

Breeding system of a plesiomorphic floral type: an investigation of small flowered *Streptocarpus* (Gesneriaceae) species

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Abstract. Six different floral morphologies can be found among the ca. 146 species of *Streptocarpus* occurring in Africa and Madagascar. One of these, a simple, small pouch type, was found to be plesiomorphic for the genus after mapping these floral types onto a molecular phylogeny. The breeding systems and population structure of three species possessing the plesiomorphic small floral morphology, *S. micranthus*, *S. ibityensis* and *S. lanatus*, have been investigated using nuclear microsatellite markers. Significant deviations from panmixia were found both at the between and within population level (*S. micranthus* $\theta = 0.708$, $f = 0.786$; *S. ibityensis*, $\theta = 0.173$, $f = 0.138$; *S. lanatus*, $\theta = 0.539$, $f = 0.646$). As a florally diverse genus, *Streptocarpus* is unusual in having a predominantly selfing, mixed mating breeding system as a plesiomorphic condition.

Key words: breeding systems, chasmogamous flowers, Gesneriaceae, microsatellite markers, population genetics, *Streptocarpus ibityensis*, *Streptocarpus lanatus*, *Streptocarpus micranthus*.

The balance between selfing and outcrossing has a profound effect on the persistence and diversification of evolutionary lineages. Selfing

lineages have been described as ‘evolutionary dead ends’ due to their possession of limited potential for adaptation and the accumulation of an increasing genetic load (Stebbins 1970), whilst outcrossing lineages are seen as a source of breeding system novelty, with the transition from outcrossing to selfing being one of the most common evolutionary transitions in plants (Grant 1981). The transition to selfing is often accompanied by floral changes such as reduced herkogamy (anther-stigma separation) (Dole 1992) and reduced corolla size (French et al. 2005). In the Gesneriaceae genus *Streptocarpus* Lindl., a preliminary mapping of floral morphology onto a molecular phylogeny by Harrison et al. (1999) indicates small flower size to be plesiomorphic, and reveals a trend of increasing floral size through evolutionary time, in parallel with a North to South range expansion in subgenus *Streptocarpus* (Möller and Cronk 2001b). Small corolla size has been suggested to be linked to autogamy in *Streptocarpus* (Harrison et al. 1999); if this hypothesis of reduced floral size being correlated with a selfing breeding system is correct, this would appear to be an unusual example of inbreeding being ancestral rather than derived.

Streptocarpus is the largest genus of Gesneriaceae in Africa, with ca. 146 species described (Hilliard and Burt 1971). The genus has a number of different floral morphologies, namely (i) keyhole type; (ii) open-tube type; (iii) personate type; (iv) *S. dunnii* type and (v) small pouch type. Further floral variation is represented by the enantiostylous and putatively buzz-pollinated *Saintpaulia* H. Wendl. (Harrison et al. 1999), which has been found to be nested within *Streptocarpus* (Möller and Cronk 1997).

Whilst all of the above floral morphologies occur on mainland Africa, *Streptocarpus* on Madagascar is relatively depauperate in terms of floral diversity. Forty-one species occur on the island, the vast majority of them being endemic (Humbert 1971). These species mainly possess variants of the simple, small pouch type flower, despite the fact that both subgenera of *Streptocarpus* (*Streptocarpus* and *Streptocarpella*) are present on the island, representing a phylogenetic assemblage of species that are nested within florally more diverse clades. The reason for this relative floral uniformity is unknown, although it has been suggested that a lack of pollinating insects may be a contributing factor (B.L. Burt, pers. comm. 2003). In contrast, the genus exhibits a wider range of vegetative variation on Madagascar than it does on the African mainland (Hilliard and Burt 1971, Möller and Cronk 2001a).

Given the investment in floral display of some of the larger flowered African species, it seems reasonable to assume that they attract pollinator visits (e.g. Potgieter and Edwards 2005) and are therefore largely outcrossing. The different floral morphologies have been putatively assigned to various pollination syndromes (Harrison et al. 1999), but confirmation of these in terms of pollinator observations is unfortunately lacking (but see Potgieter and Edwards 2005). In contrast, many of the smaller flowered species of *Streptocarpus* set seed readily in cultivation in the absence of any obvious pollinator, which is evidence that these spe-

cies may use self pollination as part of their breeding strategy in the wild. A small number of these are cleistogamous, and hence obligately selfing. In addition, there are many taxa which possess small flowers (corollas < 10 mm long) of a pouch or bucket morphology which are chasmogamous (flowers open at the time of pollination) and therefore possibly receptive to pollinator activity. Such taxa are common on the African mainland and make up the bulk of species on Madagascar. To what extent species possessing this small floral type manage to attract pollinators and maintain heterozygosity is unknown. This paper presents an investigation of three small-flowered chasmogamous *Streptocarpus* species from both Africa (*S. micranthus* C.B. Clarke) and Madagascar (*S. lanatus* MacMaster and *S. ibityensis* Humbert). Firstly, we aim to understand the breeding systems of these three species, using nuclear microsatellite markers in order to investigate the possible link between small flower size and inbreeding. Secondly, we aim to examine in further detail the hypothesis stating that the small, unspecialised floral type is plesiomorphic in *Streptocarpus*, and hence whether the genus really does represent an unusual case of selfing not being an 'evolutionary dead end'.

Material and methods

Population genetics

Study species. *S. micranthus* occurs in South Africa (Mpumalanga province) and Swaziland, along the Drakensberg escarpment from Mariepskop to Havelock (Fig. 1). It grows on moss covered rocks under the cover of a forest canopy or in sheltered crevices in rock outcrops, such as dolomite sandstone plateau in the Gods Window and Mariepskop vicinity. The species is unifoliate and has small cup shaped flowers formed from a white, cylindrical corolla tube ca. 6mm long and 4 mm wide (Fig. 2C). *S. lanatus* and *S. ibityensis* are endemic to Mt. Itremo and Mt. Ibity respectively, in the centre of Madagascar (Fig. 1) (MacMaster et al. 2005). Mt. Itremo and Mt. Ibity are quartzite outcrops which are

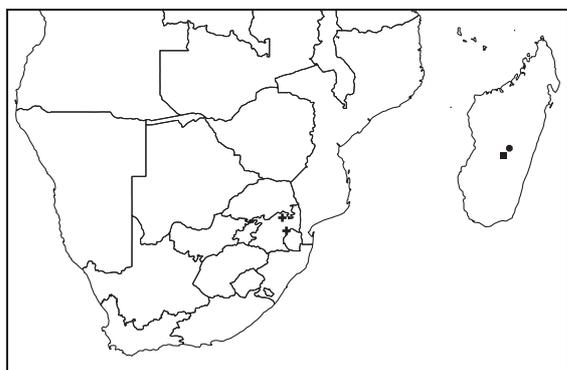


Fig. 1. Collection sites for *S. micranthus* (+) in South Africa and for *S. ibityensis* (●) and *S. lanatus* (■) in Madagascar

respectively 1700 m and 1900 m in elevation, and have sandy soils in contrast to the highly weathered laterites more commonly found on the central plateau. A large part of the precipitation at these high altitudes comes in the form of mists rather than direct rainfall. Both species are small rosulate herbs with a dense leaf indumentum, and small flowers without (*S. ibityensis*) or with purple markings (*S. lanatus*) (Fig. 2A, B). They grow in scattered populations in deep shade in sheltered places such as in deep rock crevices or small caves in an otherwise fairly open rocky habitat dominated by low herbaceous vegetation.

Population sampling of *S. lanatus* and *S. ibityensis* was carried out in March 2001, and of *S. micranthus* in January 2004, with samples being collected as leaf tips placed immediately in a ziplock bag with silica gel. The grid references for each population are listed in Table 1. Voucher specimens were also collected and deposited in the herbarium at the Royal Botanic Garden Edinburgh (E).

Molecular techniques. Microsatellite markers detailed in Hughes et al. (2004) were tested for applicability, and for each species a subset of the 9 primers (Table 2) amplified products that could be interpreted as polymorphic single loci; these were used to screen all individuals. DNA extraction, PCR amplification and scoring of the loci was carried out as detailed in Hughes et al. (2004).

Data analysis. Allele size data were formatted for analysis using the Microsatellite Toolkit (Park

2001). Basic descriptive population genetic statistics (n , number of individuals sampled per locus; A , number of alleles per locus; H_E , expected heterozygosity; H_O , observed heterozygosity) were calculated using GDA (Lewis and Zaykin 2001). Allelic richness (Ar , a measure of the number of alleles per locus independent of sample size), and Weir and Cockerham's (1984) estimates of the population differentiation parameters F_{IT} (F) and F_{ST} (θ) and the inbreeding coefficient F_{IS} (f) were calculated using FSTAT (Goudet 2001). Allelic richness was calculated from a subsample of 15 individuals in *S. micranthus*, and seven individuals in *S. ibityensis* and *S. lanatus*. Permutation tests were used (10000 permutations, randomising genotypes among populations) to test whether the estimates of F_{IT} and F_{ST} were significantly different from a null hypothesis of panmixia. Deviations from Hardy-Weinberg equilibrium within populations were tested for significance at the $P=0.001$ level and above by randomising alleles between individuals. A sequential Bonferroni correction was applied to probability estimates in all cases of multiple tests (Rice 1989). Under the assumption that populations were at inbreeding equilibrium and that all deviation from panmixia within populations was due to selfing, the outcrossing rate, t , can be estimated from the inbreeding coefficient following Allard et al. (1969) using the equation $t = (1-F_{IS})/(1+F_{IS})$.

Floral evolution

Species selection, molecular techniques and phylogenetic analysis. The ITS matrix (including 5.8S) of Möller and Cronk (2001a, b) was extended to include duplicate accessions of *S. ibityensis*, *S. lanatus* and *S. itremensis* using sequences from MacMaster et al. (2005), and a single accession for *S. micranthus* (DUB581; locality Graskop, Mpumalanga) obtained for this study following the procedure in MacMaster et al. (2005). Phylogenetic analysis implementing maximum parsimony was carried out following the protocol of Möller and Cronk (2001a, b).

Data analysis. The floral morphology of all taxa included in the ITS phylogeny was categorised following Harrison et al. (1999) into pouch, open tube, personate, keyhole, bird and *Saintpaulia*-type. This approach groups flowers

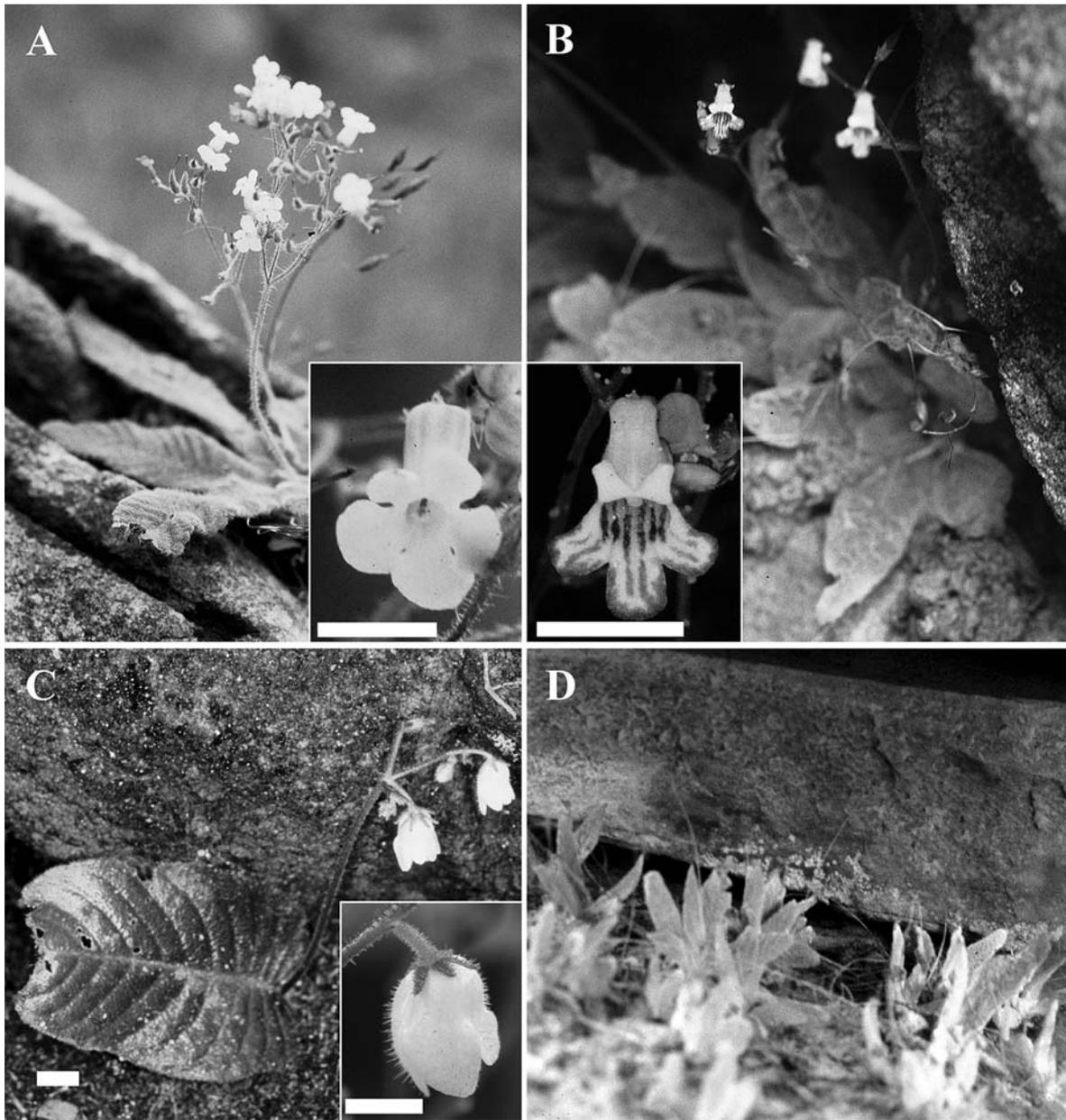


Fig. 2. Habit and flower shape (inset) of *S. ibityensis* (A), *S. lanatus* (B) and *S. micranthus* (C). (D) Seedlings of *S. lanatus* establishing *en masse* under rocks. Scale bars = 5 mm

according to their degree and type of specialisation, not necessarily relying on size alone. Character state changes were optimised onto the majority rule consensus tree under DELTRAN optimization criterion with polytomies resolved randomly in MacClade 4.06 (Maddison and Maddison 2003). Only one polytomy involving

three species *S. fanniniae*, *S. pusillus* and *S. davyi* affected the outcome of the character state change analysis, and will be described in more detail below.

As an independent test of flower specialisation and to illustrate trends in the evolution of flower size in *Streptocarpus* we plotted the tube lengths

Table 1. Location of the population sample sites

Species	Population	<i>n</i>	Latitude (°S)	Longitude (°E)	Altitude (m)
<i>S. ibityensis</i>	IbitA	10	20° 3' 48.8"	47° 0' 31.4"	1730
	IbitB	11	20° 3' 48.8"	47° 0' 31.4"	1750
	IbitC	18	20° 3' 48.8"	47° 0' 31.4"	1800
	IbitD	18	20° 3' 48.8"	47° 0' 31.4"	1830
	IbitE	21	20° 3' 55.5"	47° 0' 21.5"	ca. 1650
	IbitF	24	20° 3' 59.6"	47° 0' 7.9"	1600
	IbitG	37	20° 3' 59.8"	47° 0' 7.5"	ca. 1680
<i>S. lanatus</i>	LanA	22	20° 35' 4.1"	46° 34' 54.3"	ca. 1550
	LanB	23	20° 35' 4.1"	46° 34' 54.3"	ca. 1550
	LanC	31	20° 35' 3.9"	46° 34' 40.1"	ca. 1560
	LanD	19	20° 34' 15.7"	46° 34' 35.6"	ca. 1580
	LanE	17	20° 34' 13.1"	46° 34' 35.7"	ca. 1580
	LanF	14	20° 34' 13.5"	46° 34' 35.6"	ca. 1580
<i>S. micranthus</i>	MicrA	24	24° 52' 5.9"	30° 53' 27.0"	1700
	MicrB	24	25° 0' 1.5"	30° 49' 26.4"	1290
	MicrC	24	24° 32' 54.6"	30° 52' 59.4"	1900
	MicrD	24	25° 56' 23.4"	31° 6' 34.2"	1380

versus clade assignment (Fig. 3), as it was shown that speciation in this genus follows a North-South cline (Möller and Cronk 2001b). Corolla tube lengths were taken from Hilliard and Burt (1971).

Results

Estimation of breeding behaviour. Most of the populations of *S. micranthus* and *S. lanatus* show a significant deviation from panmixia,

Table 2. Per locus population genetic statistics^a

Locus	<i>n</i>	<i>A</i>	H_E	H_O	<i>F</i>	θ	<i>f</i>
<i>S. micranthus</i>							
PR239	56	7	0.765	0.143	0.856***	0.706***	0.509***
JH448	75	4	0.592	0.000	1.000***	0.861***	1.000***
K17L	88	2	0.314	0.000	1.000***	0.627***	1.000***
JH432	88	6	0.754	0.045	0.949***	0.613***	0.868***
All loci	77.8	4	0.485	0.038	0.937***	0.708***	0.786***
<i>S. ibityensis</i>							
CTG16	97	2	0.021	0.021	-0.006ns	-0.004ns	0.002ns
IB511	123	9	0.719	0.545	0.268***	0.198***	0.088ns
JH432	97	6	0.469	0.309	0.364***	0.226***	0.178ns
PR239	96	14	0.833	0.625	0.267***	0.126***	0.161*
All loci	103	7.8	0.510	0.375	0.287***	0.173***	0.138***
<i>S. lanatus</i>							
CTG16	106	4	0.561	0.085	0.866***	0.622***	0.646**
IB511	115	3	0.330	0.009	0.978***	0.890***	0.798*
JH432	104	5	0.394	0.144	0.653***	0.277***	0.521**
PR239	104	2	0.400	0.067	0.842***	0.348***	0.758**
All loci	107	3.5	0.421	0.076	0.837***	0.539***	0.646***

^a*n*, number of individuals sampled per locus; *A*, number of alleles per locus; H_E , expected heterozygosity; H_O , observed heterozygosity; *F*, θ & *f*, Weir & Cockerham's (1984) estimate of F_{IT} , F_{ST} and F_{IS} respectively. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant

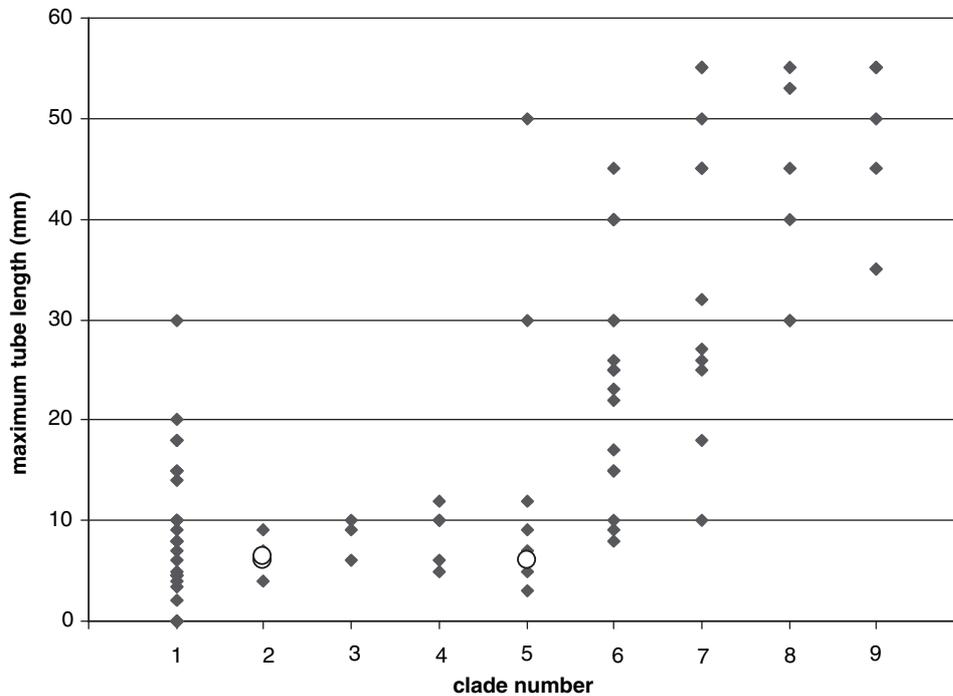


Fig. 3. Scatter plot of maximum flower tube lengths vs. clade association of 92 taxa included in the phylogenetic analysis. Clade assignment is as indicated in Figure 4. Data points for *S. lanatus* and *S. ibityensis* (both clade 2) and *S. micranthus* (clade 5) are depicted as open circles

with population level estimates of the inbreeding coefficient F_{IS} ranging between -0.028 to 1.000 for *S. micranthus* and -0.030 and 1.000 for *S. lanatus* (Table 3). The two populations (one per species) that did not deviate from panmixia had a very small number of heterozygotes due to rare alleles in an otherwise entirely homozygous background. In contrast only two of the seven populations of *S. ibityensis*, IbitF and IbitG, gave significant estimates of inbreeding ($f = 0.268$, $P < 0.05$ and $f = 0.215$, $P < 0.01$ respectively). Two populations of *S. ibityensis* showed an excess of heterozygotes (IbitB, $f = -0.154$; IbitC, $f = -0.238$), although neither of these estimates were significant at $P = 0.05$ level. At the species level, all three taxa show significant values of inbreeding; $f = 0.786$ ($P < 0.001$) for *S. micranthus*, $f = 0.646$ ($P < 0.001$) for *S. lanatus* and $f = 0.138$, ($P < 0.001$) for *S. ibityensis* (Table 2); the value for *S. ibityensis* is signifi-

cantly lower than for the other two species (t -test; $P < 0.05$). The corresponding outcrossing rates derived from these F_{IS} estimates are *S. micranthus*, $t = 0.13$; *S. lanatus*, $t = 0.22$ and *S. ibityensis*, $t = 0.76$.

Population genetic structure and descriptive statistics. All species show significant deviations from panmixia at the population level (Table 2). A significantly higher degree of population structure (10,000 permutations, randomising populations between species; $P < 0.001$) is shown by *S. micranthus* ($\theta = 0.708$, $P < 0.001$) and *S. lanatus* ($\theta = 0.539$, $P < 0.001$) than *S. ibityensis* ($\theta = 0.173$, $P < 0.001$). The same set of four loci was used in both of the Malagasy species, which permits direct comparisons to be made of locus-dependent parameters. In *S. ibityensis*, the mean population level values for allelic richness ($A_r = 2.8$) and gene diversity ($H_E = 0.396$) were significantly higher (10,000 permutations, randomising genotypes

Table 3. Population level statistics for *S. ibityensis* and *S. lanatus*^b

Population	<i>n</i>	<i>A</i>	<i>A_r</i>	<i>H_E</i>	<i>H_O</i>	<i>F</i>	<i>P</i>
<i>S. micranthus</i>							
MicrA	20.2	1.4	1.2	0.039	0.040	-0.028ns	2
MicrB	17.4	2	1.5	0.276	0.094	0.660*	2
MicrC	21.6	2.2	2.1	0.175	0.000	1.000*	4
MicrD	18.6	1.6	1.8	0.255	0.000	1.000*	2
Mean	19.5	1.8	1.7	0.186	0.034	0.658	2.5
<i>S. ibityensis</i>							
IbitA	9.3	2.0	2.0	0.394	0.342	0.137ns	3
IbitB	8.3	2.0	2.0	0.220	0.250	-0.154ns	2
IbitC	17.5	2.8	2.4	0.436	0.517	-0.238ns	3
IbitD	11.3	3.5	3.0	0.418	0.329	0.220ns	4
IbitE	12.3	3.0	2.8	0.341	0.282	0.179ns	3
IbitF	17.8	3.8	3.0	0.396	0.292	0.268*	3
IbitG	27	5.8	4.3	0.570	0.450	0.215**	4
Mean	14.8	3.3	2.8	0.396	0.352	0.090	3.1
<i>S. lanatus</i>							
LanA	19.5	3.3	2.5	0.483	0.120	0.757***	4
LanB	20	1.8	1.5	0.109	0.015	0.868***	3
LanC	27.8	2.3	1.9	0.271	0.173	0.368**	4
LanD	15	1.3	1.2	0.075	0.000	1.000**	1
LanE	11.5	1.5	1.4	0.063	0.064	-0.030ns	2
LanF	13.5	1.3	1.3	0.116	0.000	1.000**	2
Mean	17.9	1.9	1.6	0.186	0.062	0.661	2.7

^b*n*, mean number of individuals sampled per locus; *A*, number of alleles per locus; *A_r*, allelic richness; *H_E*, expected heterozygosity; *H_O*, observed heterozygosity; *f*, Weir & Cockerham's (1984) estimate of *F_{IS}*; *P*, number of polymorphic loci. **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *ns*, not significant

between species; *P* < 0.02 and *P* < 0.05 respectively) than in *S. lanatus* (*A_r* = 1.6 and *H_E* = 0.186).

Phylogenetic reconstruction. Parsimony analysis of the ITS and 5.8S matrix resulted in six most parsimonious trees of 1127 steps length, CI of 0.4978 and RI of 0.7563. The topology of the tree was identical to previously published phylogenies of *Streptocarpus* (Möller and Cronk 2001a, b), except for minor differences, namely (i) an extended polytomy in the personate clade, (ii) the position of a basal position of *S. papangae* in the Madagascan clade 2 and (iii) the placement of *S. exsertus* outside the Madagascan clades (Fig. 4). These differences were found to have no effect on the outcome of the character state optimisation.

Flower evolution. The small 'pouch' floral type was shown to be plesiomorphic for the genus (Fig. 4), and occurs in the majority of species in Clade I, the herbaceous caulescent group. It was found in all species from Madagascar in this clade, except *S. hilsenbergii*, as well as in the three basal clades within Clade II, including the two Madagascan clades (clade 2 and 3, except for the African *S. burundiensis*). For three mainland Africa clades (clades 3, 4 and 5) following the Madagascan clade the pouch type was ancestral. For clade 6 resolving alternatives of the polytomy in the sub-clade marked with an arrow in Fig. 4 (Maddison and Maddison 2003) can change the ancestral type to open tube for this clade. However, all study species were placed in clades which had the small pouch type as the ancestral state;

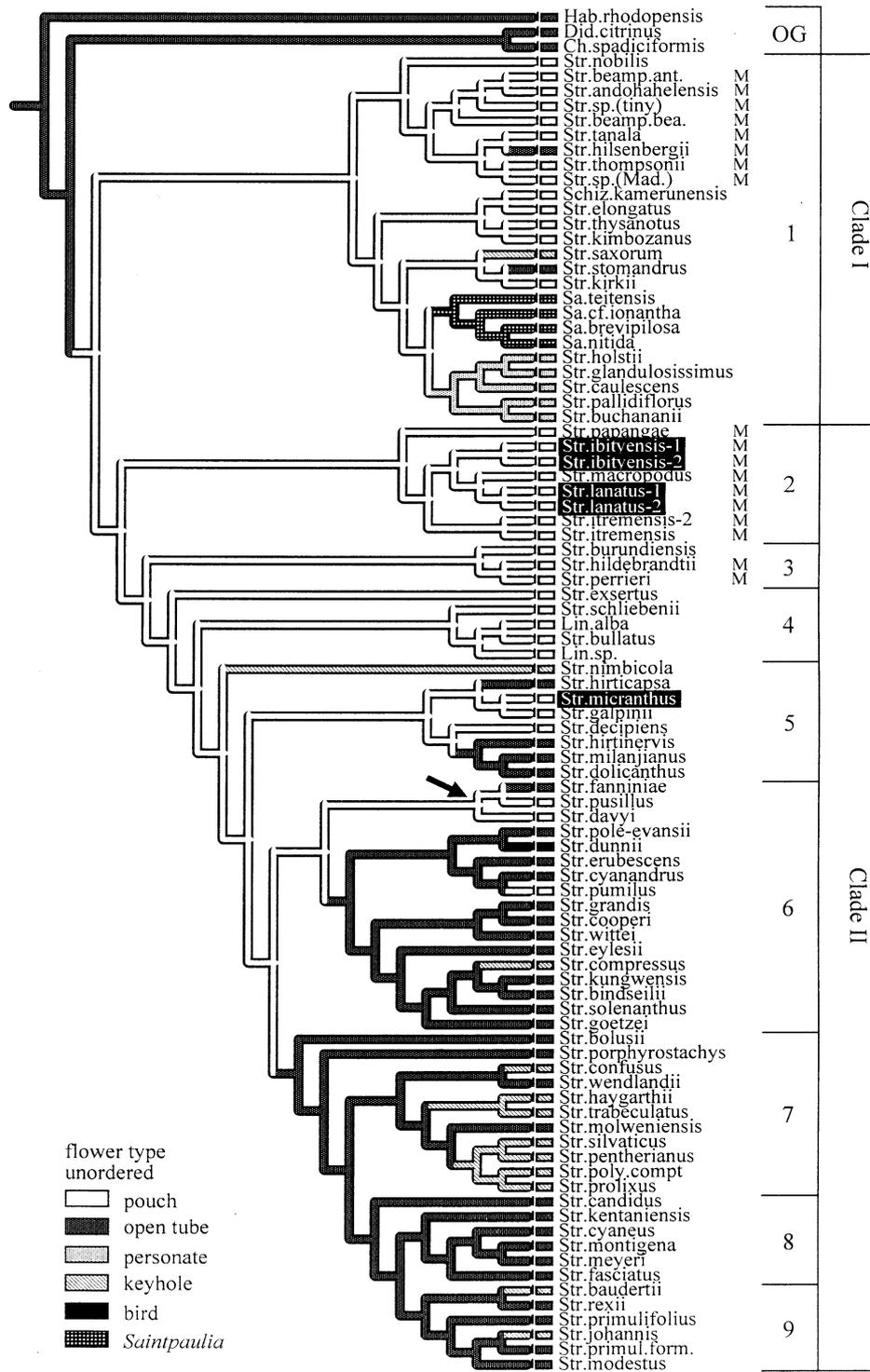


Fig. 4. Majority rule consensus tree based on ITS sequences with floral type changes optimised under DELTRAN using MaClade (Maddison and Maddison 2003). Polytomies were randomly resolved; a single polytomy which affected character state changes depending upon the topology chosen is indicated by an arrow. ‘M’ indicates species endemic to Madagascar. The species used for the population genetic and breeding system analysis are highlighted in black. Clade numbers are shown to the right

Table 4. Number of unambiguous character state changes under DELTRAN optimisation over the phylogenetic ITS tree for 92 taxa. Polytomies were randomly resolved

		to:					
		pouch	open tube	personate	keyhole	bird	<i>Saintpaulia</i>
from:	pouch	-	7	1	2	0	1
	open tube	2	-	0	6	1	0

S. ibityensis and *S. lanatus* in the Madagascan clade 2, and *S. micranthus* in the African clade 5, as sister to *S. galpinii* (Fig. 4).

Across the entire tree shown in Fig. 4 a total of 20 transitions between floral types occurred (Table 4). The majority of transitions were from pouch to open tube (7 times), with a reversal of this transition occurring only twice, and from the open type to the keyhole type (6 times). Only changes in one polytomy (Fig. 4, arrow) affected changes from pouch to open tube depending on which random topology was chosen, with the alternative topology resulting in 5 changes from pouch to open tube and 3 reversals. The personate, keyhole, bird and *Saintpaulia* floral types were all derived from either the pouch or open tube type (Table 4, Fig. 4), with no reversals or transitions from these syndromes being detected.

A scatter plot of floral tube length vs. clade assignment as designated in Fig. 3 gives further evidence for a trend of increase in floral size through evolutionary time. Our study species fall in clades with either only small flowered taxa (*S. lanatus* and *S. ibityensis* in clade 2) or predominantly small flowered (*S. micranthus* in clade 5).

Discussion

All three species show within-population deviation from panmixia, which is congruent with small flower size in *Streptocarpus* being linked with a mixed mating breeding system that includes a significant degree of self-pollination. Furthermore, two of the three species, *S. micranthus* and *S. lanatus*, are characterised

by predominantly selfing breeding systems, with inferred outcrossing rates of $t = 0.13$ and $t = 0.22$ respectively. Given that populations were collected over very small areas, especially for the Malagasy species for which population samples were restricted to a single rock (e.g. Fig. 2D), it would seem unlikely a high degree of unsuspected within-population structure could account for these figures. Although *S. micranthus* and *S. lanatus* had a high degree of variance in population level estimates of F_{IS} , ranging from $f = -0.028$ – 1.000 and $f = -0.030$ – 1.000 respectively (Table 3), the lower extremes were in each case caused by a single population with very low amounts of polymorphism in an otherwise entirely homozygous background. It is possible that whilst outcrossing events involving rare alleles were picked up, inbreeding due to self pollination remained undetectable due to the lack of polymorphic loci within the population. All other populations for these two species gave marked and significant estimates for F_{IS} . *S. ibityensis* also had a high variance in per-population F_{IS} estimates, due to the two populations which have an excess (although not significant) of heterozygotes. Artifactual heterozygote excesses can be caused by micro-satellite locus duplication or scoring errors, and the impact on the overall F_{IS} figures of these two populations was assessed by removing them from the analysis. This resulted in a slightly higher estimate of $f = 0.223$ ($P < 0.001$), which however is still significantly lower than in the other two species (t -test, $P < 0.01$).

It would seem that all species rely on self pollination to some degree, but that *S. ibityensis* is able to maintain a higher degree of

outcrossing. The cause of this difference is puzzling. *S. lanatus* has vivid purple markings on the lobes and floor of the corolla, which presumably function to attract and guide pollinators, and yet is predominantly inbreeding. Also, examination of mature flowers under a dissecting microscope showed the two Malagasy species produce similar, although minute, quantities of nectar through a nectiferous disc at the base of the ovary. One possible factor that may influence the degree of selfing could be the attitude of the corolla, i.e., whether it is horizontal or vertical, as the latter may facilitate the dropping of loose pollen from the anthers directly onto the stigma. In *S. ibityensis* the flowers are presented horizontally, in a more or less random orientation around the inflorescence (Fig. 2A). In contrast, the flowers of *S. lanatus* and *S. micranthus* are always positioned in a ‘nodding’ or pendulous fashion (Fig. 2B, C), which places the stigma directly beneath the anthers. Thus, in the latter two species the potential for self-pollination through pollen falling onto the stigma is potentially greater than in *S. ibityensis*. However, it is difficult to speculate further on the cause of the difference in inbreeding without field experiments and observations on pollinators.

All three species in this study show significant levels of population structure according to estimates of F_{ST} . This reflects their patchy distribution, as they are restricted to small pockets of favourable sheltered habitat under rocks and in crevices surrounded by unsuitable open grassland. The high degree of population structure suggests that seed dispersal between populations is limited and that the seeds, although dust-like, may not be commonly transported long distances on air currents. *S. micranthus* has the highest estimate of F_{ST} ($=0.708$) which is not surprising given its sample range of ca. 150 km. However, the figure for *S. lanatus* ($\theta=0.539$) is also remarkably high considering the range of the populations sampled is within only 1 km²; the estimate for *S. ibityensis* which was sampled over a over a similar spatial scale is $\theta=0.173$.

Given the patchy distribution and archipelago-like nature of the habitat these *Streptocarpus* species prefer, it would be advantageous for them to ensure at least a portion of their seed production is locally dispersed in the vicinity of the parent, and patterns of seedling recruitment in *S. lanatus* are congruent with this hypothesis, as shown in Fig. 2D. Given the relative uniformity of the seed throughout the genus, and the non-continuous distribution of many of its members, it would seem that a high degree of population structure could be a feature of many *Streptocarpus* species. The lowest estimate of population structure was found in the species which also showed the highest level of outcrossing (*S. ibityensis*; $\theta=0.173$). Given that seed and fruit morphology (and hence presumably seed dispersal syndrome) are similar in both the Malagasy species, which were also sampled over a similar range size, it is tempting to speculate that this comparatively low estimate of population structure is due to pollinator-mediated gene flow. However, this species also has comparatively high levels of gene diversity ($H_E=0.396$; Table 3), which biases estimates of F_{ST} downwards and hence makes comparisons to other species difficult. The reduced amount of gene diversity and allelic richness in *S. lanatus* compared to *S. ibityensis* could potentially be an effect of reduced effective population size due to a high level of self-pollination.

Studies of the floral evolution of other genera in the Gesneriaceae (Roalson et al. 2002, Perret et al. 2003, San Martin-Gajardo and Sazima 2004) have shown floral morphology to be labile, and this study is no exception, with a total of 20 transitions between our designated floral types in *Streptocarpus*. Despite the observed lability, there is a trend of increasing floral size and complexity through evolutionary time (Figs. 3 and 4). The most frequent transition was from small pouch to open tube, with the next most frequent being open tube to keyhole. Further, the simplest floral type (small pouch) is shown to be the ancestral state for the genus. Thus, this study confirms that the plesiomorphic floral morphology in *Strepto-*

carpus is associated with a breeding strategy that allows a significant degree of selfing. None of the species studied were exclusively selfing however, and it seems probable that all species with chasmogamous small flowers can manage at least some level of outcrossing due to pollinator visits. We would not go so far as to claim that we have an example of a study that provides “unequivocal support for a transition from highly selfing to outcrossing species” (Takebayashi and Morrell 2001). However, we nonetheless confirm that in some cases the ancestral floral type in this florally diverse genus is characterised by a predominantly selfing mixed mating system, and it has given rise to more complex floral types on several occasions. The lack of a completely selfing breeding system and the maintenance of some pollinator attraction at relatively low cost (small corolla and minute amount of nectar) means that small flowered and predominantly selfing *Streptocarpus* species can remain receptive to selection pressures on floral morphology due to pollinator preferences.

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