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Nectar robbers pollinate flowers with sexual organs hidden within corollas in distylous *Primula secundiflora* (Primulaceae)

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Nectar robbers are thought rarely to pollinate flowers, especially those with sexual organs hidden within corollas. In this study, we examined whether robbers pollinate flowers of distylous *Primula secundiflora*. Distylous plants have two floral morphs. Pin flowers have long styles and short stamens, and thrum flowers have short styles and long stamens. Flowers of *P. secundiflora* were commonly robbed by bumble-bees, and robbing holes were always situated between high and low sexual organs for both floral morphs. We observed that pollen grains of pin flowers were removed while thrum flowers received fresh pollen grains immediately after flowers were robbed. We manipulated flowers so that only nectar robbers could visit them. This resulted in 98 per cent of thrum flowers and 6 per cent of pin flowers setting fruit, and seed number per thrum fruit was also significantly higher than per pin fruit. Our findings suggest that nectar robbers transfer pollen from pin flowers to thrum flowers effectively, and consequently increase male fitness of the pin morph and female fitness of the thrum morph. Such asymmetrical pollen flow caused by nectar robbers may act as an important selective agent in floral fitness and evolution of distyly.

Keywords: nectar robbing; distyly; reciprocity; robber-like pollinator

1. INTRODUCTION

Nectar robbing occurs commonly in flowering plants, especially those with tubular flowers or flowers with nectar spurs (Irwin & Maloof 2002). Nectar robbers usually obtain reward without providing a pollination service (Inouye 1983; Maloof & Inouye 2000), so they have frequently been described as cheaters in the plant–pollinator mutualism system since Darwin (1872). However, some studies have shown that robbers can directly pollinate flowers (Macior 1966; Koeman-Kwak 1973; Waser 1979; Higashi *et al.* 1988; Scott *et al.* 1993; Guitian *et al.* 1994; Navarro 2000; Zhang *et al.* 2007). Such robbers were described as robber-like pollinators by Higashi *et al.* (1988). To our knowledge, in all cases of robber-like pollination, the stamens and pistils protruded from corollas, and pollination occurred when nectar robbers (usually bumble-bees or carpenter bees) unintentionally came into contact with these sexual organs. It remains unclear whether nectar robbers could pollinate flowers with sexual organs deeply hidden within corollas.

In this study, we examined the possibility of robber-like pollination in distylous *Primula secundiflora*. Distylous plants have two floral morphs: pin flowers with long styles and short stamens, and thrum flowers with short styles and long stamens. The height of stigmas in pin flowers corresponds to the height of anthers in thrum flowers, and vice versa. Most distylous plants are self- and intra-morph incompatible and distyly has traditionally been viewed as an adaptive trait to promote inter-morph pollen transfer (Ganders 1979). Recent studies have shown that the effect of nectar robbing on host plants varies among species with different mating systems (Burkle *et al.* 2007; Zhang *et al.* 2009), and between different floral types of sexually polymorphic plants (Zhang *et al.* 2008). However, nectar robbing has been reported only in a limited number of distylous species, and little is known about the effect of nectar robbing on the evolution and ecology of distyly (Irwin & Adler 2006). Flowers of *P. secundiflora* are frequently robbed by bumble-bees. We found that robbing holes were positioned between high and low sexual organs for both floral morphs (figure 1). We suggest that nectar robbers touch both high and low sexual organs, and thus pollinate both floral morphs. We tested this mechanistic hypothesis using detailed observations and measuring seed production of manipulated flowers that were visited only by robbers.

2. MATERIAL AND METHODS

(a) *Study system*

Primula secundiflora is a distylous perennial widely distributed in alpine regions of southwestern China, and the study was conducted at the Shangri-la Alpine Botany Garden, Yunnan, China (SABG; 27°54′20″ N, 99°38′11″ E; altitude, 3260 m a.l.s.). Within this site, individual plants produce 20.0 ± 7.2 (mean ± s.d.) flowers on a single main stem (31.4 ± 7.9 cm), and 3.9 ± 2.0 flowers are open simultaneously (X.-F. Zhu 2007, unpublished data). The flowers are pendulous bell-shaped (see Zhu *et al.* (2009) for details of the floral morphology), and each flower lasts 8.0 ± 1.4 days. Like most other distylous species, this species has a strict self- and intra-morph incompatibility system (Wedderburn & Richards 1990). The number of ovules per flower in the pin morph and thrum morph is not significantly different (167.4 ± 29.5 versus 172.3 ± 33.1; $t = 0.66$, d.f. = 68, $p = 0.51$), and the ratio of pin morph to thrum morph does not statistically differ from 1 : 1 ($\chi^2 = 0.76$, d.f. = 1, $p = 0.30$).

Bombus richardsi was the most abundant visitor, accounting for 50.1 per cent of total visits to *P. secundiflora* (1319 of 2634 visits in 2007 and 2008). Other visitors included two other bumble-bee species, *Bombus securus* (36.6%) and *Bombus festivus* (5.2%), and a small unidentified bee species (7.7%). *Bombus richardsi* acted mainly as a non-robbing pollinator. However, in 32.8 per cent of visits, they acted as nectar robbers, chewing a hole through the corolla tube or reusing an existing hole, and inserting their tongues to remove nectar produced by nectaries below the ovaries (figure 1*a*). Although *B. richardsi* was active throughout the flowering seasons, it did not rob flowers until the peak season. Our observations showed that robbers had no preference for pin versus thrum flowers (Wilcoxon signed ranks test, $Z = -1.125$, $p = 0.26$).

(b) *Manipulation experiment*

To examine whether *B. richardsi* robbers transfer pollen between pin and thrum flowers, and thus serve as effective pollinators, we chose 62 and 60 newly opened pin and thrum flowers, respectively, and immediately tied their corollas with a fine thread. This treatment impeded legitimate visits, but nectar robbers could get nectar by piercing holes through flower tubes. We monitored the flowers

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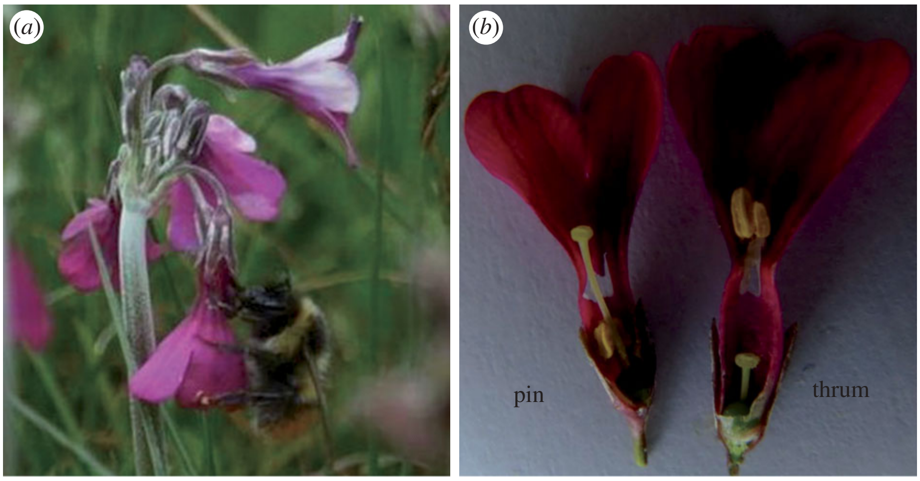

Figure 1. (*a*) *Bombus richardsi* robbing a flower; (*b*) robbed pin and thrum flowers.

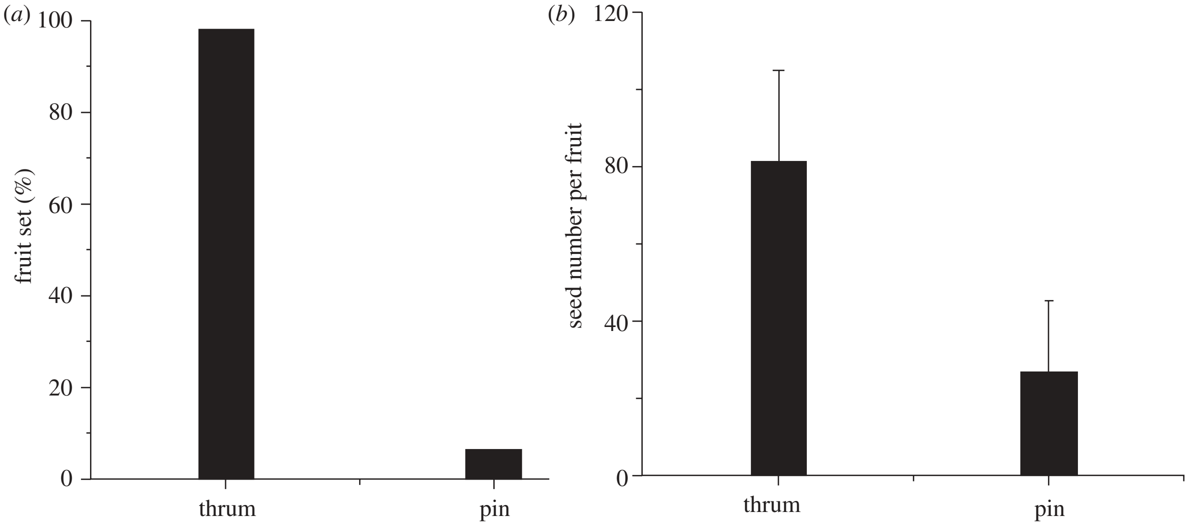

Figure 2. (*a*) Fruit and (*b*) seed set of manipulated thrum and pin flowers that could only be pollinated by nectar robbers, not legitimate visitors (both $p < 0.01$).

until their corollas shed to judge whether or not they were robbed. One month later, we measured fruit and seed sets of robbed and non-robbed flowers. Fruit set and seeds per fruit were compared between morphs using a Fisher's exact test and a Mann–Whitney *U*-test.

3. RESULTS

Anthers of pin flowers showed clear scrapes, indicating pollen had been removed, while thrum flowers had fresh pollen grains deposited on their stigmas immediately after flowers were robbed. Among all the tied flowers robbed by bumble-bees, thrum flowers had a higher probability of setting fruit than pin flowers (Fisher's exact test, $p < 0.01$). Almost all thrum flowers (49/50, 98%) set fruit while only a small proportion of pin flowers set fruit (3/49, 6%). Seeds per thrum fruit were also significantly higher than that averaged from the three pin fruits (thrum: 81.1 ± 23.8, pin: 26.7 ± 18.6; Mann–Whitney *U*-test, $p < 0.01$) (figure 2). For tied flowers that were not robbed, neither thrum flowers (12) nor pin flowers (11) set fruit.

4. DISCUSSION

Because *P. secundiflora* is self- and intra-morph incompatible, successful reproduction relies on pollen transfer between two floral morphs by insect visitors. Our study showed that, even when 'robbing' nectar by cutting holes though the corolla, the bumble-bee *B. richardsi* could still be an effective pollinator. However, their effectiveness is asymmetrical, with high success in pollinating thrum flowers but low success in pollinating pin flowers (figure 2). This result suggests that it is mechanically unlikely for the nectar robbers to contact stigmas of pin flowers and anthers of thrum flowers. In fact, we directly observed legitimate bumble-bee visitors occasionally trying to visit manipulated flowers through tied corollas. Since stigmas of pin flowers are close to the corolla mouth, this behaviour could potentially result in a few tied pin flowers being pollinated, as indicated by the 6 per cent fruit set of pin flowers (figure 2*a*). In contrast, stigmas of thrum flowers are positioned at the bottom of corolla tubes, and it is unlikely that corolla-probing bumble-bees could contact stigmas when corollas were tied. The only explanation for the high fruit set of robbed thrum flowers is that they are pollinated by nectar robbers. Moreover, the seed set per fruit of robbed thrum flowers was 88.5 per cent of natural thrum fruit set during the same period (81.1 ± 23.8 versus 91.7 ± 27.8). Therefore, bumble-bee nectar robbers appear to be an important part of this pollination system.

Unlike other species in the Sect. *Prolifera* whose flowers are goblet-shaped and horizontal-faced, *P. secundiflora* has pendulous flowers with bell-shaped corollas (Richards 2002). Legitimate visitors have to hang under corollas when visiting pendulous flowers, which requires more experience. We observed that some legitimate bumble-bee visitors fell off flowers owing to unskilled landings. However, the inverted bell-shaped flowers are well-suited to nectar robbers (figure 1*a*). Thus, robbing is apparently easier for bumble-bees than legitimate visiting.

Nectar robbing is assumed to be common in flowering plants, especially those with tubular flowers or flowers with nectar spurs (Irwin & Maloof 2002), and most distylous species have tubular flowers (Ganders 1979). However, to our knowledge, this is the first report of robber pollination in distylous plants. It is likely that nectar robbing in other distylous species could similarly result in pollination. Our results further suggest that robber pollination promotes asymmetrical pollen flow between different floral morphs of distylous species. This scenario, occurring commonly in distylous species, has been suggested as a driver of gender specialization of floral morphs (Lau & Bosque 2003). Because nectar robbers promote asymmetrical pollen flow, resulting in increased male fitness of pin morphs and female fitness of thrum morphs, nectar robbing may act as an important selective agent in floral fitness and floral evolution.

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