

CONDITIONAL EXPLOITATION AND CONTEXT-DEPENDENT FITNESS
CONSEQUENCES OF POLLINATION MUTUALISMS

by

Sarah K. Richman

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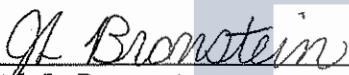
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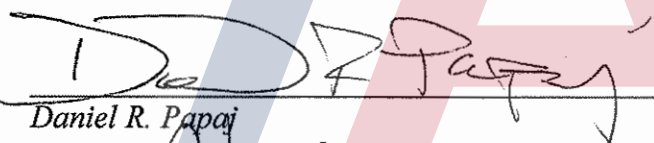
Judith L. Bronstein Date: (March 20, 2018)



Katrina M. Dlugosch Date: (March 20, 2018)



Rebecca E. Irwin Date: (March 20, 2018)




Daniel R. Papaj Date: (March 20, 2018)



D. Lawrence Venable Date: (March 20, 2018)

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ABSTRACT

Mutualisms are classically modeled as obligate, pairwise interactions in which individuals increase each other's fitness through the exchange of resources or services. Theoretically, the interaction should be unstable because the positive feedback of reciprocal fitness exchange leads to unchecked population growth. Furthermore, the benefit gained by cheating (taking a mutualistic benefit without reciprocating) should be so great that the cheating strategy takes over and the mutualism ceases to persist. Because mutualism is found across the tree of life, identifying the mechanisms permitting mutualism persistence has been a goal for ecologists and evolutionary biologists. In nature, mutualisms are rarely as simplistic as described in the classical models. In particular, they are often complicated by the many direct and indirect interactions within which they are embedded. For example, a species may interact with more than one mutualist simultaneously, and the interaction between those two species will feed back to its own performance. Mutualisms are also prone to exploitation by additional species that render resources or services not provided for them. These individuals directly and indirectly affect the performance of mutualist species. The complexity of the larger network of interactions creates context-dependent outcomes of mutualism: that is, the relative costs and benefits of being a mutualist changes in response to the environment. In order to identify mechanisms of mutualism persistence, it is necessary to understand the context-dependent processes that shape mutualistic interactions.

A classic example of mutualism embedded within a larger interaction network is plant-pollinator interactions. Many pollination systems are facultative, and plants and

pollinators often interact with more than one species. Floral larcenists, including nectar robbers, exploit floral resources provided to pollinators. The exploitation of floral resources is variable in space and time, making the costs (and relative benefits) to plants and pollinators variable as well. In this study, I examine the context-dependency of pollination mutualisms by quantifying costs and benefits to plants, pollinators, and nectar robbers in different environments. First, I measure the consequences of secondary nectar robbing (foraging for nectar through perforations in floral tissue created by another organism) for male and female reproduction in a hummingbird-pollinated plant, finding significant decreases in female function but not male function. Second, I examine the degree to which primary nectar robbers (organisms that perforate floral tissue) facilitate secondary nectar robbers, given that they are also competing for nectar. I show that the benefit of facilitation does not overcome the cost of competition for secondary nectar-robbing bumble bees, because secondary robbing is in general a more costly behavior than pollinating. Finally, I quantify the cost of inter- and intraspecific competition between a pure pollinator species and a species that acts as a secondary robber in certain contexts. I show that intraspecific competition in each species is stronger than interspecific competition, indicating that the species can coexist. This work advances our knowledge of mutualism by taking into account the net fitness effects for mutualist individuals embedded within a larger interaction network. Understanding these fitness differences allows us to make predictions about resulting population dynamics and make inferences about the stability of mutualism.

INTRODUCTION

Costs, benefits, and context-dependency of mutualism

The outcome of species interactions plays a key role in individual fitness. Pairwise interactions can be positive, negative, or neutral, and are often context-dependent; that is, they change in response to changes in the biotic or abiotic environment (Thompson 1988, Chamberlain and Holland 2008). Mutualisms, interactions in which individuals exchange fitness benefits, usually in the form of resources or services, are thought to be particularly context-dependent (Bronstein 1994, Chamberlain et al. 2014). This is because there is an inherent cost of cooperation (incurred by providing a resource or service to another individual), which may be high or low relative to the fitness benefit received (Bronstein 2001a). In other species interactions, such as predation and competition, the relative costs and benefits are more clear-cut; for example, becoming a prey item will always confer zero benefit, and competition will always be costly (Chamberlain et al. 2014).

The context-dependent nature of mutualism creates a great deal of theoretical complexity that is challenging for researchers to reconcile. However, it likely also provides an explanation for a fundamental question in the study of mutualism: How is it able to persist? Mutualism presents a paradox: it is exceedingly common in nature; however, theory tells us the interaction should not be sustainable (May 1973, Sachs 2015). From an ecological perspective, the reciprocal fitness benefits of pairwise, obligate mutualisms should drive positive density-dependent population growth to the point that the interaction is destabilized (Johnson and Amarasekare 2013, Lee 2015).

From an evolutionary perspective, mutualisms are thought to be plagued by the “temptation to cheat”, i.e., to derive benefits from another individual without reciprocating. As cheaters arise in the system, the cost of mutualism increases for the mutualist and decreases for the cheater (Addicott 1996, Bronstein 2001b, Jones et al. 2015). However, because mutualisms exist within a larger web of interactions and are spatially and temporally variable, differences in the net costs and benefits of mutualism across contexts have been shown to provide stability to mutualism in the face of unchecked population growth or rampant cheating (Ringel et al. 1996, Harcombe et al. 2016, Frederickson 2017). Taking these factors into account, the major questions posed in the study of mutualism become less about how it can persist, and more about how we can develop unifying principles about how and when context-dependent outcomes of mutualism should affect individual fitness and population dynamics.

Moving beyond pairwise mutualism

Factors that alter the relative costs and benefits of mutualism include third-party actors and multispecies effects (Bronstein 2001b, Stanton 2003, Jones et al. 2012, Afkhami et al. 2014). Third-party actors are individuals or species that do not directly participate in the “focal” mutualism, but directly or indirectly affect the fitness or performance of the individuals involved in the focal mutualism (Biere and Honders 2006, Vannette et al. 2013). These may include predators, competitors, or facilitators of mutualists, for instance, co-flowering plant species that increase the abundance of pollinators for another plant species but that may also compete for pollination benefits (Rathcke 1983, Bell et al. 2005). They also include individuals or species that usurp

resources or services provided between mutualists, termed here as “exploiters” (but see Bronstein 2001b, Ghoul et al. 2014 for alternative terminology related to unreciprocated benefactors of mutualism). There are numerous examples of exploitation in the literature. For example, pollinating wasp species *Pleistodontes imperialis* will parasitize only the inner ovules of fig (*Ficus rubiginosa*) trees, allowing for successful seed development of the outer ovules; however, these become parasitized by non-pollinating wasps in the genera *Philotrypesis* and *Sycosapter* (Dunn et al. 2008). In *Cecropria obtusa* seedlings, *Pseudocamiba* moth larvae will usurp domatia provided for ants in exchange for herbivore protection (Roux et al. 2011). Exploiters generally directly reduce the performance of one mutualist, thereby indirectly reducing the performance of the other.

Third-party actors present a challenge to the study of mutualism because they require consideration of individuals outside the interaction (Hoeksema and Bruna 2000). In order to quantify the net benefits of the focal mutualism, the direct and indirect effects of interacting with the third-party actor must also be quantified (Wootton 1994, Holland et al. 2013). Consideration of multispecies effects presents a similar challenge. Most mutualistic interactions are diffuse, with individuals interacting with a “guild” of partners (Stanton 2003). For example, a legume may host numerous *Rhizobia* bacterial strains (Heath and Tiffin 2007), and plants may rely on multiple species of seed dispersers (Larson 1996). Multispecies effects are different from interactions with third-party actors, because individual species of a guild provide *and* receive mutualistic benefits, while third-party actors will do only one of these. However, many of the same principles of quantifying the effects of direct and indirect interactions in order to quantify the net effects of mutualism can be applied to quantifying so-called “multiple mutualist effects”

(Afkhami et al. 2014). It is particularly important to consider intraguild interactions, i.e., interactions between species that are providing and receiving the same type of benefit (for example, two pollinator species of the same plant species). Intraguild competition in mutualism has gained attention in the past 15 years (Stanton et al. 2002, Mitchell et al. 2009, Jones et al. 2012). Specifically, it has been hypothesized as a mechanism for dampening positive density-dependent population growth in pairwise mutualisms, because competing for mutualistic benefits potentially incurs a large fitness cost (Jones et al. 2012). A number of conceptual and mathematical models describing these dynamics exist, and there is an increasing number of studies testing hypotheses generated from the models (Adam 2010, Jones et al. 2012, Johnson and Amarasekare 2013).

Incorporating exploiters into multispecies mutualism

Just as an individual may interact with multiple mutualists, they may also interact with multiple exploiters (Bronstein and Ziv 1997). Compared to the conceptual and empirical development of intraguild interactions between mutualists, there has been very little exploration of multiple exploiter effects. It is plausible that exploiter-exploiter interactions function similarly to intraguild mutualist interactions; that is, individuals would compete for (unreciprocated) mutualistic benefits. Furthermore, exploiters and mutualists in search of the same resource or commodity likely also compete, adding even more complexity to the network of direct and indirect interactions that govern the outcomes of mutualism. For example, different species of vigilant and non-vigilant ant defenders of *Passiflora* spp. all compete for extrafloral nectar provided by the plant (Xu

and Chen 2010). Incorporating exploitation from one or more species into models of multispecies mutualism increases the scope for context-dependent outcomes.

A final complicating factor in multispecies mutualism and exploitation is that exploitation is, in itself, context-dependent. Individuals of many species only act as exploiters under certain conditions, and are thus called “conditional exploiters” (Bronstein 2001b). They are often individuals that would act as mutualists in a different context. For example, *Tegeticula* moths do not pollinate *Yucca* flowers in which they oviposit when another individual has already done so (Tyre and Addicott 1993). Conditional exploiters choose one strategy over the other, possibly based on their relative costs and benefits, which, as described above, can change in response to the biotic or abiotic environment (Bronstein et al. 2017). In a multiply exploited mutualism, one species can indirectly increase the benefit of exploitation for another through facilitation. In the same ant-*Cecropia* mutualism described above, *Pseudocamiba* moth larvae also promote the growth of a fungal pathogen that further reduces *Cecropia* fitness (Roux et al. 2011).

As the conceptualization of mutualism moves beyond one-to-one interactions to include multiple mutualist effects, multiple exploiter effects, and all possible pairwise interactions, the potential for biotic and abiotic factors to affect any single, focal interaction dramatically increases. Working toward the goal of developing unifying principles of the costs and benefits of mutualism and exploitation in a multispecies system, the major questions addressed in this study are (1) To what extent does interacting with multiple exploiter species increase costs for mutualists? (2) What are the relative costs and benefits of conditional exploitation when it is facilitated by another

exploiter species? (3) How does conditional exploitation fit into current models of intraguild competition for mutualistic benefits? To answer these questions, I studied a multispecies plant-pollinator-nectar robber system in subalpine habitat in the Colorado Rocky Mountains.

Secondary nectar robbing: Conditional and facilitated exploitation of pollination mutualisms

Pollination mutualisms are often multispecies interactions that also have multiple exploiters. Many plant-pollinator systems are generalized: a single plant species interacts with more than one pollinator species, and vice versa (Johnson and Steiner 2000). Plants are also subjected to exploitation by floral larcenists that consume floral resources (nectar and pollen) without providing a pollination service to the plant in return (Inouye 1980). These include nectar thieves, which bypass floral reproductive structures while consuming nectar from the floral opening; pollen thieves, which remove pollen from floral anthers without depositing it onto stigmas; and nectar robbers, which consume nectar through holes in floral tissue, often avoiding floral reproductive structures (Inouye 1980). Organisms termed “primary” nectar robbers create robber holes in flowers (Inouye 1980). After doing so, “secondary” nectar robbers can further exploit floral resources by foraging from the same holes after flowers refill with nectar (Inouye 1980). Nectar robbing is common in plants that produce tubular flowers, flowers with nectar spurs, or flowers with nectar that is otherwise difficult to access, and has been widely observed in plants by an array of nectar-feeding organisms (reviewed in Irwin et al. 2010). Nectar robbing can negatively affect plant reproduction, although this is not always the case

(Maloof and Inouye 2000, Burkle et al. 2007). Mechanisms driving the reduction in plant fitness include direct damage to floral tissue and/or reproductive organs, selective abortion of ovules in flowers that have been robbed, and indirect effects driven by changes in pollinator behavior, as pollinators often avoid robbed flowers (McDade and Kinsman 1980, Irwin and Brody 1998, Zhang et al. 2011). In systems where nectar robbing has been shown to have a net neutral or positive effect on plant fitness, nectar robbing does not deter pollinators, and in some cases visitors effectively transfer pollen while robbing (Higashi et al. 1988, Burkle et al. 2007, Fumero-Cabán and Meléndez-Ackerman 2013).

Plant-pollinator-nectar robber interactions are ideal systems in which to study context dependency and the relative costs and benefits of mutualism and exploitation. Secondary nectar robbing is context-dependent by definition, as it cannot occur until primary robbers create robber holes in flowers (Irwin et al. 2010). Furthermore, many secondary robbers are conditional exploiters that sometimes act as mutualists (pollinators) instead (Irwin et al. 2010). Studying the ecology of secondary nectar robbing allows us to quantify the relative costs and benefits of conditional exploitation and the ecological contexts that promote or discourage the behavior. It also allows us to investigate how mutualists interact with exploiters, as well as how different exploiter species interact. Finally, we can ask about the consequences of multiple exploiter effects on plants and pollinators. I address these questions in the following study. First, I ask whether secondary robbing imposes a fitness cost to plants beyond that imposed by primary robbing alone, including both male and female components of plant reproduction. Second, I ask whether facilitation by primary robbers provides enough

benefit to secondary robbers to overcome the cost of competing for floral nectar, and compare the costs and benefits of secondary robbing versus acting as a pollinator. Third, I examine the dynamics of competition between a species that only pollinates and a species where individuals alternatively pollinate and secondary-rob the same plant. I ask how conditional exploitation by secondary robbers affects the ability of the two species to coexist on a shared plant resource.

PRESENT STUDY

In this section, I present the major topics covered in this study and summarize the key findings. Detailed information about this work can be found in the appended manuscripts.

Study System

This study focuses on a system of long-lived perennial plants, their pollinators, and their nectar robbers in a subalpine habitat in the West Elk Mountains of the Colorado Rocky Mountains, at and around the Rocky Mountain Biological Laboratory (RMBL) in Gothic, CO, USA. The habitat is characterized by conifer and aspen forest interspersed with open wildflower meadows, which are dominated by over 120 non-graminoid plant species. Floral morphological diversity in the meadows is high, including many species with tubular flowers and nectar spurs, which are commonly primary-robbed by the bumble bee species *Bombus occidentalis* and *B. mixtus*, and secondary-robbed by other *Bombus* species. common pollinators include bumble bees, solitary bees, and hummingbirds, and flies. Appendices A and B examine the effect of secondary robbing on female and male components of plant fitness, respectively, using *Ipomopsis aggregata* (Polemoniaceae, hereafter *Ipomopsis*) as a focal species. *Ipomopsis* is monocarpic, living as a vegetative rosette for up to 10 years before flowering in the last year of its life (Waser and Price 1989). Hummingbirds (*Selasphorus platycerus* and *Selasphorus rufus*) feed on *Ipomopsis* floral nectar and are the dominant pollinator species. Bumble bees (*Bombus* spp.) are the most abundant primary and secondary nectar robbers, although

they occasionally forage from the floral opening (Irwin and Brody 1998). *Bombus occidentalis* possesses toothed mandibles and acts as a primary robber, while other *Bombus* species that lack toothed mandibles sometimes act as secondary robbers given the opportunity. Appendices C and D take the perspective of the nectar robbers and pollinators, asking how foraging strategy affects intra- and interspecific interactions. These studies use *Linaria vulgaris* (Plantaginaceae, hereafter *Linaria*) and *Corydalis caseana* (Fumariaceae, hereafter *Corydalis*) in Appendix C and D, respectively, as floral resources for bumble bee foraging experiments. *Linaria* is pollinated by a broad range of bumble bee species and experiences primary robbing by *B. occidentalis* (Newman and Thomson 2005a). Multiple bumble bee species act as secondary robbers, including *B. bifarius*, which is the focal species in Appendix C (Newman and Thomson 2005a). *Linaria* is an invasive species in North America; however, the ecology of its invasion or the implications of invasion for pollination mutualisms lie beyond the scope of this study. *Corydalis* is also visited by broad range of bumble bee species acting as pollinators (along with occasional visits by hummingbirds) and nectar robbers (Maloof 2000). Two common pollinator species, *B. appositus* and *B. flavifrons*, served as focal species in Appendix D. While *B. appositus* always pollinates, *B. flavifrons*, which does not have toothed mandibles, will conditionally act as a secondary robber when facilitated by *B. occidentalis* and *B. mixtus*, which act as primary robbers (Heiling et al. 2018).

Plant reproductive consequences of facilitated exploitation of pollination mutualisms
(Appendices A and B)

Within mutualisms, individuals exchange resources or services that provide each with an fitness increase. Exploiters of mutualism obtain resources or services without reciprocating, negatively impacting the fitness of individuals with which they interact (Bronstein 2001b). Just as many species simultaneously engage with more than one mutualist species, they often pay simultaneous fitness costs to more than one exploiter species (Bronstein and Ziv 1997, Roux et al. 2011). As researchers expand the study of mutualism to include examination of multiple mutualist effects (Afkhami et al. 2014), it is also important to deepen our understanding of multiple exploiter effects, in order to predict the total fitness costs and benefits of multispecies mutualisms. In Appendix A and Appendix B, I test for effects of facilitated exploitation of plants in a pollination mutualism. Many plant species are subjected to exploitation by nectar robbers that consume floral nectar through perforations cut into the floral tissue, rather than from the floral opening. This behavior generally causes nectar robbers to miss contacting floral anthers and/or stigmas and precludes pollination (Irwin et al. 2010). While nectar robbing has been observed in a wide array of species, only a subset of them are able to pierce through floral tissue (Irwin et al. 2010). These “primary” nectar robbers leave behind the holes they create in flowers, creating opportunities for “secondary” robbers (individuals that cannot necessarily pierce through floral tissue) to use the robber holes to consume nectar (Inouye 1980). Nectar robbing in general is thought to have a negative effect on plant reproduction, although this is not true in all systems (Malooof and Inouye 2000).

However, almost no study of the effects of nectar robbing has examined the individual vs. combined the effects of primary and secondary robbing.

In Appendices A and B, I quantified the costs of primary vs. secondary robbing to female and male reproduction in *Ipomopsis aggregata* (Polemoniaceae). Over the course of two growing seasons, I simulated whole-plant primary plus secondary robbing to natural populations and potted arrays of *Ipomopsis* plants, observed hummingbird pollinator foraging to manipulated plants and unrobbed controls, and compared components of female fitness (pollen receipt, fruit and seed set) and male fitness (pollen (dye) donation) across the three treatments. Negative effects of primary plus secondary robbing were only identified in components of female fitness. Plants in the secondary robbing treatment received significantly fewer pollen grains per stigma and produced significantly fewer fruits and seeds than plants in either the primary robbing or control treatments. I attribute the difference in fitness between robbing treatments to changes in hummingbird behavior. Hummingbirds were more likely to avoid plants in the secondary robbing treatment than in the primary robbing or control treatments; however, the difference was not statistically significant. While nectar robbing in general reduced per-flower pollen (dye) donation, there was no detectable difference between plants in the primary and secondary robbing treatments. I attribute the difference in male fitness between robbed and unrobbed flowers to hummingbird avoidance of robbed flowers as well. In a separate test, hummingbirds foraged from floral arrays that had been manipulated to simulate either secondary robbing, no robbing, or nectar removal through the floral opening, with no robber hole present. While plants that had nectar removed from flowers experienced significantly lower hummingbird visitation rates than plants

with no floral nectar removed, there was no detectable difference in visitation rate between the two removal treatments, suggesting that nectar removal of any type (by nectar robbers, nectar thieves, or pollinators) results in pollinator avoidance. This work illuminates the total fitness costs for plants of exploitation by nectar robbers by taking into account whole-plant (i.e., male and female) fitness, and by disentangling the effects of primary and secondary nectar robbing. This work also provides insight into the putative mechanisms by nectar robbers reduce plant fitness. This work also shows that the total costs of exploitation in mutualism can be greater than those inflicted by an initial exploitation event.

Multispecies mutualism and exploitation: Intraguild interactions and implications for mutualism persistence (Appendices C and D)

The fitness consequences of engaging in a multispecies mutualism depend in part on the outcomes of the component interactions. In plant-pollinator mutualisms, these include interactions between pollinators and exploiters of floral resources, such as nectar robbers, as well as interactions between different exploiter species, e.g., primary and secondary nectar robbers (Newman and Thomson 2005b, Irwin et al. 2010, Zhang et al. 2014). These species all compete for floral resources; however, interactions between them can also be positive for one or both species. For example, primary robbers facilitate the efficient extraction of nectar by secondary robbers that either cannot create robber holes, or save time by not having to create them. While there has been recent development of mathematical models (summarized in the introduction) describing three-species population dynamics resulting from plant-pollinator nectar robber interactions

(Holland et al. 2013, Lee 2015), there has been very little empirical study of the consequences of these interactions for the pollinator or nectar robber species.

Furthermore, there has been almost no conceptual development (but see Barker and Bronstein 2016) or empirical study of interactions between primary and secondary nectar robbers.

Appendices C and D take the perspective of pollinators and secondary robbers, asking how different component interactions affect individual foraging success, which is used as a proxy for forager fitness. In Appendix C, I examine the interaction between primary and secondary nectar robbers. For nectar foragers that cannot create robber holes in flowers, primary robbers facilitate secondary robbing by other individuals (Irwin et al. 2010). These individuals generally receive a resource benefit in the form of either nectar from a newly available plant species or a new way to consume nectar from a species they already visit. Often, these individuals act as pollinators until robber holes appear in flowers, at which point they switch their behavior and begin secondary nectar robbing (Free 1968). This behavior is common in individuals and species that have difficulty consuming nectar from the floral opening due to a morphological mismatch (Newman and Thomson 2005a). While there are benefits of facilitation for secondary robbers, they also face the cost of visiting flowers that have clearly already been visited, which may mean a lower reward payoff per flower (Barker and Bronstein 2016). In order to understand the fitness consequences of switching behaviors, it is necessary to quantify the costs and benefits of each. I performed an experimental study of *Bombus bifarius* foraging on *Linaria vulgaris* (Plantaginaceae), assessing the foraging efficiency (proportion of foraging bout spent consuming nectar) of secondary robbing versus

foraging from the floral opening (termed legitimate foraging) of individuals in a flight cage. *Linaria* holds nectar in a nectar spur, which is difficult for *B. bifarius* to access from the floral opening. It lacks toothed mandibles and cannot chew holes in flowers, but will act as a secondary robber if robber holes already exist. I manipulated arrays of *Linaria* to reflect high and low (80% and 20%) levels of primary robbing and whether robbed flowers were unrewarding (nectarless) or rewarding (containing nectar) in a crossed design. Contrary to expectations, *B. bifarius* spent a significantly higher proportion of its foraging bout consuming nectar when foraging from the floral opening. Furthermore, foraging tactic was the sole predictor of foraging success, rather than frequency of primary robbing or whether robbed flowers were rewarding. These results suggest that switching tactics may not be as beneficial as once thought.

In Appendix D, I examine the costs and benefits of secondary robbing in the context of competition and coexistence between mutualists and conditional exploiters. Interspecific competition and coexistence between mutualists for a shared resource or service is often invoked as a mechanism for stabilizing the positive density-dependent population growth predicted in a given pairwise mutualism (Palmer et al. 2003, Jones et al. 2012). Similarly, pure mutualists and pure exploiters can coexist by indirectly affecting each other's performance via their shared resource (Holland et al. 2013). However, we lack knowledge about the ability of mutualists to coexist with conditional exploiters. I quantified the strength of competition between two bumble bee species that forage for nectar on the same plant species. *Corydalis caseana* (Fumariaceae) provides floral resources for *Bombus appositus*, which acts solely as a pollinator, and *B. flavifrons*, which sometimes acts as a pollinator and other times as a secondary nectar robber.

Lacking toothed mandibles, it cannot primary-rob. Using a response surface experimental design, I allowed individuals of each species to forage on patches of *Corydalis* in a controlled setting, varying the total and relative species abundance in different treatments. I quantified a number of measures of foraging efficiency, including per-flower payoff (time spent consuming nectar relative to searching for nectar and handling flowers), as a proxy for fitness for both species and for both foraging strategies employed by *B. flavifrons*. While competition for nectar was generally weak between the two species, intraspecific competition for each species was stronger than interspecific competition, suggesting that they can coexist (Chesson 2000). However, some measures *B. flavifrons* performance decreased as the density of *B. appositus* increased, suggesting that *B. appositus* is more effective at acquiring resources than *B. flavifrons*. These findings align with theoretical predictions about multispecies mutualism persistence positing that the superior mutualist must perform better than the inferior mutualist for all three populations to persist (Holland et al. 2013).

Conclusions

This work makes advances to the study of pollination mutualisms in three ways. First, it shows for the first time that facilitated exploitation (in this case, secondary nectar robbing) can affect plant reproductive success beyond that of the initial exploitation event. Second, it takes a whole-plant perspective by considering female and male components of fitness, while the majority of studies report effects on female fitness only (Irwin et al. 2010). The female fitness component of this study (Appendix A) was published in *Journal of Ecology* in 2017; the male fitness component (Appendix B) was

published in *American Journal of Botany*. Third, my research delves into the contexts that promote or discourage conditional exploitation by quantifying the relative costs and benefits for the conditional exploiter species of being facilitated by a pure exploiter species. This work (Appendix C) was published in *Oikos* in 2017. Finally, my research examines how conditional exploitation may change the outcome of competition between mutualists for shared benefits. Current models of competition in mutualism only consider pure strategies. This work (Appendix D) is presented here as a manuscript in preparation for submission to an academic journal.

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APPENDIX A

FACILITATED EXPLOITATION OF POLLINATION MUTUALISMS: FITNESS
CONSEQUENCES FOR PLANTS

Sarah K. Richman*^{1,2}, Rebecca E. Irwin^{2,3,4}, Cherie J. Nelson^{2,5}, Judith L. Bronstein^{1,2}

¹Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ
85721 USA

²Rocky Mountain Biological Laboratory, Crested Butte, CO 81224 USA

³Biology Department, Dartmouth College, Hanover, NH 03755 USA

⁴Current address: Department of Applied Ecology, North Carolina State University,
Raleigh, NC 27695 USA

⁵Department of Biology, California State University, San Bernardino, San Bernardino,
CA 92407 USA

*Correspondence Author: srichman@email.arizona.edu

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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 behavior 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; plant fitness; hummingbird pollination; Ipomopsis aggregata; multiple exploiters; generalized mutualism; nectar robbing; 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; 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 *et al.* 2016b). Usually, the term “nectar robber” is applied to the individual or species of nectar feeder that initially creates holes in floral tissue, i.e. 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 behavior 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 behavior (Irwin & Brody 1998; Irwin 2000; Irwin & Brody 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 1) additional nectar removal by secondary robbing would lead to reduced female fitness in *Ipomopsis* beyond that inflicted by primary robbing alone, and that 2) 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 *Ipomopsis 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 (Pyke 1978). 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 $\mu\text{L}/\text{day}$ (Pleasants 1983). *Ipomopsis* is primarily pollinated by broad-tailed (*Selasphorus platycercus*) 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 0-100% 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. *B. occidentalis* can secondary-rob *Ipomopsis* flowers, as can other bumble bee 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 m x 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). (1) *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 (see below). (2) *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. (3) *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/week throughout the flowering season; the two 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 μ L microcapillary tube (Microcap, Drummond Scientific, Broomall, PA) 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 bumble bees 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 (Figure A.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 (six 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 3 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/plant and an average of 145 stigmas/treatment. Collecting stigmas at this stage does not interfere with fruit development (Waser and 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 \pm 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.

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 behavior (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 bumble bee 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/week for 2.5 weeks during peak flowering season. However, we applied treatments 6 days/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 m x 6 m array in a meadow with naturally flowering *Ipomopsis*, each treatment plant 1 m apart. We recorded pollinator visitation to these plants for 3h/day. 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 ($\chi^2_2 = 18.81$, $P < 0.001$; Figure A.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 experienced no robbing (C treatment). A post-hoc test showed no significant difference in pollen receipt between P- and C-treatment plants ($P = 0.122$; Figure A.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 ($F_{2,117} = 21.87$, $P < 0.001$; Figure A.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 ($F_{2,117} = 5.31$, $P = 0.006$; Figure A.3b) and total seed production per plant ($F_{2,117} = 11.48$, $P < 0.001$; Figure A.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 (*Selasphorus platycerus*), except for one visit by a rufous hummingbird (*Selasphorus rufus*). Hummingbird visitation rate to plants in the C treatment tended to be higher than to plants in the P and PS treatments. Mean \pm SE visitation rate to the C, P and PS treatments were 0.076 ± 0.02 , 0.050 ± 0.01 and 0.048 ± 0.007 flowers/plant/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 ($F_{1,18} = 0.947$, $P = 0.34$).

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 *Ipomopsis 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 behavior

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-hour observation period, contained a negligible amount of nectar (based on measurements of *Ipomopsis* nectar production rate (Pleasants 1983a)). 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 *Arctostaphylos 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 behavior.

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 tradeoff 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 & Brody 1999). 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 bumble bee 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 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 vigor 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 et al. 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 et al. 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 behavior 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 bumble bee pollinator behavior in *Corydalis ambigua* (Fumariaceae) (Zhang et al. 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. Honey bees that cannot primary-rob will legitimately forage from *Vaccinium ashei* (Ericaceae) until carpenter bees primary-rob flowers (Dedaj & Delaplane 2004). At this point, honey bees switch to secondary robbing because it enables them to forage more efficiently, and the change in behavior leads to a reduction in seed set (Dedaj & 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 2005).

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:

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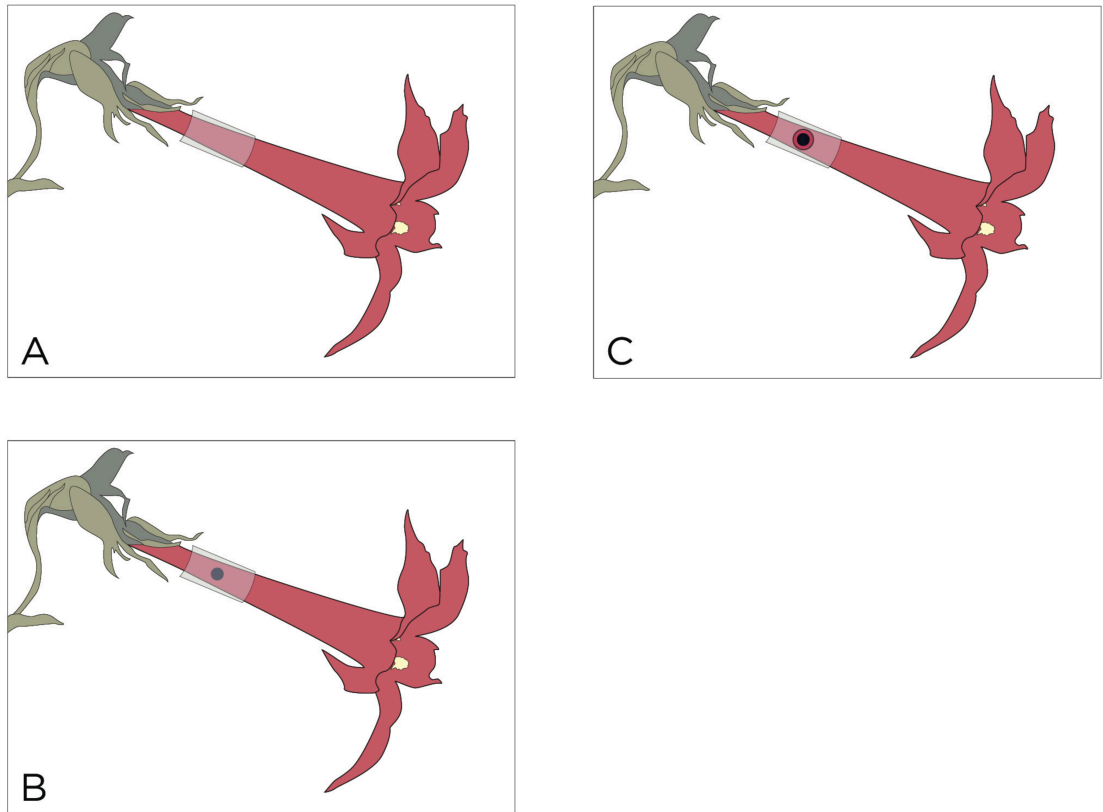


Figure A.1: Illustration of nectar robbing treatments. In A, a collar is placed over the corolla without a robbing hole (control). In B, a robbing hole is made in the corolla and nectar is removed, and a collar is placed over the hole to prohibit any additional robbing (primary robbing only). In C, a 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.

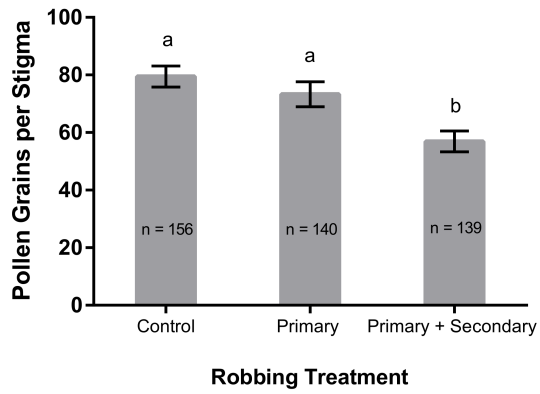


Figure A.2: The effect of robbing treatments on mean (\pm SE) pollen grains per stigma per plant. Numbers inside bars represent the total number of stigmas collected over the entire season for each treatment group. Different letters represent significantly different ($P < 0.05$) mean values based on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

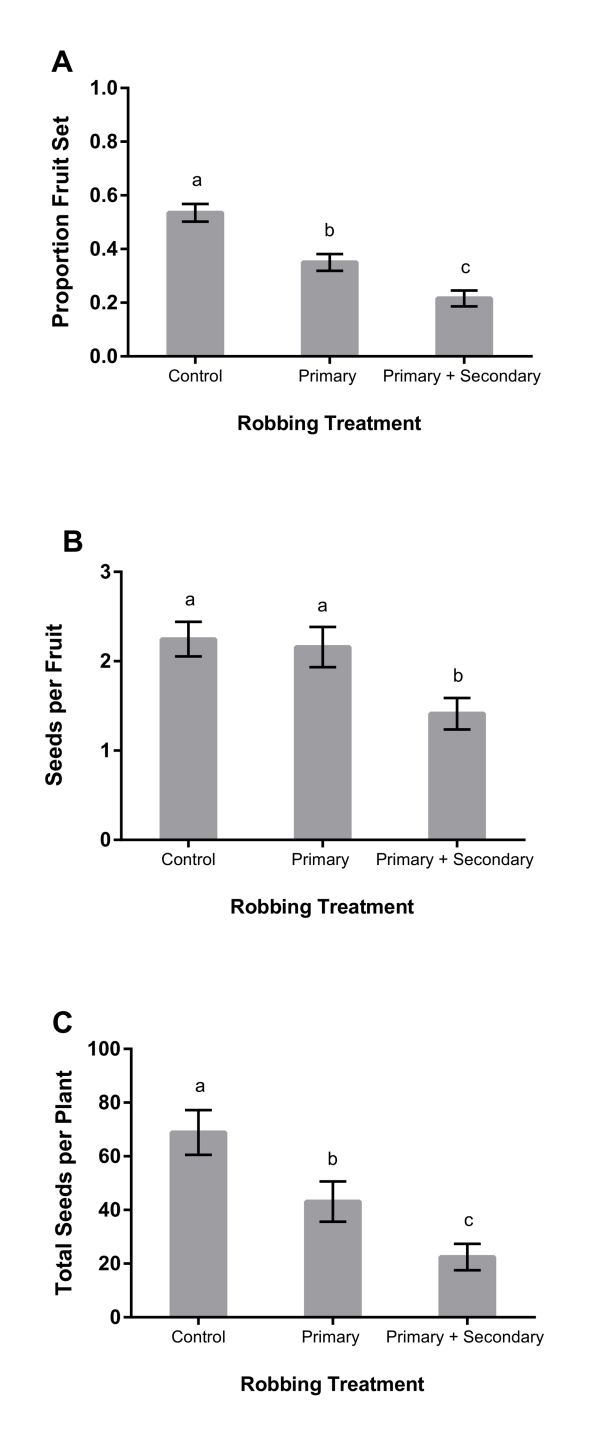


Figure A.3: The effect of robbing treatments on fruit and seed production. Panels represent treatments applied at the whole-plant level ($n = 40$ plants/treatment): A) Proportion fruit set per plant, B) average number of seeds per fruit, C) total number of seeds per plant. Bars and values as in Figure A.2.

APPENDIX B

CONSEQUENCES OF SECONDARY ROBBING FOR MALE COMPONENTS OF
PLANT REPRODUCTION

Sarah K. Richman*^{1,2}, Rebecca E. Irwin^{2,3}, John T. Bosak^{1,2}, Judith L. Bronstein^{1,2}

¹Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ
85721 USA

²Rocky Mountain Biological Laboratory, Crested Butte, CO 81224 USA

³Department of Applied Ecology, North Carolina State University, Raleigh, NC 27695
USA

* Corresponding author: Sarah Richman

srichman@email.arizona.edu

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Abstract

Premise of the study: Organisms engage in multiple species interactions simultaneously. While pollination studies generally focus on plants and pollinators exclusively, secondary robbing, a behavior that requires other species (primary robbers) to first create access holes in corollas, is common. It has been shown that secondary robbing can reduce plants' female fitness; however, we lack knowledge about its impact on male plant fitness.

Methods: We experimentally simulated primary and secondary robbing in the monocarpic perennial *Ipomopsis aggregata* (Polemoniaceae), quantifying indirect effects on pollinator-mediated pollen (dye) donation. We also assessed whether continual nectar removal via the floral opening has similar effects on hummingbird pollinator behavior as continual secondary robbing through robber holes.

Key Results: We found no significant indirect effects of secondary robbing on a component of *Ipomopsis* male fitness. Although robbing did reduce pollen (dye) donation due to avoidance of robbed plants by pollinating hummingbirds, pollen donation did not differ between the two robbing treatments. The effects of secondary robbing on hummingbird behavior resembled effects of chronic nectar removal by pollinators. Our results indicate that hummingbird pollinators may use a combination of cues, including cues given by the presence or absence of nectar, to make foraging decisions.

Conclusions: Combined with prior research, this study suggests that secondary robbing is less costly to a component of male fitness than to female fitness in *Ipomopsis*, broadening our knowledge of the overall costs of mutualism exploitation to total plant fitness.

Key words: cheating; dye donation; hummingbird pollination; *Ipomopsis aggregata*; male plant fitness; mutualism; nectar robbing

INTRODUCTION

Mutualisms are prone to exploitation, in which organisms obtain benefits or rewards while providing no benefits to the partner in return (Bronstein 2001b). Just as organisms may interact with multiple mutualists simultaneously or sequentially, they may also interact with multiple exploiters. For example, *Pseudocabima* caterpillars usurp territory on *Cecropia* saplings from mutualistic ant defenders and simultaneously promote colonization by a fungal pathogen that persists inside of *Cecropia* domatia in the absence of ants (Roux et al. 2011). Similarly, pollination mutualisms can experience exploitation from nectar larcenists that obtain nectar from flowers using behaviors that generally do not lead to pollination (Irwin et al. 2010). Primary nectar robbers feed on nectar through holes they make in flowers, in turn opening opportunities for subsequent exploitation by secondary robbers, species that can remove nectar through those holes (Inouye 1980). These organisms generally remove nectar through the corolla hole in a similar way as primary robbers, but may directly or indirectly inflict fitness costs beyond those inflicted by primary robbers. Secondary robbing is common, with at least 315 reports from 117 plant species in 34 families reported thus far, and many more species are reported as secondary robbers than primary robbers (Irwin et al., 2010 and *unpubl. data*). Additionally, nectar thieves collect nectar in a manner that precludes pollination but that does not damage floral tissue (Inouye, 1980). In general, multispecies interactions can result in additive and in some cases non-additive fitness effects (Morris et al. 2007), and sometimes differential effects through components of male and female plant reproduction (Schaeffer et al. 2013). However, in contrast to a growing understanding of multispecies interactions (Strauss and Irwin, 2004; Nunn et al., 2014),

we know comparatively little about the frequency or importance of multispecies exploitation of mutualism.

Multispecies exploitation may lead to unexpected consequences for whole-plant fitness via differential effects on female and male functions. The majority of flowering species are hermaphroditic. Nonetheless, most studies quantify the effects of species interactions on whole-plant reproduction using female components of reproduction as a surrogate (Stanton et al. 1986). While male and female components of reproduction often respond similarly to abiotic and biotic interactions (Schaeffer et al., 2013), there are also cases of sexual conflict (Barrett 2002), in which environmental contexts that make plants better male parents do not make them better female parents, and vice versa (Contreras and Ornelas 1999, Madjidian 2009). In particular, although this generalization has been debated (Wilson et al. 1994), theory predicts that female components of plant reproduction should be more limited by resources, whereas male components should be more limited by mating opportunities (Bateman 1948). Given that nectar robbing can indirectly affect plant reproduction via changes in pollinator behavior and subsequent mating opportunities, and that several studies show that robbing results in reductions in male components of plant reproduction (Irwin et al., 2010, and references therein), there is reason to suspect that secondary robbing may have additional effects on male components of plant reproduction beyond that of primary robbing alone. This may be especially true in cases where pollinator behavior is strongly affected by nectar availability. The degree to which secondary robbing affects male components of plant reproduction beyond that of primary robbing is unknown.

The goal of this work was to assess if and how additional exploitation by secondary nectar robbing affects male components of plant reproduction. We studied the hummingbird-pollinated plant *Ipomopsis aggregata* (Polemoniaceae) which experiences primary robbing by bumble bees and secondary robbing by bumble bees, flies, and wasps. Nectar robbing has been shown to reduce *Ipomopsis* female fitness: it discourages pollinator visitation, which leads to reduced fruit and seed set (Irwin and Brody 1998, 1999). Moreover, secondary robbing of *Ipomopsis* results in a greater female fitness reduction than primary robbing alone (Richman et al. 2017). Nectar robbing also reduces estimates of male reproduction in *Ipomopsis*, including pollen donation and the number of seeds sired, due to hummingbird-pollinator avoidance of robbed plants and flowers (Irwin and Brody 1999, 2000). However, Irwin and Brody (1999, 2000) did not separate the effects of primary and secondary robbing on male components of plant reproduction, which we do here. First, we asked whether secondary nectar robbing affected pollinator visitation and subsequent pollen (dye) donation beyond that of primary robbing. Hummingbird pollinators have been shown to avoid unrewarding flowers in this system, although the exact cue they use remains a mystery (Irwin, 2000). Because robbing of *Ipomopsis* indirectly decreases pollinator visitation via decreasing nectar rewards (Irwin and Brody 1998) and secondary robbed flowers generally receive fewer visits than primary robbed flowers (Richman et al., 2017), we predicted that plants with secondary-robbed flowers would donate less pollen. Second, finding effects of robbing in general on pollinator behavior, we then explored whether the effects of robbing on pollinator visitation are mechanistically equivalent to chronic nectar removal by any other visitor, or if robbing inflicts unique additional effects on pollinator visitation that cannot be

predicted simply from the removal of nectar. Secondary robbing can result in chronic nectar removal from flowers, as can high visitation rates by pollinators, although the former does not result in pollen deposition in this system whereas the latter does (Irwin et al. 2015). Observing hummingbird pollinator foraging on these different flower types may provide additional clues as to the cues they use while making foraging decisions. By addressing the effects of primary as well as secondary robbing on components of male fitness, which is underexplored relative to female fitness, this work, combined with prior research, provides empirical insight into the total plant fitness costs associated with multispecies exploitation of pollination.

MATERIALS AND METHODS

Study System—

We studied *Ipomopsis aggregata* (Polemoniaceae; hereafter *Ipomopsis*) at the Rocky Mountain Biological Laboratory (RMBL, elevation 2895 m), Gothic, Colorado, USA. *Ipomopsis* is a monocarpic, perennial, self-incompatible herb that produces approximately 50 red, tubular flowers on (usually) a single stalk from mid-June to mid-August (Waser 1978). The hermaphroditic flowers are protandrous, with male phase lasting 1-2 days and female phase lasting 2-3 days (Waser, 1978). Pollen is dispersed an average of 1.27-2.63 m from the parent plant (Campbell and Waser 1989); because seed dispersal is limited, pollen movement is thought to be a major component of gene flow (Levin and Kerster 1974, Campbell and Waser 1989). Flowers continually produce nectar at a rate of up to 5 μ L/day and nectar removal does not affect subsequent nectar production rate (Pleasants, 1983). Broad-tailed (*Selasphorus platycercus*) and rufous

(*Selasphorus rufus*) hummingbirds visit *Ipomopsis* flowers for nectar rewards and are the primary pollinators (Mayfield et al. 2001). The bumble bee *Bombus occidentalis* (Apidae) primary-robs *Ipomopsis* flowers by piercing the corolla tissue using toothed mandibles, generally removing all available nectar, and will pierce holes in up to 80% of flowers per plant (Irwin and Brody, 1998). Nectar production continues following a primary robbing event (Irwin et al. 2015), which encourages secondary robbing by other bumble bee species, including *Bombus bifarius*, as well as by other wasp and fly species that may lack the mouthparts required to act as primary robbers.

Field methods—

1) How does secondary robbing indirectly affect hummingbird pollinator visitation and pollen (dye) donation beyond that of primary robbing?

We potted 60 budding *Ipomopsis* from a population south of the RMBL (GPS: 38.7806 N, -106.8703 W) on 7 June 2016 and maintained plants in an enclosure. We measured the height of each plant to the nearest cm to use as a covariate in statistical analyses, as prior research has shown that taller *Ipomopsis* are more likely to be visited by pollinators (Brody and Mitchell 1997). We randomly assigned 20 plants each to three treatments applied at the whole-plant level: (1) *Primary robbing* (all flowers on each plant were primary robbed one time), (2) *Primary and secondary robbing* (all flowers on each plant were primary robbed and then secondary robbed once daily), and (3) *Control* (no robbing). We applied treatments for six consecutive days and started pollinator observations on the second day of treatments. All flowers in all treatments were physically handled to control for effects of touching flowers.

To simulate primary robbing, we cut a ~1 mm hole in the side of the corolla with dissecting scissors and removed all available nectar with a 10 μ L microcapillary tube (Drummond Scientific, Broomall, Pennsylvania, USA) inserted into the hole. These experimental robbing techniques do not damage nectar-producing structures in flowers (Irwin and Brody 1998, Irwin et al. 2015) and simulate natural robbing in terms of effects on hummingbird pollinator visitation and plant reproduction (Irwin and Brody, 1998). To simulate secondary nectar robbing, we inserted a 10 μ L microcapillary tube into the primary robbing holes to remove any additional nectar that was produced once daily (as in Richman et al., 2017). Every day that robbing treatments were performed, we also recorded the number of open flowers on each plant

Following robbing treatments, we placed plants into the field in a 6 m x 10 m array with 1-m spacing between plants, matching spacing of *Ipomopsis* individuals in natural populations. Treatments and plants were assigned randomly to array positions at the start of the experiment and were kept in those same positions daily. After placing plants in the field each day, we observed pollinator behavior for at least 3 hr. Observations began on the second day of treatment applications, to allow for a difference in nectar volume between primary and secondary robbed flowers. For each hummingbird that entered the array, we recorded species and sex, plants visited, and the number of flowers probed per plant. Afterwards, we returned plants to the enclosure until the next day of treatments and observations.

To estimate pollen donation, we used powdered fluorescent dyes as pollen analogues (Series JST-300, Radiant Color, Richmond, California, USA). In *Ipomopsis*, mean dye donation provides a reliable estimate of mean pollen donation (Waser and Price

1982). We used three dye colors, each assigned at random to one of the treatments. On 14 June 2016, half of the plants in each treatment were randomly chosen to act as dye donors and the other half of the plants in each treatment as recipients. Dye was applied to the anthers of flowers in male phase with dehiscing pollen using a flathead toothpick. We recorded the number of flowers dyed per donor plant as well as the number of flowers open. Dye was applied in the morning just after placing plants into the field. At the end of the approx. 3-hr pollinator observation period, we collected stigmas from 20% of the female-phase flowers from recipient plants. We counted the number of dye particles of each color on each stigma using a dissecting microscope (as in Irwin and Brody, 1999). We repeated this procedure on 17 June 2016, switching the donor and recipient plants, and re-assigning treatments at random to dye colors. *Ipomopsis* flowers on the experimental plants were lasting approx. 3 d (R. E. Irwin, *per obs*); thus, with at least 3 d between dye applications, we ensured that any dye from the previous application was no longer in the array on dehiscing flowers or open stigmas. For each recipient plant, we calculated the mean number of dye particles donated to recipient plants per treatment per flower dyed (similar to Dudash et al., 2011). Calculating dye donation on a per-flower dyed basis controlled for any differences in the number of flowers dyed in the three treatments (Campbell, 1989).

Statistical analyses— To test whether robbing treatments affected hummingbird foraging behavior, we calculated visitation rate as the number of times plants were visited multiplied by the mean proportion of flowers probed. We used ANCOVA to test if robbing treatment affected hummingbird visitation rate with plant height and mean floral

display size as covariates. Neither covariate had a significant effect on hummingbird visitation rate ($F_{1,55} \leq 1.17$, $P \geq 0.28$) and were removed from the final analysis.

To test whether robbing treatments affected pollen (dye) donation per flower dyed, we used a linear mixed model with robbing treatment, round of dye application, and their interaction as fixed factors. The interaction between robbing treatment and round of dye application was not statistically significant ($F_{2,104} = 1.86$, $P = 0.16$) and so was removed from the final model. Because recipient plants could receive dye from all three donor colors, we included plant ID as a random effect in the analysis to account for observations of multiple dye colors donated to recipient stigmas. Analyses were performed using JMP Pro version 13.0.0.

2) Are the effects of secondary robbing on pollinator visitation equivalent to chronic nectar removal by pollinators?

We conducted whole-plant manipulations to simulate nectar robbing and chronic nectar removal in late June and early July, 2016. We transplanted 60 single-stalked, budding *Ipomopsis* from a single population at the RMBL (GPS: 38.9585 N, -106.9875 W) into individual pots. Plants were subsequently maintained in an enclosure. We measured the height of each plant to the nearest cm to use as a covariate in statistical analyses.

We randomly assigned 20 plants each to one of three treatments: (1) *Primary and secondary robbing*, (2) *Chronic nectar removal*, (3) *Control*. Nectar robbing was performed as described above. In the chronic nectar removal treatment, we removed nectar from all open flowers daily through the floral opening as a pollinator would using

10 μ L microcapillary tubes. Treatments were performed daily in the morning before placing plants into the field, and we counted the number of open flowers.

Plants were placed into a field array as described above. Pollinators were observed for 9 d, starting at 0830 until 1500 or until 10 foraging bouts had been observed. We define a foraging “bout” as the time interval between a hummingbird’s first approach to any plant in the array and its departure from the array. We limited daily observations to 10 foraging bouts to ensure that hummingbird pollinators experienced assigned treatments and not emptied flowers even in the control treatment. We used a digital voice recorder to record the species and sex of the floral visitors, which plants were visited, and the number of flowers probed per plant. At the end of daily observations, the plants were returned to the enclosure.

Statistical analyses— We used ANCOVA to test whether treatments affected hummingbird pollinator visitation rate (number of times plants were visited per day multiplied by the mean proportion of flowers probed) with plant height (cm) used as a covariate. Finding significant effects of nectar treatment on hummingbird visitation rate (see Results), we then used similar ANCOVAs to assess the degree to which the number of times plants were visited or the proportion of flowers probed was driving the behavioral results. All significant ANCOVAs were followed by Tukey HSD test to assess pairwise treatment comparisons. Analyses were performed in R version 3.3.1 (R Core Team 2016).

RESULTS

1) How does secondary robbing indirectly affect hummingbird pollinator visitation and pollen (dye) donation beyond that of primary robbing?

We recorded 53 hummingbird foraging bouts over 5 observation days. All but one were made by male broad-tailed hummingbirds (at least two individuals) with the remaining bout by a female broad-tailed hummingbird. Robbing treatment had a significant effect on hummingbird visitation rate ($F_{2,57} = 4.86$, $P = 0.01$), with plants in the *Control* treatment experiencing at least 20% higher pollinator visitation rate than either of the robbing treatments (Figure B.1a). A post-hoc analysis revealed no significant difference in pollinator visitation rate between plants in the *Primary* vs. *Primary and secondary* treatments ($P > 0.05$). The difference in pollinator visitation rate between *Control* and robbing treatments was driven by a reduction in the number of times that robbed plants were visited (Mean \pm SE = 8.90 ± 0.60 , 6.65 ± 0.59 , 6.45 ± 0.61 visits for *Control*, *Primary robbing*, and *Primary and secondary robbing*, respectively; $F_{2,57} = 37.02$, $P = 0.009$); there was no difference in the mean proportion of flowers probed per visit (Mean \pm SE = 0.53 ± 0.03 , 0.5 ± 0.04 , 0.50 ± 0.04 for *Control*, *Primary robbing*, and *Primary and secondary robbing*, respectively; $F_{2,57} = 0.17$, $P = 0.84$). Plants displayed a Mean \pm SE number of flowers of 15.02 ± 1.30 , 12.18 ± 0.85 , 11.09 ± 1.38 for *Control*, *Primary robbing*, and *Primary and secondary robbing*, respectively.

Changes in pollinator visitation to robbed plants produced changes in pollen (dye) donation. We found a significant effect of robbing treatment on pollen (dye) donation, with plants in the *Control* treatment donating at least 78% more dye per flower compared to plants in either robbing treatment ($F_{2,106} = 3.14$, $P = 0.047$). Plants in the *Primary*

robbing treatment donated twice the dye as plants in the *Primary and secondary robbing* treatment, but a post-hoc analysis revealed that this difference was not statistically significant ($P > 0.05$; Figure B.1b). Finally, plants in the second round of dye application donated 89% more dye per dyed flower than plants in the first round of dye application ($F_{1,52} = 5.81, P = 0.02$).

2) *Are the effects of secondary robbing on pollinator visitation equivalent to chronic nectar removal by pollinators?*

We observed 214 foraging bouts by broad-tailed hummingbirds. Three bouts were made by female hummingbirds with the remaining bouts by males. Hummingbird visitation rate was 44% higher in the *Control* treatment than either the *Primary and secondary robbing* and *Chronic nectar removal* treatments ($F_{2,55} = 5.98, P = 0.004$; Figure B.2a). A post-hoc analysis revealed both pairwise comparisons with the *Control* treatment to be statistically significant (*Control* vs. *Primary and secondary robbing*, $P = 0.01$; *Control* vs. *Chronic nectar removal*, $P = 0.01$). However, there was no difference in visitation between the *Primary and secondary robbing* treatment and the *Chronic nectar removal* treatment ($P > 0.05$), suggesting that nectar removal in both treatments yielded similar effects on pollinator visitation rate. For the covariate, taller plants experienced a higher visitation rate ($F_{1,55} = 8.19, P = 0.006$).

The effect of the treatment on visitation rate was driven by a difference in the mean proportion of flowers probed per plant ($F_{2,55} = 3.94, P = 0.02$). Plants in the *Control* treatment experienced a 27% and 35% higher proportion of flowers probed compared to the *Primary and secondary robbing* and *Chronic nectar removal* treatments, respectively

(Figure B.2b). A post-hoc analysis revealed the difference between the *Control* and *Chronic nectar removal* to be the only significant pairwise comparison ($P = 0.02$). The effect of treatment on number of visits per day showed the same general pattern as the proportion of flowers probed, with the *Control* plants receiving more visits than either robbing treatment (mean \pm SE = 0.60 ± 0.06 , 0.47 ± 0.05 , and 0.47 ± 0.06 for *Control*, *Chronic nectar removal*, and *Primary and secondary robbing*, respectively). However, this pattern was not statistically significant ($F_{2,55} = 2.58$, $P = 0.09$). The covariate plant height revealed that taller plants experienced more visits overall ($F_{1,55} = 17.00$, $P < 0.001$). Plants displayed a mean \pm SE number of flowers of 4.93 ± 0.35 , 4.68 ± 0.34 , 3.74 ± 0.27 for *Control*, *Chronic nectar removal*, and *Primary and secondary robbing*, respectively).

DISCUSSION

Exploitation of mutualism via primary nectar robbing can result in opportunities for additional exploitation via secondary nectar robbing; however, studies documenting the combined effects of multispecies exploitation are lacking. Furthermore, despite the high frequency of secondary nectar robbing in nature (Irwin et al., 2010), its fitness consequences for male components of plant reproduction and the mechanisms that might underlie such effects have been minimally explored. Here, we report that the effects of additional exploitation by secondary nectar robbers may be strong for some components of plant fitness (i.e., female function; Richman et al., 2017), but weaker for others (i.e., as shown here, male function). Robbing did reduce per-flower pollen donation relative to control plants, but we did not detect a significant difference between primary and

secondary robbing. Additionally, hummingbird pollinators were no more likely to avoid secondary-robbed flowers than they were to avoid intact flowers that had experienced chronic nectar removal, suggesting that robbing per se does not affect pollinator visitation rate. Hummingbirds generally rely on a variety of cues to make foraging decisions, and possess the ability to learn and remember rewarding plants and flowers from complex information and signals (González-Gómez et al., 2011). Our results indicate that several different cues may influence hummingbird foraging decisions in the presence of nectar robbers and other organisms that reduce nectar standing crops.

Effect of nectar robbing on estimates of male plant reproduction

We found no evidence that secondary nectar robbing affected male components of plant reproduction beyond the effect of primary robbing. While robbed flowers donated significantly less pollen on a per-flower basis relative to the control, there was no statistically significant difference between primary vs. primary plus secondary robbing on pollen (dye) donation per flower dyed. There was also a difference in overall dye donation between the two rounds of dye application, with lower overall visitation during the first round. This may be attributed to risk-averse behavior of hummingbirds when first presented with a new resource patch, i.e. the floral array (Valone, 1992). Other factors that affect within-season variation in hummingbird pollinator foraging frequencies, such as weather, may also have played a role in the differences observed between rounds. The daily high temperature was approximately 10°C lower on the day we applied dye the first round compared to the day we applied dye the second round;

however, temperatures were similar for each following day, when hummingbirds were also foraging (*R.E. Irwin, pers. obs.*)

The difference in dye donation across treatments matches expectations based on our measurements of hummingbird-pollinator behavior, in which pollinators reduced rates of visitation to both robbing treatments relative to the control. However, there was no difference in visitation rate between the two robbing treatments. Thus, this work suggests that within the context of our experiment, hummingbird pollinators were most sensitive to initial nectar removal (via primary robbing), and avoid plants they deem as unrewarding after an initial, unsuccessful visit. Visual and spatial cues are likely driving this behavior, either independently or combined. Hummingbirds could use the presence or absence of robber holes as a visual cue that provides information about rewarding or unrewarding plants or flowers. Additionally, because plants were returned to the same location in the array each day, hummingbirds might have used spatial memory to locate rewarding and unrewarding plants. Visual reward association has been shown in *Hylocharis leucotis* hummingbirds foraging on natural *Penstemon* populations. Pérez, et al. (2011) found that birds were more likely to locate rewarding flowers when floral nectar guides were present. However, spatial memory of rewarding flowers tended to override decisions made using visual cues, as has been seen in other systems (Hurly and Healy 1996). In another study, *Selasphorus rufus* hummingbirds were better able to learn rewarding (artificial) flowers based on location rather than color or pattern cues (Hurley and Healy, 2002). We did not explicitly test reward association with visual or spatial cues, as we did not keep track of individual bird behavior during the experiment. Given our results, future studies that assess the mechanisms by which hummingbirds locate and

remember rewarding plants and flowers in the context nectar robbing will be valuable for predicting the effects of pollinator foraging patterns on male plant fitness.

Primary and secondary robbing resulted in similar effects on pollen (dye) donation (an estimate of male plant reproduction; Schaeffer et al. 2013). In contrast, prior work found that secondary robbing resulted in additional reductions in female plant reproduction relative to primary robbing alone (Richman et al., 2017). In both studies, the fitness reductions between robbing treatments or relative to control (unrobbed) plants can be attributed to fewer mating events via changes in pollinator behavior. These differences in results between studies are intriguing in the context of Bateman's principle, which predicts male fitness should be more sensitive to missed mating opportunities than female fitness (Bateman, 1948). However, alternative hypotheses posit that this need not always be the case (Wilson et al., 1994). For instance, it has been argued that missed mating opportunities should negatively affect female fitness as well, if the species experiences pollen limitation (Burd 1994). There is evidence that *Ipomopsis* is pollen-limited, although this may be spatiotemporally variable (Hainsworth et al. 1985). In other systems, effects on male versus female fitness can be highly variable, with no clear pattern of one sexual function performing better than the other, although floral attractiveness to pollinators seems to be a common factor. For example, components of male and female success were correlated with unique floral morphological characteristics (which presumably contributed to pollinator attraction) in *Polemonium viscosum* (Polemoniaceae), and therefore did not experience differential male-female performance (Galen and Stanton 1989). Third-party interactions with herbivores (which can affect floral displays) have been shown to either increase (Carper et al. 2016) or decrease

(Mutikainen and Delph 1996) male performance relative to female performance, due to indirect effects via pollinator behavior and pollen tube growth limitation, respectively. Future work that measures the effects of secondary robbing on male and female function on the same plants will yield additional insight, especially if male and female performance as a function of robbing is spatiotemporally variable. It will also be important to measure the key pollinator behaviors that drive each component of plant reproduction.

Nectar robbing versus other forms of nectar removal

Hummingbird pollinators foraging on *Ipomopsis* visited plants at the same rate whether flowers had been secondary robbed or whether flowers without robber holes had been chronically drained from the corolla opening. This was true for both components of calculated visitation rate (proportion flowers probed per visit, number of visits per day), although we only detected a statistically significant difference between control and robbing treatments for the proportion of flowers probed. Interestingly, these results contrast with our experiment testing hummingbird foraging responses to different levels of nectar robbing, in which differences in visitation rate were which driven by the number of visits per plant per day rather than the proportion of flowers probed. Given these contrasting results, hummingbirds may be making foraging decisions at the flower level as well as the plant level when flowers without robbing holes do not consistently offer nectar rewards. Another reason for the contrasting results between studies may have to do with the difference in total number of observations and number of days of the experiment (53 observations over 5 days in Experiment 1 vs. 214 observations over 9

days in Experiment 2). Assuming the same individual birds were foraging within experiments, foraging over more days may provide experience from which hummingbirds can draw to make more complex or hierarchical decisions, i.e. at the flower level rather than the plant level (Bateson et al. 2003). However, it is important to note that we have no way of knowing the relative amount of experience in birds observed in our experiments.

Hummingbirds in this experiment visited both unrewarding treatments at the same rate, even though robber holes were present in only one of the treatments. This lends support to the idea that, at least at the flower level, hummingbirds are not using the presence of a robber hole to discriminate between rewarding and unrewarding flowers, although they may do so at the plant level, as was discussed in the first experiment. The result that hummingbirds avoided plants with empty flowers, although not statistically significant, lends further support to the idea that they may be using spatial learning and memory to locate floral rewards, at least initially. Upon making a decision to visit a given plant, they may cue in on lack of nectar to avoid unrewarding flowers, as has been previously proposed in this and other systems. In *Ipomopsis*, prior experiments have shown that hummingbird pollinators can select unrobbed, rewarding plants and flowers even in the absence of spatial location cues and robbing holes (Irwin, 2000). It has also been shown that hummingbirds will depart inflorescences more readily upon encountering unrewarding flowers (Wolf and Hainsworth 1986), which is consistent with our result of hummingbirds probing fewer flowers on unrewarding plants. The same pattern has been shown in *Selasphorus rufus* hummingbirds foraging from artificial inflorescences (Biernaskie et al. 2002).

Hummingbirds may benefit from making reward-based foraging decisions, as many factors can affect levels of nectar standing crops. For instance, continual visitation not only by pollinators but also by nectar thieves will affect the proportion of rewarding flowers on a plant. Nectar reduction by nectar thieves reduced hummingbird visitation and seed production in *Bouvardia turnifolia* (Rubiaceae) (Torres et al. 2008). Conversely, nectar-thieving mites stimulated nectar production in *Moussonia deppeana* (Gesneriaceae), resulting in increased visitation by hummingbirds and increased seed production (Lara and Ornelas 2002). In both of these cases, changes in nectar availability seem to be the cue that affects bird foraging decisions and behaviors. Furthermore, if additional visitation by nectar thieves does not reliably affect the likelihood that a given flower is rewarding, hummingbirds may be more likely to evaluate individual flowers than entire plants. Given the challenges hummingbirds face in meeting their daily energetic requirements (Wolf et al. 1972), there may be strong selection for them to cue in on nectar to make foraging decisions, particularly if the presence vs. absence of robber holes is not a reliable indicator of nectar rewards. Future mechanistic experiments that determine the proximate cues that hummingbird pollinators use to avoid robbed plants and flowers will yield important ecological insight, as will experiments that explore the sensitivity of hummingbirds to small differences in nectar standing crops. It would also be valuable to assess pollinator foraging in response to finer-scale differences in nectar volume, such as between-flower, within-plant differences.

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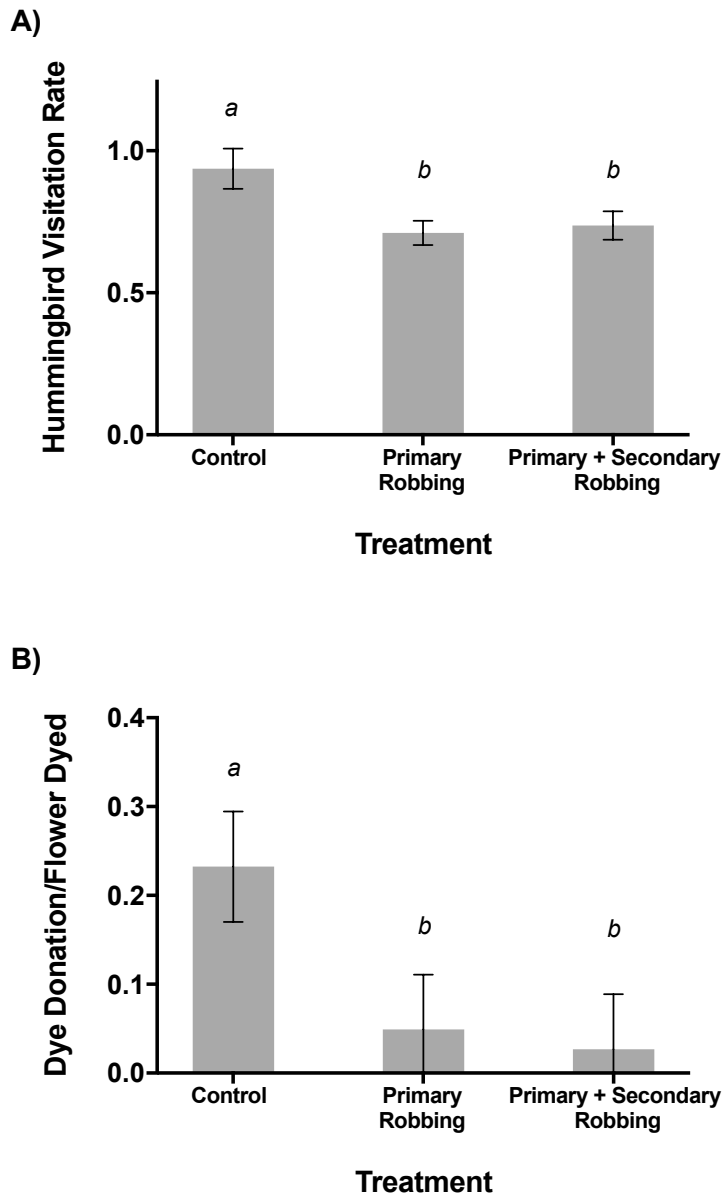


Figure B.1. The effect of nectar-robbing treatments on (A) mean (\pm SE) hummingbird visitation rate and (B) mean (\pm SE) number of pollen grains (dye) donated per flower dyed. Hummingbird visitation rate is calculated as the number of times plants were visited multiplied by the mean percentage of flowers probed. Different letters represent significantly different ($P < 0.05$) mean values based on a Tukey HSD test.

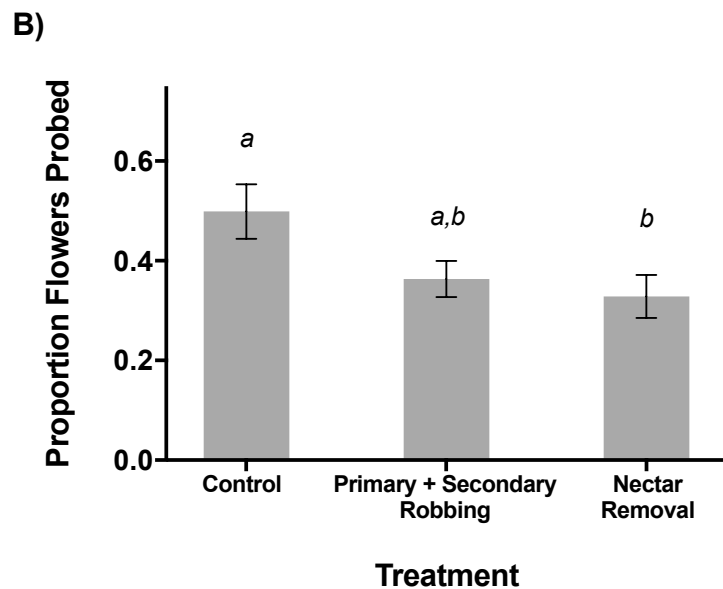
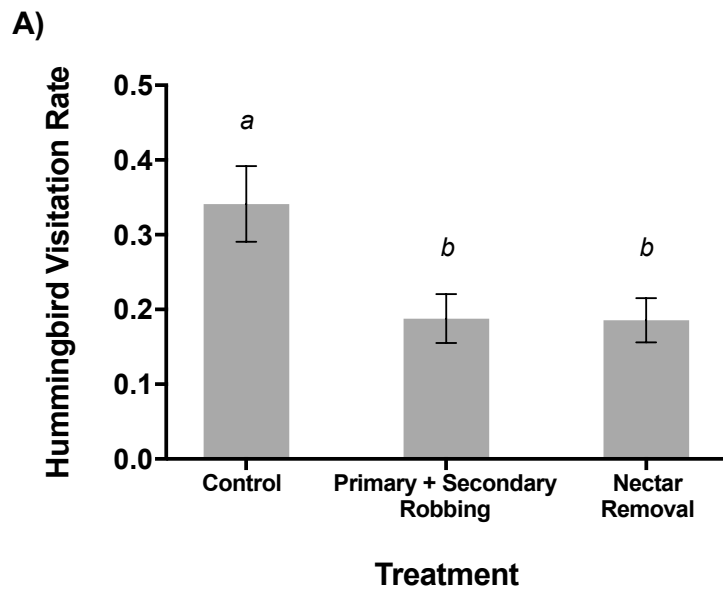


Figure B.2. The effect of simulated secondary nectar robbing (with holes in the corollas) and chronic nectar removal (without holes in the corollas) on (A) hummingbird visitation rate and (B) the proportion of flowers per plant probed by hummingbirds per day. Bars and error bars represent means \pm SEs. Hummingbird visitation rate is calculated as in Figure 2. Different letters represent significantly different ($P < 0.05$) mean values based on a Tukey HSD test.

APPENDIX C

FORAGING STRATEGY PREDICTS FORAGING ECONOMY IN A FACULTATIVE
SECONDARY NECTAR ROBBER

1) Sarah K. Richman*^{1,2}, srichman@email.arizona.edu, Orcid ID: 0000-0003-1987-1140

2) Rebecca E. Irwin^{2,3}, reirwin@ncsu.edu

3) Judith L. Bronstein^{1,2}, judieb@email.arizona.edu

¹Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ
85721 USA

²Rocky Mountain Biological Laboratory, Crested Butte, CO 81224 USA

³Department of Applied Ecology, North Carolina State University, Raleigh, NC 27695
USA

*Correspondence Author

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Abstract

In mutualistic interactions, the decision whether to cooperate or cheat depends on the relative costs and benefits of each strategy. In pollination mutualisms, secondary nectar robbing is a facultative behavior employed by a diverse array of nectar-feeding organisms, and is thought to be a form of cheating. Primary robbers create holes in floral tissue through which they feed on nectar, whereas secondary robbers, which often lack chewing mouthparts, feed on nectar through existing holes. Because primary robbers make nectar more readily available to secondary robbers, primary robbers facilitate the behaviors of secondary robbers. However, the net effect of facilitation on secondary robber fitness has not been empirically tested: it is unknown whether the benefit secondary robbers receive is strong enough to overcome the cost of competing with primary robbers for a shared resource. We conducted foraging experiments using the bumble bee *Bombus bifarius*, which can alternatively forage “legitimately” (from the floral opening) or secondary-rob. We measured the relative foraging efficiencies (handling time per flower, flowers visited per minute, proportion of foraging bout spent consuming nectar) of these alternative behaviors, and tested whether the frequency of primary robbing and nectar standing crop in primary-robbed flowers of *Linaria vulgaris* (Plantaginaceae) affected foraging efficiency. Surprisingly, there was no effect of primary robbing frequency on the foraging efficiency of secondary-robbing *B. bifarius*. Instead, foraging strategy was a major predictor of foraging efficiency, with legitimate foraging being significantly more efficient than secondary robbing. Legitimate foraging was the more common strategy used by *B. bifarius* in our study; however, it is rarely used

by *B. bifarius* foraging on *L. vulgaris* in nature, despite indications that it is more efficient . Our results suggest the need for deeper investigations into why bees adopt secondary robbing as a foraging strategy, specifically, the environmental contexts that promote the behavior.

Introduction

Nectar robbers disrupt many pollination mutualisms by usurping floral rewards intended for pollinators (Inouye 1980, Irwin et al. 2010). Two distinct nectar robbing strategies can be identified. Primary nectar robbers feed on floral nectar through holes they chew or pierce into the side or at the base of flowers with long, tubular corollas or otherwise recessed nectaries, whereas secondary robbers feed on nectar through holes created by primary robbers (Inouye 1980). Nectar robbers, unlike visitors that feed through the floral opening (henceforth termed “legitimate” foragers), usually bypass floral reproductive structures and thus typically do not act as pollen vectors (Irwin et al. 2010; however, see, e.g., Higashi et al. 1988 and Navarro 2000 for exceptions). Primary robbing requires specific morphology, usually sharp or toothed mouthparts, to puncture the floral tissue. Secondary robbing, however, does not (Irwin et al. 2010). For this reason, secondary robbing is an additional behavior, available to any nectar feeder, that can only be conducted in the presence of primary robbers (Irwin et al. 2010). Often, legitimate foragers will switch to secondary robbing on the same plant species once robbing holes have accumulated in flowers (Rust 1979, Dedej and Delaplane 2004, Kjonaas and Rengifo 2006).

Why bees shift away from foraging legitimately is unknown. Nor are the environmental conditions that promote secondary robbing clear (Irwin et al. 2010). It is commonly thought that secondary robbing is a more efficient strategy relative to legitimate foraging (Dedej and Delaplane 2005). Specifically, because secondary robbing allows nectar robbers to circumvent a morphological mismatch with a flower that would otherwise make access to nectar difficult, it can reduce flower handling times and lead to

increased nectar removal (Olesen 1996, Newman and Thomson 2005a, Dedej and Delaplane 2005). If adopting a secondary robbing over a legitimate foraging strategy is more energetically efficient, it should alleviate some of the fitness costs associated with foraging, including energetic costs, predation risk and missed opportunity costs, such as those incurred when missing a mating opportunity (Brown et al. 1988). However, few studies have tested this conventional wisdom (but see Thomson 2004).

Floral visitors that secondary-rob, but cannot primary-rob, are dependent upon the activities of primary robbers. As robbing holes accumulate in a floral patch, progressively more opportunities are made available for visitors that can potentially secondary rob to adopt that strategy. However, as the number of flowers with robbing holes in a patch approaches 100%, primary robbers will re-visit flowers through the robbing holes they have previously created. These “secondary” visits further reduce overall nectar standing crop. Competition between primary and secondary robbers may be fierce, as robbed flowers tend to have less available nectar than unrobbed flowers (Stout et al. 2000, Temeles and Pan 2002, Newman and Thomson 2005a). Therefore, in order to understand the net effect of primary robbers’ facilitation of secondary robbers, we must compare the presumed benefit of increased foraging efficiency associated with the switch to secondary robbing, with the potential cost of competition for nectar. Determining whether primary robbers indirectly increase secondary robbers’ foraging efficiency will provide ecological insight into the costs and benefits of secondary nectar robbing, as well as how interactions between nectar robbers (i.e., exploiters of mutualism) potentially affect the functioning of plant-pollinator mutualisms.

By observing the foraging behavior of facultative secondary robbing bumble bees within floral arrays manipulated to mimic different effects of primary robbing (primary robbing rate and extent of nectar drainage from robbed flowers due to primary robbing), we tested the hypothesis that shifting to secondary robbing from legitimate foraging leads to higher individual foraging efficiency. From this initial hypothesis, three predictions follow. (1) If secondary robbing is a more efficient foraging strategy, we predict that secondary robbers will achieve higher foraging efficiency in patches with higher rates of primary robbing, as long as nectar standing crop in primary-robbed flowers is high, due to limited “secondary” visits from primary robbers. In this case, we would expect that the benefits of primary robbers’ feeding activities (i.e., creating robbing holes) would outweigh the cost of competing with them for nectar, leading to facilitation (Figure C.1a). (2) If nectar standing crop in primary-robbed flowers is instead low, due to a high number of “secondary” visits from primary robbers, we predict that secondary robbers will experience reduced foraging efficiency in the presence of primary robbers, as a result of having to spend more time finding rewarding flowers. In this case, the cost of competing with primary robbers should outweigh the benefit of access to robbing holes (Figure C.1a). (3) If legitimate foraging is a more efficient strategy than secondary robbing regardless of primary robbing rate or nectar drainage in flowers, foragers should be more likely to adopt this strategy. In this case, we predict that there will not be a facilitative benefit due to primary robbing, as legitimate foragers do not utilize robbing holes. Rather, we expect that legitimate foragers will only pay a competitive cost from interacting with primary robbers (Figure C.1b).

Materials and Methods

Study site and system

Experiments were performed in a 2.5 m x 3 m x 2.1 m flight cage at the Rocky Mountain Biological Laboratory (RMBL, elevation 2895 m), Gothic, CO USA. The flight cage was outdoors, and was made of a metal frame covered with white canvas (WeatherPort Shelter Systems, Delta, CO). Mesh windows on all sides allow for ambient light and temperature inside. We observed individual foraging bouts of the facultative secondary robbing bumble bee *Bombus bifarius* on arrays of the host plant *Linaria vulgaris* (Plantaginaceae, henceforth *Linaria*). *Linaria* is a long-lived, rhizomatous perennial that produces racemes of 15-30 zygomorphic flowers (Arnold 1982). Flowers are yellow with an orange palate and a nectar spur 15-20 mm in length (Stout et al. 2000). Nectar is continually produced, with flowers producing up to 2.8 μ L in 48 hr (Nepi et al. 2003). *Linaria* was introduced by European settlers into North America and is now widespread (Arnold 1982). In its North American range, *Linaria* has been incorporated into the foraging diet of a variety of pollinators, including bumble bees (Burkle et al. 2007). In addition, *Linaria* experiences primary nectar robbing by the bumble bee *Bombus occidentalis*, which uses its toothed mandibles to create holes in the nectar spurs. *Bombus bifarius* cannot primary rob but can secondary rob flowers primary-robbed by *B. occidentalis*. Both species exhibit flexibility in foraging behavior: *B. occidentalis* often switches from primary robbing to secondary robbing as the rate of primary robbing holes in flowers increases, whereas *B. bifarius* can also legitimately forage from *Linaria* flowers for nectar and pollen (Newman and Thomson 2005). However, there is preliminary evidence that secondary robbing is a more efficient strategy for *B. bifarius* in

field conditions (Newman and Thomson 2005a). Workers possess short probosces, making access to nectar through legitimate foraging difficult; furthermore, workers can consume more nectar per unit time by foraging from robbing holes (Newman and Thomson 2005a).

Collection of individuals for foraging observations

We conducted foraging observations 5 d/wk from 25 July 2014 to 26 August 2014. Each morning and afternoon, we collected 10-15 individual *B. bifarius* workers as they foraged from natural *Linaria* populations within the vicinity of the RMBL. Bees were collected in vials and immediately placed into a cooler before being transferred to a 10°C refrigerator for 1 hr. Upon capture, we recorded the foraging strategy being employed (legitimate foraging or secondary robbing). All bees used the foraging strategy recorded at capture during the experimental trials (see Results).

Experimental treatments

We collected stalks 15-20 cm in length from *Linaria* populations within the vicinity of the RMBL. The stalks had been enclosed in mesh cages for 24 hr to allow flowers to refill with nectar. After collecting, we inserted stalks into florist picks filled with water. Picks were placed into 10 cm pots, filled with soil to keep the picks stable. When necessary, we removed flowers from stalks so that each stalk displayed 20 flowers, none of which had pre-existing robbing holes. We then performed manipulations to reflect the pattern of floral damage stalks would typically encounter after a bout of primary robbing by *B. occidentalis*. We used five experimental treatments. The first four

treatments were a 2x2 cross of primary robbing frequency (low vs. high) by nectar availability (rewarding vs. unrewarding). In the low primary robbing treatment, we made robbing holes in 20% (low primary robbing treatment) or 80% (high primary robbing treatment) of flowers. Nectar was either removed from the artificially robbed flowers (unrewarding treatment) or was left in them (rewarding treatment). Finally, in a Control treatment, no flowers received robber holes and all flowers were rewarding. Low and high robbing rates were chosen based on observations of natural variation in primary robbing rates in the field (S. Richman, personal observation). We chose to remove all available nectar from flowers with robbing holes, as field studies indicate that robbed *Linaria* are more likely to be empty than flowers without robbing holes (Newman and Thomson 2005). In all treatments, no nectar was removed from intact (unrobbed) flowers. All treatments were applied at the stalk level; that is, each stalk used in the array received the same treatment. For example, in a low primary robbing treatment, where 20% of flowers had robbing holes, we made holes in 20% of the flowers on each stalk.

We created primary robbing holes by piercing the nectar spur with a pair of fine-tipped forceps, creating the characteristic “u”-shaped perforation made by *B. occidentalis* (S. Richman, personal observation). When necessary for the experimental treatment, we removed all available nectar from flowers using a 10 uL microcapillary tube (Microcap, Drummond Scientific, Broomall, PA) inserted into the robbing hole. This method removes nectar using capillary action and does not damage the floral tissue or reproductive structures (Irwin and Brody 1998, Irwin et al. 2015).

Foraging bout observations

After treatments were applied, we assembled arrays of 30 flowering *Linaria* stalks in a 1 m x 1 m grid, each stalk having received the same treatment. For each foraging bout, we set out a new array of stalks, numbering each stalk from 1-30. We placed one control stalk in the center of the array. Bees were introduced from the refrigerator onto this control stalk before visiting the array to ensure they had time to warm up and would readily forage from the treatment stalks.

We observed foraging bouts of individual *B. bifarius* on *Linaria* arrays, $n = 10$ bees/treatment, each bee given one array. Treatments were randomized by day of the week and time of day (morning or afternoon). Once a bee began foraging from the treatment stalks, we recorded its behavior using both a video recorder (Sony HDR SR-11) and a hand-held digital voice recorder (Olympus VN-702PC). For each floral visit, we recorded the stalk number, how many flowers were visited per stalk, the time spent nectar-feeding from each flower, and the foraging strategy employed at each flower (legitimate foraging or secondary robbing). When possible, we recorded the reward collected at each visit (pollen or nectar). After a bee finished its foraging bout, signified by leaving the array and flying to the side or ceiling of the flight cage, we captured it and removed it from the arena. It has been shown in another system that across species, body size can predict bumble bees' decisions to legitimately forage or secondary rob, with smaller bees more likely to secondary rob (Ishii 2013). Consequently, we measured radial cell length (mm) of the right forewing using digital calipers (to the nearest 0.01 mm) as an estimate of bee size (Harder 1982). Before releasing a bee back to the field at its point of collection, we marked it with a unique numbered tag (The Bee Works, Oro-Medonte,

ON, Canada) glued to its thorax using non-toxic glue. This method ensured that we did not re-use bees for foraging observations.

Statistical analyses and interaction strength calculations

To test whether treatment predicted foraging strategy, we generated a contingency table containing the number of bouts observed per foraging strategy, broken down by treatment. We performed a Chi-squared Test of Independence based on this contingency table. To test the hypothesis that foraging efficiency differed by foraging strategy, and that primary robbing frequency and nectar availability (whether primary robbed flowers were rewarding or unrewarding) affected foraging efficiency (predictions 1, 2), we calculated the following response variables: (1) Feeding time per flower (s), calculated as (total time spent nectar feeding at flower (s))/total # flowers visited; this time does not include time spent searching or probing for a robbing hole. (2) Flowers visited per minute, calculated as (total # flowers visited/total bout time (min)). (3) Proportion of bout spent nectar feeding, calculated as (total time spent nectar feeding at flower (s))/total bout time (s)). For feeding time per flower and flowers visited per minute, we compared primary robbing frequency (low/high) and nectar availability (rewarding/unrewarding) using a linear model with foraging strategy, primary robbing frequency, nectar availability, and the interaction of primary robbing frequency and nectar availability as fixed effects and bee size (radial cell length) as a covariate. We excluded the two-way interaction of strategy with either primary robbing frequency or nectar availability, as well as the three-way interaction between all three variables, because initial analyses yielded all interactions as non-significant, and AIC comparison indicated the simpler

models as the best fit. For proportion of bout spent nectar-feeding, we compared primary robbing frequency and nectar availability using GLM (binomial family) with the same fixed effects and interactions as in the linear model analyses. We excluded two bouts from the analyses that were < 1 min in length; it was unclear in these cases whether bees had completed a foraging bout or had probed flowers only briefly before deciding to leave the array. Of the 38 treatment bouts, 25 bees foraged legitimately, 10 secondary robbed and 3 were inconstant, i.e., they employed each of the two strategies at least once. We assigned the inconstant bouts to either legitimate or secondary robbing foraging strategies as follows. In the first inconstant bout, assigned to legitimate foraging, 91% (19 of 22) of the visits were legitimate; in the second, assigned to secondary robbing, 96% (27 of 28) of the visits were secondary robbing. In the third bout, the bee switched strategies multiple times, although it secondary robbed the majority of the time (91%, 31 of 34 visits); it was thus assigned secondary robbing as its overall foraging strategy. Excluding the inconstant bouts from the analysis yielded the same results (data not shown). Bouts from the control treatment were excluded from the linear model analyses, but were used to calculate interaction strength (see next). Effects of individual model terms were calculated using Type II ANCOVA for feeding time per flower and number of flowers visited per minute, and using a likelihood ratio test for proportion of bout spent nectar feeding. All analyses were performed using R version 3.2.3 (R Core Team 2016).

We used Relative Interaction Intensity (R_{II}) (Armas et al. 2004) to compare the costs and benefits of primary robbing on *B. bifarius* foraging efficiency, and to determine whether the cost/benefit ratio led to primary robbers facilitating secondary robbers (Prediction 3). R_{II} is typically used to calculate the effect of interspecific interactions,

where the R_{II} value indicates the effect of species A on species B (Armas et al. 2004). We consider R_{II} to be valuable for this study, as we can use R_{II} values to extend our results and further quantify the strength of the interaction. We compared all experimental treatment conditions relative to the control for all three response variables, calculated as:

$$R_{II_{\text{Response}}} = (\bar{x}_{\text{Treatment}} - \bar{x}_{\text{Control}}) / (\bar{x}_{\text{Treatment}} + \bar{x}_{\text{Control}}).$$

R_{II} ranges from -1 to 1, where the effect of primary robbing on *B. bifarius* is more costly (i.e., the competitive effect of primary robbing on *B. bifarius* outweighs its facilitative effect) as the value approaches -1, and more beneficial (i.e., facilitative) as the value approaches 1. R_{II} values that do not differ from zero are considered to be neutral in effect. After calculating R_{II} for each treatment condition relative to the control, we performed a randomization test to generate 95% confidence intervals around each value in order to determine whether values significantly differed from zero. We interpret R_{II} values that are significantly different from zero (positive or negative) to point to a significant effect of primary robbing (i.e., the simulated effect of *B. occidentalis*) on *B. bifarius* foraging efficiency.

Results

Foraging efficiency of secondary nectar robbing vs. legitimate foraging

We observed a total of 48 bouts of *B. bifarius* foraging on 1,009 flowers across the 5 treatments, 38 of which were from the four manipulative treatments. Average bout time was 6.37 ± 0.72 min. Opposite to our initial prediction, we found that legitimate foraging was a more efficient foraging strategy than secondary robbing. Bees that legitimately foraged visited an average of 0.75 more flowers/min than did those that

secondary robbed ($F_{1,28} = 5.89, P = 0.02$; Figure C.2b). There was a significant, positive effect of body size on the number of flowers visited/min (adjusted $R^2 = 0.14, P = 0.02$). Legitimately foraging bees spent an average of 34.6% more time per bout nectar-feeding (as opposed to searching for flowers, searching for robbing holes, or grooming) than did secondary robbers ($\chi^2_1 = 10.47, P = 0.001$, Figure C.2c), with no significant effect of body size on time spent nectar-feeding ($P = 0.08$). There was no effect of treatment on foraging strategy ($\chi^2_3 = 2.68, P = 0.44$).

Effect of primary robbing on foraging efficiency

Primary robbing frequency and nectar availability within the array did not significantly affect the length of time that bees spent nectar-feeding at flowers, the number of flowers they visited per minute (Tables C.1 and C.2), nor the proportion of each bout they spent nectar-feeding (primary robbing frequency $\chi^2_1 = 1.23, P = 0.26$; nectar availability $\chi^2_1 = 0.10, P = 0.75$). Nor was the interaction between primary robbing rate and nectar availability statistically significant for any of the three response variables (Table C.1 for average time/flower, number of flowers visited/minute; proportion bout spent nectar feeding $\chi^2_1 = 1.18, P = 0.28$). Overall, neither foraging strategy nor body size significantly affected feeding time (Figure C.2a for foraging strategy).

Relative interaction intensity (R_{II})

There was an overall weak negative effect of primary robbing on R_{II} associated with average feeding time per flower (s), indicating that competitive effects of primary

robbing on *B. bifarius* outweighed its facilitative effects (Table C.3, Figure C.3a). There was a weak positive effect of primary robbing on R_{II} associated with number of flowers visited/min, although R_{II} was not significantly different from zero in the Low robbing, Rewarding treatment (Figure C.3b). There was no effect on R_{II} associated with the proportion of the bout spent nectar feeding, except in the Low robbing, Unrewarding treatment, where there was a weak negative effect (Figure C.3b).

Discussion

We tested the prediction, emerging from limited field studies (Dedej and Delaplane 2005, Newman and Thomson 2005), that the advantage of secondary robbing is that it allows a bee to make more frequent floral visits and consume more nectar per unit time than would foraging legitimately, resulting in increased foraging efficiency. Higher foraging efficiency, in turn, should decrease the costs and increase the benefits of secondary robbing, indicating that primary robbers facilitate secondary robbers (Figure C.1a). Interestingly, after testing this prediction under controlled conditions, we found the opposite. For the facultative secondary-robber *Bombus bifarius* foraging on *Linaria vulgaris*, secondary-robbing individuals visited significantly *fewer* flowers per minute and spent a *lower* proportion of their foraging bout consuming nectar than did legitimate foragers of the same species. Furthermore, 71% of bees tested chose legitimate foraging over secondary robbing. Consistent with our prediction for a scenario in which legitimate foraging is more efficient (Figure 1b), we did not find a benefit for *B. bifarius* of increased foraging efficiency in response to primary robbing. In this scenario, the lack of benefits provided by primary robbers should produce an outcome of no facilitation of

secondary robbers (Figure C.1b). Indeed, our findings indicate that primary robbers' feeding activities do not facilitate the success of species that can secondary rob. Negative R_{II} values for average feeding time per flower and the proportion of the foraging bout spent nectar-feeding indicate the potential for high competition with primary robbers for *B. bifarius*. While positive R_{II} values for the number of flowers visited per minute indicate that primary robbing can benefit *B. bifarius*' foraging efficiency, the interpretation of the results is confounded by the fact that bees that visited more flowers per minute also spent less time per flower nectar-feeding. This finding, that legitimate foraging is more efficient than secondary robbing, spurs interesting questions about the ecology of secondary robbing, particularly given that *B. bifarius* secondary robs *Linaria* commonly in nature.

Why be a secondary nectar robber?

If secondary robbing does not always maximize foraging efficiency, it becomes essential to consider other factors that could contribute to adoption of the behavior. One hypothesis is that, due to cognitive constraints (Gegear and Lavery 2001), secondary robbing is ultimately more efficient, even though individuals can increase short-term efficiency if they foraged legitimately. For instance, robbing holes may serve as a search image for secondary robbers. Use of a search image can improve foraging efficiency by creating a "run" effect, allowing foragers to find the same resource repeatedly (Tinbergen 1960, Bond and Riley 1991, Gegear and Lavery 2001), although no empirical evidence of this phenomenon exists for nectar robbers. Additionally, robbing holes may provide a visual stimulus that encourages learning of secondary robbing as a foraging strategy.

Bombus terrestris legitimate foragers can learn to secondary rob through social transmission after foraging among flowers with robbing holes in a laboratory setting, and subsequently visit the nectary (rather than the floral opening) faster than bees that do not learn to rob (Leadbeater and Chittka 2008). Secondary robbers in our experiment often flew directly to the spur, sometimes making the mistake of probing an intact spur, indicating that they have at least made an association between the nectar spur and access to floral rewards. Similar behavior has been observed in other *Bombus* species foraging on the long-spurred plant *Corydalis caseana* in the field (S. K. Richman, pers. obs.).

Another explanation for why bees secondary-rob although it is less efficient than legitimate foraging is that asymmetric inter- or intraspecific competition forces individuals to choose secondary robbing, although they would prefer to forage legitimately. Body size, which was a significant factor affecting foraging efficiency in our study, as well as proboscis length have been shown to be important factors in structuring competitive hierarchies in *Bombus* spp., with longer-tongued species often competitively dominant (Inouye 1978, Ishii et al. 2008). Moreover, there is evidence that nectar-robbing *Bombus* individuals tend to have shorter tongues than legitimate foraging individuals (Ishii 2013). It is also plausible that individuals adopt secondary robbing as a foraging strategy in response to simultaneously interacting with a high density of foragers in a patch or on a stalk, and utilizing robbing holes rather than floral openings is a resource partitioning strategy that does not require the cognitive effort involved in switching to a new plant species. There is evidence for partitioning of this kind in the field: Newman and Thomson (2005) report that *Bombus* spp. individuals spatially segregate on floral stalks by foraging strategy (legitimate foraging and secondary

robbing). Our experimental design prohibited us from being able to detect any effect of conspecific or heterospecific forager presence on *B. bifarius* foraging strategy because bees foraged alone. If *B. bifarius* experiences higher foraging efficiency by foraging legitimately, we would expect to see a high proportion of individuals using this strategy in the absence of other foragers. Our results are consistent with this expectation. Approximately 80% of bees used in the experiment foraged legitimately, a much higher proportion than is found in nature (Newman and Thomson 2005). However, it is unknown whether the difference in proportion of legitimate foragers in the flight cage versus in nature is indeed in response to other foragers being absent, or due to another factor, such as one connected with foraging in an artificial setting. We have no reason to expect that bees would forage differently in the flight cage than in nature, but it would be valuable to conduct a similar study in a field setting and compare foraging behaviors. Finally, in order to fully understand the dynamics of competition between forager types, it would be necessary to understand the behavior of facultative secondary nectar robbers in response to overall competitor density as well as the relative densities of con- and heterospecifics.

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Response Variable	Model Variable	SS	F	<i>P</i>
Visit time/flower (s)	Primary rob rate	0.13	0.03	0.86
	Nectar available	13.76	3.17	0.09
	Interaction	1.62	0.37	0.55
Flowers visited/minute	Primary rob rate	2.11	0.82	0.37
	Nectar available	6.57	2.57	0.12
	Interaction	1.14	0.44	0.51

SS = Type II Sum of Squares

Table C.1. Effect of primary robbing frequency (low or high) and nectar availability (whether or not primary robbed flowers are rewarding) on metrics of *B. bifarius* foraging efficiency. Primary robbing frequency and nectar availability were used as fixed effects in a linear model followed by an F-test. Table reports F-values and *P*-values for each fixed effect as well as their interaction, degrees of freedom = 1 (model), 28 (residual) for all rows.

Independent variable	Visit time/flower (s)	Flowers visited/minute	Proportion bout nectar feeding
	Mean \pm SE	Mean \pm SE	Mean \pm SE
Primary rob frequency - High	6.47 \pm 0.98	3.72 \pm 0.54	0.33 \pm 0.03
Primary rob frequency - Low	6.15 \pm 0.47	2.97 \pm 0.29	0.29 \pm 0.03
Nectar available - No	5.21 \pm 0.45	3.70 \pm 0.49	0.29 \pm 0.03
Nectar available - Yes	7.30 \pm 0.89	3.03 \pm 0.38	0.23 \pm 0.03

Table C.2. Mean \pm SE values for primary robbing frequency and nectar availability for all *B. bifarius* foraging efficiency response variables. Both independent variables have two levels: high and low primary robbing frequency; nectar in robbed flowers present (available) or absent (unavailable). For all response variables, n = 48 bouts. Results shown combine both foraging strategies, as primary robbing and nectar availability treatments had no significant effect on strategy.

Response Variable	Treatment	Value, Mean \pm SE	R _{II} , Mean \pm SE	95% CI, Low	95% CI, High
Visit time/flower (s)	Low, No	5.05 \pm 0.63	- 0.28 \pm 0.03	-0.33	-0.22
	Low, Yes	6.95 \pm 0.57	- 0.13 \pm 0.03	-0.17	-0.08
	High, No	5.34 \pm 0.66	- 0.26 \pm 0.03	-0.31	-0.21
	High, Yes	7.73 \pm 1.91	- 0.15 \pm 0.04	-0.22	-0.08
Flowers visited/minute	Low, No	4.70 \pm 0.51	0.18 \pm 0.03	0.13	0.23
	Low, Yes	3.69 \pm 0.41	0.04 \pm 0.03	-0.02	0.10
	High, No	5.19 \pm 0.74	0.20 \pm 0.02	0.15	0.25
	High, Yes	4.08 \pm 0.64	0.08 \pm 0.03	0.02	0.14
Proportion bout nectar feeding	Low, No	0.24 \pm 0.04	- 0.19 \pm 0.04	-0.27	-0.12
	Low, Yes	0.38 \pm 0.09	- 0.04 \pm 0.03	-0.11	0.03
	High, No	0.34 \pm 0.03	- 0.01 \pm 0.03	-0.06	0.05
	High, Yes	0.31 \pm 0.04	- 0.07 \pm 0.04	-0.15	0.00

Table C.3. Mean \pm SE values of *B. bifarius* foraging efficiency for all experimental treatment conditions. Treatments indicate the combinations of crossed factors: primary robbing frequency (low or high), nectar availability (whether primary robbed flowers are rewarding; yes or no). Mean \pm SE values for the control condition are 9.68 \pm 1.37, 3.35 \pm 0.44 and 0.35 \pm 0.04 for feeding time/flower (s), flowers visited/minute and proportion bout nectar feeding, respectively. R_{II} (Relative Interaction Intensity) is a measure of interaction strength ranging from -1 to 1. Positive values represent a net positive effect (facilitation); negative values represent a net negative effect (competition). The effect is stronger as the value approaches 1 or -1. Table shows R_{II} and 95% CI for all treatments.

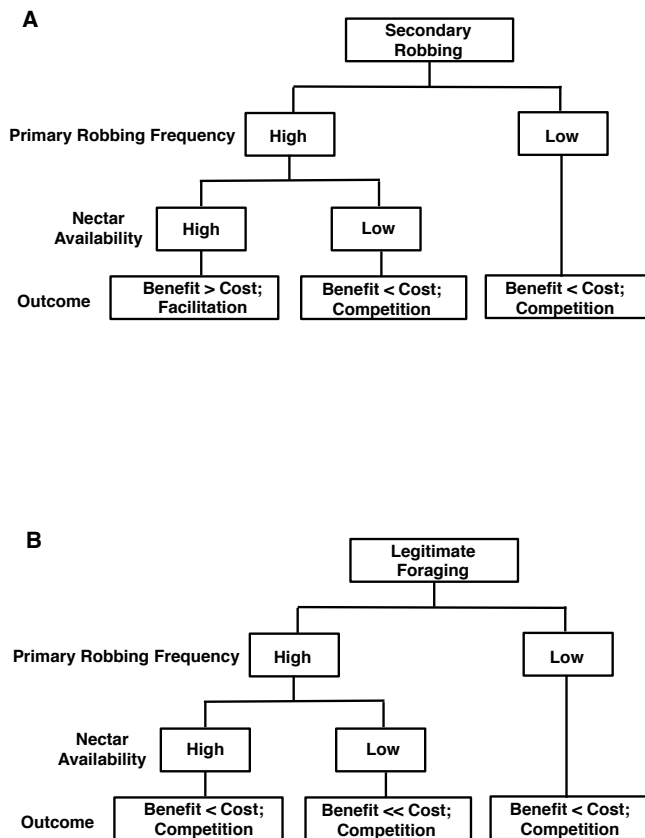


Figure C.1. Decision tree showing the possible outcomes for nectar foragers under the conditions that (A) secondary robbing or (B) legitimate foraging is a more efficient foraging strategy. Upper boxes show environmental variables: primary robbing frequency (the frequency of robbing holes in flowers) and nectar availability (whether primary robbed flowers have nectar). Lowest boxes show outcomes weighing benefits and costs. Outcomes in which the benefit > cost suggest that primary robbers facilitate secondary robbers; outcomes in which the benefit < cost suggest that facilitation does not exist. In panel (B), legitimate foragers are not facilitated by primary robbers because they do not utilize robbing holes. Outcomes show strong (benefit << cost) and weak (benefit < cost) competition.

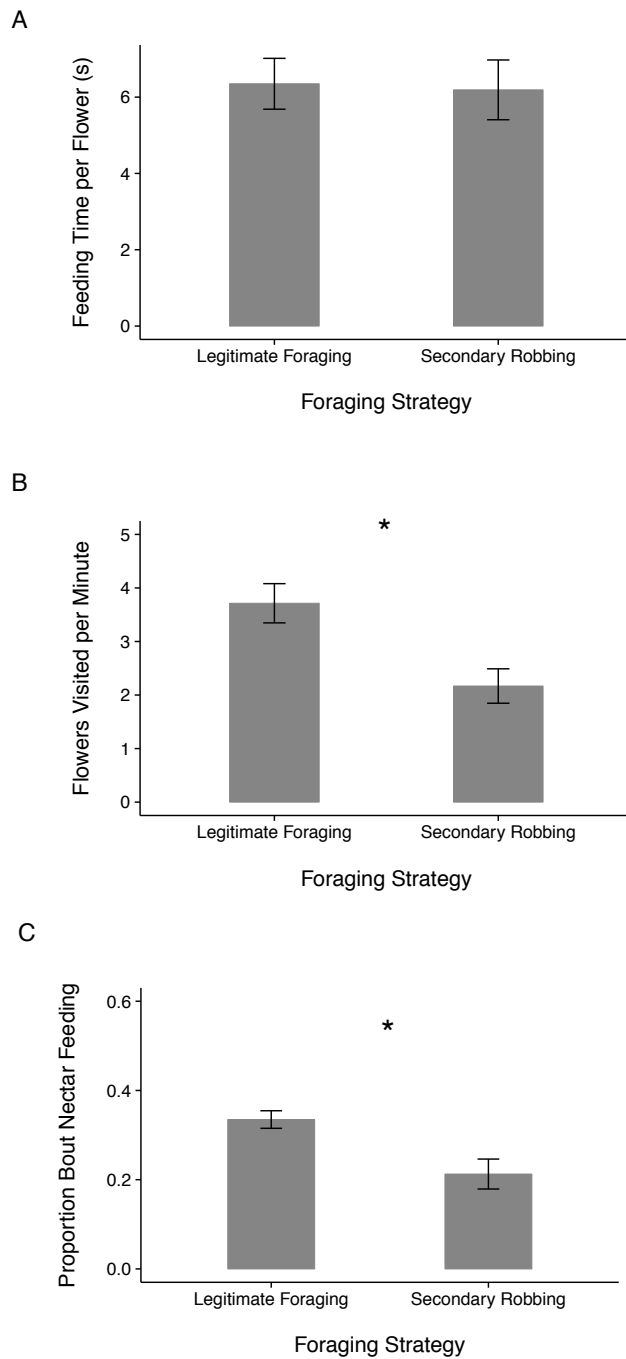


Figure C.2. The effect of foraging strategy on mean \pm SE values for (A) Feeding time/flower, (B) Flowers visited/minute, and (C) Proportion bout nectar feeding. For all graphs, $n = 39$ and 9 for the number of individual *B. bifarius* foragers that adopted a legitimate foraging or secondary robbing strategy, respectively. Asterisks indicate statistically significant ($p < 0.05$) values between foraging strategies.

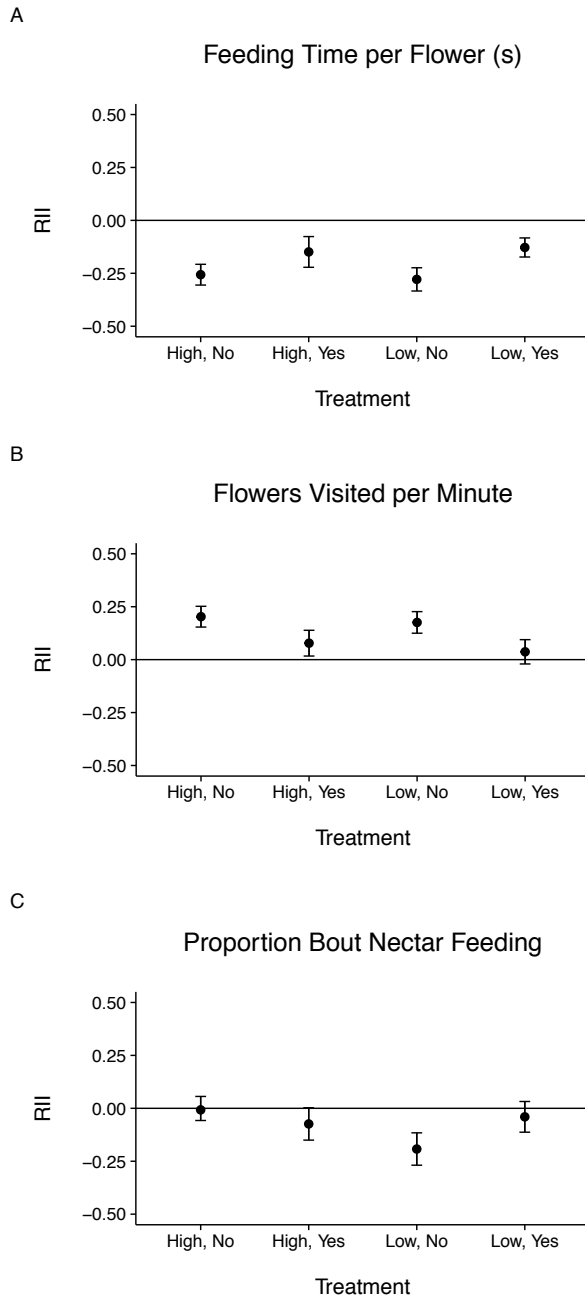


Figure C.3. Mean \pm 95% CI R_{II} Values for each experimental treatment relative to the control. 95% CI was generated using a randomization test. Panels represent R_{II} values for (A) Feeding time/flower, (B) Flowers visited/minute, and (C) Proportion bout nectar feeding. Negative R_{II} values significantly different from zero indicate a net negative effect of treatment on *B. bifarius* foraging efficiency; Positive R_{II} values significantly different from zero indicate a net positive effect of treatment on *B. bifarius* foraging efficiency.

APPENDIX D

EVIDENCE FOR COEXISTENCE BETWEEN A MUTUALIST AND A
CONDITIONAL EXPLOITER IN A MULTISPECIES POLLINATION MUTUALISM

Sarah K. Richman*^{1,2}, Christopher A. Johnson³

¹Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ
85721 USA

²Rocky Mountain Biological Laboratory, Crested Butte, CO 81224 USA

³Department of Integrative Biology, ETH Zurich, 8092 Zurich, Switzerland

* Correspondence author: sarahkrichman@gmail.com

Abstract

Towards the goal of identifying mechanisms that promote mutualism persistence, a great deal of theoretical development and empirical research has been devoted to describing the dynamics of competition and coexistence between mutualists. The outcome of mutualism is often context-dependent: it can change in response to the biotic or abiotic environment. One factor that changes the outcome of mutualism is conditional exploitation, when mutualists choose not to cooperate, presumably based upon the relative costs and benefits of doing so. In a multispecies mutualism, conditional exploiters have the potential to affect the performance of pure mutualists that compete for benefits provided by a shared partner. Adopting an exploiter strategy may increase individual competitive ability but potentially destabilize mutualism. However, conditional exploitation is rarely considered in these studies. We evaluated the strength of competition between pure mutualists and conditional exploiters in a pollination mutualism in alpine Colorado, USA. Secondary nectar robbers feed on nectar through holes in floral tissue created by other organisms. Many secondary robber species are conditional exploiters, acting as pollinators in the absence of robber holes. We quantified foraging efficiency in two bumble bee species, *Bombus appositus* (which always pollinates) and *B. flavifrons* (which switches between pollination and secondary robbing) on a shared nectar resource, *Corydalis caseana* (Fumariaceae). Using a response surface experimental design, we tested the prediction that foraging efficiency would decrease in response to increased intra- and interspecific competitor forager density. Results generally supported these predictions; per-flower foraging efficiency decreased in response to increased intra- and interspecific density. In both species, intraspecific

competition was stronger than interspecific competition, suggesting that these species are able to coexist. Finally, *B. flavifrons* foraged more efficiently as a pollinator than as a secondary nectar robber. Taken together, our results indicate that coexistence of mutualists and conditional exploiters in this system may be mediated by the costs of being an exploiter.

Introduction

Mutualistic interactions present conceptual challenges. The reciprocal exchange between individuals has been believed to drive positive density-dependent population dynamics that are ultimately destabilizing (May 1973, Johnson and Amarasekare 2013, Lee 2015). Yet mutualisms are in fact exceedingly common and so a major research goal has been to identify mechanisms that might stabilize them ecologically. Two common mechanisms studied that help stabilize mutualisms are intraguild competition and third-party exploitation. In the majority of mutualisms, individuals interact with a guild of partner species (species that provide the same type of benefit) simultaneously or over the course of their lifetime (Stanton 2003, Palmer et al. 2010). Assuming those benefits are a limiting resource, it follows that members of a guild should compete for benefits provided by their shared partner. Intraguild competition is commonly invoked as a stabilizing mechanism in mutualisms, because competing individuals are expected to incur a fitness cost large enough to dampen the positive feedback expected in pairwise mutualism (Palmer et al. 2003, Jones et al. 2012, Johnson and Amarasekare 2013). There is evidence for competition for mutualist benefits in several systems, including competition for services provided by cleaner fish and by pollinators (Bell et al. 2005, Adam 2010). Third-party exploitation is another common feature in mutualisms. Third-party exploiters extract mutualistic benefits but never provide any benefits in return (Bronstein 2001). Starting from an assumption that these individuals receive a net fitness benefit, current ecological models of three-species mutualist-mutualist-exploiter systems show that long-term coexistence is possible by periodic dampening of mutualist and

exploiter populations driven by changes in abundance of their shared partner (Yu et al., 2001; Bronstein et al., 2003; Morris et al., 2003; Holland et al., 2013).

The outcome of mutualism is usually highly context-dependent, i.e., the net benefit conferred to individuals can change in response to the biotic or abiotic environment (Thompson 1988, Bronstein 1994, Chamberlain and Holland 2008, Chamberlain et al. 2014). One form of context dependence occurs when certain species, or certain individuals within species, exploit the mutualism under some conditions but not others. These individuals, called conditional exploiters (Bronstein 2001), may be either third-party actors that would otherwise not interact with a focal mutualist, or individuals that would otherwise provide a benefit to a focal mutualist. In the context of intraguild competition in mutualism and its consequences for interaction persistence, conditional exploiters may behave like competing mutualists or third-party exploiters, potentially resulting in unpredictable population dynamics. Depending on the extent to which conditional exploiters cooperate or not, competition with pure mutualists may more closely resemble competition with another pure mutualist or competition with a third-party exploiter. Additionally, the extent to which conditional exploiters cooperate may affect the strength of intraspecific competition for the conditional exploiter species. For example, if exploitation allows individuals to acquire benefits faster or in a greater quantity, intraspecific competition among conditional exploiters should be strong. Conversely, if exploiters and mutualists are able to partition benefits because of how they are acquired, intraspecific competition between conditional exploiters should be weak. Changes in the relative strengths of intra- and interspecific competition will have consequences for the coexistence of pure mutualists and conditional exploiters, and thus,

will have consequences for the stability of the overall interaction. The goal of this study was to evaluate the strength of competition between a pure mutualist and conditional exploiter in order to make inferences about the ecological stability of mutualism in the presence of conditional exploitation.

The best-documented example of conditional exploitation is secondary nectar robbing in pollination mutualisms. Nectar robbers are individuals that consume floral nectar from the outside of the flower through a hole that has been cut through the floral tissue (usually in flowers with tubular corollas or nectar spurs; Irwin and Maloof 2002), rather than through the floral opening (Inouye 1980). Generally, nectar robbers fail to contact floral reproductive structures while foraging (Irwin et al. 2010). Primary robbers cut holes in flowers themselves, whereas secondary robbers consume nectar through holes created by primary robbers (Inouye 1980). Nectar robbing can reduce plant reproductive success by a number of mechanisms, including causing pollinators to avoid flowers that have been robbed (Irwin et al. 2010). While considerably less investigated, secondary robbing can also reduce plant reproduction, probably by causing pollinators to avoid the flower (Richman et al. 2017a). In addition, many secondary robber species cannot create holes in flowers, and will otherwise act as pollinators (i.e., forage from the floral opening) until the actions of primary robbers permit a switch from pollination to secondary robbing (Richman et al. 2017b). This interaction forms an ideal model system to examine competition between mutualists (visitors that cannot rob) and conditional exploiters (visitors that can switch between robbing and visiting flowers legitimately in the same or different floral visits).

In this study, we quantified the strength of intra- and interspecific competition in a plant-pollinator-secondary robber system in which a pure mutualist species (which only forages from the floral opening, here called a pollinator) and a conditional exploiter species (which switches between pollination and secondary robbing) compete for floral nectar. First, we measured foraging efficiency of two bumble bee (*Bombus*) species that were allowed to forage among different densities of conspecific and heterospecific competitors, examining how changes in competitor abundance affected foraging patterns expected if the bees were foraging optimally. We predicted that increased competitor abundance should drive a decrease in foraging efficiency. Assuming that foraging efficiency is a proxy for competitive ability, we predicted that the more efficient nectar forager species should be the superior competitor for nectar (Thomson 2004). Second, we estimated competition coefficients using a two-species competition model which we parameterized using field-collected data. We used the coefficient estimates to compare the strengths of intra- and interspecific competition in both species and ask whether the conditions for two-species coexistence are met, allowing us to make inferences about the stability of the multispecies interaction. Third, we asked whether conditional exploiters have higher foraging efficiency when acting as secondary robbers compared to when acting as pollinators. We predicted that secondary robbing would provide a higher per-flower payoff, assuming that the decision to exploit is based on weighing the benefits versus costs of doing so. We also ask whether the propensity of bees to act as secondary robbers is influenced by the availability of robbing holes.

Materials and Methods

Study site and species

We conducted fieldwork in 2015 and 2016 from late June to early August at Kebler Pass, an alpine meadow site approximately 16 km from the Rocky Mountain Biological Laboratory (RMBL) in Crested Butte, CO, USA (coordinates: 38.86577, -107.10608 W; elevation: 3114m). Meadows were dominated by *Corydalis caseana* (Fumariaceae), a long-lived perennial, self-compatible herbaceous plant that grows 1-2m tall and produces 10-20 flowering stalks (inflorescences), each containing 50-100 zygomorphic flowers with nectar spurs (Malooof 2000). Flowers remain open for up to 8 days, although corollas become noticeably withered by the fourth day (Malooof 2000). Flowers can accumulate up to 2 μ L of nectar (35-40% sugar concentration) over a 24-hour period, and contain an average of 0.5 μ L of nectar (Pleasant 1981, Zimmerman 1988, Malooof 2000). The main floral visitors in the Colorado Rockies are bumble bees (*Bombus* spp.), and hummingbirds occasionally visit flowers (Malooof 2000). *Bombus appositus* and *B. flavifrons* visit *Corydalis* flowers for nectar and pollen. *Bombus appositus* is a long-tongued species that always forages from the floral opening and is known to be an effective pollinator. *Bombus flavifrons* has a shorter tongue than *B. appositus*, and sometimes acts as a pollinator and sometimes consumes floral nectar by secondary robbing. Neither species possesses toothed mandibles, and therefore do not act as primary robbers.

Response surface experimental design

We evaluated foraging efficiency of *B. appositus* and *B. flavifrons* in response to conspecific and heterospecific abundance experimentally using a response surface design. An alternative to additive or substitution designs, a response surface design allows effects of total and relative abundance to be tested by varying the density of each species across a number of treatment combinations (Inouye 2001). We conducted foraging efficiency trials in which *B. appositus* and *B. flavifrons* were allowed to forage for *Corydalis* floral rewards (nectar and pollen) in the presence of conspecific and heterospecific individuals in a controlled field setting. Using a 3m x 3m x 2.5m portable field cage (E-Z Up Sierra II, E-Z Up, Norco, CA, USA), we designated a defined area where bees were allowed to forage in a given trial. In each trial, we varied the number of individuals per species to be 0, 1, 3, or 5, giving 15 total density combinations (Figure D.1). Thus, our design allowed us to assess the effects of intraspecific competition on foraging efficiency when the density of the competitor species = 0 (Figure D.1). We conducted 5 replicate foraging trials for each density combination (120 bouts across 75 trials). However, we only analyzed bouts where bees foraged only for nectar (94 bouts across 67 trials), as we found that individuals that foraged for pollen spent a significantly longer time foraging per flower than individuals that foraged for nectar ($F_{2,103} = 30.21$, $p < 0.001$). These bouts biased our data toward longer foraging times for *B. appositus* and *B. flavifrons* that foraged from the floral opening, and were therefore removed.

Field protocol, foraging trials

The day before a trial, we haphazardly selected a *Corydalis* individual and enclosed all of its flowering stalks in drawstring organza bags to exclude floral visitors. Between 0900 and 1000 on the day of a trial, we captured at least 10 *B. appositus* and 10 *B. flavifrons* workers seen foraging locally on *Corydalis* flowers and placed them on ice. After bees were collected, we placed the field cage over the bagged plant and removed bags from the stalks. We labeled each stalk with a numbered tag. We estimated the percentage of primary-robbed flowers per plant by counting the number of flower per stalk with 1, 2, 3, and 4+ robbing holes, divided by the total number of flowers per stalk, averaged across 3 stalks. Holes in flowers were pre-existing, likely made by the primary robber species *B. occidentalis* or *B. mixtus*; neither species used in the experiment can make holes in flowers. We multiplied the average number of flowers per stalk by the total number of stalks per plant to estimate the total number of flowers per plant.

We then removed bees from the ice and placed them on flowering stalks evenly spaced across the plant. The number of individuals per species corresponded with the density combination we were testing, which was randomly assigned each day. We allowed all bees to warm up on the flowering stalks and complete a foraging bout. Typical behavior inside the field cage consisted of bees contracting their flight muscles to generate heat (i.e., shivering behavior) before lifting off from the flowering stalk, and hovering before beginning to probe and feed from flowers, either on the stalk they had lifted off from or after flying to a new stalk. We defined a foraging bout as successful when the bee displayed these behaviors, visited at least three flowers, and foraged for a minimum of 30 seconds before flying to the side of the cage, signifying a departure from

the plant. In each trial, an observer was assigned a single focal individual per species to observe over its entire foraging bout. The assigned bee was selected by choosing the individual closest to the observer. After the first three floral visits, we recorded each subsequent visit using a digital voice recorder, noting the number of floral visits, number of floral stalks visited, and reward collected (pollen or nectar) for individuals both species, plus foraging tactic (pollination or secondary robbing) for *B. flavifrons* individuals. All individuals stayed constant to a tactic within a foraging bout. For all visits, we used a stopwatch to record the amount of time an individual consumed floral rewards and the amount of time it spent probing flowers and flying between flowers. *Corydalis* corollas are translucent; therefore, it was possible to see when a bee inserts its glossa into floral nectar or begins collecting pollen. We considered “consumption” to begin when a bee inserted its glossa into a flower, and to end when the glossa was withdrawn. We consider “non-consumption activities” to include when a bee flew between flowers, landed on and initially probed a flower, or exhibited another behavior such as grooming. After bees completed their foraging bouts, we re-captured them and measured radial cell length (mm) of the left forewing (to the nearest 0.01 mm) using digital calipers as a proxy for body size (Harder 1982, Cariveau et al. 2016). Finally, to ensure that we did not re-use bees in a subsequent trial, we marked them on the thorax with indelible ink before releasing them outside of the cage. We completed 2-4 trials each day.

Statistical analyses

To compare foraging performance of each species across the response surface, we analyzed the following four response variables: (1) Per-flower payoff, calculated as average reward consumption time per flower [s]/average non-consumption foraging time per flower [s]. (2) Per-stalk payoff, calculated as average nectar consumption time per stalk [s]/average non-consumption foraging time per stalk [s] (3) Number of visits per stalk, calculated as number of floral visits/number of stalks visited, and (4) Total foraging time per stalk, calculated as total bout length (s)/number of stalks visited. All variables were log-transformed to improve normality. For each response variable, we first fit a linear mixed-effects model, with interspecific density and intraspecific density as crossed fixed effects and year as a random effect. The amount of variance explained by year was not significantly different from zero in any of the models, and was removed from all analyses. Foraging tactic was included as a third fixed effect in the *B. flavifrons* models. Possible covariates included the percentage of primary robbed flowers, forager body size, number of stalks per plant, and number of flowers per plant; we chose the best fit model based on AIC criterion, using the model with the fewest variables when competing model AICs differed by < 2 . In all models, we tested the significance of discrete variables (when applicable) using an F Test. Because the response surface experimental design sacrifices replication across density treatments for a higher number of density combinations, the design does not lend itself to classical ANOVA approaches that test the presence or absence of an effect (Inouye 2001). We evaluated results of the models using effect sizes and their standard errors. However, we do include F and p-values for model main effects in Table 1.

To test the assumption that foragers experienced diminishing returns as the length of their foraging bout increased, we then analyzed per-flower payoff (calculated as described above and log-transformed to improve normality) in response to total bout length using a linear model. We originally performed a linear mixed-effects model with year as a random effect. However, the amount of the variance explained by year was not significantly different from zero; therefore, it was removed from the final model.

To test whether secondary robbing in *B. flavifrons* was correlated with increased intra- or interspecific density, we defined foraging tactic (pollination or secondary robbing) as a binary response variable and performed a generalized linear model (binomial error distribution), with the number of *B. appositus* and *B. flavifrons* as crossed fixed effects. We included the percentage of primary robbed flowers as a covariate. Body size and the number of flowers and stalks per plant were originally added as covariates, but were excluded from the final models after comparing AICs of all competing models. Significance of individual model terms was determined using a Wald test.

To evaluate the outcome of competition, we fit the following functions describing nectar acquisition (f) as a function of the abundance of *B. appositus* (N_a) and *B. flavifrons* (N_f) to data on foraging performance (calculated as per-flower payoff); i.e.,

$$f_a = c_a(1 - \alpha_{aa}N_a - \alpha_{af}N_f) \quad (1a)$$

$$f_f = c_f(1 - \alpha_{ff}N_f - \alpha_{fa}N_a) \quad (1b)$$

where c and α parameters quantify the maximum foraging performance and competitive coefficients, respectively. Subscripts denote species ($a = B. appositus$; $f = B. flavifrons$). Parameters c and α were fit via a nonlinear least-squares estimation. See Table D.2 for

parameter estimates. All analyses were performed using R version 3.3.1 (R Core Team 2016).

Results

Foraging performance of pure mutualists

The pure mutualist species, *B. appositus*, experienced a decrease in per-flower payoff (ratio of consumptive time to non-consumptive time) in response to increased intra- and interspecific density; the effect of intraspecific density was larger than the effect of interspecific density (Figure D.2A, Table D.1). When payoffs were calculated at the per-stalk level, individuals experienced a reduction in payoff in response to increased intraspecific density, but an increase in payoff in response to increased interspecific density (Figure D.2B, Table D.1). Visits per stalk and time per stalk (*s*) both increased in response to intra- and interspecific density, although only effect sizes of interspecific density differed significantly from zero (Figure D.2C and D.2D, Table D.1).

Foraging performance of conditional exploiters

Bombus flavifrons, the conditional exploiter species, experienced reduced per-flower payoff in response to both increased intra- and interspecific density, with the effect of intraspecific density being stronger (Figure D.3A, Table D.1). Individuals that acted as pollinators showed a significantly higher per-flower payoff than individuals that acted as secondary robbers ($F_{1,47} = 4.367$, $p = 0.042$). Mean \pm SE per-flower payoff (*s*) was 0.116 ± 0.028 and 0.064 ± 0.022 for pollinators and secondary robbers, respectively. Of the 52 *B. flavifrons* individuals included in the analysis, 20 (39.5%) acted as

secondary nectar robbers. When payoffs were calculated at the per-stalk level, increased interspecific density negatively affected payoff, whereas increased intraspecific density positively affected it (Figure D.2B, Table D.1). Pollinating individuals experienced a higher per-stalk payoff than did secondary robbing individuals ($F_{1,47} = 15.667$, $p < 0.001$). Mean \pm SE per-stalk payoff (s) was 0.442 ± 0.066 and 0.174 ± 0.038 for pollinators and secondary robbers, respectively. Individuals visited more flowers per stalk as the density of intra- and interspecific competitors increased; however, individuals spent more time per stalk foraging only in response to increased intraspecific density, and effect sizes of time per stalk did not differ from zero (Figure D.2C and D.2D, Table D.1). There was no statistically significant difference in visits per stalk or time spent per stalk between the two foraging tactics ($F_{1,43} = 0.238$, $p = 0.608$; $F_{1,47} = 1.157$, $p = 0.221$ for visits per stalk and time per stalk, respectively). Mean \pm SE visits per stalk was 7.391 ± 1.095 and 4.519 ± 0.763 , mean \pm SE time per stalk was 62.545 ± 11.732 and 44.446 ± 4.976 for pollination and secondary robbing, respectively. The percentage of flowers with primary robbing holes did not influence the number of visits made per stalk ($F_{1,43} = 0.119$, $p = 0.732$); however, body size was positively correlated with the number of visits per stalk ($F_{1,43} = 4.414$, $p = 0.042$).

Quantification of competition coefficients

All model coefficients calculated from the experimental data are shown in Table D.2. For each species, the intraspecific competition coefficient was larger than the interspecific competition coefficient, suggesting that pure mutualists and conditional

exploiters are able to coexist at ratios of abundance similar to those used in the experiment.

Diminishing payoff returns and probability of conditional exploitation

Per-flower payoff decreased in response to increased foraging time ($F_{1,92} = 112.01$, $p < 0.001$), with an adjusted R^2 of 0.54. Bees were not more likely to adopt a secondary robbing tactic in response to intra- or interspecific density, or in response to the proportion of flowers per plant that were primary-robbed ($\chi^2_1 = 0.236$, $p = 0.627$; $\chi^2_1 = 0.021$, $p = 0.884$; $\chi^2_1 = 2.588$, $p = 0.101$ for intraspecific density, interspecific density, and proportion of primary robbed flowers, respectively). The interaction between intra- and interspecific density was not statistically significant ($\chi^2_1 = 0.610$, $p = 0.435$).

Discussion

Competition for benefits and coexistence between competing mutualists is commonly invoked as an ecological stabilizing mechanism in multispecies mutualisms (Jones et al. 2012). However, whether conditional exploitation would alter predictions about the outcome of competition for mutualistic benefits is unknown. We evaluated the foraging efficiency of two pollinator species, one of which is a conditional exploiter (sometimes acting as a secondary nectar robber), asking whether increasing conspecific and heterospecific forager density affected the cost-to-benefit foraging payoff ratio for focal individuals. We identified a decrease in performance in response to increased conspecific and heterospecific density in both bee species, although the signature was stronger for the conditional exploiter than for the pure mutualist. When we compared

individuals of the conditional exploiter species that used mutualistic vs. exploitative behaviors, individuals that adopted the mutualistic tactic consistently performed better in three of our four response variables. In both species, the strength of intraspecific competition exceeded the strength of interspecific competition, suggesting that they are able to coexist.

Conditional exploitation and the outcome of competition

The low per-flower payoff obtained by secondary robbers may explain the outcome of our competition models, which showed that the relative strengths of intra- and interspecific competition in *B. appositus* and *B. flavifrons* allows the species to coexist. In our study, *B. flavifrons* individuals that acted as pollinators (mutualists) performed better than those that acted as secondary robbers (exploiters), indicating that secondary robbers did not gain a competitive advantage by switching tactics. Theory predicts that a mutualism in which two species compete for benefits from a shared partner should persist if the superior competitor species provides the higher benefit to that partner (Jones et al. 2009, Afkhami et al. 2014). Our results are consistent with this expectation. However, the reduced payoff of secondary robbing suggests that the incentive to switch tactics should be low. Interestingly, 40% of *B. flavifrons* tested used a secondary robbing tactic. Why individuals secondary-rob even though it is a relatively more costly tactic remains a mystery.

Coexistence between pure mutualists and conditional exploiters might also be explained by other factors that reduce the strength of interspecific competition relative to intraspecific competition. For example, pollinators have been shown to partition floral

resources by corolla length, with long-tongued species visiting plant species with deeper floral corollas and short-tongued species visiting plant species with shallower floral corollas (Alanära et al. 2006). For *B. appositus* and *B. flavifrons* foraging on *Corydalis*, there may be interspecific partitioning of corolla length within a single plant species. Interestingly, primary nectar robbers themselves generate the conditions for more competition for long-corolla flowers by creating robber holes, as this is what makes secondary robbing possible. However, the benefit of switching strategies must still exceed the cost, and it may not be efficient to forage from a flower previously visited by a primary robber (Bronstein et al. 2017).

The role of competition in observed changes in foraging patterns.

Consistent with our predictions, we observed a reduction in per-flower payoff (ratio of consumption time to non-consumption time) in response to increased forager density. However, this pattern was not consistent at the stalk (inflorescence) level. Both species experienced increased per-stalk energetic payoff in the presence of *B. flavifrons* only. For conspecifics, this pattern may be due to social transmission of foraging behavior or information about resource quality. Although conspecifics are competing for floral resources, they also may benefit from watching other individuals forage. Benefits of social information have been documented in another bumble bee species, *B. terrestris*, in a laboratory setting, where per-flower profitability was maximized under low conspecific density, as opposed to high conspecific density or when bees foraged alone (Geslin et al. 2014). While the extent to which social transmission occurs across species

boundaries is less well known, there is evidence to suggest that it does occur in some bees (Dawson and Chittka 2012).

Generally, bees foraged less efficiently in the presence of competitors. This is consistent with our expectations, and has been seen in other systems (e.g., Makino and Sakai 2005, Balfour et al. 2015). We predicted that bees would increase time and visits per stalk as competition for nectar increased. This prediction assumes that bees avoid foraging on the same stalk at the same time as a competitor. Generally, bees did do this. If individuals flew to a stalk where another bee was present, they usually moved to a new stalk quickly, or avoided it altogether. These observations are anecdotal; we were not able to quantify the probability of avoiding a stalk due to competitor presence. Similarly, although we observed occasional interference between individuals, sometimes leading a bee to end its foraging bout (e.g., to immediately fly to the side of the cage), we did not quantify this phenomenon. Interference competition is poorly studied in pollination systems, although it has been observed in stingless bees (Dworschak and Blüthgen 2010) and in interactions between pollinators and nectar thieves (other exploiters of mutualism, Hanna et al. 2014).

Factors affecting the strength of competition

Overall, intra- and interspecific competition reduced *B. appositus* and *B. flavifrons* foraging performance. However, the biological significance of the observed competition coefficients is not entirely clear. While effect sizes were generally small, and some did not differ significantly from zero, they may be strong enough to alter population dynamics. Future work simulating population dynamics based upon the measured

competition coefficients will shed light on their biological significance. Conservatively assuming the measured competition coefficients reflect a weak biological effect on one or both species, this could be due to a number of factors. First, we cannot reject the possibility that floral resource (nectar) availability was not limiting enough in our experiment to lead to strong competition. Preliminary evidence shows that the proportion of nectar removed per trial increased with overall bee density and was often 1 or close to 1 at high forager density (data not shown). However, nectar production per flower is highly variable in *Corydalis* (Heiling et al. 2018), and the variability may have influenced our nectar measurements. It is also possible that, even if bees had removed all or almost all available nectar per plant, they would have had to forage for a longer amount of time than we observed them to begin to experience the effects of competition. Second, we observed a great deal of individual variation in foraging performance, which may have obscured our results. We could not control for individual forager experience prior to a competition trial. Consequently, more experienced foragers may have been able to forage more efficiently than inexperienced foragers, regardless of the competitive environment. In a laboratory setting, experienced *Bombus impatiens* foragers consumed more nectar per unit time than inexperienced competitors (Ohashi et al. 2008).

Another cause of weak competition is that floral resources may not be limiting for *Bombus* populations. Nectar is required mainly for fueling individual flight, and to some extent for larval growth and queen development; however, nutrition from pollen is more important for the latter processes (Sutcliffe and Plowright 1990). Thus, competition for pollen, which was not captured in our study, may be stronger than competition for nectar. Queens also compete intra- and interspecifically for nest sites at the beginning of the

growing season, which may prove to have a larger effect on population dynamics than competition for floral resources (Harder 1986, Strange 2010). There is strong evidence that *Bombus* populations are affected by top-down factors, such as parasites and pathogens, and these top-down factors may play a larger role in limiting population growth (Colla et al. 2006, Goulson et al. 2008). It would be interesting, however, to investigate the combined effects of these factors. For instance, pathogens or parasites may indirectly affect competitive ability, as there is evidence that individuals with high pathogen and parasite loads are weaker foragers (Gegear et al. 2006).

Conclusions

Conditional exploitation has the potential to change the outcome of competition between species that compete for mutualistic benefits, particularly if exploiter individuals are better able to acquire benefits than mutualist individuals and therefore become competitively superior. This has implications for persistence of both mutualistic partners, and thus of the mutualism itself. We have shown here that conditional exploitation between pollinators and conditional secondary nectar robbers in a plant-bumble bee pollination mutualism does allow persistence of both species, likely due to a number of reasons. First, competition for nectar was generally weak, suggesting that other factors may be limiting bee populations. Further work addressing how competition for other resources such as pollen or nest sites affects mutualism persistence will yield predictive insight. Second, pollinators foraged more efficiently than conspecific secondary robbers of the same species. This result leads to the question of why individuals choose a less efficient foraging tactic. It also motivates the question of whether contexts in which

exploitation is more beneficial than cooperation change competitive outcomes, and, by extension, affect mutualism persistence.

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Species	Response Variable	Explanatory Variable	Estimate	Std. Error	F	p
<i>Bombus appositus</i>	Per-flower payoff (s)	(Intercept)	-2.252	0.520		
		Intraspecific density	-0.188	0.147	1.960	0.170
		Interspecific density	-0.147	0.169	0.780	0.382
		Interaction	0.025	0.051	0.240	0.630
	Per-stalk payoff (s)	(Intercept)	-1.022	0.531		
		Intraspecific density	-0.111	0.150	1.740	0.195
		Interspecific density	0.111	0.173	0.990	0.327
		Interaction	-0.008	0.052	0.030	0.874
	Visits per stalk	(Intercept)	1.230	0.345		
		Intraspecific density	0.077	0.097	0.010	0.938
		Interspecific density	0.258	0.112	8.210	0.007
		Interaction	-0.033	0.034	0.960	0.334
	Time per stalk (s)	(Intercept)	3.506	0.359		
		Intraspecific density	0.020	0.101	0.890	0.351
		Interspecific density	0.218	0.117	3.400	0.073
		Interaction	-0.038	0.035	1.150	0.290
<i>Bombus flavifrons</i>	Per-flower payoff (s)	(Intercept)	-1.829	0.466		
		Intraspecific density	-0.244	0.134	4.370	0.042
		Interspecific density	-0.185	0.158	2.050	0.159
		Interaction	0.026	0.048	0.300	0.588
	Per-stalk payoff (s)	(Intercept)	-1.143	0.391		
		Intraspecific density	0.040	0.112	0.110	0.743
		Interspecific density	-0.030	0.132	0.610	0.440
		Interaction	-0.007	0.041	0.030	0.859
	Visits per stalk	(Intercept)	1.204	0.414		
		Intraspecific density	0.289	0.094	10.520	0.002
		Interspecific density	0.152	0.112	0.560	0.460
		Interaction	-0.039	0.034	1.300	0.261
	Time per stalk (s)	(Intercept)	53.037	23.647		
		Intraspecific density	3.037	6.796	1.170	0.285
		Interspecific density	-2.305	7.988	0.010	0.910
		Interaction	0.968	2.451	0.160	0.695

Table D.1. Results of linear models testing the effect of intraspecific density, interspecific density, and their interaction on foraging performance of *Bombus appositus*, the pure mutualist species and *Bombus flavifrons*, the conditional exploiter species. Foraging performance was measured using four response variables, which are listed in the table. Model estimates and standard errors were used to evaluate the effect size of each explanatory variable; F-values and p-values were used to determine statistically significant ($p < 0.05$). Numerator and denominator degrees of freedom are 1, 38 and 1, 47 for *B. appositus* and *B. flavifrons*, respectively.

Parameter	Biological Description	Estimate	Std. Error	p
C_a	Maximum foraging performance of <i>B. appositus</i>	0.134	0.032	< 0.001
C_f	Maximum foraging performance of <i>B. flavifrons</i>	0.168	0.045	< 0.001
α_{aa}	Intraspecific competitive effect of <i>B. appositus</i>	0.081	0.043	0.072
α_{af}	Interspecific competitive effect of <i>B. flavifrons</i> on <i>B. appositus</i>	0.063	0.044	0.159
α_{ff}	Intraspecific competitive effect of <i>B. flavifrons</i> on <i>B. flavifrons</i>	0.114	0.052	0.033
α_{fa}	Interspecific competitive effect of <i>B. appositus</i> on <i>B. flavifrons</i>	0.054	0.045	0.330

Table D.2. Coefficient parameter estimates for a two-species competition model describing the outcome of competition between *Bombus appositus*, the pure mutualist species, and *Bombus flavifrons*, the conditional exploiter species. Estimates were calculated using nonlinear least-squares regressions. Estimates and standard errors for α values represent the strength of intra- or interspecific competition for each species; subscripts denote species ($a = B. appositus, f = B. flavifrons$). Parameter C represents the y-intercept of each regression.

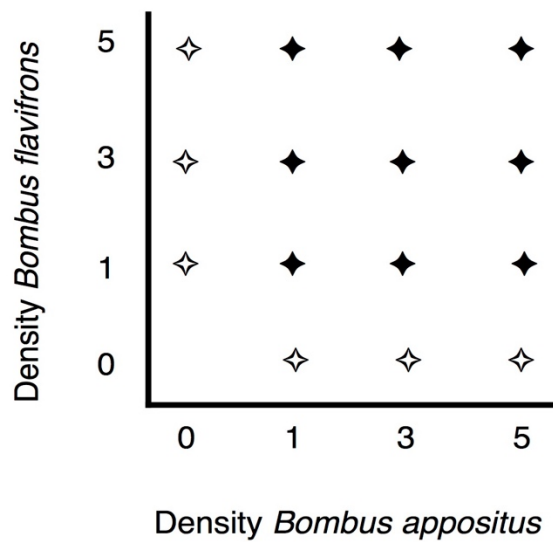


Figure D.1. Diagram of response surface experimental design, used to evaluate competition for nectar between *Bombus appositus*, the pure mutualist species, and *Bombus flavifrons*, the conditional exploiter species. Axes represent the number of each species allowed to forage simultaneously in a given experimental trial. Diamonds represent all possible density combination treatments; total density for any given combination = number *B. appositus* + number *B. flavifrons*. Filled diamonds represent trials testing intra- and interspecific competition (individuals of both species foraging at the same time); open diamonds represent trials testing intraspecific competition (only individuals of the same species foraging at the same time).

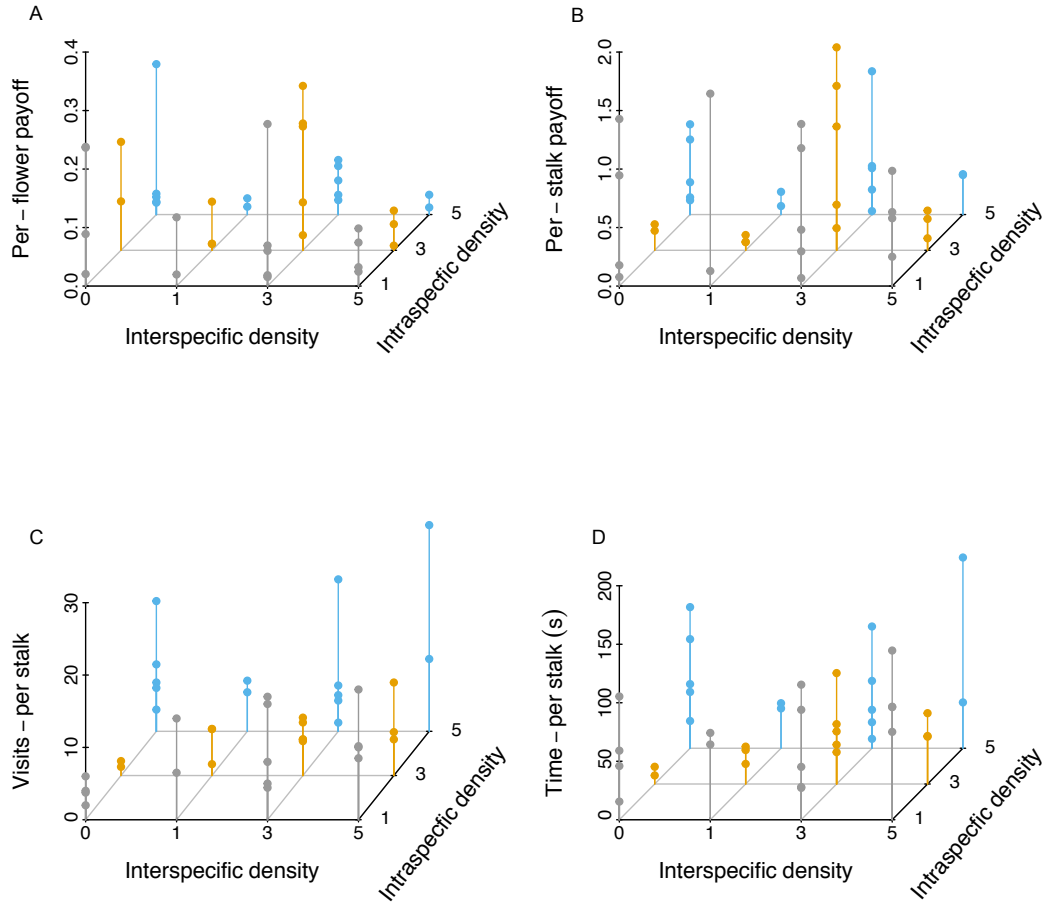


Figure D.2. Individual values of *Bombus appositus* (pure mutualist) foraging performance in response to intra- and interspecific density for all four measured response variables: (A) Per-flower payoff (s), (B) Per-stalk payoff (s), (C) Visits per stalk, (D) Time per stalk (s). Grid represents the response surface; x- and z-axes represent interspecific and intraspecific density, y-axis measures the value of the response variable. Different colored lines and point represent different intraspecific densities (grey = 1, yellow = 3, blue = 5).

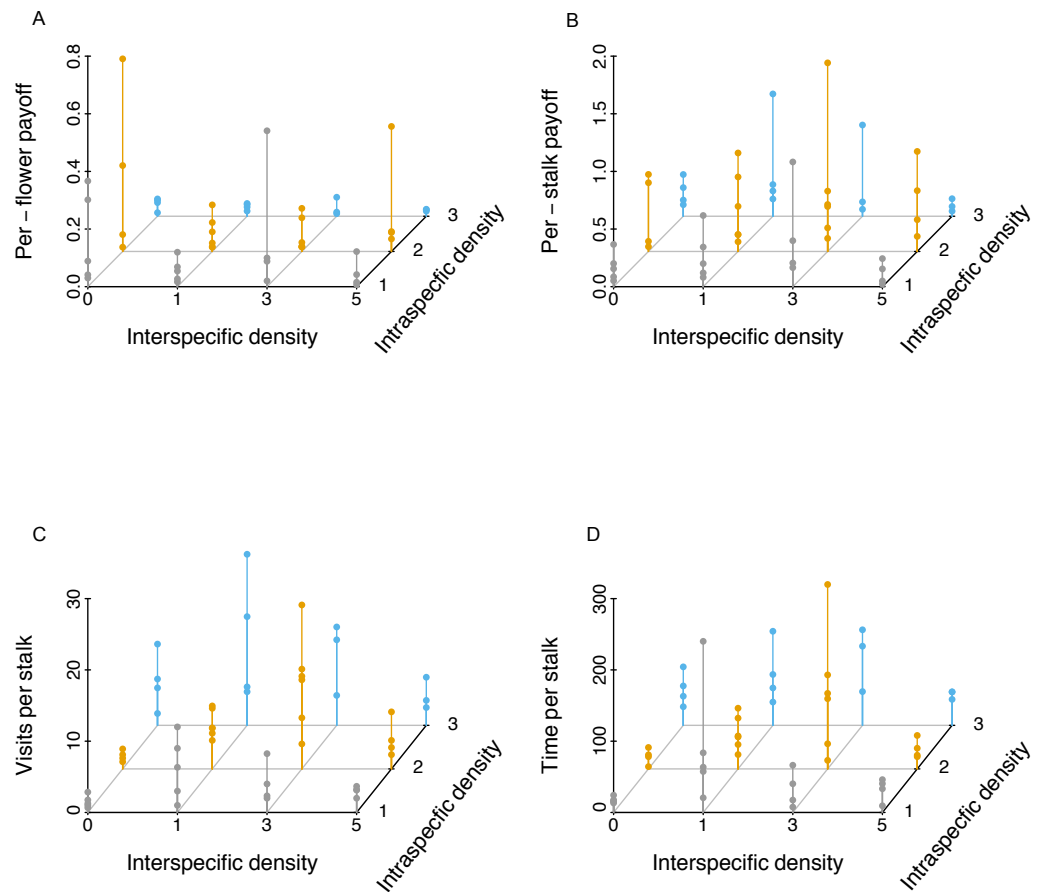


Figure D.3. Individual values of *Bombus flavifrons* (conditional exploiter) foraging performance in response to intra- and interspecific density for all four measured response variables: (A) Per-flower payoff (s), (B) Per-stalk payoff (s), (C) Visits per stalk, (D) Time per stalk (s). Grid represents the response surface; x- and z-axes represent interspecific and intraspecific density, y-axis measures the value of the response variable. Different colored lines and point represent different intraspecific densities (grey = 1, yellow = 3, blue = 5).