



Density-Dependent Effects Of Ants On Selection For Bumble Bee Pollination In *Polemonium Viscosum*

By: Candace Galen and Jennifer C. Geib

Abstract

Mutualisms are commonly exploited by cheater species that usurp rewards without providing reciprocal benefits. Yet most studies of selection between mutualist partners ignore interactions with third species and consequently overlook the impact of cheaters on evolution in the mutualism. Here, we explicitly investigate how the abundance of nectar - thieving ants (cheaters) influences selection in a pollination mutualism between bumble bees and the alpine skypilot, *Polemonium viscosum*. As suggested in past work with this species, bumble bees accounted for most of the seed production ($78\% \pm 6\%$ [mean \pm SE]) in our high tundra study population and, in the absence of ants, exerted strong selection for large flowers. We tested for indirect effects of ant abundance on seed set through bumble bee pollination services (pollen delivery and pollen export) and a direct effect through flower damage. Ants reduced seed set per flower by 20% via flower damage. As ant density increased within experimental patches, the rate of flower damage rose, but pollen delivery and export did not vary significantly, showing that indirect effects of increased cheater abundance on pollinator service are negligible in this system. To address how ants affect selection for plant participation in the pollination mutualism we tested the impact of ant abundance on selection for bumble bee - mediated pollination. Results show that the impact of ants on fitness (seed set) accruing under bumble bee pollination is density dependent in *P. viscosum*. Selection for bumble bee pollination declined with increasing ant abundance in experimental patches, as predicted if cheaters constrain fitness returns of mutualist partner services. We also examined how ant abundance influences selection on flower size, a key component of plant investment in bumble bee pollination. We predicted that direct effects of ants would constrain bumble bee selection for large flowers. However, selection on flower size was significantly positive over a wide range of ant abundance (20–80% of plants visited by ants daily). Although high cheater abundance reduces the fitness returns of bumble bee pollination, it does not completely eliminate selection for bumble bee attraction in *P. viscosum*.

Galen, C. and Geib, J.C. (2007), DENSITY - DEPENDENT EFFECTS OF ANTS ON SELECTION FOR BUMBLE BEE POLLINATION IN POLEMONIUM VISCOSUM. *Ecology*, 88: 1202-1209. <https://doi.org/10.1890/06-1455>. Publisher version of record available at: <https://esajournals.onlinelibrary.wiley.com/doi/10.1890/06-1455>

DENSITY-DEPENDENT EFFECTS OF ANTS ON SELECTION FOR BUMBLE BEE POLLINATION IN *POLEMONIUM VISCOSUM*

CANDACE GALEN¹ AND JENNIFER C. GEIB

Division of Biological Sciences, University of Missouri, Columbia, Missouri 65211-7400 USA

Abstract. Mutualisms are commonly exploited by cheater species that usurp rewards without providing reciprocal benefits. Yet most studies of selection between mutualist partners ignore interactions with third species and consequently overlook the impact of cheaters on evolution in the mutualism. Here, we explicitly investigate how the abundance of nectar-thieving ants (cheaters) influences selection in a pollination mutualism between bumble bees and the alpine skypilot, *Polemonium viscosum*. As suggested in past work with this species, bumble bees accounted for most of the seed production ($78\% \pm 6\%$ [mean \pm SE]) in our high tundra study population and, in the absence of ants, exerted strong selection for large flowers. We tested for indirect effects of ant abundance on seed set through bumble bee pollination services (pollen delivery and pollen export) and a direct effect through flower damage. Ants reduced seed set per flower by 20% via flower damage. As ant density increased within experimental patches, the rate of flower damage rose, but pollen delivery and export did not vary significantly, showing that indirect effects of increased cheater abundance on pollinator service are negligible in this system. To address how ants affect selection for plant participation in the pollination mutualism we tested the impact of ant abundance on selection for bumble bee-mediated pollination. Results show that the impact of ants on fitness (seed set) accruing under bumble bee pollination is density dependent in *P. viscosum*. Selection for bumble bee pollination declined with increasing ant abundance in experimental patches, as predicted if cheaters constrain fitness returns of mutualist partner services. We also examined how ant abundance influences selection on flower size, a key component of plant investment in bumble bee pollination. We predicted that direct effects of ants would constrain bumble bee selection for large flowers. However, selection on flower size was significantly positive over a wide range of ant abundance (20–80% of plants visited by ants daily). Although high cheater abundance reduces the fitness returns of bumble bee pollination, it does not completely eliminate selection for bumble bee attraction in *P. viscosum*.

Key words: alpine skypilot; *Bombus kirbyellus*; bumble bee pollinators; cheaters; density dependence; exploitation; flower size; *Formica neorufibarbus gelida*; mutualism; nectar-thieving ants; *Polemonium viscosum*; selection.

INTRODUCTION

Mutualisms, characterized by the reciprocal exchange of benefits between interspecific partners, are ubiquitous in nature. Theoreticians have long debated the conditions necessary for the evolution and persistence of mutualism. Much of the debate concerns the impact of antagonist species in general and cheaters specifically on the fate of mutualism. High rates of exploitation are thought to drive mutualist populations to extinction and impede the evolution of mutualism (Schwartz and Hoeksema 1998, Bronstein 2001a, b, Holland et al. 2002). Yet, with the exception of a few model systems involving obligate mutualist species (e.g., yucca and yucca moth [Pellmyr et al. 1996]) there is a notable dearth of empiricism that addresses the impact of exploiters on the evolution of mutualism (Hoeksema

and Bruna 2000). For example, surprisingly few studies of mutualism have demonstrated a fitness cost associated with exploiter activity (Bronstein 2001a). Here, we address this gap by exploring the impact of nectar-thieving ants (*Formica neorufibarbus gelida*) that act as cheaters on natural selection in a pollination mutualism between the alpine wildflower, *Polemonium viscosum* and its primary pollinator, bumble bees of the species *Bombus kirbyellus* (Galen 1996b).

If the capacity of cheaters to disrupt reciprocal selection between mutualists is density dependent, then the gap between theoretical predictions that mutualisms should collapse under exploitation and the empirical observation that mutualisms are ubiquitous despite widespread pressure from exploiters may be easily resolved. Under density dependence, mutualisms should have the capacity to tolerate exploiter species at low but not high density (Schwartz and Hoeksema 1998, Holland et al. 2004). This scenario has been explored in obligate mutualisms where density determines the nature of the relationship between the two partner

Manuscript received 3 September 2006; revised 30 October 2006; accepted 1 November 2006. Corresponding Editor: D. H. Feener, Jr.

¹ E-mail: galenc@missouri.edu

species (e.g., Pellmyr et al. 1996), but not in mutualisms or interaction webs where unique species take on mutualist and antagonist roles. Simulations suggest that spatial refugia may play a pivotal role in the persistence of such mutualisms by providing “low exploiter” neighborhoods in which investment in the mutualism is favored and from which mutualists may spread to repopulate cheater-dominated habitats (Bronstein et al. 2003). Yet, data clarifying what is meant by “low” and “high” exploiter density are not known for any system and most studies of cheater impact are limited to presence/absence comparisons (e.g., Rudgers and Strauss 2004, Irwin 2006). In this study, we manipulate the abundance of cheaters experimentally with the aim of characterizing their impact across the range of spatial and temporal variation in density found in natural populations (Galen 1983, 1999).

Few empirical studies have addressed the impact of multiple species interactions on selection or trait evolution (Strauss and Irwin 2004). In pairwise interactions, fitness consequences of antagonistic interactions increase with antagonist density (Stachowicz 2001). If losses inflicted by cheaters on the mutualist species with which they interact increase similarly, then mutualist fitness should decline proportionally to cheater abundance. This “diminishing benefits” hypothesis predicts that the opportunity for selection in a mutualism will decrease as cheaters reach sufficiently high density to constrain one or both partner’s fitness directly. According to this hypothesis, cheaters will restrict the evolutionary advantage of mutualist services and selection on traits rewarding those services by constraining fitness benefits of interacting with the mutualist species.

Cheaters may also disrupt mutualism indirectly by escalating the costs of engagement with partner species relative to benefits of partner service (Bronstein 2001a). Escalating costs arise when cheaters compete with legitimate partners, reducing the supply of goods or rewards for partner service. For example, under nectar robbery, a higher rate (cost) of nectar production may be required to offset losses to cheaters and sustain a pool of legitimate pollinators. This “escalating cost” hypothesis predicts that as cheater density increases, selection for more rewarding phenotypes increases concomitantly, at the expense of greater production costs.

Addressing density dependent effects of cheaters on pollination mutualisms under natural conditions requires knowledge of the contribution of each pollinator species to plant fitness and selection on floral traits. Past research indicates that bumble bees are the most effective pollinators of *P. viscosum* and account for nearly all seed production at high altitudes (Galen 1996a). However, flies and solitary bees also pollinate flowers of *P. viscosum* (Galen and Kevan 1980). Since pollinator abundances can vary widely from year to year (e.g., Price et al. 2005, Irwin 2006), the first objective of our study was to verify the importance of bumble bee pollinators for seed production and selection on floral

traits in our study population. We focus on corolla size, a physiologically and demographically costly trait that is highly correlated with nectar rewards in *P. viscosum* (Cresswell and Galen 1991, Galen 2000). Next, we manipulated ant abundance experimentally to address the following hypotheses:

1) Density dependence. As cheater abundance increases, direct and/or indirect fitness costs imposed by cheaters will increase proportionately.

2) Diminishing benefits. As cheater abundance increases selection on mutualist (bumble bee) service and for floral traits that represent rewards for that service will decline due to cheater driven constraints on fitness.

3) Escalating costs. As cheater abundance increases, selection on floral traits associated with rewards for pollinator service will increase due to competition between cheaters and legitimate pollinators.

MATERIALS AND METHODS

Study system

Experiments reported in this study were conducted in June–July 2004 in a large high tundra meadow at 3700 m altitude spanning the east-facing slope of Pennsylvania Mountain (Park County, Colorado, USA). Pennsylvania Mountain is located near the geographic center of the range for *P. viscosum* and has been the site of a long-term ecological and evolutionary study of the species since 1975.

Plants of *P. viscosum* are long-lived herbaceous perennials with self-incompatible, weakly protandrous flowers. Plants produce about 13 showy blue to purple flowers each and flower for one to two weeks. Individuals depend completely on insects for outcross pollination (Galen and Kevan 1980, Galen and Butchart 2003). *P. viscosum* has two floral scent morphs: sweet and skunky. Skunky-flowered plants are much less attractive to bumble bee pollinators and nectar-thieving ants than are sweet-flowered plants (Galen 1983, Galen and Kevan 1983). Because experiments reported in this paper use the bumble bee–skypilot–ant interaction web to explore the role of cheaters in the evolution of mutualism, they were conducted in a high altitude tundra habitat (~3700 m) where the skunky scent morph is uncommon (Galen 1983). Only sweet-flowered plants were used, and inflorescences were removed from the few skunky-flowered plants in our experimental plots.

Near timberline at its lower range limit, *P. viscosum* is pollinated by a generalized complement of visitors that includes bumble bees, flies and small bees (Galen 1996a). However, the pollination niche of *P. viscosum* becomes increasingly specialized in high alpine tundra populations where queens of a single bumble bee species, *Bombus kirbyellus*, account for nearly all skypilot pollination (Macior 1974, Galen 1996a). Bumble bee queens visit skypilot flowers solely for nectar rewards and not for pollen. Bumble bees are highly efficient pollinators, picking up 44% of the pollen available per

flower of *P. viscosum* and depositing up to 300 pollen grains in each flower of the next plant visited (Galen and Stanton 1989). Because skypilot flowers each contain only 10–15 ovules, one bumble bee visit is enough to saturate seed production per flower. Past surveys suggest that bumble bees at our high tundra study site account for about 75% of pollinator visits and 90% of seed production in *P. viscosum* (Galen 1996a).

Ants of the species *Formica neorufibarbus gelida* also collect nectar from skypilot flowers. *F. n. gelida* nests under rocks and is extremely abundant above timberline in the Colorado Rocky Mountains. At our study site, ant nests are surrounded by flowering *P. viscosum* (average distance from an ant nest to the nearest flowering *P. viscosum* plant is 1.4 ± 0.11 m [$n = 30$ nests; C. Galen, J. J. Rabenold, and A. McKinley, unpublished data]). To access nectar at the base of the skypilot flower, ants often, though not always, detach the style from the ovary. In nature, the total number of flowers damaged per plant per day depends strongly on the number of ants observed on the inflorescence ($R^2 = 0.42$, $F_{1,20} = 14.56$, $P < 0.0011$) but the slope for the relationship between damage and visitation frequency is only 0.3 ± 0.07 , suggesting that several ant visits occur for every flower damaged (C. Galen, J. J. Rabenold, and A. McKinley, unpublished data). Consequently, while odds of ant damage in the population at large vary from 0.1–0.52 yearly (Galen 1983, 1999), each incidence of damage probably reflects at least three ant visits. Plants protected experimentally from ant visits have higher annual seed production than unprotected neighbors (Galen 1999). Ant visitation likely also reduces male fitness since exposure of anthers to ants reduces pollen germinability (Galen and Butchart 2003). Indirect effects of *F. n. gelida* on pollination of *P. viscosum* are less clear. Ants and bumble bees exhibit similar floral preferences, choosing skypilot plants with large, highly rewarding flowers over plants with smaller, less rewarding flowers (Cresswell and Galen 1991, Galen and Cuba 2001). Although these overlapping preferences should promote ant/bumble bee interactions, the small (3–4 mm length) ants of *F. n. gelida* forage singly from skypilot flowers and have limited crop capacity. Aggressive behavior of *F. n. gelida* reportedly deters pollinators from flowers of some Rocky Mountain plant species (e.g., *Frasera speciosa* [Norment 1988]), but ants move quickly away when large queen bumble bees of *B. kirbyellus* alight on skypilot flowers. In choice trials, flowers of control plants (lacking ants) and flowers of ant addition plants (each inflorescence receiving one ant just before presentation to bumble bees) received similar visitation from captive bumble bees ($F_{1,14} = 1.88$, $P > 0.19$; C. Galen, unpublished data).

Verification of bumble bee pollination

In this study, pollen delivery per stigma and pollen export per flower are used as surrogates for bumble bee visitation (see also Irwin 2006). Using components of

pollination in this way assumes that bumble bees are the major pollinators of *P. viscosum* at our study site. To test this assumption, we measured the contribution of bumble bees to seed set in our study population concurrently with the experimental analysis of cheater impacts. Plants in clusters of four located a few meters from each of the 20 patches of *P. viscosum* used in the ant addition experiment (see *Materials and Methods: Impact of cheaters on selection*) were assigned randomly among the following treatments: bumble bee exclusion with natural pollination, bumble bee exclusion with hand-supplemented pollination, control with natural pollination, and control with hand-supplemented pollination. Before the flowers opened, stems of all plants were surrounded with tanglefoot-coated tubes to exclude ants (Galen 1983). Plants excluded from bumble bees (large *B. kirbyellus* queens) were enclosed individually in cages of hardware cloth mesh (1.25×1.25 cm²) that allowed visits from smaller co-pollinators (solitary bees and flies). Pollen supplementation tested for a direct impact of caging on seed production due to shading. Flowers were pollinated by hand on two successive days with pollen collected from different donors daily. We counted the total number of flowers per plant and collected one fully expanded flower for measurement of flower size (corolla surface area). Flowers were pressed, dried, and measured in the laboratory using a CID 202 leaf area meter (CID Instruments, Inc., Camas, Washington, USA). After flowering, plants were protected from elk with taller cages made of Diamond Link polyvinyl fencing (Ben Meadows Company, Janesville, Wisconsin, USA). Despite these efforts, a few individuals were grazed or trampled reducing sample size to 17–19 plants per treatment. Fruits were harvested in September to tally total seed set per plant.

We used mixed-model analysis of covariance (SAS Version 6.12, general linear models procedure [GLM]; SAS Institute 1996) with treatment as a fixed effect, cluster as a random effect, and flower size as the covariate to test the impact of bumble bees on seed set and selection on flower size (the relationship between flower size and seed set).

Impact of cheaters on selection

To determine how the density of *F. neorufibarbus gelida* affects selection for bumble bee pollination in *P. viscosum*, we experimentally varied the abundance of ants in 20 spatially isolated patches of *P. viscosum*. Patches 10×10 m in size were established before the onset of flowering for *P. viscosum*. In each patch, 25 randomly selected sweet-flowered *P. viscosum* were included in the experiment and inflorescences in bud were removed from other skypilot plants within the patch and for 1.0 m beyond it. On average, patches were separated by a minimum of 12.6 m from other patches and plants in each patch were separated from one another by 0.6 ± 0.06 m. Patches were assigned at random among four levels of ant density: five, 10, 15, or

20 ants per patch per day, each placed on flowers of a plant in the ant addition treatment. Plants destined to receive ants were selected at random in each patch and each individual received one new ant daily over the course of flowering (7–10 d). Ants were collected from plants near timberline (3500 m) about 1.5 km away each afternoon, cooled overnight in the refrigerator, transported individually in vials on ice to the field site early the following morning, and inserted into open flowers of designated plants by quickly inverting the vial over the corolla mouth. Ants handled in this manner visit similar numbers of flowers as ants foraging in nature from *P. viscosum* (6.1 ± 0.92 and 5.8 ± 1.1 [mean \pm SE] flowers per plant, respectively) and spend from one to 22 minutes in each flower (Galen and Butchart 2003). No effort was made to keep naturally foraging ants from visiting plants in the ant addition treatment, so the experimental rate of one ant visit per plant per day represents a lower boundary for actual visitation frequency to ant addition plants. We did not measure ant activity on experimental plants because we wished to minimize experimental interference with ant and pollinator visitation. The long corolla tubes of *P. viscosum* flowers obscure ant visibility from afar so that reliable counts of ants in flowers require shaking or twisting the inflorescence stem. Remaining (exclusion) plants in each patch were protected experimentally from ants with tanglefoot. This simulates natural variation in ant density, with plants near nests rapidly discovered and exploited while those further from nests remain spatially isolated from foraging ants (C. Galen, A. McKinley, and J. Rabenold, unpublished data). This design yielded a range of 0.2–0.8 ant visits per plant at least once daily, approximating visitation rates in natural populations of *P. viscosum* (frequency of damage from 0.1 to 0.52, reflecting a visitation rate of 0.3–1.5 ants per plant per day). Plants in experimental patches flowered from 24 June to 14 July 2004.

For each plant, we counted the total number of flowers, collected one flower to measure corolla area, and scored two components of pollinator service: pollen delivery, the amount of outcross pollen received per pistil and pollen export, the fraction of pollen removed from the flower's anthers daily. Past experiments with captive bumble bees have shown that visitation rate is highly correlated with both measures of pollination success (Galen and Stanton 1989). Pollen delivery was scored by collecting the style from a flower that had opened during peak bloom at the onset of wilting, storing the style in 3:1 (ethanol:acetic acid) fixative, and staining it in the laboratory with aniline blue dye. Self-incompatible pollen fails to germinate and is washed from the stigma during staining, leaving germinated outcross (pollinator-transported) grains for visualization under fluorescence microscopy (Galen and Cuba 2001). Pollen export was estimated by marking calyces of two adjacent buds per plant before the onset of flowering. Two of the five total unopened anthers were collected from one flower in bud and two fully dehisced anthers

TABLE 1. Linear regression analysis for direct and indirect effects of ant density on reproductive success in *Polemonium viscosum*.

Effect type	Mode of impact	t †	df	Model F	R^2	r
Direct	flower damage	2.59	1, 19	4.002	0.32	0.28
Indirect	pollen receipt	1.14	1, 19	1.28	0.13	0.07
Indirect	pollen export	0.64	1, 19	0.25	0.03	0.02

Notes: Day of year, included in the regression models to account for phenological variation in insect activity over the course of the experiment, had a marginally significant negative effect on flower damage rate ($r = 0.17$; $t = -1.865$, $P < 0.08$) and nonsignificant effects on pollen receipt and export ($P > 0.47$ for both). Boldface values show effects with $P < 0.05$. Values for r indicate partial correlations of specific effects with ant density.

† For regression coefficient $\beta = 0$.

were collected from the other flower after it had been open for two days. Total pollen was counted for the anthers from each flower using an Elzone model 180PC particle counter (Micrometrics Instrument Corporation, Norcross, Georgia, USA; methods follow Ashman [1998]). The fraction of pollen taken from the anthers per day was estimated as $0.5([\text{pollen per bud} - \text{pollen remaining per flower}]/\text{pollen per bud})$. Temperatures rise over the summer months in temperate alpine habitats, affecting insect activity. Style collection date was recorded to account for the impact of flowering phenology on seed production, pollen delivery, pollen export, and ant damage. As before, all experimental plants were fenced after flowering to prevent elk from grazing the inflorescences. Fruit were collected in September and the total number of seeds counted for each individual.

Regression analysis was used to test for effects of cheater density, scored as the frequency of plants visited per patch by ants daily (range: 0.20–0.80), on the average number of flowers damaged per plant per patch (direct effect on female fitness), average pollen delivered per plant (indirect effect on female fitness), and average pollen exported per plant per patch (indirect effect on male fitness) (Table 1). To control for phenological variation in insect activity over the course of the experiment, average day of year for stigma collection in each patch was included as a covariate in the regression models. Analyses were conducted on patch means for dependent variables, since the 25 individuals in each patch were not deemed statistically independent ($n = 20$ patches, $df = 1, 18$ for all regression analyses). Means for flower damage and pollen delivery were square root transformed prior to analysis to correct for deviations from normality and for heteroschedasticity.

We tested whether ant abundance affects selection for bumble bee pollinator service and large flower size in *P. viscosum* by calculating selection gradients on pollen delivery (β_P) and flower size (β_{FS}) in each patch. β_P was calculated for each patch as the slope of the regression line between relative fitness (based on total seed set per plant) and pollen delivery. β_{FS} was calculated for each

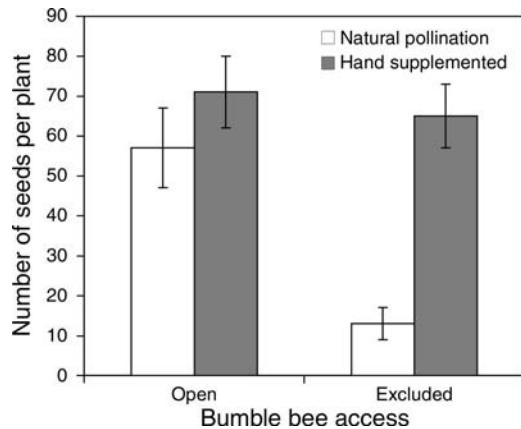


FIG. 1. Seed set per plant for *Polemonium viscosum* in treatment combinations of bumble bee access (open vs. caged from bumble bees) and pollination regime (natural vs. hand supplemented). Pollen supplementation had a significant impact on seed set in the absence of bumble bees ($P < 0.0001$), but not when plants were open to bumble bee visits ($P > 0.3$).

patch as the slope of the regression line between relative fitness and flower size. Here, as elsewhere, patches are the experimental unit since neighboring plants within them were not deemed statistically independent. We subjected patch specific selection gradients to linear regression analysis with the frequency of plants visited per patch by ants daily (cheater density) as the explanatory variable.

RESULTS

Verification of bumble bee pollination

Seed production varied significantly among treatments, but not clusters in the bumble bee exclusion experiment ($F_{3,46} = 3.78$, $P < 0.017$ and $F_{19,46} = 1.25$, $P > 0.27$, respectively). Seed set was strongly pollination limited inside bumble bee exclusion cages, but not outside of cages ($F_{1,46} = 34.3$, $P < 0.0001$ and $F_{1,46} = 2.38$, $P > 0.13$, respectively; Fig. 1). The contribution of bumble bee pollination to fecundity in *P. viscosum* plants in our study area averaged $78 \pm 6\%$, with seed set of naturally pollinated plants increasing approximately fourfold with access to bumble bees (planned contrast, $F_{1,46} = 23.80$, $P < 0.0001$). For plants with supplemented pollination, seed production was comparable inside and outside of cages (planned contrast, $F_{1,46} = 0.34$, $P > 0.56$). The relationship between flower size and seed set varied significantly among treatments (interaction, $F_{1,46} = 2.96$, $P < 0.042$). Only for naturally pollinated plants with flowers open to bumble bees did seed set increase with flower size (regression, $R^2 = 0.34$, slope = 4.1 ± 1.4 , $t = 2.96$, $P < 0.0088$). In all other treatments, flower size had no effect on seed set ($P > 0.59$).

Impact of cheaters on selection

Pollinators delivered an average of 32 ± 3 compatible pollen grains per pistil over a skypilot flower's lifetime

and exported an average of 8.5% of the 33 000 pollen grains produced per flower daily. However, neither pollen delivery nor export declined with cheater density, the frequency of plants visited by ants per patch per day ($P > 0.27$ for both tests, Fig. 3). In contrast, as cheater density increased in experimental patches, the average number of flowers damaged per plant increased concomitantly (for the partial correlation of ant frequency with damage, $r = 0.28$, $P < 0.02$; Fig. 2). Results suggest that ants constrain reproductive fitness in *P. viscosum* through direct effects on flower integrity rather than indirect effects on pollinator service. This trend is supported when averages for seed set, pollen delivery, and pollen export are computed separately for ant-exclusion and ant-addition treatments in each patch and compared statistically. On average, ant addition decreased seed set per flower by 20%, causing a loss of 0.7 ± 0.25 seeds (significant direct effect, paired $t = 2.80$, $P < 0.0115$; $n = 20$ patches), but had little impact on pollinator service (nonsignificant indirect effects on pollinator service; for both pollen receipt and export, $P > 0.53$).

Phenotypic selection analysis showed that as cheater density increases, selection (β_P) on pollen delivery declines significantly ($R^2 = 0.29$, $F_{1,18} = 7.53$, $P < 0.0133$; Fig. 3). This result holds, even after the two high values for β_P at ant frequencies of 0.2 are removed from

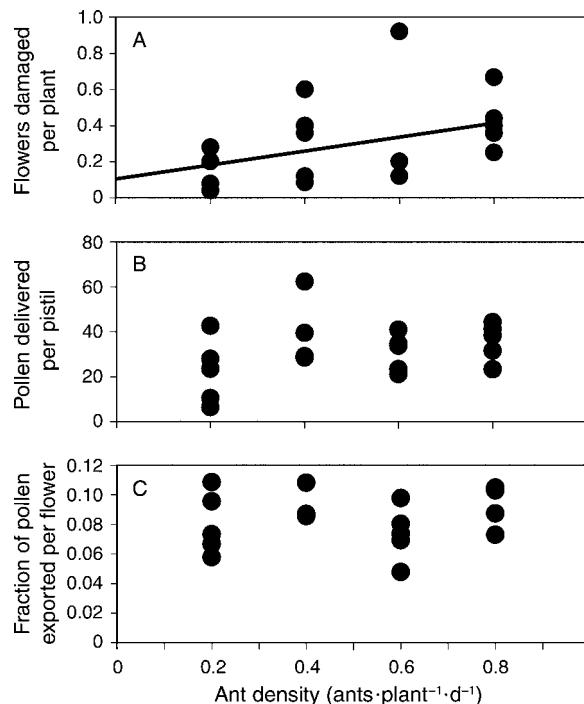


FIG. 2. Impact of ant density on patch averages for (A) the number of flowers damaged per plant, (B) the amount of outcross pollen delivered per pistil, and (C) the fraction of pollen exported daily from the anthers. Ant density had a significant effect on flower damage ($P < 0.02$), but not on pollen delivery or export ($P > 0.27$).

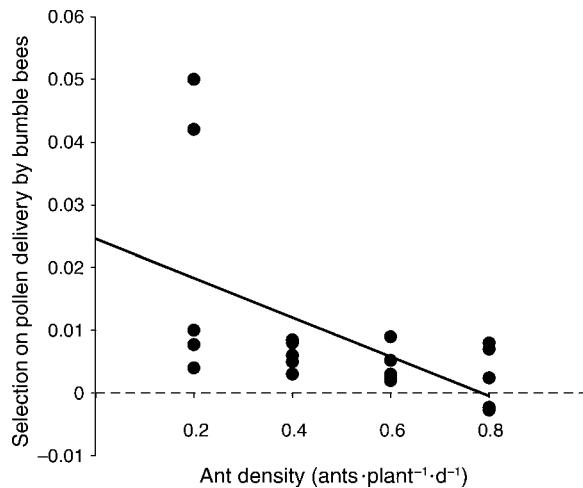


FIG. 3. Impact of ant density on selection for bumble bee-mediated pollen delivery (β_p) in experimental patches of *P. viscosum*. The equation for the best-fit line is $\beta_p = 0.0245 - 0.031(\text{ant density})$ ($R^2 = 0.30$, $P < 0.0133$). The relationship remains significant after removal of the two extreme positive values for β_p at ant density of 0.2. The strength of selection on pollen delivery (β_p) was calculated for each patch as the slope of the regression line between relative fitness (based on total seed set per plant) and pollen delivery.

the analysis ($R^2 = 0.23$, $F_{1,18} = 4.959$, $P < 0.0407$) supporting the diminishing benefits hypothesis. According to the best-fit regression model, $\beta_p = 0.0246 - 0.0312a$, where a represents ant density. The equation predicts that if ants visit more than 79% of the skypilot population, selection for bumble bee service should fall to zero (Fig. 3). Ant abundance had a negative, but nonsignificant impact on selection for large flowers in experimental patches of *P. viscosum* ($R^2 = 0.04$, $F_{1,19} = 0.70$, $P > 0.39$; Fig. 4). While β_{FS} varied in strength and sign among patches, the selection gradient on flower size was positive across replicates, averaging 0.37 ± 0.15 ($n = 20$, $t = 2.38$, $P < 0.028$; Fig. 4).

DISCUSSION

Results of the bumble bee exclusion experiment supported the assumption that compatible pollinator service and pollinator-mediated selection on flower size under experimental manipulation of cheater abundance could be attributed mainly to *Bombus kirbyellus* queens. As expected based on past studies at this tundra field site (Galen 1996a), in the absence of ants, bumble bees accounted for most (78%) of the seeds produced in our study population and were the major source of positive directional selection on flower size (Fig. 1). Addition of cheaters, made the pattern of spatial variation in plant fitness more complex. Ants reduced seed set per flower by 20% on average via damage to pistils. Consequently, as local ant abundance increased in experimental patches, the number of flowers benefiting from pollinator service declined, despite equivalent pollinator visitation (Fig. 2). Results show that direct effects of

cheaters on flower integrity predominate in this system, leading to diminishing benefits of the pollination mutualism at high ant density (Fig. 3). Nonetheless, on average, benefits of bumble bee pollination persist across a wide range of ant density (Fig. 3) and investment in the mutualism is favored (Fig. 4). Results concur with earlier models predicting a limited role for ants in the evolution of floral traits within bumble bee-pollinated populations of *P. viscosum* (Galen and Cuba 2001).

Our results provided little evidence of indirect effects of ants on pollinator behavior. Average pollen delivery and removal rates were similar for plants protected from ants and neighbors to which ants were added on a daily basis. This trend is unchanged even when the analysis is conducted at the level of the individual plant, rather than on patch averages for protected and ant exploited plants (for the impact of cheating on pollen delivery and export, respectively, $P > 0.33$ and $P > 0.84$, $df = 1, 436$). Conversely, the direct impact of ant addition on seed set per flower remains highly significant at the plant level ($P < 0.011$). Results contrast with other studies showing an impact of nectar robbers on pollination success (e.g., Irwin and Brody 2000). Experimental design may have contributed to this discrepancy. Because we added cheaters rather than experimentally simulating removal of floral rewards, the impact of cheating probably varied more broadly within and among patches due to variation in cheater (ant) residence time and nectar consumption in our study. Such variation would increase “error” variance in the magnitude of ant effects on floral attractiveness, reducing our ability to detect ant effects on pollination success if present.

We reasoned that if ants, by usurping nectar rewards, create conditions in which even larger rewards are

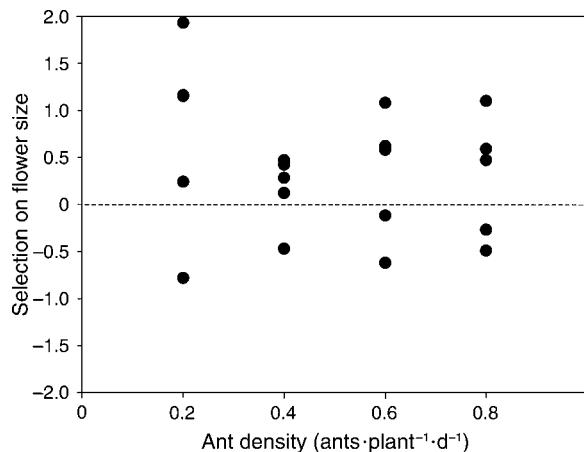


FIG. 4. Relationship between ant density and selection on flower size (β_{FS}) in experimental patches of *P. viscosum*. Ant density had no significant impact on selection for large flowers in this bumble bee-pollinated population ($R^2 = 0.04$, $P > 0.39$). The strength of selection on flower size (β_{FS}) was calculated for each patch as the slope of the regression line between relative fitness (based on total seed set per plant) and corolla surface area.

necessary to sustain bumble bee pollinators, bumble bee mediated selection on flower size should increase with ant density. Our results fail to support this idea and instead show a negligible relationship between selection on flower size and ant density. Positive selection on flower size was the norm in experimental patches of *P. viscosum*, and selection did not vary significantly in strength with the frequency of ant addition (Fig. 4). However, a couple of qualifying remarks may be in order. First, it is possible that our technique for manipulating ant abundance (ant addition) limited nectar consumption by cheaters in this study and decreased our ability to detect indirect effects. As individual foragers, *Formica* ants have very small crop sizes, and likely remove only a small fraction of the nectar reward during a flower visit. Though *Formicine* ants typically forage individually (Carroll and Janzen 1973), to the extent that ants of *F. n. gelida* engage in trail-following behavior, our method of adding individual ants to flowers may have under-represented nectar consumption by *F. n. gelida* in nature. Experiments in progress, comparing nectar standing crop of *P. viscosum* before and after ant visitation, will provide a more detailed view of potential competition between *Formica* ants and bumble bees.

Next, we assumed that bumble bee pollinators were equally abundant across the experimental patches. Spatial variation in bumble bee density could obscure cheater impacts on the pollination mutualism. Pollinator abundance varies spatially over small scales, creating patches of rewarding and empty flowers (Zimmerman and Pyke 1986, 1988). Spatial patchiness in pollinator visitation may reflect territorial foraging, underlying heterogeneity in vegetation structure, nest site limitation or myriad other environmental factors (Herrera 1997, Steffan-Dewenter et al. 2001, Price et al. 2005). Time constraints did not allow us to survey pollinators in the present study; however lack of strong spatial patterning in stigma pollen loads supports the assumption that pollinator density did not vary much among experimental replicates: variation among patches in outcross pollen delivery was weak and non-significant (mixed-model ANOVA, $Z = 1.69$, $P > 0.091$).

Mutualism is favored when members of each partner species provide a cheap commodity to the other and in return receive a resource or service that is more costly if not impossible to otherwise obtain (Schwartz and Hoeksema 1998). Though cheaters are usually thought to weaken mutualism by exacerbating its costs, our results show that they can instead reduce the benefits of interactions with legitimate partners (Bronstein 2001b). Consistent with the diminishing benefits hypothesis, ants visiting skypilot flowers interfered directly with seed production, constraining the fitness impact of bumble bee pollination without reducing bumble bee service. Though average damage rates were low (Fig. 1), ants likely also have less obvious effects on flower integrity (Galen and Butchart 2003). For example, ants frequent-

ly contact the stigma surface in flowers of *P. viscosum*, with potential inhibitory effects on pollen germinability.

Whether cheaters inflate costs or reduce benefits of mutualist service is an important distinction, because compensation for lost benefits is more expensive, if not impossible than compensation for usurped rewards. Our results suggest that like the parasitic cheating insects that exploit mutualisms between figs and fig wasps or yucca and yucca moths, exploitation by ants has no impact on female or male components of pollination success in skypilots, but reduces the likelihood that pollinator service will translate into plant fitness gains (Pellmyr and Huth 1994, Weiblen et al. 2001).

Comparisons of exploited and unexploited (experimentally protected) individuals in a broad array of animal-pollinated plant species have revealed the potential for cheaters to disrupt pollination mutualisms by reducing fitness returns of partner services (e.g., Wyatt 1980, Galen 1999, Irwin and Brody 1999, Ashman and King 2005). However, in natural populations, cheating is not absolute. Instead, some plants always escape exploitation. Our study illustrates how an understanding of the ecological and evolutionary impacts of cheaters on mutualism can gain from manipulating cheater density rather than presence/absence. Bronstein et al. (2003) propose that local variation in cheater abundance, by creating spatial refugia from exploitation, may play a critical role in stabilizing populations of mutualists. Experiments described in this paper support their explicit spatial framework by showing that natural selection for mutualist service depends on local exploiter density.

Theory predicts disruption as a likely outcome of exploitation in mutualism (Schwartz and Hoeksema 1998, Bronstein 2001a, b, Holland et al. 2002). Yet our findings suggest that disruption of selection between mutualist partners by cheaters is not certain, but instead represents a density dependent risk. Results concur with recent models suggesting that specialized pollination mutualisms are remarkably resilient in the face of exploitation, perhaps because services of highly effective pollinators translate into fitness returns over a wide range of exploiter density (Bronstein et al. 2003). In another well-studied facultative mutualism between plant and pollinator, Irwin (2006) also finds that the evolutionary impact of exploiters depends strongly on pollinator effectiveness.

In conclusion, density-dependent effects of cheater species can help explain why, despite the ubiquity of exploitation, mutualisms occur globally in all ecological communities. Density dependence provides a point of consensus between theoretical arguments that cheaters disrupt mutualism and the view that they are instead a tolerable if not stabilizing force.

ACKNOWLEDGMENTS

We thank S. Youngstrom, J. J. Rabenold, B. Goff, D. Cole, and A. J. Dona for help in the field and laboratory; J. Rudgers, R. Cocroft, J. N. Holland, and two anonymous reviewers for

critical comments on the manuscript; and NSF Grant DEB 0316110 for funding.

LITERATURE CITED

- Ashman, T. L. 1998. Is relative pollen production or removal a good predictor of relative male fitness? An experimental exploration with a wild strawberry (*Fragaria virginiana*, Rosaceae). *American Journal of Botany* 85:1166–1171.
- Ashman, T. L., and E. A. King. 2005. Are flower-visiting ants mutualists or antagonists? A study in gynodioecious wild strawberry. *American Journal of Botany* 92:891–895.
- Bronstein, J. L. 2001a. The exploitation of mutualisms. *Ecology Letters* 4:277–287.
- Bronstein, J. L. 2001b. The costs of mutualism. *American Zoologist* 41:127–141.
- Bronstein, J. L., W. G. Wilson, and W. F. Morris. 2003. Ecological dynamics of mutualist/antagonist communities. *American Naturalist* 162:S24–40.
- Carroll, C. R., and D. H. Janzen. 1973. Ecology of foraging by ants. *Annual Review Ecology and Systematics* 4:231–257.
- Cresswell, J. E., and C. Galen. 1991. Frequency-dependent selection and adaptive surfaces for floral character combinations: the pollination of *Polemonium viscosum*. *American Naturalist* 138:1342–1353.
- Galen, C. 1983. The effects of nectar thieving ants on seedset in floral scent morphs of *Polemonium viscosum*. *Oikos* 41:245–249.
- Galen, C. 1996a. The evolution of floral form: insights from an alpine wildflower, *Polemonium viscosum* (Polemoniaceae). Pages 273–291 in D. G. Lloyd and S. C. H. Barrett, editors. *Floral biology*. Chapman and Hall, New York, New York, USA.
- Galen, C. 1996b. Rates of floral evolution: adaptation to bumble bee pollination in an alpine wildflower, *Polemonium viscosum*. *Evolution* 50:120–125.
- Galen, C. 1999. Flowers and enemies: predation by nectar-thieving ants in relation to variation in floral form of an alpine wildflower, *Polemonium viscosum*. *Oikos* 85:426–434.
- Galen, C. 2000. High and dry: drought stress, sex-allocation trade-offs, and selection on flower size in the alpine wildflower *Polemonium viscosum* (Polemoniaceae). *American Naturalist* 156:72–83.
- Galen, C., and B. Butchart. 2003. Ants in your plants: effects of nectar thieves on pollen fertility and seed-siring capacity in the alpine wildflower, *Polemonium viscosum*. *Oikos* 101:521–528.
- Galen, C., and J. Cuba. 2001. Down the tube: pollinators, predators, and the evolution of flower shape in the alpine skypilot, *Polemonium viscosum*. *Evolution* 55:1963–1971.
- Galen, C., and P. G. Kevan. 1980. Scent and color floral polymorphisms and pollination ecology in *Polemonium viscosum* Nutt. *American Midland Naturalist* 104:281–289.
- Galen, C., and P. G. Kevan. 1983. Bumble bee foraging and floral scent dimorphism: *Bombus kirbyellus* Curtis (Hymenoptera: Apidae) and *Polemonium viscosum* Nutt. (Polemoniaceae). *Canadian Journal of Zoology* 61:1207–1213.
- Galen, C., and M. L. Stanton. 1989. Bumble bee pollination and floral morphology: factors influencing pollen dispersal in the alpine sky pilot, *Polemonium viscosum* (Polemoniaceae). *American Journal of Botany* 76:419–426.
- Herrera, C. M. 1997. Thermal biology and foraging responses of insect pollinators to the forest floor irradiance mosaic. *Oikos* 78:601–611.
- Hoeksema, J. D., and E. M. Bruna. 2000. Pursuing the big questions about interspecific mutualism: a review of theoretical approaches. *Oecologia* 125:321–330.
- Holland, J. N., D. L. DeAngelis, and J. L. Bronstein. 2002. Population dynamics and mutualism: functional responses of benefits and costs. *American Naturalist* 159:231–244.
- Holland, J. N., D. L. DeAngelis, and S. T. Schultz. 2004. Evolutionary stability of mutualism: interspecific population regulation as an ESS. *Proceedings of the Royal Society of London B* 271:1807–1814.
- Irwin, R. E. 2006. The consequences of direct versus indirect species interactions to selection on traits: pollination and nectar robbing in *Ipomopsis aggregata*. *American Naturalist* 167:315–329.
- Irwin, R. E., and A. K. Brody. 1999. Nectar-robbing bumble bees reduce the fitness of *Ipomopsis aggregata* (Polemoniaceae). *Ecology* 80:1703–1712.
- Irwin, R. E., and A. K. Brody. 2000. Consequences of nectar robbing for realized male function in a hummingbird-pollinated plant. *Ecology* 81:2637–2643.
- Macior, L. M. 1974. Pollination ecology of the Front Range of the Colorado Rocky Mountains. *Melandria* 15:1–59.
- Norment, C. J. 1988. The effect of nectar-thieving ants on the reproductive success of *Frasera speciosa* (Gentianaceae). *American Midland Naturalist* 120:331–336.
- Pellmyr, O., and C. J. Huth. 1994. Evolutionary stability of mutualism between yuccas and yucca moths. *Nature* 372: 257–260.
- Pellmyr, O., J. N. Thompson, J. Brown, and R. G. Harrison. 1996. Evolution of pollination and mutualism in the yucca moth lineage. *American Naturalist* 148:827–847.
- Price, M. V., N. M. Waser, R. E. Irwin, D. R. Campbell, and A. K. Brody. 2005. Temporal and spatial variation in pollination of a montane herb: a seven-year study. *Ecology* 80:2106–2116.
- Rudgers, J. A., and S. Y. Strauss. 2004. A selection mosaic in the facultative mutualism between ants and wild cotton. *Proceedings of the Royal Society of London Biological Sciences* 271:2481–2488.
- SAS Institute. 1996. SAS/STAT software: changes and enhancements through Release 6.11. SAS Institute, Cary, North Carolina, USA.
- Schwartz, M. W., and J. D. Hoeksema. 1998. Specialization and resource trade: biological markets as models of mutualisms. *Ecology* 79:1029–1038.
- Stachowicz, J. J. 2001. Mutualism, facilitation, and the structure of ecological communities. *BioScience* 51:235–246.
- Steffan-Dewenter, I., U. Munzenber, and T. Tschardtke. 2001. Pollination, seed set and seed predation on a landscape scale. *Proceedings of the Royal Society of London B* 268:1685–1690.
- Strauss, S. Y., and R. E. Irwin. 2004. Ecological and evolutionary consequences of multispecies plant–animal interactions. *Annual Review of Ecology, Evolution, and Systematics* 35:435–466.
- Weiblen, G. D., G. W. Yu, and S. A. West. 2001. Pollination and parasitism in functionally dioecious figs. *Proceedings of the Royal Society of London B*. 268:651–659.
- Wyatt, R. 1980. The impact of nectar-robbing ants on the pollination system of *Asclepias curassavica*. *Bulletin of the Torrey Botanical Club* 107:313–321.
- Zimmerman, M., and G. H. Pyke. 1986. Reproduction in *Polemonium*: patterns and implications of floral nectar production and standing crops. *American Journal of Botany* 73:1405–1415.
- Zimmerman, M., and G. H. Pyke. 1988. Pollination ecology of Christmas bells (*Blandfordia nobilis*): patterns of standing crop of nectar. *Australian Journal of Ecology* 13:301–309.