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## Evolution and Development of Floral Diversity in *Streptocarpus* and *Saintpaulia*

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Floral diversity in *Streptocarpus* and *Saintpaulia* can be classified into six distinct types on the basis of quantitative variation. Species are differentiated by overall flower size and by corolla lobe size in relation to corolla tube opening size, characteristics implicated in pollination ecology. *Saintpaulia* has a very short corolla tube and yellow protruding anthers, strikingly different from *Streptocarpus*, and probably associated with buzz pollination. Some species of *Streptocarpus* and all species of *Saintpaulia* are enantiostylous, a feature often linked to buzz pollination. Mapping of these floral characters onto a molecular phylogeny based on ITS sequence data showed that in *Streptocarpus* flower size evolved from small to large, with reversals in four species. The putatively bee pollinated 'open-tubed' type had two independent origins, and the putatively lepidopteran-pollinated 'keyhole' type had four separate origins. Enantiostyly had one or two origins. The degree of zygomorphy varies within *Streptocarpus/Saintpaulia*. The most extreme difference is between *Saintpaulia ionantha* and a *Saintpaulia* peloric cultivar. The developmental basis of floral variation was compared using SEM in three very different types: *Streptocarpus primulifolius*, *Saintpaulia ionantha* and a *Saintpaulia* peloric cultivar. Differences in corolla tube length and zygomorphy are established very early in flower development. Small, early changes in flower development establish major changes in mature floral morphology.

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**Key words:** African Violet, Cape Primrose, *CYCLOIDEA*, floral development, floral diversity, enantiostyly, Gesneriaceae, inflorescence architecture, *Saintpaulia*, *Streptocarpus*, sympetaly.

### INTRODUCTION

Within many large genera a wide range of floral form can be found (Grant and Grant, 1965; Ornduff, 1969; Stebbins, 1970; Johnson, Linder and Steiner, 1998). It has been suggested that diversity has arisen through adaptive radiation to different pollinators (Grant and Grant, 1965; Faegri and Van der Pijl, 1966; Ornduff, 1969; Stebbins, 1970). Recent work mapping pollinators and floral characters onto molecular phylogenies has allowed explicit testing of this hypothesis (Barrett and Graham, 1998; Hapeman and Inoue, 1998; Johnson *et al.*, 1998). The mapping approach shows a remarkable variation in floral form through time, which is coupled to pollinator changes (Hapeman and Inoue, 1998; Johnson *et al.*, 1998). Variation in floral form is also linked to differences in breeding system (Ornduff, 1969; Graham and Barrett, 1995; Ortega-Olivencia *et al.*, 1997; Barrett and Graham, 1998). Large flower size may promote outcrossing, and small flower size is often associated with selfing, particularly by cleistogamy (Ornduff, 1969; Ortega-Olivencia *et al.*, 1997). Outcrossing and inbreeding rates are also affected by the respective positions of the anthers and stigma at anthesis.

*Streptocarpus* Lindl. is a genus of Gesneriaceae encompassing approx. 130 species in Africa and Madagascar (Hilliard and Burt, 1971). *Streptocarpus* has zygomorphic (bilaterally symmetric) tubular flowers which are variable in shape, giving a wide range of floral types (Hilliard and Burt, 1971). These types are separated on the basis of their

corolla tube openings, which may be open (the flower is funnel-shaped), keyhole-shaped, or masked by the palate of the lower lip (personate) as in *Antirrhinum* L. (Hilliard and Burt, 1971). *Streptocarpus* flowers are nectariferous, and also vary conspicuously in size, colour and scent, all features associated with pollination ecology in other genera (Grant and Grant, 1965; Ornduff, 1969).

*Saintpaulia* H. Wendl. also occurs in tropical Africa, but has flowers strikingly different from *Streptocarpus*. They almost completely lack a corolla tube, and have large, bright yellow, protruding anthers that contrast with the violet petals. The anthers contain creamy/white light, dry pollen, and persist after pollen is dispensed. All of these features are commonly associated with buzz pollination (Buchmann, 1983). Another feature commonly associated with buzz pollination is enantiostyly (Dulberger, 1981; Buchmann, 1983), and *Saintpaulia* is enantiostylous (Willis, 1973). Enantiostyly has been defined as the strong deflection of the style to the left or right of the main floral axis at anthesis, with flowers of different morphs occurring at the same frequency within a plant (Dulberger and Ornduff, 1980; Buchmann, 1983; Simpson, 1990). The reciprocal deflection of the anthers is usually included in this definition (Dulberger, 1981; Buchmann, 1983; Fenster, 1995; Graham and Barrett, 1995). Both forms occur in *Streptocarpus/Saintpaulia*; in *Saintpaulia* only the style is displaced, but in some *Streptocarpus* there is reciprocal deflection of the anthers. Enantiostyly also occurs in other Gesneriaceae (e.g. *Didymocarpus podocarpus* C. B. Cl.). How enantiostyly

affects plant breeding systems remains unclear, although several hypotheses have been proposed (Dulberger, 1981; Buchmann, 1983; Fenster, 1995; Graham and Barrett, 1995).

Despite the marked difference between *Saintpaulia* and *Streptocarpus*, recent molecular phylogenetic work suggests that *Saintpaulia* has evolved from *Streptocarpus* (Möller and Cronk, 1997a, unpubl. res.). The clade including both *Streptocarpus* and *Saintpaulia* encompasses a very wide range of floral types and traits. The molecular phylogeny offers an opportunity to discover how often different floral types and other floral characters evolved in *Streptocarpus*.

In some diverse genera floral variation is apparent early during floral ontogeny, in others it is apparent later (Crisci and Stuessy, 1980; Tucker, Stein and Derstine, 1985; Bowman, 1997). Variation in flower form has a genetic and developmental basis (Bowman, 1997). Investigating floral development allows fuller understanding of variation in form, potentially revealing homology between traits which may be masked at anthesis (Crisci and Stuessy, 1980; Tucker et al., 1985).

Recent work combining developmental and genetic approaches has elucidated the genetic basis of floral morphology in *Arabidopsis* Heynh. and *Antirrhinum* (Coen and Meyerowitz, 1990). This work has shown that flower structure is determined by the whorl-specific expression of genes in the MADS box family (the ABC genes), which are highly conserved throughout the angiosperms (Bowman, 1997; Irish and Kramer, 1998). This conservation is reflected in the basic similarity of all angiosperms in flower structure (Bowman, 1997). However, variation in flower shape is generated at lower levels of organization by different genes. Particularly relevant to this study is the influence of *CYCLOIDEA*, a gene which affects zygomorphy (Cronk and Möller, 1997). The action of *CYCLOIDEA* has been studied in *Antirrhinum* (Scrophulariaceae, closely related to Gesneriaceae) (Carpenter and Coen, 1990; Luo et al., 1996). *Cycloidea* loss of function mutants in *Antirrhinum* have semipeloric flowers characterized by the appearance of a six floral organ in the sepal, petal and stamen whorls, and ventralized petals and stamens (Luo et al., 1996). Fully peloric flowers form when *CYCLOIDEA* acts in conjunction with another gene, *DICHOTOMA* (Luo et al., 1996). *Saintpaulia* has peloric cultivars that share some features with *cycloidea* loss of function mutants, and it is possible that the cultivars are mutants in a *CYCLOIDEA* orthologue.

Within the angiosperms, inflorescence architecture is also variable, particularly in the number and distribution of flowers. The presence or absence of bracts subtending flowers is normally a constant feature important in distinguishing inflorescences (Coen and Nugent, 1994). *Saintpaulia* and *Streptocarpus* are unusual in their inflorescence architecture in that the number and position of their bracts is highly variable (Weber, 1982). Coen and Nugent (1994) favoured the bractless condition as an evolutionary loss. They postulated the acquisition of bract primordia cells by the floral meristem, offering flowers an accelerated development. This possibility is open to investigation in *Streptocarpus* and *Saintpaulia*.

The wide range of floral morphology in *Streptocarpus* is

set against a diverse vegetative backdrop. Subgenus *Streptocarpella* Fritsch (and the genus *Saintpaulia* nested within it) has a normal stem, a characteristic thought to be plesiomorphic. Subgenus *Streptocarpus* lacks a true stem. Its large, unusual leaves (phyllomorphs) are either solitary (unifoliate) or serially replicated to form a pseudo-rosette (rosulate). The habitat and range of the two subgenera also differ. Subgenus *Streptocarpella* grows mainly on shady forest floors, banks, outcrops or moss-covered rocks near streams in tropical Africa and Madagascar, whereas subgenus *Streptocarpus* typically grows on rock outcrops from tropical East Africa to the Cape of Good Hope (Hilliard and Burtt, 1971). Subgenus *Streptocarpus* is also found in Madagascar. Despite its wide geographical range, *Streptocarpus* occurs in patches due to the scattered distribution of suitable habitats. Where conditions are favourable, several different species may grow together (Hilliard and Burtt, 1971). Floral types are not distributed evenly between species of the two subgenera, suggesting that there may be some interplay between vegetative and floral morphology.

This study aims to describe the range of floral diversity in *Streptocarpus* including *Saintpaulia*, to determine the extent to which small changes in early development can explain the diversity of floral form in the genus, and to examine these changes within a phylogenetic and biogeographical context to elucidate the evolutionary pressures that are ultimately responsible for change.

## MATERIALS AND METHODS

### *Morphometric procedure*

To classify *Streptocarpus* flowers according to their shape, and to define how they vary, multivariate measurements of the flowers were taken, analysed, and plotted in the form of an ordination. Forty-four measurements from single flowers of 39 species in cultivation at the Royal Botanic Garden Edinburgh, UK were used; the complete list is given in Table 1. Data were analysed with the R Package 3.0.1 principal components option (Legendre and Vaudor, 1991). To determine intraspecific variability five flowers of two species (*S. saxorum* and *S. candidus*) were also measured, and included in the analysis as supplementary objects, as were two hybrids (*S. rexii* × *S. dumii* and *S. rexii* × *S. wittei*). Full details of the taxa used and their provenance are given in Table 2. The general appearance of each flower was also noted.

### *Buzz pollination*

It has previously been suggested that *Saintpaulia* is buzz pollinated (Vogel, 1978; Dafni, 1992). Buzz pollinated flowers usually extrude pollen from their anthers in response to vibrations in the range of 50–2000 Hz (Buchmann, 1983). A frequency-dependent expulsion of pollen could therefore be taken as evidence of a buzz pollinated system. In *Saintpaulia*, the anthers are fused at the top, and mounted on sturdy filaments (Weberling, 1989). When they are

TABLE 1. Characters scored for morphometric analysis

Character number	Measurement (mm)
1	Length of adaxial petals from corolla tube mouth to tip
2	Width of adaxial petals at widest point
3	Length of middle petals from corolla tube mouth to tip
4	Width of middle petals at widest point
5	Length of abaxial petal from corolla mouth to tip
6	Width of abaxial petal at widest point
7	Length of flower face
8	Width of flower face at widest point
9	Width of flower face adaxial side widest point
10	Length of corolla tube opening
11	Width of corolla tube opening
12	Length between adaxial sinuses
13	Length between abaxial sinuses
14	Length of corolla tube from base to tip of lip, lip relaxed
15	Length of corolla tube from base to tip of lip, lip extended
16	Length of corolla tube from base to tip of adaxial petals, relaxed
17	Length of corolla tube from base to tip of adaxial petals, extended
18	Length of adaxial calyx lobe
19	Length of corolla tube from base to sinus
20	Depth of corolla tube at base
21	Width of corolla tube at base
22	Depth of corolla tube halfway between base and lateral sinus
23	Width of corolla tube halfway between base and lateral sinus
24	Depth at position of anthers
25	Width at position of anthers
26	Length of anthers
27	Width of anthers
28	Length of filament
29	Width of filament at base
30	Width of filament at widest point
31	Width of filament at tip
32	Length of stamen from corolla base
33	Distance between bases of filaments
34	Length of staminodes
35	Length of staminodes from corolla base
36	Distance between two lateral staminodes
37	Length of gynoecium
38	Length of ovary in mature flower
39	Width of ovary at base
40	Width of ovary at widest point
41	Length of style
42	Width of style; narrowest point
43	Width of stigma
44	Length of stigma

depressed slightly from their adaxial side, they separate, exposing the locules. To see how pollen was released, the anthers of several flowers were separated by pushing them gently upwards against a mounted vibrator (Bridage Scientific Instruments), and vibrated at different frequencies within the natural range reported by Buchmann (1983). Pollen was captured on a 5 cm squared piece of Saran<sup>®</sup> wrap (Dow Chemical Company) placed directly underneath the anthers. This was subsequently wrapped around a microscope slide to give an approximate gauge of the amount of pollen released.

### Flower development

To understand the basis of shape variation within the *Streptocarpus/Saintpaulia* clade, the development of representative and contrasting inflorescences and flowers of *Streptocarpus primulifolius*, *Saintpaulia ionantha* and a peloric cultivar of *Saintpaulia* were surveyed. The induction and general pattern of inflorescence development in *Saintpaulia* has previously been studied by Faust and Heins (1994). Mature inflorescences were partially dissected to yield buds of varying size. Buds were fixed in Copenhagen Mixture (3.5 parts methylated spirits, 5.5 parts water, and 0.5 parts glycerol) overnight, and dehydrated through an ethanol/acetone series into 100% acetone dried with a molecular sieve. Material was then dried in an Emitech K850 critical point dryer. Dried parts were mounted with silver in methyl isobutyl ketone (Electrodag 1415M) on 1.25 cm Agar Scientific aluminium stubs, and further dissected. Stubs were sputter coated with gold-palladium using an Emscope sc500. Specimens were viewed using a Zeiss DSM962 SEM at a working distance of 8–11 mm, and operating at 5 kV. Photographs were taken using Kodak 25ASA Technical Pan F film.

### Phylogenetic analysis and mapping

To understand the evolutionary origins of variation within *Streptocarpus*, floral characters were mapped onto a molecular phylogeny of *Streptocarpus* based on internal transcribed spacer (ITS) sequence data. The majority rule topology depicted here is reduced from a fuller analysis of *Streptocarpus* (Möller and Cronk, unpubl. res.) compiled as described previously by Möller and Cronk (1997a, b). Some species included in the morphometric analysis were omitted from the phylogeny due to unavailability of sequence data. Analysis of character-state transitions was performed in MacClade Version 3 (Maddison and Maddison, 1993), using Acctran to resolve equivocal nodes.

## RESULTS

### Morphology

The designation of three types of *Streptocarpus* flower by Hilliard and Burt (1971) was supported by the morphometric analysis (Fig. 1A). Three further types were defined: a *Saintpaulia* type, a small pouch type and a *dunnii* type (Fig. 1A). The main aspects of variation differentiating species were size, and corolla lobe size in relation to corolla tube opening size, both of which are important in floral display (Fig. 1B). The morphological extremes are illustrated in Fig. 1B.

### Vibration-dependent release of pollen in *Saintpaulia*

When the anthers were vibrated in the range of 70–100 Hz, a large part of the pollen content sprayed out. At lower frequencies a small amount of pollen was also dispensed.

TABLE 2. *Streptocarpus* (S.) and *Saintpaulia* (Sa.) taxa used in this study

Species	Authority	Abbreviation	Accession number	Distribution
<i>S. baudertii</i>	L. L. Britten	BAU	19961858	S. Africa
<i>S. bindseilii</i>	E. Fischer	BIN	19972934	Rwanda
<i>S. buchananii</i>	C. B. Clarke	BUC	19972911	Malawi
<i>S. candidus</i>	Hilliard	CAN	19771204	S. Africa
<i>S. caulescens</i>	Vatke	CAU	19731215	Tanzania
<i>S. cyaneus</i>	S. Moore	CYA	19911950	Swaziland
<i>S. dunnii</i>	J. D. Hooker	DUN	19972038	Transvaal
<i>S. floribundus</i>	T. J. Weigend and M. Edwards	FLO	19962085	S. Africa
<i>S. gardenii</i>	W. J. Hooker	GAR	19912180	S. Africa
<i>S. glandulosissimus</i>	Engler	GLA	19961868	Central Africa
<i>S. goetzei</i>	Engler	GOE	19972033	Tanzania
<i>S. grandis</i>	N. E. Brown	GRA	19971210	S. Africa
<i>S. hirticapsa</i>	B. L. Burt	HIR	19932793	Zimbabwe
<i>S. holstii</i>	Engler	HOL	19592272	Tanzania
<i>Sa. ionantha</i>	Wendl.	ION	19970185	Tanzania
<i>Saintpaulia peloric</i> cultivar	Horticultural origin	CUL	19990062 19990063	
<i>S. johannis</i>	L. L. Britten	JOH	19923042	S. Africa
<i>S. kentaniensis</i>	Britten and Story	KEN	19961877	S. Africa
<i>S. kirkii</i>	J. D. Hooker	KIR	19961879	Kenya
<i>S. michelmorei</i>	B. L. Burt	MIC	19972913	Zimbabwe
<i>S. modestus</i>	L. L. Britten	MOD	19943058	S. Africa
<i>S. pallidiflorus</i>	C. B. Clarke	PAL	19691121	Tanzania
<i>S. parviflorus</i>	J. D. Hooker	PAR	19961883	S. Africa
<i>S. penterianus</i>	Fritsch	PEN	19972034	S. Africa
<i>S. polyanthus</i> ssp. <i>verecundus</i>	Hilliard	POL	19912548	S. Africa
<i>S. porphyrostachys</i>	Hilliard	POR	19972916	S. Africa
<i>S. primulifolius</i> ssp. <i>formosus</i>	Hilliard and Burt	PRI	19690444	Zimbabwe
<i>S. prolixus</i>	C. B. Clarke	PRO	19772035	S. Africa
<i>S. pumilus</i>	B. L. Burt	PUM	19932787	S. Africa
<i>S. rexii</i>	(Hook.) Lindl.	REX	19870333	S. Africa
<i>S. saxorum</i>	Engler	SAX	19721499 19701898 19721495 19961901 19751855	Kenya
<i>S. solenanthus</i>	Mansfeld	SOL	19972037	Tanzania
<i>S. sp.</i>	/	SP1	19972883	Madagascar
<i>S. sp.</i>	/	SP2	19931445	Madagascar
<i>S. stomandrus</i>	B. L. Burt	STO	19711392	Tanzania
<i>S. tanala</i>	Humbert	TAN	19972882	Madagascar
<i>S. thompsonii</i>	R. Brown	THO	19923189	Madagascar
<i>S. trabeculatus</i>	Hilliard	TRA	19972032	S. Africa
<i>S. variabilis</i>	Humbert	VAR	19972932	Madagascar
<i>S. wendlandii</i>	Sprenger	WEN	19970108	S. Africa
<i>S. wittei</i>	De Wildeman	WIT	19871695	Zaire/Malawi
<i>S. rexii</i> × <i>S. dunnii</i>	/	HY1	19972905 no. 2	
<i>S. rexii</i> × <i>S. wittei</i>	/	HY2	19972906 no. 5	

The relative amount of pollen released (estimated visually) depended on the frequency at which the anthers were vibrated. In contrast, the open-tubed *Streptocarpus* species do not require vibration for pollen release. In these species slight pressure on the bow-shaped anthers is sufficient to swing apart the two stamens, allowing the pollen to drop under gravity through the longitudinal dehiscence slits located between the appressed stamens.

#### Phylogeny and flower diversity

Mapping of floral characters onto a molecular phylogeny of *Streptocarpus* revealed their origins and direction of change. This suggested a trend of increasing flower size

through time, with reversal to small flowers in some species (*S. pumilus*, *S. prolixus*, *S. penterianus* and *S. kentaniensis*: data not shown). The largest flowers are restricted to subgenus *Streptocarpus*. A trend of increasing corolla lobe size through time was also indicated (data not shown). Mapping of the flower types suggests that the pouch type is plesiomorphic, and it occurs in both subgenera; other types were by definition apomorphic. The open-tubed type had two origins and was restricted to subgenus *Streptocarpus* with one exception (*S. stomandrus* in subgenus *Streptocarpella*). The keyhole type had four independent origins, and was again restricted to subgenus *Streptocarpus* with one exception (*S. saxorum* in subgenus *Streptocarpella*). The personate type had one origin in subgenus *Streptocarpella*,

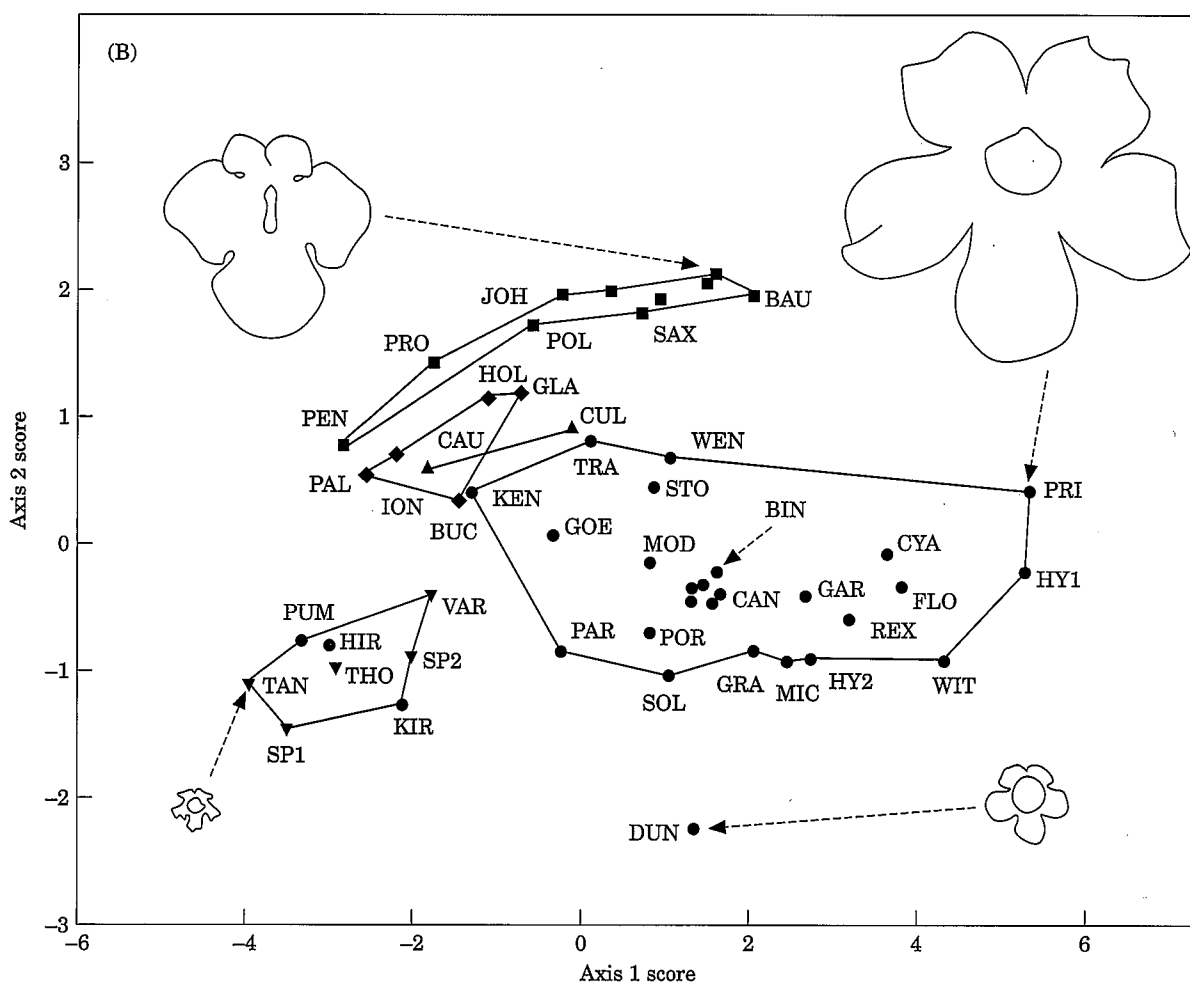
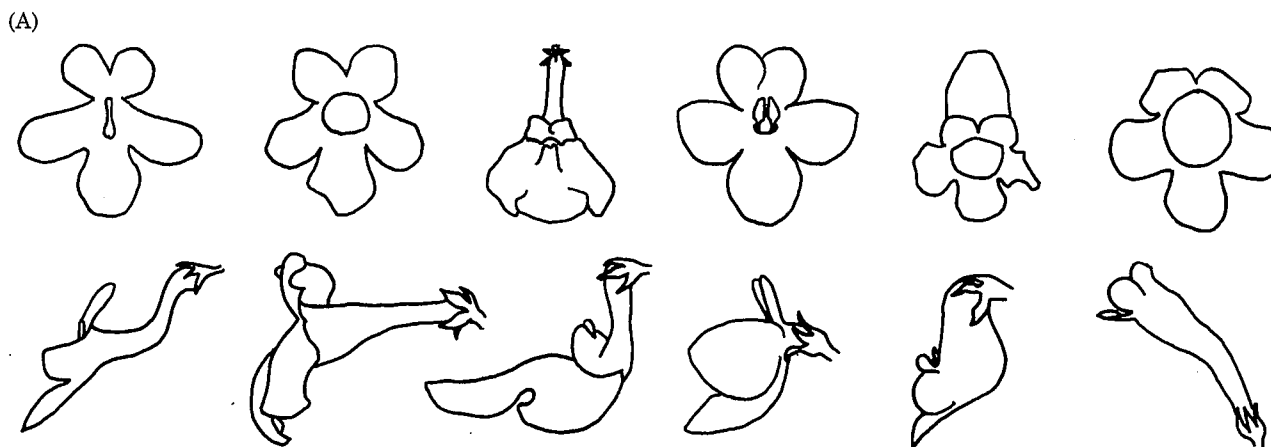


FIG. 1. A. Front (top row) and side (bottom row) views of the floral types of *Streptocarpus* (not to scale). From left to right: keyhole type (*S. johannis*); open-tubed type (*S. candidus*); personate type (*S. holstii*); *Saintpaulia* type (*Sa. ionantha*); pouch type (unnamed Malagasy species); *S. dumii*, which warrants its own type based on its distinct shape. The different types are probably adapted to different pollinators. B. Principal components plot of *Streptocarpus* and *Saintpaulia*. The floral types shown in Fig. 1A are distinguished: the top group are keyhole type (■), followed by the personate type (◆) and the *Saintpaulia* type (▲) nested within it, the open-tubed type (●) are next, followed by the pouch type (● or ▼), and finally the *S. dumii* type (●). Within the pouch type there are Malagasy (▼) and a few African (●) species. Size and shape extremes are illustrated by the outline drawings. Taxa are denoted as shown in Table 2. Axes 1 and 2 accounted for 68.19% (Eigenvalue 5.59) and 12.83% (Eigenvalue 1.05), respectively, of the variation.

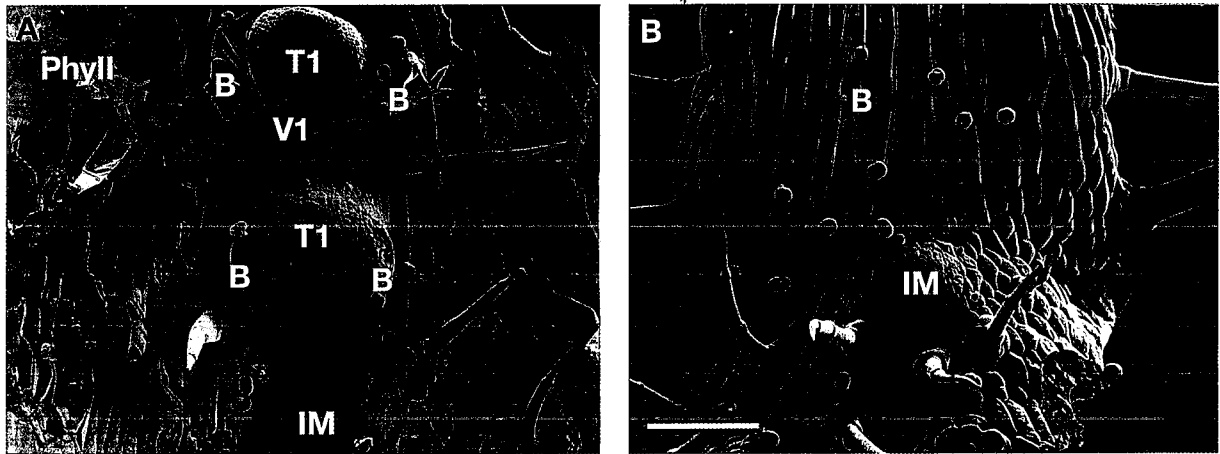


FIG. 2. A, Groove and inflorescence meristem in *Streptocarpus primulifolius*; B, inflorescence meristem in *Saintpaulia ionantha* (SEM micrographs). The meristematic region is far larger and more sheltered in *Streptocarpus* than in *Saintpaulia*. The phyllomorph, terminal flowers, front flowers, inflorescence meristem and bracts are denoted as Phyll, T1, V1, IM and B, respectively. Bar = 100  $\mu$ m.

and the *Saintpaulia* type had one origin, from subgenus *Streptocarpella* (Fig. 4).

The evolution of enantiostyly in *Streptocarpus* is also particularly interesting (Fig. 4). Two forms of enantiostyly occur: stylar displacement alone occurs in *Saintpaulia* and *S. pallidiflorus*, and stylar displacement with reciprocal anther displacement occurs in the remaining personate species. Enantiostyly probably has one or two independent origins in *Streptocarpus*, in the personate species and *Saintpaulia*. In the personate species reciprocal enantiostyly appears to have evolved from stylar displacement alone. Of the remaining species, approximately a third showed some stylar deflection in bud. Usually this was simply to the left or right of the main floral axis, with the style avoiding the anthers. In some species, the stigma touches the anthers before lateral displacement. This phenomenon occurs across both subgenera, and may have predisposed *Streptocarpus* to enantiostyly.

Enantiostyly has traditionally been associated with buzz pollination in plants with porose anther dehiscence (Buchmann, 1983). *Saintpaulia* may be buzz pollinated (Vogel, 1978; Dafni, 1992; this study). The association with buzz pollination cannot be made for the personate species, and neither *Streptocarpus* nor *Saintpaulia* have poricidal anthers. Enantiostyly in *Streptocarpus* is therefore particularly intriguing.

#### Development

Flower development may be influenced by vegetative and inflorescence morphology. The major vegetative differences between *Streptocarpus primulifolius* and *Saintpaulia* are that the former has no stem and has phyllomorphic leaves. The inflorescences arise from a groove meristem (Jong and Burt, 1975), which is an enlarged and elongated meristematic region at the base of each leaf (Fig. 2A). Inflorescence development also differs between the species. The bracts subtending flowers are always greatly reduced in *S. primulifolius*, whereas they are usually well developed in *S. ionantha* (Fig. 3, stage 1).

Flower development was divided into seven stages to permit comparison between taxa (Fig. 3). In *S. primulifolius* the stages of development were far less distinct than in *Sa. ionantha*, implying an accelerated floral development.

Variation in development is apparent before the floral organs begin to differentiate, as the floral meristems of *S. primulifolius* and *Sa. ionantha* differ in size (stage 1); *S. primulifolius* has slightly larger meristems than *Sa. ionantha*. This difference in size persists to anthesis in *Streptocarpus*. Early floral meristems in *S. primulifolius* are also shaped differently to those of *Sa. ionantha* (stage 1 and 2). The differences in meristem size and shape may be because the inflorescence in *S. primulifolius*, and other unifoliate and rosulate, arises from a groove meristem rather than an axillary meristem (Fig. 2A).

Developing flowers also diverge in shape very early in ontogeny (Fig. 3, stages 5–7). The major feature distinguishing *Streptocarpus* and *Saintpaulia* is corolla tube length; *Streptocarpus* has a pronounced corolla tube, *Saintpaulia* has virtually none. In *Streptocarpus* the corolla initiates as a ring at stage 5 (early sympetaly, Fig. 3), and the lobes begin to differentiate later on at stage 6, whereas in *Saintpaulia* the corolla lobes initiate first (late sympetaly, stage 5), and tube development is greatly retarded (Fig. 3).

Another aspect of flower shape that varies in *Streptocarpus* is zygomorphy, as indicated by the ratio of dorsal to ventral corolla lobe length between species. At anthesis, *Sa. ionantha* is more strongly zygomorphic than *S. primulifolius*. Again, this difference is evident very early; in *Saintpaulia* the lateral and ventral corolla lobes establish and begin to grow before the dorsal lobes (Fig. 3, stages 5 and 6), whereas in *S. primulifolius* all the petals establish together. Zygomorphy is also more pronounced in the stamen whorl in *Sa. ionantha* (Fig. 3, stages 5 and 6), although stamen growth is affected in the opposite way to petal growth. Although they are tiny at anthesis, the staminodes initiate and enlarge before the stamens. In *S. primulifolius* the stamens and lateral staminodes initially have similar size, and the dorsal staminode is smaller.

Zygomorphy differs most strongly between *Saintpaulia*

and its peloric cultivar (Fig. 3). Differences between these types first became apparent at stage 3. The first major difference is that a sixth floral organ sometimes develops in the sepal and petal whorls of the cultivar (Fig. 3, stage 5). The second major difference is that in the stamen whorl the two ventral stamens, and two-three dorsal staminodes of *Saintpaulia* (Burt, 1958) are replaced by five stamens in the cultivar (Fig. 3, stages 5–7). The third main difference is that primordia within petal and stamen whorls grow approximately equally in the cultivar, rather than showing dorsiventrality, although sometimes a slight residual dorsiventral asymmetry remains (Fig. 3, stages 5–7). Rarely, this asymmetry is pronounced as in Fig. 3, stage 7b. A final conspicuous difference between *Sa. ionantha* and its peloric cultivar is that the arrangement of the petals in bud is disrupted in the cultivar. In *Sa. ionantha* the two lateral petals always envelop the dorsal and ventral petals, whereas in the cultivar aestivation is random (Fig. 3, stage 7a).

Overall, the comparative developmental survey showed that large differences in flower size and shape at anthesis, which separate species taxonomically, are apparent very early during ontogeny.

## DISCUSSION

### *Morphology and phylogeny*

Floral diversity in the angiosperms has arisen as a result of the adaptation of flowers to diverse pollinators (Grant and Grant, 1965; Faegri and Van der Pijl, 1966; Stebbins, 1970). Flowers favoured by particular pollinators can often be identified by a suite of associated floral characters, termed a syndrome (Faegri and Van der Pijl, 1966). Variation in floral form and its relationship to pollination ecology has been demonstrated in other genera (Grant and Grant, 1965; Barrett and Graham, 1998; Johnson *et al.*, 1998). This link has been made by observing pollination in the field. Although field observations of *Streptocarpus* and *Saintpaulia* pollination are lacking, it is likely that the six floral types defined by the morphometric analysis represent four different pollination syndromes. Putatively these are small fly pollination (pouch type), bee pollination (open-tubed, personate and *Saintpaulia* types), moth or butterfly pollination (keyhole type), and bird pollination (*S. dunnii*). Fly pollination is suggested in the pouch type by the small size, pale colours, and relatively wide tubes of these flowers. Bee pollination is suggested in the open-tubed type by its white/lilac colour and deep purple markings, and the flowers are probably worked by bees crawling into the corolla tube. In the deep purple personate type, the corolla lobes must be prised apart before nectar can be accessed. In *Saintpaulia* the reward offered by the flower is pollen, not nectar, and the flowers are probably buzz pollinated. Bird pollination is suggested in the *dunnii* type by its red colour (unique in *Streptocarpus*) and by its long curved tubes. Lepidopteran pollination is suggested in the keyhole type by the pale colour, very thin corolla tubes and narrow entrance of these flowers. Although *S. trabeculatus* is not classified as keyhole type, it closely resembles the keyhole species in limb shape, and may also be lepidopteran pollinated. It is possible that

the keyhole species *S. baudertii* and *S. johannis* arose as hybrids between the *rexii* group and the *polyanthus* group.

The evolution of several flower types, and repeated evolution of two flower types within the *Streptocarpus* clade suggests that it has radiated in response to different pollinators. In subgenus *Streptocarpella* where variation in shape is greatest, these pollinators are probably more specialized.

In *Streptocarpus*, flower size appears to have increased through time. African species in subgenus *Streptocarpus* show the greatest variation in size, with larger flowers predominating in the south. Flower size has previously been linked to floral rewards, with larger flowers offering more copious nectar, thereby favouring outcrossing (Ortega-Olivencia *et al.*, 1997). The evolution of large floral size in *Streptocarpus* subgenus *Streptocarpus* has probably occurred as an adaptive response to larger pollinators. Small-flowered species have previously been linked to autogamy, particularly by cleistogamy (Ornduff, 1969; Ortega-Olivencia *et al.*, 1997; Johnson *et al.*, 1998). Semi-cleistogamous (partly open) flowers have previously been recorded in *Streptocarpus* (Hill, 1941; Ponniah, 1971), and cleistogamy is frequently observed in many small flowered species cultivated at the Royal Botanic Garden Edinburgh (M.M. pers. obs). Small flower size in *Streptocarpus* may also be linked to autogamy. This is particularly likely in the tiny flowered species *S. hirticapsa*, which has semi-cleistogamous (only partly open) flowers with protruding stigmas (C.J.H. pers. obs.).

Enantiostyly may also link to breeding system, allowing differential pollen transfer between left- and right-handed floral morphs within and between plants (Dulberger, 1981; Buchmann, 1983; Graham and Barrett, 1995). Several other functions of enantiostyly have previously been proposed. Enantiostyly usually occurs in buzz pollinated flowers, which are vibrated by heavy bees (Buchmann, 1983). It may protect the style from damage by removing it from the path of the bee (Dulberger, 1981; Fenster, 1995). The greater distance between anthers and stigma may also reduce within-flower selfing (Fenster, 1995). Enantiostyly could increase pollination precision and efficiency, beneficially reducing resource allocation on pollen (Dafni, 1992; Fenster, 1995).

In *Saintpaulia* several adaptive interpretations of enantiostyly are plausible. To work the anthers of *Saintpaulia* a bee must apply mechanical force to their adaxial side, effecting their separation. The mechanical force required is sufficiently slight to be given by the weight of a bee as it clasps the anthers. Subsequent buzzing by a bee would cause pollen release. The pollen ejected by the anthers when they are vibrated would be intercepted by its body, and pollen harvesting would follow grooming (Buchmann, 1983). Although this task is relatively simple, it presumably takes a little practice on the part of the bee. As a bee moves around the flower clasping the anthers, different parts of its body will contact the displaced stigma. From previous forays, the bee will have already received pollen, which in some areas will remain ungroomed. A stigma that is remote from the anthers in this system is probably less likely to receive pollen from its own anthers. An alternative



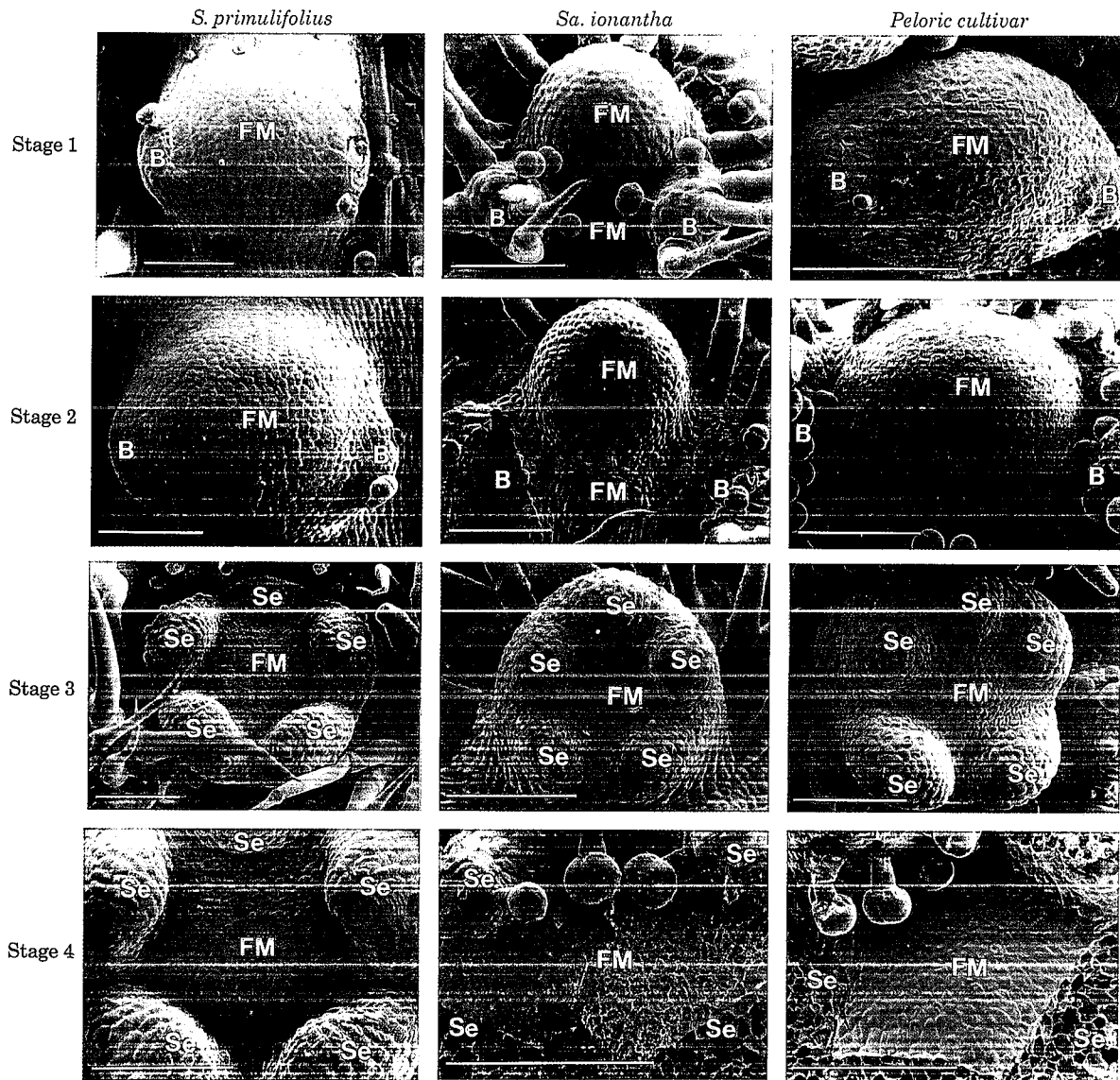


FIG. 3. For legend see opposite.

hypothesis regarding enantiostyly in *Saintpaulia* is that it functions to remove the style and stigma from the path of the bee, reducing the risk of premature departure, or damage to the flower.

Similar speculation can be applied to the personate species. The corolla tube is almost perpendicular to the face of the flower, and to insert its proboscis to feed, the head of the insect would have to press against the sexual organs of the flower. Slight displacement of the anthers in the personate species causes a paste-like extrusion of the glutinous pollen (C.J.H. pers. obs.). The personate species in this survey are mainly small flowered, (though *S. glandulosissimus* is a medium sized exception). Small flowers are correlated with small anthers (Ortega-Olivencia *et al.*, 1997), and produce correspondingly small quantities of pollen. Personate species are also specialized in form, and their specific mode of pollination may necessitate efficient pollen use by plants. In the personate forms, enantiostyly

could represent one way of ensuring this efficiency, with specific placement of pollen to one side or the other of a pollinator's head.

#### Development

As stated previously, the unusual leaves of subgenus *Streptocarpus* may be serially replicated in the form of a pseudo-rosette. This is the case in *S. primulifolius*, in which inflorescences arise acropetally from a large elongated meristematic region at the base of each leaf, called the groove meristem (Fig. 2A). This arrangement provides a sheltered environment minimizing desiccation and predation. In *Saintpaulia*, and other stemmed species of *Streptocarpus*, the developing inflorescence arises in the leaf axils, and is protected only by the leaves (Fig. 2B). This may explain why inflorescences in *Saintpaulia* and other caulescent species have far more conspicuous bracts than *S.*

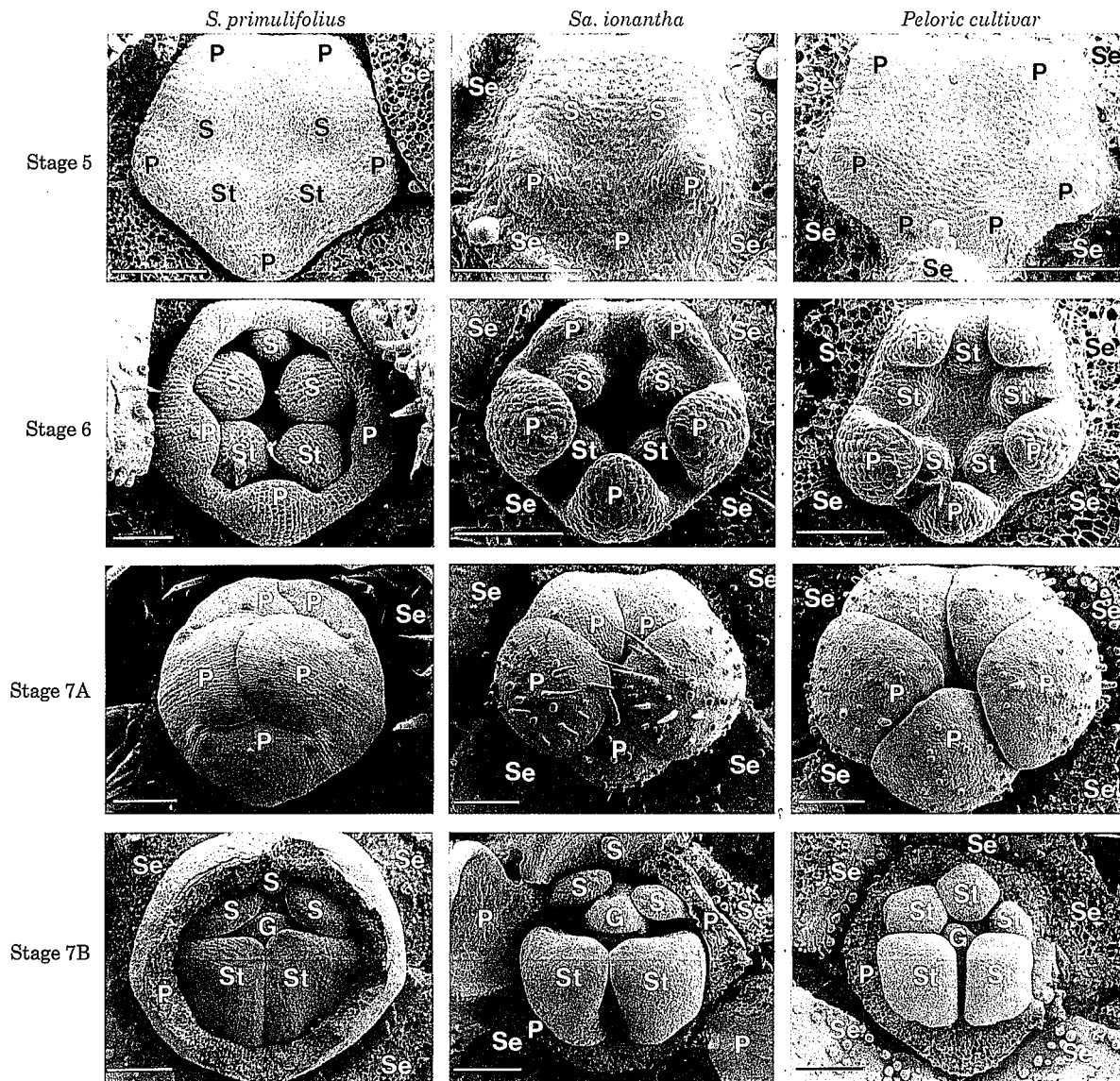


FIG. 3. Floral organogenesis in *S. primulifolius*, *Sa. ionantha* and a *Saintpaulia* peloric cultivar (SEM micrographs). Floral meristems are denoted by FM, and developing bracts, sepals, petals, stamens, staminodes, and gynoecium by B, Se, P, St, S, and G, respectively. Bars (Stages 1–6) = 100  $\mu$ m, (Stage 7A and B) = 200  $\mu$ m. Seven stages of floral development are shown: (1) dome: the floral meristem comprises a hemispherical dome; (2) pentagon: five-fold symmetry is established, although no floral primordia are visible; (3) the sepals form as bulges at the points of the pentagon; (4) the sepals grow, while the floral meristem remains undifferentiated; (5) the corolla and androecium initiate simultaneously; (6) the corolla and androecium grow, and the gynoecium initiates; and (7) A, the corolla grows, enfolding the inner whorls, and becomes hirsute; B, at the same time, the androecium and gynoecium grow and differentiate. The main differences between species are in size (*S. primulifolius* is larger than *Sa. ionantha* from stage 1), corolla tube establishment (in *S. primulifolius* the corolla initiates as a tube, whereas in *Sa. ionantha* the petals initiate separately), and zygomorphy (*S. primulifolius* is less strongly zygomorphic than *Sa. ionantha*). The main differences between *Sa. ionantha* and the peloric cultivar are in organ number (the cultivar sometimes has a sixth sepal and petal: stage 5 onwards), and in zygomorphy (the cultivar is usually actinomorphic: stage 5 onwards).

*primulifolius*. The protection normally provided by bracts in caulescent *Streptocarpus* may be transferred to the leaves in acaulescent *Streptocarpus*, allowing flexibility in their growth; if the bracts are redundant, their cells are available for recruitment by floral meristems (Coen and Nugent, 1994), which could account for the accelerated floral development of *S. primulifolius* in comparison to *Sa. ionantha*.

Another notable difference between the two species examined here was that *Sa. ionantha* had much better

developed sepals than *S. primulifolius* at the early stages of floral development. In *Sa. ionantha* the sepals completely enclose the floral meristem before the inner floral organs initiate. In *S. primulifolius* the inner floral organs initiated before the floral meristems were enclosed by the sepals (Fig. 3D). It may be that the protection provided by the bracts and sepals in *Sa. ionantha* has been assumed by the phyllomorphs themselves in *S. primulifolius*, and possibly in other species of subgenus *Streptocarpus*. If this is the case then the favourable environment in which the inflorescence

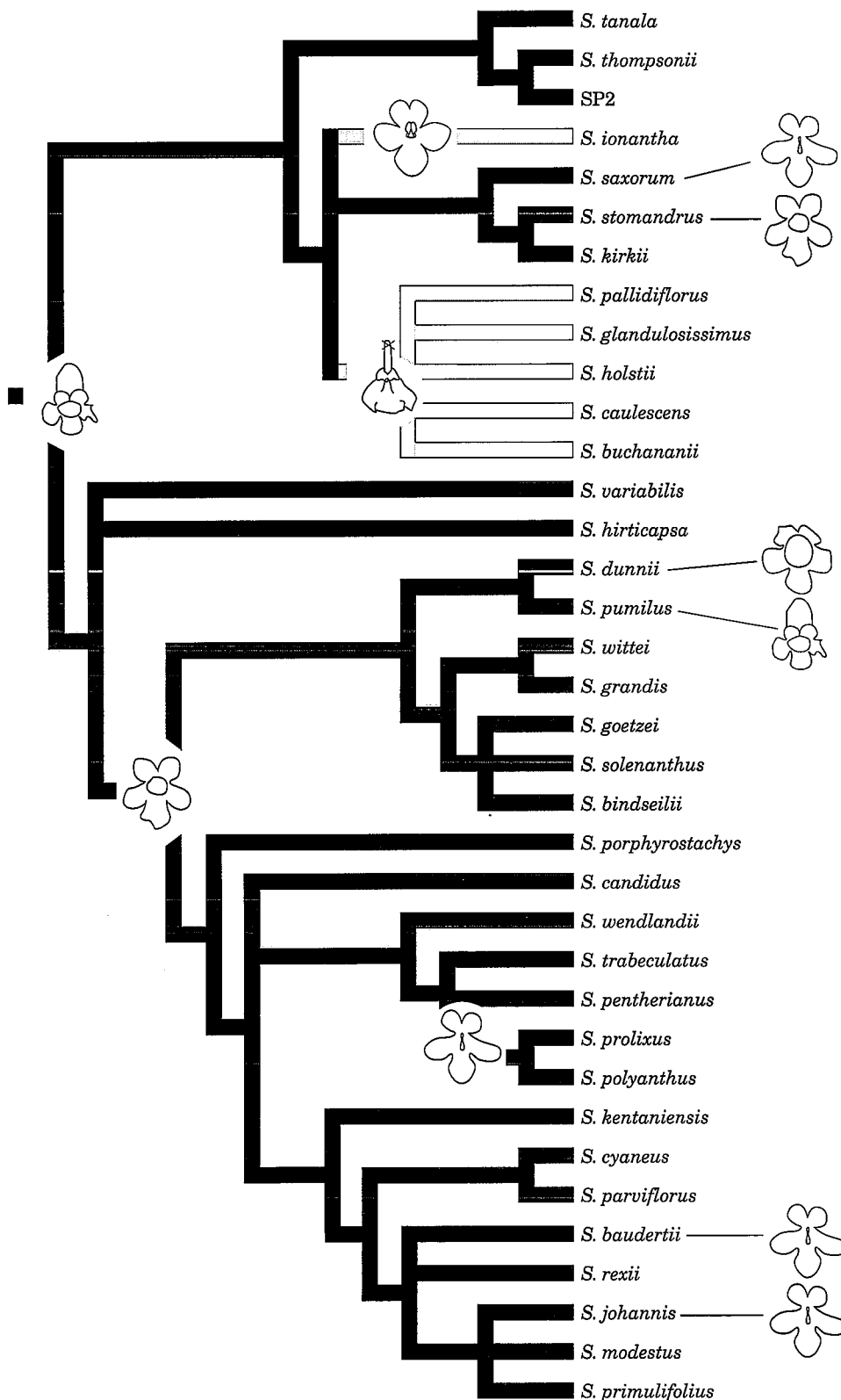


FIG. 4. Molecular phylogeny of *Streptocarpus* based on ITS sequence data, showing the evolution of enantiostyly. Enantiostyly had two origins in *Saintpaulia* and the personate species (◻). Reciprocal enantiostyly evolved from enantiostyly in the personate group (◻). The evolution of the open-tubed, keyhole, personate, *Saintpaulia*, and *dunnii* floral types from the pouch type is also shown. The open-tubed type has two independent origins (African acaulescent clade; *S. stomandrus*), the keyhole type has four independent origins (*S. saxorum*; *S. prolixus*, *S. polyanthus* and *S. pentherianus*; *S. baudertii*; *S. johannis*), and there is one reversal to pouch type in *S. pumilus*.

arises in subgenus *Streptocarpus*, coupled with the release of functional constraint on the bracts and sepals could explain how subgenus *Streptocarpus* has been able to attain its larger floral size.

As well as offering some insight into floral size, the developmental part of this study offered clues as to how and when floral shape might be altered. The first of these was shown by comparison of the early development of *S. primulifolius* with that of *Sa. ionantha*. At anthesis in *Streptocarpus* the corolla tube is pronounced, and encloses the anthers. The corolla initiates as a ring (i.e. sympetaly is early), previously unrecorded in the Gesneriaceae. In *Saintpaulia* the corolla tube is reduced and the anthers are shielded by the petal lobes. The corolla lobes initiate before the corolla tube forms (late sympetaly). The occurrence of early and late sympetaly in such closely related taxa suggests that sympetaly is more evolutionarily labile than previously suggested (Erbar, 1991; Erbar and Leins, 1996). Phylogenetic mapping shows the reduction of the corolla tube in *Saintpaulia* to be an evolutionary loss, which may result from a simple switch in the timing of initiation of the corolla tube.

Important aspects of development involved in determining shape were also shown by the comparison of *Saintpaulia ionantha* to the peloric cultivar. The occasional establishment of a sixth floral organ is shown from stage 3 of floral development, and the absence or reduction of zygomorphy is shown from stage 5 of floral development.

Each of these examples illustrates the importance of small changes during early floral ontogeny in altering morphology at anthesis. How these small changes are effected remains unknown. Analysis of loss of function mutants like *CYCLOIDEA* has revealed some of the genes that affect floral shape in *Antirrhinum*. Loss of function phenotypes can be severe. To understand the evolution of floral form on a mechanistic level, it will be necessary to look at the behaviour of alleles involved in producing natural variance. The genetic changes that give rise to a more natural array of variation remain unknown. One important process by which small changes in development can produce large phenotype effects is heterochrony—the alteration in timing of developmental processes (Bateman, 1994; Mosbrugger, 1995; Bowman, 1997). This change in the behaviour of genes may be effected by their transcriptional regulators, and is a potentially crucial process behind the generation of diversity (Doebley and Lukens, 1998; Stern, 1998; Wang et al., 1999). It is very apparent from this work that these changes need only be minute at the early developmental level to generate considerable diversity at anthesis.

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