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THE IMPACT OF POLLINATION SYNDROME AND HABITAT ON GENE FLOW: A COMPARATIVE STUDY OF TWO *STREPTOCARPUS* (GESNERIACEAE) SPECIES[1]

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Gene flow through pollen and seed dispersal is important in terms of population differentiation and eventually speciation. Seed and pollen flow are affected in turn by habitats and pollen vectors. We examined the effect of different pollinators and habitats on gene flow by comparing two species of *Streptocarpus*, using microsatellite and chloroplast RFLP markers. Populations of the forest-dwelling *S. primulifolius* were highly differentiated according to nuclear microsatellite data and had mutually exclusive chloroplast haplotypes. This result is congruent with infrequent seed dispersal and limited between-population foraging by the long-tongued fly pollinator *Stenobasipteron wiedemanni*. In contrast, populations of *S. dunnii* growing in exposed crags had lower levels of population differentiation according to both nuclear and chloroplast data, congruent with a hypothesis of more effective between population seed dispersal and greater pollen-mediated gene flow due to the sunbird pollinator *Nectarinia famosa*. The population genetic behavior of these species is reflected in their taxonomy and phylogenetic position; *S. primulifolius* belongs to a taxonomically complex clade in which recent speciation is evident, while the clade containing *S. dunnii* is characterized by taxonomically well-defined species on longer phylogenetic branches. Our study shows that pollinator movements and seed dispersal patterns are a major determinant of the evolutionary trajectories of these species.

Key words: gene flow; microsatellites; pollination; seed dispersal; South Africa; *Streptocarpus*.

The movement of pollen and seed between plant populations is of fundamental evolutionary importance. The resulting exchange of genes is essential for maintaining heterozygosity and fitness through the spread of favorable alleles, and in preventing allele loss through genetic drift. The degree of connectivity between populations also governs the size of units that evolve in unison, which in the absence of gene flow would become the population rather than the species (Ehrlich and Raven, 1969; Morjan and Rieseberg, 2004). Conversely, gene flow can also cause outbreeding depression and prevent populations from adapting optimally to local conditions (Lynch, 1991; Barton, 2001). Further, gene flow in terms of seed or propagule dispersal into new sites allows the expansion of species ranges and permits their continued existence in times of environmental change. To what extent populations remain in genetic contact depends on seed dispersal ranges and the movements of pollinators. These factors are of special interest in *Streptocarpus*, which tend to exist as discrete populations separated by areas of unfavorable habitat. An understanding of the population connectivity in this genus and how it is affected by pollinators and habitat variables could give us a greater understanding of the evolution of its biodiversity.

To examine these issues, we have chosen to study two species of *Streptocarpus*, namely *S. primulifolius* Gand. and *S. dunnii* Hook. *f. Streptocarpus primulifolius* is a rosulate, polycarpic species with long, mauve, tubular flowers (Fig. 1) pollinated by the nemestrinid fly *Stenobasipteron wiedemanni* Lichtwardt (Potgieter and Edwards, 2005). It is distributed in subtropical forest patches on the eastern South African seaboard, from the midlands of Kwazulu-Natal toward East London in the Eastern Cape. The forest in this area is naturally fragmented because of the presence of surrounding grasslands, which are a fire-dominated habitat; the forest patches are relictual and are likely to have been important Pleistocene refugia (Hughes et al., 2005). *Streptocarpus dunnii* is a unifoliate monocarpic species with curved, red, tubular flowers (Fig. 1) amassed in a densely packed inflorescence, which is pollinated by the malachite sunbird [*Nectarinia famosa* (L.)] (Vogel, 1954; M. Möller, personal observation). This species occurs in the Steenkampsberg and northern Drakensberg in Mpumalanga, where it grows in the rocky and treeless habitat of the North Eastern Mountain Sourveld and North Eastern Sandy Highveld. The behavior of the two different pollinators could profoundly affect the population structure of the study species. Sunbirds have larger ranges than *Stenobasipteron* (Daniels, 1987) and hence have the potential to bridge different habitats and contribute to the connection of disparate populations. In contrast, *Stenobasipteron* is restricted to a forest habitat (Potgieter and Edwards, 2005) and may not have the ability to connect populations of *Streptocarpus primulifolius* in neighboring forest patches, which are separated by open grassland.

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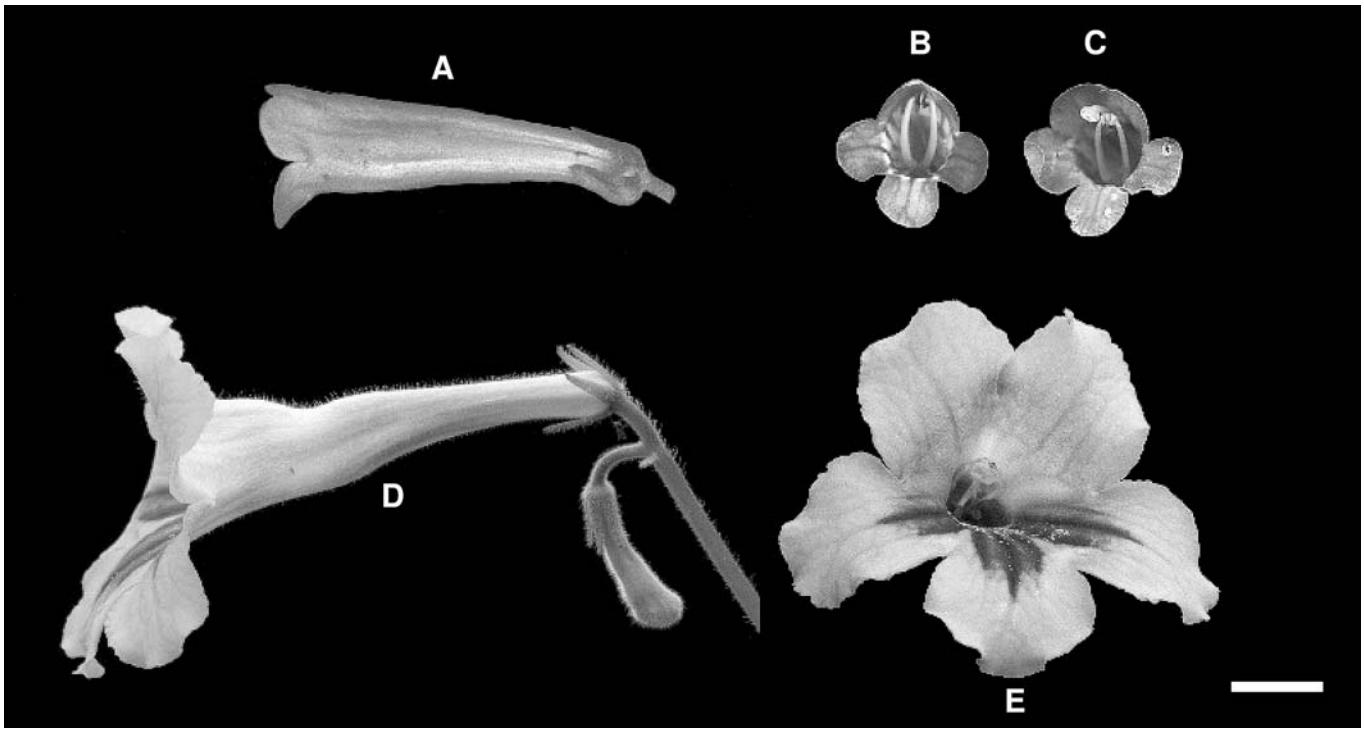

Fig. 1. Flowers of *Streptocarpus dunnii* (A, side view; B, face view, male phase; C, face view, female phase) and *S. primulifolius* (D, side view; E, face view). Scale bar = 10 mm.

The amount of seed dispersal between populations of the study species will also greatly influence their differentiation. They produce tiny seeds of a similar shape and size, typical for the genus *Streptocarpus* (0.5–0.8 mm long), which are released from a spirally twisted, dehiscent fruit. They differ slightly in the surface ornamentation of their seed coat, with *Streptocarpus primulifolius* having reticulate patterning while *S. dunnii* has verruculose patterning (Hilliard and Burtt, 1971; Beaufort-Murphy, 1983). Such tiny seeds are potentially capable of being carried on the wind. However, this has been considered to be unlikely in sheltered forest localities (Möller and Cronk, 2001), and true wind dispersal mechanisms are thought not to occur in this type of habitat (Killeen et al., 1998), which is the home of many species of *Streptocarpus*. The true capacity for wind dispersal of this small seed type and its relative efficacy in exposed and sheltered habitats is unknown. The habitats in which the two study species grow represent two extremes of the niches occupied by *Streptocarpus* in South Africa, namely low-altitude (10–100 m a.s.l.), closed canopy evergreen forest (*S. primulifolius*) and high-altitude (1700–2200 m a.s.l.) afromontane, exposed, rocky crags (*S. dunnii*) in open grassland. Within these areas, the two species occupy specific microhabitats: shaded, damp, south-facing slopes in the case of *S. primulifolius* and at the base of large boulders or in rock clefts in the case of *S. dunnii*. Seed dispersal in *S. primulifolius* is potentially passive and highly local; in *S. dunnii*, it could operate over much longer distances, solely because of the different environment the seeds are released into.

The influence of differences in pollen vectors and habitat on gene flow between populations of these two *Streptocarpus* species was investigated using a combination of nuclear microsatellite and chloroplast RFLP genetic markers. Specifically, we aimed to (1) examine the degree of population differentiation in both species and the geographic scale at which it occurs, (2) compare to what extent differentiation is mediated by pollinator behavior, and (3) investigate the comparative efficacy of seed dispersal in closed forest and open montane habitats. Using a combination of maternally (chloroplast) (Möller et al., 2004) and biparentally (nuclear microsatellite) inherited markers allows the investigation of the proportion of gene flow due to seed and pollen exchange. If dispersal of the same seed type strongly depends on landscape context, then we expect a significant difference in maternal F_{ST} (population differentiation measured with choloplast markers) between the two species. The extent to which pollinators connect populations of the study species will be revealed by population structure once seed dispersal effects have been controlled for; if one pollinator is significantly more vagile than the other, we expect to see a comparatively lower paternal F_{ST}.

MATERIALS AND METHODS

Sampling strategy—Population samples were collected as leaf tips (dried in powdered silica gel) from sites in the Port Saint Johns vicinity, Transkei, Eastern Cape (*S. primulifolius*; January 2003), and Mpumalanga (*S. dunnii*; February 2004) (Table 1; Fig. 2). Population sizes for each species were broadly similar, generally consisting of ca. 50–100 individuals. In the case of *S. primulifolius*, individuals tended to be concentrated in an area several meters square at the most; in the case of *S. dunnii*, individuals were spread over a larger area (ca. 50 m^2). Because we anticipated the possibility of higher levels of gene flow in *S. dunnii*, this species was sampled over a slightly wider geographic range than *S. primulifolius* to ensure the detection of population structure. The populations of *S. primulifolius* we sampled have been considered conspecific by all authors, although they have previously been recognized as *S. insignis* (Hilliard and Burtt, 1971).

TABLE 1. Collection localities for *Streptocarpus primulifolius* and *S. dunnii* in South Africa. *N*, number of individuals collected. Collector initials: DUB = D. U. Bellstedt, MH = M. Hughes. Voucher specimens are deposited in both NU (Bews Herbarium, University of Natal) and E (Royal Botanic Garden Edinburgh).

Locality	Coll. num.	*N*	Latitude	Longitude	Altitude (m a.s.l.)
S. dunnii					
Verloren Vallei	DUB570	24	−25.2996	30.1152	2200
Steenkampsberg	MH1239	24	−25.2281	30.1567	2250
Uitvlugt	MH1268	24	−25.4599	30.0371	1800
Sabie	MH1226	24	−25.1684	30.6425	1700
Slaaihoek	MH1273	24	−25.7244	30.4789	1900
S. primulifolius					
Endliniyokozi	MH1126	20	−31.5198	29.6731	50
Dwalana	DUB587	16	−31.6046	29.4658	20
Mt. Sullivan	MH1119	32	−31.5971	29.5298	20
Silaka	MH1133	24	−31.6493	29.5055	10
Bulolo Gorge	MH1135	30	−31.5573	29.5143	10

Nuclear microsatellites—Nine polymorphic nuclear microsatellite loci were used for the population genetic surveys, using fluorescently labeled primers and PCR conditions as detailed in Hughes et al. (2004). Microsatellite allele data were formatted for analysis using the Microsatellite Toolkit (Park, 2001). Descriptive parameters (A = mean number of alleles per locus; H_E = expected heterozygosity, and H_O = observed heterozygosity) were calculated using the program Genetic Data Analysis (GDA; Lewis and Zaykin, 2001). Weir and Cockerham's (1984) estimates of Wright's F statistics F_{IS} (f; deviation from panmixia attributable to nonrandom mating within populations), F_{ST} (θ; deviation from panmixia attributable to nonrandom mating among populations), and the inclusive measure F_{IT} (F; deviation from panmixia attributable to nonrandom mating within and between populations) were estimated using the program FSTAT (Goudet, 2002). The per locus estimates of F_{ST} were tested for significant difference from the null hypothesis of panmixia by jack-knifing over populations. The global estimate of F_{ST} was tested for significance by jack-knifing across loci. Permutation tests (1000

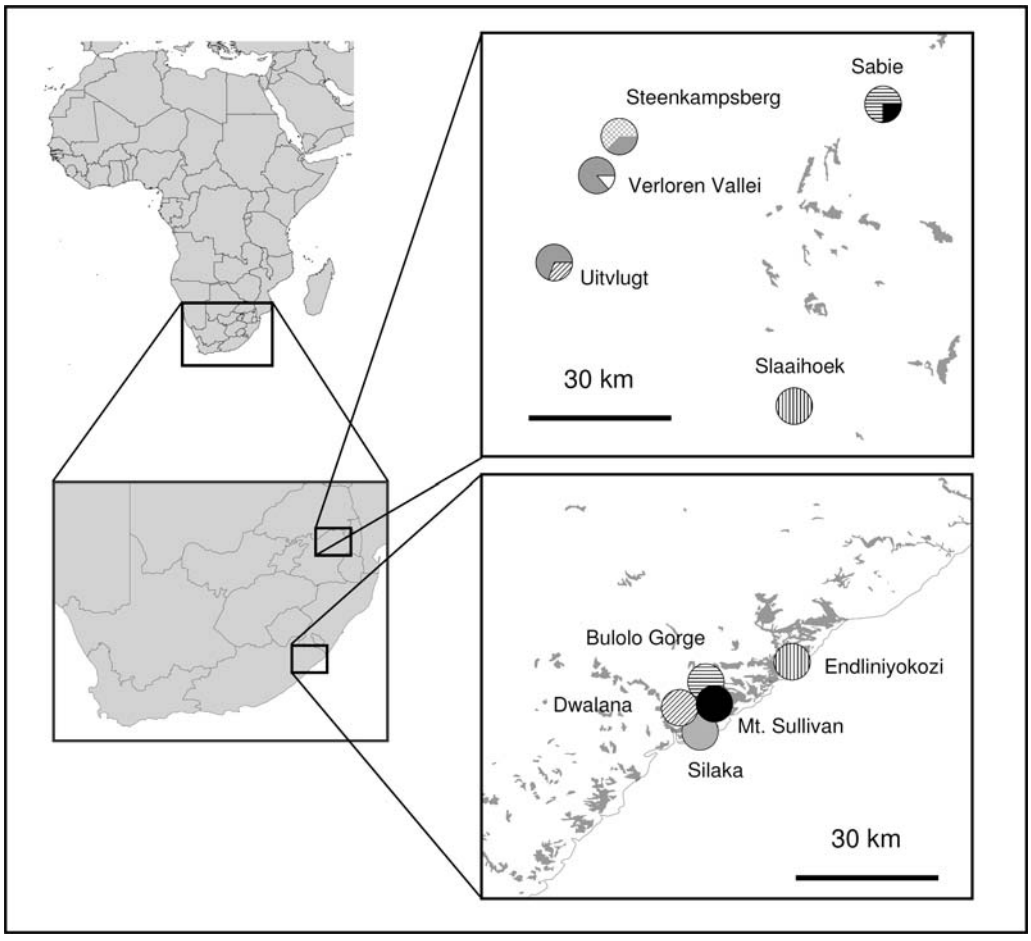

Fig. 2. Distribution of chloroplast haplotypes in *Streptocarpus dunnii* (top) and *S. primulifolius* (bottom). Both maps are to the same scale; shading represents the present day extent of indigenous forest.

TABLE 2. Per-locus statistics for *Streptocarpus primulifolius* and *S. dunnii* derived from nuclear microsatellite loci. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ns, not significant; n, mean number of individuals sampled per locus; A, total number of alleles per locus; H_E, expected heterozygosity; H_O, observed heterozygosity; F, Weir and Cockerham's summary estimate of deviation from panmixia F_{IT}; f, Weir and Cockerham's estimate of the inbreeding coefficient F_{IS}; θ, Weir and Cockerham's estimate of the population differentiation parameter F_{ST}.

Locus	n	A	H_E	H_O	F	f	θ
S. dunnii							
Ctg16	103	5	0.500	0.311	0.389***	0.340***	0.074**
PR239	103	6	0.520	0.447	0.164*	0.041ns	0.128***
JH448	78	29	0.940	0.666	0.307***	0.235***	0.094***
DN110	109	5	0.277	0.183	0.366***	0.202*	0.205***
D14	106	3	0.055	0.037	0.323*	0.320ns	0.004ns
K17L	113	6	0.567	0.478	0.174**	0.090ns	0.093***
PR241	108	16	0.789	0.490	0.387***	0.344***	0.065***
JH432	116	14	0.748	0.647	0.146**	0.094ns	0.057***
All	**104.5**	**10.5**	**0.550**	**0.408**	**0.273*****	**0.201*****	**0.090*****
S. primulifolius							
Ctg16	108	6	0.394	0.306	0.056ns	−0.133*	0.167***
PR239	118	9	0.693	0.678	0.621***	0.509***	0.229***
JH448	96	13	0.883	0.219	0.603***	0.421***	0.314***
DN110	111	34	0.944	0.802	0.194**	−0.006ns	0.199***
D14	97	6	0.625	0.515	0.202***	0.020ns	0.218***
K17	118	6	0.325	0.220	0.349***	−0.090ns	0.403***
PR241	98	16	0.854	0.286	0.751***	0.583***	0.402***
JH432	100	13	0.864	0.360	0.173***	0.053ns	0.127***
B22	107	11	0.794	0.626	0.194**	0.019ns	0.178***
All	**105.9**	**12.7**	**0.708**	**0.446**	**0.379*****	**0.176*****	**0.246*****

permutations, randomizing alleles) were used to assess the significance of F_{IS} and F_{IT} estimates. A Mantel test to assess the significance of isolation by distance patterns was carried out using Arlequin version 2.000 (Schneider et al., 2000), with the two matrices being the natural log of straight-line geographic distances (calculated from GPS data) and $F_{ST}/1 - F_{ST}$ (Rousset, 1997).

Chloroplast haplotype genotyping—To assess chloroplast haplotype variation, we screened each individual for restriction site polymorphism in the *trnC-trnD* region as follows. The region was amplified using the PCR primers and conditions in Demesure et al. (1995), and digested with *Taq*I (New England Biolabs, Massachusetts) according to the manufacturers instructions. The resulting digests were run on 20 cm 8% polyacrylamide gels using Tris/Borate/EDTA TBE buffer and visualized using ethidium bromide staining. Standards of each different haplotype were included in each gel for comparison. The haploid population genetic parameters h_S (average within-population diversity), h_T (total diversity), and G_{ST} (population differentiation) were calculated using the program Haplodiv (Pons and Petit, 1995).

A representative of each different haplotype was sequenced for the regions *trnC-trnD* and *rpl20-rps12*, using the primers from Hamilton (1999) and Demesure et al. (1995). Networks for each species were built from the aligned sequences using the program TCS (Clement et al., 2000).

RESULTS

Nuclear microsatellite loci—*Descriptive statistics*—Eight of the nine microsatellite locus primer pairs amplified products in *S. dunnii*, with locus B22 not giving a product. All nine loci amplified products in *S. primulifolius*. The means for the per-locus values of expected heterozygosity using all amplifiable loci (H_E, Table 2) are 0.550 (*S. dunnii*) and 0.708 (*S. primulifolius*). These values are not significantly different, whether the estimate for B22 from *S. primulifolius* is included (*t* test; $t = -1.29$, df = 15, $P = 0.108$) or excluded to generate a directly comparable figure using an identical set of loci for both species ($H_E = 0.698$; *t* test; $t = -1.18$, df = 15, $P = 0.129$). The population level estimates of H_E (Table 3) vary between 0.383–0.602 (*S. dunnii*) and 0.422–0.639 (*S. primulifolius*). All populations studied harbored private alleles, with the number per population ranging from 3–7 in *S. dunnii*, and 7–20 in *S. primulifolius*.

Inbreeding—Both species deviated significantly from panmixia within populations. Considering all loci, the estimates of the inbreeding coefficient F_{IS} are $f = 0.201$ ($P < 0.001$) (*S. dunnii*) and $f = 0.176$ ($P < 0.001$) (*S. primulifolius*) (Table 2). There is quite a large variance in the per-locus estimates, particularly for *S. primulifolius*, and the mean figures for the two species are not significantly different (*t* test across per-locus estimates; $t = 0.50$, df = 15, $P = 0.68$). The per-population estimates of F_{IS} vary between $f = 0.076$–0.380 (*S. dunnii*) and $f = 0.067$–0.284 (*S. primulifolius*) (Table 3).

Population structure and isolation by distance—Both species have a significant degree of population structure. Per-locus estimates of the population differentiation coefficient F_{ST} ranged from θ = 0.004–0.205 (*S. dunnii*) and θ = 0.127–0.403 (*S. primulifolius*) (Table 2). Considering all microsatellite loci, the species level figures are θ = 0.090 (*S. dunnii*, $P < 0.001$) and θ = 0.246 (*S. primulifolius*, $P < 0.001$) (Table 2). Because the level of gene diversity in both species is not significantly different, it is possible to meaningfully compare the estimates of F_{ST} between the two species directly (Hedrick, 2005); the values of θ are significantly different from one another (*t*-test; $t = -3.99$, df = 15, $P = 0.001$). Plots of population pairwise genetic (Slatkin's linearized F_{ST}) vs. geographic distance (natural log distance in km) reveal a positive trend in both species (Fig. 3), although neither species showed a statistically significant pattern of isolation by distance according to the Mantel test (*S dunnii*, $P = 0.107$; *S. primulifolius*, $P = 0.087$). The scatter plots highlight that pairwise comparisons between populations of *S. primulifolius* have a higher degree of differentiation over a shorter distance than observed in *S. dunnii*. Our comparison of *S. dunnii* population differentiation over a similar geographic scale to all the *S. primulifolius*

TABLE 3. Per-population statistics for *Streptocarpus primulifolius* and *S. dunnii* derived from nuclear microsatellite loci. ***$P < 0.001$; **$P < 0.01$; ns, not significant; *N*, total number of individuals sampled; *n*, mean number of individuals sampled per locus; *f*, Weir and Cockerham's estimate of the inbreeding coefficient F_{IS}; H_E, expected heterozygosity; H_O, observed heterozygosity; *A*, mean number of alleles per locus; *A*p, total number of private alleles; *p*, proportion of polymorphic loci.

Population	*N*	*n*	*f*	H_E	H_O	*A*	*A*p	*p*
S. dunnii								
Verloren Vallei	24	21.9	0.124**	0.541	0.476	5.3	3	1.00
Sabie	24	22.1	0.380***	0.602	0.376	5.8	7	1.00
Steenkampsberg	24	19.3	0.076ns	0.494	0.458	5.4	7	0.88
Uitvlugt	24	18.9	0.192**	0.383	0.313	3.6	5	0.75
Slaaihoek	24	22.4	0.223***	0.497	0.388	5.3	7	1.00
S. primulifolius								
Endliniyokozi	20	18.0	0.160**	0.588	0.461	5.0	12	1.00
Silaka	16	13.6	0.192*	0.422	0.341	3.9	7	0.89
Mt. Sullivan	32	30.7	0.067ns	0.639	0.549	5.6	20	1.00
Dwalana	24	18.6	0.284***	0.552	0.399	4.6	12	1.00
Bulolo Gorge	30	21.6	0.238**	0.560	0.429	5.0	8	1.00

populations (Steenkampsberg vicinity only; the three leftmost populations in Fig. 2, spread over a distance of ca. 30 km) gave an estimate of $\theta = 0.055$ ($P < 0.001$), compared to $\theta = 0.246$ ($P < 0.001$) for all populations of *S. primulifolius*. Comparing the two closest populations of *S. primulifolius* (Bulolo Gorge and Mt. Sullivan, 5 km apart, Fig. 2) gave a value of $\theta = 0.197$ ($P < 0.001$).

Chloroplast haplotype population structure—A total of six RFLP haplotypes were detected in *S. dunnii*, and a further five were detected in *S. primulifolius* (Figs. 2 and 4, Table 4). In *S. primulifolius*, all haplotypes were restricted to a single population ($h_T = 0.800$, $G_{ST} = 1.000$), and no within population diversity was detected ($h_S = 0.000$). This is in contrast to the situation in *S. dunnii*, in which one of the haplotypes was shared between three populations from the Drakensberg area ($h_T = 0.883$, $G_{ST} = 0.645$), and most of the populations were polymorphic for two different haplotypes ($h_S = 0.313$).

The obtained chloroplast haplotype networks separated the *S. dunnii* haplotypes into two groups that differ by seven changes. One group included the two private haplotypes from Steenkampsberg and Uitvlugt plus the shared haplotype between these localities and Verloren Vallei, while the remaining haplotypes formed a group with the private haplotype from Verloren Vallei included, suggesting that this haplotype has closer affinities among these samples. The *S. primulifolius* haplotype network shows the southern population at Silaka separated from the remaining samples by a long

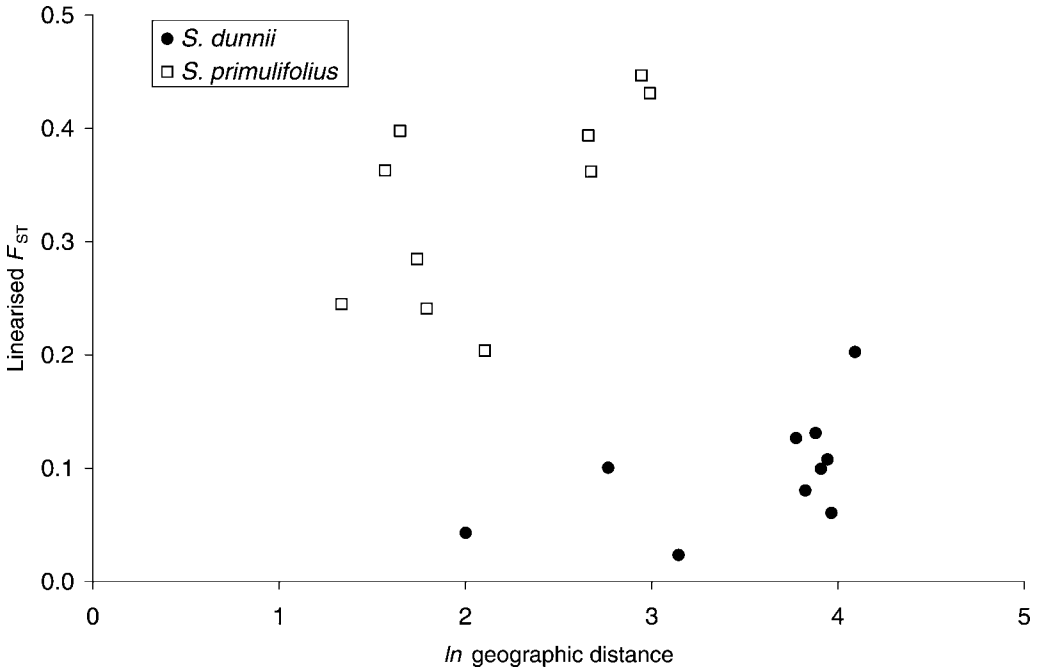

Fig. 3. Linearized F_{ST} [$= F_{ST}/(1 - F_{ST})$] derived from nuclear microsatellite data vs. natural log of geographic distance in km for pairwise comparisons of *Streptocarpus dunnii* and *S. primulifolius* populations. In neither of the species was the pattern of isolation by distance statistically significant ($P = 0.107$, $R^2 = 0.31$; $P = 0.087$, $R^2 = 0.39$, respectively).

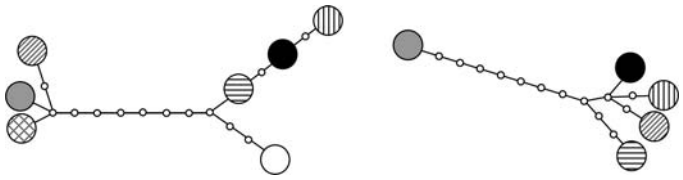

Fig. 4. Chloroplast haplotype networks for *Streptocarpus dunnii* (left) and *S. primulifolius* (right). The haplotype shading for each species is equivalent to that in Fig. 2. Small white circles represent intermediate haplotypes separated from the next by one mutation. (Note that all haplotypes are only found in one species; the use of the same shading in both species does not mean the haplotypes are shared.)

branch of eight steps. The latter populations form a close group, with the Bulolo Gorge population being most divergent.

Seed and pollen gene flow ratio—Because there are estimates of population structure from both the chloroplast (maternally inherited and dispersed via seed in *Streptocarpus*; Möller et al., 2004) and nuclear genome (biparentally inherited and dispersed via seed and pollen), it becomes possible to obtain some insight into the relative amount of seed and pollen flow between populations by using the equation derived by Ennos (1994). Taking into account the degree of within-population inbreeding found in each of the species, the ratio of pollen to seed flow between populations is 32 : 1 for *S. dunnii* and, because maternal $G_{ST} = 1$ for *S. primulifolius*, we conclude that all recent gene flow is due to pollen exchange for this species.

Considering populations or groups of populations with mutually exclusive haplotypes (i.e., with no seed dispersal occurring between them), it becomes possible to compare estimates of the amount of pollinator-mediated gene flow between the two species. In *S. primulifolius*, all populations have unique haplotypes, and hence the degree of population structure observed ($\theta = 0.246$, $P < 0.001$) is mediated entirely by pollinator activity. If we combine the populations of *S. dunnii* sharing a common haplotype (Steenkampsberg, Verloren Vallei, and Uitvlugt) into one unit, then we can generate a comparative figure for this species ($\theta = 0.093$, $P < 0.01$), which is significantly different from the value obtained for *S. primulifolius* (*t* test across per-locus estimates; $t = -3.48$. df = 15, $P = 0.001$).

DISCUSSION

Gene flow through the movement of pollen and seed between plant populations is responsible for maintaining species cohesion. The degree of genetic communication between populations dictates whether populations evolve in unison, or whether they follow separate evolutionary paths and diverge, eventually becoming different species. Hence, pollinator behavior and seed dispersal patterns are of great importance in angiosperm evolution.

Our results show that differences in habitat and pollination ecology between *S. dunnii* and *S. primulifolius* have a significant impact on gene flow, with the latter species showing significantly higher levels of population structure despite the closer geographical proximity of its populations. The lack of seed dispersal between populations of *S. primulifolius* is congruent with a hypothesis of wind dispersal not operating in a sheltered forest environment (Möller and Cronk, 2001; Killeen et al., 1998). The ecology of *S. primulifolius* is also best suited to a highly local and passive seed dispersal syndrome, as sites suitable for seedling establishment are most likely to be found only in small patches of suitable microhabitat near the parent plant. How long the current distribution of chloroplast haplotypes has persisted is open to speculation; it might reflect contemporary genetic drift and consequent sorting of haplotypes in a metapopulation-like manner, with patches becoming colonized by rare between-patch dispersal events. However, the high nuclear gene diversity statistics and high level of private alleles are not congruent with a "weedy" habit; neither is the fact that none of the sampled populations share a chloroplast haplotype. It is possible that each of the populations represents a stable and refugial collection of individuals. The high diversity could be influenced in part by the perennial habit of *S. primulifolius*, which will increase mating possibilities and hence the effective population size, consequently reducing the effects of genetic drift.

TABLE 4. Summary of chloroplast population genetic statistics for *Streptocarpus primulifolius* and *S. dunnii*. h_S, average within-population diversity; h_T, total diversity; G_{ST}, Pons and Petit's (1995) estimate of the haploid population differentiation statistic.

Statistic	*S. dunnii*	*S. primulifolius*
h_S	0.313	0.000
h_T	0.883	0.800
G_{ST}	0.645	1.000

The relative rate of pollen and seed migration between plant populations varies greatly according to the life history of the species concerned (Squirrell et al., 2001). The proportion of gene flow via seed dispersal compared to pollen dispersal is higher in herbaceous insect-pollinated plants (especially in orchids; pollen to seed flow ratio 1.43 : 1) than in many trees, which have a very strong bias toward gene flow via pollen dispersal, especially in wind-pollinated trees with animal-dispersed fruits (ratio ca. 100–600 : 1) (Squirrell et al., 2001). The ratios we have calculated for *S. primulifolius* and *S. dunnii* fall near the extremes of these values. The pollen to seed gene flow ratio for *S. dunnii* was 32 : 1, while for *S. primulifolius* pollen exchange accounts for all interpopulation gene flow. Estimating gene flow from measurements of contemporary population structure relies on a number of assumptions, which are arguably unlikely to be fulfilled (Whitlock and McCauley, 1999). We therefore stress that it is not possible to disentangle the historical and contemporary effects of gene flow on our estimates for population structure, and hence our ratios for maternal/paternal gene flow should be interpreted as useful for comparison rather than strictly representing current gene exchange. However, we feel that in our comparative study of two species using the same loci with similar levels of gene diversity, there is a biological reality to the differences observed. *Streptocarpus dunnii* contrasts with *S. primulifolius* in that seed dispersal is likely to account for at least some of the gene flow between populations, as evidenced by the sharing of a haplotype between the three populations sampled from the Steenkampsberg vicinity (Fig. 2). This indicates that true wind dispersal can occur with this seed type, but that such dispersal

depends on an exposed habitat. Strong "berg winds," which occur during the fruiting season for *Streptocarpus* in South Africa (autumn and winter months of May–September) could effectively disperse the seeds. However, two of the more isolated populations in the east (Slaaihoek and Sabie) have haplotypes not shared with any other population and hence indicate the absence of recent, effective longer distance migration via seed into or out of these populations. The historical isolation of the eastern populations from the three clustered in the west (Steenkampsberg, Verloren Vallei and Uitvlugt, Fig. 2) is also evident from the relationship between the chloroplast haplotypes. The chloroplast network (Fig. 4) shows two groups in which geographically proximal haplotypes tend to cluster together, with the exception of the haplotype found at a low frequency in Verloren Vallei (coloured white in Figs. 2 and 4), which indicates historical dispersal between eastern and western populations.

Because of the lack of seed exchange between populations of *S. primulifolius*, the pollinator *S. wiedemanni* is primarily responsible for maintaining the genetic cohesion of the species. This fly species is pivotal in a recently described pollination guild limited to forest patches on the eastern seaboard of South Africa (Potgieter and Edwards, 2005). The highly local nature of seed dispersal in *S. primulifolius* has no doubt contributed significantly to the high levels of population differentiation observed. However, the degree of divergence between populations remains high even over short distances (Fig. 3) and so does not appear to be strongly mediated by pollinator behavior. The higher degree of population differentiation in *S. primulifolius* is possibly due to (1) the patchy, colonial nature of the distribution of this species, which will promote within-population pollen transfer by pollinators working that particular patch, and (2) higher altitude savannah and grassland areas between the valley populations, restricting insect movement. The evergreen forest patches to which *S. primulifolius* is endemic are considered to be refugial because of the seasonal climate of the subcontinent (Meadows and Linder, 1989; Hughes et al., 2005) and are restricted to rocky gorges from which fire is excluded. These sandstone gorges lie parallel to each other and provide very limited opportunity for intergorge foraging by *Stenobasipteron*.

Conversely, the comparatively low genetic differentiation observed in *S. dunnii*, as well as being influenced by the greater ease of seed dispersal in an open habitat, is also a product of the greater vagility of its sunbird pollinator. The malachite sunbird has a wide distribution from the high altitude areas of southern Sudan and Eritrea to the southern tip of Africa; in Mpumalanga, it is essentially limited to the Drakensberg (Skead, 1967). The species feeds on a range of nectariferous plant genera including *Aloe*, *Crocosmia*, *Erica*, *Erythrina*, *Gladiolus*, *Greyia*, *Halleria*, *Kniphofia*, *Leucospermum*, *Melianthus*, *Mimetes*, *Protea*, *Strelitzia*, *Tecomaria*, and *Watsonia* (Cyrus and Robson, 1980; Brown and Barnes, 1984; Daniels, 1987). The malachite sunbird moves freely across open habitat (Protea Savannah, Highland Sourveld), and discontinuities in its range are in large part due to the patchy distribution of nectariferous plants. Thus, the birds are not restricted to the narrow rocky niches of *S. dunnii* because other nectariferous plants often span the habitats between adjacent populations of *S. dunnii*, which may facilitate interpopulation pollen transfer. Although only one visit to a flower of *S. dunnii* by a sunbird was observed during recent fieldwork by the authors, approximately 50% of the stigmas per inflorescence of most plants were observed with the unaided eye to have pollen deposited, indicating significant, yet elusive, pollinator activity.

Pollinator behavior will also affect the amount of inbreeding as estimated by F_{IS}; both species have a predominantly outcrossing breeding system with a significant level of inbreeding in most populations. Although the degree of inbreeding does not differ significantly between the species, there was a high level of variation in population level estimates of F_{IS} observed in *S. dunnii*. Outcrossing in this species may be facilitated by the strong spatial and temporal separation of male and female phases of *S. dunnii* flowers, which is mainly due to a long delay in style development and stigma maturation (Fig. 1). The population with the highest estimates of F_{IS} (Sabie) was not flowering at the time of sampling, and because of the lack of other nectar sources, no sunbirds were observed at this site. For the other sites with lower levels of inbreeding (Verloren Vallei, Steenkampsberg, and Uitvlugt), numerous flowering individuals were present, and sunbirds were regularly observed in the vicinity, in particular at the Verloren Vallei and Steenkampsberg sites where large stands of *Crocosmia* and *Protea*, respectively, were attracting sunbirds; the level of inbreeding in these two sites is significantly lower than that in Sabie (t test; Sabie vs. Verloren Vallei, $t = 2.60$, df $= 15$, $P = 0.01$; Sabie vs. Steenkampsberg, $t = 3.19$, df $= 15$, $P = 0.003$). However, whether this is a direct case of pollinator facilitation (Ghazoul, 2006) is debatable, because the peak of the *S. dunnii* flowers approximately 1 mo earlier than *Crocosmia*, possibly to avoid competing for pollinators with this comparatively nectar-rich species. The timing of reproduction will also affect the population structure of *S. dunnii*, as its monocarpic habit will restrict mating possibilities and hence reduce effective population size, in theory leading to an increased propensity for drift and population differentiation. However, given the observed levels of genetic diversity and population structure, this does not seem to be a strong factor in this species.

Streptocarpus primulifolius differs significantly from *S. dunnii* in population genetic behavior, as evidenced by a higher number of private alleles (Table 3), higher differentiation over shorter geographic distances at nuclear loci (Fig. 3), and a complete lack of shared chloroplast haplotypes between populations (Fig. 2). Our study suggests that sunbird pollination leads to more population connectivity than nemestrinid fly pollination, and that dispersal of the small seeds of *Streptocarpus* depends strongly upon habitat. The lower levels of population connectivity in *S. primulifolius* could contribute to the taxonomic complexity of this species (plants from the Port St. Johns vicinity were originally described as *S. insignis* [Burtt and Sealy, 1946], *S. primulifolius* subsp. *formosus* from Umtamvuna has been given specific rank [Weigend and Edwards, 1994], and populations in the KwaZulu-Natal midlands may represent yet another new taxon). This is further evidenced by recent and ongoing diversification on a small geographic scale in the forest-dwelling cape-primrose clade to which *S. primulifolius* belongs (Hughes et al., 2005). In contrast, the clade containing *S. dunnii* is characterized by taxonomically well-defined species on longer phylogenetic branches (Möller et al., 2001), with pollinator selection likely to have played a significant part in their divergence. Hence, pollinator movements and seed dispersal patterns, as well as strongly influencing population structure, are ultimately a strong influence on the evolutionary trajectories of *Streptocarpus* species.

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