Nectar robbing impacts pollinator behavior but not plant reproduction

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Trait-mediated indirect effects (TMIEs) refer to interactions in which the effect of one species on another is mediated by the behavior of a third species. A mechanistic approach that identifies the direction and impact of TMIEs can shed light on why different net outcomes are observed in the same general phenomena across systems. Nectar robbing has variable net effects through TMIEs on animal-pollinated plants across systems, but the mechanistic steps underlying this range of outcomes are often unclear. To address this knowledge gap, we assessed linkages between nectar robbing, pollinator behavior and plant reproductive success in the Andean tree, *Oreocallis grandiflora*. We found that robbing in this system led to lower nectar volumes, higher nectar sucrose concentration, and higher nectar viscosity, which together negatively impact nectar quality. This drop in nectar quality was associated with decreased visitation rates by hummingbirds, which might be expected to impact plant reproduction negatively by pollen limitation. However, it was also associated with increased diversity (Shannon's) and evenness in the pollinator community due to reduced visitation by a territorial hummingbird, which might be expected to impact reproduction positively via enhanced genetic diversity of pollen as non-territorial pollinators forage over greater areas. We measured seed set and mass to distinguish the relative intensity of these two possible outcomes, but found no detectable effect. We tentatively conclude that these two consequences of TMIEs may have balanced each other out to yield a neutral net effect of nectar robbing on plant reproduction, though other explanations are also possible. This study highlights ways in which ecologically important TMIEs may act in opposing directions to mask important ecological forces, and underscores the continued need for detailed study of the mechanisms through which TMIEs operate.

Ecologists find it useful to distinguish between direct effects of one species on another, for example predation, and indirect effects, in which non-consumptive interactions mediated by a third species impact net outcomes (Schmitz et al. 2004, Walsh 2013). Given the relative complexity of quantifying indirect effects, the mechanisms by which they shape the net outcome of ecological processes are often obscure. Yet, quantifying the magnitude and direction of indirect effects is an important goal in ecology because of the insights it can provide on how various factors might shift the net impact of species interactions under differing or changing contexts (Relyea and Hoverman 2006), which is relevant in the context of the anthropogenic perturbation of ecological processes like pollination (Biesmeijer et al. 2006, Anderson et al. 2011, Spiesman and Inouye 2013).

Indirect effects can generally be described as either trait- or density-mediated (TMIE and DMIE respectively) (Schmitz et al. 2004). DMIEs refer to the indirect effects on two species that result due to changes in the relative density of an intermediate species. TMIEs refer to effects that result from changes in the behaviors of one of the species, or on an intermediate species, as a result of the activities of the other.

For example, the presence of a predator can cause lower rates of communication displays in bees, therefore lowering food acquisition rates, which then has a negative effect on pollen movement and plant reproduction (Bray and Nieh 2014). TMIEs have been shown to impact species diversity (Steffan and Snyder 2010), demography (Schmitz et al. 1997), and evolution (Walsh 2013). Behaviorally mediated indirect effects on demography depend on a suite of variables including species-level behavioral responses (Steffan and Snyder 2010). Thus, their net impact may represent a balance of opposing forces, such that neutral net responses may mask important processes that could lead to distinctive outcomes if their relative intensities were altered.

Animal-mediated pollination is an ecological process that may be subject to TMIEs mediated by behavioral response of pollinators to interspecific interactions, such as the presence of predators (Bray and Nieh 2014). In the case of nectar robbing as defined by Inouye (1980), nectar may be altered, causing changes in pollinator behavior that might scale up to influence plant reproduction (Irwin and Brody 1998). TMIEs involving three or more species, such as the putative linkage among nectar robbers, pollinators and plants,

are complex to study, but can provide important insights into the mechanisms underlying net outcomes. Indeed, reconciling the wide range of net effects of nectary robbing on plant reproduction reported across systems (Irwin et al. 2010) may depend on unraveling the behavioral responses of pollinators.

Nectar robbers can elicit TMIEs on plant reproduction via scramble competition with legitimate pollinators or via changes in floral traits, such as nectar properties (Irwin et al. 2010), that alter foraging behavior of true pollinators. Nectar robbing can reduce nectar volumes via consumption or evaporation through the incision made by robbers in the flower corolla (Pleasants 1983), both of which may increase nectar viscosity and reduce foraging efficiency (Kim et al. 2011), leading to avoidance of robbed flowers by pollinators (Irwin and Brody 1998, Irwin 2000, Zhang et al. 2014; but see Lasso and Naranjo 2003). These changes in pollinator behavior might influence plant reproduction in different ways. Some pollinators respond to robbing by increasing inter-flower flight distances (Maloof 2001), which could improve outcrossing. However, decreased overall pollinator visitation may lead to pollen limitation (Knight et al. 2005) and decreased plant reproduction (Irwin and Brody 1998). Changes in nectar properties could also lead to shifts in composition of pollinator communities, which could lead to changes in pollen delivery (Ne'eman et al. 2010). For example, a community of pollinators dominated by a single territorial species as opposed to a diversity of trap-lining or transient species may have negative effects on plant reproduction due to more limited pollen movement and possible subsequent inbreeding effects (García-Meneses and Ramsay 2012). In contrast, a shift in pollinator community towards pollinators that forage over greater distances may improve plant reproduction through the benefits of genetic outcrossing (Loveless and Hamrick 1984, Waser and Price 1994), though negative impacts of expanded pollinator community are also possible (Ne'eman et al. 2010). The degree to which robbing elicits TMIEs via pollen limitation vs. improved outcrossing is poorly resolved, and these opposing forces may cancel each other out and lead to neutral net effects on plant reproduction (Fig. 1).
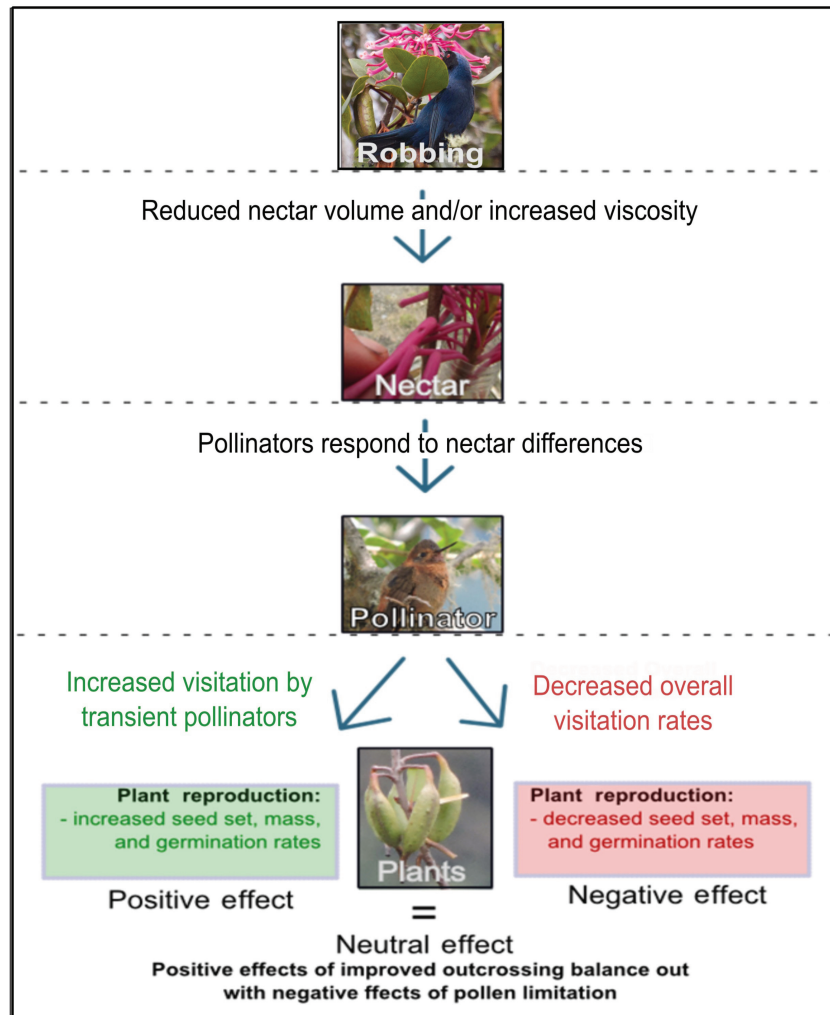

Figure 1. The cascade of putative causes and effects of trait-mediated indirect effects from nectar robbing to plant reproduction. Labels on arrows indicate the mechanism by which one level of the cascade may impact another. Green indicates a positive effect on maternal plant reproduction, while red indicates a negative effect on maternal plant reproduction.

In this study we investigated the net reproductive outcomes of TMIEs instigated by avian nectar robbing on the hummingbird-pollinated Andean tree *Oreocallis grandiflora* as moderated by pollinator behavior. In this system, avian flower-piercers (family: Thraupidae) are the only confirmed robbers and the territorial hummingbird *Aglaeactis cupripennis* is the dominant pollinator. We predicted that robbing would: 1) have a negative impact on nectar properties due to nectar consumption and evaporation through the incision made by the birds; that this in turn would 2) reduce pollinator visitation overall, and 3) change the composition of the pollinator community by reducing relative visitation of the dominant territorial hummingbird while increasing relative visitation rates by non-territorial pollinators. We then measured seed set and seed mass to assess whether these predicted changes impact plant reproduction positively (if outcrossing benefits outweigh pollen limitation), or negatively (if the converse is true). Predicted changes to nectar properties and pollinator behavior were observed, but there was no detectable effect on seed set or seed mass, consistent with the idea that costs of pollen limitation and benefits of outcrossing might balance might balance each other out in this system.

Methods

Study system

Fieldwork was conducted from July–September (2013–2014) at the Wayqecha Biological Station (43°38′N, 116°14′W) in Manu National Park, Peru in cloud forest habitat at 3900 m a.s.l. *Oreocallis grandiflora* is a small tree that produces terminal inflorescences of 10–30 bright pink tubular flowers (6–7 cm corolla length) that open sequentially from the base of the inflorescences towards the top. Flowers are hermaphroditic and have a pollen presenter, wherein the flower opens with its own pollen deposited on its own stigma (Prance et al. 2007) and has been observed to produce seed autogamously (Hazlehurst unpubl.). At the start of the study it was unknown if *O. grandiflora* was capable of autogamous selfing, as many species with pollen presenters are not. The dominant pollinator is a territorial hummingbird species, *Aglaeactis cupripennis*, which comprised over 60% of all visits recorded during 200 h of observation (Hazlehurst unpubl.). Six other species of hummingbirds have also been seen visiting *O. grandiflora* at this study site (Table 1). There are three resident species of avian nectar robbers, all of which rob *O. grandiflora*: *Diglossa cyanea*, *D. brunneiventris* and *D. mysticalis*. Surveys of nectar robbing in *O. grandiflora* at

the site revealed a mean robbing rate of $21 \pm 0.30\%$ (mean $\pm$ SE; n = 110) of flowers robbed per inflorescence.

Effects of simulated robbing on nectar properties

We calculated the proportion of nectar *Diglossa* robbers extract from flowers during a robbing event in order to calibrate our simulated robbing experiments by conducting an aviary experiment in which captive *Diglossa* were offered flowers of *O. grandiflora* with 15 µl of 30% brix sucrose solution (based on averages of random sampling of *O. grandiflora* at the time). We conducted six successful aviary trials with all species of the genus *Diglossa* occurring at the study site, and in all six trials the birds extracted all nectar present and did not damage floral ovaries. To understand how nectar robbing by *Diglossa* impacts nectar properties in a natural setting, we established plots consisting of three individuals of *O. grandiflora* of similar size and structure and standardized all plants to five terminally located inflorescences, each with five flowers that were about to open. Within each plot, each individual tree was randomly assigned one of the following three treatments: Unrobbed (flowers left un-manipulated), Robbed (an artificial robbing incision was made and robbing by *Diglossa* simulated), and Closed (flowers excluded from all visitors with mesh bags) (Table 2). Each plot was monitored for five days, and nectar volume and sucrose concentration by weight (% Brix) was measured every morning from 8 to 10 a.m. from one flower from each inflorescence. Different flowers were measured each day. All statistical analyses were conducted in R ver. 3.1.3 (<www.r-project.org>). To analyze how nectar volume responded to treatment, we conducted two, complementary analyses to accommodate a large number of zero-volumes. First, we ran a binomial generalized linear mixed model (GLMM) on the entire data set (*glmer* function from package *lme4*; Bates et al. 2015) considering the presence or absence of nectar as the response variable, treatment as the predictor variable, and day and individual tree nested within plot as the random variables ($n_{observations} = 252$, $n_{trees} = 42$, $n_{plots} = 14$). Secondly, we analyzed only non-zero data using a Gaussian linear mixed-effects model (LMM) (*lme* function from package *nlme*; Pinheiro et al. 2009). We considered the average nectar volume (µl of nectar) from the five flowers (square-root transformed) sampled from each individual tree daily as the response variable, treatment as the predictor variable, and day and individual tree nested within plot as random variables ($n_{observations} = 199$, $n_{trees} = 42$, $n_{plots} = 14$). The model was weighted by plot to improve model fit, and a correlation structure was added to mitigate autocorrelation.

Table 1. The proportion of identified visitors belonging to each species observed at robbed versus unrobbed treatment plots.

Hummingbird species	Robbed	Unrobbed
Aglaeactis cupripennis	20.9%	63.4%
Boissonneaua matthewsii	9.3%	13.4%
Colibri coruscans	32.6%	13.4%
Coeligena violifer	8.1%	2.4%
Heliangelus amethysticollis	5.8%	1.0%
Metallura tyrianthina	14.0%	2.4%

Table 2. Descriptions of the treatments used in the nectar and visitation experiments, with the exception of the second nectar experiment in which visitors were excluded to isolate the effect of nectar evaporation from nectar consumption. An X indicates that the column description apples to that treatment, whereas a line indicates that it does not.

Treatment	Pollinator access?	Nectar properties	Pollinator visitation	Plant reproduction
Unrobbed	Yes	X	X	X
Robbed	Yes	X	X	X
Closed	No	X	—	X

To assess the effects of simulated robbing on nectar properties independently of pollinator visitation, we selected 20 random trees of *O. grandiflora* with similar height and structure, identified two inflorescences per tree, pruned the number of flowers to four, and applied our artificial Robbing treatment to two of the flowers while leaving the other two un-manipulated. The inflorescences were then bagged with light mesh bags to exclude all visitors. In the context of this experiment, these treatments will be referred to as 'Robbed' and 'Unrobbed', however they are different from those used above because they are also closed off from visitors (Table 2). We measured the nectar volume and sucrose concentration (% Brix) in these flowers at 6 a.m. every day to calculate 24-h nectar accumulation rates. We analyzed these data using a two-step model as above. Due to the nested nature of the data, the raw data means we present in the results may differ from the fitted model results which are those reported in the figures for all of our analyses. We used a binomial GLMM to measure the effect of the robbing treatment on the presence or absence of nectar in the flowers (*glmer* function in package *lme4*; Bates et al. 2011) with day, and inflorescence nested within individual tree as random factors ($n_{observations} = 318$, $n_{inflorescences} = 40$, $n_{trees} = 20$). We then used a Gaussian LMM (*lme* function in package *nlme*; Pinheiro et al. 2009) with square-root transformed nectar volume (µl nectar) as the response variable, treatment as the predictor, and day, and inflorescence nested within individual tree as random factors ($n_{observations} = 256$, $n_{inflorescences} = 40$, $n_{trees} = 20$). The model was weighted by treatment and a polynomial term was added to improve model fit and autocorrelation.

To analyze how sucrose concentration responded to treatment, we conducted a Gaussian linear mixed-effects model (*lme* function from package *nlme*; Pinheiro et al. 2009). We considered the average sucrose concentration by weight (% Brix) across the five flowers sampled from each individual tree daily as the response variable, the interaction of treatment and day of experiment as the predictor variable, and individual tree nested within tree plot as random variables ($n_{observations} = 144$, $n_{trees} = 36$, $n_{plots} = 12$). Sucrose concentration was square-root transformed for the analysis, and the LMM was weighted by tree and a correlation structure added to improve model fit.

Effects of simulated robbing on pollinator visitation and community

To assess how robbing may impact the number of visits by pollinators and the pollinator community in *O. grandiflora*, we set up a separate set of experimental plots identical to those described in our methods for studying the effects of simulated robbing on nectar properties, with the difference that there was no closed treatment (Table 2). We set up digital camcorders at each tree every day for five days and recorded all visitors for at least 1 h. The videos were then reviewed manually and the identity of visitors, duration of visits, and number of flowers probed per visit were recorded. Statistical analysis of count data was again conducted with a two-step model. In both steps we included an offset of the log-transformed number of hours of observation at each tree. First, the effects of robbing treatment on the presence or absence of hummingbird visits was analyzed using a binomial GLMM (*glmer* function in the package *lme4*; Bates et al. 2015). We considered the interaction of treatment and day as fixed effects, and individual tree nested within plot as random factors ($n_{observations} = 100$, $n_{trees} = 20$, $n_{plots} = 10$). We then analyzed the non-zero visitation data using a Gaussian LMM (function *lme* in package *nlme*; Pinheiro et al. 2009). We considered the log-transformed number of visits as the response variable and treatment and day as fixed effects and individual tree nested within plot as the random variable ($n_{observations} = 91$, $n_{trees} = 20$, $n_{plots} = 10$). The model was weighted by individual tree and a correlation structure was added to improve model fit and to mitigate autocorrelation.

To analyze how nectar robbing affected the pollinator community, we lumped the visitors across observations at each individual tree (from 18 trees) and calculated the Shannon's diversity index (SDI) and Pielou's evenness (E) index of the pollinator community at each tree, treating each tree as though it were a different 'site' in a traditional diversity analysis. We then used a nested ANOVA (*aov* function in package *stats*; <www.r-project.org>) considering SDI or E as the response variable, treatment as the predictor variable, total observation time as a covariate, and treatment nested within plot as the error term ($n_{observations} = 18$, $n_{plots} = 9$). To analyze how nectar robbing impacted visitation by the dominant and highly territorial pollinator of *O. grandiflora*, the hummingbird *Aglaeactis cupripennis*, we used a Gaussian LMM (function *lme* in package *nlme*; Pinheiro et al. 2009) to analyze the effect of robbing treatment on the log-transformed count of *A. cupripennis* visits with plot as a random factor ($n_{observations} = 72$, $n_{plots} = 10$). The model was weighted by plot and a polynomial term was added to improve model fit and mitigate autocorrelation.

Effects of simulated robbing on plant reproduction

To quantify the impacts of nectar robbing on plant reproduction, we waited for seeds to develop from the treatment plots and calculated the resulting seed set per pod and mean seed mass per pod. We distinguished between pods that developed before or after our treatments by tying small pieces of flagging directly on to the stem of the inflorescence above and below our treatment flowers. We used a Gaussian LMM (function *lme* in package *nlme*; Pinheiro et al. 2009) to analyze the effect of treatment on the mean seed set from each seed pod with each pod nested within inflorescence within tree within plot ($n_{observations} = 124$, $n_{plots} = 16$, $n_{trees} = 30$, $n_{inflorescences} = 60$). We included an offset of the log-transformed number of seed pods produced by each tree and weighted the model by plot to improve fit. We then used a Gaussian LMM (function *lme* in package *nlme*; Pinheiro et al. 2009) with the square-root transformed seed mass as the response variable, and individual pod nested within inflorescence within tree within plot as the random variable ($n_{observations} = 124$, $n_{plots} = 16$, $n_{trees} = 30$, $n_{inflorescences} = 60$). We included offsets for the log-transformed number of seeds in each pod and the number of pods collected.

Data deposition

Results

Effects of simulated robbing on nectar properties

Simulated nectar robbing had no detectable impact on nectar presence or absence when pollinators were present (Fig. 2; slope $\pm$ SE $= -0.35 \pm 0.55$, z $= -0.62$, p $= 0.53$), or on nectar volume between the Robbed treatment and Unrobbed treatment (Fig. 2; slope $\pm$ SE $= 0.04 \pm 0.17$, t-value $= 11.15$, p $= 0.80$, DF $= 26$). However the Closed treatment had significantly greater nectar volume than the other treatments (Fig. 2; slope $\pm$ SE $= 0.61 \pm 0.17$, t-value $= 3.63$, p < 0.001, DF $= 26$, marginal $r^2_{GLMM} = 0.12$). When visitors were excluded from flowers, the robbing treatment had a significant negative effect on both the presence and absence of nectar (Fig. 2; slope $\pm$ SE $= -1.50 \pm 0.34$, z-value $= -4.43$, p < 0.001, DF $= 309$) and on the volume of nectar present (Fig. 2; slope $\pm$ SE $= -1.21 \pm 0.12$, t-value $= -9.64$, p < 0.001, DF $= 213$, marginal $r^2_{GLMM} = 0.26$). Simulated robbing also had a significant positive effect on the sucrose concentration of nectar in flowers open to visitation by pollinators (Fig. 2; slope $\pm$ SE $= 1.11 \pm 0.23$, t-value $= 4.79$, p < 0.001, DF $= 22$, marginal $r^2_{GLMM} = 0.77$). In summary, simulated robbing had a positive effect on nectar sucrose concentration and a neutral effect on nectar volume and presence or absence in the presence of pollinators but a negative effect on both nectar volume and presence or absence when visitors were excluded.

Effects of simulated robbing on pollinator visitation

We documented 253 hummingbird visits during 332 hours of recorded video. Robbing treatment had no effect on the presence or absence of pollinator visits (Fig. 3; slope $\pm$ SE $= 0.40 \pm 0.82$, z-value $= 0.49$, p $= 0.625$, DF $= 9$), but there was a significant negative effect of simulated robbing on the number of visits during observations in which one or more visit was recorded (Fig. 3; slope $\pm$
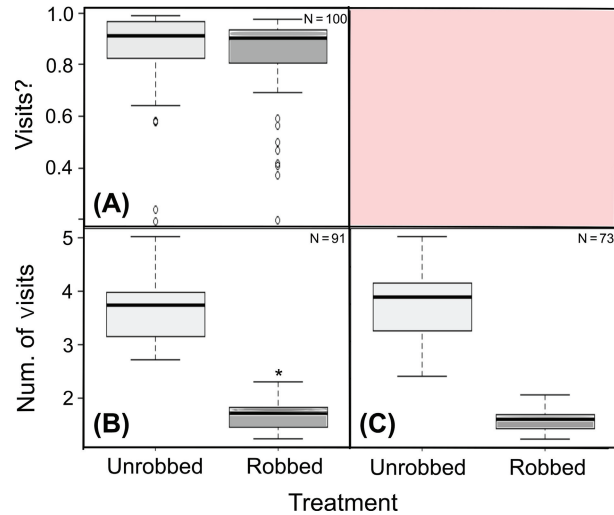

Figure 3. Simulated nectar robbing impacts pollinator behavior in terms of (A) no effect on the presence or absence of hummingbird visits, (B) a negative effect on the number of visits by hummingbirds and (C) a negative effect on the number of visits by *Aglaeactis cupripennis*. Open dots represent outliers. Stars represent significant results. The sample size refers to the number of observation periods. The relative means of the raw data for number of visits were as follows: Robbed treatment (mean $\pm$ SE $= 0.53 \pm 0.34$) and Unrobbed treatment (1.07 ± 0.56). The relative means of the raw data for number of visits by *A. cupripennis* were as follows: Unrobbed treatment (2.3 ± 0.23) and Robbed treatment (0.51 ± 0.09).

SE $= -0.78 \pm 0.10$, t-value $= -7.80$, p < 0.001, DF $= 9$, marginal $r^2_{GLMM} = 0.30$).

Effects of simulated robbing on pollinator community

There was a significant positive effect of robbing on the Shannon's diversity index (Fig. 4; $F_{1,7} = 20.98$; p < 0.001)
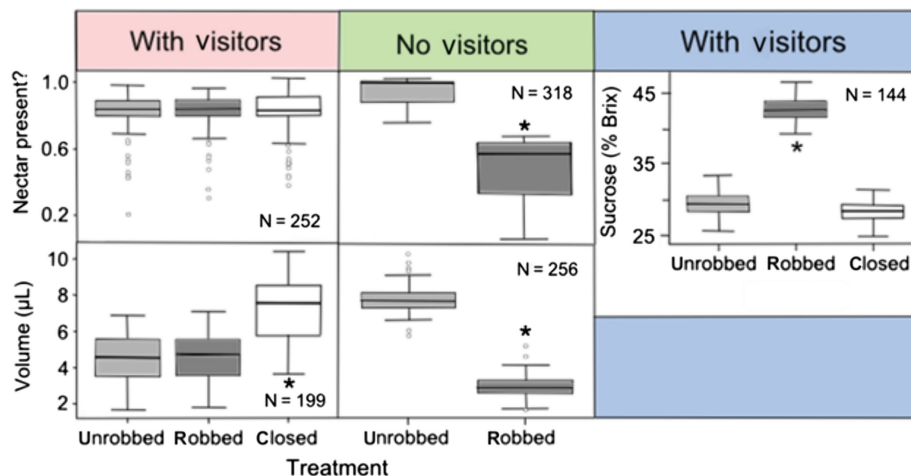

Figure 2. Simulated nectar robbing impacts both nectar volume and the presence or absence of nectar in the Andean tree *Oreocallis grandiflora* only when visitors are excluded, while sucrose concentration is positively impacted with visitors. Shown are the effects of robbing treatment on nectar properties when visitors are included and excluded. Stars represent significant results. The relative means of the raw data for nectar volume in the presence of visitors were as follows: Unrobbed treatment (mean $\pm$ SE $= 4.82$ μl ± 0.45), Robbed treatment (4.88 μl ± 0.52), and Closed treatment (7.5 μl ± 0.63). The relative means of the raw data for nectar volume in the absence of pollinator visitation were as follows: Unrobbed treatment (11.75 μl ± 0.68), Robbed treatment (10.86 μl ± 0.46). The relative means of the raw data for nectar sucrose concentration (% Brix) in flowers open to visitation by pollinators were as follows: Unrobbed treatment (31.13% ± 2.27), Robbed treatment (43.33% ± 11.46), and Closed treatment (30.15% ± 1.79).
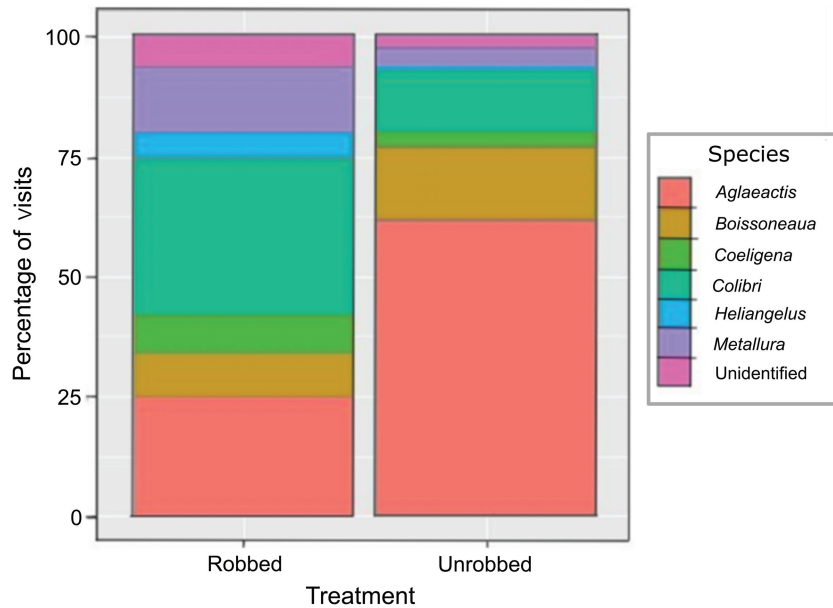
Figure 4. Simulated nectar robbing is associated with improved pollinator community diversity and evenness and reduced visitation by the territorial pollinator *Aglaeactis cupripennis*. There was only one observed species for each genus listed in the key; for full species names and values see Table 1. The relative means of the raw data for SDI were as follows: Unrobbed treatment (mean $\pm$ SE = 0.91 $\pm$ 0.03) and Robbed treatment (1.26 $\pm$ 0.06), while those for E were as follows: Unrobbed treatment (0.75 $\pm$ 0.03) and Robbed treatment (0.91 $\pm$ 0.02).

and on Pielou's evenness in Robbed plots (Fig. 4; $F_{1,7}$ = 10.88; $p < 0.01$). We also found significantly more visits by the dominant pollinator, *Aglaeactis cupripennis* in Unrobbed plots (Fig. 3; slope $\pm$ SE = 1.35 $\pm$ 0.44, t-value = 3.06, $p < 0.001$, DF = 72, marginal r^2_{GLMM} = 0.57).

Effects of simulated robbing on maternal plant reproduction

Neither the Robbed treatment (Fig. 5; slope $\pm$ SE = 0.35 $\pm$ 0.42, t-value = 0.83, p = 0.43, DF = 12, marginal r^2_{GLMM} = 0.01, conditional r^2_{GLMM} = 0.31) nor the Closed (Fig. 5; slope $\pm$ SE = 0.25 $\pm$ 0.45, t-value = 0.55, p = 0.58, DF = 12, marginal r^2_{GLMM} = 0.01, conditional r^2_{GLMM} = 0.31) treatments had a significant effect on seed set. The Robbed treatment had no effect on mean seed mass either (Fig. 5; slope $\pm$ SE = –0.002 $\pm$ 0.006, t-value = –0.31, p = 0.77, DF = 12, marginal r^2_{GLMM} = 0.26). In contrast, the Closed treatment did have a significant negative effect on seed mass (Fig. 5; slope $\pm$ SE = 0.03 $\pm$ 0.01, t-value = –4.34, $p < 0.001$, DF = 12, marginal r^2_{GLMM} = 0.26). It appears that simulated robbing treatment had no effect on seed set or seed mass and that *Oreocallis grandiflora* may experience negative effects of selfing on seed mass but not on seed set.

Discussion

Our results indicate that nectar robbing of the Andean tree *Oreocallis grandiflora* has a neutral net effect on seed set and mass, despite having a negative effect on pollinator visitation rates. Neutral net effects of nectar robbing on maternal reproduction have been documented in other studies, but so have positive and negative effects. Past studies that have shown a neutral effect of nectar robbing on maternal plant

reproduction invoked either an inability of pollinators to distinguish between robbed and unrobbed flowers (Maloof
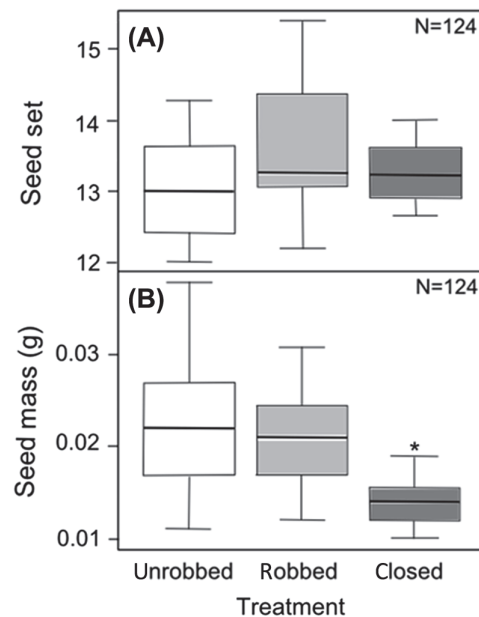

Figure 5. Simulated nectar robbing has no detectable impact on seed set and seed mass in the Andean tree *Oreocallis grandiflora*. Shown are the effects of simulated nectar robbing on maternal reproduction in terms of (A) a neutral effect on seed set and (B) a neutral effect on seed mass, while the selfing (Closed) treatment had no effect on seed set and a negative effect on seed mass. The relative means of the raw data for seed set were as follows: Unrobbed treatment (mean $\pm$ SE = 12.98 $\pm$ 0.23), Robbed treatment (13.70 $\pm$ 0.25) and Closed treatment (13.22 $\pm$ 0.19). The relative means of the raw data for mean seed mass were as follows: Unrobbed treatment (0.023 $\pm$ 0.001), Robbed treatment (0.021 $\pm$ 0.001) and Closed treatment (0.014 $\pm$ 0.000).

2001, Lasso and Naranjo 2003), low-efficiency pollination by nectar robbers offsetting declines in pollinator visitation rates (Arizmendi et al. 1996), or self-pollination (Zhang et al. 2009). Generally only the male aspect of plant reproductive output, pollen movement, is positively impacted by nectar robbing (Zimmerman and Cook 1985, Maloof 2001), and even so this is not a direct measure of male reproductive success but rather a proxy. However, Singh et al. (2014) also found a positive effect of nectar robbing on fruit and seed set, which they attributed to improved genetic outcrossing due to increased inter-flower flight distance. In cases where robbing has a negative net effect on plant reproduction, identified mechanisms include direct damage of floral reproductive structures (Askins et al. 1987, Traveset et al. 1998), aggressive interactions of nectar robbers against pollinators (Roubik 1982), or decreased attractiveness or profitability of flowers due to changes in nectar properties as a result of robbing (Irwin and Brody 1998, 1999). One intrinsic factor driving the variety of responses to robbing, at least in terms of maternal plant reproduction, is plant mating system. Burkle et al. (2007) found that pollen-limited, self-incompatible plants were much more likely to suffer negative consequences of nectar robbing in terms of fruit or seed set.

On the surface, our findings seem to corroborate parts of previous studies, though there is a great diversity of findings in the literature. We found a change in nectar properties of *O. grandiflora* in terms of both decreased volume and increased sucrose concentration, both of which may make flowers less attractive to pollinators (Pleasants 1983). Then, we found that robbed flowers experienced lower pollinator visitation rates, probably as a result of the changes in nectar properties, as has been documented in other hummingbird-pollinated systems (Irwin 2000) wherein pollinators can either visually identify robbed flowers or learn to avoid them by experience. While in some systems this decrease in pollinator visitation should lead to decreased maternal reproductive output in terms of seed or fruit set, we saw no decline in seed set or mass. This finding is consistent with the predictions of Burkle et al. (2007) and Zhang et al. (2009) since selfing plants, especially autogamously selfing plants, are less susceptible to pollen limitation and therefore less likely to suffer negative effects as a result of nectar robbing. In this study we are unable to tease apart the contribution of selfing pollen versus outcrossed pollen to seed production. We could have done so by including a treatment wherein flowers were emasculated, however the morphology of the pollen presenter, which is tightly conjoined with the anthers until dehiscence, at which point the anthers become defunct, would make such a treatment impractical. Had we included such a treatment, we may have seen more signal from either pollen limitation or outcrossing in our seed set and mass results. However, even with self-pollen in the picture, previous studies do not consider the potential negative inbreeding effects that partially selfing species may experience as a result of lower pollinator visitation rates in response to nectar robbing. If self-compatible species are less vulnerable to nectar robbing in terms of seed set, it is probably because they are compensating for any decreases in pollinator visitation by increasing the proportion of self-pollinated ovules, which could lead to increased negative effects of inbreeding in offspring. The negative effects of

inbreeding are not limited to seed set alone, and may also include lower seed mass, germination rates, seedling growth and survival (Montalvo 1994). In our 'closed' treatment, in which only self-pollination of *O. grandiflora* was possible, we found no effect on seed set but a significant negative effect on seed mass, suggesting that in the absence of imported pollen *O. grandiflora* may suffer some negative inbreeding effects. However, despite lower pollinator visitation rates in our robbing treatments, there was no effect of robbing on either seed set or seed mass. It is therefore possible that trait-mediated indirect effects of robbing are having a positive effect via a different pathway which is countering the negative effects of inbreeding.

Why did we fail to detect a significant effect of robbing on seed mass despite a decrease in pollinator visitation rates? It is possible that enough pollen arrived at stigmas despite the drop in pollinator visitation to fertilize available ova without increasing the proportion of selfing that occurred. It is also possible that the observed shifts in pollinator community composition improved genetic outcrossing rates, which could offset the increase in selfing as a result of decreased pollinator visitation. We cannot discount that the former is occurring, because we did not directly measure the proportion of ovules fertilized by self-pollen. However, pollen limitation has been reported in hummingbird-pollinated, non-selfing plants that experienced declines in pollinator visitation rates as a result of nectar robbing (Irwin and Brody 1998), so it is not unreasonable to think that decreases in pollinator visitation in our system would necessitate an increased rate of selfing. In our system nectar robbing may indirectly increase inter-flower flight distances and outcrossing rates as in Singh et al. (2014), because of the decline in visits by territorial pollinators, specifically by *Aglaeactis cupripennis*. Irwin (2000) found that hummingbirds used a combination of visual and experience-based spatial cues to identify robbed flowers, and there is also evidence that territorial hummingbirds can recall flower-specific nectar concentrations as well as nectar renewal rates (González-Gómez et al. 2011). This suggests that territorial hummingbirds can remember and avoid robbed flowers within their territories, which may have a positive effect on outcrossing rates given the generally small foraging area of territorial hummingbirds. Territorial pollinators move pollen over very small distances compared to other species, and tend to be less effective pollinators as a result because their limited foraging range increases the chances that inbreeding will occur (Franceschinelli and Bawa 2000). Preliminary radio telemetry of *A. cupripennis* showed that they defend small territories that are a fraction of the size of transient species at our site like *Colibri coruscans* and *C. violifer* (Hazlehurst unpubl.). To our knowledge, no studies to date have considered shifts in pollinator community as a mechanism for transmitting indirect effects of nectar robbing on plant reproduction. Rather, past work has focused instead on inter-flower flight distance alone. Future studies should consider pollinator community in addition to overall behavior as potential mechanisms for transmitting TMIEs of nectar robbing on plant reproduction and would benefit from the use genetic analysis to quantify the amount of outcrossing that occurs as a result of nectar robbing.

Conclusion

Our findings highlight that ecologically important but opposing forces may yield neutral net responses (Facelli 1994, Rand 2004) of the effect of nectary robbing on pollen delivery and seed set. Teasing apart the mechanisms of TMIE transmission paves the way for future research with practical application for conservation by identifying extrinsic and intrinsic factors that could shift net response of species to perturbing forces. In the course of our study, for example, we identified pollinator community as a previously unexplored and potentially important mechanism of nectar robbing-induced TMIEs in the pollination process. We recommend that future study of TMIEs caused by nectar robbing take into account pollinator community, and also that genetic methods be used to quantify outcrossing effects more precisely.

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