New Phytologist

Research

Selective seed abortion induced by nectar robbing in the selfing plant *Comastoma pulmonarium*

Chan Zhang[1,2], Rebecca E. Irwin[3], Yun Wang[1,4], Ya-Ping He[5], Yong-Ping Yang[1,4] and Yuan-Wen Duan[1,4]

[1]Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences, Lanhei Road 132, Heilongtan, Kunming 650204, Yunnan, China; [2]Graduate University of the Chinese Academy of Sciences, Beijing 100049, China; [3]Biology Department, Dartmouth College, Hanover, NH 03755, USA; [4]Institute of Tibetan Plateau Research at Kunming, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China; [5]Sichuan Academy of Forestry, Chengdu 610081, China

Authors for correspondence:
Yong-Ping Yang
Tel: +86 871 522 3231
Email: yangyp@mail.kib.ac.cn

Yuan-Wen Duan
Tel: +86 871 522 3231
Email: duanyw@mail.kib.ac.cn

Received: *26 February 2011*
Accepted: *3 May 2011*

New Phytologist (2011) **192**: 249–255
doi: 10.1111/j.1469-8137.2011.03785.x

Key words: *Comastoma pulmonarium* (Gentianaceae), the inbreeding depression, nectar robbing, Qinghai–Tibet Plateau, resource limitation.

Summary

- Self-pollination often provides plants with the benefit of reproductive assurance; thus, it is generally assumed that species' interactions that alter floral attractiveness or rewards, such as nectar robbing, will have little effect on the seed production of selfing species. We challenge this view with experimental data from *Comastoma pulmonarium*, a selfing annual experiencing a high ratio of nectar robbing in the Qinghai–Tibet Plateau.
- We manipulated robbing (robbed or netted) and pollination mode (hand-selfed or hand-outcrossed) in a factorial design and measured the number of developing ovules and mature seeds, together with seed weight and seed germination, in each treatment.
- Robbing decreased the number of mature seeds, but not the number of developing ovules, suggesting a negative influence of robbers through indirect effects via selective seed abortion. We found no evidence for early-acting inbreeding depression, but found later-acting inbreeding depression. Our data also suggested that later-acting inbreeding depression of progeny from robbed flowers could be reduced in comparison with that from unrobbed flowers.
- We suggest that nectar robbing can have both negative and positive effects on the quantity and quality, respectively, of progeny produced in selfing plants, and challenge the view that robbing has no effect on selfing species.

Introduction

Plant mating systems can have a profound influence on how species' interactions translate into effects on plant fitness (e.g. Burkle *et al.*, 2007; Zhang *et al.*, 2009a; Irwin *et al.*, 2010). For example, for plants that are self-incompatible and require pollinators to vector pollen, biotic and abiotic factors can strongly affect plant reproduction via changes in pollinator visitation, assuming that plants are pollen- or pollinator-limited for seed set (Herrera, 1995; Aizen, 2003; Burkle *et al.*, 2007; Zhang *et al.*, 2009a). Alternatively, for plants that are self-compatible and do not require pollinators for pollen transfer, biotic and abiotic factors that alter pollinator visitation are presumed not to strongly affect the seed production of selfing species. Here, we challenge this paradigm by suggesting that resource limitation and reallocation within selfing plants can influence how species' interactions that typically alter pollination can affect the seed production of a selfing species. To do so, we studied the effects of nectar robbing bumble bees on the reproductive success of a selfing plant.

Nectar robbers are floral visitors that remove nectar through holes pierced or bitten in flower tissue (Inouye, 1980). Nectar robbing can have a range of effects on plant fitness, including negative, neutral and positive effects (reviewed in Maloof & Inouye, 2000; Zhang *et al.*, 2006; Irwin *et al.*, 2010). The effect of robbers on plant reproduction can occur through both direct and indirect pathways of species' interactions. Direct effects can occur when robbers damage floral reproductive structures (Traveset *et al.*, 1998; Deng *et al.*, 2004) or act as pollinators (i.e. robber-like pollinators) in the same or different floral visits (Higashi *et al.*, 1988; Arizmendi *et al.*, 1996; Zhu *et al.*, 2010). Indirect effects of robbers have most often been cited via changes in pollination, for example, by altering pollinator behavior and patterns of plant and flower visitation (Zimmerman & Cook, 1985; Richardson, 1995; Irwin & Brody, 1998, 1999, 2000; Maloof, 2001; Irwin, 2003, 2006). These changes in pollinator behavior could have positive or negative effects on pollen flow and plant reproduction (Irwin *et al.*, 2010).

The majority of studies on the effects of robbing on plant reproduction have focused on outcrossing species and, to our knowledge, less attention has been given to the effects of nectar robbers on selfing plant species. This lack of study of the effects of robbing on selfing species may be a bias driven by the assumption that floral visitation of any type (legitimate or larcenous) does not affect seed production of selfing plants, assuming little early-acting inbreeding depression (Hauser & Loeschcke, 1996; Daehler, 1999). Thus, it is generally assumed that nectar robbers will have neutral effects on the reproduction of predominantly selfing plants (e.g. Zhang *et al.*, 2009b). Nevertheless, scenarios exist in which nectar robbers could affect the reproduction of selfing species, although they are rarely tested. For example, if we assume that resources allocated to flowers are limited, subsequent nectar secretion induced by nectar robbing could consume part of the resources that should have been used for the development of fertilized ovules. Studies have shown that nectar removal and subsequent replenishment can exact a cost on seed production (e.g. Pyke, 1991; Ornelas & Lara, 2009). In this case, nectar robbing could induce seed abortion as a result of resource limitation caused by nectar replenishment. Alternative scenarios also exist whereby nectar robbers could affect the reproduction of selfing species, including direct damage caused by robbers to floral reproductive structures. One caveat is that, if early-acting inbreeding depression does occur in selfing species, experiments are required that disentangle the effects of robbing vs early-acting inbreeding depression; doing so requires experiments that manipulate both robbing and selfed vs outcrossed pollination and measure the effects on plant reproduction.

In this study, we compared the numbers of developing ovules and mature seeds in hand-selfed and outcrossed flowers under netting (unrobbed) and open (robbed) conditions in *Comastoma pulmonarium* (Turczaninow) Toyokuni (Gentianaceae), a selfing annual of the Qinghai–Tibet Plateau that is nectar robbed by bumble bees. In addition, we compared the magnitude of inbreeding depression in progeny from unrobbed and robbed flowers. First, we measured pollinator visitation, nectar production and robbing for *C. pulmonarium* to document the lack of pollinator visitation and the commonality of nectar robbing. We then used our experiment to address the following questions: Does nectar robbing affect the number of developing ovules and/or mature seeds? Does early-acting inbreeding depression influence the degree to which robbing affects seed production? Compared with progeny from unrobbed flowers, does the magnitude of inbreeding depression change in progeny from robbed flowers?

Materials and Methods

Study site

Our studies were carried out at the Haibei Alpine Meadow Ecosystem Research Station, Chinese Academy of Sciences, from July to September in 2001, 2009 and 2010. This field station is located on the northeast Qinghai–Tibet Plateau (latitude 37°29′–37°45′N, longitude 101°12′–101°23′E and altitude 3200 m) and, at this station from 1957 to 1997, the average annual air temperature was −1.7°C with extremes of 27.6°C (maximum) and −37.1°C (minimum); the average annual precipitation ranged from 426 to 860 mm, 80% of which fell in the summer from late May to early September (Zhao & Zhou, 1999).

Study species

Comastoma pulmonarium is an alpine annual inhabiting meadow slopes, alpine meadows and river banks at altitudes ranging from 2170 to 4800 m, and is 5–30 cm in height. *Comastoma pulmonarium* flowers in July and August, and produces two to seven flowers per plant (mean ± SE, 3.6 ± 0.1 flowers per plant, n = 125 plants). The flowers are tubular and pale blue with five corolla lobes. There are two whorls of fringed white fimbriae at the base of elliptic lobes, covering the anthers and stigma completely. Ten nectaries are situated at the base of the corolla tube. At our study site, *C. pulmonarium* is fully self-compatible, and a single flower lasts *c.* 4 d. Almost no seed is produced after emasculation in the bud stage, and there is no significant difference in seed set between netted flowers and naturally pollinated flowers (C. Zhang *et al.*, unpublished), suggesting predominantly autonomous selfing in *C. pulmonarium.*

Field methods

Observations of floral visitors Floral visitors to *C. pulmonarium* were observed during peak bloom in one population in 2001 and in three populations in 2009. Each day before observations, 20 open flowers on different individual plants were labelled. We observed these flowers from 09:00 h to 17:00 h. In total, we carried out observations for 20 h across 5 d in 2001 and 60 h across 12 d in 2009. During these observations, we stayed 2 m away from the focal flowers to observe all the floral visitors without disturbing their foraging behaviors. For pollinating visits, we only recorded visits from species that appeared to contact the anthers and/or stigmas. During all observation periods, the weather was warm and without strong wind.

Survey of flowers for nectar robbing At the end of the flowering season in 2001 and 2010, we haphazardly selected plants and examined all the flowers on these plants to determine whether they had been robbed and how many holes there were per robbed flower. In total, we examined 223 flowers in 2001 and 445 flowers in 2010. In addition, we examined the specimens of *C. pulmonarium* in four herbaria [Institute of Botany (PE), Northwest Institute of Plateau Biology (HNWP), Kunming Institute of Botany (KUN) and Northwest Agricultural and Forestry University of Science and Technology (WUK)] to determine the occurrence of nectar robbing on the specimens collected in different sites in China from 1918 to 2008. Robbed flowers and the number of robbing holes can be easily viewed on herbarium specimens (C. Zhang *et al.*, pers. obs.).

Nectar production We examined the nectar production of *C. pulmonarium* in 2009. We netted 60 flowers at 08:00 h to exclude floral visitors, and measured the nectar volume using microcapillary tubes 24 h later.

Effects of nectar robbing and pollination on plant reproduction To test the effects of nectar robbing and pollination on the reproduction of *C. pulmonarium*, we selected 240 flowers each on different plants in 2010. We randomly assigned flowers to one of four treatments (60 flowers per treatment), representing a factorial cross of robbing treatment (robbed or unrobbed) by pollination mode (selfed or outcrossed). All flowers were emasculated as buds to prevent self-pollination, and only the terminal flowers were used for this experiment to control for any effect of flower position on ovule number. For the robbing treatment, flowers were either netted with fine nylon bags with 1-mm mesh to exclude robbers or left open for robbers to visit them naturally. For the pollination mode, self-pollinated flowers were hand-pollinated using pollen grains from the same plant. Outcrossed flowers were hand-pollinated with pollen grains from another plant 10 m away from the receptive plant. Pollen grains used in the hand-pollinations were from recently dehisced anthers to ensure pollen viability.

Undeveloped ovules (including aborted and unfertilized ovules) disappear when fruits are mature; therefore, we collected half of the flowers in each treatment (n = 30 flowers per treatment) to determine the number of developing ovules 2 wk after hand-pollination. The other half of the flowers in each treatment (n = 30 flowers per treatment) were collected when fruits were mature, but just before dehiscence, to determine the number of mature seeds per fruit. For the group exposed to robbers, if no hole was found in the corolla when we were collecting fruits for the number of ovules or seeds, we did not include the fruit. In total, we discarded six fruits. Occasionally, grazing by sheep occurred in the study site, and we could not find some labelled flowers in both the netting and robbing treatments. Collectively, the total sample size decreased to 223.

To examine whether nectar robbing reduced female fitness estimates, we used a two-way ANOVA, with robbing treatment (robbed and unrobbed) and pollination mode (selfing and outcrossing) as fixed factors, to compare the numbers of developing ovules and mature seeds. If a significant and negative effect of robbing on the number of developing ovules and seeds was found, this would suggest a direct damaging effect of robbing on female reproduction. However, if it was found that robbing only affected seed production and not developing ovules, this would suggest that any effect of robbing is more indirect, potentially through changes in selective seed abortion and resource limitation during seed development. If selfing negatively affects plant reproduction, we would expect to find an effect of pollination mode on the number of developing ovules and/or mature seeds, with selfed progeny producing fewer developing ovules and mature seeds than outcrossed progeny. An interaction between robbing and pollination mode would suggest that robbing alters how pollen quality (selfed vs outcrossed) affects developing ovules and/or seeds.

Inbreeding depression In the laboratory, all seeds from the robbing and pollination treatments above were combined within treatment and air dried. To assess whether robbing and pollination mode affected seed weight, we weighed 12 replicates of 100 seeds from each treatment to the nearest 0.1 mg. To test whether robbing and pollination mode affected seed germination, all seeds were kept at −20°C for 1 month to break dormancy, and seeds from each treatment were placed in three small Petri dishes with wet filter paper. The three small Petri dishes were placed in a larger Petri dish with water inside to prevent seed dehydration, and the seeds were germinated under 20°C in an incubator with a 12-h light : 12-h dark photoperiod. We recorded the number of germinated seeds each day. If there was no increase in the number of germinated seeds for five consecutive days in the Petri dishes, we ceased observation and recorded the number of seeds that did not germinate in the Petri dishes to calculate the seed germination rate per dish.

We used a two-way ANOVA, with robbing treatment (robbed and unrobbed) and pollination mode (selfing and outcrossing) as fixed factors, to compare the seed mass and germination rate. An interaction between robbing and pollination mode would suggest that robbing alters the magnitude of inbreeding depression. Seed germination rates were power-transformed using Box-Cox methods before comparisons. Inbreeding depression (δ) under netting and robbing conditions was estimated at three stages: seed number, seed mass and seed germination. The magnitude of inbreeding depression at each stage was calculated using

$\delta = 1 - (W_s/W_o)$, where W_s and W_o are the mean fitness of selfed and outcrossed progeny, respectively. Cumulative inbreeding depression was calculated by multiplying fitness values for each treatment across the life stage and then applying the formula above.

Results

Observations of flower visitors, survey of flowers for nectar robbing and measurements of nectar production

We observed no pollinators visiting *C. pulmonarium* flowers during 80 h of observations to 240 flowers in 2 yr. The only frequent floral visitors observed were workers of *Bombus kashmirensis*, which chewed a hole in the base of the tubular corolla to rob the flowers of nectar without touching the anthers and/or stigma.

We also surveyed flowers at the end of the flowering season for nectar robbing holes and found that *C. pulmonarium* experienced a high ratio of robbing in 2001 and 2010. More than 70% of surveyed flowers were robbed at least once, and a small fraction of flowers had multiple robbing holes (two to five times; Fig. 1). From the herbarium records, we found that nectar robbing occurred in 59 collecting sites at altitudes from 2170 to 4800 m in Qinghai, Sichuan, Xizang and Yunnan, covering most of the distribution range of *C. pulmonarium* in China.

The average nectar production of netted flowers was 5.84 ± 0.27 µl per 24 h (mean ± SE, $n = 60$ flowers).

Effects of robbing and pollination on plant reproduction

The number of developing ovules was not affected significantly by robbing treatment (Table 1; Fig. 2a,b), suggesting that robbing did not damage the stigma, style or ovary directly. However, the number of mature seeds was affected significantly by robbing (Table 1). Relative to netted flowers, robbing reduced seed production by 10.4% and 10.3% in selfed and outcrossed flowers, respectively (Fig. 2b). This result suggests that the negative effect of robbing on the number of mature seeds is more indirect, occurring during seed development.

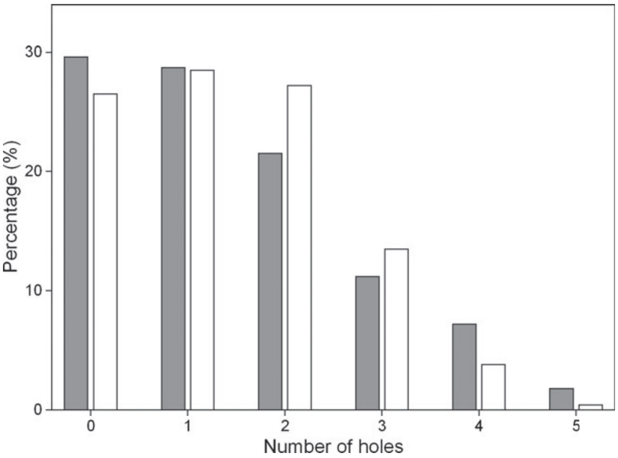

Fig. 1 Frequency of the number of nectar-robbing holes in flowers of *Comastoma pulmonarium* in 2001 (closed bars) and 2010 (open bars).

We found no effect of pollination mode (selfed vs outcrossed) on the number of either developing ovules or mature seeds (Table 1; Fig. 2), suggesting that selfing does not negatively affect these two measures of plant reproduction. In addition, we found no robbing treatment by pollination mode interaction (Table 1), suggesting that robbing did not modify how pollen quality (selfed vs outcrossed) affected the number of developing ovules or mature seeds.

Inbreeding depression

We found evidence of later-acting inbreeding depression, measured at the seed mass and seed germination stages (Table 1; Fig. 3). Seed mass and seed germination were significantly negatively affected by inbreeding, but not by robbing treatment and the pollination by robbing interaction (Table 1). However, the magnitude of later-acting inbreeding depression was higher for netted (unrobbed) flowers relative to robbed flowers (Fig. 3), although a two-way ANOVA with robbing and pollination mode did not detect a statistically significant interaction between the two factors (Table 1). Interestingly, the cumulative inbreeding depression was much greater than 0.5 under both netting and robbing conditions.

Discussion

We found that *C. pulmonarium*, an alpine selfing annual in the Qinghai–Tibet Plateau, produces nectar, and the volume of nectar produced is comparable with that of animal-pollinated plants (e.g. Fenster *et al.*, 2006; Huang & Fenster, 2007). However, we observed no pollinators visiting the flowers of this species during peak blooming of the population. Although one could argue that we missed important pollinators that could have foraged at the beginning or end of the flowering season, the lack of pollinator visitation supports our unpublished findings that *C. pulmonarium* is primarily a selfing species. The only observed flower visitors at our study site were nectar robbers who consumed the nectar without pollinating. Because of the wide occurrence and high ratio of nectar robbing of *C. pulmonarium* measured at the end of the flowering season, we wanted to determine whether nectar robbing had any effect on the reproduction of this selfing species. Our results showed that nectar robbing was associated with a

Table 1 Two-way ANOVAs of ovule number, seed number, seed mass and seed germination rate from *Comastoma pulmonarium* flowers subjected to different pollination modes (selfing and outcrossing) and robbing treatments (netted and robbed)

	Ovule number				Seed number				Seed mass				Seed germination rate			
Source	Sum Squ.	df	*F*	*P*	Sum Squ.	df	*F*	*P*	Sum Squ.	df	*F*	*P*	Sum Squ.	df	*F*	*P*
Pollination	0.02	1	< 0.01	0.99	0.20	1	< 0.01	0.97	307.9	1	134.5	< 0.01	0.22	1	25.9	< 0.01
Robbing	10.15	1	0.4	0.85	1339.5	1	6.95	0.01	0.03	1	0.01	0.91	0.02	1	2.12	0.18
Pollination × robbing	0.003	1	< 0.01	0.99	< 0.01	1	< 0.01	0.99	2.07	1	0.90	0.35	< 0.01	1	< 0.01	0.98
Total	566 263	114			470 356	109			34 031	48			3.36	12		

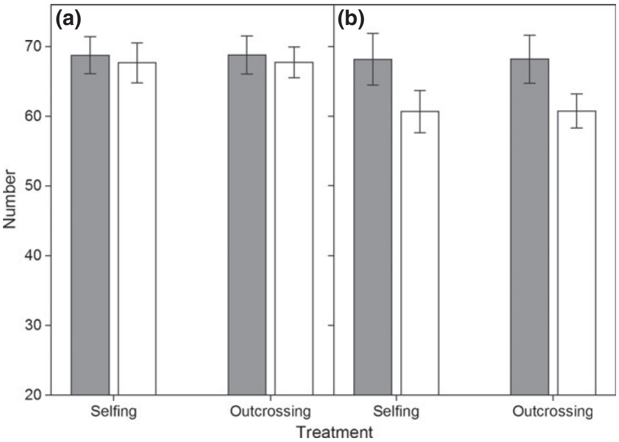

Fig. 2 Number of ovules (closed bars) and seeds (open bars) from netted (a) and robbed (b) *Comastoma pulmonarium* flowers that were either hand-selfed or hand-outcrossed. Data are shown as mean ± SE.

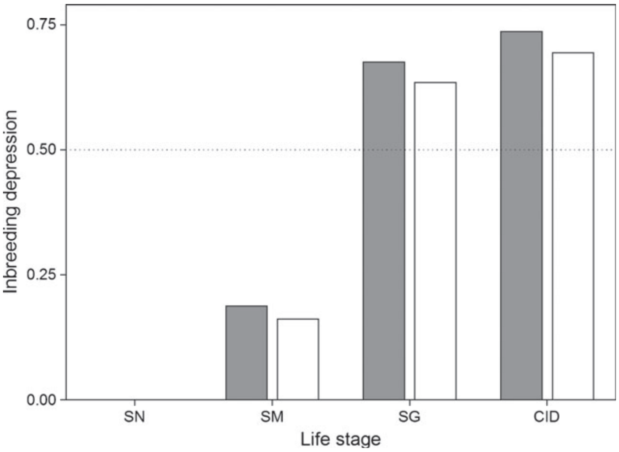

Fig. 3 Inbreeding depression of *Comastoma pulmonarium* from netted (closed bars) and robbed (open bars) flowers. CID, cumulative inbreeding depression; SG, seed germination; SM, seed mass; SN, seed number. The dotted line indicates the theoretically predicted threshold below which selfing should evolve.

reduction in the number of mature seeds, but not developing ovules, suggesting that the influence of robbing was not driven by any direct effects of damage on floral reproductive structures. Moreover, because all flowers were hand-pollinated and pollinator visitation was scarce, the reduction in seed set was not driven by changes in pollination. Instead, we speculate that the decrease in the number of mature seeds following robbing may be an indirect effect associated with selective seed abortion and resource limitation, which may have consequences for the magnitude of inbreeding depression in robbed vs netted flowers. We outline our rationale below, first discussing early-acting inbreeding depression and then linking the effects associated with nectar robbing. In addition, we highlight important additional experiments to dissect mechanistically our hypothesized causal structure.

Early-acting inbreeding depression can be an important factor in reducing fruit and seed production (Charlesworth & Charlesworth, 1987; Liao *et al.*, 2009), resulting in the abortion of homozygous offspring during embryo development because of the presence of deleterious recessive alleles (Krebs & Hancock, 1990; Husband & Schemske, 1996). The incidence of early-acting inbreeding depression can be examined by comparing embryo abortion following controlled selfing and outcrossing (Liao *et al.*, 2009). In our study, if early-acting inbreeding depression occurred in *C. pulmonarium*, the number of mature seeds should be less than that of the developing ovules in hand-selfed flowers under both netting and robbing conditions. However, this was not the case; therefore, we can conclude that early-acting inbreeding depression did not occur in *C. pulmonarium*.

Resource limitation is an important factor inducing selective abortion of flowers, fruits and seeds in plant species (Burd, 1998; Goto *et al.*, 2010). Under resource limitation, it is assumed that the developing embryos have to compete with each other for limiting resources, and selective seed abortion will increase the survival and performance of the remaining offspring (Melser & Klinkhamer, 2001; Mena-Ali & Rocha, 2005). For *C. pulmonarium*, the decrease in the number of mature seeds in hand-selfed and outcrossed flowers with robbing may have resulted from this selective seed abortion. Although we cannot evaluate the available resources in both netted and robbed flowers of *C. pulmonarium*, the disappearance of nondeveloping ovules (including aborted and unfertilized ovules) when seeds were mature indicated that the resources of the nondeveloping ovules might have been reallocated to the developing ovules, suggesting resource limitation during the development of seeds in this alpine species. To test our hypothesis experimentally would require crossing a resource manipulation treatment (water and nutrients) with our robbing and pollination treatments.

Although we can speculate on how robbing reduces the number of mature seeds, it remains unclear why robbing has this effect. In *C. pulmonarium*, nectar robbing results in a hole in the side of the corolla and the removal of nectar, but, to our knowledge, no damage to floral reproductive organs or the nectary. Would any type of damage to floral petal tissue induce the same type of plant response as robbing, or does nectar robbing, in particular, result in this particular plant response? Or, does the removal of nectar induce subsequent nectar production, which could reduce resource availability for seed production? In preliminary studies, we observed that plants would replenish nectar after hand removal of nectar in the field, suggesting that robbers may induce subsequent nectar replenishment (C. Zhang *et al.*, pers. obs.). However, detailed studies are needed to analyze quantitatively the dynamics of nectar secretion following nectar removal in *C. pulmonarium* (as in Ordano & Ornelas, 2004) and the potential costs of repeated nectar removal for seed production (as in Pyke, 1991; Ornelas & Lara, 2009). Moreover, to understand mechanistically why robbing reduces the number of mature seeds would require the manipulation of petal tissue damage and nectar removal in a factorial design and the measurement of plant response. Doing so would provide important mechanistic insights that may be applicable to the effects of robbing in other plant systems.

Although nectar robbing was associated with a reduction in the number of mature seeds, interestingly, inbreeding depression was lower in robbed than in netted flowers, estimated as seed weight and seed germination. One interpretation of this finding is that selective seed abortion in robbed flowers may buffer the magnitude of inbreeding depression. Nevertheless, the cumulative inbreeding depression was much greater than 0.5 in robbed flowers, which is above the theoretically predicted threshold below which predominant selfing should evolve in plants (Charlesworth & Charlesworth, 1987; Jarne & Charlesworth, 1993). Therefore, the decreased inbreeding depression in the seeds produced from robbed flowers was not sufficiently strong to facilitate the evolution of selfing in *C. pulmonarium.* Four caveats are important to consider in the interpretation of these inbreeding results. First, we did not detect a significant robbing by pollination mode interaction for seed weight or germination in a two-way ANOVA, which would suggest that robbing altered how pollen quality affected seed mass and germination. Thus, although our calculations of inbreeding depression were lower for robbed relative to netted flowers, the result should be viewed with caution. Our measurements of seed weight and germination had lower sample sizes than those for developing ovules and mature seeds. Future studies should increase the sample sizes for response variables at later life-history stages to assess the validity and strength of the effect of robbing on inbreeding depression. Second, flowers in the robbing treatment were open and accessible to potential pollinators. Although we did not observe any pollinators visiting flowers at our study site, we cannot rule out the possibility that rare pollination events somehow reduced later-acting inbreeding depression. We believe that this is unlikely, however, given that all flowers were hand-pollinated with selfed or outcrossed pollen in excess. Third, robbed flowers did not have netting, whereas unrobbed flowers did. Although the netting was made of fine, relatively clear mesh, we cannot rule out the possibility that the netting itself could have increased later-acting inbreeding depression as a result of changes in the plant photosynthetic rate. We believe that this result is unlikely, but could include a netting control treatment in future studies to remove any minor potential effects of the netting. Fourth, we measured the seed germination rate in the laboratory and did not examine the fitness of selfed and outcrossed progeny from robbed and netted flowers in later life stages (e.g. seedling survival, flowering time and biomass) in the field. Thus, the magnitude of inbreeding depression in the harsh environment of the Qinghai–Tibet Plateau could have been underestimated in our results.

In summary, this study examined the effects of nectar robbing on an alpine selfing plant, *C. pulmonarium*, in the Qinghai–Tibet Plateau. The results suggest that nectar robbing reduces the number of mature seeds, potentially via selective seed abortion. However, the reduction in mature seeds with robbing comes with a potential benefit, namely reduced magnitude of inbreeding depression at the seed weight and seed germination stages. How common are these results in other predominantly selfing species that experience robbing warrants further investigation.

Acknowledgements

We are grateful to two anonymous reviewers and Dr David Ackerly (Editor) for their suggestions and comments on the manuscript, Professor Ying-Nian Li for his support at the Haibei Station and Professor Jian-Quan Liu for his kind guidance in recent years. This work was financially supported by the Chinese Academy of Sciences (CAS) (Key Innovation Plan: KSCX2-YW-Z-1019), National Natural Science Foundation of China (NSFC) (31070337, 40930209), Major State Basic Research Development Program (2010CB951700) and Special Grants to CAS President Scholars (to Y.W.D.).

References

Aizen MA. 2003. Influences of animal pollination and seed dispersal on winter flowering in a temperate mistletoe. *Ecology* **84**: 2613–2627.

Arizmendi MC, Dominguez CA, Dirzo R. 1996. The role of an avian nectar robber and of hummingbird pollinators in the reproduction of two plant species. *Functional Ecology* **10**: 119–127.

Burd M. 1998. "Excess" flower production and selective fruit abortion: a model of potential benefits. *Ecology* **79**: 2123–2132.

Burkle LA, Irwin RE, Newman DA. 2007. Predicting the effects of nectar robbing on plant reproduction: implications of pollen limitation and plant mating system. *American Journal of Botany* **94**: 1935–1943.

Charlesworth D, Charlesworth B. 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* **18**: 237–268.

Daehler CC. 1999. Inbreeding depression in smooth cordgrass (*Spartina alterniflora*, Poaceae) invading San Francisco Bay. *American Journal of Botany* **86**: 131–139.

Deng XB, Ren PY, Gao JY, Li QJ. 2004. The striped squirrel (*Tamiops swinhoei hainanus*) as a nectar robber of ginger (*Alpinia kwangsiensis*). *Biotropica* **36**: 633–636.

Fenster CB, Cheely G, Dudash MR, Reynolds RJ. 2006. Nectar reward and advertisement in hummingbird-pollinated *Silene virginica* (Caryophyllaceae). *American Journal of Botany* **93**: 1800–1807.

Goto R, Okamoto T, Kiers ET, Kawakita A, Kato M. 2010. Selective flower abortion maintains moth cooperation in a newly discovered pollination mutualism. *Ecology Letters* **13**: 321–329.

Hauser TP, Loeschcke V. 1996. Drought stress and inbreeding depression in *Lychnis floscuculi* (Caryophyllaceae). *Evolution* **50**: 1119–1126.

Herrera CM. 1995. Microclimate and individual variation in pollinators: flowering plants are more than their flowers. *Ecology* **76**: 1516–1524.

Higashi S, Ohara M, Arai H, Matsuo K. 1988. Robber-like pollinators – overwintered queen bumblebees foraging on *Corydalis ambigua*. *Ecological Entomology* **13**: 411–418.

Huang SQ, Fenster CB. 2007. Absence of long-proboscid pollinators for long-corolla-tubed Himalayan *Pendicularis* species: implications for the evolution of corolla length. *International Journal of Plant Sciences* **168**: 325–331.

Husband BC, Schemske DW. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* **50**: 54–70.

Inouye DW. 1980. The terminology of floral larceny. *Ecology* **61**: 1251–1252.

Irwin RE. 2003. Impact of nectar robbing on estimates of pollen flow: conceptual predictions and empirical outcomes. *Ecology* **84**: 485–495.

Irwin RE. 2006. The consequences of direct versus indirect species interactions to selection on traits: pollination and nectar robbing in *Ipomopsis aggregata*. *The American Naturalist* **167**: 315–328.

Irwin RE, Brody AK. 1998. Nectar robbing in *Ipomopsis aggregata*: effects on pollinator behavior and plant fitness. *Oecologia* **116**: 519–527.

Irwin RE, Brody AK. 1999. Nectar-robbing bumble bees reduce the fitness of *Ipomopsis aggregata* (Polemoniaceae). *Ecology* **80**: 1703–1712.

Irwin RE, Brody AK. 2000. Consequences of nectar robbing for realized male function in a hummingbird-pollination plant. *Ecology* **81**: 2637–2643.

Irwin RE, Bronstein JL, Manson JS, Richardson L. 2010. Nectar robbing: ecological and evolutionary perspectives. *Annual Review of Ecology, Evolution and Systematics* **41**: 271–292.

Jarne P, Charlesworth D. 1993. The evolution of the selfing rate in functionally hermaphrodite plants and animals. *Annual Review of Ecology and Systematics* **24**: 441–466.

Krebs SL, Hancock JF. 1990. Early-acting inbreeding depression and reproductive success in the highbush blueberry, *Vaccinium corymbosum* L. *Theoretical and Applied Genetics* **79**: 825–832.

Liao WJ, Hu Y, Zhu BR, Zhao XQ, Zeng YF, Zhang DY. 2009. Female reproductive success decreases with display size in monkshood, *Aconitum kusnezoffii* (Ranunculaceae). *Annals of Botany* **104**: 1405–1412.

Maloof JE. 2001. The effects of a bumble bee nectar robber on plant reproductive success and pollinator behavior. *American Journal of Botany* **88**: 1960–1965.

Maloof JE, Inouye DW. 2000. Are nectar robbers cheaters or mutualists? *Ecology* **81**: 2651–2661.

Melser C, Klinkhamer PGL. 2001. Selective seed abortion increases offspring survival in *Cynoglossum officinale* (Boraginaceae). *American Journal of Botany* **88**: 1033–1040.

Mena-Ali JI, Rocha OJ. 2005. Selective seed abortion affects the performance of the offspring in *Bauhinia ungulata*. *Annals of Botany* **95**: 1017–1023.

Ordano M, Ornelas JF. 2004. Generous-like flowers: nectar production in two epiphytic bromeliads and a meta-analysis of removal effects. *Oecologia* **140**: 495–505.

Ornelas FJ, Lara C. 2009. Nectar replenishment and pollen receipt interact in their effects on seed production of *Penstemon roseus*. *Oecologia* **160**: 675–685.

Pyke GH. 1991. What does it cost a plant to produce floral nectar? *Nature* **350**: 58–59.

Richardson SC. 1995. Nectar-robbing bees as cheaters on a plant–pollinator mutualism. *Bulletin of the Ecological Society of America* **76**: 380–381.

Traveset A, Willson MF, Sabag C. 1998. Effect of nectar-robbing birds on fruit set of *Fuchsia magellanica* in Tierra del Fuego: a disrupted mutualism. *Functional Ecology* **12**: 459–464.

Zhang YW, Wang Y, Guo YH. 2006. The effects of nectar robbing on plant reproduction and evolution. *Chinese Journal of Plant Ecology (formerly Acta Phytoecologica Sinica)* **30**: 695–702.

Zhang YW, Yang CF, Zhao JM, Guo YH. 2009a. Selective nectar robbing in a gynodioecious plant (*Glechoma longituba*) enhances female advantage. *Journal of Evolutionary Biology* **22**: 527–535.

Zhang YW, Yu Q, Zhao JM, Guo YH. 2009b. Differential effects of nectar robbing by the same bumble-bee species on three sympatric *Corydalis* species with varied mating systems. *Annals of Botany* **104**: 33–39.

Zhao XQ, Zhou XM. 1999. Ecological basis of alpine meadow ecosystem management in Tibet: Haibei Alpine Meadow Ecosystem Research Station. *Ambio* **28**: 642–647.

Zhu XF, Wan JP, Li QJ. 2010. Nectar robbers pollinate flowers with sexual organs hidden within corollas in distylous *Primula secundiflora* (Primulaceae). *Biology Letters* **6**: 785–787.

Zimmerman M, Cook S. 1985. Pollinator foraging, experimental nectar-robbing and plant fitness in *Impatiens capensis*. *American Midland Naturalist* **113**: 84–91.