Hughes, Möller et al. 2005

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Molec. Ecol. 14: 4415-4426.

REFNO: 3486

KEYWORDS:

Africa, Biogeography, Conservation, Ecology, South Africa, Streptocarpus

Molecular Ecology (2005) 14, 4415–4426 doi: 10.1111/j.1365-294X.2005.02756.x

Refugia, dispersal and divergence in a forest archipelago: a study of *Streptocarpus* in eastern South Africa

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Abstract

We describe a scenario of plant speciation across a relict forest archipelago in South Africa involving Pleistocene habitat expansion-contraction cycles, dispersal and adaptation to lower temperatures. This is the first population level study using molecular data in South African forests and has significant implications for conservation efforts in this area. Populations of the mesophytic forest floor herbs *Streptocarpus primulifolius sensu lato* and *Streptocarpus rexii* were sampled throughout their range in the naturally fragmented forests of eastern South Africa in order to investigate population genetic and phylogenetic patterns within the species complex, using nuclear microsatellites, nuclear ribosomal ITS (internal transcribed spacer) sequences and chloroplast genome sequences. *S. primulifolius* harbours high levels of genetic diversity at both the nuclear (mean $H_E = 0.50$) and the chloroplast level (each population fixed for a unique haplotype). This is consistent with populations of these coastal species being Pleistocene relicts. In contrast, populations of *S. rexii* in cooler habitats at higher altitudes and lower latitudes harbour little or no nuclear genetic diversity (mean $H_E = 0.09$) and most share a common chloroplast haplotype. The split of *S. rexii* from populations intermediate between the two species (*S.* cf. *primulifolius*) occurred between 0 and 0.44 million years ago according to the calibrated ITS phylogeny of the taxa. The low genetic diversity and homogeneity of *S. rexii* is congruent with this species having reached its current range during the Holocene. We found no evidence of monophyly of any of the taxa in this study, which we consider a consequence of recent evolution in a fragmented habitat.

Keywords: indigenous forest, microsatellites, refugia, South Africa, *Streptocarpus*

Received 24 June 2005; revision accepted 24 August 2005

Introduction

Biological refugia persisting during Pleistocene climatic cycles have been shown to be crucial in shaping the present-day distribution of plant biodiversity (Hewitt 2000). Refugial areas and Holocene migration routes have been well characterized for the intensively studied yet relatively species poor floras of Europe and North America (e.g. Kremer & Goenaga 2002), but comparatively little is known about such refugia in species-rich areas at lower latitudes (Hewitt 2000, 2001). Indigenous forest in South Africa is one such species-rich biome, which contains 7.1% of the country's vascular plant species biodiversity (Geldenhuys 1992), whilst only occupying 0.3% of the land area (Mucina *et al.* 2003). There is an increasing awareness of the importance of forest conservation in the region due to growing pressures from agricultural expansion, changing fire regimes and the unregulated removal of plant products, hence pinpointing which of the fragments harbour high levels or unique aspects of biodiversity is important in conservation management (Eeley *et al.* 2001).

There is evidence that forest cover in South Africa has been greatly affected by past climatic change (Eeley *et al.* 1999), giving rise to range changes, disjunctions and population size fluctuations of native forest taxa. Very few studies have

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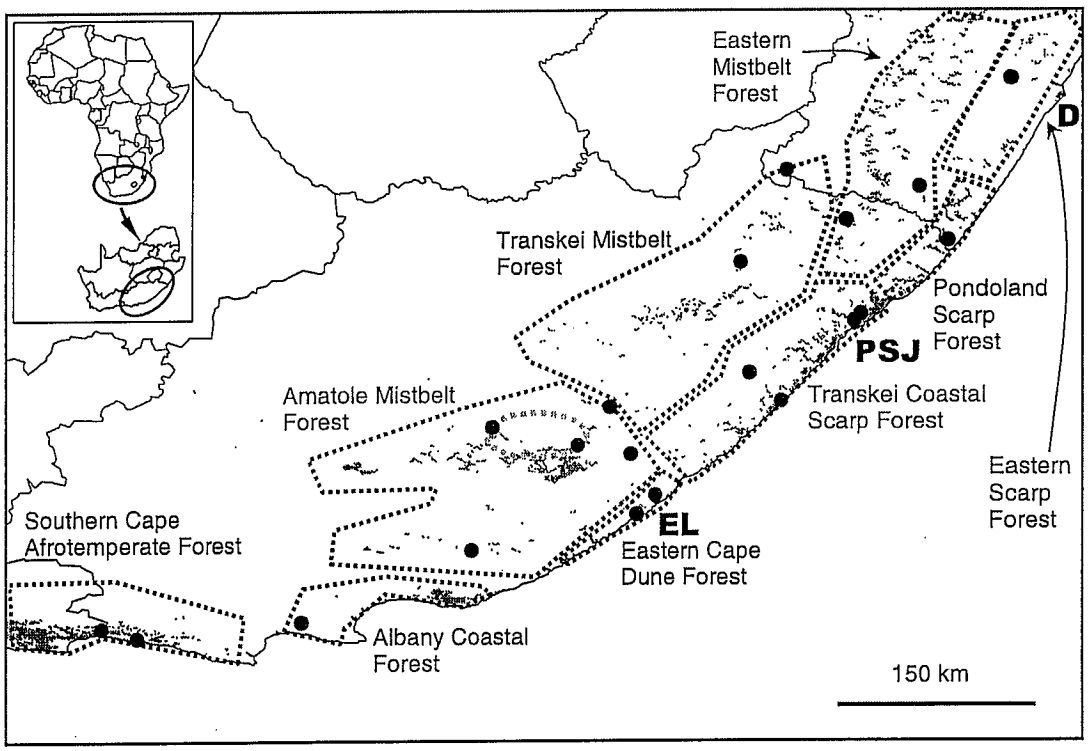

Fig. 1 Position of collection sites for *Streptocarpus* agg. *primulifolius* in South Africa. Dashed black lines indicate the classification of forest types in the study area according to Mucina *et al.* (2003). Green indicates the current extent of forest cover in the region (CSIR 1996). The Amatole Mountains are indicated by the orange dashed line. D, Durban; PSJ, Port St Johns; EL, East London.

attempted to document the effect of these factors on the evolution and divergence of plant taxa within the biogeographically complex and biodiversity-rich forest biome of South Africa. In order to investigate these issues, a molecular population genetic and phylogenetic study of the obligate forest-dwelling herbs *Streptocarpus primulifolius* Gandoger and *Streptocarpus rexii* (Hooker) Lindley was undertaken. We included material from populations that are considered to be intermediate between these two species (Hilliard & Burtt 1971; Möller *et al.* 2003), which we term *S.* cf. *primulifolius*. Further, the population of *S. primulifolius* ssp. *formosus* sampled from Umtamvuna is currently considered to belong to *S. formosus* (Hilliard & Burtt) T.J. Edwards. For convenience, all the taxa listed above are collectively referred to throughout this study as *S.* agg. *primulifolius*.

The forest habitat of *S.* agg. *primulifolius* has a highly fragmented distribution (Fig. 1), which has previously been attributed to anthropogenic clearance and increased fire frequency (Acocks 1953). However, this view has been challenged by recent evidence (Meadows & Linder 1993; Bond *et al.* 2003) suggesting the grassland currently interspersed between forest fragments is a natural and relatively ancient habitat, which began to spread in the Pliocene–Pleistocene following the evolution of C4 grasses, thus considerably pre-dating human influences on the landscape. The current extent of forest is presently understood to be an expansion of an even more restricted and fragmented distribution at the last glacial maximum (Meadows & Linder 1989; Eeley *et al.* 1999).

A molecular phylogeny of *Streptocarpus* suggests a north–south migration for the genus in Africa, with southern Africa witnessing a recent burst of speciation (Möller & Cronk 2001a). The taxa chosen for this study belong to the Cape Primrose clade (Möller & Cronk 2001b) and their distribution (Figs 1 and 2) represents the most southerly extension of the genus. *S. primulifolius* (*sensu lato*, including *S. formosus*) occurs mainly in the Eastern, Pondoland and Transkei scarp forests. The taxonomically intermediate populations of *S.* cf. *primulifolius* occur in the Amatole Mist Belt Forest and neighbouring Eastern Cape Dune Forest. Finally, *S. rexii* is found in the Eastern, Transkei and Amatole Mist Belt forests, as well as in coastal forest types in the south of its range, namely Albany Coastal and Southern Cape Afromontane Forest. Within these forest types, the *Streptocarpus* taxa are restricted to shaded and rocky banks of a southerly aspect, often near water.

The ranges of the taxa in *S.* agg. *primulifolius* will have been affected by Pleistocene climatic upheavals, and therefore their current distributions have been influenced by colonization events following climatic amelioration. In order to

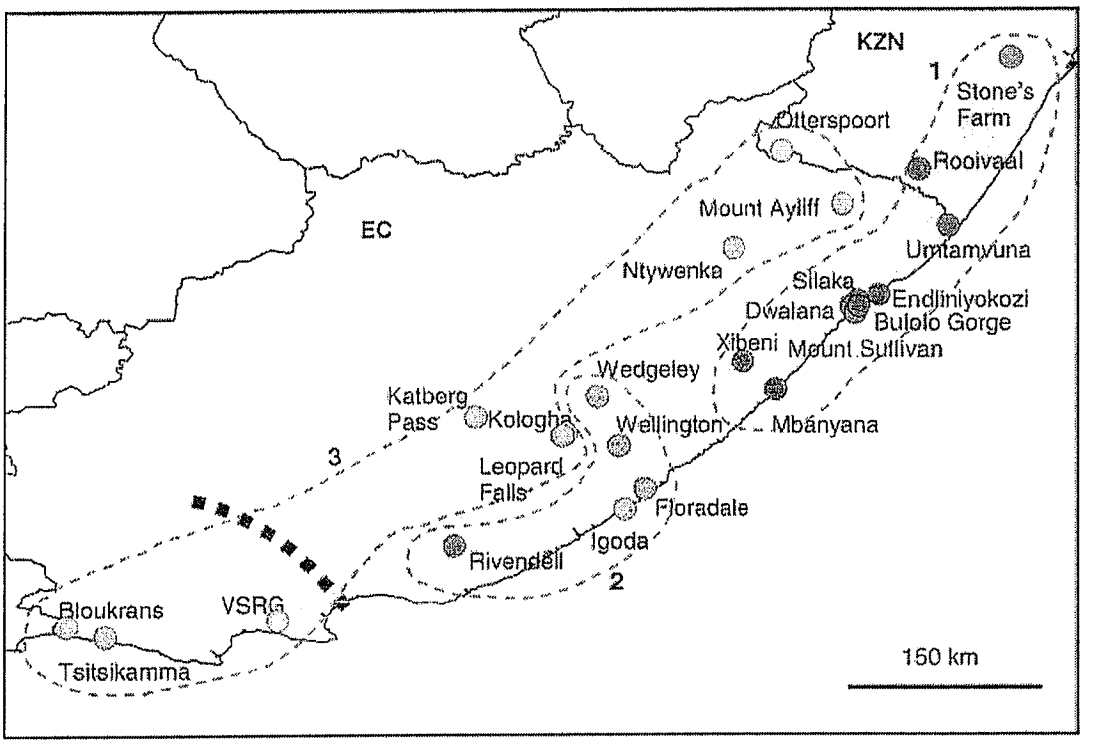

Fig. 2 The distribution of sampled populations of *Streptocarpus primulifolius sensu lato* (1), *Streptocarpus* cf. *primulifolius* (2) and *Streptocarpus rexii* (3). The colour of the circles represents gene diversity, ranging from 0.00 (blue) to 0.60 (red). The grey arrow indicates a generalized route of migration of *S.* agg. *primulifolius* from an origin in southern KwaZulu-Natal (KZN) through the Eastern Cape (EC). Blue dashed line indicates the position of the Sundays River valley.

gain a more complete picture of the population genetic history of *S.* agg. *primulifolius*, we utilized a combination of microsatellite allele frequency data, nuclear ribosomal ITS sequences and chloroplast genome sequence data. Specifically, this study aims to investigate (i) the age and locale of the split between the constituent taxa, (ii) which areas harbour refugial populations and (iii) the route and mode of colonization of any recently founded populations.

Materials and methods

Population sampling

Populations were sampled in January and February 2003 from forest fragments throughout eastern South Africa (Figs 1 and 2, Table 1), as silica-dried leaf tips. Where flowering material was available, voucher specimens were taken for each population, and deposited in herbaria E, NU and KEI.

Chloroplast and nuclear ribosomal ITS phylogenies

The chloroplast gene spacers *rpl*20-*rps*12, *trn*C-D and *trn*L-F were amplified using primers and polymerase chain reaction (PCR) conditions in Hamilton (1999), Demesure *et al.* (1995) and Taberlet *et al.* (1991), respectively. The presence of the lone polymorphism in the *trn*L-F spacer (a 65-bp deletion) was screened for on 2% agarose gels. The ITS primer combination for PCR amplification of the entire ITS region were '5P' (Möller & Cronk 1997) and '4' (White *et al.* 1990). The ITS PCR cycle parameters were as previously published in Möller & Cronk (1997). Intra-individual polymorphic sites in ITS were confirmed by sequencing five individuals from each population.

The PCR products were purified prior to sequencing using QIAGEN PCR purification kits according to the manufacturer's instructions. Sequencing was performed using a CEQ DTCS kit and a Beckman CEQ 8000 automated sequencer (Beckman Coulter) according to the manufacturer's protocol. Sequencing primers were identical to those used for PCR, with the addition that internal primers 'ycf6F' and 'psbmR' (Heinze 2002) were also used to sequence the *trn*C-D spacer. Sequence alignments were performed manually. Sequence characteristics were calculated using PAUP 4.0b10 (Swofford 2002). Sequence boundaries were determined by comparison with retrieved sequence data from GenBank for *Streptocarpus*.

Phylogenetic trees were generated from unordered, equally weighted characters using PAUP 4.0b10 (Swofford 2002). In

Table 1 Locality, altitude and population genetic statistics of *Streptocarpus* agg. *primulifolius* populations in Natal and the Eastern Cape

	Latitude	Longitude	Altitude (m)	*n*	*f*	H_E	H_O	P_L	*A*	Pr_A
S. primulifolius										
Stone's Farm	29.7654	30.6402	460	24.8	0.236**	0.27	0.21	5	2.7	7
Rooivaal	30.5876	29.9549	800	19.4	−0.071 NS	0.45	0.48	9	3.9	4
Umtamvuna*	31.0020	30.1730	20	18.1	0.126 NS	0.45	0.40	8	5.2	8
Endliniyokozi	31.5198	29.6731	50	18.0	0.160*	0.56	0.47	9	4.7	6
Dwalana	31.6046	29.4658	20	18.6	0.284**	0.55	0.40	9	4.6	6
Mt.Sullivan	31.5971	29.5298	20	30.7	0.067 NS	0.61	0.57	9	4.9	7
Silaka	31.6493	29.5055	10	13.6	0.192 NS	0.44	0.36	8	3.7	4
Bulolo Gorge	31.5573	29.5143	10	21.6	0.238**	0.56	0.43	9	5.0	4
Mbanyana Falls	32.2181	28.9048	40	21.9	0.143*	0.57	0.49	8	5.7	7
Xibeni	32.0063	28.6558	600	20.6	0.110 NS	0.52	0.47	9	6.0	11
Mean				**20.7**	**0.149**	**0.50**	**0.43**	**8.3**	**4.6**	**6.4**
S. cf. *primulifolius*										
Igoda	33.0931	27.7704	10	15.6	0.145 NS	0.24	0.21	6	2.1	0
Floradale	32.9406	27.9222	20	15.0	0.360**	0.36	0.23	8	2.7	1
Rivendell	33.3560	26.5055	500	19.9	0.330**	0.42	0.29	8	3.4	4
Post Wellington	32.6288	27.7256	650	24.9	0.393**	0.33	0.20	8	2.7	1
Wedgeley	32.2673	27.5690	1150	22.1	0.298**	0.36	0.25	7	2.6	1
Mean				**19.5**	**0.305**	**0.34**	**0.24**	**7.4**	**2.7**	**1.4**
S. rexii										
Ntywenka	31.1702	28.5810	1100	15.9	0.472**	0.12	0.06	3	1.4	0
Mt.Ayliff	30.8467	29.4045	1500	15.2	0.000 NS	0.01	0.01	1	1.1	0
Otterspoort	30.4583	28.9454	1500	13.0	NA	0.00	0.00	0	1.0	0
Katberg Pass	32.4619	26.6584	1300	18.4	0.497**	0.14	0.07	3	1.4	0
Leopard Falls	32.5586	27.3152	950	21.6	0.145 NS	0.28	0.24	7	2.4	0
Kologha	32.5377	27.3414	990	21.3	0.417*	0.14	0.09	4	1.6	0
VSRG	33.9100	25.1939	50	14.8	NA	0.00	0.00	0	1.0	1
Tsitsikamma	34.0202	23.9049	50	5.2	0.608 NS	0.10	0.04	2	1.4	0
Bloukrans Pass	33.9480	23.6267	50	9.8	1.000 NS	0.02	0.00	1	1.1	0
Mean				**15.0**	**0.448**	**0.09**	**0.06**	**2.3**	**1.4**	**0.1**

n, number of individuals; *f*, Weir & Cockerham's estimate of the inbreeding coeifficient F_{IS}; H_E, expected heterozygosity; H_O, observed heterozygosity; P_L, number of polymorphic loci; *A*, mean number of alleles per locus; Pr_A, total number of private alleles. **S. formosus sensu stricto.*

view of the relatively small number of taxa included in the matrices HEURISTIC searches were employed, using 100 000 random addition sequences with TBR, MULTREES and STEEPEST DESCENT options turned on. Branch support was estimated using bootstrap analyses (Felsenstein 1985) with FULL HEURISTIC searches in PAUP, using 10 000 replicates with TBR on and MULTREES off. Descriptive statistics reflecting the amount of phylogenetic signal in the parsimony analyses were given by the consistency index (CI: Kluge & Farris 1969) and retention index (RI; Farris 1989).

Two analyses, on the combined nuclear ITS 1, 5.8S and ITS 2 sequence data and the combined chloroplast *rpl*20–5′*rps*12 and *trn*C-D spacer sequences plus the single character for the *trn*L-F deletion, were subjected to phylogenetic analyses. Indels were scored as a separate presence/absence character and added to the sequence data matrix (Simmons & Ochtorena 2000). A 12-bp inversion at the 5′ end of the *rpl*20-*rps*12 spacer was offset (Schill *et al.* 2004) and coded as a single character to avoid overestimation of genetic distances. The data matrices each contained 25 sequences, representing 24 ingroup populations of *Streptocarpus rexii*, *Streptocarpus* cf. *primulifolius* and *Streptocarpus primulifolius*. Sequences of *Streptocarpus polyanthus* were used as an outgroup, based on results by Möller & Cronk (1997, 2001a, b) on which the trees were rooted.

Divergence time dating

To obtain an approximate dating of branching events between species and populations, the average sequence divergence values were calculated from the uncorrected mean pairwise distances. The average divergence time across all previously reported rates for ITS as summarized by Richardson *et al.* (2001) was used (Yuan *et al.* 2003). Evenly weighted MP branch lengths may be biased as they are not corrected for multiple hits (Sanderson 2002). However, a likelihood-based correction was not performed because of the very low level of sequence divergence (Yuan *et al.* 2005).

Nuclear microsatellites

Nine polymorphic microsatellite loci were used for the population genetic surveys, using fluorescently labelled primers and protocols as detailed in Hughes *et al.* (2004).

Microsatellite allele data was formatted for analysis using the Microsatellite Toolkit (Park 2001). Descriptive parameters (A, mean number of alleles per locus; Pr_A, number of private alleles; H_E, expected heterozygosity; and H_O, observed heterozygosity) were calculated using GDA (Lewis & Zaykin 2001). Weir & Cockerham's (1984) estimate of F_{IS} (f, deviation from panmixia attributable to nonrandom mating within populations), was calculated using FSTAT (Goudet 2002), using a permutation test (1000 permutations, randomising alleles) to assess significance.

Unlinked markers such as microsatellites can theoretically be used to construct phylogenies of populations or taxa (Takezaki & Nei 1996), and many studies have shown this to be practically possible (Harr *et al.* 1998; Petren *et al.* 1999; Richard & Thorpe 2001; Koskinen *et al.* 2002; Perez *et al.* 2002). Richard & Thorpe (2001) also showed that even as few as five loci gave a phylogeny congruent with other estimates based on organelle DNA sequences. However, when interpreting phylogenies based on a number of unlinked microsatellite loci, one should be aware that (i) the phylogeny may differ according to the genetic distance used to construct it, and (ii) it may be confounded by ongoing migration between populations. We were careful in this case to only draw general conclusions that were supported by alternative phylogenetic hypotheses. Further, our study species are characterized by very high levels of population differentiation and are distributed in isolated forest patches surrounded by otherwise unfavourable habitat, and hence very low rates of ongoing migration between populations are expected. MICROSAT (Minch *et al.* 1995) was used to create population pairwise genetic distance matrices for $1 - P_{SA}$ (Bowcock *et al.* 1994) and chord distance (Cavalli-Sforza & Edwards 1967). POPULATIONS (Langella 2002) was used to create a Nei's standard genetic distance (D_a; Nei *et al.* 1983) matrix. NEIGHBOUR, part of the PHYLIP package (Felsenstein 2002), was used to create neighbour-joining trees from these distance matrices, which were visualized using TREEVIEW (Page 1996). Bootstrap values were calculated by exhaustively resampling over loci.

Results

Nuclear ribosomal ITS sequences

Phylogenetic analysis. The nuclear ribosomal ITS sequences gave a total aligned length of 642 bases, with 19 of these sites being polymorphic (2.96%) and 5 of these being parsimony informative. Four equally most parsimonious trees were found of 19 steps length (CI = 1.0, RI = 1.0), one of which is presented in Fig. 3. Two main clades were present in all trees, namely (i) a clade containing the northerly populations of *Streptocarpus primulifolius sensu lato* from Stone's Farm, Rooivaal Farm and Umtamvuna (BS = 86%), and (ii) a clade containing all the other sampled populations (BS = 64%). Within this second larger clade was a smaller subclade containing the three northernmost populations of *Streptocarpus rexii*, with the remaining populations of *Streptocarpus primulifolius*, *S.* cf. *primulifolius* and *S. rexii* being present in two grades. Equally parsimonious arrangements of the Mbanyana and Xibeni populations between these grades are responsible for the four alternative tree topologies found. The two populations are either placed (alone or together) in the grade as shown in Fig. 2, or in the grade containing the *S. primulifolius* populations from Port St Johns. The different arrangements depend on how the intra-individual polymorphic site at position 604 is interpreted (character states shown in Fig. 3). Position 70 was also polymorphic within individuals (Fig. 2), with most of the populations of *S.* cf. *primulifolius* (except Igoda) and the two southerly populations *S. primulifolius* from Mbanyana and Xibeni possessing the polymorphism at that site. The phylogeny shows a broad pattern of north–south migration within *S. primulifolius*, with the populations at the south of the range (Xibeni and Mbanyana) being genetically intermediate between *S. primulifolius* and *S. rexii* when considering the polymorphic site 604. The phylogeny is not resolved enough to shed any light on the biogeography of *S. rexii*, although the three northern populations from Ntywenka, Otterspoort and Mount Ayliff possess a synapomorphy at position 201 which indicates a common origin for these three localities.

Dating results. Applying a precalibrated mutation rate to the tree places a maximum divergence time between the northern populations of *S. primulifolius* (Stone's Farm ITS type) and the northern populations of *S. rexii* (Ntywenka ITS type) as 1.11 (± 0.1 SE) million years (Myr) (5 steps; 0.78%). The divergence of *S. rexii* from *S.* cf. *primulifolius* can be dated to between 0 and 0.44 (± 0.04 SE) million years ago (Ma) (0–2 steps; 0–0.31%). However, given the small number of mutations separating the ITS types and the stochastic nature of mutation, and also the fact that the mutation at position 70 is not homogenized, this estimate is tentative.

Chloroplast sequence data

The combined alignment of *rpl*20-*rps*12 and *trn*C-*trn*D was 3190 bp in length, with 40 of these sites being polymorphic (1.25%) and 7 being parsimony informative, including the 65-bp indel found in the *trn*L-F intergenic spacer. Two most parsimonious trees were obtained with 42 steps (CI = 0.95, RI = 0.95). Two supported clades could be identified (Fig. 3), one consisting of most of the *S. primulifolius* populations from

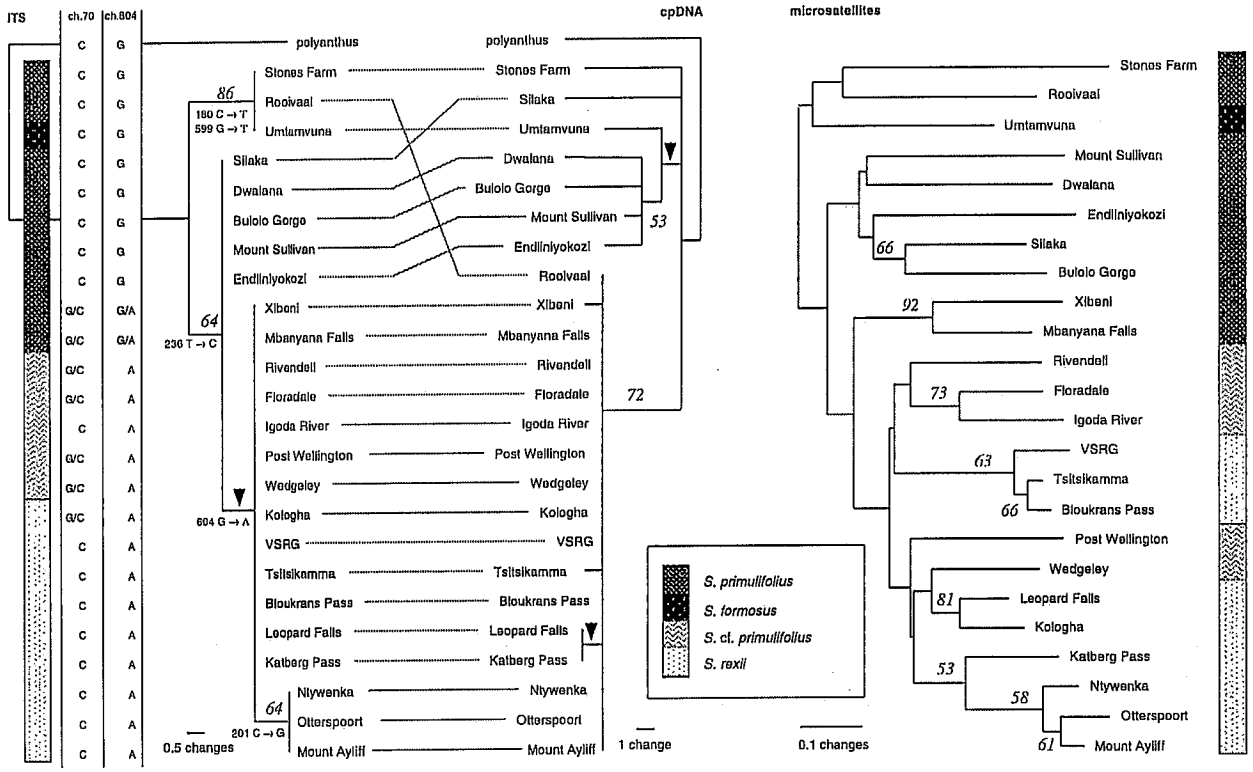

Fig. 3 Most parsimonious trees for ITS (left, one of four) and chloroplast data (middle, one of two). Characters showing intra-individual polymorphisms in ITS sequences (chars. 70 and 604) are indicated. Right, neighbour-joining tree from nuclear microsatellite data based on $1 - P_{SA}$. Arrows indicate branches which collapse in strict consensus trees.

Port St Johns (Dwalana, Mount Sullivan, Bulolo Gorge and Endliniyokozi) (BS = 53%), and the other of all the remaining populations (BS = 72%) with the exception of the populations of *S. primulifolius sensu lato* from Stone's Farm, Silaka and Umtamvuna (*Streptocarpus formosus*) which take up an unresolved position at the base of the ingroup.

Within the first clade, all of the populations had distinct haplotypes separated by between three and eight steps (0.09–0.25%) (Fig. 3). Among the populations from Port St Johns, Silaka was most distant from the others with a distance of 10–11 steps (0.32–0.37%). Further analysis of all individuals in these populations by restriction fragment length polymorphism (RFLP) of the *trn*C-*trn*D region using *Taq*I revealed each population in this clade to be homogenous for a unique haplotype.

The second clade is far less genetically diverse, with most of the populations of *S.* cf. *primulifolius* and *S. rexii* sharing the same haplotype. Within this clade, some populations have autapomorphies (Xibeni with a unique point mutation at position 511; Tsitsikamma with a unique 7-bp duplication at position 1007 in *trn*C-*trn*D), and Leopard Falls and Katberg Pass have a synapomorphy which is a homoplastic occurrence of a 12-bp inversion in *trn*C-*trn*D, also found in all of the populations from the Port St Johns vicinity except Silaka. The haplotype possessed by Rooivaal belongs in this clade, and hence is identical to the haplotype found in the majority of populations of *S.* cf. *primulifolius* and *S. rexii*. The marked incongruence between nuclear (both ITS and microsatellite) and chloroplast data for this population can be interpreted as a chloroplast capture event.

Nuclear microsatellite data

Descriptive statistics and diversity indices. The mean number of samples genotyped per population varied between 5.2 (*S. rexii*, Tsitsikamma) and 30.7 (*S. primulifolius*, Mount Sullivan). The small sample number for some of the southernmost *S. rexii* populations such as Tsitsikamma was caused by a high phenolic content in the genomic DNA extractions, which made the samples extremely reticent to PCR. This effect was observed across all loci in these populations, and hence is not likely to be a problem caused by null alleles.

Mean estimates of the per-population inbreeding coefficient F_{IS} for the three taxa were $f = 0.149$ (*S. primulifolius*), $f = 0.305$ (*S.* cf. *primulifolius*) and $f = 0.448$ (*S. rexii*). The estimate for *S. primulifolius* is significantly lower when compared to the per-populations estimates for the other two

taxa (*t*-test, $P < 0.007$); the estimates for *S.* cf. *primulifolius* and *S. rexii* do not differ significantly from each other. Although there does appear to be a significantly higher level of inbreeding in populations of *S.* cf. *primulifolius* and in particular *S. rexii*, these results must be interpreted in the light of a high per-locus and per-population variance in these estimates. High variance in F_{IS} estimates can be caused by the presence of null alleles, which cause an upward bias due to heterozygote detection failure. In rare cases, individuals were found which did not give a product for a particular locus despite amplifying normally for the remainder (homozygous nulls). Hence, it is likely that null alleles have contributed to the observed variance. However, there was no evidence to suggest that the higher estimate of F_{IS} in *S. rexii* was due to nulls being more prevalent in this species than in *S. primulifolius*. Given the very low gene diversity in this species, any nulls present are likely to have generated a large number of homozygotes, leading to large-scale PCR failure for that particular locus; this was not observed in *S. rexii*. However, the highest variance in estimates of F_{IS} was seen in *S. rexii*, which is probably due to the fact that in some cases they were drawn from very small amounts of polymorphism in an otherwise entirely homozygous background. In some populations of *S. rexii*, no estimation of F_{IS} was possible due to all individuals being homozygous at all loci.

The three measures of genetic diversity calculated (gene diversity, H_E; number of polymorphic loci, P_L; number of alleles per locus, *A*) all show a significant decreasing trend from *S. primulifolius* to *S. rexii* (Table 1). All of these indices show values that are significantly different between all taxa at the $P < 0.01$ level, with the exception of the comparison of the number of polymorphic loci between *S. primulifolius* and *S.* cf. *primulifolius* which is not significant. The number of alleles per locus must be interpreted in the light of sample sizes which are not identical; allelic richness, a measure of number of alleles per locus standardized for sample size, was not calculated due to the amount of missing data in a small number of populations which reduced the standardized sample size to a very small level. However, the mean sample sizes for *S. primulifolius* and *S.* cf. *primulifolius* are very similar ($n = 20.7$ and $n = 19.5$, respectively), and the mean sample size for *S. rexii* is only slightly smaller at $n = 15.0$.

The number of private alleles (Pr_A) per population showed a similar trend to the diversity indices, with $Pr_A = 6.4$, 1.4 and 0.1 for *S. primulifolius*, *S.* cf. *primulifolius* and *S. rexii*, respectively (Table 1). The figures are significantly different between all the taxa at $P < 0.001$ level, with the exception of the comparison between *S.* cf. *primulifolius* and *S. rexii* which is significant at the $P < 0.05$ level.

Neighbour-joining analysis. The neighbour-joining tree produced from a proportion of shared alleles distance matrix gave a topology which was broadly congruent with that found in the phylogenetic analysis of the ITS data and with the tree derived from chloroplast sequence data. Three main groups can be defined which correspond to the ITS phylogeny: (i) the northerly populations of *S. primulifolius sensu lato* (Stone's Farm, Rooivaal Farm and Umtamvuna; the latter is *S. formosus sensu stricto*), (ii) the *S. primulifolius* from the Port St Johns vicinity (Mount Sullivan, Dwalana, Endliniyokozi, Silaka and Bulolo Gorge) and (iii) a large group consisting of the southerly populations of *S. primulifolius* (Xibeni and Mbanyana), plus all populations of *S.* cf. *primulifolius* and *S. rexii*. The northernmost population sampled, Stone's Farm, is on the longest branch of any population in the microsatellite neighbour-joining tree, having pairwise genetic distances to other populations of *S. primulifolius* of up to 0.996. Bootstrap values based on resampling of loci gave little support for basal nodes in the tree, although higher values were obtained for some population groups (support values of > 50% are given on the tree in Fig. 3).

Trees produced using Nei's D_a produced the same three groups, with minor re-arrangments within the groups. Chord distance differed from the displayed topology mainly in that the two southernmost populations of *S. primulifolius* (Xibeni and Mbanyana) became sister to the *S. primulifolius* from the Port St Johns vicinity instead of being sister to *S.* cf. *primulifolius* and *S. rexii*.

Within the group consisting of *S.* cf. *primulifolius* and *S. rexii*, some groupings were consistently recovered independent of the genetic distance measure used. These tend to be geographically proximal groups of populations, namely Rivendell, Floradale and Igoda River from the Grahamstown vicinity and coastal areas near East London; VSRG, Tsitsikamma and Bloukrans Pass from the coast between Port Elizabeth and Plettenberg Bay; and Otterspoort, Ntywenka and Mount Ayliff from the southern Drakensberg foothills. The populations of *S.* cf. *primulifolius* and *S. rexii* did not form respective single groups in any analysis, there always being some intermixing of the two, with *S.* cf. *primulifolius* populations being on longer branches, branching off nearer the base of the group than the *S. rexii* populations. There is some incongruence between the microsatellite-derived tree and the chloroplast phylogeny with respect to the position of the Katberg and Leopard Falls populations. In the chloroplast tree, these populations share a synapomorphy, whilst in the microsatellite tree they are in different groups.

Discussion

All three sources of data (nuclear ribosomal ITS sequences, chloroplast genome sequences and nuclear microsatellites) give a congruent picture of the recent history of the study species and their constituent populations, in which *Streptocarpus primulifolius* is the older and more genetically diverse progenitor of *Streptocarpus rexii*.

Pleistocene refugia

The maximum divergence time of 1.11 Myr between *S. primulifolius* and *S. rexii*, as calculated from the ITS phylogeny, places the split between these two species in the early Pleistocene. The microsatellite and chloroplast data are consistent with the populations of *S. primulifolius sensu lato* being Pleistocene relicts, each harbouring a high level of nuclear genetic diversity and in most cases unique and divergent chloroplast haplotypes. The Stone's Farm population is particularly divergent, and is restricted to a very small area of habitat, which is a shaded rocky cliff approximately 20 m² with plentiful groundwater seepage. This suggests that this and other similar areas may have been important as microrefugia, allowing the persistence of highly mesophytic taxa such as *Streptocarpus* during the climatic upheavals of the Pleistocene.

The large amount of genetic diversity held by the populations in the Port St Johns vicinity with respect to both nuclear and chloroplast diversity is also remarkable. With one exception (Silaka), these populations are polymorphic at all loci, and have amongst the highest estimates of H_E found in this study. Each population also has a considerable number of private alleles, despite being sampled from a relatively confined area, which along with the chloroplast data indicates that significant diversity is partitioned between, as well as within, populations. The maintenance of such high levels of diversity, in particular the number of divergent and unique chloroplast haplotypes, indicates that the Port St Johns vicinity has been able to support sizeable populations of these forest-dwelling plants throughout recent periods of climate change. This is consistent with the date of divergence of these populations from the more northerly populations of *S. primulifolius* (0.67 Myr, ± 0.062 SE). The maintenance of forest cover and suitable habitat for *Streptocarpus* in this area has probably been influenced by two main factors. First, the Port St Johns forest complex is the largest forest block in eastern South Africa, which indicates currently favourable climatic conditions, with high rainfall, humidity and stable temperature. During periods of climatic deterioration, the most favourable areas for forest persistence are likely to be in such areas near the coast where conditions will be ameliorated by the Mozambique Current. Second, the highly dissected terrain means that sheltered gorges and south-facing cliffs are common, and these provide protection from both fire and prolonged exposure to drying sunlight. The area is also the confluence for a drainage basin for a *c.* 200-km stretch of the southern Drakensberg Mountains, ending in the Mzimvubu River. This could potentially provide sufficient groundwater to sustain forest through the diversion of orographic precipitation in the Drakensberg, even in the absence of sufficient local rainfall.

The southernmost populations of *S. primulifolius* sampled for this study were sampled from the Mbashe River Valley, one upstream (Xibeni) and the other downstream near the coast (Mbanyana). Both populations also have very high levels of genetic diversity (as indicated by H_E, P and A; Table 1). Xibeni also has the highest number of private alleles of any population in the study ($Pr_A = 11$), which along with the high diversity indices indicates that this is not a population that has been recently founded from a coastal refuge such as Mbanyana. The pairwise distance between these two populations is relatively low (Fig. 2) and the relationship between them is supported by a high bootstrap value (92%) in the microsatellite neighbour-joining tree. As well as reflecting common ancestry, this may reflect the relative ease of gene flow along a river course, possibly via unsampled populations along the valley.

These results confirm hypotheses about the refugial nature of many of the coastal scarp forest fragments and some sheltered mist belt areas (Lawes 1990). In particular, we highlight the forest patches around Port St Johns, Mbanyana (The Haven) and Umtamvuna as being important in this respect.

Migration and ecological differentiation

Although the extensive use of ITS sequence data in angiosperm phylogenetic research has allowed us to produce a calibrated tree and obtain divergence time estimates, the resolution found between our study taxa was low using this phylogenetic marker. Phylogenetic estimates using chloroplast and microsatellite data have given more resolution at the population level, and the latter has been especially useful in giving insight into the divergence of *S. rexii* from *S.* cf. *primulifolius*. Considering the microsatellite data in terms of phylogenetic inference informed also by the genetic diversity indices, *S. rexii* appears to be a very recent and genetically depauperate entity which has arisen out of *S.* cf. *primulifolius*. Our data are congruent with a hypothesis of a southerly coastal migration of *S. primulifolius*, followed by migration with speciation from the coast inland towards the Amatole Mountains (*S.* cf. *primulifolius* × *S. rexii*). From an origin in the centre of its range, *S. rexii* has migrated northwards through the Transkei mist belt forests, and southwards into the southern Cape Afrotemperate forests (Fig. 2).

Streptocarpus *cf.* primulifolius. The populations designated to belong to *S.* cf. *primulifolius* (Rivendell, Floradale, Igoda River, Post Wellington and Wedgeley) are intermediate between *S. primulifolius* and *S. rexii* in terms of H_E, P and A (Table 1). They do not form a single group in the neighbour-joining tree derived from the nuclear microsatellites, but cluster with all the sampled populations of *S. rexii* (Fig. 2). *S.* cf. *primulifolius* is also morphologically intermediate between *S. primulifolius* and *S. rexii*, with the flowers appearing like a large and deeply coloured *S. rexii*; there was a range of floral variation which according to field observations was mostly partitioned between populations.

Some of the populations we have assigned to *S.* cf. *primulifolius* from the East London vicinity have previously been described as 'the same species as that at Port St Johns (*S. primulifolius*), but tending towards, most probably introgressed by, *S. rexii*.' (Hilliard & Burtt 1971, p. 271). Based on information from the *trn*L-F intergenic spacer deletion, the population from Igoda River was also described as being of hybrid origin (*S. rexii* × *S. primulifolius*) by Möller *et al.* (2003). However, using three data sets with comprehensive population sampling, we here consider the populations of *S.* cf. *primulifolius* to represent an intermediate form between *S. primulifolius* and *S. rexii*, resulting from the evolution of *S. rexii* from within *S.* cf. *primulifolius*. Although it is probable that during climatic fluctuations there will have been some contact between neighbouring populations as forest cover expanded, to account for the presence of *S.* cf. *primulifolius* across its range of over 150 km by hybridization events alone would require extensive gene flow between *S. rexii* and *S. primulifolius*. Calculating Weir & Cockerham's (1984) estimate of F_{ST} from the microsatellite data gives values for *S. rexii* and *S. primulifolius* of $\theta = 0.756$ and $\theta = 0.370$, respectively. As these poorly dispersed species are also growing in restricted favourable areas within an already highly fragmented forest habitat, extensive population admixture and hybridization at this scale seems unlikely.

The inland migration of *S.* cf. *primulifolius* is likely to have been aided by the fact that the region between the Amatole Mountains and East London is a meeting point for the inland Afrotemperate mistbelt forests (Amatole Mistbelt) and small fragments of the subtropical coastal forests (Eastern Cape Dune Forest) along a comparatively gentle environmental gradient (L. Mucina, personal communication). The presence of several Afrotemperate elements [e.g. yellowwoods (*Podocarpus*)] in the Eastern Cape dune forests has been suggested as evidence for a riverine link between coastal and montane forest (Burns & Raal 1993). These forest types do meet to some extent further north (Transkei Mistbelt Forest and Transkei Coastal Scarp Forest), but the forest patches are smaller and more scattered, making dispersal and migration more difficult. Also, the two types occur on separate and parallel escarpments and hence lack a gradual environmental gradient between them.

Streptocarpus rexii. It is tempting to speculate that *S. rexii* has arisen out of its relatively polymorphic and genetically diverse progenitor *S.* aff. *primulifolius* due to selection for (i) cold tolerance and (ii) an increased capacity for autogamy.

The mean minimum winter temperature for these taxa is shown in Fig. 4. *S. primulifolius* is a plant of subtropical coastal forests and does not fare well at temperatures below 5 °C (D.U. Bellstedt, personal observation). *S. rexii* can cope with the lower temperatures found at higher altitudes (e.g. Mount Ayliff) and lower latitudes (e.g. Tsitsikamma). The acquisition of tolerance for lower

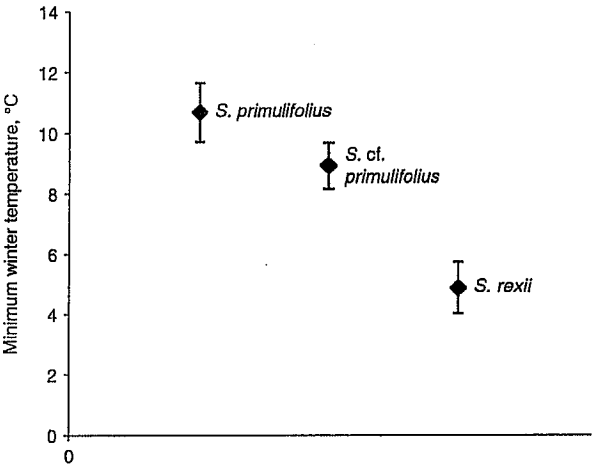

Fig. 4 Minimum winter temperature averaged over all collection localities for the three taxa (temperature data obtained from WORLDCLIM 1.3; Hijmans *et al.* 2004).

temperatures could have been facilitated by the relatively gentle environmental gradient between the Amatole mist belt and coastal dune forests.

The smaller, paler corolla in *S. rexii* appears to be related to an increased selfing rate. Although there is quite a high variance in estimates of F_{IS}, and reliable estimates were difficult to obtain from *S. rexii* due to a lack of gene diversity, this is congruent with greenhouse observations. Seeds of *S. primulifolius* from Mount Sullivan were sown at the same time as seeds of *S. rexii* from Tsitsikamma and the resulting progeny flowered at the same time in November. In the absence of pollinators, the corollas on *S. primulifolius* plants withered and dropped and all the fruits aborted. Flowers on *S. rexii* plants however, revealed close to 100% fruit set. Selfing in this species is apparently aided by increased proximity of the anthers and stigma, with the stamens elongating during flower maturation until brought into contact with the stigma. Selection for a floral morphology which aids autogamy is likely to have occurred during colonization of new sites, in a manner analagous to that of Bakers' Law (Baker 1955; Stebbins 1957).

Considering both the microsatellite neighbour-joining tree derived and the genetic diversity indices, we can gain insight into the dispersal of *S. rexii* from its central area of origin. The three southernmost populations (VSRG, Tsitsikamma and Bloukrans) and the three northernmost (Mount Ayliff, Ntywenka and Otterspoort) each form respective groups on long branches within the *S.* cf. *primulifolius*/*S. rexii* group, with very little genetic distance between the neighbouring populations (Fig. 3). This topology, and the fact that these populations harbour extremely low levels of genetic diversity as indicated by all indices, is consistent with these edge of range population groups each having a monophyletic

origin from a very small number of individuals (Ibrahim *et al.* 1996).

In the south, this is congruent with the fact that there is a biogeographical barrier to mesic taxa such as *Streptocarpus* in the vicinity of the Sundays River Valley (Fig. 1). There is a finger of the Karoo Biome that extends from the interior down towards the coast between East London and Port Elizabeth. This relatively low-lying region, termed the 'Bedford Gap' (Lawes 1990), has a more pronounced summer dry period than the surrounding area, conditions which are thought to have persisted since the late Pliocene (Geldenhuys 1992). Thus, the southern populations of *S. rexii* cannot have migrated there with increasing forest cover; they must have colonized the area via long-distance dispersal. This could conceivably have been assisted through stepping stone opportunities offered by the sheltered southern slopes of the Zuurberg, which currently harbour sparse forest remnants.

Depending on the genetic distance measure used, the three northerly populations are most similar to either Katberg (P_{SA} and Chord distance) or Wedgeley (Nei's D_a). Thus, it is likely that these populations have originated from an origin somewhere in the northern Amatole Mistbelt Forests. There is some incongruence between the microsatellite results and the ones obtained from the chloroplast phylogeny (Fig. 3), as the Katberg Pass population shares a synapomorphy with Leopard Falls; thus, one would expect any populations founded from the Katberg Pass area to share this maternally inherited polymorphism. However, the occurrence of the observed chloroplast polymorphism (a 12-bp inversion in *trn*C-*trn*D) was not possible to screen with an RFLP analysis, and so the extent of within-population polymorphism for this character is unknown. The extent of the forest cover along the inland Transkei mistbelt during the Holocene altithermal is likely to have been more extensive than at present. Our results do not suggest that forest cover became continuous, facilitating the wholesale migration of *S. rexii*, but are congruent with a hypothesis of reduced distance between patches, increasing the probability of successful dispersal events.

These results highlight the importance of rare long-distance dispersal events in shaping species distributions (Cain *et al.* 1998). Given reasonable estimates of mutation rates of 10^{-2} to 10^{-4} for microsatellite loci in angiosperms (Udupa & Baum 2001; Thuillet *et al.* 2002; Vigouroux *et al.* 2002) and the extremely low or absent genetic diversity found at the extremes of the distribution of *S. rexii*, it is likely that this species reached its current range during the Holocene. The recent northward migration of *S. rexii* along the Drakensberg escarpment brought this species into contact with *S. primulifolius* in southern KwaZulu-Natal, as evidenced by the chloroplast capture observed in the population from Rooivaal, which shares a common haplotype with *S. rexii*. There is no evidence in the data for ongoing nuclear introgression however. The contact between Rooivaal Farm in southern KwaZulu-Natal and the current nearest populations with the acquired haplotype (*S. rexii* in the Mount Ayliff vicinity) may have been facilitated by the expansion of mist belt forests during the Holocene altithermal as modelled by Eeley *et al.* (1999).

Species concepts

The Umtamvuna population is homogenous for a unique chloroplast haplotype nested within the variation observed in *S. primulifolius sensu lato* (Fig. 3). This population belongs to *S. formosus* (Hilliard & Burtt) T.J. Edwards, which was raised to specific rank from *S. primulifolius* ssp. *formosus* Hilliard & Burtt (Weigend & Edwards 1994). Hence, *S. primulifolius* as currently circumscribed is paraphyletic. *S. formosus* has a narrow distribution on the Pondoland coast, where it is restricted to a small number of river gorges. As only one population of the species was sampled, the monophyly of *S. formosus* itself is unknown, although a single individual sampled from Oribi Gorge was found to have an identical chloroplast haplotype to the Umtamvuna population. *S. primulifolius* is also paraphyletic with respect to *S.* cf. *primulifolius* and *S. rexii*, according to all three data sets. Further, there is no evidence to suggest that either of these latter two taxa are themselves monophyletic; *S.* cf. *primulifolius* is likely to be paraphyletic and there is evidence to suggest *S. rexii* is polyphyletic, as the populations of this species do not cluster together as a single unit in any of the trees generated from the nuclear microsatellite data. Hence, one should be flexible in applying species concepts when dealing with recently diverged entities. Paraphyletic progenitors may be prevalent when dealing with speciation in fragmented habitats; this may be a factor contributing to the taxonomic complexity seen in many *Streptocarpus* species, and in other forest-dwelling herbs such as *Plectranthus*.

Conclusions

This is the widest ranging plant population genetic study ever undertaken in South Africa, and the first to cover a species complex over several different forest types. By using three different genetic data sources, we have been able to gain an understanding of forest history and refugia in eastern South Africa, and confirm hypotheses about the relictual nature of the coastal scarp forests. *S. primulifolius* is likely to have existed for about 1.11 Myr in this habitat, which hence represents a minimum age for these forests, and the high level of within-species variation found in its populations should be considered in conservation efforts (Matolweni *et al.* 2000). Some of the collections of this species have been from very small habitat remnants, which indicate the considerable conservation value of these as microrefugia.

Acknowledgements

This research was funded by the Leverhulme Trust (grant no. F/00 771 A-0735), and carried out at the Royal Botanic Garden Edinburgh, which is supported by the Scottish Executive Environment and Rural Affairs Department. Financial support is also acknowledged from the South African National Research Foundation and the Carnegie Trust for the Universities of Scotland. The authors would like to thank Dr M. Hollingsworth, A. Clark and Dr E. Haston for technical support. The following landowners are thanked for permission to collect from their farms: Johann Botha, Cedarville and Otterspoort; Mervyn Hart, Post Wellington Farm; Nicolas James, Rivendell Farm; Neil and Carmen Potter, Wedgeley Farm; Steven Rennie, Rooivaal Farm; 'Klippy' Stone(†), Stone's Farm. The following conservation authorities are thanked for issuing permits: KZN Permits Office, Cascades, KwaZulu Natal; South African National Parks, Conservation Services, Sedgefield; the Western Region, Central Region, Umtata and Kokstad offices of the Department of Economic Affairs, Environment and Tourism, Eastern Cape; Western Cape Nature Conservation, Cape Town. Tony Abbott, Elize Cloete, Ben Dekker, Tony Dold, Siyabulela Nonjinge, Cameron MacMaster and Avril and Victor Sonemann are thanked for assistance in the field.

References

Acocks JPH (1953) Veld types of South Africa. *Memoirs of the Botanical Survey of South Africa*, **28**, 1–192.

Baker HG (1955) Self-compatability and establishment after 'long-distance' dispersal. *Evolution*, **9**, 347–348.

Bond WJ, Midgely GF, Woodward FI (2003) What controls South African vegetation — climate or fire? *South African Journal of Botany*, **69**, 79–91.

Bowcock AM, Ruiz-Linares A, Tomfohrde J, Minch E, Kidd JR, Cavalli-Sforza LL (1994) High resolution of human evolutionary trees with polymorphic microsatellites. *Nature*, **368**, 455–457.

Burns MER, Raal PA (1993) Dune forests of the Eastern Cape. In: *South African Forestry Handbook* (ed. Van der Sijde HA), pp. 639–646. Southern African Institute of Forestry, Pretoria, South Africa.

Cain ML, Damman H, Muir A (1998) Seed dispersal and the Holocene migration of woodland herbs. *Ecological Monographs*, **68**, 325–347.

Cavalli-Sforza LL, Edwards AWF (1967) Phylogenetic analysis: models and estimation procedures. *American Journal of Human Genetics*, **19**, 233–257.

CSIR (1996) *South African National Land-Cover GIS Data*. Council for Scientific and Industrial Research, Pretoria, South Africa.

Demesure B, Sodzi N, Petit RJ (1995) A set of universal primers for the amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. *Molecular Ecology*, **4**, 129–131.

Eeley HAC, Lawes MJ, Piper SE (1999) The influence of climate change on the distribution of indigenous forest in KwaZulu-Natal, South Africa. *Journal of Biogeography*, **26**, 595–617.

Eeley HAC, Lawes MJ, Reyers B (2001) Priority areas for the conservation of subtropical indigenous forest in southern Africa, a case study from KwaZulu-Natal. *Biodiversity and Conservation*, **10**, 1221–1246.

Farris JS (1989) The retention index and homoplasy excess. *Systematic Zoology*, **38**, 406–407.

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39**, 783–791.

Felsenstein J (2002) *PHYLIP (Phylogeny Inference Package) version 3.57c*. http://evolution.genetics.washington.edu/phylip.html.

Geldenhuys CJ (1992) Richness, composition and relationships of the floras of selected forests in southern Africa. *Bothalia*, **22**, 205–233.

Goudet J (2002) *FSTAT: a program to estimate and test gene diversities and fixation indices (version 2.9.3)*. www2.unil.ch/izea/softwares/fstat.html.

Hamilton MB (1999) Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. *Molecular Ecology*, **8**, 521–523.

Harr B, Weiss S, David JR, Brem G, Schlotterer C (1998) A microsatellite-based multilocus phylogeny of the *Drosophila melanogaster* species complex. *Current Biology*, **8**, 1183–1186.

Heinze B (2002) *A database for primers in the chloroplast genome*. http://bfw.ac.at/200/1859.html.

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

Hewitt G (2001) Speciation, hybrid zones and phylogeography — or seeing genes in space and time. *Molecular Ecology*, **10**, 537–549.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2004) *The WORLDCLIM interpolated global terrestrial climate surfaces. Version 1.3*. http://biogeo.berkeley.edu/.

Hilliard OM, Burtt BL (1971) Streptocarpus: *An African Plant Study*. University of Natal Press, Pietermaritzburg, South Africa.

Hughes M, Möller M, Bellstedt DU, Edwards TJ (2004) EST and random genomic nuclear microsatellite markers for *Streptocarpus*. *Molecular Ecology Notes*, **4**, 36–38.

Ibrahim KM, Nichols RA, Hewitt GM (1996) Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. *Heredity*, **77**, 282–291.

Kluge AG, Farris JS (1969) Quantitative phyletics and the evolution of anurans. *Systematic Zoology*, **18**, 1–32.

Koskinen MT, Nilsson J, Veselov AJ, Potutkin AG, Ranta E, Primmer CR (2002) Microsatellite data resolve phylogeographic patterns in European grayling, *Thymalus thymallus*, Salmonidae. *Heredity*, **88**, 391–401.

Kremer A, Goenaga X (2002) Editorial. *Forest Ecology and Management*, **156**, 1–3.

Langella O (2002) *POPULATIONS 1.2.28. Population genetic software*. www.pge.cnrs-gif.fr/bioinfo/populations/index.php

Lawes MJ (1990) The distribution of the Samango monkey (*Cercopithecus mitis erythrarchus* Peters, 1852, and *Cercopithecus mitis labiatus* I. Goeffroy, 1843) and forest history in southern Africa. *Journal of Biogeography*, **17**, 669–680.

Lewis PO, Zaykin D (2001) *GENETIC DATA ANALYSIS: Computer program for the analysis of allelic data. Version 1.0 (d16c)*. http://hydrodictyon.eeb.uconn.edu/people/plewis/software.php.

Matolweni LO, Balkwill K, McLellan T (2000) Genetic diversity and gene flow in the morphologically variable, rare endemics *Begonia dregei* and *Begonia homonyma*. *American Journal of Botany*, **87**, 431–439.

Meadows ME, Linder HP (1989) A reassessment of the biogeography and vegetation history of the southern Afromontane region. In: *Biogeography of the Mixed Evergreen Forest of South Africa* (ed. Geldenhuys CJ), pp. 15–30. CSIR, Pretoria, South Africa.

Meadows ME, Linder HP (1993) A paleoecological perspective on the origin of South African grasslands. *Journal of Biogeography*, **20**, 345–355.

Minch E, Ruiz-Linares A, Goldstein D, Feldman M, Cavalli-Sforza LL (1995) MICROSAT *(version 1.4d): A computer program for calculating various statistics on microsatellite allele data.* http://hpgl.stanford.edu/projects/microsat/.

Möller M, Cronk QCB (1997) Origin and relationships of *Saintpaulia* H.Wendl. (Gesneriaceae) based on ribosomal DNA internal transcribed spacer (ITS) sequences. *American Journal of Botany*, **84**, 956–965.

Möller M, Cronk QCB (2001a) Phylogenetic studies in *Streptocarpus* (Gesneriaceae): reconstruction of biogeographic history and distribution patterns. *Systematics and Geography of Plants*, **71**, 545–555.

Möller M, Cronk QCB (2001b) Evolution of morphological novelty: a phylogenetic analysis of growth patterns in *Streptocarpus* (Gesneriaceae). *Evolution*, **55**, 918–929.

Möller M, Brooks KJ, Hughes M (2003) Plastid inheritance in *Streptocarpus* (Gesneriaceae) and an inferred hybrid origin for a population of *S.* aff *primulifolius* from Igoda River, South Africa. *Edinburgh Journal of Botany*, **60**, 389–408.

Mucina L, Geldenhuys C, Lawes M *et al.* (2003) *Classification System for South African Indigenous Forests. An Objective Classification for the Department of Water Affairs and Forestry.* CSIR Environmentek, Pretoria, South Africa.

Nei M, Tajima F, Tateno Y (1983) Accuracy of estimated phylogenetic trees from molecular data. II. Gene frequency data. *Journal of Molecular Evolution*, **19**, 153–170.

Page RDM (1996) TREEVIEW: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences*, **12**, 357–358.

Park SDE (2001) *The Excel Microsatellite Toolkit.* http://oscar.gen.tcd.ie/~sdepark/ms-toolkit/index.php.

Perez T, Albornoz J, Dominguez A (2002) Phylogeography of chamois (*Rupicapra* spp.) inferred from microsatellites. *Molecular Phylogenetics and Evolution*, **25**, 524–534.

Petren K, Grant BR, Grant PR (1999) A phylogeny of Darwin's finches based on microsatellite DNA length variation. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **266**, 321–329.

Richard M, Thorpe RS (2001) Can microsatallites be used to infer phylogenies? Evidence from population affinities of the western Canary Island Lizard (Gallotia galloti). *Molecular Phylogenetics and Evolution*, **20**, 351–360.

Richardson JE, Pennington RT, Pennington TD, Hollingsworth PM (2001) Rapid diversification of a species rich genus of Neotropical rain forest trees. *Science*, **293**, 2242–2245.

Sanderson MJ (2002) Estimating absolute rates of molecular evolution and divergence time: a penalized likelihood approach. *Molecular Biology and Evolution*, **19**, 101–109.

Schill D, Long DG, Möller M, Squirrel J (2004) Phylogenetic relationships between Lophoziaceae and Scapaniaceae based on chloroplast sequences. *Monographs in Systematic Botany from the Missouri Botanical Garden*, **98**, 141–149.

Simmons MP, Ochtorena H (2000) Gaps as characters in sequence based phylogenetic analyses. *Systematic Biology*, **49**, 369–381.

Stebbins GL (1957) Self-fertilization and population variability in the higher plants. *American Naturalist*, **41**, 337–354.

Swofford DL (2002) PAUP*. *Phylogenetic Analysis Using Parsimony (* and Other Methods). Version 4.* Sinauer Associates, Sunderland, Massachusetts.

Taberlet P, Gielly L, Patou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*, **17**, 1105–1109.

Takezaki N, Nei M (1996) Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics*, **144**, 389–399.

Thuillet AC, Bru D, David J *et al.* (2002) Direct estimation of mutation rate for 10 microsatellite loci in durum wheat, *Triticum turgidum* (L.) Thell. ssp. *durum* Desf. *Molecular Biology and Evolution*, **19**, 122–125.

Udupa S, Baum M (2001) High mutation rate and mutational bias at $(TAA)_n$ microsatellite loci in chickpea (*Cicer arietinum* L.). *Molecular Genetics and Genomics*, **265**, 1097–1103.

Vigouroux Y, Jaqueth JS, Matsuoka Y *et al.* (2002) Rate and pattern of mutation at microsatellite loci in maize. *Molecular Biology and Evolution*, **19**, 1251–1260.

Weigend M, Edwards TJ (1994) Notes on *Streptocarpus primulifolius* (Gesneriaceae). *South African Journal of Botany*, **60**, 169–174.

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

White TJ, Burns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols* (eds Innis MA, Gelfand DH, Sninsky JJ, White TJ), pp. 315–322. Academic Press, London.

Yuan YM, Wohlhauser S, Möller M *et al.* (2003) Monophyly and relationships of the tribe *Exaceae* (Gentianaceae) inferred from nuclear ribosomal and chloroplast DNA sequences. *Molecular Phylogenetics and Evolution*, **28**, 500–517.

Yuan YM, Wohlhauser S, Möller M, Klackenberg J, Küpfer P (2005) Phylogeny and biogeography of *Exacum* (Gentianaceae): a disjunctive distribution in the Indian Ocean basin resulted from long distance dispersal and rapid radiation. *Systematic Biology*, **54**, 1–14.

This study is part of a larger collaborative project using *Streptocarpus* as a model to investigate influences on the evolution of plant biodiversity in South Africa. Dr Mark Hughes is interested in factors affecting the distribution of genetic diversity within species and species distributions as a whole and is currently a MacIntyre *Begonia* research fellow. Dr Michael Möller is coordinator of the project and interested in reconstructing phylogenies in relation to biogeography and species delimitation. Prof. Dirk Bellstedt studies plant biodiversity in relation to biogeography in southern Africa using molecular systematic and population genetic techniques. Trevor Edwards curates the Herbarium at the University of KwaZulu-Natal (NU) and has strong research interests in the flora of the eastern seaboard of southern Africa. Margaret de Villiers is a PhD student working on the population genetics and phylogenetics of selected South African *Streptocarpus* species.
