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Facilitated exploitation of pollination mutualisms: fitness consequences for plants

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Summary

1. Mutualisms are only rarely one-to-one interactions: each species generally interacts with multiple mutualists. Exploitation is ubiquitous in mutualisms, and we would therefore expect that each mutualist interacts with multiple exploiters as well. Exploiter species may also interact with one another. For example, the action of one exploiter species might open the opportunity for exploitation by a second species.

2. Exploitation is common in many plant–pollinator mutualisms: 'primary' nectar robbers feed through holes they make in flowers, which can be subsequently used by 'secondary' nectar robbers unable to create holes themselves. The overall effect of nectar robbing on plant fitness is often (although not always) negative. No study has separated the effects of interacting with primary vs. secondary robbers.

3. Here, we examine the effects of primary vs. secondary nectar robbing on pollinator visitation rate and female fitness in *Ipomopsis aggregata*. Manipulating the type of nectar robbing that flowers experienced, we found that secondary nectar robbing inflicted fitness costs to plants beyond that inflicted by primary robbing alone. Secondary nectar robbing significantly reduced pollen receipt to flowers, as well as fruit and seed production. Although the causes are elusive, the effect may be attributed to changes in pollinator behaviour at these plants.

4. *Synthesis.* Our findings provide evidence that interacting with multiple exploiters can lead to increased negative effects for mutualists, and highlight the importance of incorporating multiple exploiters into the conceptual framework of mutualism.

Key-words: facilitated exploitation, generalized mutualism, hummingbird pollination, *Ipomopsis aggregata*, multiple exploiters, nectar robbing, plant fitness, reproductive success

Introduction

Central to an understanding of mutualism is the concept of exploitation. An exploiter profits from an interaction between mutualists by usurping benefits being produced by one mutualist species for its partner(s) (Bronstein 2001). When exploitation takes place, the exchange of benefits between the mutualist and its partner is disrupted (Jones *et al.* 2015; Sachs 2015). Exploitation is ubiquitous, and many mutualisms are exploited by multiple species simultaneously (Bronstein & Ziv 1997; Vannette, Gauthier & Fukami 2013). However, it is most often studied by examining consequences to a mutualist of interacting with a single exploiter. There has been minimal investigation of the fitness effects of interacting simultaneously with multiple exploiters (but see Zhang, Zhao & Inouye 2014).

A potential consequence of having multiple exploiters is that interactions among exploiter species might act to alter the fitness costs that exploitation inflicts upon a mutualist. One way in which exploiters can interact is when one organism opens the opportunity for additional exploitation by another (here termed 'facilitated exploitation'). A first step in understanding the evolutionary ecology of facilitated exploitation requires testing the fitness consequences on the focal mutualist using controlled experiments. If through simple manipulation we can detect patterns of fitness consequences of facilitated exploitation, it would warrant deeper investigation of this phenomenon within a larger, community context.

Pollination mutualisms provide ideal systems for exploring facilitated exploitation and its effects on plants. Nectar robbing is extremely common in plant species that produce flowers with tubular corollas or long nectar spurs (Lara & Ornelas 2001;

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Rojas-Nossa, Sánchez & Navarro 2016a). Nectar robbers exploit pollination mutualisms by feeding on floral nectar through holes punctured or chewed in the floral tissue, usually at the base of the flower or in the nectar spur (Inouye 1980). By accessing nectar in this way, the nectar robber can bypass the floral reproductive structures (Inouye 1980). Robbing is generally thought to be costly to plant fitness (reviewed in Irwin *et al.* 2010), although there is evidence that some nectar robbers pollinate flowers (e.g. Higashi *et al.* 1988; Navarro 2000; Zhu, Wan & Li 2010) and others have no measurable effects on pollination mutualisms (Morris 1996; Richardson 2004; Rojas-Nossa, Sánchez & Navarro 2016b). Usually, the term 'nectar robber' is applied to the individual or species of nectar feeder that initially creates holes in floral tissue, that is to a 'primary' nectar robber (Inouye 1980). However, it is common for plants to experience additional visits from 'secondary' nectar robbers that feed through the same holes (Rust 1979; Inouye 1980; Irwin *et al.* 2010). Unlike primary robbers, secondary robbers do not require specific morphology such as toothed or piercing mouthparts to create robbing holes (Irwin *et al.* 2010). It follows that species lacking this type of morphology cannot rob until a primary robber has visited a flower. Thus, secondary robbing is a form of facilitated exploitation. Secondary nectar robbing is common in nature: a review of robbing reports at least 315 accounts of secondary robbing to 113 plant species across 34 families (Irwin *et al.* 2010). However, very few studies have examined secondary nectar robbing outside of a natural history context. Moreover, while most studies of the effect of nectar robbing on plant fitness likely report the combined effects of primary and secondary robbing, no study to our knowledge has teased apart these two effects, nor tested the separate effects of primary and secondary robbing on pollinator behaviour and plant fitness.

Here, we assess the effects of secondary robbing in one system by examining whether primary and secondary robbing together affect plant fitness and pollinator visitation rate more than does primary robbing alone. We studied scarlet gilia *Ipomopsis aggregata* (Polemoniaceae, hereafter referred to as *Ipomopsis*), which experiences primary nectar robbing by bees and secondary robbing by bees, flies and wasps. Prior studies have shown that nectar robbing in *Ipomopsis* can reduce male and female components of plant fitness indirectly via changes in hummingbird pollinator behaviour (Irwin & Brody 1998, 2000; Irwin 2000). Pollinators avoid nectar robbed plants and flowers (Irwin & Brody 1998) due to lower nectar availability (Irwin 2000), and deposit less pollen per flower in robbed compared to unrobbed flowers (Irwin, Howell & Galen 2015). However, these studies combined the effects of primary and secondary nectar robbing without differentiating their effects. Here, we experimentally manipulated primary vs. secondary nectar robbing and measured their separate effects on estimates of female plant reproduction and pollinator visitation rate. We predicted that (i) additional nectar removal by secondary robbing would lead to reduced female fitness in *Ipomopsis* beyond that inflicted by primary robbing alone and that (ii) this can be attributed to continued pollinator avoidance of secondary compared to primary-robbed plants and flowers. However, if pollinator avoidance of robbed plants and flowers is driven by the initial nectar exploitation of primary robbing, secondary robbing may inflict no additional effect on either pollinator visitation rate or plant fitness, assuming that secondary robbing does not damage floral reproductive structures.

Materials and methods

STUDY SYSTEM

We studied *I. aggregata* (Polemoniaceae) near the Rocky Mountain Biological Laboratory (RMBL, elevation 2895 m), Gothic, CO, USA. At this location, *Ipomopsis* is a monocarpic herb that flowers from mid-June through mid-August (Waser & Price 1989). It lives as a vegetative rosette for up to 10 years (Waser & Price 1989). In a single season, it bolts into typically a single flowering stalk, producing approximately 50 red, tubular flowers (Waser 1978). Because flowering occurs in a single year (Waser 1978; Waser & Price 1989), it is possible to measure lifetime reproductive output for a single plant in one flowering season. Flowers are obligately outcrossing, hermaphroditic and protandrous, with male phase lasting 1–2 days and female phase 2–3 days (Pleasants 1983). Male- and female-phase flowers occur on the same plant at the same time. Flowers produce nectar continuously at a rate of up to 1–5 μL day^{-1} (Pleasants 1983). *Ipomopsis* is primarily pollinated by broad-tailed (*Selasphorus platycerus*) and rufous (*Selasphorus rufus*) hummingbirds (Waser 1978).

Ipomopsis flowers experience both primary and secondary robbing. *Bombus occidentalis* acts as a primary robber, using its toothed mandibles to make a hole at the base of the corolla. Primary robbing rates range from 0% to 100% of flowers robbed per plant within a season, with a mean of 66% of flowers robbed at peak robbing activity at this site (Irwin & Maloof 2002). Primary robbing does not result in pollination, nor does it damage male or female floral sexual organs (Irwin, Howell & Galen 2015). A single primary robbing event typically results in all available nectar being removed, but because robbers do not damage nectar-producing structures, nectar production can and does continue. *Bombus occidentalis* can secondary-rob *Ipomopsis* flowers, as can other bumblebee species, as well as wasps and flies that cannot primary-rob but do secondary-rob *Ipomopsis* flowers (R. E. Irwin, pers. obs.).

EXPERIMENTAL DESIGN

We conducted whole-plant manipulations to simulate nectar robbing of *Ipomopsis* in 2013 at a 25 × 25 m field site near the RMBL (coordinates: 38.989235, −107.007481). We selected 120 *Ipomopsis* that had newly grown from a vegetative rosette to a single, flowering stalk for manipulation. We haphazardly assigned plants to one of three treatments spread out across the site (n = 40 plants per treatment). (i) *Primary robbing only (P):* all flowers on each plant were experimentally primary-robbed one time; subsequent natural secondary robbing was prevented on all flowers by applying plastic collars to floral corollas. (ii) *Primary and secondary robbing (PS):* all flowers on each plant were experimentally primary-robbed, then experimentally secondary-robbed once daily until corollas abscised; natural secondary robbing visits were permitted. (iii) *No robbing (control) (C):* robbing was prevented on all flowers on each plant. Natural pollinator visitation was permitted to all flowers in all treatments, and all flowers in all treatments were handled to control for flower handling. We applied treatments 5 days per week throughout the flowering season;

the 2 days per week that treatments were not applied were not consecutive. Flowers that opened on days when treatments were not applied received treatments the following day, ensuring that every flower on every plant received treatment.

In both robbing treatments, we simulated primary robbing by puncturing a small hole at the base of the corolla using dissecting scissors and removing all available nectar from the hole via capillary action, using a 10 μL microcapillary tube (Microcap; Drummond Scientific, Broomall, PA, USA) inserted into the hole. This method of robbing does not damage floral reproductive or nectar-producing structures (Irwin & Brody 1998; Irwin, Howell & Galen 2015). Moreover, our experimental nectar robbing technique simulates the effects of natural nectar robbing by bumblebees on *Ipomopsis* reproduction (Irwin & Brody 1998). Using experimental robbing as opposed to natural robbing offers the advantage of removing any co-variation between robber preference for plants and plant health and fitness. In the PS treatment, we simulated additional secondary robbing by inserting a microcapillary tube into the pre-existing robbing hole and removing all available nectar once per day on all days that flowers were open, an average of 4 days. We prevented robbing in the P and C treatments with the use of plastic collars made from 1 cm pieces of clear drinking straws (Fig. 1). Collars were applied to all newly opened flowers in the C treatment and to all newly opened flowers after primary robbing in the P treatment. All flowers on plants in the PS treatment were primary-robbed once, then fitted with collars with windows to allow experimental and natural secondary robbing through the existing robbing hole. By including collars on the flowers of all treatment plants, we controlled for any unexpected effects of collars on pollinator visitation and plant reproduction. Moreover, a prior nectar robbing study using *Ipomopsis* found no effects of collaring on pollen receipt or on fruit or seed production (Irwin & Brody 1999).

EFFECT OF ROBBING TREATMENTS ON FEMALE FITNESS

Pollen receipt

Once per week over the course of the flowering season (6 weeks total), we collected stigmas from all focal plants in order to examine pollen receipt as a function of robbing treatment. In *Ipomopsis*, pollen receipt serves as a proxy for pollinator visitation, as pollinator visitation rates increase with increased pollen deposition per stigma (Engel & Irwin 2003; Price *et al.* 2005). Each week, we collected up to three available stigmas (no more than 20% of the available stigmas per plant) from flowers with freshly abscised corollas on all treated plants. In total, we collected up to 10 stigmas per plant and an average of 145 stigmas per treatment. Collecting stigmas at this stage does not interfere with fruit development (Waser & Fugate 1986). Stigmas were mounted on microscope slides and stained with basic fuchsin gel (Kearns & Inouye 1993). Using a compound microscope, we counted all conspecific (*Ipomopsis*) and heterospecific (other species) pollen grains on each stigma.

Fruit production

At the end of the reproductive season, we collected all mature fruits from all plants. At the same time, we counted aborted fruits on all plants. For each plant, we calculated the proportion of successful fruits set by dividing the total number of mature fruits by the total number of mature and aborted fruits.

Seed production

To estimate total seeds produced per focal plant, we dissected all mature fruits and counted all seeds. *Ipomopsis* suffers seed predation from *Hylemya* spp. flies, so we also counted the total number of partially damaged seeds, and noted when seeds were fully destroyed (indicated by the presence of frass without any intact seed tissues). We calculated the average number of seeds per fruit per focal plant by dividing the total number of undamaged seeds per focal plant by the number of successful fruits that plant produced. We calculated the proportion of fruits damaged by seed predators by dividing the total number of partially or fully damaged fruits by the total number of fruits produced. Seed predation was low across treatments. Mean ± SE proportion of seeds receiving seed predation in the C, P and PS treatments was 0.07 ± 0.01, 0.05 ± 0.009 and 0.03 ± 0.006, respectively. Given the low level of attack, seed predation was not considered further in analyses.

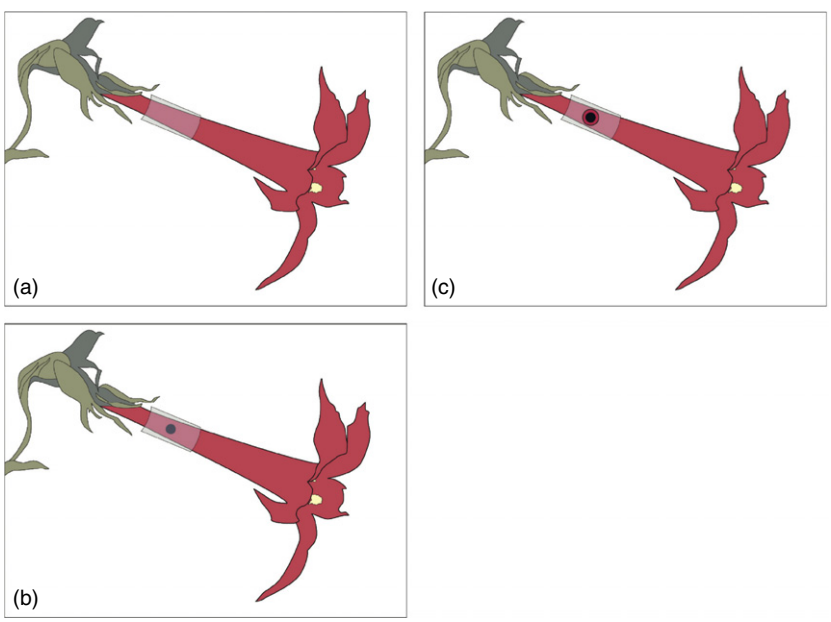

Fig. 1. Illustration of nectar robbing treatments. In (a), a collar is placed over the corolla without a robbing hole (control). In (b), a robbing hole is made in the corolla and nectar is removed, and a collar is placed over the hole to prohibit any additional robbing (primary robbing only). In (c), a hole is made in the corolla and nectar is removed, and a collar with a window is placed over the corolla to prohibit additional primary robbing visits while allowing additional secondary robbing visits (primary plus secondary robbing). Illustration by Claire Seizovic. [Colour figure can be viewed at wileyonlinelibrary.com]

EFFECTS OF ROBBING ON POLLINATOR VISITATION RATE

To test the predictions that pollinators avoid plants with flowers that experience primary robbing and that secondary robbing inflicts additional fitness costs through further reductions in pollinator visitation, we conducted an experiment in which we observed hummingbirds foraging on plants with P, PS and C treatments. In early July 2015, we selected 30 single-stalked flowering *Ipomopsis* of approximately equal size and similar flowering phenology growing at the RMBL and transplanted them into 15-cm diameter circular pots. Plants were maintained in a robber- and pollinator-free screened enclosure and given root stimulator for 3 days followed by an NPK fertilizer thereafter. After 3 days, we randomly assigned plants to the P, PS and C treatments ($n = 10$ plants per treatment). Treatments were applied using the methods described above, although collars were not applied to flowers. Previous work has shown that the presence vs. absence of collars does not affect hummingbird behaviour (Irwin & Brody 1999). Furthermore, *B. occidentalis* nectar robbers were rare from areas in and around the RMBL in 2015. Over the period 13–29 July when this study was conducted, we conducted 163.5-person hours of bumblebee observations, specifically looking for robbing by *B. occidentalis*, and observed no *B. occidentalis* (R. E. Irwin & J. L. Bronstein, unpubl. data). Moreover, we did not observe any secondary robbers near the flowers while they were in the field. All treatment plants were in full view for the duration of the observation periods, which allowed us to verify that primary and secondary robbers were absent from the array. For these reasons, the use of collars was not necessary during observations.

Hummingbird visitation was observed 5 days per week for 2.5 weeks during peak flowering season. However, we applied treatments 6 days per week, thus ensuring that hummingbirds were exposed to plants in which all open flowers on those plants were treated with the correct treatment. Each morning beginning at 0630, we counted the number of flowers on each plant and manipulated all new flowers in the P treatment, all new flowers in the PS treatment and re-robbed all PS flowers that were still open. Plants were then immediately moved from the screened enclosure to a 5 × 6 m array in a meadow with naturally flowering *Ipomopsis*, each treatment plant 1 m apart. We recorded pollinator visitation to these plants for 3 h day^{-1}. Each time a pollinator entered the array, we used a digital voice recorder to record the sex and species of the pollinator (all pollinators observed were hummingbirds), which plants it visited and how many flowers per plant were probed (defined by observing a hummingbird inserting its bill into the corolla). At the end of the observation period, all plants were collected and returned to the screened enclosure until the next morning. Plants were returned to the same position in the array on each observation day. Because flowers are usually open 3–5 days, individual flowers were exposed to pollinators on multiple days.

STATISTICAL ANALYSES

All analyses were performed using the R software program version 3.1.0 (R Core Team 2014). To determine the effect of primary and secondary robbing on mean *Ipomopsis* pollen receipt per stigma per week (square-root-transformed to improve normality), we constructed a linear mixed-effects model using the lmer() function from the LME4 package (Bates *et al.* 2015). We included plant treatment (P, PS and C) as a fixed effect, the mean number of open flowers on the focal plant the week before stigma collection as a covariate and plant and week as random effects. Effect sizes of individual model terms were tested using likelihood ratio tests using the drop1() function (type III SS), and treatment levels were compared using a Holm test using the testInteractions() function. Heterospecific pollen deposition was rare (present in <5% of all stigma samples) and was therefore excluded from the analysis.

To test how robbing treatments affected estimates of female plant reproduction, we first used a MANOVA to test how robbing treatment affected proportion fruit set per plant (arcsine-square-root-transformed), mean seed set per fruit per plant and total seeds per plant (square-root-transformed). A significant MANOVA was followed by univariate ANOVAS (type I SS) for each response variable. Because we used plant as the unit of replication in this analysis, we could proceed with a general linear model rather than a random effects model with plant as a repeated measure. In all three univariate ANOVAS, we performed *post hoc* comparisons of the three treatment levels (P, PS and C) using a Tukey HSD test.

To test the prediction that pollinators visited plants with flowers in the PS treatment less frequently than those in the P or C treatments, we calculated pollinator visitation rate as (number of visits per plant/number of observation days) × (mean number of flowers visited/mean number of open flowers on the day of visit). We used one-way ANOVA to test how robbing treatment affected visitation rate. Previous work has shown that floral display size and plant height can affect visitation rates in this system (Brody & Mitchell 1997), so we added plant height (cm) and mean number of flowers open as covariates.

Results

EFFECT OF PRIMARY AND SECONDARY ROBBING ON FEMALE FITNESS

Pollen receipt

Nectar robbing significantly reduced the number of conspecific pollen grains received ($\chi^2_2 = 18.81$, $P < 0.001$; Fig. 2). Plants that experienced both primary and secondary nectar robbing (PS treatment) received 23% fewer pollen grains than did plants that experienced primary robbing only (P treatment) and 28% fewer pollen grains than plants that

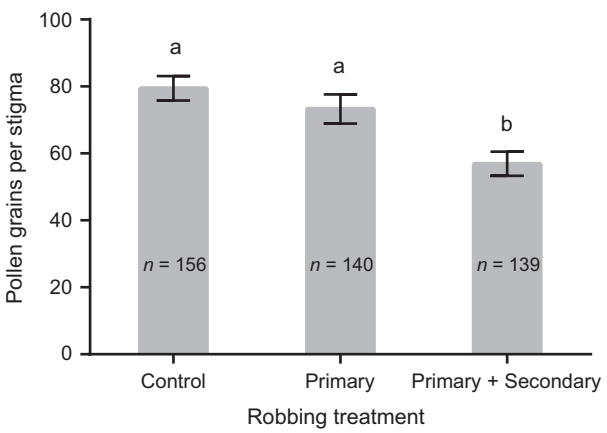

Fig. 2. The effect of robbing treatments on mean (±SE) pollen grains per stigma per plant. Numbers inside bars represent the total number of stigmas collected over the entire season for each treatment group. Different letters represent significantly different ($P < 0.05$) mean values based on a Holm test.

experienced no robbing (C treatment). A *post hoc* test showed no significant difference in pollen receipt between P and C treatment plants ($P = 0.122$; Fig. 2), but PS differed significantly from all other treatments (C vs. PS: $P < 0.001$, P vs. PS: $P = 0.010$). The number of open flowers per plant did not significantly affect pollen receipt ($\chi^2_1 = 0.1526$, $P = 0.696$).

Fruit and seed production

Nectar robbing significantly affected fruit and seed production (MANOVA: Wilk's $\lambda = 0.66$, $F_{2,117} = 8.809$, $P < 0.001$). Univariate analyses revealed that nectar robbing significantly reduced proportion fruit set ($F_{2,117} = 21.87$, $P < 0.001$; Fig. 3a). Plants in the PS treatment set 21.6% fruit, compared to 35% in the P treatment and 53.5% in the S treatment. A *post hoc* analysis showed that all three treatments differed significantly from one another ($P \leq 0.011$ in all pairwise comparisons).

Robbing treatment also significantly affected mean seeds produced per fruit ($F_{2,117} = 5.31$, $P = 0.006$; Fig. 3b) and total seed production per plant ($F_{2,117} = 11.48$, $P < 0.001$; Fig. 3c). Secondary nectar robbing reduced mean seeds per fruit by 35% compared to plants that were primary-robbed only ($P = 0.025$) and by 37% compared to control plants ($P = 0.010$). However, there was no significant difference between P and C treatments ($P = 0.947$). For total seeds per plant, a *post hoc* test showed significant differences among all treatments, although the difference between P and C treatments was marginal (PS vs. P: $P = 0.042$, PS vs. C: $P < 0.001$, P vs. C: $P = 0.054$). Plants in the PS treatment produced 49% and 65% fewer seeds overall than plants in the P and C treatments, respectively.

EFFECTS OF PRIMARY AND SECONDARY ROBBING ON POLLINATOR VISITATION RATE

Over 12 mornings of observations, we observed a total of 445 floral visits to 28 of the 30 observation plants. All visits were made by broad-tailed hummingbirds (*S. platycerus*), except for one visit by a rufous hummingbird (*S. rufus*). Hummingbird visitation rate to plants in the C treatment tended to be higher than to plants in the P and PS treatments. Mean ± SE visitation rates to the C, P and PS treatments were 0.076 ± 0.02, 0.050 ± 0.01 and 0.048 ± 0.007 flowers per plant per day visited, respectively. However, these differences were not statistically significant ($F_{2,18} = 0.857$, $P = 0.44$). Plant height significantly affected hummingbird visitation rate ($F_{1,18} = 7.413$, $P = 0.01$), with taller plants receiving higher visitation rate, while the number of open flowers did not ($F_{1,18} = 0.947$, $P = 0.34$).

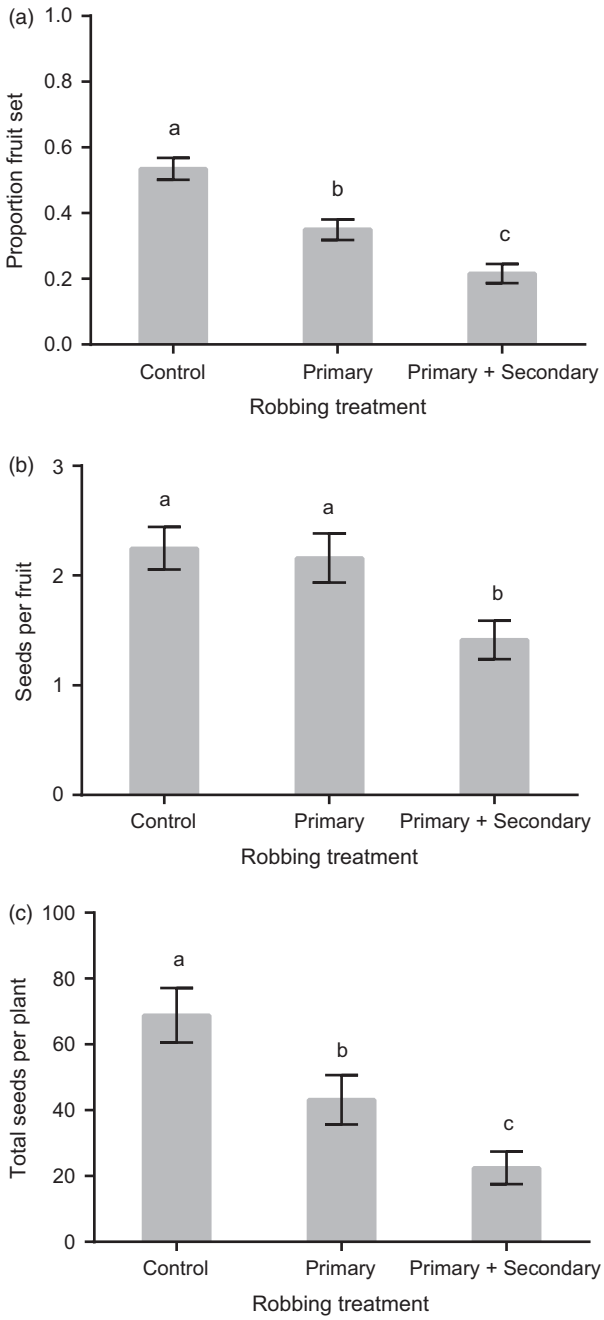

Fig. 3. The effect of robbing treatments on fruit and seed production. Panels represent treatments applied at the whole-plant level ($n = 40$ plants per treatment): (a) Proportion fruit set per plant, (b) average number of seeds per fruit, (c) total number of seeds per plant. Bars and values as in Fig. 2. Different lowercase letters indicate statistically significant differences ($P < 0.05$).

Discussion

Mutualistic interactions are rarely pairwise, as most mutualists interact with multiple partner species (Stanton 2003), and the ecological and evolutionary consequences of interacting with single vs. multiple partners have been investigated in depth (Bronstein 2015). Species engaged in mutualism also commonly interact with multiple exploiter species sequentially and/or simultaneously (Bronstein & Ziv 1997; Vannette, Gauthier & Fukami 2013). However, there has been minimal recognition of this phenomenon, nor measurement of its effects. Here, we examined how interactions between two forms of exploitation might generate effects on a shared mutualist. Towards this end, we simulated interactions among

plants, pollinators and nectar robbers to examine whether exploitation by secondary nectar robbers, which can only take place if primary nectar robbers have been active, imposes fitness costs to plants over that imposed by primary robbing alone. We found strong evidence for such fitness costs in *I. aggregata*. There was a significant negative effect of secondary robbing on all plant reproduction response variables measured. Furthermore, plants that were secondary- as well as primary-robbed were the only ones that experienced reduced pollen deposition and reduced number of seeds per fruit compared to a control. These results suggest that facilitated exploitation may exact fitness costs on the mutualist, and thus, this form of exploitation should be given closer attention to elucidate its role in affecting mutualist performance in natural communities.

SECONDARY ROBBING, POLLEN DEPOSITION AND POLLINATOR BEHAVIOUR

Secondary nectar robbing resulted in a significant reduction in pollen receipt to flowers. In *Ipomopsis*, pollen receipt tends to increase with pollinator visitation rate (Engel & Irwin 2003). If hummingbirds avoid secondary-robbed flowers, we would have expected to see such a reduction in pollen deposition. There is evidence that avian pollinators avoid visiting robbed flowers in *Puya coerulea* (Bromeliaceae) and *Tristerix aphyllus* (Loranthaceae) when robbers remove substantial amounts of nectar (Gonzalez-Gomez & Valdivia 2005; Caballero *et al.* 2013), and that simulated and natural robbing reduces nectar standing crop (Lasso & Naranjo 2003; Newman & Thomson 2005). Previous work in *Ipomopsis* has shown that hummingbirds do avoid robbed flowers (which have been drained of nectar) and are able to distinguish a nectar-full from an empty flower (Irwin 2000). In our study, secondary-robbed flowers were always empty or, by the end of the 3-h observation period, contained a negligible amount of nectar [based on measurements of *Ipomopsis* nectar production rate (Pleasants 1983)]. In contrast, primary-robbed flowers could be either empty (on the day of the initial manipulation) or have nectar available (on subsequent observation days, given that nectar was allowed to accumulate following primary robbing). While it is unlikely that primary- or secondary-robbed flowers will always be fully drained, there are many reports in other species of decreased nectar volume in flowers with natural robbing holes compared to unrobbed flowers in the same population. Stout, Allen & Goulson (2000), Maloof (2001) and Richardson & Bronstein (2012) found this pattern in the plants *Linaria vulgaris* (Scrophulariaceae), *Corydalis caseana* (Fumariaceae) and *Arctostaphylous pungens* (Ericaceae), respectively. Thus, it is plausible that if hummingbirds use the presence of nectar to decide whether or not to probe a flower, plants with flowers that have been both primary- and secondary-robbed would be visited less frequently overall. However, our study does not provide statistically significant evidence that this process is at work. Hummingbirds showed a slight but not statistically significant tendency to probe fewer secondary-robbed flowers than flowers in either of the other treatments. A *post hoc* power analysis revealed that we had only a 12% probability of detecting a significant robbing treatment effect on pollinator visitation rate. We would have needed to increase our sample size almost 10-fold to have found a significant effect of robbing treatment on pollinator visitation rate. Furthermore, pollinator visitation rate is not the only factor that affects pollen receipt to flowers. Another possibility is that hummingbirds spend less time probing individual flowers that do not contain nectar, leading to lower pollen deposition for these flowers. Mitchell & Waser (1992) found reduced pollen removal and pollen deposition as the number of probes to a flower declined. Measuring per-probe pollen deposition by pollinators to treatment flowers was beyond the scope of this study, but could provide additional mechanistic insight for future research. Interestingly, Lara & Ornelas (2002) report the opposite pattern in *Moussonia deppeana* (Gesneriaceae) when flowers were manipulated to mimic nectar thievery (consumption of nectar without subsequent pollination). Hummingbird pollinators made longer visits to flowers that experienced nectar theft and were more likely to revisit these flowers, resulting in additional probing, which was correlated with increased seed set (Lara & Ornelas 2002). These findings inspire questions about the potential for interaction between two putative floral antagonists, nectar robbers and nectar thieves, as well as the potential for differential outcomes on plant fitness due to the net indirect effects via changes in pollinator behaviour.

CONSEQUENCES OF SECONDARY ROBBING FOR PLANT FITNESS

Fruit and seed production in *Ipomopsis* is often pollen-limited (Hainsworth, Wolf & Mercier 1985). Therefore, when fewer pollen grains are deposited onto stigmas in response to secondary robbing, fruit and seed output should be lower (Hainsworth, Wolf & Mercier 1985; Campbell & Halama 1993). Our results are consistent with this expectation. Plants that experienced secondary robbing produced just one-third as many seeds as plants that did not experience robbing, and half as many seeds as plants that only experienced primary robbing. *Ipomopsis* does not have a seed bank, so all germination in a given year is dependent upon seed production the previous year (Campbell 1997; Price *et al.* 2008). Thus, secondary robbing not only has the potential to affect seed production but also seedling numbers the subsequent year, depending on the magnitude of seedling density dependence (Price *et al.* 2008). Campbell (1997) hypothesized a trade-off between seed production and survival to adulthood, as the probability of surviving increases with seed mass. Nectar robbing does not affect seed mass in *Ipomopsis* (Irwin & Brody1999). Thus, reduced seed production by plants that experienced secondary robbing may reduce the number of seedlings that survive to reproduce. Indeed, Price *et al.* (2008) report pollen limitation contributing to reduced germination and seedling survival. Additionally, a population growth model of *Ipomopsis* predicts population growth to be most sensitive to early survival (i.e. years 1–3), followed by reproduction, with low survival and reproduction causing population growth (λ) to

fall below 1 (Price *et al.* 2008). Taken together, we can reasonably hypothesize that secondary nectar robbing could lead to negative population growth in this species.

One caveat is that we only measured the effects of primary and secondary robbing on female fitness. The effect of nectar robbing on male plant fitness has been less studied than female fitness (reviewed in Irwin *et al.* 2010), although existing studies suggest that robbing may have differential effects on male fitness. Mayer *et al.* (2014) found that bumblebee pollinators of *Aconitum napellus* ssp. *lusitanicum* (Ranunculaceae) visited more flowers per plant and spent less time foraging per flower when foraging from robbed flowers, leading to an increase in male fitness. Previous studies on *Ipomopsis* have shown that robbing reduces male fitness, measured as pollen (dye) donation (Irwin & Brody 1999) and number of seeds sired (Irwin & Brody 2000). As with female fitness, these results have been attributed to pollinator avoidance of robbed (i.e. unrewarding) flowers (Irwin & Brody 1999, 2000). In the light of this mechanism, we might expect reduced seeds sired from secondary-robbed flowers through reduced pollen export and donation. However, *Ipomopsis* is protandrous and only in male phase for 1–2 days. Thus, the degree to which secondary robbing will affect male plant function beyond that of primary robbing alone may be contingent on how long flowers stay in male phase, how often and how quickly secondary robbing occurs following primary robbing, and the rate of nectar production. There is also the possibility that nectar robbing affects how long flowers are in male phase. Experimental robbing shortens the duration of male phase in *Impatiens capensis*, presumably because robbing reduces pollinator attractiveness, leading to a greater investment in female fitness than male fitness (Temeles & Pan 2002). We advocate that more work be done on the effects of primary and secondary robbing on male function, as it may change our understanding of the overall fitness consequences of secondary nectar robbing.

Our experimental design allowed us to test the effect of repeated nectar removal without pollination on plant female fitness. Using simulated rather than natural robbing was advantageous as it allowed us to remove any confounding effects associated with robber preference for individual plants, which may covary with plant vigour or reproductive potential. However, by simulating primary and secondary robbing rather than allowing natural robbing to occur, we were unable to identify any potential direct effects secondary robbers may have on *Ipomopsis* reproduction. A number of potential direct effects could occur: for instance, continual probing of robbing holes may increase their size, forcing plants to allocate resources away from reproduction in order to compensate for damage. In a study of florivory in *Isomeris arborea* (Cleomaceae), damaged flowers produced significantly less nectar than undamaged flowers (Krupnick, Weis & Campbell 1999). Conversely, if robbing holes are larger, more water in nectar may evaporate out of holes or may evaporate more quickly, which could stimulate additional nectar production. Production of excessive floral nectar has been shown to be costly for plants (Pyke 1991). Moreover, more concentrated sugars in nectar are more preferred by some pollinators up to a point, such as bees (Cnaani, Thomson & Papaj 2006; Nicolson *et al.* 2013), but less preferred by other pollinators, such as hummingbirds which feed by capillary action (Baker 1975). Furthermore, secondary robbers might jostle anthers and stigmas while nectar feeding from robbing holes, dislodging pollen in the process. No study to our knowledge has tested for these potential effects, but they warrant future investigation. Finally, because we did not quantify natural secondary robbing in our experiment, we can only conclude that effects on plant fitness and pollinator behaviour are in response to additional nectar removal from flowers, which could be attributed to secondary nectar robbing or chronic nectar removal by any sort of nectar thief. Nectar thieves influence bumblebee pollinator behaviour in *Corydalis ambigua* (Fumariaceae) (Zhang, Zhao & Inouye 2014), leading to decreased plant reproductive success. The degree to which secondary nectar robbing vs. chronic nectar theft affect pollinator visitation in *Ipomopsis* warrants further investigation.

FACILITATED EXPLOITATION AND INTRAGUILD INTERACTIONS

This study demonstrates a clear pattern of secondary exploitation reducing mutualist fitness. This phenomenon is worthy of deeper investigation in natural settings, as it could lead to generalizable hypotheses about how exploiters interact and their consequences on mutualist fitness. In nectar robbing systems, it is assumed that primary robbers provide a fitness benefit to secondary robbers because secondary robbers are more easily able to forage from robbing holes than to legitimately forage from floral openings (Irwin *et al.* 2010). Previous studies have shown that there is some incentive for legitimate foragers to switch to secondary robbing once use of the strategy is an option. Honeybees that cannot primary-rob will legitimately forage from *Vaccinium ashei* (Ericaceae) until carpenter bees primary-rob flowers (Dedej & Delaplane 2004). At this point, honeybees switch to secondary robbing because it enables them to forage more efficiently, and the change in behaviour leads to a reduction in seed set (Dedej & Delaplane 2004, 2005). The same phenomenon is seen in hummingbirds switching from legitimate foraging to secondary robbing in *Cavendishia pubescens* (Ericaceae), although the incentive at work in this system was not detected (Kjonaas & Rengifo 2006).

In facilitated exploitation, one exploiter has a positive effect on another. However, exploiters may more commonly interact with each other antagonistically, as is seen in interactions between species that compete for a common mutualist partner. Researchers have argued that competition among species that share a mutualist should mitigate the positive density dependence characteristic of one-to-one mutualism and thus play an important role in the persistence of the interaction (Palmer, Stanton & Young 2003; Jones, Bronstein & Ferriere 2012). Whether the same can be said for competition among exploiters is unknown. Further investigation into competition among exploiter species and between mutualists and exploiters is

warranted in order to generate predictions about the outcome of multi-species interactions on mutualist fitness and the potential for coexistence among exploiters, mutualists and their partners.

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Data accessibility

All data used in this study are archived at the Dryad Digital Repository http://dx.doi.org/10.5061/dryad.tf71f (Richman *et al.* 2016).

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