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The Pollination Ecology of *Paraboea rufescens* (Gesneriaceae): a Buzz-pollinated Tropical Herb with Mirror-image Flowers

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• *Background and Aims* Gesneriaceae is a pantropical plant family with over 3000 species. A great variety of pollination mechanisms have been reported for the neotropical members of the family, but the details of buzz-pollination and enantiostyly for the family have not been described. We investigated the floral biology and pollination ecology of *Paraboea rufescens* in Xishuangbanna, south-west China, considering three aspects: (1) the type of enantiostyly exhibited; (2) whether the species is self-compatible; and (3) whether pollinator behaviour could enhance the precision of pollen transfer between flowers of contrasting stylar orientation.
• *Methods* Flowering phenology was monitored once a month during vegetative growth, and once a week during flowering both in the field and under cultivation. Pollination manipulations and pollinator observation in the field were conducted.
• *Key Results* Anthesis occurred early during the morning, and flowers remained open for 1–5 d, depending on weather conditions. Controlled pollinations revealed that *P. rufescens* is self-compatible, and exhibited inbreeding depression in seed set. Plants were pollinator limited in natural populations. The similar stylar deflection among flowers within a plant limits autonomous self-pollination as well as pollination between flowers. Two species of bumble bees (*Bombus* spp.), *Amegila malaccensis* and *Nomia* sp. effectively pollinated *P. rufescens*. These pollinators visited flowers in search of pollen with almost the same frequency. None of the pollinators appeared to discriminate between left- or right-handed flowers.
• *Conclusions Paraboea rufescens* exhibits monomorphic enantiostylous flowers and a buzz-pollination syndrome. Floral morphology in *P. rufescens* and pollinator foraging behaviour seems likely to reduce self-pollination and pollinations between flowers of the same stylar deflection.

Key words: Buzz-pollination, enantiostyly, Gesneriaceae, mirror-image flowers, *Paraboea rufescens*, reproductive biology, *Xishuangbanna*.

INTRODUCTION

Enantiostyly (mirror-image flowers) is a sexual polymorphism in which left-styled flowers have anthers deflected to the right, and right-styled flowers have the opposite arrangement (Todd, 1882; Webb and Lloyd, 1986; Jesson and Barrett, 2003). This form of reciprocal herkogamy occurs in at least a dozen unrelated families of flowering plants (Jesson and Barrett, 2003). Enantiostyly occurs in two distinct forms (Barrett *et al*., 2000). In monomorphic enantiostyly, which is most common, left- and right-styled flowers occur on the same plant. In contrast, in dimorphic enantiostyly which has been reported for only seven species, the flowers of individual plants are entirely either left- or right-styled (Barrett *et al*., 2000; Jesson and Barrett, 2003). Enantiostyly is generally considered to promote insect-mediated cross-pollination by reducing sexual interference between female and male function (Wilson, 1887; Iyengar, 1923; Ornduff and Dulberger, 1978; Dulberger and Ornduff, 1980; Dulberger, 1981; Webb and Lloyd, 1986; Fenster, 1995; Barrett *et al*., 2000; Barrett, 2002*a*). Moreover, recent work has detailed the inheritance, developmental basis, evolution and adaptive significance of enantiostyly (Barrett *et al*., 2000; Barrett, 2002*a*, *b*; Jesson and Barrett, 2002*a–c*; Jesson and Barrett, 2003; Jesson *et al*., 2003). Nevertheless, the basic reproductive biology of most enantiostylous species remains unstudied.

Enantiostyly is often associated with buzz-pollination (Buchmann, 1983), a pollination mechanism in which bees must buzz flowers by rapidly vibrating their indirect flight muscles to release pollen from the anthers, which dehisce only at the tip so that their pollen remains hidden (Buchmann, 1983). Floral traits commonly associated with buzz-pollination include a bowl-shaped perianth of suitable size for pollinator activity, reflexed petals, lack of nectar, and brightly coloured anthers (Buchmann, 1983). These traits combined with specific pollinator behaviour may lead to precise pollen transfer (Harder, 1990; Harder and Barclay, 1994; King and Buchmann, 1996). Several species in unrelated enantiostylous families, for example, *Solanum rostratum* in the Solanaceae (Bowers, 1975) and *Chamaecrista fasciculata* in the Leguminosae (Fægri and van der Piji, 1979), have independently evolved the buzz pollination syndrome. Because the details of pollen dispersal by pollinators affects the mating patterns of animal-pollinated plants (Harder and Barrett, 1996), it is not unexpected that buzz pollination would reduce the levels of geitonogamy (pollination between different flowers of the same individual), especially in species with enantiostyly.

The Gesneriaceae is a large tropical family with about 3000 animal-pollinated species in 133 genera (Wiehler, 1983). This family exhibits extensive diversity in floral structure, even within genera, which appears to have

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resulted from adaptive changes in pollination mode (Burtt, 1970; Wiehler, 1983; Endress, 1994). In the 1500 neotropical species of Gesneriaceae, 60 % are pollinated by hummingbirds, 30 % are pollinated by nectar-feeding euglossine bees and 10 % are pollinated by bats, butterflies, hawkmoths, flies or male euglossine bees in search of nectar (Wiehler, 1983). In contrast, little is known about the pollination ecology of Gesneriaceae in China.

During work on the pollination biology of tropical Chinese plants casual observations suggested that *Paraboea rufescens* is enantiostylous and buzz pollinated. In this paper are reported the results of subsequent studies concerning three aspects of the pollination ecology of *P. rufescens*: (1) the type of enantiostyly exhibited; (2) whether this species is self-compatible; and (3) whether pollinator behaviour could enhance the precision of pollen transfer between flowers of contrasting stylar orientation.

MATERIALS AND METHODS

Research site and plant material

Paraboea rufescens (Franchet) B. L. Burtt is a small herb distributed in Guangdong, south-western Guangxi, southern Guizhou, eastern and southern Yunnan provinces in China, Thailand and northern Vietnam (Wang *et al*., 1998). It usually grows in sunny cracks and crevices of calcareous rocks with little soil. This species was studied on the peak of a limestone mountain in the monsoon rain forest in Xishuangbanna National Nature Reserve, south Yunnan Province, China (21°41′N, 101°25′E; 580 m a.s.l.), 5 km (south-east) from Xishuangbanna Tropical Botanical Garden (XTBG). At this site, *P. rufescens* is the main species in crevices and is restricted to very sunny mountain tops with sparse trees and shrubs, including *Ficus pisocarpa*, *F. orthoneura*, *Pistacia weinmaniifolia* and *Agapetes burmanica* (Zhu *et al*., 2003). Some observations of flowering phenology were also made on potted plants at XTBG.

Floral phenology and morphology

Flowering phenology was monitored once a month from May 2002 to September 2003, and once a week during the flowering season. The time of flower anthesis and withering, the region of stigmatic receptivity, and the position of the stamens and the style were observed and recorded. Also the longevity of pollinated and non-pollinated flowers was compared, and the number of left- and right-handed flowers produced each day on 20 marked plants recorded on 1–3 Jul. 2003.

Pollination experiments

To examine the effect of self- and cross-pollination of *Paraboea rufescens*, the duration of style receptivity, and the contribution of insect visitors to effective pollination, five pollination treatments were carried out on 115 randomly selected plants: (1) bagging—14 plants were bagged without pollination; (2) self-pollination—first-day flowers of 24 plants were hand-pollinated with pollen from flowers of the opposite stylar orientation from the same plant; (3) cross-pollination of first-day flowers—first-day flowers of 21 plants were hand-pollinated with pollen of the opposite stylar orientation from another individual; (4) cross-pollination to second-day flowers—second-day flowers of 16 plants were hand-pollinated with pollen of the opposite stylar orientation from another individual: and (5) open-pollinaton—40 plants were left for unmanipulated natural pollination. Inflorescences subjected to hand-pollination were bagged with nylon mesh before anthesis and bagged again after hand-pollination. The number of inflorescence and open flowers was recorded for all treatments. These experiments were conducted during 5–17 Jul. 2003, and fruit set of each treatment was counted 5 weeks later (on 25 Aug.). The seeds produced per fruit were counted, but undeveloped ovules were not counted because each ovary contains >1500 tiny ovules, which are stuck together by a glue-like substance. The fruit and seed set data for the open-pollinated and bagged flowers were analysed using a one-way ANOVA (SPSS ver. 9·0; Norusis, 1999) with pollination treatment as fixed effects. Fruit set data were arcsine transformed and seed set data were square root transformed prior to analysis.

Pollinator visitation and behaviour

Pollinators were identified and their behaviour observed between 0700 and 1900 h on 29–30 Jun. and 4 Jul. 2003. During a bee's visit, its position was observed while entering and exiting a flower, and whether it buzzed the anthers and collected pollen grains to determine if pollinator visits could result in self- or cross-pollination. Also pollinator movements were recorded among flowers on individual plants to assess whether they discriminated between left- and right-handed flowers. Observations were made synchronously by several observers posted at three sites in the population. After observations were completed, at least three individuals of each pollinator morphospecies were captured and identified. All species are deposited in the insect collections of XTBG.

RESULTS

Floral traits and flowering phenology

Paraboea rufescens is a deciduous herb, which is dormant from late October to the end of April during the dry season of the Xishuangbanna area. Flowering begins in June, after a month of vegetative growth, and continues for 3 months.

Paraboea rufescens produces flowers in cymes. Five united blue petals form a cup-shaped blossom containing one style, two stamens and two staminodes. The style, which is deflected either to the left or right of the main floral axis, places the stigma just beyond the stamens and parallel to the edge of corolla. Two white fertile anthers and two yellow staminodes are coherent and deflected in the opposite direction from the style (Fig. 1). The flower produces no nectar and the anther dehisces by an apical pore. *Paraboea rufescens* inflorescences bear both left- and right-styled flowers (Fig. 1), so this species exhibits monomorphic enantiostyly. On average ($\pm$ standard error), plants produced 8·45 $\pm$ 1·1 inflorescences (range 2–20, n = 20),

FIG. 1. Left- and right-styled flowers, inflorescence and visitors of *Paraboea rufescens*: (A) an inflorescence bearing a left- and a right-styled flower; (B) *Amegila malaccensis* visiting a flower; (C) *Amegila malaccensis* entering or exiting a flower; (D) *Trigona pagdeni* visiting a flower.

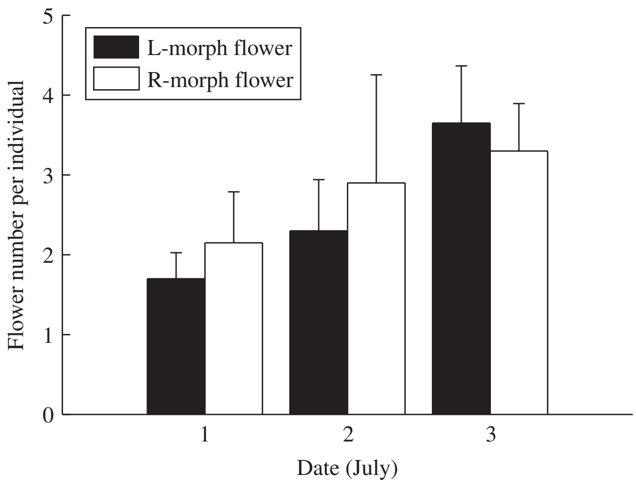

FIG. 2. The mean (± standard error) number of left- and right-styled flowers produced by *Paraboea rufescens* on 1, 2 and 3 Jul. 2003. Each plant produces the same frequencies of left- and right-handed flowers each day. Sample size = 20 plants with a total of 169 inflorescences.

with one to three left- and right-handed flowers open per inflorescence per day (mean 0·46 and 0·48, respectively; n = 169 inflorescences of 20 individuals) from 1 to 3 Jul. 2003 (Fig. 2).

Flowers opened during very early morning (0400–0500 h) and remained open for 1–5 d depending on environmental conditions. Once a flower was pollinated, the corolla and attached anthers fell off during the morning after pollination, if touched by a visitor, or during the following late afternoon. In contrast, unpollinated flowers at the study site remained open for 2–3 consecutive days and for up to 5 d in shade at XTBG, before withering gradually during over hours.

Experimental pollinations

The pollination treatments revealed that *P. rufescens* is self-compatible, but does not self-pollinate autonomously (Figs 3 and 4). Bagged inflorescences that were not hand-pollinated set no fruit. The three main treatments (selfed, outcrossed and open) had significant fruit sets (Fig. 3; one-ANOVA on arcsine-transformed fruit set, $F_{3,97} = 24{\cdot}45$, $P = 7{\cdot}21 \times 10^{-12} < 0{\cdot}001$), in which, self-pollinated inflorescences had the highest fruit set. In addition, cross-pollination during a flower's first or second day resulted in equivalent fruit set, and hand cross-pollination flowers had significantly higher fruit set than open-pollinated flowers. In contrast, open-pollinated flowers produced significantly more seeds than hand cross-pollinated flowers, which in turn produced more seeds than self-pollinated flowers (Fig. 4, one-way ANOVA on square root-transformed seed set, $F_{2,87} = 57{\cdot}14$, $P = 1{\cdot}42 \times 10^{-16} < 0{\cdot}001$).

Pollinator visitation and behaviour

Six bee species (two species of *Bombus*, *Amegilla malaccensis*, *Nomia* sp., *Apis florea* and *Trigona pagdeni*)

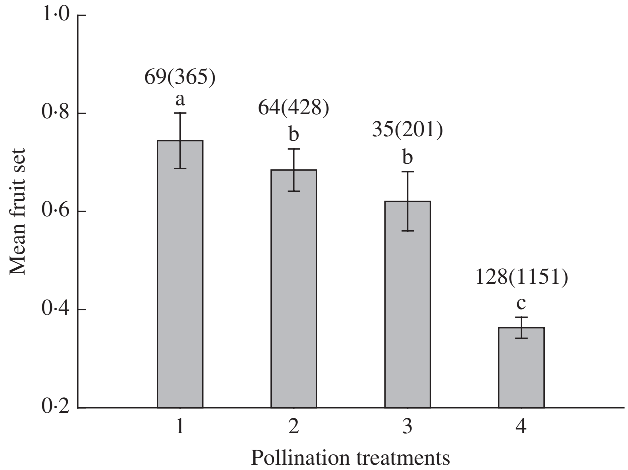

FIG. 3. Mean (± standard error) fruit set of *Paraboea rufescens* inflorescences subjected to four pollination treatments: 1, self-pollination; 2, cross-pollination of first-day flowers; 3, cross-pollination of second-day flowers; and 4, open-pollination. Sample sizes are given below the bars [inflorescence number (flower number)], and statistically homogeneous groupings based on a one-way analysis of variance are indicated by the same letter (a, b or c) above the bars.

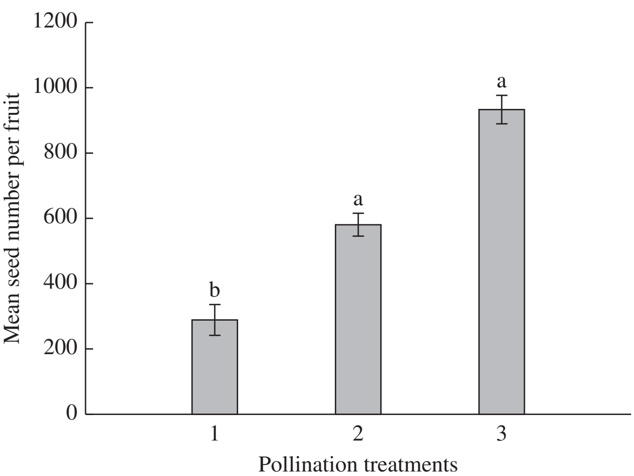

FIG. 4. Mean (± standard error) seed number per *Paraboea rufescens* fruit resulting from one of three pollination treatments: 1, self-pollination; 2, cross-pollination of first-day flowers; and 3, open-pollination. Statistically homogeneous groupings based on a one-way analysis of variance are indicated by the same letter (a or b) above the bars.

visited *Paraboea rufescens* flowers during the observations. Of the bees that visited *P. rufescens* flowers regularly, bumble bees, *Amegila malaccensis*, and *Nomia* sp. are considered the primary pollinators, because their bodies contacted the stigma and they were observed to buzz flowers, which caused pollen grains to stream out of the anthers and collect on one side of their body. (A video recording is available from the senior author on request.) *Apis florae* and *Trigona pagdeni* are poor pollinators because their small bodies rarely touch the stigma (Fig. 1D). Instead, these species generally gleaned displaced pollen grains from the corolla.

During a total of 108 h of observations, bumble bees (*Bombus* spp.) made 128 visits (53·6 %, $n = 239$) and *Amegilla malaccensis* and *Nomia* sp. made 111 visis (46·4 %). All of these major pollinators visited primarily during the morning, reaching a peak at noon, and declining during the afternoon (Fig. 5). They entered *P. rufescens* flowers with their thorax upwards, curled their body and grasped the anthers and staminodes (Fig. 1B), and then buzzed. Because the stamens bend in the opposite direction from the style and the pollinator's body occupied almost the entire flower, pollen was dispatched on one side of a pollinator's body while the stigma touched the corresponding position on the other side (Fig. 1C). Pollinators did not discriminate between right-styled and left-styled flowers. They visited flowers randomly and sometimes visited all flowers of an individual one by one. During 3 d of observation, in which 239 visits to 717 flowers were observed, 376 were to right-styled flowers, and 341 were to left-styled flowers ($\chi^2 = 1{\cdot}612$, $P > 0{\cdot}1$, $n = 1$).

DISCUSSION

A prominent feature in the Gesneriaceae is the four postgenitally united anthers either in pairs or combined together (Lamond and Vieth, 1972; Endress, 1994). Many gesneriad species also show marked protandry combined with herkogamy, and many are pollinated by birds (Wiehler, 1983). As far as is known, the present work on *Paraboea rufescens* represents the best-documented case in the Gesneriaceae of enantiostyly combined with buzz-pollination. In this family, some species of *Streptocarpus* and all species of *Saintpaulia* are enantiostylous and usually buzz-pollinated by bees (Harrison *et al*., 1999; Jesson *et al*., 2003), but no detailed studies on the basic reproductive biology have been reported. Although enantiostyly has evolved independently in at least 12 unrelated angiosperm families (Jesson and Barrett, 2003), convergence in floral morphology occurs among enantiostylous species. For example, enantiostyly is often associated with heteranthery, the specialization of anthers into brightly coloured feeding anthers and a cryptically coloured pollinating anther (Graham and Barrett, 1995). In *P. rufescens*, two yellow staminodes appear to serve as an attractant to insect visitors, whereas the two white anthers produce pollen.

Our pollination experiments provided insights into the floral biology of *P. rufescens*. The lack of fruit production in the bagging treatment indicates that *P. rufescens* is dependent upon insects for pollination; spontaneous self-pollination in this species does not occur. Fruit set was significantly higher in hand pollinations than in flowers visited by natural pollinators, suggesting that fruit production in natural populations is pollen limited. As in other enantiostylous species (Bowers, 1975; Dulberger, 1981; Fenster, 1995), floral morphology and pollinator foraging behaviour in *P. rufescens* seems likely to reduce self-pollination and pollinations between flowers of the same stylar deflection. However, pollen can be transferred between the two flower types within the same individual, resulting in geitonogamy. The relative degree of geitonogamy and xenogamy (pollination between different

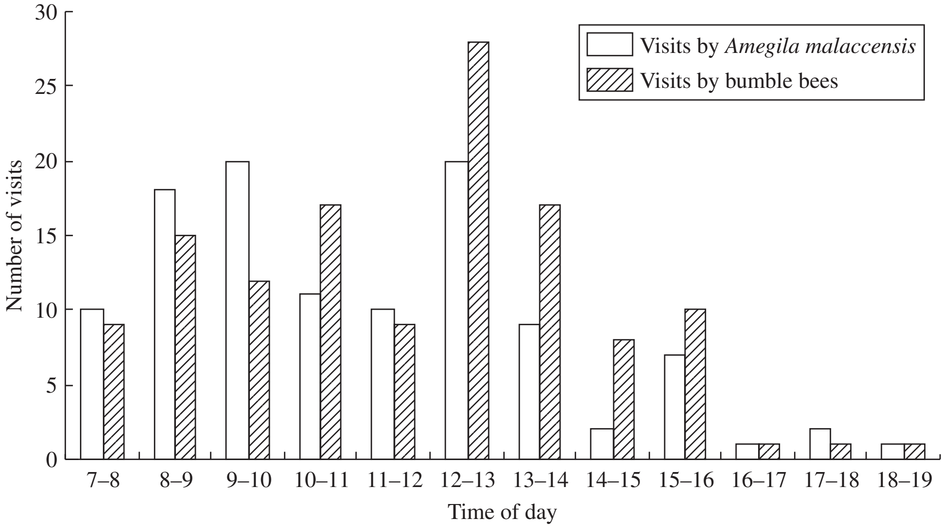

FIG. 5. Frequency of visits by bumble bees and *Amegila malaccensis* to flowers of *Paraboea rufescens* during 108 h of observation of 20 plants on 29 and 30 Jun. and 4 Jul. 2003.

individuals) depends on the flight pattern of pollinators, the number of open flowers per plant and population size (Bowers, 1975). However, in buzz-pollinated *Solanum rostratum*, Jesson and Barrett (2002*b*) demonstrated that monomorphic enantiostyly can significantly reduce levels of geitonogamy compared with levels of geitonogamy characteristic of straight-styled flowers. It seems likely that in *P. rufescens*, monomorphic enantiostyly also serves this role.

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