

Sarah C. Richardson

## Are nectar-robbers mutualists or antagonists?

Received: 15 May 2003 / Accepted: 14 January 2004 / Published online: 7 February 2004  
© Springer-Verlag 2004

**Abstract** As “exploiters” of plant-pollinator mutualisms, nectar-robbers remove rewards (nectar) without providing pollination services. Though one might expect nectar-robbing to be costly to plants, it may instead benefit plants by indirectly increasing pollen dispersal. I investigated the direct effects of nectar-robbing bees (*Xylocopa californica*) on floral rewards and behaviors of pollinators visiting desert willow (*Chilopsis linearis*) and indirect effects of robbing on the reproductive success of the plant. Nectar-robbers reduced nectar; while unrobbed and robbed flowers were equally likely to contain nectar, nectar volumes were smaller in robbed flowers with nectar. *Apis mellifera* (honeybees), ineffective pollinators in terms of pollen deposition, avoided robbed flowers. In contrast, *Bombus sonorus* (bumblebees), effective pollinators, did not avoid robbed flowers. While bumblebees tended to spend less time in robbed flowers, the time that they spent in flowers was not correlated with pollen deposition. Using powder mimicking pollen, I found that on some days, powder was dispersed farther or to more flowers from robbed flowers, indicating that robbing may sometimes benefit plants by increasing male reproductive success. Powder movement suggested that the effect of robbing on male reproductive success ranged from costly to beneficial. The outcome for flowers that were marked early each morning was a function of prevalence of robbing and abundances of effective pollinators, but not a function of spatial variability among trees in prevalence of robbing or the abundance of ineffective honeybees. Unlike powder dispersal, female reproductive success, measured by fruit set and the number of pollen tubes growing in styles, was not affected by robbing. Thus, robbers did not reduce plants’ female reproductive success either directly by damaging flowers or indirectly by reducing pollen

deposition by pollinators. Overall, this study indicates that nectar-robbers were not often costly to plants, and sometimes even benefited plants.

**Keywords** Conditional outcome · Indirect effects · Mutualism · Nectar-robbing · Reproductive success

### Introduction

Recently, researchers have become interested in the balance between conflict and cooperation in mutualisms. Mutualists exchange rewards or services, but may only benefit each other under certain conditions (Bronstein 1994; Bronstein 2001a) or may switch back and forth from antagonism to mutualism (Pellmyr et al. 1996; Johnson et al. 1997; Lara and Ornelas 2001). Given that mutualists can both cooperate and exploit each other, how will the intrusion of other species affect the interaction? For example, how will mutualists be affected if a species from outside of the mutualism removes a reward that a mutualist offers its partner?

Some theories of the evolution of mutualism predict that this “exploiter” of a mutualism (sensu Bronstein 2001b) will have a negative effect on one mutualist by competing with its partners for rewards, thus indirectly reducing services provided to it (reviewed in Pellmyr 1997; Bronstein 2001b; Yu 2001). Alternatively, some means of “punishing” the exploiter (Bshary and Grutter 2002), controlling the exploiter without excluding mutualists (Noë and Hammerstein 1994; Pellmyr and Huth 1994; Schwartz and Hoeksema 1998; Agrawal 2000; Denison 2000), remembering partner quality (Bshary and Schaffer 2002), or competition between mutualists and exploiters (Letourneau 1991; Ferriere et al. 2002) may limit the negative effects of an exploiter.

As exploiters of the plant-pollinator mutualism, nectar-robbers take nectar from a flower by cutting a slit in the corolla and removing nectar, usually without pollinating. Emerging evidence suggests that the effect of nectar-robbers on plants can range from costly to beneficial

S. C. Richardson (✉)  
Indiana University, Department of Biology,  
Jordan Hall 015,  
Bloomington, IN, 47405, USA  
e-mail: srichard@bio.indiana.edu  
Fax: +1-812-8556705

depending on the identity of nectar-robbers and the breeding system of plants (reviewed in Maloof and Inouye 2000; Irwin et al. 2001). Only the combined effects of robbing mediated by pollinator communities as a whole have been investigated. However, for a generalist plant, visitors may differ in their effectiveness at depositing pollen. If these visitors also differ in their behaviors in response to robbing, the outcome of interaction between nectar-robbers and the plant may also differ, depending on the identity of pollinators. Thus, spatial or temporal variation in the outcome of the interaction may be produced by changes in pollinators' abundances.

In the Sonoran desert in Arizona, USA, the abundant carpenter bee, *Xylocopa californica*, robs nectar from the desert willow tree, *Chilopsis linearis* (Bignoniaceae). Two other common visitors, *Apis mellifera*, honeybees, and *Bombus sonorus*, bumblebees, differ in their effectiveness at depositing pollen; honeybees usually do not deposit measurable pollen, whereas bumblebees are very effective. In addition, honeybees can cost the plant by causing stigmas to close, preventing later pollen deposition (Richardson, unpublished data). This system is particularly well suited to investigate the effects of nectar-robbing for several reasons. (1) The plant is pollen-limited (Richardson, unpublished data), so pollen delivery by pollinators is important for its reproductive success; (2) the plant is self-incompatible (Richardson, unpublished data) and cornucopia flowering (sensu Gentry 1974), producing hundreds of flowers at one time. Hence, there is a potential conflict between mutualists: benefits to a plant are greater if pollinators visit a few flowers and then leave the plant (Klinkhamer et al. 1994). However, pollinators have the option of foraging at multiple flowers at one plant, reducing the cost of flight between plants. For this reason, any effect that will cause pollinators to leave a plant with its pollen will potentially increase the male reproductive success of the plant; (3) *Chilopsis* does not possess an obvious mechanism to exclude or punish nectar-robbing bees. Gentry (1990) hypothesized that other members of Bignoniaceae have clustered flowers or stiff calyces in order to protect flowers from nectar-robbers. However, *Chilopsis* flowers are not tightly clustered and do not have calyces that prevent robbing; and (4) robbers of *Chilopsis* do not cost the plant by damaging ovaries or benefit it by pollinating as they rob. Thus, effects of nectar-robbing on reproductive success in *Chilopsis* can only occur indirectly through changes in pollinator behavior.

To determine the effect of the nectar-robbing bees on the pollination mutualism, I addressed four questions: (1) Do nectar-robbers reduce the nectar available to pollinators? (2) Does removal of this nectar affect the behavior of "legitimate" pollinators (those visitors that enter flowers and potentially transfer pollen)? (3) Is the net effect of robbing to the plant costly or beneficial for male or female components of reproductive success? If pollinators encounter robbed flowers with less nectar, male reproductive success may increase in two ways. First, pollinators leaving plants from flowers with reduced rewards may fly longer distances to subsequent plants, which could result

in pollen moving farther from close relatives of the plants and could thus decrease inbreeding (Inouye 1983; Fenster 1991). For *Bombus bimaculatus*, a single visit to a less rewarding flower is enough to affect the flight distance to the next flower (Dukas and Real 1993). Second, male reproductive success may increase if pollinators leave plants more often after encountering low-rewarding robbed flowers. In this case, pollinators may move pollen to more flowers on other plants, rather than within the same plant where it cannot fertilize flowers. Plants would also benefit because self pollen (and thus the opportunity to produce a fruit) would not be lost when stigmas closed from touches by pollinators (Richardson, unpublished data). In this second scenario, more flowers on other plants would be recipients of pollen from robbed flowers than from unrobbed flowers. (4) Is the effect of nectar-robbing on pollen dispersal correlated with either the prevalence or spatial variability of robbing? Whether robbing is costly or beneficial, one might predict that a low prevalence of exploitation may not measurably affect a mutualism, but a greater prevalence will. One might also predict that robbing only will benefit plants if its spatial distribution among trees is variable enough for pollinators to profit from searching for trees with more unrobbed flowers. Thus, if male reproductive success increases with robbing, the indirect benefit should increase with spatial variability in the prevalence of robbing among trees.

---

## Materials and methods

### Background

The tree *Chilopsis linearis* (desert willow) blooms from late May to early June in southwestern USA and Mexico. The two sites used in this study were in S. Arizona, Cochise Co., near Portal, Arizona, USA. "Desert Site" (DS) was located 6.9 km N of Portal on the San Simon Road (elevation 1,387 m, 31°57'N, 109°07'W) near the Chiricahua Mountains. At this site, trees grew in two parallel lines along the sides of a wash running through desert flats that contained nests of *Xylocopa californica*, nectar-robbing carpenter bees. Experiments were conducted at DS unless noted. The other site, "Cave Creek" (CC), was at slightly higher elevation (1,463 m, 31°54'N, 109°08'W), about 9 km from DS. Over a flowering season from 1 to 3 weeks at each site, trees produced hundreds of trumpet-shaped flowers. Trees at CC bloomed slightly later than those at DS. Trees at DS bloomed from 20 May–7 June 1993, 12–31 May 1994, 18 May–16 June 1995, and 15–22 May 1996. At CC, trees bloomed 1–13 June 1994, 13–23 June 1995, and 21 May–1 June 1996. The length of flowering season at DS, but not at CC, probably was affected by the previous years' precipitation: October–May rainfall measured at Portal (1,642 m elevation, NWS PRTA3) was 17.33 inches during 1994–5 and 2.59 inches during 1995–6 (NCDC 1999).

At both sites, the most common visitors to *Chilopsis* were bumblebees (*Bombus sonorus*), honeybees (*Apis mellifera*), and nectar-robbing bees, *Xylocopa californica* (Richardson 1995, unpublished data). Black-chinned (*Archilochus alexandri*) and broad-tailed hummingbirds (*Selasphorus platycercus*) occasionally visited flowers at DS and CC. Occasional magnificent hummingbirds (*Eugenes fulgens*) also visited flowers at CC.

Nectar in *Chilopsis* was produced as buds opened late in the afternoon, during anthesis. Nectar did not refill (Whitham 1977). The protandrous flowers usually lasted ca. 2 days. Little other floral

nectar was available until *Ipomopsis longiflora* bloomed near the end of the flowering season of CC.

When nectar-robbing bees (*X. californica*) visited *Chilopsis* flowers, they landed on the outside of the corolla without touching anthers or stigmas. They slit the corolla with the sharp galeae that covered their proboscides and removed nectar through the slit. Other visitors foraged "legitimately" through the entrance of the flower, with the exception of an occasional butterfly that visited slits made by nectar-robbers.

#### Prevalence and spatial variability of robbing

I evaluated changes in the frequency and among-tree variability of robbing over flowering seasons. To do this, I counted the percentage of robbed flowers every 2 days at 1030 hours during 1995 and 1996. I determined the percentage of robbed flowers in a sample of 100 haphazardly chosen flowers. For samples, I usually used three trees that had the most flowers open at one time. At the end of the season, I checked flowers on more trees to complete the survey of 100 flowers. In order to determine the among-tree variability of robbing, I calculated the CV of numbers of flowers on each tree for each day of the survey on which only three trees were needed for the survey.

#### Measurement of nectar

I compared amounts of nectar remaining in robbed and unrobbed flowers by sampling nectar in both types of flowers with a micropipette at several times over the day. Even though the number of visits to each type of flower was unknown, I predicted that as a group, robbed flowers would contain less nectar than unrobbed flowers measured at the same time of day because robbed flowers must have been visited at least once, whereas unrobbed flowers may have been unvisited. I sampled nectar during the first morning that flowers were open to visits by legitimate visitors (day 1) on six days in 1993, 1994 and 1995 at DS. On two of those days and on two additional days in 1994 at CC, I sampled nectar from bagged, unvisited flowers in order to compare the amount accumulated in 1-day-old flowers. On one additional day in 1993, I sampled open flowers of unknown age.

I determined whether nectar continued to be produced during mornings using two methods because many flowers had no nectar. First, I compared proportions of unvisited, bagged flowers with nectar present over time, since I observed that some flowers never held nectar. I hypothesized that this desert plant could have been producing empty flowers or reabsorbing nectar from older flowers. For this test, I used a *G*-test and grouped unvisited samples into 2-h intervals. Second, I determined whether amounts of nectar in unvisited flowers that contained nectar continued to increase during the first morning the flowers were open. For this test, I used a one-way Kruskal-Wallis test for each 2-h interval. I also used two methods to test for differences in amounts of nectar between robbed and unrobbed flowers. First, for each 2-h interval, I compared the proportion of robbed and unrobbed flowers that contained nectar, using a *G*-test. Second, for those flowers that contained nectar, I compared amounts of nectar present in robbed and unrobbed flowers, grouping all time periods and using a Mann-Whitney U-test.

#### Pollinator behavior

From 1993 to 1996 at DS and CC, I determined the behavior of pollinators at robbed flowers. First, I tested the hypothesis that legitimate visitors spent less time during visits to robbed flowers than to unrobbed flowers. In 34 half-hour observation periods, I used colored thread on flower pedicels to mark approximately 100–200 robbed and unrobbed flowers in one part of a tree (i.e., a patch). During observation six, I stood on a six foot ladder about 2–3 m

away. For each species of legitimate visitor, I measured length of visits to robbed and unrobbed flowers and compared them using a Mann-Whitney U-test.

Second, I hypothesized that legitimate visitors avoided robbed flowers within patches. For each of the 34 observation periods, I determined the proportion of unrobbed flowers that visitors entered and compared that to the proportion available in the patch. I assumed that if visitors avoided robbed flowers, then individuals visiting fewer robbed flowers than expected from their representation in the patch would outnumber individuals visiting more robbed flowers than expected. I tested this hypothesis using a log-likelihood ratio goodness of fit test for each species of visitor. For this hypothesis, I focused on honeybees and bumblebees because they were the most common species of legitimate visitors during the years studied (Richardson 1995, unpublished data).

In a third experiment, I further investigated the behavior of honeybees at robbed flowers. After finding that honeybees avoided robbed flowers (see Results), I tested whether they used the presence of slits in flowers as a cue to identify robbed flowers. On eight days over two seasons, I bagged about 100 buds in a patch on a tree. Two days later, I unbagged the open, unvisited flowers and removed other flowers within the patch. I slit the flowers of one-half of the patch with a razor blade in order to simulate robbing. I touched all the flowers on the other half of the patch, but did not slit them. I did not remove nectar from any flowers. Over 1-h observation periods, I observed the number of flowers on each side of the patch that each honeybee visited. I hypothesized that if honeybees avoided robbed flowers using visual or odor cues from slits, then the proportion of bees that visited more slit (robbed) flowers would be less than expected by chance alone. I tested this hypothesis using a Wilcoxon signed-rank test with each visitor as an observation.

In a fourth experiment, I tested whether visit length was correlated with amount of pollen deposited by bumblebees. At CC, I bagged flowers as buds in order to prevent visitation, then unbagged them 2 days later when stigma lobes were open and receptive. I recorded the length of time that bumblebees spent visiting each flower. After each visit, I collected the stigma and stained it with Alexander's stain (Alexander 1980) in order to count pollen grains on the stigmas. I tested whether there was a correlation between visit length and pollen deposition using a Spearman rank correlation.

Finally, in the fifth experiment, I tested one prediction from the hypothesis that robbing increases pollen dispersal because pollinators are more likely to depart after encountering robbed flowers. One prediction of this hypothesis is that the last flower that a pollinator visits before leaving the plant should be a robbed flower more often than expected. To test this prediction, I observed bumblebees foraging on *Chilopsis* trees at DS and CC during 17 observation periods of 0.5 h. During each observation period, I observed departing bumblebees leaving a tree and determined whether the last flower visited was robbed or unrobbed. If bees' behaviors were not affected by robbery, the proportion of bees departing from robbed flowers should equal the proportion of robbed flowers available on the plant. I tested this prediction using a paired sign test that compared the predicted and observed proportions of bees that departed from robbed flowers during each observation period. Thus, each observation period was a data point.

#### Effects of robbing on dispersal of powder mimicking pollen

If pollinators are more likely to depart trees after visits to robbed flowers, pollen from robbed flowers should pollinate more flowers on neighboring trees, thus increasing male reproductive success of the source plant. If pollinators travel farther from robbed flowers, pollen from robbed flowers should disperse farther. I investigated these predictions using fluorescent powders to mimic pollen dispersal. Powder may underestimate long distance pollen dispersal, but it does respond similarly to experimental manipulations (Thomson et al. 1986). Thus, it is likely that comparisons of powder movement from unrobbed and robbed flowers yielded the same pattern as dispersal of real pollen.



In the mornings of eight days over 1995 and 1996, I placed different colors of powder on anthers of equal numbers of robbed and unrobbed flowers on one tree (the “source”). Between 50 and 72 flowers were treated each morning during ca. 2 h. Treated flowers (robbed and unrobbed) were located at similar heights and positions on branches. I used two colors of powder for robbed and unrobbed flowers during the first hour of marking (“early”) and two different colors for the second hour (“late”). Flowers were left open to all visitors. After dusk, I used a black light to look for powder on stigmas and corollas of flowers on neighboring *Chilopsis* trees leading away from the source tree in both directions along the wash. I recorded the color of powder I found on each flower and the distance that the recipient tree was from the source tree. I exhaustively searched every flower on all trees in both directions, stopping in each direction when I found a tree with no powder on any flower. Using this stopping point may have underestimated rare long-distance pollen dispersal. For each repetition of this experiment, a different source tree was used and the four colors were rotated through the treatments.

For each day, I compared dispersal distances from robbed and unrobbed flowers using Mann-Whitney U-tests; I compared numbers of flowers that powder reached from each using *G*-tests. To investigate factors that might explain the changes in effects of robbing among days, I used stepwise regressions to determine which factors most influenced among-day variation in *Y*, which was either the difference between robbed or unrobbed flowers in median distance powder dispersed or difference between robbed and unrobbed source flowers in number of recipient flowers powder reached. This technique allowed me to determine the factors with greatest influence on *Y* among multiple independent variables that covaried. Each day was treated as an independent observation. Factors used were total number of flowers marked, prevalence of robbing, spatial variability of robbing (CV, square root transformed), abundances of the most common pollinators (visitation rates per flower-hour, arcsine transformed), and combined abundances of rare pollinators. Visitation rates for each pollinator on the two days preceding and the two days following each day of this experiment were averaged to determine the abundances. Thirty minute observation periods for 75–100 flowers and 0.5–2 h observation periods for 3–13 flowers were used. All factors were crossed except the number of flowers marked. I repeated the regressions after restricting analyses to observations of powder dispersed from flowers that were marked early each day.

#### Effects of robbing on fruits set and pollen tubes per style

In order to investigate the effects of robbing on female reproductive success of the plant, I first examined whether robbed and unrobbed flowers set fruits in equal proportions. At DS and CC, I marked 216 buds over 5 days and returned to the flowers twice a day until corollas fell to observe whether they had been robbed. After corolla abscission, I returned weekly to determine whether fruits remained. I compared the numbers of fruits set from robbed and unrobbed flowers using Fisher’s exact test.

Because the rate of flower and fruit abortion was very high (93.1% of flowers failed to mature fruits past one week,  $n=216$ ), I also determined the number of pollen tubes that grew in stigmas in another set of flowers in order to compare female reproductive success of robbed and unrobbed flowers at an earlier stage of reproduction. I marked 64 buds over 7 days and returned twice a day to determine whether they had been robbed. After the corollas fell, I collected the stigmas and placed them in either FAA or FPA (formalic acid). Following methods of Dafni (1992) to stain for pollen tubes, I fixed stigmas in FAA or FPA for 31 h, softened in NaOH for 7 h, rinsed in tap water for 15 h, and stained with aniline blue for 4.5 h. Using a UV microscope, I determined numbers of pollen tubes in each stained, squashed stigma at the junction of the stigma lobes. Using a Mann-Whitney U-test, I compared the number of pollen tubes growing in robbed and unrobbed flowers.

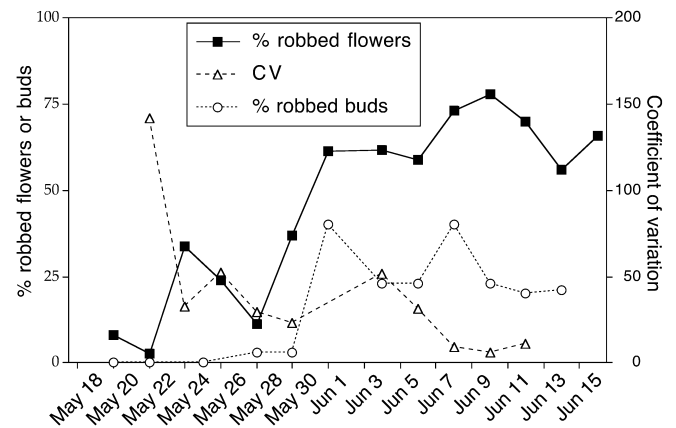
## Results

### Seasonal trends in prevalence and spatial variability of robbing

*Xylocopa californica*, the nectar-robbers, were common (Richardson, unpublished data) and were some of the first visitors to arrive at plants each year during 1993–6. The prevalence of robbing of flowers increased quickly over the season at DS in 1995 (Fig. 1) and 1996. The prevalence did not have a temporal pattern at CC in 1995 and decreased over the season at CC in 1996. Spatial variability of robbing, measured by CV of robbing among trees, decreased over the season in 1995 at DS (Fig. 1) and 1996 at DS. Pollinators such as bumblebees started visiting plants either at the same time as the nectar-robbers, or a few days later. As the seasons progressed at DS, robbers began to rob buds in the late afternoon and removed nectar before corollas opened to pollinators. Subsequently, the prevalence of robbing of buds increased (Fig. 1). At CC, where the flowering season began later than at DS, a large percentage of buds was robbed on the first day of flowering.

### Nectar

The proportion of unvisited, bagged flowers that contained nectar did not change over mornings ( $G^2=3.62$ ,  $P<0.20$ ; Table 1), and the amount of nectar in bagged flowers with nectar also did not change ( $H=0.22$ ,  $P<0.90$ ; Table 1). Only 81% of unvisited flowers contained nectar, so some flowers were empty before being robbed or visited. Robbed and unrobbed flowers were equally likely to contain nectar at the same time (Table 1). However, among flowers with nectar, robbed flowers contained less nectar than unrobbed ones ( $U=969$ ,  $P<0.05$ ,  $n=139$ ; Table 1).



**Fig. 1** Percentage of robbed flowers and robbed buds (prevalence), and coefficient of variation (spatial variability) of robbing on three trees from 1995 at DS ( $n=100$  for each date)

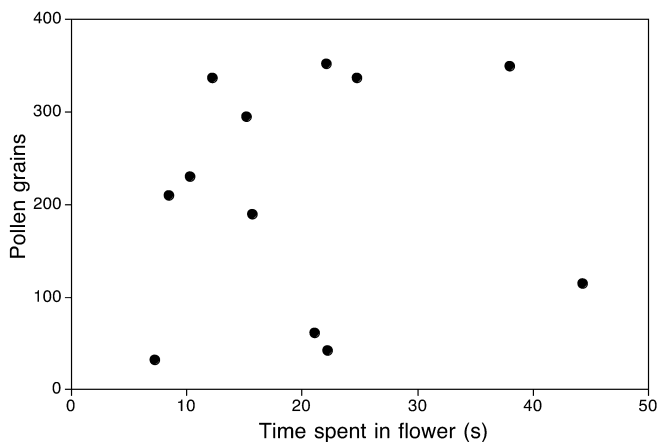
**Table 1** Proportions of flowers containing nectar and mean volume in flowers with nectar for unrobbed, robbed, and unvisited, bagged flowers. Proportions of robbed and unrobbed flowers containing nectar were compared using *G*-tests for each 2-h interval

Time of day	Proportion of flowers containing nectar ( <i>n</i> )			<i>P</i> (unrobbed vs robbed)	Mean nectar volume ( $\mu$ ) in flowers containing nectar, $\pm$ SE ( <i>n</i> )		
	Unrobbed	Robbed	Unvisited		Unrobbed	Robbed	Unvisited
0500–0700 hours	0.74 (65)	0.57 (7)	0.81 (21)	0.37	1.7 $\pm$ 0.33 (42)	0.5 $\pm$ 0.16 (4)	5.6 $\pm$ 0.88 (17)
0700–0900 hours	0.52 (117)	0.60 (10)	0.74 (31)	0.63	1.9 $\pm$ 0.40 (61)	0.7 $\pm$ 0.57 (6)	5.2 $\pm$ 0.91 (23)
0900–1100 hours	0.38 (66)	0.20 (6)	0.94 (18)	0.27	0.7 $\pm$ 0.29 (25)	0.7 (1)	5.8 $\pm$ 1.4 (17)

### Pollinator behavior

The two most common legitimate visitors to *Chilopsis* from 1993–1996 were honeybees and bumblebees (Richardson, unpublished data). Less common were *Anthophora* spp, bees intermediate to the others in pollen deposition and induction of stigma closure. Because honeybees and bumblebees accounted for most visits (31.3% and 36.7%, respectively,  $n=788$ ), I focused on their responses to robbing. Honeybees spent less time in robbed flowers than in unrobbed flowers, a median of 5.6 s (SE of  $\mu=0.89$ ) versus 7.8 s (SE of  $\mu=0.73$ ;  $U'=693$ ,  $P<0.05$ ,  $n=82$ ). There was a trend, though not significant, that bumblebees also spent less time in robbed flowers, 4.6 s (SE of  $\mu=0.34$ ) versus 5.0 s in unrobbed flowers (SE of  $\mu=0.37$ ;  $U'=4975$ ,  $P=0.07$ ,  $n=196$ ). Bumblebees were the most effective visitors at depositing pollen (Richardson, unpublished data). When presented with flowers that had not been previously visited, bumblebees did not deposit fewer pollen grains during shorter visits ( $\sigma=0.24$ ,  $P<0.45$ ; Fig. 2). During half-hour observation periods, the longest time that I observed bumblebees spend in a flower was 20 s, so I removed the two longest visits from the data. There still was no relationship between pollen deposition and visit length by bumblebees ( $\sigma=0.29$ ,  $P<0.39$ ).

I investigated whether pollinators responded to robbing by avoiding robbed flowers. Honeybees visited fewer robbed flowers than expected ( $G=9.2$ ,  $P<0.002$ ; Fig. 3),

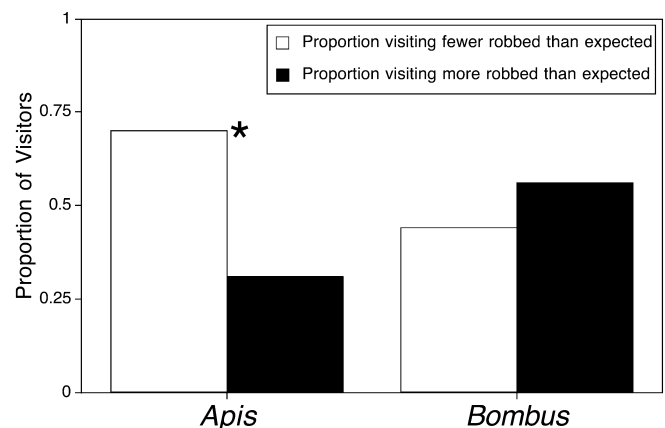
**Fig. 2** Numbers of pollen grains deposited by *Bombus* visiting *Chilopsis* flowers compared with time that each bee spent in a flower ( $n=12$ ). Flowers were not previously visited

but bumblebees did not ( $G=0.97$ ,  $P<0.35$ ; Fig. 3). The presence of an artificial slit, with its accompanying visual and odor cues, was not sufficient to cause honeybees to avoid flowers ( $Z=-0.50$ ,  $P<0.65$ ,  $n=24$ ).

To summarize, among flowers with nectar, robbed flowers contained less than unrobbed flowers at the same time of day but robbed flowers were equally likely to have some nectar. Bumblebees, the most effective pollen-depositing species, did not avoid robbed flowers. I followed up these results by determining whether bumblebees left trees more often than expected from robbed flowers, which would also have indicated that bumblebees avoided robbed flowers. Contrary to my prediction, bumblebees did not leave more often than expected from robbed flowers (11<predicted, 6>predicted,  $P<0.35$ ,  $n=17$  observation periods).

### Effect of robbing on dispersal of powder mimicking pollen

On eight days, I tested whether nectar-robbing indirectly benefited trees by causing pollinators to disperse powder mimicking pollen from robbed flowers either (1) farther away or (2) to more flowers than from unrobbed flowers. (1) For the first question, on two of eight days, powder

**Fig. 3** Proportion of *Apis* ( $n=59$ ) and *Bombus* ( $n=66$ ) visiting more and fewer robbed flowers than expected during a foraging bout, based on the ratio of robbed flowers available in the observation area at that time. The null hypothesis was that number of visitors visiting more robbed flowers than expected in a bout = number of visitors visiting fewer robbed flowers = 0.50. The asterisk indicates a significant difference in a log-likelihood ratio goodness of fit test for each species of visitor ( $P<0.05$ )

mimicking pollen from robbed flowers dispersed significantly farther than powder from unrobbed flowers (Table 2). On five other days, robbing did not significantly affect distance of dispersal. On one day, no powder dispersed from robbed flowers. (2) On two days, powder from robbed flowers reached significantly more recipients. However, on three days, powder from unrobbed flowers reached significantly more flowers. Of the remaining three days, robbing did not affect the number of recipients of powder (Table 2). Thus, experiments using powder suggested that the effect of robbing varied over the season. These experiments also suggested that on some days robbing benefited the plant through increased pollen dispersal and on some days it was costly.

I used stepwise regressions to determine what factors influenced variation in the effect of robbing on powder dispersal over the days it was studied. When observations of powder dispersal from flowers marked both early and late were included in analyses, no factor explained changes over days in the effect of robbing on distances powder dispersed or numbers of flowers it reached; neither changes in prevalence of robbing, nor among-tree spatial variability of robbing, nor floral visitation rates of pollinators influenced changes in the effect of robbing.

However, when analyses were restricted to powder dispersed from flowers that were marked early each day, the abundance of bumblebees, abundance of *Anthophora*, the interaction between the two, and the prevalence of robbing influenced the effect of robbing on the distance that powder dispersed from the source flower (Table 3a). In particular, when bumblebees were more common or the prevalence of robbing was high, powder dispersed farther from robbed flowers. Also, when only powder from flowers marked early was included, the same factors explained variation in the effect of robbing on the numbers of flowers that powder reached (Table 3b). In particular, as prevalence of robbing increased, the difference between robbed and unrobbed flowers in the number of flowers that powder reached also increased. Overall, these results suggest that the abundance of the most effective pollinators, bumblebees and *Anthophora*, and the prevalence of robbing did affect dispersal from robbed flowers, but only in the early morning when robbed flowers held less nectar (Table 1). However, contrary to predictions, among-tree spatial variability of robbing did not affect powder dispersal. I also found no evidence that abundance of honeybees affected dispersal (Table 3).

Effect of robbing on fruits set and pollen tubes per style

For 130 flowers that opened from 216 buds marked, 10.4% of robbed flowers and 12.7% of unrobbed flowers produced fruits. Thus, robbing did not have a significant effect on fruit set ( $P < 0.80$ ;  $n = 67, 63$ ). Robbed and unrobbed flowers also did not differ in the number of pollen tubes in their styles ( $U' = 594, P < 0.10$ ;  $n = 64$ ). In fact, robbed flowers tended to have more pollen tubes than

**Table 2** Distance and numbers of flowers at which powder that mimicked pollen was deposited. Different colors of powder were used to mark anthers of equal numbers of robbed (R) and unrobbed (NR) source flowers. Powder dispersal from flowers marked both early and late in the morning is included. Significant differences are indicated by asterisks. Distances at which powder was deposited were compared using Mann-Whitney U-tests for each date. Numbers of flowers that powder reached were compared by G-tests for each date

Date	Is powder from robbed flowers deposited farther?	Level of significance	Does powder from robbed flowers reach more flowers?	Level of significance	CV of robbing among trees	Prevalence of robbing (%)	Most abundant pollinators
21 May 1995	NR>R	$P < 0.85, Z = -21$	NR>R*	$P < 0.065, G = 3.4$	29.4	11.4	<i>Anthophora</i>
31 May 1995	R>NR	$P < 0.90, Z = 0.14$	NR>R	$P < 0.0002, G = 13.7$	29.0	49.3	<i>Anthophora, Apis</i>
4 June 1995	R>NR*	$P < 0.05, Z = -2.28$	R>NR*	$P = 0.055, G = 3.7$	51.5	61.6	<i>Anthophora, Apis</i>
8 June 1995	R>NR	$P < 0.85, Z = -0.23$	R>NR*	$P < 0.0001, G = 52.4$	8.9	73.3	<i>Megachile, Anthophora</i>
12 June 1995	R>NR*	$P < 0.004, Z = 2.92$	NR>R*	$P = 0.056, G = 3.7$	10.9	70.0	<i>Bombus, Megachile</i>
17 May 1996	NR>R	$P < 0.10, Z = -1.77$	NR>R	$P < 0.15, G = 2.3$	110.6	19.0	<i>Bombus</i>
20 May 1996	no powder found from R	-	NR>R*	$P = 0, G = 0$	48.8	43.8	<i>Bombus, Megachile</i>
23 May 1996	R>NR	$P < 0.15, Z = -1.46$	R>NR	$P < 0.10, G = 3.1$	-	33.7	<i>Bombus</i>

**Table 3** Stepwise multiple regression of factors influencing **a**) The difference between robbed and unrobbed flowers in median distance that powder representing pollen traveled to each day and **b**) The difference between robbed and unrobbed flowers in the number of flowers that powder traveled to on each day. Factors tested were

Step number	Variable entered	Regression coefficient	Partial $r^2$	Model $r^2$	$F$	$P$
a 1	<i>Bombus</i> × <i>Anthophora</i>	605.72	0.76	0.76	105.55	0.0095
	<i>Bombus</i>	6.46			16.87	0.0545
	<i>Anthophora</i>	-130.11			38.33	0.0251
a 2	<i>Anthophora</i> × prevalence of robbing	2.81	0.13	0.99	54.76	0.0178
	Prevalence of robbing	-0.15			27.63	0.0343
b 1	<i>Bombus</i> × prevalence of robbing	-9.28	0.74	0.74	30.40	0.0117
	<i>Bombus</i>	309.67			28.87	0.0126
	Prevalence of robbing	0.89			18.17	0.0237
b 2	<i>Anthophora</i>	-95.67	0.17	0.91	6.14	0.0894

unrobbed flowers did; robbed flowers had a mean of 59.9 pollen tubes per style (SE=12.45,  $n=41$ ) and unrobbed flowers had a mean of 32.0 per style (SE=5.19,  $n=23$ ). Incompatible pollen does generate pollen tubes which do not penetrate ovules (personal observation), but I assumed that total numbers of pollen tubes would be proportional to tubes from outcrossed pollen. Together, the fruit set data and pollen tube data indicate that robbing did not reduce plants' female reproductive success.

## Discussion

Recent interest has focused on mechanisms that could control exploiters and maintain the stability of mutualisms (reviewed in Pellmyr 1997; Bronstein 2001b; Yu 2001). For example, *Piper* ants defend their plants' reward-producing domatias against beetles that can induce plant rewards and kill ants if they occupy the plants. In this case, the exploiting beetles are controlled by their competitors, the ants (Letourneau 1991). In another example, *Yucca kanabensis* is dependent on seed-eating moths for pollination. The yucca controls costly non-pollinating yucca moths by aborting fruits; in this case, the mutualist host controls exploiters (Humphries and Addicott 2000). In both of these cases, the exploiters are costly to the mutualists.

A priori, nectar-robbers in the *Chilopsis*-pollinator mutualism might also be expected to be costly to plants because robbing was prevalent (Fig. 1). In addition, robbers gained an advantage later in the season by robbing buds before pollinators had access to nectar. Nectar-robbers did reduce the amount of reward available to pollinators (Table 1) and pollinators' behaviors were affected (Fig. 3). However, reduction of nectar rewards by nectar-robbers was only rarely linked to a decrease in indicators of reproductive success for plants. In fact, female reproductive success of the plant, measured by fruit set and pollen tube number, was not reduced. Experiments with powder mimicking pollen suggested that male

number of flowers marked on each day, prevalence of robbing (%), CV of robbing among trees (spatial variability), and visitation rates of *Bombus*, *Apis*, *Anthophora*, and rare pollinators (no. visits/fl-h). Only flowers marked early in the morning were included.  $n=8$  days

reproductive success occasionally may even have been greater for robbed flowers (Table 2).

When bumblebees were abundant, robbing may have indirectly benefited plants by decreasing rewards below the point at which bumblebees would finish foraging and return to nests, but still above the threshold where foraging on robbed flowers was unprofitable. Foraging on artificial flowers, *Bombus bimaculatus* choose to leave patches of flowers based on the average volume of the last three flowers (Dukas and Real 1993). Thus, I expected that *B. sonorus* would leave trees more often after robbed flowers because they would be likely to have less nectar. However, bumblebees tended to leave trees more often after they visited unrobbed flowers. When bumblebees left trees, they often flew away from the wash in which the trees grew. A possible explanation for this behavior is that bumblebees filled up on nectar and left for their nests more often from unrobbed flowers. This behavior may have been costly to plants, because pollen that was carried to bees' nests may have been removed from circulation. In this way, being robbed may have benefited plants not by causing pollinators to leave a tree, but by causing pollinators to continue foraging.

A combination of factors explain why a reduction in reward did not usually translate into a reduction of benefit for plants, the mutualists providing the reward. First, robbers did not consume entire nectar rewards, so the probability of pollinators encountering rewarding robbed flowers was the same as for unrobbed flowers. In a similar way, pollinators themselves also leave less easily extracted nectar in flowers, making later visits marginally profitable for bumblebees (Whitham 1977). However, with a larger sample size, a difference between robbed and unrobbed flowers in the percentage of empty flowers may become apparent. Nectar measurements during this study happened to occur on days when few flowers were robbed. Second, alternative nectar sources were not available to pollinators, so they could not switch to less robbed species of plants. This system contrasts with *Ipomopsis aggregata*, for which nectar robbing is costly partly because pollina-



tors can switch to more rewarding plants (Irwin and Brody 1998). Third, *Chilopsis* was more generalized in its partners than are *Piper* or yucca, so multiple potential partners existed that could provide necessary benefits for the plant as long as some did not avoid robbed flowers.

For *Chilopsis* in particular, pollinators' responses to robbing combined with their effectiveness in a way that made nectar-robbing less costly: honeybees usually were not very effective at depositing pollen grains (Richardson, unpublished data), so their reduced time visiting and avoidance of robbed flowers may not have been costly (Fig. 3). In contrast, bumblebees tended to spend less time visiting flowers that contained little nectar, but pollen deposition by bumblebees was not correlated with time spent in flowers. Thus, bumblebees' potential benefit to the plant's female reproductive success would not have been affected by robbing.

There are several possible reasons, not mutually exclusive, why honeybees avoided robbed flowers and bumblebees did not (Fig. 3; Richardson 1995). First, honeybees may have identified less rewarding flowers by detecting marking scents that nectar-robbers left after they depleted flowers, whereas bumblebees could not detect them. What signal honeybees used to identify robbed *Chilopsis* is unclear, but the visual cue of the slit or the odor coming from the plant damage alone was not sufficient to cause honeybees to avoid robbed flowers. It is possible that the honeybees could detect the real levels of nectar in robbed flowers and artificially slit flowers, rather than using the associated cue. Whether scent is deposited by nectar-robbers is unknown, but nectar-robbers visiting *Chilopsis* did avoid flowers recently visited by conspecifics, which suggests that they may deposit scent. Honeybees and a few bumblebee species avoid flowers emptied and scent-marked by heterospecifics (Stout and Goulson 2001), but whether they can detect scents deposited by genera other than each other is also unknown. Second, honeybees may avoid robbed flowers because they are not able to reach nectar in grooves of flowers. Some of the nectar in *Chilopsis* is contained in deep grooves in the corolla (Whitham 1977) and may be available to bumblebees but not honeybees (Brown et al. 1981). Third, bumblebees may have lower or slower learning abilities than honeybees, though they have not been compared. Finally, honeybees have greater metabolic requirements than bumblebees. *Apis* expend more metabolic energy at rest than *Bombus vosnesenskii* up to about 30°C (Heinrich 1979; Moritz and Southwick 1992). Thus, robbed flowers may have been too unprofitable for honeybees, but not bumblebees, to visit.

Experiments with powder dispersal suggested that the interaction between *Chilopsis* and its nectar-robbers led to a conditional outcome in powder dispersal as a measure of male reproductive success. For flowers that were marked early in the day, the outcome varied with changing abundances of effective pollinators and prevalence of robbing but not with ineffective pollinators or spatial variability of robbing (Table 3). Whether powder dispersed farther or to more flowers from unrobbed flowers

or from robbed flowers depended on the abundance of bumblebees and *Anthophora*. Probably because these bees were the two most effective visitors at depositing pollen, when they were abundant, their responses to robbing affected the outcome of robbing (Table 3). In particular, when bumblebees were absent and prevalence of robbing was high or when bumblebees were common and prevalence was low, then more powder dispersed from robbed flowers. Thus, the effect of bumblebees' abundance on the number of flowers that powder reached from robbed flowers was mediated by the prevalence of robbing.

Changing abundances of *Apis* did not affect variation in the outcome of robbing, perhaps because they were so ineffective at depositing pollen that their responses to robbed flowers did not translate to an effect on powder dispersal. Also, the difference between robbed and unrobbed flowers marked late in the morning may not have been affected by any factor because at that time both types held little nectar (Table 1). Thus, no effect on behaviors of pollinators would be expected as long as they were tracking actual rewards, rather than relying on the presence of slits to identify less rewarding flowers.

I originally predicted that a low prevalence of exploitation would not measurably affect a mutualism, but a greater prevalence would, which was supported by a stepwise regression. However, the benefit of robbing increased with its prevalence, contrary to what would be expected if nectar-robbers were viewed as detrimental parasites. Finally, spatial variability of robbing among trees did not affect the outcome of robbing on dispersal, contrary to predictions.

Nectar-robbing is widespread, affecting at least 59 plant families (Irwin and Maloof 2002). However, plant defenses against robbers are not common, though rare examples of clustered flowers (Barrows 1976), thickened corollas (Gentry 1990), ant defenders (Barrows 1976), sap from damaged flowers (Inouye 1983), or toxic nectar (Stephenson 1982; Adler 2000) have been proposed as defenses against robbing. Spatiotemporal variation in the prevalence of robbing has been suggested as a factor that limits plants' abilities to respond to selection from robbing (Irwin and Maloof 2002). For *Chilopsis*, changes in the spatial distribution of robbing were not a factor in the outcome of the interaction between nectar-robbers and plants. Instead, the outcome varied from positive to negative with changing abundances of effective pollinators and changing prevalence of robbing over time. This variation in the indirect effects of nectar robbing may limit the potential for the plant to respond to selection from negative effects with some kind of control or "punishment" of robbers.



**Acknowledgements** Financial support for this work came from two grants from the Theodore Roosevelt Memorial Fund of the American Museum of Natural History, by the 1994 Cranwell Smith Pollination Award from the University of Arizona, by the Southwestern Research Station Student Support Fund, and by grants from the Department of Ecology and Evolutionary Biology at the University of Arizona. The Knight family of Paradise, Ariz., the Ritchins families of Cotton City, N.M. and Animas, N.M., J. Caron of Crown Dancer Estates, M. Kraft, and A. and J. Dominic of Portal, Ariz. were kind in allowing research to be conducted on their land. I thank N. Plotkin, J. Chesnick, S. McGraw, P. Murphy, D. Banks, N. Compton, K. Toal, K. Spörns, A. Gove, and M. Brown for assistance in the field, and S. Buchmann for instruments, dye, and advice on techniques. The comments of J. Bronstein, L. McDade, J. Rudgers, R. Hudson, S. Kinsman, S. Forsyth, A. Boyle, J. Weeks, H. Harvey, H. Reynolds, T. Rajaniemi, and J. Bever improved this study and manuscript.

## References

- Adler LS (2000) The ecological significance of toxic nectar. *Oikos* 91:409–420
- Agrawal AA, Rudgers J, Botsford LW, Cutler D, Gorin JB, Lundquist CJ, Spitzer BW, Swann AL (2000) Benefits and constraints on plant defense against herbivores: spines influence the legitimate and illegitimate flower visitors of yellow star thistle, *Centaurea solstitialis* L. (Asteraceae). *Southwest Nat* 45:1–5
- Alexander MP (1980) A versatile stain for pollen, fungi, yeast, and bacteria. *Stain Tech* 55:13–18
- Barrows, EM (1976) Nectar robbing and pollination of *Lantana camara* (Verbenaceae). *Biotropica* 8:132–135
- Bronstein JL (1994) Conditional outcomes in mutualistic interactions. *Trends Ecol Evol* 9:214–217
- Bronstein JL (2001a) The costs of mutualism. *Am Zool* 41:825–839
- Bronstein JL (2001b) The exploitation of mutualisms. *Ecol Lett* 4:277–287
- Brown JH, Kodric-Brown A, Whitham TG, Bond HW (1981) Competition between hummingbirds and insects for the nectar of two species of shrubs. *Southwest Nat* 26:133–145
- Bshary R, Grutter AS (2002) Asymmetric cheating opportunities and partner control in a cleaner fish mutualism. *Anim Behav* 63:547–555
- Bshary R, Schaffer D (2002) Choosy reef fish select cleaner fish that provide high-quality service. *Anim Behav* 63:557–564
- Dafni A (1992) *Pollination ecology*. Oxford University Press, New York
- Denison RF (2000) Legume sanctions and the evolution of symbiotic cooperation by rhizobia. *Am Nat* 156:567–576
- Dukas R, Real LA (1993) Effects of recent experience on foraging decisions by bumble bees. *Oecologia* 94:244–246
- Fenster CB (1991) Gene flow in *Chamaechrista fasciculata* (Leguminosae). *Evolution* 45:398–409
- Ferriere R, Bronstein J, Rinaldi S, Law R, Gauduchon M (2002) Cheating and the evolutionary stability of mutualisms. *Proc R Soc London B* 269:773–780
- Gentry AH (1974) Coevolutionary patterns in Central American Bignoniaceae. *Ann Mo Bot Gard* 61:728–759
- Gentry AH (1990) Evolutionary patterns in neotropical Bignoniaceae. *Mem NY Bot Gard* 55:118–129
- Heinrich B (1979) *Bumblebee economics*. Harvard University Press, Cambridge, Massachusetts
- Humphries S, Addicott J (2000) Regulation of the mutualism between yuccas and yucca moths: intrinsic and extrinsic factors affecting flower retention. *Oikos* 89:329–339
- Inouye DW (1983) The ecology of nectar robbing. In: Bentley B, Elias T (ed) *The biology of nectaries*. Columbia University Press, New York, pp 153–173
- Irwin RE, Brody AK (1998) Nectar robbing in *Ipomopsis aggregata*: effects on pollinator behavior and plant fitness. *Oecologia* 116:519–527
- Irwin RE, Maloof JE (2002) Variation in nectar robbing over time, space, and species. *Oecologia* 133:525–533
- Irwin RE, Brody AK, Waser NM (2001) The impact of floral larceny on individuals, populations, and communities. *Oecologia* 129:161–168
- Johnson NC, Graham JH, Smith FA (1997) Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytol* 135:575–586
- Klinkhamer PGL, de Jong TJ, Metz JAJ (1994) Why plants can be too attractive—a discussion of measures to estimate male fitness. *J Ecol* 82:191–194
- Lara C, Ornelas JF (2001) Preferential nectar robbing of flowers with long corollas: experimental studies of two hummingbird species visiting three plant species. *Oecologia* 128:263–273
- Letourneau DK (1991) Parasitism of ant-plant mutualisms and the novel case of *Piper*. In: Huxley CR, Cutler DF (ed) *Ant-plant interactions*. Oxford University Press, Oxford, pp 390–396
- Maloof JE, Inouye DW (2000) Are nectar robbers cheaters or mutualists? *Ecology* 81:2651–2661
- Moritz RFA, Southwick EE (1992) *Bees as superorganisms*. Springer, Berlin Heidelberg New York
- National Climate Data Center (NCDC; Updated 19 July 1999) <http://lwf.ncdc.noaa.gov/oa/climate/online/coop-precip.html#intro>. US On-line Monthly Precipitation Data for Cooperative and NWS Sites
- Noë R, Hammerstein P (1994) Biological markets: supply and demand determine the effect of partner choice in cooperation, mutualism, and mating. *Behav Ecol Sociobiol* 35:1–11
- Pellmyr O (1997) Stability of plant-animal mutualisms: keeping the benefactors at bay. *Trends Plant Sci* 2:408–409
- Pellmyr O, Huth CJ (1994) Evolutionary stability of mutualism between yuccas and yucca moths. *Nature* 372:257–260
- Pellmyr O, Leebens-Mack J, Huth CJ (1996) Non-mutualistic yucca moths and their evolutionary consequences. *Nature* 380:155–156
- Richardson SC (1995) Temporal variation in pollinator abundance and pollinator foraging behavior in response to robbed flowers. In: DeBano LF, Ffolliott PF, Ortega-Rubio A, Gottfried GJ, Hamre RH, Edminster CB (ed) *Biodiversity and management of the Madiran Archipelago: the Sky Islands of southwestern United States and northwestern Mexico*. U.S. Department of Agriculture, Forest Service, Fort Collins, Colo., pp 311–316
- Schwartz MW, Hoeksema JD (1998) Specialization and resource trade: biological markets as a model of mutualisms. *Ecology* 79:1029–1038
- Stephenson AG (1982) Iridoid glycosides in the nectar of *Catalpa speciosa* are unpalatable to nectar thieves. *J Chem Ecol* 8:1025–1034
- Stout JC, Goulson D (2001) The use of conspecific and interspecific scent marks by foraging bumblebees and honeybees. *Anim Behav* 62:183–189
- Thomson JD, Price MV, Waser NM, Stratton DA (1986) Comparative studies of pollen and fluorescent dye transport by bumblebees visiting *Erythronium grandiflorum*. *Oecologia* 69:561–566
- Whitham TG (1977) Coevolution of foraging in *Bombus* and nectar dispensing in *Chilopsis*: a last dreg theory. *Science* 197:593–596
- Yu DW (2001) Parasites of mutualisms. *Biol J Linn Soc* 72:529–546