992). Statistical parsimony networks were generated using the program TCS 1.2.1 (Clement et al., 2000) with default options. As the option in TCS 1.2.1 which "treats gaps as a 5th state" treats a single multiple base indel as multiple single-base indels, all indels were shortened to a single base prior to analysis. Neighbour-net (NN) splits graphs were generated for all *B. cuneata* nDNA sequences using SplitsTree 4.8 (Huson and Bryant, 2006) and polymorphism p-distances (Potts et al., 2013c); the NN splits algorithm can accommodate the intra-individual

Table 1
Details of settings used for model fitting for species distribution modelling of *Berkheya cuneata*.

Model class	Method	Reference	Settings and notes
Profile	Bioclim	Nix (1986)	Used R[1] and function *bioclim* from the dismo library.
	Domain	Carpenter et al. (1993)	Used R[1] and function *domain* from the dismo library.
Regression	Generalized linear models	McCullagh and Nelder (1989)	Used R[1] and function *glm*. Created all possible subsets of models using the *step.gam* function from the gam library with the options for each variable being: linear, quadratic or cubic fits. Used AIC to select the best model.
	Generalized additive models	Hastie and Tibshirani (1990)	Used R[1] and function *gam* from the mgcv library. The smoothing parameter estimation was solved using generalized cross validation and best models were selected using null space penalization.
Machine learning	Random forests	Breiman (2001)	Used R[1] and function *randomForest* from the randomForest library to build an ensemble of classification trees. Models used here had 500 trees with 2 variables randomly selected from 7 candidates at each split. No class weights.
	Maximum entropy	Phillips et al. (2006)	Used version 3.3.3e from within R[1] with the *maxent* function in the dismo library. All settings were the defaults except that the background samples were the sampled 8000 absence points combined with presence points.

[1] R Development Core Team 2011.

site polymorphisms present in the nDNA sequences whereas the SP algorithm cannot.

In order to test the monophyly of nDNA sequences of *B. cuneata* within the genus, we conducted a phylogenetic analysis of these sequences with the readily available accessions from the *Berkheya-Cullumia* clade identified by Funk and Chan (2008) using maximum parsimony analyses conducted in PAUP* version 4b10 (Swofford, 2002). Maximum parsimony analyses were undertaken with intra-individual site polymorphisms treated as informative characters (Potts et al., 2013c). Heuristic searches were performed with 100 random sequence addition replicates, tree bisection-reconnection branch swapping, and MAXTREES set to 1000. Assessment of MP bootstrap support followed the suggestions of Müller (2005), with 10,000 bootstrap replicates composed of a single random sequence replicate and tree bisection-reconnection branch swapping. *Berkheya spinosissima*, *Didelta spinosa* and *Didelta carnosa* were treated as an outgroup clade for this analysis following the results from Funk and Chan (2008).

3. Results

A total of 60 from 169 locality records were available for distribution modelling after filtering to the resolution of the environmental grid layers (2.5 arc-minutes; ~4 × 4 km). The combination of three locality datasets (20 localities each with a minimum of 10 km between localities), six climate variable datasets and six different methods produced a total of 108 species distribution models. The majority of AUC values (87%) across these models were considered 'good' (>0.80; mean = 0.87, SD = 0.06, min = 0.68, max = 0.95), following Swets (1988), indicating that most models have high specificity (true positive rate) and sensitivity (false positive rate) (but see Lobo et al., 2008). Comparisons between

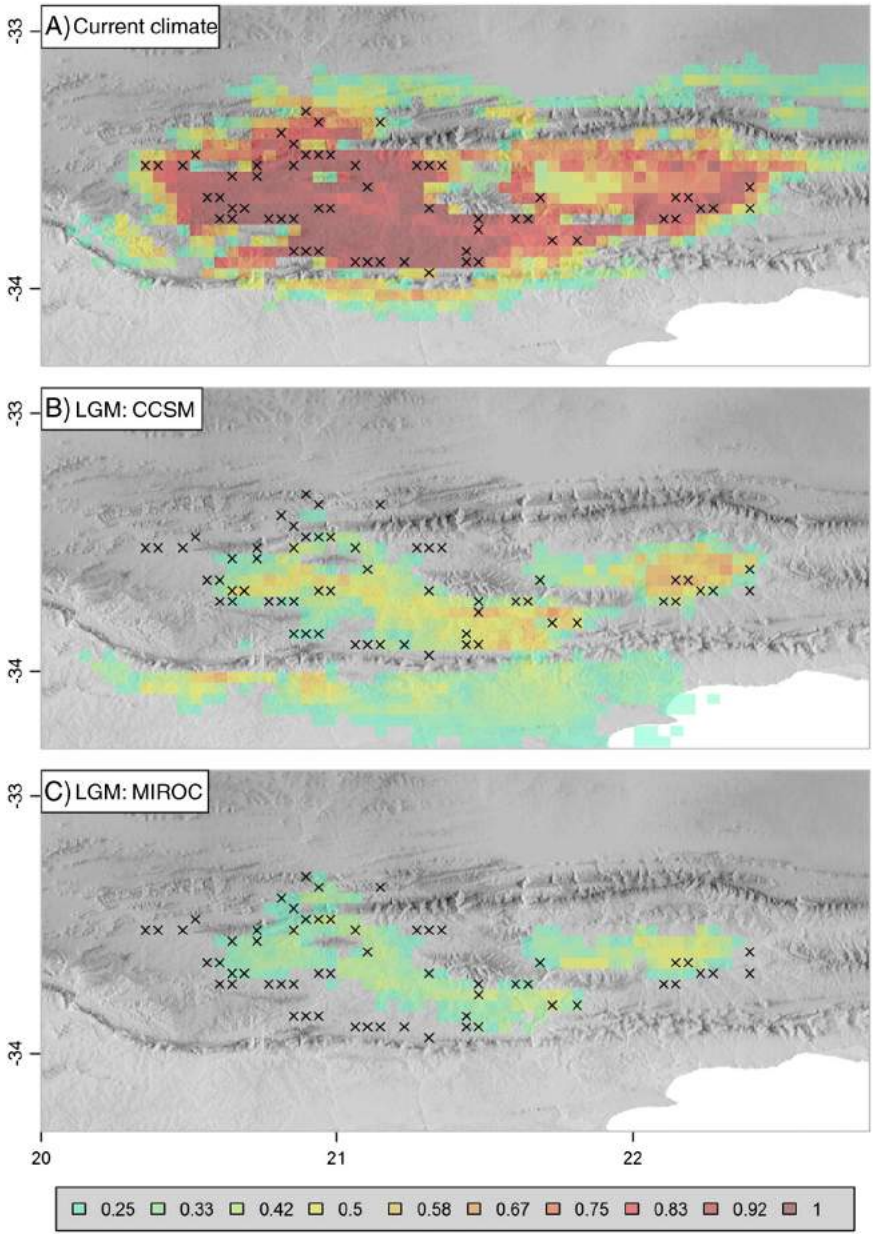

Fig. 2. Maps showing the percentage agreement of the 108 distribution models of *Berkheya cuneata* under current and Last Glacial Maximum (LGM) climate conditions using a threshold of maximum sensitivity plus specificity. Locality samples used for modelling are shown using '×'. LGM conditions are generated from two downscaled global climate models (CCSM and MIROC). The 108 maps are generated using a combination three unique locality datasets, six environmental parameter sets and six different modelling algorithms (GLM, GAM, Domain, Bioclim, Random Forests and Maxent; see Table 1 for details).

the ensemble SDM of current and LGM distributions of *B. cuneata* suggest that this species suffered a severe range reduction with an overall decline in elevation and concomitant fragmentation (Fig. 2). Nonetheless, the species is predicted to have persisted in both the western and eastern Little Karoo. The LGM climates estimated by the CCSM and MIROC simulations were broadly similar over the study region, with both indicating cooler and drier conditions. However, the MIROC simulations predicted colder temperatures than the CCSM simulations (Potts et al., 2013b). These cooler temperatures likely explain why the hindcasted ensemble SDM has a smaller range under the MIROC than the CCSM simulated climate. The Rooiberg represents an obstacle of unsuitable climate and soils under present conditions splitting the species' distribution in the Little Karoo. However, under LGM conditions and the predicted range fragmentation, this mountain range becomes a notable climatic barrier between the western and eastern distribution.

Our sampling for genetic analyses included 47 individuals from 34 sites across the known geographic range of *B. cuneata*. The *B. cuneata* chloroplast dataset comprised 1234 base pairs (bp) from the *psbD-trnT*$^{(GGU)}$ region (Genbank Accessions: GQ220831-5) and 983 bp from the *trnQ*$^{(UUG)}$*-5 rps16* regions (Genbank Accessions: GQ220840-4) (total 2217 bp) (Table D.2). This dataset contained 16 polymorphic sites; 4 transitions, 6 transversions, and 5 indels (see Table D.2 for a summary of the cpDNA sequence alignment). The GC content of the combined cpDNA data was 30.1%.

The SP network of the cpDNA dataset revealed five haplotypes that formed two divergent lineages within *B. cuneata* that are separated by a large number of mutations (Fig. 3A). These two clades were identified as unique genetic populations as they correspond to allopatric populations located in the western and eastern Little Karoo, separated by the Rooiberg mountain range (Fig. 3B). Note that the chloroplast network arrangement and sequence relationships were identical under both SP and NN network algorithms.

The *B. cuneata* ITS1-5.8S-ITS2 dataset comprised 46 individuals (AJP040 failed to sequence despite multiple attempts) and 683 base pairs which yielded 25 variable nucleotide sites (Genbank Accessions: KF640823-72), the majority of which were intra-individual site polymorphisms (2ISPs); no indels were detected in the dataset (see Table D.3 for a summary of the nDNA alignment). The GC content of the nDNA data was 56.1%.

All alignments used for cpDNA and nDNA analyses are stored in Dryad (http://dx.doi.org/10.5061/dryad.d18r8). Maximum parsimony bootstrap analyses resolved high support values (100%) for monophyly of *B. cuneata* ITS sequences (Appendix E) when analysed in conjunction with those from other *Berkheya* species sampled in this study and or from those obtained from the study by Funk and Chan (2008) (Figs. 4 and E.1). However, there was no supported resolution of the relationships among accessions within this *B. cuneata* clade. In contrast, the NN phylogenetic network of the *B. cuneata* accessions showed a significant non-random distribution of samples (clusters 1 and 2) that mirrors the west–east split observed in the cpDNA dataset (Fig. 4, Table 2; Pearson's $\chi^2 = 28.7$, df = 1, $p < 0.001$; $\Phi = 0.79$).

4. Discussion

Globally, the Pleistocene glacial–interglacial cycles often had severe impacts on species' range dynamics and evolutionary history (Dynesius and Jansson, 2000; Jansson and Dynesius, 2002). However, one hypothesis for the outstanding richness of the flora of the Cape region is that it was climatically buffered during these cycles (Cowling et al., 2009; Dynesius and Jansson, 2000; Hopper, 2009). This hypothesis, and the role of topography, has remained largely untested in the GCFR in terms of range dynamics and genetic structuring of plant species. In this study we utilised range-wide sampling of a species that is narrowly distributed and endemic to the Little Karoo to generate an ensemble of LGM distribution models, which are then compared to phylogeographic patterns.

The ensemble SDM predictions under LGM conditions suggest that the species experienced a dramatic range reduction, but managed to persist in both the western and eastern Little Karoo drainage basins during this and, likely, other Pleistocene glacial periods. With such population isolation we can predict that long distance seed and pollen dispersal between the western and eastern distributions would have been dramatically hindered by the Rooiberg mountain range. The large tumbling and dried flowerheads are not conducive to long-distance dispersal as they are easily trapped (JHJ Vlok, RM Cowling and AJ Potts, Pers. Obs.). Also, the geographic distances and altitudinal changes between western and eastern populations would impede long distance dispersal of both flowerheads and pollinators. This

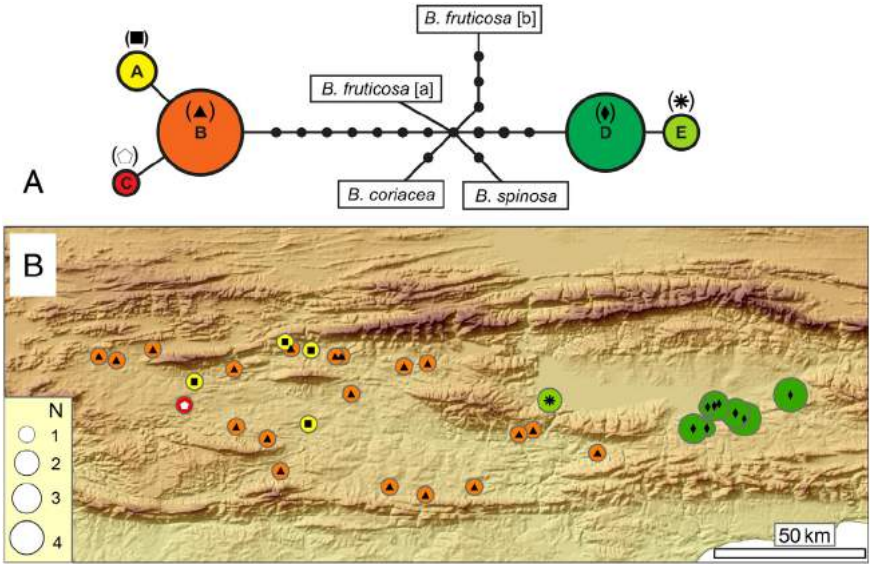

Fig. 3. The genealogical relationships A) and distribution B) of haplotypes from two merged chloroplast loci (*trnQ*$^{(UUG)}$*-5 rps16* and *psbD-trnT*$^{(GGU)}$) as inferred from the statistical parsimony algorithm. Black ovals indicate 'missing' haplotypes, whilst haplotypes connected by single lines differ by a single mutational difference. Areas of circles are proportional to haplotype frequencies. Outgroup species included are: *Berkheya fruticosa* ([A] AJP434, [B] AJP435), *B. coriacea* (AJP464) and *B. spinosa* (AJP356). Note that the NeighbourNet splits algorithm (used to analyse the ITS sequence data — see Fig. 4) produces an identical network.

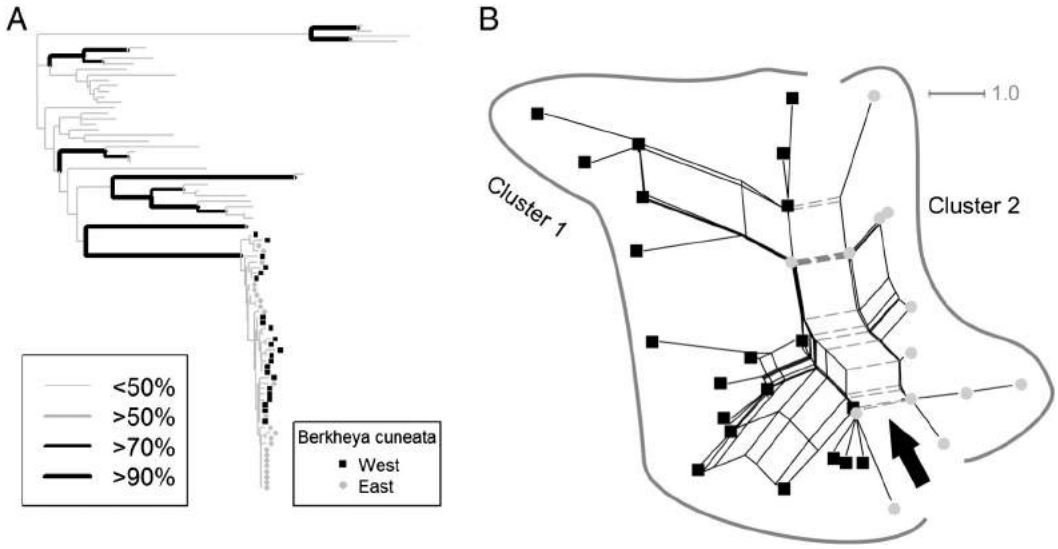

Fig. 4. (A) One of the most parsimonious trees based on ITS sequence data (nDNA) for the *Berkheya-Cullumia* clade (Funk and Chan, 2008) including the *Berkheya cuneata* samples from this study. Bootstrap support is shown using branch width and colour. See the supplementary material for a more detailed figure (Fig. E.1). (B) The NeighbourNet splits phylogenetic network of *Berkheya cuneata* based on ITS sequence data (nDNA). Genetic distances are calculated using polymorphism p-distances. Samples west and east of the Rooiberg Mountain are shown. A primary edge bundle that separates the majority of eastern samples from western samples is highlighted (see arrow; grey dashed edges).

persistence and isolation of populations in the western and eastern Little Karoo is mirrored in the phylogeographic patterns observed in the genetic data. The chloroplast haplotypes are highly structured into two genetic populations (Fig. 3), and this is reflected in the nuclear data as significant clustering in the splits graph (Fig. 4). The genetic data contain two idiosyncrasies that warrant further discussion.

The first relates to the degree of sequence divergence between the cpDNA *B. cuneata* phylogeographic clusters. Only five haplotypes were detected from the cpDNA dataset. This low diversity of chloroplast haplotypes from sequence data is not unusual, and has been observed in other studies (*e.g.* Cloutier et al., 2005; Comes and Abbott, 2001; Schonswetter et al., 2006). This is an acknowledged problem in plant phylogeography due to the slow rate of mutation and low effective population size (in comparison to the nuclear genome) of the chloroplast genome (Schaal et al., 1998). Despite this, the cpDNA data reveal an unusually high degree of sequence divergence between the haplotype clusters found in western and eastern Little Karoo; for example, other members of this genus are more genetically similar to one another and the eastern phylogeographic cluster than the eastern and western clusters are to each other (Fig. 3b). The chloroplast sequences lack sufficient signal to support or oppose monophyly of *B. cuneata*; the monophyly would be challenged if the other *Berkheya* accessions were nested within, or formed clades with, the *B. cuneata* clusters. The star phylogeny of the haplotype network means that no relationships between the *B. cuneata* clusters and other species can be inferred. However, it is unlikely that these clusters represent different species as the nuclear data strongly supports the monophyly of all *B. cuneata* samples; the dual peaks observed in the ITS electropherograms are most likely due to incomplete lineage sorting and not hybridisation (hybridisation would have lowered the bootstrap support; Potts et al., 2013c) and the interspecies genetic distances far outweigh the fairly low intraspecific distances within *B. cuneata* (Fig. 4, Table D.3). Therefore, this remarkable sequence divergence between regional populations could be caused by i) genetic drift during exceptionally long-term cessation of seed flow or during periods of very small population sizes, ii) lineage sorting of divergent ancestral haplotypes during isolation, or iii) introgression and subsequent fixation of a chloroplast genome from another species into one of the regional populations (*i.e.* chloroplast capture). Introgression results in a blurring of species history in the genetic record. However, even if one of these populations has captured a chloroplast from another, as yet unidentified, *Berkheya* species, the fact that the two regions share no cpDNA haplotypes still suggests long term cessation of seed flow between them since any possible capture and fixation event. Thus, we consider this phylogeographic pattern a result of isolation in terms of seed flow between the western and eastern Little Karoo.

The second idiosyncrasy relates to the genetic diversity of the *B. cuneata* nDNA. The nDNA dataset has a higher sequence diversity and a less pronounced, but still significant, phylogeographic structure in comparison to the haploid chloroplast dataset (Figs. 3 and 4). This, however, is expected given the different effective population sizes between cytoplasmic and nuclear genomes (McCauley, 1995; Moore, 1995). Nuclear genes have a larger effective population size and hence, under a neutral model of evolution, the rate of lineage sorting occurs more slowly than in the chloroplast genome. Despite this, there is significant clustering of the eastern samples which supports the population differentiation observed in the cpDNA data (Fig. B.1). Theoretical models of genetic divergence suggest that one immigrant per generation is considered sufficient to prevent differentiation between populations (Ellstrand and Elam, 1993; Slatkin, 1987). Thus, the observed phylogeographic patterns can be attributed to very low to absent levels of pollen flow and a lack of seed flow across the barrier between the western and eastern Little Karoo.

Unique cpDNA lineages and nDNA clusters, along with similar patterns of nucleotide diversity, suggests that these populations have

Table 2
Comparison of *Berkheya cuneata* accessions assigned to chloroplast DNA haplogroups and nuclear DNA clusters. The haplogroups and clusters are strongly ($\Phi = 0.79$) and significantly (Pearson's $\chi^2 = 28.7$, df = 1, $p < 0.001$) associated.

	nDNA clusters	
cpDNA haplogroups	1	2
A–C[1]	25	0
D–E[1]	4	17

[1] Haplotypes A–C are restricted to the western Little Karoo whereas D–E are restricted to the eastern Little Karoo.

persisted in the western and eastern Little Karoo for a substantial evolutionarily period. As the chloroplast sequences lack sufficient genetic variation for more advance molecular dating approaches (*e.g.* using the coalescent isolation-with-migration model; Won and Hey, 2005) we utilise a simple clock-based approach (Sarich and Wilson, 1973) with a wide range of biologically plausible mutation rates for cpDNA (0.024%/Myr to 0.116%/Myr, Zhang and Hewitt, 2003). This provides divergence estimates between the western and eastern *B. cuneata* populations that range from 87291 ± 30208 to 18060 ± 6250. Such dates can be treated as rough estimates of maximum and minimum divergence times (assuming that chloroplast capture has not occurred). Thus, these largely overlap and support a Pleistocene divergence between these populations. These dates rule out the possibility that the vicariance of western and eastern populations was related to the moderate uplift events that occurred throughout the Cape Fold Mountains during the Miocene (Cowling et al., 2009).

The general pattern of range contraction and fragmentation of lowland vegetation into drainage basins along the southern coastal lowlands, as observed in *B. cuneata* into the western and eastern sub-basins of the Gouritz basin, has been observed in other studies. The Albany Subtropical Thicket biome which occurs in the lowlands of the Little Karoo, but is centred to the east of this region, is predicted to have dramatically contracted and fragmented into drainage basins during the Last Glacial Maximum (Potts et al., 2013b) and two tree species, *Pappea capensis* and *Nymania capensis*, display similar phylogeographic patterns of chloroplast DNA populations isolated within primary drainage basins along the coastal lowlands (Potts et al., 2013a). A cicada species that is a near endemic to the Little Karoo, *Platypleura breedeflumensis*, also shows genetic structuring with haplotypes restricted to Gouritz sub-basins (Price et al., 2010). This pattern likely reflects tracking of lowland host plant species that also experienced range reductions and fragmentations, but not local extinctions, within sub-basins during the Pleistocene climate cycles.

The Little Karoo contains over 2000 plant species occurring in 23,000 km^2 (van Wyk and Smith, 2001; Vlok et al., 2005; Vlok and Schutte-Vlok, 2010), which is lower than the density of plant species found in the strong winter-rainfall region in the west (Cowling and Lombard, 2002). Nonetheless, this region remains a succulent biodiversity hotspot as it has higher species richness than other semi-arid regions across the globe (Cowling et al., 1998) with most of the species, including *B. cuneata*, associated with the Succulent Karoo biome (Fig. A.2) (van Wyk and Smith, 2001; Vlok and Schutte-Vlok, 2010). The ensemble SDMs of the LGM and the phylogeographic patterns show that the Pleistocene climatic cycling has played an important role in fragmenting populations and strengthening topographic barriers during glacial periods for *B. cuneata* and has been responsible for driving population divergence. This scenario of restricted gene flow over mountains coupled with 'mild' climatic shifts (in terms of local extinctions) on such a fine scale (~100 km) during the Pleistocene is in stark contrast to the Northern hemisphere where range dynamics were largely of a broad/regional scale (~1000s of km; *e.g.* Hewitt, 2000; Soltis et al., 2006; Taberlet et al., 1998). Thus, the climate stability and resultant decline in the extinction rate (fewer local extinctions) are likely to have been prominent drivers of the observed biodiversity richness in the lowlands of the Little Karoo.

Authors contributions

AJP designed the study, conducted the fieldwork, carried out the molecular genetic studies and analyses, drafted the manuscript and submitted the sequences to GenBank. TAH and RMC conceived the project, and directed its design and coordination. JHJV participated in the design of the study and in the fieldwork. All authors commented on the drafted manuscript and have read and approved the final version.

Acknowledgements

The financial assistance of the South African National Research Foundation (NRF), University of Cape Town (UCT), and Darwin Initiative (DI) in support of this research is gratefully acknowledged. Opinions expressed and conclusions arrived at, are those of the authors and are not necessarily to be attributed to the NRF, UCT or DI. Per Ola Karis and Nicola Bergh provided the material for the outgroup *Berkheya* species. Nicola Bergh provided comments that greatly improved an early version of this manuscript.

Appendix A. Habit and habitat photographs of *Berkheya cuneata*

Fig. A.1. *Berkheya cuneata* has succulent spiny leaves and yellow asteraceous flowers. The bracts harden to form a tumbling flowerhead that is dispersed by wind.

Fig. A.2. *Berkheya cuneata* is a low growing shrub that is strongly associated with the Succulent Karoo biome, but is also found growing in biome mosaics as seen here (Succulent Karoo/Albany Thicket).

Appendix B. The description, clustering and selection of the Bioclim variables

Table B.1
Variable description, clustering and selection of the 19 Bioclim variables (Busby 1991). The selected variables were used for species distribution modelling of the impacts of past climates on the distribution *Berkheya cuneata* within the Little Karoo, Western Cape. See Fig. SB.2 for the hierarchical clustering of variables.

		Climate variable set					
Climate variables	BIOCLIM code	1	2	3	4	5	6
Cluster 1							
Annual precipitation (mm)	BIO12				X	X	
Precipitation in the warmest quarter (mm)	BIO18						X
Precipitation in the wettest month (mm)	BIO13		X	X			
Precipitation in the wettest quarter (mm)	BIO16	X					
Cluster 2							
Precipitation in the coldest quarter (mm)	BIO19	X				X	
Precipitation in the driest month (mm)	BIO14			X	X		
Precipitation in the driest quarter (mm)	BIO17						X
Cluster 3							
Mean diurnal range (mean of monthly maximum temperature minus minimum temperature)	BIO02					X	
Temperature seasonality (standard deviation of annual mean temperature × 100)	BIO04	X	X	X			
Temperature annual range	BIO07				X		X
Cluster 4							
Minimum temperature of the coldest month (°C)	BIO06	X			X	X	
Mean temperature of the coldest quarter (°C)	BIO11		X	X			
Unclustered							
Maximum temperature of the warmest month (°C)	BIO05	X		X			X
Mean temperature of the wettest quarter (°C)	BIO08	X	X				
Precipitation seasonality (standard deviation of monthly precipitation values)	BIO15	X		X	X	X	
Annual mean temperature	BIO01		X	X	X	X	X
Mean temperature in the warmest quarter (°C)	BIO10		X			X	
Mean temperature in the driest quarter (°C)	BIO09		X				X
Isothermality	BIO03				X		X

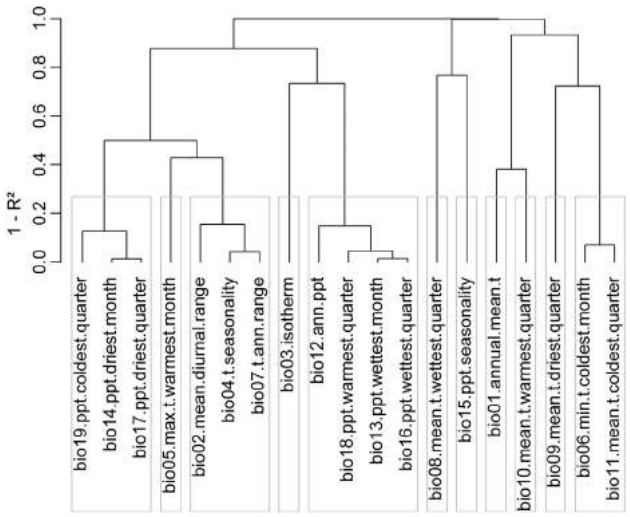

Fig. B.1. Hierarchical clustering of the 19 climate variables using a dissimilarity dendrogram. Clusters shown in grey boxes are highly intercorrelated ($r^2 > 0.7$). Variable descriptions and codes are shown in Table S2.1.

Appendix C. PCR amplification and DNA sequencing

The cpDNA target regions were PCR-amplified from gross cellular DNA extracts in volumes of 36 μl containing 1.2 μl of template DNA, 3.6 μl of 10× KAPATaq polymerase reaction buffer (Kapa Biosystems, Boston, Massachusetts, United States), 0.72 μl MgCl2 (50 mM), 1.2 μl of each primer (10 μM), 1.44 μl of dNTPs (10 mM), 0.24 μl Taq polymerase and sterile H_2O up to 36 μl. PCRs were conducted with an initial 2 minute (min) denaturing step at 94°C followed by 28 cycles of 94°C for 1 min, 50°C for 30 seconds (s), and 72°C for 1 min; this was followed by a final extension of 6 min at 72°C.

Nuclear variation was sampled for the ITS-1, 5.8S and ITS-2 region using the primers ITS5m (Sang et al., 1995) and ITS4 (White et al., 1990). PCR reactions were performed in 25 μl, with 5 μl 1× KAPA HiFi Buffer, 0.75 mM dNTPs, 0.75 mM forward primer, 0.75 mM reverse primer, 0.4 μl of the proofreading KAPA HiFi DNA polymerase (2 Units) and 1.2 μl template DNA (~1–5 ng). PCR was conducted using the following conditions: initial denaturation and polymerase activation at 98°C for 20 s followed by 30 cycles of 94°C for 45 s, 58°C for 30 s, and 72°C for 30 s; this was followed by a final extension at 72°C for 1 min.

All PCRs were performed on a GeneAmp 2700 PCR System (Applied Biosystems, USA). PCR products were cleaned and sequenced by either Macrogen (Korea) or University of Stellenbosch Sequencing Facility. All PCR products were sequenced in both directions to verify base calling.

Appendix D. Summary tables of the *Berkheya cuneata* chloroplast and nuclear DNA sequence alignments and collection details

Table D.1
Source of sequence data and Genbank Accession numbers. All herbarium samples are stored at the Bolus Herbarium (BOL), University of Cape Town. Chloroplast regions are $trnQ^{(UUG)}$-5´*rps16* and *psbD*-$trnT^{(GGU)}$.

Political subdivision	Species	Collection number	Date	South	East	Herb	ITS	trnQ	psbD
WC	*Berkheya cuneata*	AJP-040	1-Jun-07	−33.7060	20.9689			A	1
WC		AJP-048	1-Jun-07	−33.6158	21.0969		KF640823	B	2
WC		AJP-052	20-Mar-07	−33.5346	21.2544	Yes	KF640824	B	2
WC		AJP-056	17-Mar-07	−33.5251	21.3247	Yes	KF640825	B	2
WC		AJP-088	3-Jun-07	−33.5041	21.0679		KF640826	B	2
WC		AJP-095	3-Jun-07	−33.5044	21.0498		KF640827	B	2
WC		AJP-100	3-Jun-07	−33.4858	20.9775		KF640828	B	2
WC		AJP-102	3-Jun-07	−33.4807	20.9173		KF640829	B	2
WC		AJP-115	3-Jun-07	−33.4612	20.8999		KF640830	B	2
WC		AJP-125	3-Jun-07	−33.5425	20.7478		KF640831	B	2
WC		AJP-142	3-Jun-07	−33.5800	20.6288		KF640832	A	1
WC		AJP-161	3-Jun-07	−33.6497	20.5997		KF640833	C	3
WC		AJP-203	3-Jun-07	−33.7144	20.7542		KF640834	B	2
WC		AJP-222	20-Mar-07	−33.7497	20.8469	Yes	KF640835	B	2
WC		AJP-225	4-Jun-07	−33.6198	22.4072		KF640836	D	4
WC		AJP-226	4-Jun-07	−33.6198	22.4072		KF640837	D	4
WC		AJP-227	4-Jun-07	−33.6198	22.4072		KF640838	D	4
WC		AJP-235	4-Jun-07	−33.6198	22.4072		KF640839	D	4

(continued on next page)

Table D.1 (*continued*)

Political subdivision	Species	Collection number	Date	South	East	Herb	ITS	trnQ	psbD
WC		AJP-308	1-Sep-07	−33.6909	22.2692	Yes	KF640840	D	4
WC		AJP-309	1-Sep-07	−33.6909	22.2692		KF640841	D	4
WC		AJP-310	1-Sep-07	−33.6909	22.2692		KF640842	D	4
WC		AJP-311	1-Sep-07	−33.6909	22.2692		KF640843	D	4
WC		AJP-313	1-Sep-07	−33.6740	22.2431		KF640844	D	4
WC		AJP-314	1-Sep-07	−33.6740	22.2431		KF640845	D	4
WC		AJP-315	1-Sep-07	−33.6740	22.2431		KF640846	D	4
WC		AJP-320	1-Sep-07	−33.6463	22.1945	Yes	KF640847	D	4
WC		AJP-321	1-Sep-07	−33.6526	22.1808		KF640848	D	4
WC		AJP-322	1-Sep-07	−33.6526	22.1808	Yes	KF640849	D	4
WC		AJP-323	1-Sep-07	−33.6526	22.1808		KF640850	D	4
WC		AJP-326	1-Sep-07	−33.6563	22.1602	Yes	KF640851	D	4
WC		AJP-328	1-Sep-07	−33.7207	22.1165		KF640852	D	4
WC		AJP-332	1-Sep-07	−33.7194	22.1579		KF640853	D	4
WC		AJP-344	1-Sep-07	−33.6361	21.6890	Yes	KF640854	E	5
WC		AJP-345	1-Sep-07	−33.6361	21.6890		KF640855	E	5
WC		AJP-360	3-Sep-07	−33.7247	21.6390	Yes	KF640869	B	2
WC		AJP-362	3-Sep-07	−33.7373	21.5970	Yes	KF640856	B	2
WC		AJP-372	3-Sep-07	−33.8940	21.4643	Yes	KF640857	B	2
WC		AJP-379	3-Sep-07	−33.9181	21.3183	Yes	KF640858	B	2
WC		AJP-381	3-Sep-07	−33.8934	21.2116	Yes	KF640859	B	2
WC		AJP-393	3-Sep-07	−33.8464	20.8862	Yes	KF640860	B	2
WC		AJP-404	1-Aug-07	−33.7199	22.1170		KF640861	D	4
WC		AJP-405	1-Aug-07	−33.7199	22.1170		KF640862	D	4
WC		AJP-406	1-Aug-07	−33.7199	22.1170		KF640863	D	4
WC		AJP-408	1-Aug-07	−33.7926	21.8309		KF640864	B	2
WC		AJP-423	2-Sep-07	−33.4835	20.5052	Yes	KF640865	A	1
WC		AJP-428	2-Sep-07	−33.5156	20.3966	Yes	KF640866	B	2
WC		AJP-431	1-Aug-07	−33.5041	20.3441		KF640867	B	2
WC	*Berkheya spinosa*	AJP-356	2-Sep-07	−33.4764	20.5296	Yes	KF640868	GQ220847	GQ220838
NC	*Berkheya fruticosa*	AJP-434	11-Sep-07	−29.7900	17.7974		KF640870	GQ220845	GQ220836
NC	*Berkheya fruticosa*	AJP-435	1-Sep-07	−28.8012	17.2037		KF640871	GQ220846	GQ220837
WC	*Berkheya coriacea*	AJP-464	1-Feb-08	−34.4777	20.5108	Yes	KF640872	GQ220848	GQ220839

Table D.2
Summary table of the chloroplast DNA sequence alignment of two gene regions (*psbD-trnT*$^{(GGU)}$ and *trnQ*$^{(UUG)}$*-5′rps16*) from *Berkheya cuneata*. The different mutation types are indicated (T — transition, V — transversions, I — indel). The sample identity and the regional population (W — west of the Rooiberg, E — east of the Rooiberg) are included.

			psbD-trnT$^{(GGU)}$								*trnQ*$^{(UUG)}$*-5′rps16*						
			0	0	0	0	0	0	0	1	1	1	1	1	2	2	2
			0	2	2	3	7	7	9	1	2	4	4	7	0	0	0
			4	1	6	8	2	8	9	7	9	2	6	8	2	2	4
Haplotype	Sample ID	Region	6	3	1	7	9	9	9	1	8	5	8	4	1	8	6
A	040;100;115;142	W	T	–	G	T	G	A	C	–	–	C	C	–	$	C	G
B	048; 052; 056; 088; 095; 102; 125; 203; 222; 360; 362; 372; 379; 381; 393; 408; 423; 428; 431	W	T	–	G	T	G	A	C	T	–	C	C	–	$	C	G
C	161	W	T	–	G	T	G	A	C	T	–	C	C	!	$	C	G
D	225; 226; 227; 235; 308; 309; 310; 311; 313; 314; 315; 320; 321; 322; 323; 326; 328; 332; 404; 405; 406	E	C	*	T	C	C	C	G	T	#	T	A	–	–	C	A
E	344; 345	E	C	*	T	C	C	C	G	T	#	T	A	–	–	T	A

* — ATCAA; # — ATAGATAATAAA; ! — TTTAATATTTT; $ — TTTA.

Table D.3
Summary table of the nuclear DNA sequence alignment of ITS from *Berkheya cuneata*. Clusters are those found in Fig. 4. The regional population (W — west of the Rooiberg, E — east of the Rooiberg) and sample identity are included.

			ITS1														5.8S				ITS2							
		Position	65	80	87	93	107	111	117	120	132	147	237	238	254		299	321	417	429	509	514	521	536	567	591	628	657
		Consensus	C	C	C	C	T	C	A	C	C	G	T	G	C		C	C	C	C	T	C	C	C	A	C	A	C
Cluster	W/E	Sample ID																										
1	W	AJP0048	.	.	Y	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	.	.	.	.	.
	W	AJP0052	.	.	.	.	.	.	R	.	.	.	Y	.	.		.	.	.	.	.	.	Y	.	.	.	.	.
	W	AJP0056	.	.	.	.	.	.	R	Y	Y	.	Y	.	.		.	S	.	.	.	.	.	.	.	Y	.	.
	W	AJP0088	.	.	.	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	.	R	.	.	.
	W	AJP0095	.	.	.	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	.	.	.	.	.
	W	AJP0100	.	.	.	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	.	.	.	.	.
	W	AJP0102	.	.	.	.	.	.	R	.	.	.	.	.	.		.	.	.	.	.	.	Y	.	.	.	.	.
	W	AJP0115	.	.	.	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	.	.	.	.	.
	W	AJP0125	.	.	.	.	.	.	R	.	.	.	.	.	.		.	.	.	.	.	.	Y	.	.	.	.	.
	W	AJP0142	.	.	.	.	.	.	R	Y	.	.	Y	.	.		Y	.	.	.	.	.	Y	.	.	.	.	.
	W	AJP0161	.	.	.	Y	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	.	.	.	.	.
	W	AJP0203	.	.	.	.	Y	.	R	Y	.	.	Y	.	.		.	.	.	.	.	.	.	.	.	Y	.	.
	W	AJP0222	.	.	.	.	.	.	R	Y	.	.	Y	.	.		.	.	.	.	.	.	.	.	.	Y	.	.
	W	AJP0344	.	.	.	.	.	.	G	Y	.	.	C	.	.		.	.	.	.	.	.	.	.	.	T	.	.
	W	AJP0345	.	.	.	.	.	.	G	Y	.	.	C	.	Y		.	.	.	.	.	.	.	.	.	T	.	.
	W	AJP0360	.	.	.	.	.	.	G	.	.	.	Y	.	.		.	.	.	.	.	Y	.	.	.	Y	R	.
	W	AJP0362	.	.	.	.	.	.	G	.	.	.	C	.	.		.	.	.	.	.	.	.	.	.	T	.	.
	W	AJP0372	.	.	.	.	.	.	R	.	.	.	.	R	.		.	.	.	.	.	.	Y	.	.	.	.	.
	W	AJP0379	.	.	.	.	.	.	G	.	.	.	.	.	.		.	.	.	.	.	.	y	.	.	.	.	.
	W	AJP0381	.	.	.	.	.	.	R	.	.	.	.	.	.		.	.	.	.	.	.	.	.	.	.	.	.
	W	AJP0393	.	.	.	.	.	.	R	.	.	.	.	.	.		.	.	.	.	.	.	.	.	.	.	.	.
	W	AJP0408	.	.	.	.	.	.	G	T	.	.	C	.	.		.	.	.	.	Y	.	.	.	.	T	.	.
	W	AJP0423	.	.	.	.	.	.	.	.	.	.	.	.	.		.	.	Y	.	.	.	Y	.	.	.	Y	.
	W	AJP0428	.	.	.	.	.	.	.	.	.	R	.	.	.		.	.	Y	.	.	.	.	.	.	.	Y	.
	W	AJP0431	.	.	.	.	.	.	.	.	.	R	.	.	.		.	.	Y	.	.	.	Y	.	.	.	Y	.
	E	AJP0308	.	.	.	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	.	.	.	.	.
	E	AJP0321	.	.	.	.	.	.	.	.	.	.	Y	.	.		.	.	.	.	.	.	.	.	.	Y	.	.
	E	AJP0322	.	.	.	.	.	.	.	.	.	.	Y	.	.		.	.	.	.	.	.	.	.	.	Y	.	.
	E	AJP0326	.	.	.	.	.	.	.	.	.	.	.	R	.		.	.	.	Y	.	.	.	.	.	.	.	.
2	E	AJP0225	.	Y	.	.	.	.	R	Y	.	.	Y	.	.		.	.	.	.	Y	.	.	.	.	Y	.	y
	E	AJP0226	.	Y	.	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	.	.	.	.	.
	E	AJP0227	.	Y	.	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	.	.	.	.	.
	E	AJP0235	.	Y	.	.	.	Y	R	.	.	.	Y	.	.		.	.	.	.	.	.	.	.	.	Y	.	.
	E	AJP0309	.	Y	.	.	.	Y	R	.	.	.	.	.	.		.	.	.	.	.	.	.	.	.	.	.	.
	E	AJP0310	.	Y	.	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	.	.	.	.	.
	E	AJP0311	.	Y	.	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	.	.	.	.	.
	E	AJP0313	.	Y	.	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	.	.	.	.	.
	E	AJP0314	.	Y	.	.	.	Y	R	.	.	.	Y	.	.		.	.	.	.	.	.	.	.	.	Y	.	.
	E	AJP0315	.	Y	.	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	.	.	Y	.	.
	E	AJP0320	.	T	.	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	Y	.	.	.	.
	E	AJP0323	.	Y	.	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	.	.	.	.	.
	E	AJP0328	.	Y	.	.	.	.	R	.	.	.	Y	.	.		.	.	.	.	.	.	.	.	.	Y	.	.
	E	AJP0332	M	Y	.	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	.	.	.	.	.
	E	AJP0404	.	Y	.	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	.	.	.	.	.
	E	AJP0405	.	T	.	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	.	.	.	.	.
	E	AJP0406	.	Y	.	.	.	.	.	.	.	.	.	.	.		.	.	.	.	.	.	.	.	.	.	.	.

Appendix E Maximum parsimony tree of *Berkheya*

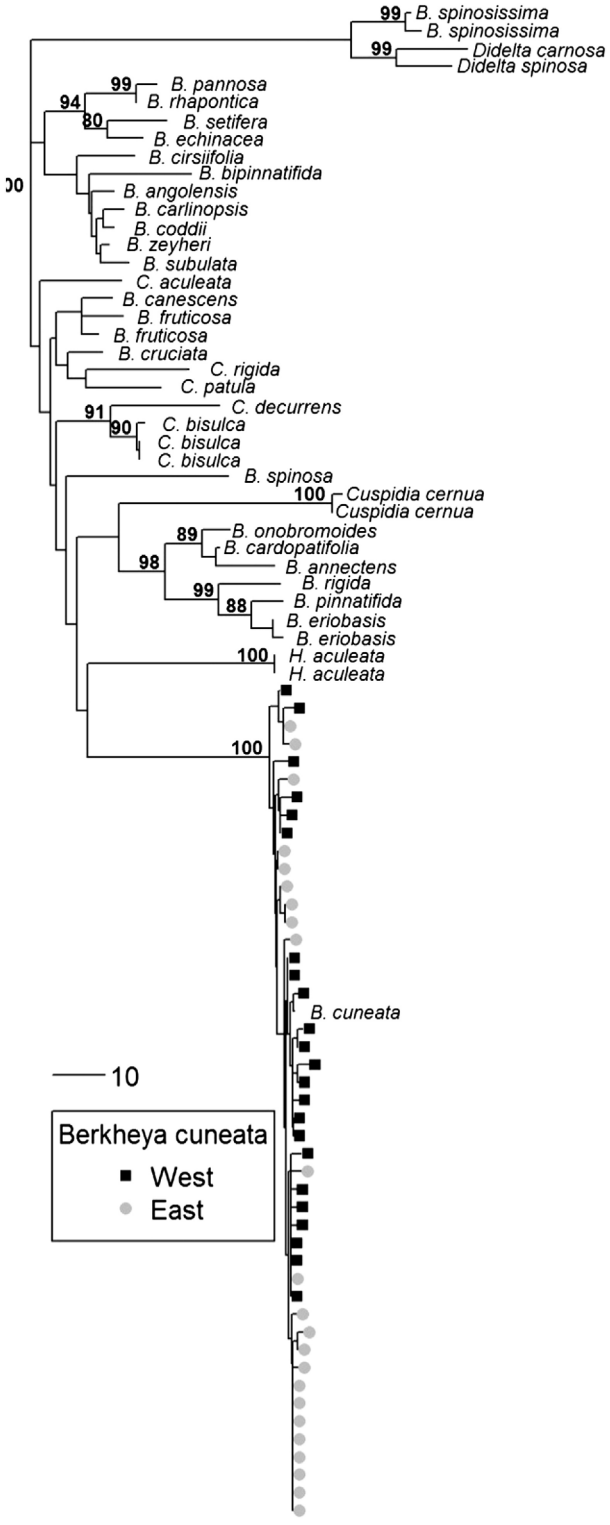

Fig. E.1. One of the most parsimonious trees on ITS sequences of the *Berkheya-Cullumia-Heterorhachis* clade (all labelled samples from Funk and Chan (2008) excluding AJP356, AJP434, AJP435 and AJP464) and the *Berkheya cuneata* accessions used in this study (symbols). Bootstrap values greater than 75% are shown, respectively.

References

Araújo, M.B., New, M., 2007. Ensemble forecasting of species distributions. Trends in Ecology & Evolution 22, 42–47.

Avise, J.C., 2000. Phylogeography: The History and Formation of Species. Harvard University Press, Cambridge, Massachusetts.

Born, J., Linder, H.P., Desmet, P., 2007. The Greater Cape Floristic Region. Journal of Biogeography 34, 147–162.

Breiman, L., 2001. Random forests. Machine Learning 45, 5–32.

Brumfield, R.T., Beerli, P., Nickerson, D.A., Edwards, S.V., 2003. The utility of single nucleotide polymorphisms in inferences of population history. Trends in Ecology & Evolution 18, 249–256.

Bryant, D., Moulton, V., 2004. Neighbor-net: an agglomerative method for the construction of phylogenetic networks. Molecular Biology and Evolution 21, 255–265.

Carpenter, G., Gillison, A.N., Winter, J., 1993. DOMAIN: a flexible modelling procedure for mapping potential distributions of plants and animals. Biodiversity and Conservation 2, 667–680.

Chase, B.M., 2010. South African palaeoenvironments during marine oxygen isotope stage 4: a context for the Howiesons Poort and Still Bay industries. Journal of Archaeological Science 37, 1359–1366.

Chase, B.M., Meadows, M.E., 2007. Late Quaternary dynamics of southern Africa's winter rainfall zone. Earth-Science Reviews 84, 103–138.

Clement, M., Posada, D., Crandall, K., 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9, 1657–1660.

Cloutier, D., Povoa, J.S.R., Procopio, L.C., Leao, N.V.M., Wadt, L.H.O., Ciampi, A.Y., Schoen, D.J., 2005. Chloroplast DNA variation of *Carapa guianensis* in the Amazon basin. Silvae Genetica 54, 270–274.

Comes, H.P., Abbott, R.J., 2001. Molecular phylogeography, reticulation, and lineage sorting in Mediterranean *Senecio* sect. *Senecio* (Asteraceae). Evolution 55, 1943–1962.

Cowling, R.M., 1992. The Ecology of Fynbos: Nutrients, Fire and Diversity. Oxford University Press, Cape Town.

Cowling, R.M., Lombard, A.T., 2002. Heterogeneity, speciation/extinction history and climate: explaining regional plant diversity patterns in the Cape Floristic Region. Diversity and Distributions 8, 163–179.

Cowling, R.M., Proches, S., Partridge, T.C., 2009. Explaining the uniqueness of the Cape flora: incorporating geomorphic evolution as a factor for explaining its diversification. Molecular Phylogenetics and Evolution 51, 64–74.

Cowling, R.M., Rundel, P.W., Desmet, P.G., Esler, K.J., 1998. Extraordinary high regional-scale plant diversity in southern African arid lands: subcontinental and global comparisons. Diversity and Distributions 4, 27–36.

Dormann, C.F., 2007. Promising the future? Global change projections of species distributions. Basic and Applied Ecology 8, 387–397.

Dynesius, M., Jansson, R., 2000. Evolutionary consequences of changes in species' geographical distributions driven by Milankovitch climate oscillations. Proceedings of the National Academy of Sciences of the United States of America 97, 9115–9120.

Ellstrand, N.C., Elam, D.R., 1993. Population genetic consequences of small population size: implications for plant conservation. Annual Review of Ecology and Systematics 24, 217–242.

Ennos, R.A., 1994. Estimating the relative rate of pollen and seed migration among plant populations. Heredity 72, 250–259.

Ewing, B., Hillier, L., Wendl, M.C., Green, P., 1998. Base-calling of automated sequencer traces using *Phred*. I. Accuracy assessment. Genome Research 8, 175–185.

Franklin, J., 2010. Mapping Species Distributions: Spatial Inference and Prediction. Cambridge University Press, Cambridge, UK.

Funk, V.A., Chan, R., 2008. Phylogeny of the Spiny African Daisies (compositae, tribe Arctotideae, subtribe Gorteriinae) based on trnL-F, ndhF, and ITS sequence data. Molecular Phylogenetics and Evolution 48, 47–60.

Gawel, N.J., Jarret, R.L., 1991. A modified CTAB DNA extraction procedure for *Musa* and *Ipomoea*. Plant Molecular Biology Reporter 9, 262–266.

Goldblatt, P., Manning, J., 2000. Cape Plants: A Conspectus of the Vascular Plants of the Cape Region of South Africa. National Botanical Institute of South Africa, Pretoria.

Goldblatt, P., Manning, J.C., 2002. Plant diversity of the Cape region of southern Africa. Annals of the Missouri Botanical Garden 89, 281–302.

Harwood, T.D., 2009. The circular definition of populations and its implications for biological sampling. Molecular Ecology 18, 765–768.

Hastie, T.J., Tibshirani, R.J., 1990. Generalized Additive Models. Chapman and Hall, London.

Hewitt, G., 2000. The genetic legacy of the Quaternary ice ages. Nature 405, 907–913.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25, 1965–1978.

Hopper, S., 2009. OCBIL theory: towards an integrated understanding of the evolution, ecology and conservation of biodiversity on old, climatically buffered, infertile landscapes. Plant and Soil 322, 49–86.

Hugall, A., Moritz, C., Moussalli, A., Stanisic, J., 2002. Reconciling paleodistribution models and comparative phylogeography in the wet tropics rainforest land snail *Gnarosophia bellendenkerensis* (Brazier 1875). Proceedings of the National Academy of Sciences of the United States of America 99, 6112–6117.

Huson, D.H., Bryant, D., 2006. Application of phylogenetic networks in evolutionary studies. Molecular Biology and Evolution 23, 254–267.

Jansson, R., Dynesius, M., 2002. The fate of clades in a world of recurrent climatic change: Milankovitch oscillations and evolution. Annual Review of Ecology and Systematics 33, 741–777.

Linder, H.P., 2003. The radiation of the Cape flora, southern Africa. Biological Reviews 78, 597–638.

Liu, C., Berry, P.M., Dawson, T.P., Pearson, R.G., 2005. Selecting thresholds of occurrence in the prediction of species distributions. Ecography 28, 385–393.

Lobo, J.M., Jiménez-Valverde, A., Real, R., 2008. AUC: a misleading measure of the performance of predictive distribution models. Global Ecology and Biogeography 17, 145–151.

McCauley, D.E., 1995. The use of chloroplast DNA polymorphism in studies of gene flow in plants. Trends in Ecology & Evolution 10, 198–202.

McCullagh, P., Nelder, J.A., 1989. Generalized Linear Models. Chapman and Hall, London.
Meadows, M.E., Sugden, J.M., 1993. The late quaternary palaeoecology of a floristic kingdom: the southwestern Cape South Africa. Palaeogeography, Palaeoclimatology, Palaeoecology 101, 271–281.
Moore, W.S., 1995. Inferring phylogenies from mtDNA variation: mitochondrial-gene trees *versus* nuclear-gene trees. Evolution 49, 718–726.
Müller, K., 2005. The efficiency of different search strategies in estimating parsimony jackknife, bootstrap, and Bremer support. BMC Evolutionary Biology 5, 58.
Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B.., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403, 853–858.
Nix, H.A., 1986. A biogeographic analysis of Australian elapid snakes. In: Longmore, R. (Ed.), Atlas of Elapid Snakes of Australia. Australian Government Publishing Service, Canberra, pp. 4–15.
Phillips, S.J., Anderson, R.P., Schapire, R.E., 2006. Maximum entropy modeling of species geographic distributions. Ecological Modelling 190, 231–259.
Potts, A.J., Hedderson, T.A., Cowling, R.M., 2013a. Testing Large-scale Conservation Corridors Designed for Patterns and Processes: Comparative Phylogeography of Three Tree Species. Diversity and Distributions. http://dx.doi.org/10.1111/ddi.12113.
Potts, A.J., Hedderson, T.A., Cowling, R.M.C., Franklin, J., 2013b. The Last Glacial Maximum distribution of Albany Subtropical Thicket inferred from community distribution modelling. Journal of Biogeography 40, 310–322.
Potts, A.J., Hedderson, T.A., Grimm, G.W., 2013c. Constructing phylogenies in the presence of intra-individual site polymorphisms (2ISPs) with a focus on the nuclear ribosomal cistron. Systematic Biology. http://dx.doi.org/10.1093/sysbio/syt052.
Price, B.W., Barker, N.P., Villet, M.H., 2010. A watershed study on genetic diversity: phylogenetic analysis of the *Platypleura plumosa* (Hemiptera: Cicadidae) complex reveals catchment-specific lineages. Molecular Phylogenetics and Evolution 54, 617–626.
Reboud, X., Zeyl, C., 1994. Organelle inheritance in plants. Heredity 72, 132–140.
Sang, T., Crawford, D.J., Stuessy, T.F., 1995. Documentation of reticulate evolution in peonies (*Paeonia*) using internal transcribed spacer sequences of nuclear ribosomal DNA: implications for biogeography and concerted evolution. Proceedings of the National Academy of Sciences of the United States of America 92, 6813–6817.
Sarich, V.M., Wilson, A.C., 1973. Generation time and genomic evolution in primates. Science 179, 1144–1147.
Schaal, B.A., Hayworth, D.A., Olsen, K.M., Rauscher, J.T., Smith, W.A., 1998. Phylogeographic studies in plants: problems and prospects. Molecular Ecology 7, 465–474.
Schonswetter, P., Popp, M., Brochmann, C., 2006. Rare arctic-alpine plants of the European Alps have different immigration histories: the snow bed species *Minuartia biflora* and *Ranunculus pygmaeus*. Molecular Ecology 15, 709–720.
Shaw, J., Lickey, E.B., Schilling, E.E., Small, R.L., 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. American Journal of Botany 94, 275–288.
Slatkin, M., 1987. Gene flow and the geographic structure of natural populations. Science 236, 787–792.
Soltis, D.E., Morris, A.B.., McLachlan, J.S., Manos, P.S., Soltis, P.S., 2006. Comparative phylogeography of unglaciated eastern North America. Molecular Ecology 15, 4261–4293.
Städler, T., Haubold, B., Merino, C., Stephan, W., Pfaffelhuber, P., 2009. The impact of sampling schemes on the site frequency spectrum in non-equilibrium subdivided populations. Genetics 182, 205–216.
Svenning, J.-C., Fløjgaard, C., Marske, K.A., Nógues-Bravo, D., Normand, S., 2011. Applications of species distribution modeling to paleobiology. Quaternary Science Reviews 30, 2930–2947.
Swets, J.A., 1988. Measuring the accuracy of diagnostic systems. Science 240, 1285-1239.
Swofford, D.L., 2002. PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods). Version 4.10b Sinauer Associates, Sunderland, Massachusetts.
Taberlet, P., Fumagalli, L., Wust-Saucy, A.G., Cosson, J.F., 1998. Comparative phylogeography and postglacial colonization routes in Europe. Molecular Ecology 7, 453–464.
Templeton, A.R., Crandall, K.A., Sing, C.F., 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data: III. Cladogram estimation. Genetics 132, 619–633.
Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Research 22, 4673–4680.
van Wyk, A.E., Smith, G.F., 2001. Regions of floristic endemism in Southern Africa. A Review with Emphasis on Succulents. CTP Book Printers, Cape Town.
Vlok, J.H.J., Cowling, R.M., Wolf, T., 2005. A vegetation map for the Little Karoo. Unpublished maps and report for a SKEP project supported by CEPF grant no 1064410304.
Vlok, J.H.J., Schutte-Vlok, A.L., 2010. Plants of the Klein Karoo. Umdaus Press, Hatfield.
Waltari, E., Hijmans, R.J., Peterson, A.T., Nyari, Á.S., Perkins, S.L., Guralnick, R.P., 2007. Locating Pleistocene refugia: comparing phylogeographic and ecological niche model predictions. PLoS One 2, e563.
White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M., Gelfand, D., Sninsky, J., White, T. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, pp. 315–322.
Whitehead, V.B., 1984. Distribution, biology and flower relationships of fideliid bees of southern Africa (Hymenoptera, Apoidea, Fideliidae). South African Journal of Zoology 19, 87–90.
Won, Y., Hey, J., 2005. Divergence population genetics of chimpanzees. Molecular Biology and Evolution 22, 297–307.
Zhang, D.-X., Hewitt, G.M., 2003. Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. Molecular Ecology 12, 563–584.