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Reproductive biology of *Alpinia blepharocalyx* (Zingiberaceae): another example of flexistyly

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Abstract. The floral biology and breeding system of *Alpinia blepharocalyx* were studied in Yunnan province, southwest China. Our results indicate that populations of *A. blepharocalyx* have two floral morphs, which differ in flowering behaviour: the cataflexistylous morph in which the stigma is held erect above the dehiscent anther when anthesis begins in the morning and becomes curved under the anther at afternoon, and the anaflexistylous morph in which the receptive stigma is curved under the indehiscent anther first and moves into a reflexed superior position above the anther as it begins to shed pollen in the afternoon; the flowering period of both floral morphs is 12 hours; the stylar movements in the two floral morphs are synchronous, and they have similar traits to those found in other *Alpinia* species previously reported. Also, on average, cataflexistylous flowers are larger than anaflexistylous flowers, especially the labellum and corolla tube length; moreover, the P/O ratio of the two floral morphs is significantly different: the cataflexistylous morph has more pollen grains and fewer ovules than the anaflexistylous morph; the evolutionary significance of this comparison is discussed. Cataflexistylous flowers were observed to produce a lower concentration of nectar than that of anaflexistylous flowers before 11:00 a.m., but they then have higher nectar concentration; cataflexistylous flowers always have more nectar than anaflexistylous flowers. Flowers of *A. blepharocalyx* were visited by fewer insects at a lower frequency, including honey bees (*Apis cerana cerana*) and two species of carpenter bees (*Xylocopa* spp.). No significant differences were found in the fruit set among the hand-pollination treatments regardless of selfing (geitonogamy and autogamy) or crossing (intermorph and intramorph); but all hand-pollination treatments exhibited much higher fruit set than the controls; meanwhile, no fruit set occurred in the unpollinated bagged plants. Pollen tube growth was examined using fluorescence microscopy following self, intramorph, and intermorph hand pollinations at different times of the day; the pollen tubes of both floral morphs have the same growth rate and the capability to reach the ovary. Both the results of pollen tube growth and fruit sets under different pollination conditions show that *A. blepharocalyx* is self-compatible and dependent upon insects for pollination. The distinctive flexistyly mechanism of *A. blepharocalyx* is likely a floral mechanism that promotes effective intermorph pollen transfer.

Key words: *Alpinia blepharocalyx*, flexistyly, floral biology, pollination, pollen tube growth, nectar secretion, Zingiberaceae.

Introduction

Flowering plants exhibit a diverse array of floral mechanisms that reduce the incidence of self-pollination and the interference between female and male sexual function (Darwin 1877, Lloyd 1965, Lloyd and Webb 1986, Webb and Lloyd 1986, Bertin 1993, Barrett 2002a). Several features of the floral biology of animal-pollinated plants are particularly important in influencing self-pollination, these include the spatial and temporal deployment of anthers and stigmas (herkogamy and dichogamy, respectively) and the size of floral displays. Flowers that present anthers and stigmas simultaneously are more likely to experience intrafloral self-pollination (Lloyd 1980) and large floral displays increase opportunities for geitonogamous self-pollination (de Jong et al. 1993, Harder and Barrett 1995). Since self-pollination can result in inbreeding depression (Charlesworth and Charlesworth 1987) and pollen discounting (Harder and Wilson 1994), it is expected that many floral adaptations have evolved to reduce these mating costs.

Zingiberaceae is a large family of animal-pollinated tropical monocotyledons (Endress 1994). Members of this family display a broad range of pollination and breeding systems (Kress and Beach 1994, Sakai et al. 1999). Our recent work has revealed that in all observed *Alpinia* species, a remarkable sexual polymorphism occurs involving the co-existence of cataflexistylous (protandrous) morphs and anaflexistylous (protogynous) morphs (Li et al. 2001a,b, 2002). We defined this gender strategy as flexistyly and this stylar polymorphism has been referred to as an example of heterodichogamy (Renner 2001, Barrett 2002b). The two floral morphs in flexistylous species change from one functional phase to the other reciprocally in the middle of the one-day flowering period. Despite the presence of many flowers in anthesis on a given day in *Alpinia* species, the change in sexual function from female to male and vice versa precludes opportunities for both intrafloral and geitonogamous self-pollination (Li et al. 2001a,b, 2002).

To further increase our understanding of the reproduction of *Alpinia* and the evolution of flexistyly itself, we have chosen to study the reproductive system of other species in the genus *Alpinia* and other ginger plants. Here we report on details of the reproductive biology of *A. blepharocalyx*, one of the most common *Alpinia* species in tropical areas of south China. The goal of this study was to understand the floral biology of *A. blepharocalyx* in which the floral dimorphism was found earlier (Cui et al. 1996). We addressed the following questions: (1) Is the stigma behavior of the *A. blepharocalyx* flowers the same as in *A. kwangsiensis* during anthesis? (2) Are there any differences in the size of floral parts between the two morphs? (3) What are the patterns of nectar secretion, sugar concentration and production? (4) What are the characteristics of the breeding systems? (5) Is stylar behavior a mechanism that reduces the incidence of intrafloral and geitonogamous self-pollination and promotes outcrossing?

Material and methods

Study species and sites. *A. blepharocalyx* K. Schum. is a perennial herb, usually 1–3 m tall. Racemes are terminal on leafy shoots and 20–30 cm long, the flower has a very special structure, a conspicuous three-lobed labellum produced by the fusion of two staminodes, flesh-colored, red with yellow center. Only one fertile stamen with 2 thecae and the style extend through the sacs. Bracteoles are green and elliptic, dry and brittle, early deciduous at anthesis. Flowering occurred from late March to late April, and by August–September the capsules are ripe.

The study was carried out in a monsoon evergreen broad-leaf forest in the Caiyanghe nature reserve of Simao, Yunnan province, southwestern China (22°30′N, 101°22′E, 1,200 m in altitude). The plants of *A. blepharocalyx* occur in the evergreen broad-leaved forest dominated by *Betula alnoides* and *Alnus nepalensis* along several valleys.

Our field work was conducted from 9 to 14 of April, 2002.

Floral biology

Duration of flowering. Some initial observations were made in a natural population of the research site for 5 days. Detailed flowering phenology was monitored in the ginger collection of Xishuangbanna Tropical Botanical Garden (XTBG) in southern Yunnan (21°45′N, 101°02′E; 580 m in altitude). Flowering and fruiting behaviors were recorded daily during the blooming seasons from 2000 to 2002.

Stylar behaviour and flower size of the two floral morphs. Flower size measurements and stylar movement observations were made in the field. We randomly selected 30 cataflexistylous and 30 anaflexistylous inflorescences, each from one individual plant, for counting the total flower numbers on each inflorescence during anthesis. Then one flower from each inflorescence was chosen for flower size measurement. Flower size was determined from separated flower parts of both floral morphs: including length and width of bract, calyx dorsal lobe, and ventral lobe, labellum, lateral staminodes, cleft depth of these flower parts, and length of the corolla tube. We also quantified pollen grain and ovule numbers for each flower. We chose one flower bud from each inflorescence, separated all the flower parts, and fixed the anther and ovary in FAA solution (formaldehyde:acetic acid:ethanol = 1:3:9) separately for pollen and ovule counting. We squeezed each anther and washed them with distilled water individually, until all pollen grains were washed out, centrifuged the liquid about 5 minutes (3,000 rpm), removed the upper clear water, suspended the pollen grains in 2ml lactic acid and glycerin solution (lactic acid: glycerin = 3:1), and counted the pollen grains under the microscope (Dafni 1992). The number of ovules in each ovary was counted with a hand lens.

We observed eighteen inflorescences over 4 days; every day, three individuals of each floral morph were observed from morning (ca. 07:30, when the flowers were opening) to late evening (ca. 19:30, when the flowers began to wilt). For each flower, we recorded the stylar position (measured as the angle with the anther's ventral face, Li et al. 2002) every 60 minutes during the day, and the time of anther dehiscence in both flower morphs.

Pollination mechanisms

Nectar secretion. We selected 3 inflorescences of each floral morph randomly every day and bagged them with nylon net bags in the last evening before anthesis. The following day we chose 3 blooming flowers in each inflorescence to measure the nectar secretion hourly from 8:00 to 19:00. We measured the nectar volumes with 10 and 20 μl SIGMA "micro-cap" calibrated capillary tubes and measured nectar sucrose concentration with a hand-held, temperature-compensated refractometer (eclipse, Bellingham + Stanley Ltd., UK). This experiment was repeated on the two following days (April 10–12, 2002), and 9 inflorescences with a total number of 27 flowers for each floral morph were measured.

Visitor observations. We observed the visitors of *A. blepharocalyx* from 08:00 to 18:00 for 3 days. All visitors were video-recorded. After observations were completed, we captured at least three individuals of each visitor morphospecies for identification.

Breeding system

Pollination treatments. To examine the effects of self- and cross-pollination of *A. blepharocalyx* and the contribution of insect visitors to effective pollination, we performed five treatments on 128 randomly selected inflorescences: (1) cata-selfing: all flowers on 17 cataflexistylous inflorescences were hand-pollinated with self-pollen in the morning (ca. 08:00); (2) cata-crossing: flowers on 25 cataflexistylous inflorescences were bagged and their pollen gently removed in the morning, and hand-pollinated with pollen from anaflexistylous flowers in the afternoon (ca. 16:00), which was released at this time; (3) cata-natural: flowers on 29 cataflexistylous inflorescences were left exposed, permitting visitation by insects; (4) ana-crossing: flowers on 26 anaflexistylous inflorescences were bagged first, and hand-pollinated with pollen from cataflexistylous flowers in the afternoon (ca. 16:00) when the stigma of anaflexistylous flowers move into a reflexed superior position above the anther, and pollen was removed at the same time; (5) ana-natural: flowers on 31 anaflexistylous inflorescences were also left exposed, permitting insects to visit them. We did not conduct selfing of the anaflexistylous morph since this does not happen under natural conditions (after anther dehiscence, the stigma protrudes beyond the anther). These experiments were done during 10–14 April; subsequently, all remaining unpollinated flowers on each inflorescence were removed. In total, 128 individual plants and 3,294 flowers were treated. Fruit set and aborted flowers were counted one month later (on May 16).

Pollen tube growth. To compare the compatibility of self- and cross-pollination between two floral morphs, we conducted experiments to study pollen-tube growth rates under different treatments. We bagged the inflorescences on the day before flowers opened and performed the following treatments: (1) cata-selfing in the morning: 36 flowers on cataflexistylous inflorescences were hand-pollinated with self-pollen at 8:00 when their stigma was held above the anther; (2) cata-selfing in the afternoon: 36 flowers on cataflexistylous inflorescences were hand-pollinated with self-pollen at 16:00 while their stigmas had curved down to the receptive position; (3) cata-crossing: 36 flowers on cataflexistylous inflorescences were bagged and their pollen gently removed in the morning; they were hand-pollinated with pollen from anaflexistylous flowers at 16:00, and bagged again after pollination; (4) ana-crossing: 36 flowers on anaflexistylous inflorescences were hand-pollinated with pollen from cataflexistylous flowers at 8:00. We did not conduct selfing in the anaflexistylous morph for the same reason as mentioned above.

At 2, 4, 8, 24 hours after pollination we picked 9 flowers per treatment, removed the styles and fixed them with FAA, transferred them to ethanol (70%) after 24 hours of fixation, and then stored them in the refrigerator.

We softened the styles for 12 h in 8N sodium hydroxide, and then rinsed them in tap-water for 1 h to remove the sodium hydroxide. We stained them with 0.1% aniline blue in 0.1N potassium acetate for 4 h, squashed them under a cover-slip, and observed them under a fluorescence microscope equipped with a filter set (of maximum transmission of 365 nm). Both pollen tube walls and the callose plugs showed a distinct bright yellow to yellow-green fluorescence. We determined the length of pollen tubes and style with a micrometer (Dafni 1992).

The growth rate of pollen tubes (v) was determined by the length of pollen tube (L_{pt}) divided by the total style length (L_s), that is: $v = L_{pt}/L_s$.

Statistical analyses. Data on flower size and pollen:ovule (P/O) ratio were compared between two flower forms using a *t*-test of means. Nectar sugar concentration and nectar volume were compared by two-way factorial ANOVA between cataflexistylous and anaflexistylous morphs hourly from 08:00 to 20:00. Data on fruit set rate were compared among the treatments and floral morphs using a two-way analysis of variance (ANOVA; Norusis 1999). We performed a multiple comparison of means with the statistic program of Biomstat (Applied Biostatistics, Inc., USA 1996) to determine which treatments differed from each other.

Results

Floral biology

Duration of flowering. Inflorescences of the two floral morphs bloom almost at the same time, and last about one month from late March to late April. Every inflorescence has a total number of 25 to 70 (43.7 ± 9.4, $n = 60$) flowers, and 1 to 10 (3.71 ± 1.04, $n = 45$) flowers blooming every day during anthesis. The number of flowers on inflorescences of the two floral morphs was not significantly different (t test, $df = 58$, $P > 0.05$). The duration of anthesis of both flower forms is twelve hours and begins before dawn.

Stylar behaviour and flower size of the two floral morphs. All the patches in the population of *A. blepharocalyx* studied have two morphs, the ratio is 1:1: anaflexistylous individuals in which the receptive stigma is first curved under the undehisced anther (Fig. 1a) and moves into a reflexed superior position above the anther as it begins to shed pollen at noon (Fig. 1b), and cataflexistylous individuals in which the stigma is held erect above the dehisced anther when anthesis begins in the morning (Fig. 1c) and becomes curved under the anther at noon (Fig. 1d). The stylar movements in the two floral morphs are synchronous and correlate with the foraging behaviour of floral visitors. Therefore, the different floral morphs seem to function primarily for the purpose of inhibiting self-pollination.

Bract, calyx, dorsal lobe, ventral lobe, and lateral staminodes show no differences between cataflexistylous and anaflexistylous flowers. The labellum of cataflexistylous flowers is larger than that of anaflexistylous flowers (t test, $p < 0.05$), and the corolla tube of cataflexistylous flowers is longer than that of anaflexistylous flowers (t test, $p < 0.05$, $n = 30$) (Table 1).

Anaflexistylous flowers have an average ($\pm$SD) ovule number of 40.2 $\pm$ 4.8 ($n = 30$), while cataflexistylous flowers have 39.6 $\pm$ 5.7 ($n = 30$) ovules; thus there is no difference in ovule number between the two morphs. Anaflexistylous flowers have 46.871 $\pm$ 9.594 ($n = 30$) pollen grains per anther, which is significantly less than that in cataflexistylous flowers with 54.295 $\pm$ 8.362 ($n = 30$) (t test,

Table 1. Floral size (cm) and number of pollen and ovules of the two floral morphs in *Alpinia blepharocalyx* (mean $\pm$ SD)

Morph	Corolla tube length	Labellum		Lateral staminodes	Pollen grains	Ovules	P/O ratio
		Length	Width				
Cata-	1.22 ± 0.26	4.37 ± 0.41	4.37 ± 0.61	0.63 ± 0.23	54.295 ± 8.362	39.6 ± 5.7	1.370
Ana-	1.05 ± 0.15	3.97 ± 0.52	4.12 ± 0.37	0.57 ± 0.22	46.871 ± 9.594	40.2 ± 4.8	1.165
N	30	30	30	30	30	30	30
t	2.0003	2.001	2.0003	1.699	2.0017	2.0017	2.0017
p	0.003	0.003	0.074	0.085	0.002	0.662	0.002

Fig. 1. Position of the stigma of the two flower forms in *Alpinia blepharocalyx* at different stages of flowering. **a** anaflexistylous flower in its female phase (before noon), in which the stigma is below the undehisced anther. **b** The same flower as in **a**, but in its male phase (afternoon), with the stigma now erect above the anther, which then sheds its pollen. **c** cataflexistylous flower in its male phase (before noon), in which the stigma is above the dehisced anther. **d** The same flower as in **c** during its female phase (afternoon), with the stigma below the anther

P < 0.05). Both flower morphs have, therefore, a higher P/O ratio: anaflexistylous flowers, 1.165, and cataflexistylous, 1.370, thus significantly different (*t* test, P < 0.005) (Table 1).

Pollination mechanisms

Nectar secretion. Daily change of nectar sugar concentration and volume are shown in Figs. 2 and 3. Nectar sugar concentration of anaflexistylous flowers was higher than that of cataflexistylous flowers before 11:00 a.m. In contrast, from 11:00 a.m. cataflexistylous flowers had higher nectar sugar concentration than anaflexistylous flowers, and it was highest in the afternoon (Fig. 2). In total, cataflexistylous flowers always have higher nectar volume than anaflexistylous flowers (Fig. 3).

Visitors. Flowers of *A. blepharocalyx* are visited mainly by native honey bees (*Apis cerana cerana* Fabricius) and several species of carpenter bees (*Xylocopa* spp.), both of them are effective pollinators, but very few pollinators are observed, less than in other *Alpinia* species we have studied, in spite of their very conspicuous flowers.

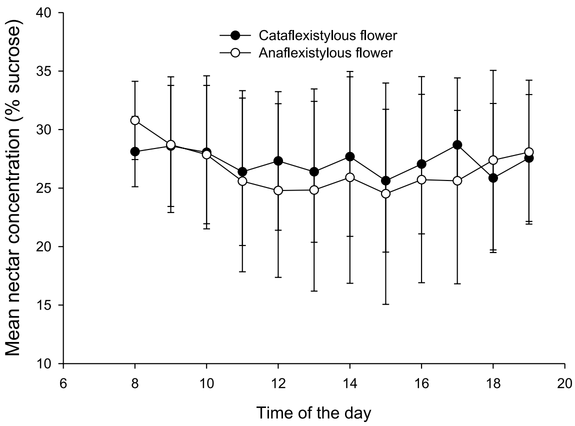

Fig. 2. Change in mean nectar concentration of *Alpinia blepharocalyx* flowers during the day (±SD, $n = 27$)

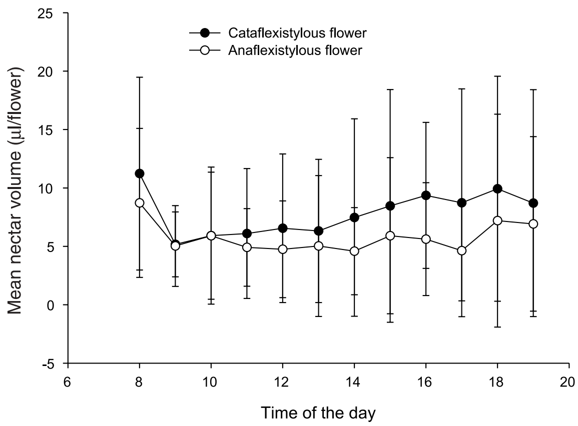

Fig. 3. Change in mean nectar volume of *Alpinia blepharocalyx* flowers during the day (±SD, $n = 27$)

Breeding system

Fruit set of different pollination treatments. The results of the different pollination treatments within and between floral morphs (Fig. 4) shows no significant difference in the fruit set by cata-selfing (87.6 ± 10.7%), by ana-crossing (86.7 ± 14.5%), and by cata-crossing (90.5 ± 13.0%) (*t* test, $P_1 = 0.8297$,

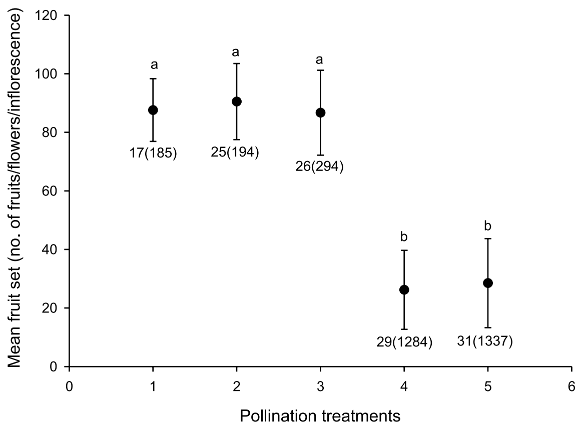

Fig. 4. The effect of pollination treatments on fruit set in the floral morphs of *Alpinia blepharocalyx*. Plotted are the means and standard errors of the following groups: 1, cataflexistylous selfing; 2, cataflexistylous ♀ × anaflexistylous♂; 3, anaflexistylous ♀ × cataflexistylous♂; 4, cataflexistylous natural; 5, anaflexistylous natural. Group sample sizes are given below the bars [inflorescence number (flower number)], and statistically homogeneous groupings based on a two-way analysis of variance are indicated by the same letter (a, b) above the bars

$P_2 = 0.4455$, $P_3 = 0.3266 > 0.05$). This is also the case for the fruit set by cata-natural (26.2 ± 13.5%) and by ana-natural (28.5 ± 15.2%) (*t* test, $P_4 = 0.5261 > 0.05$); but the fruit set by any hand pollination is much higher than that under natural condition (Fig. 1). This means that *A. blepharocalyx* is self-compatible and dependent upon insects for fertilization.

Pollen tube growth. Pollen tube growth rate depends upon the position of the stigma, regardless of the pollination treatments. When the stigma is located at the receptive position (curved under the anther), the pollen grains begin to germinate and the tubes penetrate into the stigma just one hour after pollination. But when the stigma is still above the anther, or when the stigma has moved away from the receptive position, pollen germination and tube penetration takes much longer (usually eight hours). After pollen tube penetration, pollen tube growth rates of different pollination treatments do not differ significantly (Fig. 5). Cataflexistylous selfing, and anaflexistylous and cataflexistylous intermorph tubes do not differ in their capability to reach the ovary; the slope of pollen tube growth rate is almost the same (4.22, 4.46 and 4.52 respectively).

Discussion

After the discovery of flexistyly in *A. kwangsiensis*, we conducted a series of studies on the reproductive biology of other *Alpinia* species. As other observed species, *A. blepharocalyx* has two floral morphs, in which the stylar behaviour and anther dehiscence is influenced by environmental factors, such as habitat, temperature, humidity, etc. The floral mechanism found in *Alpinia* appears to reduce self-

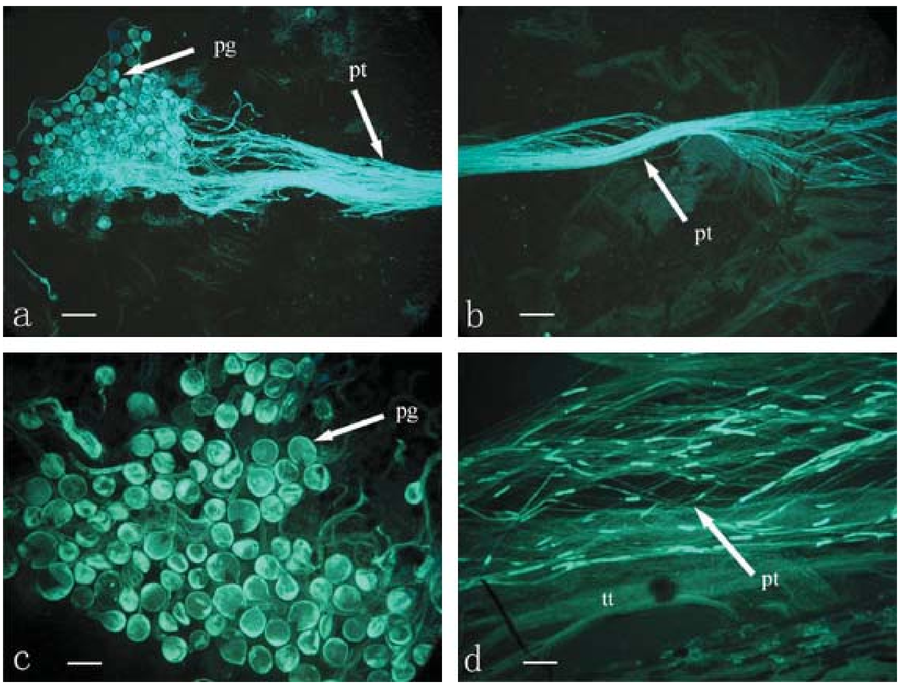

Fig. 5. Pollen tube growth of *Alpinia blepharocalyx* after various pollination treatments, as seen in fluorescence micrographs of longitudinal sections. **a** Stylar region of anaflexistylous flower 24 h after intermorph pollination. Pollen grains on the stigma surface and pollen tubes penetrating the stylar tissue. **b** Bundle of pollen tubes in anaflexistylous style 24 h after intermorph pollination. **c** Pollen grains on cataflexistylous stigma 4 h after selfing. **d** Bundle of pollen tubes in cataflexistylous style 24 h after selfing. Pollen tube walls and callose plugs show a bright yellow fluorescence. *pg* pollen grains; *pt* pollen tubes; *tt* transmitting tissue. Scale bars: **a**, **b** = 200 μm; **c**, **d** = 100 μm

pollination within a flower (autogamy) and among flowers within inflorescences or an individual (geitonogamy), thereby increasing the pollen available for export to other individuals. We believe that this elaborate floral mechanism results in a low level of inbreeding despite the presence of self-compatibility in the populations.

Labellum size and corolla tube length of the two floral morphs show a significant difference: cataflexistylous flowers have a longer corolla tube and labellum than anaflexistylous flowers (Table 1). This result predicts that cataflexistylous flowers have more nectar than anaflexistylous flowers. In addition to the difference in corolla size, cataflexistylous flowers have significantly more pollen grains than anaflexistylous flowers. The same pattern of dimorphism of floral size and number of pollen grains was observed in other *Alpinia* species, such as *A. kwangsiensis* (Li et al. unpublished data). The evolutionary significance of this phenomenon needs further investigation, including research on reproductive biology as well as phylogenetic studies of *Alpinia* and other Zingiberaceae (such as *Amomum*) (Zhang and Li 2002).

P/O ratios are a better predictor of breeding systems than other morphological characteristics (Cruden 1977). The P/O ratios of *A. blepharocalyx* are 1,370 in the cataflexistylous morph and 1,165 in the anaflexistylous morph. This result is congruent with the evidence that *A. blepharocalyx* is an allogamous and pollinator-dependent plant species.

At the research site, the visiting frequency of the effective pollinators was rather low. Insufficient pollinator service arises whenever plants receive too few visits, resulting in pollen limitation of seed production. According to the flower design they should be effectively pollinated by birds. Although we observed bird pollination in *A. kwangsiensis* (*Arachnothera longirostra*) (Li et al. unpublished data), we did not observe any bird visits in *A. blepharocalyx*. It is possible that fragmentation of the forest has resulted in the lack of birds.

Fruit set was higher in hand pollinations than in flowers visited by natural pollinators (Fig. 4), suggesting that natural populations of *A. blepharocalyx* are pollinator-limited. In the hand-pollination treatments, fruit set showed no difference between cata-selfing, cata-crossing and ana-crossing. Moreover, in natural pollination, there was no difference between cataflexistylous and anaflexistylous plants, but the difference between hand pollination and natural pollination was significant. The index of self-incompatibility (ISI) (Zapata and Arroyo 1978) value of this species was 96.8%, indicating it had a 96.8% possibility of self-compatibility. Our results suggest that *A. blepharocalyx* is a self-compatible and primarily insect-pollinated species, and selfing appears to be prevented by flexistyly.

The period of nectar production is generally correlated with the period in which pollinators are active (Cruden et al. 1983). This is also the case in *A. blepharocalyx*. In the morning, anaflexistylous flowers have higher nectar volume than cataflexistylous flowers, thus, they have more chances to attract pollinators when the receptive stigma is curved under the undehisced anther. After 11:00, cataflexistylous flowers have higher nectar volume, making them more attractive to pollinators than anaflexistylous flowers. But, in general, daily change of nectar volume and sugar concentration is relatively small.

The result that there is no difference among pollen tube growth rate in crossing and selfing treatments indicates that: (1) out-crossing is the preferential means of fertilization; (2) in lieu of out-crossing, the species is self-compatible. These results are consistent with the fruit set data reported here.

Flexistyly is a newly-discovered floral dimorphism in angiosperms; it is the only sexual polymorphism that combines reciprocal herkogamy and heterodichogamy (Li et al. 2001a). Thus, it involves both spatial (herkogamy) and temporal (dichogamy) features of sexual function. These are associated in a single polymorphism. It would be worthwhile to determine the order in which these two separate components became associated with the evolution of flexistyly. For this reason, it is necessary to study in detail the reproductive biological traits in more flexistylous species.

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References

Barrett S. C. H. (2002a) Sexual interference of the floral kind. Heredity 88: 154–159.

Barrett S. C. H. (2002b) The evolution of plant sexual diversity. Nat. Rev. Genet. 3: 237–284.

Bertin R. I., Newman C. M. (1993) Dichogamy in angiosperms. Bot. Rev. 59: 112–152.

Charlesworth D., Charlesworth B. (1987) Inbreeding depression and its evolutionary consequences. Annual Rev. Ecol. Syst. 18: 237–268.

Cruden R. W. (1977) Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. Evolution 31: 32–46.

Cruden R.W., Hermann S.M., Peterson S. (1983) Patterns of nectar production and plant-pollinator coevolution. In: Bentley B., Elias T. (eds.) The biology of nectaries. Columbia University Press, New York, pp. 80–125.

Cui Xiao-Long, Wei Rong-Chen, Huang Rui-Fu (1996) A study on the breeding system of Amomum tsao-ko. Proceedings 2nd Symposium Family Zingiberaceae 1996: 288–296. South China Institute of Botany.

Dafni A. (1992) Pollination ecology: a practical approach. Oxford University Press, New York.

Darwin C. (1877) The different forms of flowers on plants of the same species. Murray, London.

de Jong T. J., Waser N. M., Klinkhamer P. G. L. (1993) Geitonogamy: the neglected side of selfing. Trends Ecol. Evol. 8: 321–325.

Endress P. K. (1994) Diversity and evolutionary biology of tropical flowers. Cambridge University Press, Cambridge, UK.

Harder L. D., Barrett S. C. H. (1995) Mating cost of large floral displays in hermaphrodite plants. Nature 373: 512–515.

Harder L. D., Wilson W. G. (1994) Floral evolution and male reproductive success: optimal dispensing schedules for pollen dispersal by animal-pollinated plants. Evol. Ecol. 8: 542–559.

Kress W. J., Beach J. H. (1994) Flowering plant reproductive systems at la Selva Biological Station. In: McDade L. A., Bawa K. S., Hespenheide H., Hartshorn G. (eds.) La Selva: Ecology and natural history of a neotropical rain forest. University of Chicago Press, Chicago, pp. 161–182.

Li Q.-J., Xu Z.-F., Kress W. J., Xia Y.-M., Zhang L., Deng X.-B., Gao J.-Y., Bai Z.-L. (2001a) Flexible style that encourages outcrossing. Nature 410: 432.

Li Q.-J., Xu Z.-F., Xia Y.-M., Zhang L., Deng X.-B., Gao J.-Y. (2001b) Study on the flexistyly pollination mechanism in *Alpinia* plants (Zingiberaceae). Acta Bot. Sin. 43: 364–369 (in Chinese).

Li Q.-J., Kress W. J., Xu Z.-F., Xia Y.-M., Zhang L., Deng X.-B., Gao J.-Y. (2002) Mating system and stylar behaviour during flowering of *Alpinia kwangsiensis* (Zingiberaceae). Plant Syst. Evol. 232: 123–132.

Lloyd D. G. (1965) Evolution of self-compatibility and racial differentiation in *Leavenworthia* (Cruciferae). Contr. Gray Herb. Harvard Univ. 195: 3–134.

Lloyd D. G. (1980) Parental strategies of angiosperms. New Zeal. J. Bot. 17: 595–606

Lloyd D. G., Webb C. J. (1986) The avoidance of interference between the presentation of pollen and stigmas in angiosperms. I. Dichogamy. New Zeal. J. Bot. 24: 135–162.

Norusis M. J. (1999) SPSS for Windows + V9.0 base manual. SPSS Inc., Chicago, IL.

Renner S. S. (2001) How common is heterodichogamy? Trends Ecol. Evol. 16: 595–597.

Sakai S., Kato M., Inoue T. (1999) Three pollination guilds and variation in floral characteristics of Bornean gingers (Zingiberaceae and Costaceae). Amer. J. Bot. 86: 646–658.

Webb C. J., Lloyd D. G. (1986) The avoidance of interference between the presentation of pollen and stigmas in angiosperms. II. Herkogamy. New Zeal. J. Bot. 24: 163–178.

Zapata T. R., Arroyo M. T. K. (1978) Plant reproductive ecology of a secondary deciduous tropical forest in Venezuela. Biotropica 40: 221–230.

Zhang L., Li Q.-J. (2002) Flexistyly and its evolutionary ecological significance. Acta Phytoecol. Sin. 26: 385–390 (in Chinese).

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