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Intrafloral differentiation of stamens in heterantherous flowers

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Abstract Flowers that have heteromorphic stamens (heterantherous flowers) have intrigued many researchers ever since the phenomenon was discovered in the 19th century. The morphological differentiation in androecia has been suggested as a reflection of "labor division" in pollination in which one type of stamens attracts pollinators and satisfies their demand for pollen as food and the other satisfies the plant's need for safe gamete dispersal. The extent and patterns of stamen differentiation differ notably among taxa with heterantherous flowers. Seven species with heteromorphic stamens in three genera were sampled from Leguminosae and Melastomataceae, and the morphological difference of androecia, pollen content, pollen histochemistry and viability, pollen micromorphology, as well as the main pollinators were examined and compared. Pollen number differs significantly between stamen sets of the same flower in most species investigated, and a correlation of pollen number and anther size was substantiated. Higher pollen viabilities were found in the long (pollinating) stamens of *Senna alata* (L.) Roxb. and *S. bicapsularis* (L.) Roxb. Dimorphic pollen exine ornamentation is reported here for the first time in *Fordiophyton faberi* Stapf. The height of stigma and anther tips of the long stamens in natural conditions was proved to be highly correlated, supporting the hypothesis that they contact similar areas of the pollinator's body.

Key words buzz pollination, heteranthery, labor division, pollen micro-morphology, pollen number, pollination, stamen differentiation.

Stamens are the male reproductive organs of flowering plants with the primary functions of pollen production and presentation (Walker-Larsen & Harder, 2000; Scott et al., 2004). Approximately 20,000 species of flowering plants that are adapted to pollination by bees offer only pollen as a reward for pollinators. These plants often face a dilemma that they need to preserve enough pollen for fertilization while maintaining their attractiveness to bees. Heteranthery, the presence in a flower of stamens that differ notably in size, colors and/or shape, and presumably with differentiation of labor (function), is widespread among such pollen-offering flowers (Buchmann, 1983). This phenomenon was first described more than one hundred years ago (Darwin, 1862; H. Müller, 1881, 1882; F. Müller, 1883). Darwin (1862) hypothesized that it reflects a "division of labor" among stamens: one set satisfies the pollinators' demand for pollen as food (the "feeding" stamens), the other the plant's need for safe gamete transport (the "pollinating" stamens). Fritz Müller, a contemporary and major supporter of Darwin who presented the "number-dependent" model of mimicry ("Müllerian mimicry"), found that a species of *Centradenia* G. Don (as "*Heeria* Meisn." , Melastomataceae) in Brazil had two kinds of stamens with different color and length (H. Müller, 1881). After that, other examples of such species with heterantherous flowers were reported, including *Tradescantia virginica* L., *Tinantia undata* Schlecht., *Commelina coelestis* Willd. (as "*Commelyna* Endl.") and *C. communis* L. (Commelinaceae), the lythraceous genera *Lagerstroemia* L. and *Lythrum* L., *Melastoma* L. (Melastomataceae), *Solanum rostratum* Dunal. (Solanaceae), *Chamaecrista fasciculata* (Michx.) Greene (as *Cassia chamaecrista* L.) and *Senna marilandica* (L.) Link (as *Cassia marilandica* L., Caesalpiniaceae) (H. Müller, 1882; Todd, 1882; F. Müller, 1883; Meehan, 1886).

Although the phenomenon was noticed early, few experimental studies concerning heteranthery had been carried out. Early studies were mainly limited to descriptions and field observations (e.g., H. Müller, 1881, 1882; Todd, 1882; F. Müller, 1883; Meehan, 1886). Lee (1961) examined pollen from the dimorphic stamens of *Tripogandra grandiflora* (Donn. Sm.) Woodson (Commelinaceae) and found several differences between the two kinds of pollen grains. Mori and Orchard (1980) found that pollen grains from different stamen sets of *Lecythis pisonis* Cambess. and *Couroupita guianensis* Aubl. (Lecythidaceae) differed

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in *in vitro* germination, and suggested that this differentiation had evolved in response to different functions. Dulberger (1981) studied the differentiation of androecia in *Senna didymobotrya* (Fresen.) H. S. Irwin & Barneby and *S. auriculata* (L.) Roxb. (as *Cassia*), and found significant difference in stamen morphology and pollen content between different stamen types. Bahadur et al. (1991) examined the pollen microcharacters of seven *Senna* (as *Cassia*) species with the aid of SEM, and discussed their relations to enantiostyly and heteranthery. Marazzi et al. (2007) and Marazzi and Endress (2008) studied the floral structure of 69 *Senna* species of the major clades retrieved by Marazzi et al. (2006). These researches, concentrating on single species or species of a single genus, however, lacked inter-family comparison and integrated examinations.

In this study, seven species with heterantherous flowers from *Senna* (Leguminosae), *Melastoma* and *Fordiophyton* (Melastomataceae) were sampled, all of which have poricidal anthers and offer pollen as the only reward for their pollinators. *Senna*, with the greatest androecial diversity among Cassiinae (Venkatesh, 1957; Lasseigne, 1979), is a prominent genus in Leguminosae for its unique androecial patterns and poricidal anthers, which have intrigued botanists for a long time (Forbes, 1882; Todd, 1882; F. Müller, 1883). *Melastoma* includes a large group of species with dimorphic stamens in Melastomataceae. The pollination biology and breeding system in relation to heteranthery of *M. affine* D. Don have been studied by Gross (1993) and Gross and Kukuk (2001). *Fordiophyton* is a small herbaceous genus with 9 species, all of which endemic to China and Vietnam (Chen & Renner, 2007).

We compare pollen number, histochemistry, viability, micro-morphology and the morphological characters of differentiated stamens in the seven species, aiming to examine the correlations among these characters, and use these correlations, which occur in phylogenetically unrelated families, as a first step to explore the functional and evolutionary significance of heteranthery.

1 Material and methods

1.1 Material

Seven species with heteromorphic stamens were sampled from three genera of Leguminosae and Melastomataceae (Table 1). All species were sampled from the South China Botanical Garden (SCBG), a 300-ha nature reserve in Guangzhou, except for *Fordiophyton faberi*, which was sampled from Dadongshan Mountain, Nanling National Nature Reserve, all in Guangdong Province.

1.2 Methods

Pollen quantification, histochemistry and micro-morphology, stamen morphology and anther tip structure were studied on all the species sampled, while pollen viability has been tested for all species except *Senna siamea* and *Fordiophyton faberi*.

1.2.1 Floral visitors Floral visitors were observed, photographed, and collected for later identification. Observations were carried out in fine days at the peak flowering period of the study species.

1.2.2 Measurements of floral parts The length and width of androecial and gynoecial organs were measured by a digital caliper (Mitutoyo, Japan). Ten flowers from five individuals of each species were bagged before anthesis to prevent pollen loss. When fully opened, the flowers were collected and numbered. For each flower, one stamen of each type was selected randomly for measurement. Anther length was measured from anther tip to the joint with filament (for Melastomataceae species, only the anther sacs were measured, not including the elongated connectives). Anther width was measured in the

Table 1 Taxa used in this study, stamen differentiation and voucher information

Taxon	Stamen differentiation	Locality	Voucher
Leguminosae			
Senna alata (L.) Roxb. (Se, II)*	long, mid, short, stn.**	Guangzhou, Guangdong	Z. L. Luo 52 (IBSC)
S. bicapsularis (L.) Roxb. (Ch, VIIa)	long, mid, short, stn.	Guangzhou, Guangdong	Z. L. Luo 70 (IBSC)
S. siamea (Lam.) H. S. Irwin & Barneby (Ch, I)	long, mid, short, stn.	Guangzhou, Guangdong	Z. L. Luo 63 (IBSC)
S. surattensis (Burm. f.) H. S. Irwin & Barneby (Ps, IV)	long, short	Guangzhou, Guangdong	Z. L. Luo 69 (IBSC)
Melastomataceae			
Melastoma dodecandrum Lour.	long, short	Guangzhou, Guangdong	Z. L. Luo 49 (IBSC)
M. sanguineum Sims	long, short	Guangzhou, Guangdong	Z. L. Luo 46 (IBSC)
Fordiophyton faberi Stapf	long, short	Lianzhou, Guangdong	L. Gu 12 (IBSC)

* Sections of *Senna* by Irwin and Barneby (1982) and clades by Marazzi et al. (2006) (Ch, *Chamaefistula* (DC.) G. Don; Ps, *Psilorhegma*; Se, *Senna*).
** Stn., Staminodes.

middle of anthers. The heights of stamens in natural positions were determined by measuring from the anther tip to the receptacle. The position of stigmas was measured in the same way. After measurement the anthers were placed separately in numbered calibrated tubes filled with FAA (formalin-acetic acid-alcohol) and kept in refrigerator for pollen quantification (see below).

1.2.3 Quantification of pollen grains The pollen grain number in single anthers was determined following the techniques of Cruden (1977) and Wang et al. (2004). One anther from each bud was carefully dissected and then crushed in a calibrated tube; the staining solution (a 1:3 mixture of glycerin and aniline-blue in lactophenol) was added to the tube up to 1 mL. The suspension was stirred with a vortex mixer for 60 s, and then 10 separate samples of 1 μL each were transferred onto slides by an Eppendorf transferpettor (Eppendorf, Germany). The number of pollen grains in each sample was counted under a microscope (Olympus BX-41; Olympus, Japan), and this number was multiplied by the dilution factor (1000) to get the total number of pollen in one anther. Pollen grain numbers from different stamen sets were calculated separately.

1.2.4 Pollen histochemistry The types of pollen reserves (starch or lipids) were considered to reflect the nutritional needs of pollinators, as well as the pollination modes of plants (Baker & Baker, 1979; Dafni, 1992). Mature pollen from dehiscing anthers in freshly-opened flowers was collected, and stained with a drop of IKI ("iodine in aqueous potassium iodide", Baker & Baker, 1979) solution or a drop of Sudan IV solution, and examined under a microscope. Pollen samples from different stamen sets were tested separately. A brown color in IKI-tests indicates the presence of starch in pollen grains, and a red color in Sudan IV-tests indicates the presence of lipids (Dafni, 1992).

1.2.5 Pollen viability MTT (dimethylthiazol-diphenyltetrazolium bromide) was used to test for the presence of dehydrogenase in pollen as an assay for pollen viability (Rodriguez-Riaño & Dafni, 2000; Dafni, 2001). The pollen grain was considered viable if it turned purple or dark pink. At least 200 grains were calculated each time. For each species, tests were performed every 4–6 h from anthesis to flower wilting. Ten flowers from 3–5 individuals were used for each test. The tests were carried out in warm and sunny days to prevent the effects of rain on pollen viability.

1.2.6 Micro-morphology of anther tips and pollen grains Fully-developed flower buds just before anthesis were collected and fixed in 4% glutaraldehyde solution, vacuumed for 2 h, and stored in a refrigerator. Anther tip materials were rinsed in 0.1 mol/L phosphate saline buffer (PSB), postfixed in 1% buffered osmium tetroxide (OsO_4) for 3 h, then rinsed in 0.1 mol/L PSB and dehydrated through an ethanol series. Tert-butyl alcohol was used to replace the ethanol before freeze-dried in a freeze drying device (JFD-310, JEOL, Japan). Pollen samples were dehydrated through an ethanol series. Samples were observed with the SEM (JSM-6360LV; JEOL, Japan) after being coated with gold-palladium in a sputter coater (JFC-1600; JEOL, Japan). Images were digitally recorded. Pollen description and terminology followed Erdtman (1952) and Faegri and Iversen (1964).

1.2.7 Data analysis One-way ANOVA test (when flowers have 3 fertile stamen sets) or *t*-test (when flowers have only 2 stamen sets) was used respectively to compare the morphological differentiations between different stamen sets, the positions of anther tips and stigma, pollen contents, pollen viability, as well as the palynological characters of pollen grains. Pearson correlation coefficients were calculated for stamen heights and stigma positions, as well as pollen contents and anther sizes.

2 Results

2.1 Morphology and differentiation of androecia

The extent and patterns of differentiation in the androecia differed among the species investigated, even in the same genus (*Senna*) (Table 2; Fig. 1: A, B, D, E). In *S. alata*, *S. bicapsulari*s and *S. siamea* (Lam.) H. S. Irwin & Barneby, the androecia have four types of stamens with notable morphological differentiations. The three adaxial stamens (staminodes) are sterile and flattened, producing no pollen (Fig. 2: D, H, L). At the center of the flower there are four short stamens with erect filaments and anthers, which have usually been suggested as "feeding" stamens with the function of providing pollen as "food for larvae". The three abaxial stamens ("pollinating" stamens) often form two sets. The two lateral abaxial stamens belong to the inner androecial whorl, while the median abaxial stamen belongs to the outer one. The two lateral stamens in *S. alata* have stout filaments with large, curved anthers, while the third centric stamen has a much smaller anther but slightly longer filament (Fig. 1: A). All the anthers and filaments are light yellow. In *S. bicapsularis*, the two lateral stamens have long, curved filaments and brown

Table 2 Floral characters, pollen number, pollen histochemistry and pollen viability of investigated species

Taxon	Stamen sets	Flower sizes (mm)	Pollen number per anther	Pollen number per flower	Sizes of anther sacs (mm)		Height of stamens (mm)	Height of stigmas (mm)	Pollen histochemistry reactions*		Pollen viability (%)	
					Length	Width			Starch	Lipid	Ant hesis	Flower wilting
Senna alata	L	20.1 ± 0.36	416,613 ± 9,196	833,227± 18,392	9.68 ± 0.06	2.62 ± 0.05	13.7 ± 0.10	14.8 ± 0.17	+	+++	76.9 ± 1.69	35.1 ± 1.41
	M		27,715 ± 2,400	27,715 ± 2,400	4.16 ± 0.07	1.54 ± 0.03	11.3 ± 0.10		+	++	39.2 ± 1.89	11.2 ± 0.64
	S		19,734 ± 1,194	78,937 ± 4,777	3.84 ± 0.05	1.50 ± 0.03	6.84 ± 0.08		+	++	19.4 ± 0.97	6.24 ± 0.27
S. bicapsularis	L	32.2 ± 0.40	78,986 ± 2,538	157,972 ± 5,075	9.33 ± 0.09	1.65 ± 0.03	17.6 ± 0.21	17.2 ± 0.25	+	+	68.7 ± 1.73	43.0 ± 0.18
	M		25,392 ± 988	25,392 ± 988	7.74 ± 0.06	1.13 ± 0.02	10.4 ± 0.10		+	+	42.4 ± 1.25	12.2 ± 0.33
	S		27,533 ± 1,072	110,133 ± 4,289	6.11 ± 0.09	1.58 ± 0.07	8.20 ± 0.02		+	+	33.4 ± 0.74	4.08 ± 0.61
S. siamea	L	21.4 ± 0.33	28,402 ± 461	56,805 ± 922	6.43 ± 0.12	1.81 ± 0.03	14.7 ± 0.24	14.6 ± 0.19	+	++	n.t. **	n.t.
	M		16,370 ± 663	16,370 ± 663	5.39 ± 0.03	1.81 ± 0.03	9.46 ± 0.06		+	++	n.t.	n.t.
	S		19687 ± 924	78,747 ± 3,697	5.28 ± 0.02	1.89 ± 0.02	9.29 ± 0.07		+	+	n.t.	n.t.
S. surattensis	L	36.2 ± 0.72	18,861 ± 774	37,722 ± 1,550	5.58 ± 0.07	1.36 ± 0.02	9.20 ± 0.09	10.5 ± 0.14	++	++	65.9 ± 1.85	8.02 ± 0.56
	S		18,534 ± 596	148,273 ± 4,767	5.36 ± 0.06	1.32 ± 0.01	7.50 ± 0.09		++	++	61.8 ± 1.96	6.13 ± 0.61
Melastoma dodecandrum	L	29.9 ± 0.85	40,724 ± 2,197	203,619 ± 10,984	5.30 ± 0.17	0.51 ± 0.03	16.1 ± 0.49	12.9 ± 0.22	+	+	72.4 ± 2.67	4.77 ± 1.19
	S		16,752 ± 1,295	83,758 ± 6,475	4.94 ± 0.09	0.39 ± 0.02	11.5 ± 0.19		+	–	66.5 ± 2.30	4.84 ± 1.66
M. sanguineum	L	82.6 ± 1.73	233,952 ± 5,199	1,169,762 ± 25,994***	14.5 ± 0.25	2.19 ± 0.03	32.5 ± 0.30	28.5 ± 0.25	+	+	57.3 ± 3.66	11.7 ± 1.17
	S		136,083 ± 2,370	680,416 ± 11,850	12.1 ± 0.22	1.75 ± 0.03	21.4 ± 0.21		+	+	54.7 ± 3.30	9.66 ± 0.87
Fordiophyton faberi	L	25.6 ± 0.54	374,826 ± 10,427	1,874,130 ± 52,133	16.3 ± 0.28	0.59 ± 0.02	19.2 ± 0.45	18.4 ± 0.34	+	+	n.t.	n.t.
	S		45,489 ± 2,114	227,447 ± 10,569	4.55 ± 0.10	0.41 ± 0.01	13.4 ± 0.22		+	+	n.t.	n.t.

* Pollen histochemistry reactions: –, negative; +, positive; ++, strong; +++, very strong. ** n.t., not tested. *** In pentamerous flowers

anthers, while the third stamen is centric and lowermost, with shorter filament and a brown anther. The four short stamens have showy yellow anthers, and two dark furrows are obvious on the lateral surfaces of each anther (Fig. 1: B). Length of the stamens of the three-stamen set differed significantly (F=1333.2, P<0.001). Anthers of the three fertile stamen sets in *S. siamea* are similar in color while differ significantly in length (F=70.6, P<0.001) but not in width (F=2.20, P=0.14). Filaments of the two lateral stamens do not curve obviously. The androecium of *S. surattensis* (Burm. f.) H. S. Irwin & Barneby does not have obvious differentiations. Anthers of the two-stamen set are subequal in length (t=2.45, P=0.026) and width (t=1.97, P=0.066), while the two abaxial stamens are longer (t=12.9, P<0.001) and deviated from the center as a result of the slightly elongated filaments. The remaining stamens are aggregated, forming a "cone" in the center of the flower (Fig. 1: E).

In the species of Melastomataceae investigated, the androecium consists of two whorls of stamens. The outer stamens (usually called as "pollinating" stamens) are longer, opposite the sepals (antesepalous stamens), while the inner ones (usually called as "feeding" stamens) are shorter, crowded in the center of the flower and opposite the petals (antepetalous stamens). The five (sometimes seven) antesepalous stamens of *Melastoma sanguineum* Sims have pale purple anthers with elongated connectives and yellow connective spurs. The five (or seven) antepetalous stamens are much shorter, with straight, bright yellow anthers, and are positioned in the flower center (Fig. 1: G). Anthers from the long and short stamens differ significantly in both length (t=7.16, P<0.001) and width (t=10.7, P<0.001). *Melastoma dodecandrum* Lour. has similar androecial structure as *M. sanguineum*, but much smaller in size (Fig. 1: F; Table 2). Anthers from the two stamen sets are similar in length

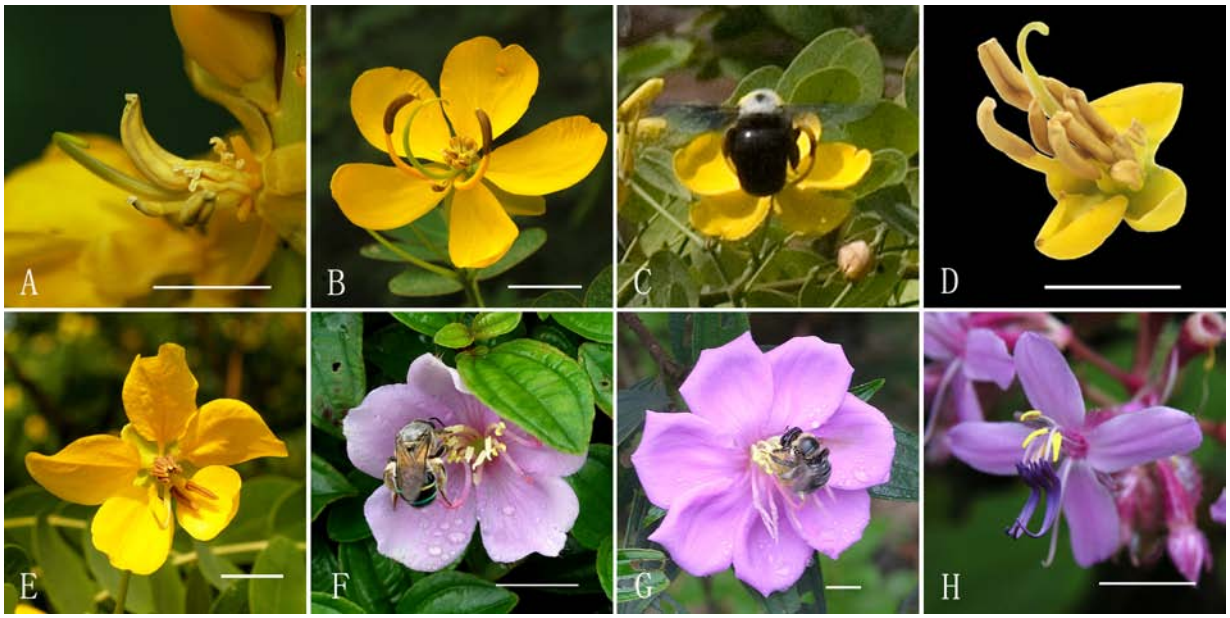

Fig. 1. Heterantherous flowers of the investigated species, showing differentiations in androecia. **A,** *Senna alata*. **B,** *S. bicapsularis*. **C,** A *Xylocopa* bee foraging on a flower of *S. bicapsularis*. **D,** *S. siamea*. **E,** *S. surattensis*. **F,** *Melastoma dodecandrum* (*Amegilla zonata* is buzzing the anthers of the short stamens). **G,** *M. sanguineum* (a *Xylocopa collaris* is buzzing the anthers of the short stamens). **H,** *Fordiophyton faberi*. Scale bars=10 mm.

(t=1.87, P=0.09), while anthers of the long stamens are wider (t=3.40, P<0.01). The flowers of *Fordiophyton faberi* are 4-merous with 8 stamens (Fig. 1: H). The four antesepalous stamens are longer and purplish red in color, with linear, curved anthers, the base of which is elongated, forming a forked spur. The four antepetalous stamens are shorter, with bright yellow, oblong anthers, and the anther base is obtuse. Significant differences exist in both anther length (t=38.9, P<0.001) and width (t=7.34, P<0.001).

In contrast to the morphological differentiation in the androecia of heterantherous species, anther dehiscence patterns (dehiscence by slits or pores) exhibit little variations in the same flower. In *Senna alata*, anthers dehisce through two separate apical slits. The lateral furrow is not continuous on the theca of long stamens, which does not reach the slits but runs only along the sides of thecae between the pollen sacs (Fig. 2: A). But on the mid and short stamens, the lateral furrow is continuous, stretching from the lower end of the slit down along the theca (Fig. 2: B, C). The two dehiscence slits of the anther in *S. bicapsularis* are confluent into a U-shaped pore. The lateral furrow is absent on the thecae of the long and mid stamens (Fig. 2: E, F). On the thecae of the short stamens, however, two furrows with coarsely-serrated edges are conspicuous. The furrow runs along the theca and is connected to the end of the slit (Fig. 2: G). Anther apical slits of *S. siamea* are also confluent, and no significant differences could be observed among the three stamen sets. No lateral furrows are present on the thecae (Fig. 2: I–K). *Senna surattensis* has narrow and distantly separated anther slits in both stamen sets, which are connected with the lateral furrows (Fig. 2: M, N). Anther dehiscence pores of *Melastoma dodecandrum* and *M. sanguineum* are confluent, forming a suborbicular pore (Fig. 2: O–R). The pollen sac wall of the short stamen in *M. dodecandrum* is slightly coarser than that of the long stamen (Fig. 2: O, P). Anthers of *Fordiophyton faberi* dehisce by single pores on the tips, which are small and round. Tissues around the anther pores of long stamens slightly protrude, forming a crater-shaped protuberance (Fig. 2: S). This structure was not observed on the anther tips of short stamens (Fig. 2: T).

In all species investigated (except for *Senna surattensis*, whose androecia have little differentiation), style and the long stamens are extended and curved in similar patterns (Fig. 1: A, B, D–H). The calculation of Pearson correlation coefficients showed that heights of stigma and the anther tips of long stamens in a flower were moderately or strongly correlated, except that in *Melastoma dodecandrum* the stigma height was correlated with the position of anther tips of short

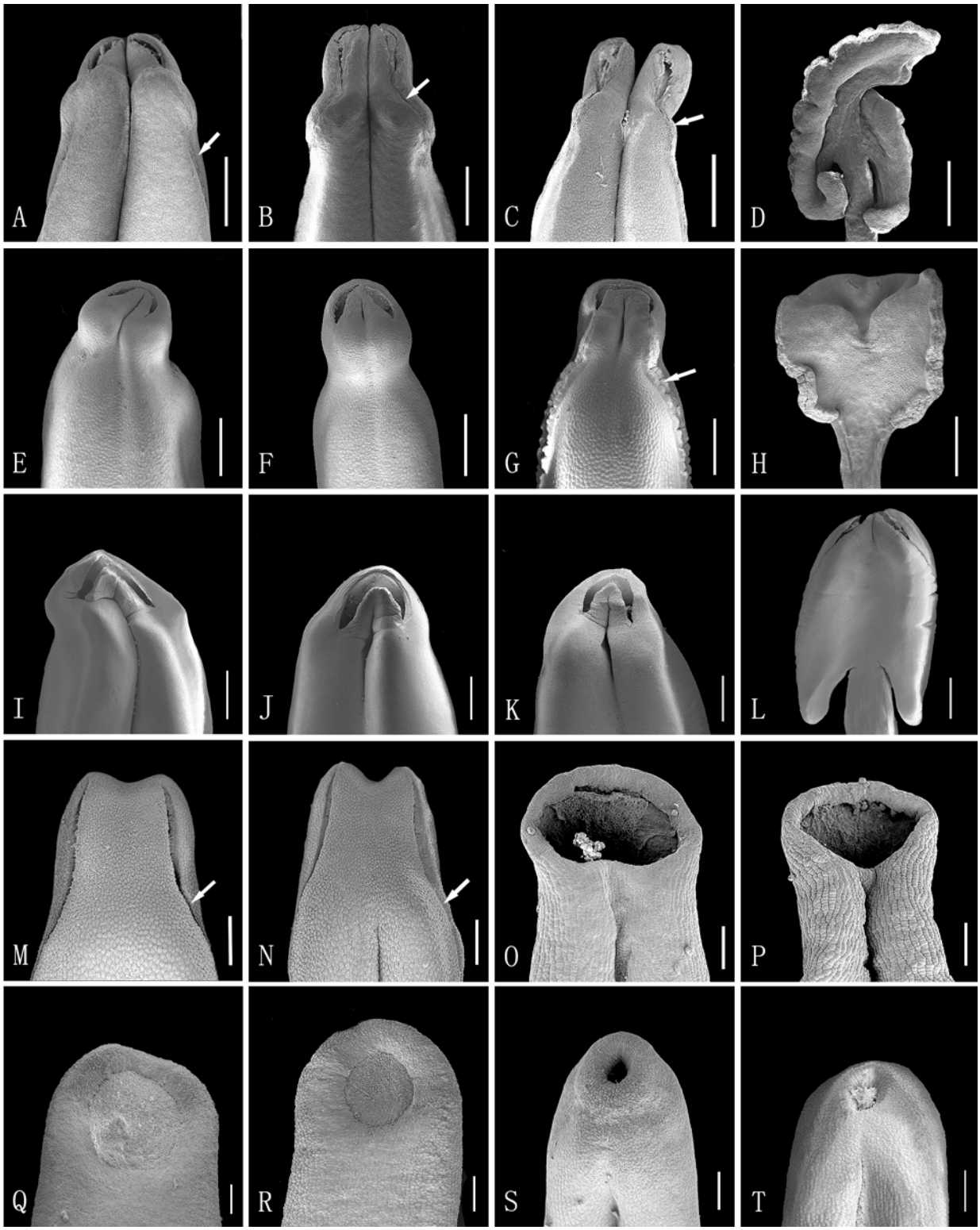

Fig. 2. Anther tips and staminodes of the investigated species. **A–D,** *Senna alata*. **A,** Long stamen. **B,** Mid stamen. **C,** Short stamen. **D,** Staminode. **E–H,** *S. bicapsularis*. **E,** Long stamen. **F,** Mid stamen. **G,** Short stamen. **H,** Staminode. **I–L,** *S. siamea*. **I,** Long stamen. **J,** Mid stamen. **K,** Short stamen. **L,** Staminode. **M, N,** *S. surattensis*. **M,** Long stamen. **N,** Short stamen. **O, P,** *Melastoma dodecandrum*. **O,** Long stamen. **P,** Short stamen. **Q, R,** *M. sanguineum*. **Q,** Long stamen. **R,** Short stamen. **S, T,** *Fordiophyton faberi*. **S,** Long stamen. **T,** Short stamen. Arrows indicate lateral furrows. Scale bars: 500 μm in A–L, 200 μm in M, N, 100 μm in O–T.

Table 3 Pearson correlation coefficients (r) between pollen contents and anther sizes, as well as stamen heights and stigma positions

Taxon	Pollen no. vs. anther size		Stamen height vs. stigma height		
	Anther length	Anther width	L	M	S
Senna alata	0.99**	0.98**	0.63*	0.22	0.06
S. bicapsularis	0.83**	0.61*	0.95**	0.25	0.57
S. siamea	0.83**	0.16	0.77*	0.17	0.57
S. surattensis	0.23	0.08	0.67*	–	0.53
Melastoma dodecandrum	0.61*	0.72**	0.61	–	0.77*
M. sanguineum	0.82**	0.90**	0.83*	–	0.28
Fordiophyton faberi	0.99**	0.93**	0.93**	–	0.27

* Correlation is significant at the 0.05 level. ** Correlation is significant at the 0.01 level.

stamens (Table 3).

2.2 Behavior of buzzing bees

At our observation sites, carpenter bees (*Xylocopa* spp.) were the principal floral visitors on the flowers of all four *Senna* species. When foraging on *S. bicapsularis*, these large bees usually landed on the four short stamens that are situated at the center of the flower, grasped them with their prothoracic and mesothoracic limbs and mandibles, and buzzed the anthers for a few seconds (usually 1–2 s) (Fig. 1: C). Vibration was then transmitted to the long stamens, and a small "pollen cloud" was noticeably ejected from the anther pores, covering the dorsal surface of the bee's abdomen. Meanwhile, the stigma, which had similar curving pattern with the long stamen, touched the bee's backs that had been covered with pollen. Anther of the mid stamen also touched the dorsal surface of the bee's abdomen. The bees flew very quickly when foraging on *Senna* flowers. A unique high-pitch buzzing sound could be heard clearly when the bees were harvesting pollen from the short stamens. Similar pollen-collecting behavior was observed when the bees foraged on the flowers of other species in *Senna*. On flowers of *S. surattensis*, however, bees often buzzed all the anthers instead of only harvesting pollen from the short ones.

The main pollinators of *Melastoma sanguineum* were *Xylocopa* at our study sites. The bees exclusively alighted on the yellow feeding stamens, grasped the anthers together with the connectives of pollinating stamens, and buzzed for 2–3 s (Fig. 1: G). They also milked the "feeding" anthers with their mandibles, which produced dark necrotic marks on anther sacs. During buzzing bouts, tips of the "pollinating" stamens touched the bees' lateral sides and back of the abdomens, while the tips of the "feeding" stamens were placed close to the bees' mouthparts. Clouds of pollen grains were emitted from the pores of the "pollinating" anthers and landing on the ventral and dorsal abdomen, which was the part that touched the stigma.

Amegilla zonata was the primary floral visitor of *Melastoma dodecandrum* (Fig. 1: F). Different from the large carpenter bees, *Amegilla zonata* buzzed the anthers of both the long and short stamens. They landed on the short stamens in the centre of a flower, and buzzed the anthers. When landing on the long stamens, the bees usually curled their bodies, gripped the anther with their limbs, and buzzed for a few seconds. When the short stamens were buzzed, the stigma often touched the bee's body, either the dorsal or ventral surface of the abdomen. However, when single (occasionally two) long stamens were buzzed, the stigma rarely touched the bee's body.

2.3 Pollen number, histochemistry and viability

The number of pollen grains from different stamen sets of the investigated species is compared in Table 2. Pollen quantity varied among stamen types and associated with anther sizes. In all species investigated, the anther of the single long stamen ("pollinating" anther) contains more pollen grains than the anther of the mid and (or) short stamen. The two long stamens contain many more pollen in total than the four short ones in *Senna alata* (t=39.7, P<0.001) and *S. bicapsularis* (t=7.20, P<0.001), while a larger proportion of pollen is present in the four short stamens of *S. siamea*. Pollen grain number and anther length are strongly correlated in most *Senna* species, except that in *S. surattensis* the correlation is not significant (n=10, r=0.23, P=0.42). Pollen number and anther width are only correlated in *Senna alata* (n=10, r=0.98, P<0.001). In *Melastoma dodecandrum*, *M. sanguineum*, and *Fordiophyton faberi*, pollen grain number correlates moderately or strongly with both anther length and width (Table 3).

The results of pollen histochemistry tests are presented in Table 2. Pollen grains from all fertile stamen sets of most investigated species contain both starch and lipid, except that no lipid was detected in pollen from the short stamens of *Melastoma dodecandrum*. In *Melastoma sanguineum* and *Fordiophyton faberi*, pollen grains from the long and short stamens

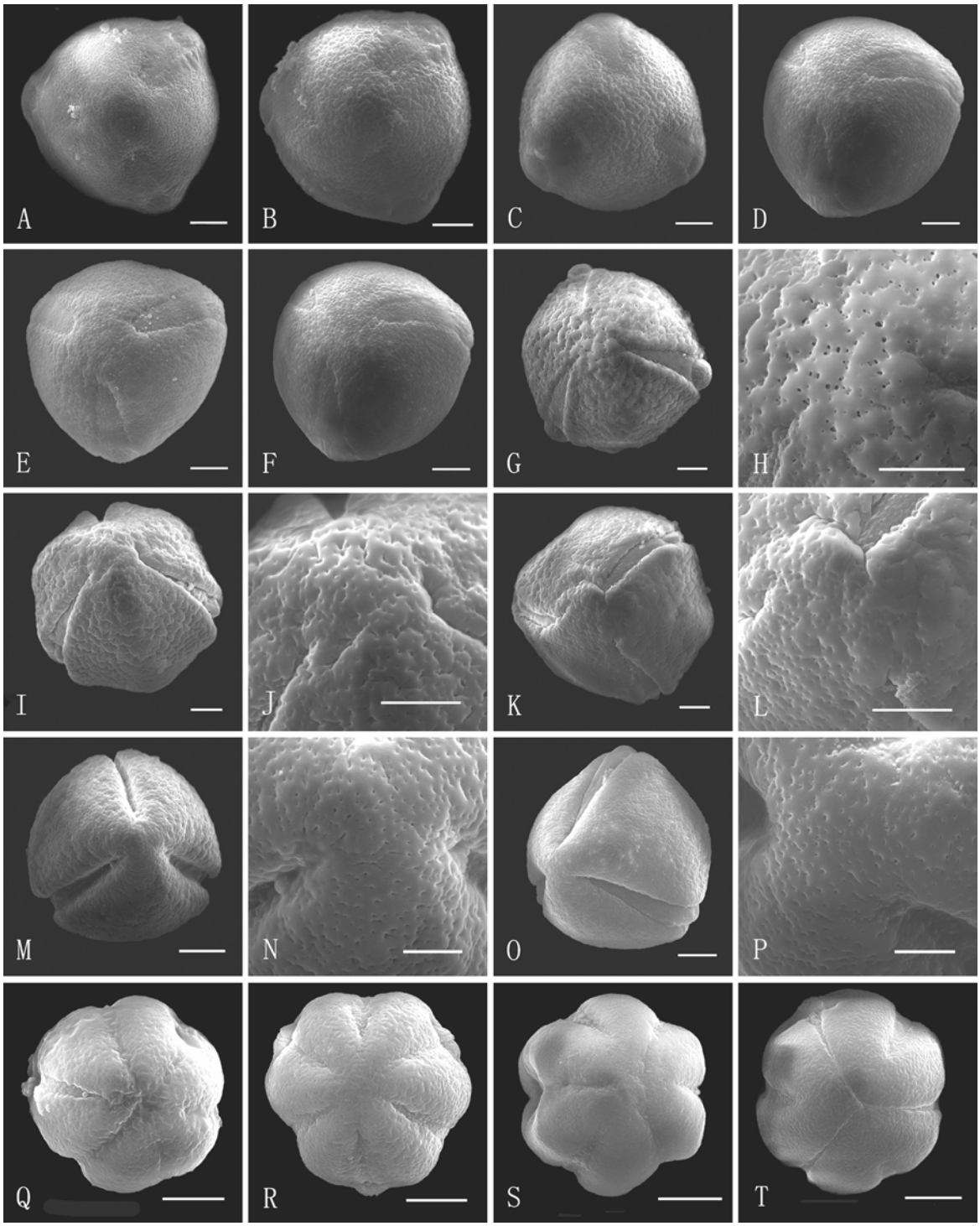

Fig. 3. Micro-morphology of pollen grains from different stamen sets of the investigated species. **A–C,** Pollen from the long (**A**), mid (**B**) and short (**C**) stamens of *Senna alata*, showing the suboblate shape and a uniformly striato-reticulate ornamentation. **D–F,** Pollen from the long (**D**), mid (**E**) and short (**F**) stamens of *S. bicapsularis*, showing the suboblate shape and a uniformly foveolate-punctate ornamentation. **G, H,** Pollen from the long stamens of *S. siamea*, showing the suboblate shape and rugosely reticulate ornamentation with irregular perforations. **I–L,** Pollen from the mid (**I, J**) and short (**K, L**) stamens of *S. siamea*, showing the suboblate shape and rugulate-foveolate ornamentation. **M–P,** Pollen from the long (**M, N**) and short (**O, P**) stamens of *S. surattensis*, showing the spherical shape and foveolate-punctate ornamentation. **Q–R,** Pollen from the long (**Q**) and short (**R**) stamens of *Melastoma dodecandrum*, showing the spherical shape and a micro-rugulate ornamentation. **S, T,** Pollen from the long (**S**) and short (**T**) stamens of *M. sanguineum*, showing the spherical shape and a micro-rugulate ornamentation near the polar.
Scale bars: 5 μm in A–L, M, O, Q–T, 2.5 μm in N, P.

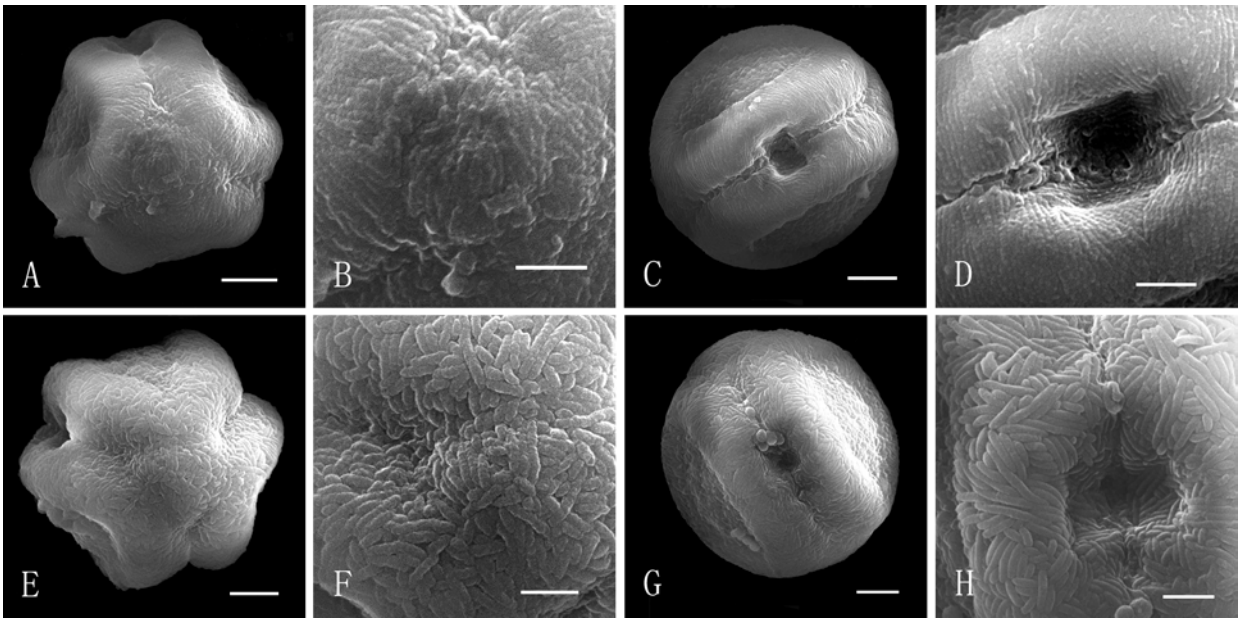

Fig. 4. Micro-morphology of pollen grains from different stamen sets of the investigated species. **A–D,** Pollen from the long stamens of *Fordiophyton faberi*. **A,** polar view, showing the three colpi and three intercolpar concavities. **B,** exine of the polar region, showing a striate-reticulate ornamentation. **C,** Equatorial view, showing a colpus with a square-shaped aperture and two intercolpar concavities. **D,** exine near mesocolpium, showing a striate ornamentation. **E–H,** Pollen from the short stamens of *Fordiophyton faberi*. **E,** polar view, showing the three colpi and three intercolpar concavities. **F,** Exine of the polar region, showing a micro-striate ornamentation. **G,** equatorial view, showing a colpus with a square-shaped aperture and two intercolpar concavities. **H,** exine near mesocolpium, showing a micro-striate ornamentation.
Scale bars: 2 μm in A, C, E, G, 1 μm in B, D, F, H.

stained similarly in both IKI and Sudan IV tests.

The percentages of viable pollen from different stamen sets are compared in Table 2. No asynchrony in pollen maturation was found among stamen types in the same flower. The proportions of viable pollen from the long stamens of *Senna alata* and *S. bicapsularis* were significantly higher than that from the mid and short stamens from anthesis to flower wilting. In *S. alata*, pollen viability varied significantly among the three stamen sets (F=349.4, P<0.001 at anthesis; F=288.8, P<0.001 at flower wilting). This phenomenon was also observed in *S. bicapsularis* (F=197.7, P<0.001 at anthesis; F=356.5, P<0.001 at flower wilting). However, pollen from the two stamen sets of *S. surattensis* stained similarly (t=1.50, P=0.17 at anthesis; t=1.40, P=0.20 at flower wilting). In *Melastoma*, pollen from both long and short stamens of *M. sanguineum* exhibited similar stainability (t=0.54, P=0.60 at anthesis; t=1.39, P=0.20 at flower wilting). The proportion of viable pollen was slightly higher at anthesis in the long stamens of *M. dodecandrum* (72.4%±2.67% vs. 66.5%±2.30%, t=1.69, P=0.14).

2.4 Pollen micro-morphology

Details of pollen micro-morphology from different stamen sets of the investigated species are shown in Fig. 3 and Fig. 4. Pollen shapes, sizes, P/E ratios and aperture types are compared in Table 4. Pollen from different stamen sets of the same species had similar shape and aperture types. The sizes of pollen grains did not differ significantly among stamen sets in *Senna* except that in *S. bicapsularis* the polar axis of pollen from mid stamens was shorter than that from short stamens (t=4.1, P<0.05). Pollen grains were similar in size between the long and short stamens in *Melastoma sanguineum*, while the equatorial axis was longer in pollen from the short stamens of *M. dodecandrum* (t=2.07, P<0.05). Pollen grains from the short stamens of *Fordiophyton faberi*, however, were much larger than those from the long stamens (polar axis, t=11.3, P<0.001; equatorial axis, t=9.63, P<0.001).

Pollen grains from the three fertile stamen sets of *S. alata* were similar in exine ornamentation (Table 4; Fig. 3: A–C). Pollen grains exhibited striato-reticulate, heterobrochate exine surface, while the surface of colpi was granular. Pollen grains of *S. bicapsularis* had a similar foveolate-punctate or small perforated exine. The colpus membrane was granular (Fig. 3: D–F). Pollen ornamentation exhibited little differences between the two stamen sets in *S. surattensis* (Fig. 3: M–P). Pollen grains from the long stamens of *S. siamea* were rugosely reticulate, with irregular

Table 4 Palynological characters of pollen from different stamen sets

Taxon	Stamen sets	Shape	Exine ornamentation	Size (P×E) (μm)*	P/E	Polar view	Aperture type
Senna alata	L	suboblate	striato-reticulate	21.7 ± 0.15 × 26.1 ± 0.18	0.83 ± 0.01	3-lobed circular	3-colporate
	M	suboblate	striato-reticulate	22.4 ± 0.23 × 26.6 ± 0.28	0.84 ± 0.02	3-lobed circular	3-colporate
	S	suboblate	striato-reticulate	22.2 ± 0.27 × 26.3 ± 0.13	0.84 ± 0.01	3-lobed circular	3-colporate
S. bicapsularis	L	suboblate	foveolate-punctate	21.8 ± 0.14 × 25.5 ± 0.22	0.86 ± 0.01	3-lobed circular	3-colporate
	M	suboblate	foveolate-punctate	21.4 ± 0.29 × 25.9 ± 0.21	0.83 ± 0.01	3-lobed circular	3-colporate
	S	suboblate	foveolate-punctate	22.5 ± 0.26 × 26.1 ± 0.41	0.86 ± 0.02	3-lobed circular	3-colporate
S. siamea	L	suboblate	rugosely reticulate with irregular perforation	27.9 ± 0.42 × 32.4 ± 0.27	0.86 ± 0.01	3-lobed circular	3-colporate
	M	suboblate	rugulate, foveolate	27.9 ± 0.38 × 32.6 ± 0.41	0.86 ± 0.02	3-lobed circular	3-colporate
	S	suboblate	rugulate, foveolate	28.2 ± 0.30 × 32.2 ± 0.39	0.87 ± 0.01	3-lobed circular	3-colporate
S. surattensis	L	spherical	foveolate-punctate	23.1 ± 0.44 × 23.8 ± 0.46	0.97 ± 0.03	3-lobed circular	3-colporate
	S	spherical	foveolate-punctate	22.1 ± 0.25 × 22.9 ± 0.27	0.97 ± 0.01	3-lobed circular	3-colporate
Melastoma dodecandrum	L	spherical	micro-rugulate	15.4 ± 0.18 × 16.7± 0.08	0.92 ± 0.01	3-lobed circular	3-colporate with 3 subsidiary colpi
	S	spherical	micro-rugulate	15.9 ± 0.19 × 17.4 ± 0.26	0.92 ± 0.01	3-lobed circular	3-colporate with 3 subsidiary colpi
M. sanguineum	L	spherical	near the polar: psilate; mesocolpia: mico-rugulate	14.7 ± 0.09 × 16.9 ± 0.09	0.88 ± 0.01	3-lobed circular	3-colporate with 3 subsidiary colpi
	S	spherical	micro-rugulate	14.5 ± 0.11 × 16.4 ± 0.12	0.89 ± 0.01	3-lobed circular	3-colporate with 3 subsidiary colpi
Fordiophyton faberi	L	spherical	striate or striate-reticulate, with parallel striations on the mesocolpia	10.4 ± 0.12 × 9.4 ± 0.46	1.11 ± 0.02	3-lobed circular	3-colporate with 3 intercolpar concavities
	S	spherical	micro-striate	12.3 ± 0.11 × 10.9 ± 0.10	1.12 ± 0.01	3-lobed circular	3-colporate with 3 intercolpar concavities

* Size values averaged across measurements of 50 grains of each anther type from 5 individuals.

perforations (Fig. 3: G, H), while pollen in the mid and short stamens exhibited rugulate, foveolate ornamentation (Fig. 3: I–L).

Melastoma dodecandrum and *M. sanguineum* exhibited little variance in pollen ornamentation between the two stamen sets. *Melastoma dodecandrum* had a micro-rugulate exine on pollen from both stamen sets (Fig. 3: Q, R). In the long stamens of *M. sanguineum*, pollen exine was psilate near the pole, while the mesocolpia were micro-rugulate or with punctate dots (Fig. 3: S). Pollen from the short stamens had micro-rugulate exine near the poles, as well as on the mesocolpia (Fig. 3: T). In contrast, pollen ornamentation of *Fordiophyton faberi* differed remarkably between different stamen types. The exine of pollen from the long stamens was striate or striate-reticulate, with parallel striations on the mesocolpia (Fig. 4: A–D); while pollen from the short stamens had a micro-striate ornamentation (Fig. 4: E–H). The surfaces of the intercolpar concavities were rugulate or scabrate-verrucate, similar on pollen from both stamen sets (Fig. 4: C, G).

3 Discussion

3.1 Morphological differentiations in stamens

The androecia of the investigated species exhibit diverse intrafloral differentiations. In *Senna*, the androecium of *S. surattensis* has little morphological differentiation, while *S. alata*, *S. bicapsularis* and *S. siamea* have four stamen types (long, mid, and short stamens and staminodes) which differ notably in length, shape, and anther size. According to Irwin and Barneby (1981, 1982), the four species belong to three different sections (Table 1). And *S. surattensis* (section *Psilorhegma* (Vogel) H. S. Irwin & Barneby), with all 10 stamens fertile and similar in size, was considered to represent the ancestral state within *Senna*. However, the results of molecular phylogenetic studies on *Senna* did not support sect. *Psilorhegma* as the basal clade, but revealed it to be a derived group in

the genus; it also revealed that *Senna siamea* is more primitive than the other three *Senna* species investigated (Marazzi et al., 2006). Our results demonstrate that those anthers of the three fertile stamen sets in *S. siamea* are similar in width, and the filaments of the two lateral stamens do not curve obviously, contrary to those of *S. bicapsularis*. The "feeding" and "pollinating" stamens of the three melastome species are also notably different. Significant difference is found in both anther length and width in *Melastoma sanguineum* and *Fordiophyton faberi*. The most significant difference between the two stamen sets of both *M. dodecandrum* and *M. sanguineum* is the specialized connectives of the long stamens which elongated and curved downwards, parallel to the style.

The micro-morphology of anther tips in *Senna* was studied by Marazzi et al. (2007). Seven anther dehiscence patterns were recognized in the two lateral abaxial stamens (the long stamens) as well as the four middle stamens (the short stamens). Marazzi and collaborators, however, did not examine the mid stamen (the median abaxial stamen). Our observations show that the anther of the mid stamen in *Senna alata* exhibits notable differences from that of the long stamen, but is similar to that of the short stamen, both of which have continuous lateral furrows on the thecae. Differences of lateral furrows between different anther sets of the same flower are reported here. In *S. alata*, the lateral furrows are continuous on the thecae of mid and short stamens, but discontinuous on the thecae of long stamens. More interestingly, in *S. bicapsularis*, lateral furrows are completely absent on the long and mid stamens, while each anther of the short stamens had two obvious lateral furrows with coarsely-serrated edges that could be noticed easily in opened flowers (Fig. 1: B). The function of such lateral furrows remain to be explored. We hypothesize that these coarsely-edged furrows may increase the friction when bees clasp the "feeding" anthers with their legs, and thus facilitate pollen collecting from the anthers. Anther tips of the long and short stamens of *Melastoma dodecandrum* and *M. sanguineum* have little difference. In *Fordiophyton faberi*, anther tip of the long stamen protrudes, while that of the short stamen slightly depresses. The adaptive significance of such morphological difference also remains to be explored.

3.2 Differentiation in pollen grains

Morphological and (or) physiological differences of pollen grains in species with heterantherous flowers have been reported by previous authors. Mori and Orchard (1980) found that different stamen sets of *Lecythis pisonis* and *Couroupita guianensis* had different *in vitro* pollen germination rates. Nepi et al. (2003) reported a relatively higher viability of pollen in the long stamens of *Lagerstroemia indica* L. at anther opening, which also remained viable for a longer time. On the contrary, Bowers (1975) found no differences in pollen viability between the two stamen types in *Solanum rostratum*. The stainability in aniline blue and germination rates of pollen from all the stamen sets in *Senna didymobotrya* were similar (Dulberger, 1981, as *Cassia*). Our data have revealed that the difference in pollen viability between different stamen sets is not a stable characteristic in species with heterantherous flowers. Pollen grains from the long stamens of *S. alata* and *S. bicapsularis* have a much higher viability than those from the mid and short stamens of the same flower. However, pollen grains from the two stamen sets of *S. surattensis*, which have little androecial differentiation, exhibit similar viability. Gross and Kukuk (2001) found that the proportion of inviable pollen differed insignificantly between the long and short stamens of *Melastoma affine*. This result is congruent with our findings. Pollen viability is similar in the two stamens sets of *M. dodecandrum* and *M. sanguineum* from anthesis to flower wilting.

Pollen morphological differences have been reported in heterantherous flowers of *Lagerstroemia* (Muller, 1981; Pacini & Bellani, 1986; Kim et al., 1994; Nepi et al., 2003), *Tripogandra grandiflora* (Lee, 1961) and *Senna* (Bahadur et al., 1991, as *Cassia*). Pollen dimorphism exists widely in *Lagerstroemia* species with dimorphic stamens. Pacini and Bellani (1986) found that the "real" pollen (pollen in the long stamens) of *L. indica* had granular ornamentation whilst the "nutritive" pollen (pollen in the short stamens) was verrucose. Muller (1981) addressed several differences of pollen grains in *L. indica* between the short and the long stamens, including larger size, absence of colpi, coarser tectum structure and thicker wall. Similar palynological differences have been found in several other species with heterantherous flowers of *Lagerstroemia* (Muller, 1981; Kim et al., 1994). Bahadur et al. (1991) examined the pollen morphology from seven *Senna* (as *Cassia*) species with the SEM, and found several differences in grain size, exine ornamentation, and (or) sexine thickness between stamen sets. For example, pollen grains from the long stamens of *S. auriculata* were larger than those in the mid stamen, and the surface of colpi from long stamen pollen in *S. tora* (L.) Roxb. was smooth but crustate in mid stamens. According to our observations, pollen

size and exine ornamentation have little variance among the fertile stamen sets of the same flower in *Senna*, except that in *S. bicapsularis* the polar axis of pollen from mid stamens was shorter. However, the exine ornamentation of pollen from long stamens in *S. siamea* was slightly different from that of the mid and short stamens, which had been reported to be similar by Bahadur et al. (1991). Moreover, pollen ornamentation of the long and short stamens was found to be similar in *Melastoma dodecandrum*, while slightly different in *M. sanguineum*. Intrafloral pollen micro-morphological difference is reported here for the first time in *Fordiophyton*. Pollen in the long stamens of *Fordiophyton faberi* has striate or striate-reticulate exine, with parallel striations on the mesocolpia, while pollen from the short stamens has a distinct micro-striate ornamentation. Nepi et al. (2003) suggested that the coarser tectum surface and larger intercolumellar space of the "feeding" pollen in *Lagerstroemia indica* would increase the interface between pollen grains and the digestive juices of the insects, which made pollen digestion more easily. In contrast, the "pollinating pollen" had a relative smoother tectum and the columellae were regular with smaller intercolumellar space. The compact structure of pollen wall may reduce water loss and protect the pollen against physical damage in pollen transfer (Punt, 1986). However, the adaptive significance of di- (or tri-) morphic pollen as well as the development of the pollen wall in heterantherous flowers still remains to be explored.

3.3 Labor-division of heteranthery

The "division-of-labor" hypothesis concerning heteromorphic stamens was proposed early, but it still lacks the support of direct evidence. Darwin (1862) attempted to test this hypothesis in a species of Melastomataceae by comparing the vigor of seedlings fathered by pollen from the long and short stamens, but no consistent difference of vigor was found despite his "enormous labor". Some authors (e.g., Gross & Kukuk, 2001) questioned the labor division within heteromorphic androecia.

Different colors of the "feeding" and "pollinating" stamens may reflect the roles that each stamen set plays in pollination. The "pollinating" stamens (or sometimes only the anthers) usually have a cryptic color or similar color with the petals, which could protect the "functional pollen" from being consumed by pollinators (F. Müller, 1883; Renner, 1989; Nepi et al., 2003). H. Müller (1882) noticed that the long stamens of melastomes which had the same color with the petals would hardly be perceived by insects (such as *Xylocopa* or *Bombus*), and bees only harvested pollen from the short yellow anthers. The similar color of "pollinating" stamens and the petals may "puzzle" the pollinators, and the insects will not perceive them as "food resource". The "feeding" stamens, on the other hand, mainly function as an attractant for pollinators. They usually are showy (often bright yellow, as the short stamens of many species in *Melastoma*) which can be easily noticed by insects. In our study, this phenomenon is present in both families. Only the anthers of *S. bicapsularis* exhibit color differentiation in *Senna*: the four mid ("feeding") anthers are yellow, while the two lateral ("pollinating") ones and the lowermost one were brown. *Senna alata*, *S. siamea*, and *S. surattensis* have similar anther color among different stamen sets. According to our observations, female *Xylocopa* bees buzzed anthers of the short stamens ("feeding" anthers) exclusively in both *S. bicapsularis* and *S. alata*, while all ten anthers were buzzed in *S. surattensis*, whose androecia are less differentiated. *Xylocopa* bees also only harvested pollen from the short showy stamens of *Melastoma sanguineum*. In small bee-pollinated *Melastoma dodecandrum*, however, such preference was not obvious. *Amegilla zonata* buzzed the anthers of both long and short stamens. This difference may have resulted from the different foraging strategies of large (like *Xylocopa*) and small bees (like *Amegilla*). Small bees that buzz only one or a few stamens can learn to preferentially empty the pollen-rich "pollinating" anthers (Gross & Kukuk, 2001), in effect acting as pollen thieves.

Differences in pollen content among anther sets have also been reported in other species with heterantherous flowers. Dulberger and Ornduff (1980) found that the lower anther of *Cyanella lutea* L. f. (Tecophilaeaceae) contained about 6 times more grains than an anther of the upper group; each of the lower anthers of *C. hyacinthodes* L. produced 14.5 times more pollen than each of the upper ones, and 3.7 times more than each of the lateral ones. The two long lateral stamens (with the largest anthers) of *Senna didymobotrya* produced 3.7 times more pollen than the five remaining ones together (Dulberger, 1981). Each large anther contained about 8.9 times more pollen grains than a short anther, and 10.7 times more than the mid (lowermost) anther. Our study found that anthers of the long stamens contain the largest amount of pollen on average, and a correlation between anther size and pollen content was substantiated. Pollen quantity variation among different stamens sets is associated with the extent of androecial differentiation. The

relative abundance of pollen source in the "pollinating" part of the androecium may be explained as a "trade off" between transferring genes to offspring and attracting pollinators in that plants tend to invest more energy in producing "functional pollen". This also occurs in *Dillenia*, a genus from another family of eudicots (Endress, 1997).

That stigmas and the anther tips of long stamens contacting similar parts of the bee's body has been reported by several authors (e.g., Forbes, 1882; H. Müller, 1882; Dulberger, 1981). Anther pores of the "pollinating" stamens usually release pollen grains to the dorsal and lower ventral parts of the bees' bodies, which are least accessible during pollen grooming, and in turn are in the proper positions for the stigmas to receive pollen during the bees' visit to the next flower (Buchmann, 1983; Michener, 2007). Here we compared the positions of stigma and anther tips of the "pollinating" stamens in natural conditions through statistical analysis. The high correlations demonstrate the structural adaptation of "pollinating stamens" for the primary function of gamete transport, and thus strongly support the "division of labor" hypothesis as first initiated by Darwin (1862).

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References

Bahadur B, Chaturvedi A, Rama Swary N. 1991. SEM studies of pollen in relation to enantiostyly and heteranthery in *Cassia* (Caesalpiniaceae). Current Perspectives in Palynological Research, Journal of Palynology Silver Jubilee Commemoration Volume: 7–22.

Baker HG, Baker I. 1979. Starch in angiosperm pollen grains and its evolutionary significance. American Journal of Botany 66: 591–600.

Bowers KA. 1975. The pollination ecology of *Solanum rostratum* (Solanaceae). American Journal of Botany 62: 633–638.

Buchmann SL. 1983. Buzz pollination in angiosperms. In: Jones CE, Little RJ eds. Handbook of Experimental Pollination Biology. New York: Van Nostrand Reinhold.

Chen C (陈介), Renner SS. 2007. Melastomataceae. In: Wu Z-Y (吴征镒), Raven PH eds. Flora of China. Beijing: Science Press; St. Louis: Missouri Botanical Garden Press. 13: 384–387.

Cruden RW. 1977. Pollen-ovule ratio: a conservative indicator of breeding systems in flowering plants. Evolution 31: 32–46.

Dafni A. 1992. Pollination ecology: a practical approach. London: Oxford University Press.

Dafni A. 2001. Field methods in pollination ecology. Course Manual, Second International Pollination Course. Haifa: University of Haifa.

Darwin C. 1862. Letter to Asa Gray [online]. Available from www.darwinproject.ac.uk [accessed 10 June 2007].

Dulberger R. 1981. The floral biology of *Cassia didymobotrya* and *C. auriculata* (Caesalpiniaceae). American Journal of Botany 68: 1350–1360.

Dulberger R, Ornduff R. 1980. Floral morphology and reproductive biology of four species of *Cyanella* (Tecophilaeaceae). New Phytologist 86: 45–56.

Endress PK. 1997. Relationships between floral organization, architecture, and pollination mode in *Dillenia* (Dilleniaceae). Plant Systematics and Evolution 206: 99–118.

Erdtman G. 1952. Pollen morphology and plant taxonomy: angiosperms. Stockholm: Almqvist and Wiksell Press.

Faegri K, Iversen J. 1964. Textbook of pollen analysis. Copenhagen: Munksgaard Press.

Forbes H. 1882. Two kinds of stamens with different functions in the same flower. Nature 26: 386.

Gross CL. 1993. The breeding system and pollinators of *Melastoma affine* (Melastomataceae): a pioneer shrub in tropical Australia. Biotropica 25: 468–474.

Gross CL, Kukuk PF. 2001. Foraging strategies of *Amegilla anomola* at the flowers of *Melastoma affine*—no evidence for separate feeding and pollinating anthers. Acta Horticulturae 561: 171–178.

Irwin HS, Barneby RC. 1981. Tribe 2: Cassieae Bronn. In: Pohlhill RM, Raven PH eds. Advances in legume systematics, pt 1. Richmond: Royal Botanic Gardens, Kew.

Irwin HS, Barneby RC. 1982. The American Cassiinae. Memoirs of the New York Botanical Garden 35: 1–918.

Kim SC, Graham SA, Graham A. 1994. Palynology and pollen dimorphism in the genus *Lagerstroemia* (Lythraceae). Grana 33: 1–20.

Lasseigne A. 1979. Studies in *Cassia* (Leguminosae–Caesalpinioideae). III. Anther morphology. Iselya 1: 141–160.

Lee RE. 1961. Pollen dimorphism in *Tripogandra grandiflora*. Baileya 9: 53–56.

Marazzi B, Endress PK, Paganucci de Queiroz L, Conti E. 2006. Phylogenetic relationships within *Senna* (Leguminosae, Cassiinae) based on three chloroplast regions: patterns in the evolution of floral symmetry and extrafloral nectaries. American Journal of Botany 93: 288–303.

Marazzi B, Conti E, Endress PK. 2007. Diversity in anthers and stigmas in the buzz-pollinated genus *Senna* (Leguminosae, Cassiinae). International Journal of Plant Sciences 168: 371–391.

Marazzi B, Endress PK. 2008. Patterns and development of floral asymmetry in *Senna* (Leguminosae, Cassiinae). American Journal of Botany 95: 22–40.

Meehan T. 1886. On the fertilization of *Cassia marilandica*. Proceedings of the Academy of Natural Sciences of Philadelphia 38: 314–318.

Michener CD. 2007. The bees of the world. 2nd ed. Baltimore: John Hopkins University Press.
Mori SA, Orchard JE. 1980. Intrafloral pollen differentiation in the new world Lecythidaceae, subfamily Lecythidoideae. Science 209: 400–403.
Müller F. 1883. Two kinds of stamens with different functions in the same flower. Nature 27: 364–365.
Müller H. 1881. Two kinds of stamens with different functions in the same flower. Nature 24: 307–308.
Müller H. 1882. Two kinds of stamens with different functions in the same flower. Nature 27: 30.
Muller J. 1981. Exine architecture and function in some Lythraceae and Sonneratiaceae. Review of Palaeobotany and Palynology 35: 93–123.
Nepi M, Guarnierim M, Pacini E. 2003. “Real” and feed pollen of *Lagerstroemia indica*: ecophysiological differences. Plant Biology 5: 311–314.
Pacini E, Bellani L. 1986. *Lagerstroemia indica* L. pollen: form and function. In: Blackmore S, Ferguson IK eds. Pollen and spores: form and function. London: Academic Press.
Punt W. 1986. Functional factors influencing pollen form. In: Blackmore S, Ferguson IK eds. Pollen and spores: form and function. London: Academic Press.
Renner SS. 1989. A survey of reproductive biology in neotropical Melastomataceae and Memecylaceae. Annals of the Missouri Botanical Garden 76: 496–518.
Rodriguez-Riaño T, Dafni A. 2000. A new procedure to assess pollen viability. Sexual Plant Reproduction 12: 241–244.
Scott RJ, Spielman M, Dickinson HG. 2004. Stamen structure and function. Plant Cell (Suppl.) 16: S46–S60.
Todd JE. 1882. On the flowers of *Solanum rostratum* and *Cassia chamaecrista*. American Naturalist 16: 281–287.
Venkatesh CS. 1957. The form, structure and special ways of dehiscence in anthers of *Cassia*. III. Subgenus *Senna*. Phytomorphology 7: 253–273.
Walker-Larsen J, Harder LD. 2000. The evolution of staminodes in angiosperms: patterns of stamen reduction, loss, and functional re-invention. American Journal of Botany 87: 1367–1384.
Wang Y-Q (王英强), Zhang D-X (张奠湘), Chen Z-Y (陈忠毅). 2004. Pollen histochemistry and pollen: ovule ratios in Zingiberaceae. Annals of Botany 94: 583–591.