

The Roles of Ants as Flower Visitors: Experimental Analysis in Three Alpine Plant Species

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## The roles of ants as flower visitors: experimental analysis in three alpine plant species

Mary N. Puterbaugh

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I investigated three co-occurring North American alpine plant species for which ants comprise at least 20% of the flower visitors. Ants of the species *Formica neorufibarbis gelida* pollinate gynodioecious *Paronychia pulvinata*, are herbivores of the gynodioecious *Eritrichum aretioides* and appear to have little effect on the hermaphroditic *Oreoxis alpina*. Given that floral nectar and lipids are important resources for alpine ants, the effect of all three plant species on the ants is positive. In *E. aretioides*, ants chew on the coronal ring of flowers but are unlikely to contact the stigmas, explaining their negative effects on seed production. In ant-pollinated *P. pulvinata*, ants affect pollination in females more than in hermaphrodites. Single ant visits to unpollinated pistillate flowers show that ants are inefficient pollinators, transferring no pollen (viable or inviable) to stigmas 55% of the time. This result is probably explained by low pollen loads and infrequent movement between plants. In contrast, single ant visits to previously unpollinated perfect flowers on hermaphrodites produce seed-set similar to that in flowers open to pollination. In *O. alpina*, the lack of an effect of ants on seed production is probably explained by autogamous seed-set and lack of pollen limitation. In both gynodioecious species, *E. aretioides* and *P. pulvinata*, female plants produce more seeds than hermaphrodites in the absence of ants, but this difference is magnified when ants are allowed access to plants, suggesting that ants can influence the evolutionary maintenance of breeding system dimorphism in these species.

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Increasingly, interspecific interactions are receiving attention as important evolutionary factors. Predators and parasites, for example, have an important role in the maintenance of sex in plants (Strauss and Karban 1994) and animals (Hamilton 1980). Biotic interactions have also been implicated in the evolution of plant breeding systems. In *Freyinetia reineckei*, a dioecious liana in Samoa, staminate flowers are more frequently attacked by floral herbivores than are pistillate flowers (Cox 1982). This finding suggests that differential herbivory can favor sexual specialization in this species such that floral herbivores promote the evolution of dioecy (Cox 1982). Mutualistic plant-pollinator interactions can also influence the evolution of plant breeding

systems (Lloyd 1974, Bawa 1980, Bierzychudek 1987, Maurice and Fleming 1995). If female plants receive too little pollen to reproduce successfully, bisexuality (hermaphroditism) can be favored over unisexuality in self-compatible species (Lloyd 1974, Maurice and Fleming 1995). Pollen limitation in female plants is not only a result of the frequency or abundance of pollinators, but also of pollinator behavior. For female plants to reproduce and persist in a population, pollinators must move frequently between individuals to transfer pollen from flowers of males or hermaphrodites to those of females (Bierzychudek 1987, Maurice and Fleming 1995). Additionally, in gynodioecious populations, the relative fitness of hermaphroditic and female plants

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depends on the rate of self-pollination in hermaphrodites and, again, on pollinator behavior. Selfing is enhanced when pollinators move within individual plants, rather than among them (Bawa 1980, Snow et al. 1996).

Flower-visiting ants have received less attention than other insect flower visitors, despite the fact that the relationships between these ants and plants are rich in the diversity of fitness outcomes for plants. Ants frequently visit flowers but rarely pollinate them (Peakall et al. 1991). The orchid *Leporella fimbriata* is specialized to ant pollination: male ants pseudocopulate with flowers (Peakall 1989). However, in most other cases of pollination by ants, the plant species are pollinated by an array of insects that include ants (e.g. Gómez and Zamora 1992, Gómez et al. 1996). In these species, the traits conducive to pollination by ants (flower shape and size) may reflect adaptation to small insect pollinators in general rather than ants specifically. One generalization that has arisen from studies of these cases is that effective ant-pollinators often show high flower visitation frequency (Gómez and Zamora 1992, Gómez et al. 1996). Until recently, however, flower visiting ants have been mostly ignored, leading to the suggestion that more thorough investigations may demonstrate that ants are more commonly pollinators than once thought (Beattie 1991). Hickman (1974) predicted that pollination by ants should be most common in hot and dry habitats because ants are usually very active in these environments and represent the best “buy” in terms of pollinators; ants are attracted to nectar-producing flowers, but require less energy than flying insects (Hickman 1974). More recent studies have documented that nectar can be an important water source to ants in deserts (Ruffner and Clark 1986) and dry tropical forests (Rico-Gray 1989), also suggesting that ant visitation and pollination might be more frequent in dry habitats. In fact, most known cases of pollination by ants involve plant species of dry environments such as deserts and arid montane, sandy, and rocky regions (Hickman 1974 and references therein, Wyatt 1981, Svensson 1985, Gómez and Zamora 1992, Gómez et al. 1996), supporting Hickman (1974) view. Seven of nine unrelated plant species studied in Mediterranean high mountains and arid lands are ant-pollinated (Gómez and Zamora 1992, García et al. 1995, Gómez et al. 1996). Petersen (1977) suggested that ant pollination might have comparable importance in North American alpine ecosystems, but he provided no experimental evidence for ant pollination in such environments. The few experimental studies that have been undertaken in the North American alpine indicate that flower-visiting ants actually represent predators, rather than pollinators (Galen 1983). One goal of the research reported in this paper is to assess experimentally whether North American alpine ants have positive or negative effects on the species they visit. A second goal

is to describe further the role of ants as floral visitors in alpine ecosystems worldwide. Clarifying the nature of such interactions is important for understanding community structure, especially when the taxa involved are as ubiquitous and abundant as ants (Hölldobler and Wilson 1990).

In this study, I document the pollination effectiveness of ant visitors to flowers of three alpine plant species that grow in central Colorado. I reasoned that, by studying several species, underlying determinants of interactions between ants as flower visitors and their associated plants would be revealed. Specifically, I consider how floral morphology and breeding system influence whether flower-visiting ants have positive, negative, or neutral effects on the plant species. Similarly, I consider how ant behavior also affects the nature of the interaction, and I ascertain whether within-species variation exists in the strength or direction of ant/plant interaction by testing for differential effects of ants on male-sterile and male-fertile plant morphs of two gynodioecious species, *Eritrichum aretioides* (Chamisso) de Condolle (Boraginaceae) and *Paronychia pulvinata* Gray (Caryophyllaceae). These data allow me to evaluate the potential for ants to influence gender dimorphism in these two cushion plant species.

The three plant species considered afford a range of breeding systems and are among the plants most frequently visited by ants in the Rocky Mountains (Petersen 1977). Two, *E. aretioides* and *Oreoxis alpina* (Gray) C. & R. (Apiaceae), were described as ant-pollinated by Petersen (1977), based on mostly anecdotal data. The third, *P. pulvinata* is viewed as potentially ant-pollinated (Petersen 1977). Both *E. aretioides* and *P. pulvinata* are gynodioecious: two morphs, female (male-sterile) and hermaphroditic (male-fertile), co-occur in populations of each of these two species. Consequently, the study species provide a range of attributes, such as flower size, shape, and ability to self, that can reveal possible determinants of ant/plant relationships.

## Methods

### Study system

Research reported in this paper was performed in fellfield habitats on Pennsylvania Mountain, in the Park Range of central Colorado, USA (Park Co., CO, 39°15'N, 106°08'W). Fellfields are characterized by rocky soils and high winds that prevent much winter snowpack from accumulating; thus, they are typically very dry throughout the year (Bliss 1985). I studied effects of ants as flower visitors to three co-occurring perennial alpine wildflowers: *P. pulvinata* (alpine nailwort); *E. aretioides* (alpine forget-me-not); and *O. alpina* (alpine parsley). All three species occur from

timberline to tundra (3500–4000 m). Two species of ants, *Formica neorufibarbis gelida* (Wheeler) (Hymenoptera: Formicidae) and *Leptothorax canadensis* Provancher (Hymenoptera: Formicidae) visit flowers of the plants in this study. Because *L. canadensis* is rare in the study area, I focused on plant interactions with *F. neorufibarbis gelida*. Nests of *F. neorufibarbis gelida* are abundant in the fellfield habitats where these three plant species occur (Taussig 1962). The workers are  $3.9 \pm 0.10$  mm ( $\bar{x} \pm \text{SE}$ ) in body length with headwidth  $0.9 \pm 0.03$  mm (see also Bernstein 1976). In the populations examined, *F. neorufibarbis gelida* represents 24% of the flower visitors to *E. aretioides*, 99% of the flower visitors to *P. pulvinata*, and at least 50% of the flower visitors of *O. alpina* (Puterbaugh 1997).

Study populations of *E. aretioides*, *P. pulvinata*, and *O. alpina* attained maximum densities of 6.2 flowering plants/m<sup>2</sup>, 3 flowering plants/m<sup>2</sup>, and 29 flowering rosettes/m<sup>2</sup> respectively (Puterbaugh 1997, Puterbaugh et al. 1997). *P. pulvinata* and *E. aretioides* are both gynodioecious cushion plants. Perfect flowers of these species are protandrous and larger than pistillate flowers on females (Puterbaugh et al. 1997). Flowers of *E. aretioides* are  $\approx 0.5$  cm in width and typically are borne 2–5 cm above the ground when open. The corollas are tubular and showy, with blue petals and a yellow coronal ring that turns white as the flower ages. Nearly all plants retain the same sex expression between years (Puterbaugh et al. 1997). Only 4% of plants in populations that I studied were inconsistent in sex expression and produced flowers of both types (Puterbaugh et al. 1997). Each flower has four ovules and produces a fruit with one to four nutlets (or seeds). Flowers of *E. aretioides* do not produce seed without insect visitation (Puterbaugh et al. 1997). Unpollinated flowers of females also set no seed (Puterbaugh et al. 1997). *Formica neorufibarbis gelida* forage on the coronal ring of *E. aretioides* flowers, probably for lipids. Because they do not forage deeper in the flowers, ants are unlikely to contact the stigmas of *E. aretioides* flowers, but when observed under a microscope, *E. aretioides* pollen is found on the head and bodies of foraging ants. Plants of *P. pulvinata* have numerous tiny ( $\approx 0.3$  cm width), solitary, yellow green flowers that lack petals. Each flower has only one ovule, and rarely extends above 1 cm from the ground. Self-incompatibility is rare in the Carvophyllaceae (East 1940). Here, I assume that hermaphrodites of *P. pulvinata* are self-compatible, although this assumption is untested in this species. Ants forage at the base of these bowl-shaped flowers for nectar, and in the process regularly contact both anthers and stigmas. Pollen of *P. pulvinata* collects on the heads of foraging ants. Plants of *O. alpina* (alpine parsley) have protogynous, perfect flowers. Umbels consist of 7–16 clustered flowers,  $\approx 1.3$  cm in width, and held 2–6 cm above the ground. Stigmas are exerted from the red buds, and several days later the

corolla opens to reveal five stamens and yellow petals. Each flower has two uniovulate carpels. *Oreoxis alpina* is unique among the three species in that it reproduces vegetatively as well as by seed. Ants forage at the base of the flowers for nectar and, in the process, contact anthers and stigmas. Pollen of *O. alpina* can be observed on the heads of foraging ants. Pollen grains of the three species are easily distinguished: *E. aretioides* pollen grains are bar-bell shaped ( $10 \pm 1.2 \times 6 \pm 0.9$   $\mu\text{m}$ , respectively,  $n = 20$ ); pollen grains of *P. pulvinata* are spherical ( $19 \pm 1.8$   $\mu\text{m}$ ,  $n = 20$ ); and pollen grains of *O. alpina* are oblong ( $26 \pm 1.4 \times 15 \pm 1.3$   $\mu\text{m}$ ,  $n = 20$ ).

## Exclusion experiments

I assessed the effects of ants on pollen receipt and seed-set using ant-exclusion experiments. Methodology was similar, but not identical, between species and years. For all species, the ant-exclusion treatment consisted of a plastic ring nailed into the ground around the plant and coated with Tanglefoot™ (Gómez et al. 1996). Unless large rocks were present below the soil, plants were centered within the ring, with 5–10 cm between the edge of the plant and the plastic ring to avoid damage to roots, leaves, etc. These rings kept ants from visiting the plants but allowed flying insects to visit.

For *E. aretioides*, the ant-exclusion experiment was replicated in 1993, 1994, and 1995 using different individuals each year. Randomly chosen plants of both sex morphs were assigned to exclusion and control (unmanipulated) treatments at 3530 m in the krummholz. Sample sizes are given in Table 1, and represent the number of plants in each group after some individuals were removed due to inconsistent sex expression, seed dispersal prior to collection, close proximity to another plant such that identity was unclear, damage by ungulate herbivores, or trampling. To ensure that the barrier was effective, ringed plants were checked for ants several times in each replicate of the experiment. In 1993, I attempted to pair similar-sized plants for the treatments. In 1994 and 1995, all plants with eight or fewer open flowers in  $\approx 625$  m<sup>2</sup> and  $\approx 550$  m<sup>2</sup> plots, respectively, were used. Plots were about 40 m apart. Variation in flower number among plants was removed statistically (see below). In 1995, pollen receipt was assessed by collecting one mature central flower (fully opened corolla, yellow coronal ring) from each plant on 18 July. Collecting flowers on the same day ensured that flowers had experienced similar conditions for pollinator foraging; collecting fully-opened flowers controlled for flower phenology. Styles were squashed and stained in basic fuchsin gel (Beattie 1971) and stigmas were examined for pollen under a light microscope ( $250\times$ ). The number of pollen grains per stigma and seeds per ovule was compared between sexes and treat-



ments (both fixed factors) using a factorial analysis of variance (ANOVA). For seed-set, year of reproduction was also included as a random effect. For this and all subsequent ANOVAs, the General Linear Models Procedure (GLM) of the Statistical Analysis System (SAS 1989) was used. To improve fit to the assumptions of ANOVA, pollen counts were square-root transformed, and the number of seeds per ovule was angular transformed before analysis.

The ant-exclusion experiment was performed on *P. pulvinata* plants in 1996. All plants with at least 10 buds were marked within a 625-m<sup>2</sup> plot at 3600 m elevation and assigned randomly to the control or exclusion treatment. Final sample sizes were smaller because ants penetrated the tanglefoot barrier on some plants and repairs were ineffective (Table 1). Treatments were applied on 7 June at which time any open flowers were removed (typically fewer than 15 flowers or <18% of the total flowers per plant). Previously initiated fruits and senesced flowers were left on the plant, in part because senesced flowers were difficult to distinguish from unopened buds. To assess pollen receipt, one open flower per plant was randomly chosen and bracts below the flower were marked with small dabs of yellow enamel paint on 17 June. All of these flowers were collected three days later, when all but three had senesced. Carpels were too fragile to clear in NaOH solution; thus, they were simply dissected from the flowers and stained overnight in 0.01% aniline blue in 0.1 M K<sub>3</sub>PO<sub>4</sub> (Kearns and Inouye 1993). Styles and stigmas were removed, squashed in Karo Syrup™, and the number of grains adhering to each stigma were counted. The number of pollen grains per stigma was compared between treatments and sexes using ANOVA. Counts were square-root transformed before

analysis to improve the fit to the assumptions of ANOVA. On 8, 9, 11, 12, 17, and 28 June, all newly opened flowers were marked with small dabs of green enamel paint on the subtending bracts. The number of flowers marked per plant ranged from 4 to 42 ( $\bar{x}$  = 21 flowers). At the end of the season, these marked flowers were collected, and the number of seeds produced by each was counted. Because fruits that initiated before the experiment had begun were not removed, I only monitored seed-set from these marked flowers. Seed-set per ovule (equivalent to seed-set per flower, as each has only one ovule) was compared between sexes and treatments using ANOVA after angular transformation.

For *O. alpina*, the high density of plants allowed for a blocked design in the ant exclusion experiment. The experiment was replicated in 1995 and 1996. In 1995, 15 random points were chosen within a 64-m<sup>2</sup> plot at 3600 m on 26 June. In 1996, 17 random points were chosen within a different 64-m<sup>2</sup> plot at 3650 m elevation on 16 June. In each year, the nearest three plants to each point having umbels from which stigmas were not yet exerted were randomly assigned to one of three treatments: ant-excluded; unmanipulated control, and full insect exclusion (test for autogamy). Cages used to exclude insects consisted of a clear plastic ring covered on top with fine nylon mesh (bridal veil) and nailed into the ground over the rosette. To assess pollen receipt in 1996, one senesced flower was collected from the edge of each umbel. Styles were excised and treated as for *P. pulvinata* styles. Flowers were removed from the edge of the umbel to avoid damaging the remaining flowers. Styles and stigmas were squashed in Karo Syrup™ on a microscope slide, and examined under a microscope with epifluorescence for germinated pollen grains on the stigma. The number of germinated pollen grains per

Table 1. Sample sizes for plants in the ant-exclusion experiment. *n* refers to the number of plants

Species	Year	Treatment	Morph	<i>n</i>
<i>E. aretioides</i>	1993	control	female	16
			hermaphrodite	18
		ant-excluded	female	10
			hermaphrodite	9
	1994	control	female	20
			hermaphrodite	13
		ant-excluded	female	20
			hermaphrodite	15
	1995	control	female	11
			hermaphrodite	18
		ant-excluded	female	9
			hermaphrodite	21
<i>P. pulvinata</i>	1996	control	female	19
			hermaphrodite	18
		ant-excluded	female	12
			hermaphrodite	13
<i>O. alpina</i>	1995	control	hermaphrodite	14
		ant-excluded	hermaphrodite	15
		all insects-excluded	hermaphrodite	15
		control	hermaphrodite	17
	1996	ant-excluded	hermaphrodite	17
		all insects-excluded	hermaphrodite	17

flower was compared between sexes and treatments using ANOVA. Counts were square-root transformed to improve normality and homoscedasticity. Fruits were collected from the remaining flowers in each umbel 7–8 weeks later. The number of seeds per umbel was compared between sexes, treatments, and years using a mixed-model ANOVA with block (a random factor) nested within year. Seed counts were square-root transformed before analysis.

## Pollination quality

The high frequency of ant visitation to flowers of *P. pulvinata* allowed for tests of the effectiveness of ants as pollinators. I was able to examine how well ants that visited flowers only once pollinated pistillate flowers of *P. pulvinata*. Using flowers from females allowed me to test the cross-pollinating ability of ants. From 13 June–1 July, 1996, 18 female plants were chosen at elevations of 3530, 3580, and 3600 m. Bracts below the buds were marked with paint as above, and buds were capped with BEEM capsules to exclude insect visitors (size 3; E. Fullum, Inc., Latham, NY) and randomly assigned to either receive a single ant visit or remain capped, as controls. The control flowers tested whether the capsules successfully excluded insects. Capsules were secured over buds with a straight pin through the hinge connecting the lid to the body. Upon opening, flowers assigned to receive a single ant visit were uncapped, individually. Typically after 3–5 min, an ant began foraging on the flower. After the ant left the flower, it was recapped. Both ant-visited and control flowers were collected the day after opening. The carpels were dissected and stained overnight in 0.01% aniline blue in 0.1 M  $K_3PO_4$  as described above. Styles and stigmas were removed, squashed in Karo Syrup™, and examined with epifluorescence microscopy for pollen tubes ( $250\times$ ). Both the number of pollen grains on the stigma and the number of pollen tubes in the style were recorded. By examining both pollen grains and pollen tubes, two factors affecting ant-pollination efficiency could be considered: whether ants were carrying and transferring pollen grains and whether the pollen grains ants transferred were viable or inviable. The number of pollen grains per stigma and the number of pollen tubes per style were compared between treatments using non-parametric, unpaired Wilcoxon tests, as the data were not normally distributed (NPARIWAY procedure; SAS 1989).

Pollination effectiveness of ants was also assessed for hermaphrodite plants as follows from 13 June–20 June, when 35 large hermaphroditic plants that could accommodate numerous capped flowers were chosen in the same locations. Female plants were not used in this experiment because of the extreme difficulty in identifying pistillate flowers in bud. On each plant, 2–9 buds

were marked with small dots of enamel paint on the sepal, capped as described above, and randomly assigned to one of three treatments: a single ant visit, all insects-excluded, and unmanipulated (open) controls. Any extra buds were assigned to the control treatment. Senesced flowers were sometimes mistaken as buds, reducing sample sizes as follows: single-ant visit ( $n = 43$  flowers, 19 plants), all insects excluded ( $n = 105$  flowers, 23 plants), unmanipulated control ( $n = 129$  flowers, 31 plants). As flowers opened, those from which all insects were excluded were simply marked to indicate successful opening and recapped. For flowers in the control treatment, caps were removed after the flower opened. For flowers that received a single ant visit, capsules were removed, and the flower was watched until an ant visited. If no ant visited within 5 min, flowers were randomly assigned to one of the other two treatments. Ideally, styles as well as seeds should have been collected. After applying treatments, however, flowers were not monitored again until after styles had withered. On 5 July, after all flowers had senesced, caps were removed from the flowers of the all-insect-excluded treatment and a straight pin (colored to identify treatment) was placed next to the flower. Four weeks later, after seeds had begun to mature, the number of flowers producing a seed was recorded. No flowers from which all insects were excluded set seed; thus, this treatment was not included in the analysis. A *t*-test was used to compare the average seed-set of flowers receiving a single-ant visit to that of controls (TTEST Procedure, SAS 1989). To improve normality, data were angular transformed before analysis.

## Results

### Exclusion experiments

The number of pollen grains received per stigma in *E. aretioides* was not significantly affected by ant-exclusion ( $F = 2.27$ ;  $df = 1,47$ ;  $P = 0.14$ ) or morph ( $F = 0.96$ ;  $df = 1,47$   $P = 0.33$ ). On average,  $24.22 \pm 3.64$  pollen grains ( $\pm$  SE;  $\approx 6$  pollen grains per ovule) were deposited per stigma ( $n = 51$ ). Stigmas of ant-excluded pistillate flowers (for which self-pollination is impossible) received an average of  $24.89 \pm 12.19$  pollen grains each ( $n = 9$ ). The number of seeds set per ovule varied among years ( $F = 93.87$ ;  $df = 1,168$ ;  $P < 0.01$ ). In all three years, there was a nonsignificant, but consistent, tendency for seed-set to be lower in the control plants than in the plants from which ants were excluded ( $F = 9.70$ ;  $df = 1,2$ ;  $P = 0.09$ ; Fig. 1). The number of seeds set per ovule did not differ between sexes ( $F = 4.94$ ;  $df = 1,2$ ;  $P = 0.16$ ) nor were any of the two- or three-way interactions significant ( $0.12 < P < 0.60$  for all).

### *Eritrichum aretioides*

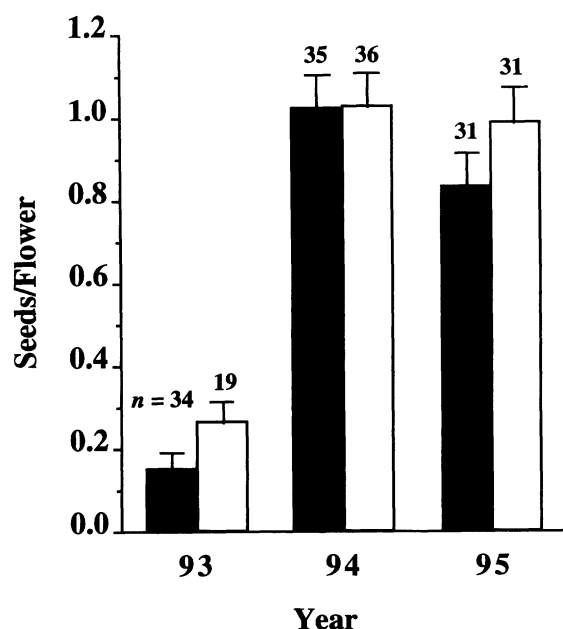


Fig. 1. Mean seed-set (+ SE) in *Eritrichum aretioides* in three years. Filled bars give means for unmanipulated control plants, and open bars give means for plants from which ants were excluded.

The number of pollen grains adhering to the stigmas of *P. pulvinata* depended on whether or not ants were excluded ( $F = 4.22$ ;  $df = 1,33$ ;  $P = 0.05$ ) and on the interaction between sex and exclusion treatment ( $F = 6.40$ ;  $df = 1,33$ ;  $P = 0.02$ ). Pistillate flowers of *P. pulvinata* from which ants were excluded had fewer pollen grains per stigma than all other groups, the remainder of which had similar numbers of pollen grains per stigma (Fig. 2). Plant sex morph alone had no effect on the numbers of deposited pollen grains ( $F = 0.09$ ;  $df = 1,33$ ;  $P = 0.76$ ). Female control plants produced three times as many seeds per ovule as did hermaphrodite plants ( $F = 20.86$ ;  $df = 1,58$ ;  $P < 0.01$ ). The exclusion of ants also reduced seed set ( $F = 4.42$ ;  $df = 1,58$ ;  $P = 0.04$ ), with control plants producing 1.5 times as many seeds per ovule as did ant-excluded plants (Fig. 3). For seed-set, there was no significant interaction between sex and treatment ( $F = 2.97$ ;  $df = 1,58$ ;  $P = 0.09$ ), but female plants tended to show greater seed-set reduction when ants were excluded than did hermaphroditic plants (Fig. 3). Ant-excluded female plants did set some seeds ( $\bar{x} = 0.23 \pm 0.06$  seeds per flower,  $n = 12$ ), showing that other pollinators visit their flowers occasionally.

No significant differences in pollen receipt or seed production were detected among the three experimental treatments for *O. alpina* ( $F = 0.80$ ;  $df = 1,40$ ;  $P = 0.46$  and  $F = 1.54$ ;  $df = 2,59$ ;  $P = 0.22$ , respectively). Stigmas had on average  $6.27 \pm 1.33$  ( $n = 43$ ) germinated pollen grains each and umbels produced  $1.33 \pm 0.23$  seeds

### *Paronychia pulvinata*

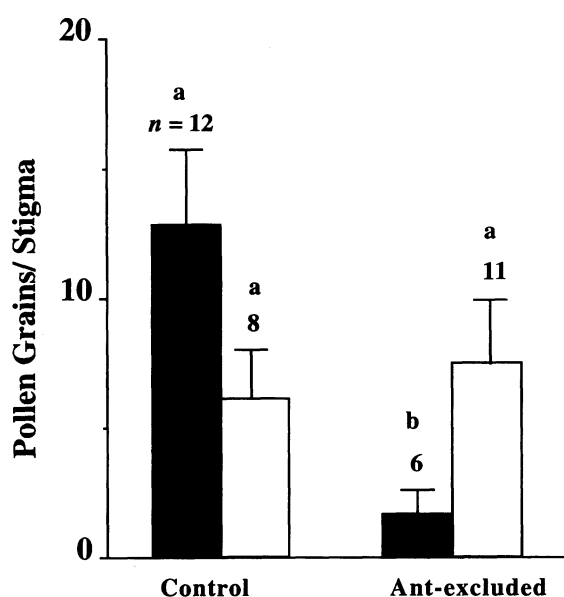


Fig. 2. Mean number of pollen grains per stigma (+ SE) in *Paronychia pulvinata* when ants were excluded from plants and when plants were unmanipulated for female plants (solid) and hermaphroditic plants (open). Letters above bars show significant differences based on Tukey's multiple comparisons test ( $P < 0.05$ ).

( $n = 95$ ). Seed-set was higher in 1995 than 1996 ( $F = 5.55$ ;  $df = 1,59$ ;  $P = 0.02$ ). No interaction between year and treatment was detected ( $F = 1.71$ ;  $df = 2,59$ ;  $P = 0.19$ ).

### *Paronychia pulvinata*

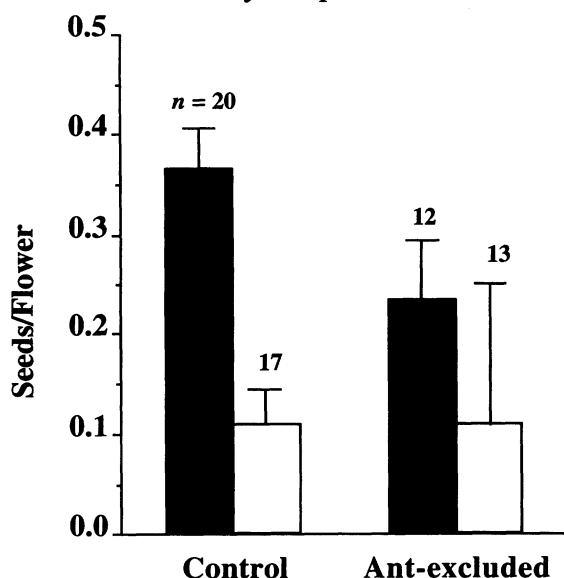


Fig. 3. Average seed-set (+ SE) in *Paronychia pulvinata* when ants were excluded from plants and when plants were unmanipulated for female plants (solid) and hermaphroditic plants (open).

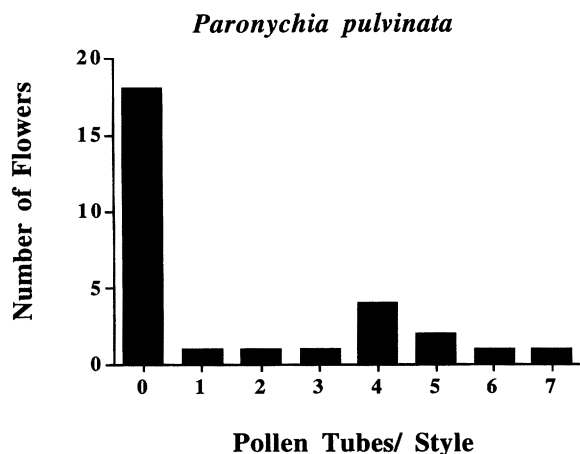


Fig. 4. Frequency distribution of the number of pollen tubes per style for previously unpollinated pistillate flowers of *Paronychia pulvinata* receiving a single ant visit.

### Pollination quality

Ants appeared to contact the stigma during nearly all flower visits to *P. pulvinata*. Only one of the 18 capped control flowers from which all insects were excluded was contaminated with pollen. Despite this minor contamination, stigmas of pistillate flowers that were visited once by an ant obtained significantly more pollen grains per stigma than unvisited flowers ( $Z = 2.64$ ;  $df = 29, 18$ ;  $P = 0.01$ ). Of those ant-visited flowers with pollen grains on the stigma, an average of 75% of the grains germinated, sending tubes down the style. Ant-visited flowers also had more pollen tubes per style than control flowers ( $Z = 2.26$ ;  $df = 29, 18$ ;  $P = 0.02$ ). Only 40% of all ant-visited flowers received pollen that germinated and sent tubes down the style. As a consequence, the frequency of flowers having pollen tubes in the style after a single ant visit was bimodal (Fig. 4). Respectively, the average numbers of pollen grains per stigma and pollen tubes per style for these ant-visited flowers were  $2.48 \pm 0.84$  and  $1.55 \pm 0.42$ .

No perfect flowers of *P. pulvinata* set seed in the absence of insect visitors ( $n = 105$  flowers, 23 plants). This result is unlikely to be due to the capping as average seed-set of flowers from which caps were removed for only 5 min was  $>0$  based on a  $t$ -test ( $t = 2.12$ ;  $df = 18$ ;  $P = 0.04$ ). Perfect flowers receiving a single ant visit had seed-set comparable to that of uncapped controls ( $t = 1.68$ ;  $df = 48$ ;  $P = 0.10$ ). Mean seed-set per flower was  $0.14 \pm 0.03$  ( $n = 50$ ).

## Discussion

### Pollination effectiveness of ants

Flower-visiting ants of *F. neorufibarbis gelida* have diverse effects on the three alpine plant species examined

in the study. For *E. aretioides*, *F. neorufibarbis gelida* is an herbivore, damaging the coronal ring of flowers and tending to reduce seed-set. For *P. pulvinata*, *F. neorufibarbis gelida* is a pollinator, and for *O. alpina*, flower visitation by ants has no detectable effect on seed production. Given that floral nectar and lipids are important resources to ants, the net effect of these plants on the ant species is positive, whereas the effect of the ants on the plants varies from negative to positive.

The negative effect of ants on fecundity in *E. aretioides* is probably due to physical damage to the flower. Workers of *F. neorufibarbis gelida* destructively seek lipids in the coronal ring, rather than probing for nectar at the base of the flower. The tendency for unmanipulated plants to have lower seed-set than ant-excluded plants was probably not the result of ants deterring effective pollinators through either harassment or reduced floral attractiveness, since pollen receipt was unaffected by the exclusion of ants. Stigmas of ant-excluded female plants received an average of  $6.2 \pm 3.05$  pollen grains per ovule, demonstrating that flying insects effectively transfer substantial outcross pollen. Petersen (1977) found that workers of *Leptothorax canadensis* were the most frequent visitors to flowers of *E. aretioides* at Niwot Ridge, Colorado. Possibly, the smaller *L. canadensis* is capable of navigating the narrow flower tube and pollinating *E. aretioides*, whereas larger individuals of *F. neorufibarbis gelida* are not. Individuals of *L. canadensis* often forage at single flowers for more than 10 min (Petersen 1977, pers. obs.). While this behavior does not negate their potential to pollinate, it probably reduces pollen transfer relative to that provided by flying insects, which move frequently from flower to flower (but see Young 1988).

*Formica neorufibarbis gelida* pollinates flowers of *P. pulvinata*, but contributes differentially to pollen receipt in the two morphs. Female plants from which ants were excluded showed a greater reduction in pollen grains per stigma than did hermaphroditic plants. Consequently, *F. neorufibarbis gelida* not only has diverse effects on different plant species, but also on morphs within species.

Hickman (1974) provided a list of features for plants which he predicted would be ant-pollinated. In essence, Hickman (1974) described what might be expected for an "ant pollination syndrome". Evidence has long existed that most plants are pollinated by multiple pollinator species and a recent paper directly questions the persistent concept of "pollination syndromes" (Waser et al. 1996). Pollinator generalization is expected to be more common than specialization (Waser et al. 1996), and "ant pollination syndromes" may be rare for the same reason that most "pollination syndromes" are rare. All three of the plant species examined in this study receive visits from multiple pollinator species. While *P. pulvinata* receives primarily ant visits at the



sites examined, this species occurs at higher elevations lacking ant populations; pollination in *P. pulvinata* is probably also generalized rather than specialized to ants. The plant features described by Hickman (1974) and occurring in *P. pulvinata*, such as small flower size and low nectar volumes, are likely to be adaptations to pollination by small insects in general rather than ants specifically. However, even in generalized systems, flower visiting ants appear to be less frequently pollinators than other flower visiting insects (Peakall et al. 1991). In both *E. aretioides* and *O. alpina*, ant visitation rates exceeded 20% of all flowers visited, but ants were not pollinators.

Why are ants visiting flowers but not pollinating them? One explanation for the rarity of pollination by ants invokes the presence of pollen-damaging substances released onto the ant body by its metapleural gland (Beattie et al. 1984). Whether workers of *F. neorufibarbis gelida* have active metapleural glands is undocumented, but, occurrence of pollen tubes in styles following ant visitation of *P. pulvinata* flowers proves that ants transfer viable pollen. In addition, most pollen was observed on the anterior portion of the ants' head, and this part of the body most frequently contacted the reproductive parts of the flower (pers. obs.). Pollen of *P. pulvinata* probably would not contact secretions from the metapleural glands (located at the posterior end of the middle portion of the ant body) if such secretions are produced by individuals of this ant species (Beattie et al. 1985).

Single ant visits to pistillate flowers demonstrated that ants are very inefficient cross-pollinators of *P. pulvinata*. Of these visits, 55% resulted in no pollen transfer and 62% in no pollen tube growth within styles. Since the germination rate of the pollen transferred was 75% (assuming ungerminated grains remained on stigmas throughout staining), the inefficient pollination of *P. pulvinata* by ants is better explained by their low rate of pollen transfer, rather than by low pollen viability. One explanation for the low rate of pollen transfer is that 87% of all ant visits are between flowers of the same plant (Puterbaugh 1997). Stated another way, one could predict that, on average, only 13% of the ants on a female plant will have just visited a perfect flower on a hermaphroditic plant. A low rate of pollen carryover could explain why 55% of ant visits to pistillate flowers resulted in no pollen transfer to stigmas. This supports the premise that the likelihood of pollination of females by ants should increase as a function of ant abundance. That ant abundances typically reach levels sufficient for effective pollination of *P. pulvinata* is consistent with data from flowers of unmanipulated control plants in the ant-exclusion experiment. These received unconstrained levels of ant-visitation and nearly all (91%) had pollen grains on their stigmas, with half of those having more than 10 pollen grains each. With only one ovule per flower,

these cumulative loads do not suggest pollen limitation. As with other examples of pollination by ants, the frequency of ant visitation helps explain why ants are effective pollinators despite their low efficiency of pollen transfer (Gómez and Zamora 1992, Gómez et al. 1996).

For hermaphrodites, however, flowers visited only once by an ant yielded only slightly reduced seed-set relative to controls that were open to unconstrained visitation. One explanation is that perfect flowers self via geitonogamy and, although not autogamous, require less pollinator activity for seed-set than do pistillate flowers. Similarly, in hermaphroditic *Blandfordia grandiflora* ant activity also contributes to selfing, but not outcrossing (Ramsey 1995). Most theoretical models show that females must have higher seed fecundity than hermaphrodites in order for the two morphs to be maintained in a population over time, although the degree of female advantage will depend on the mode of inheritance of sex (Lewis 1941, Lloyd 1974, Charlesworth and Charlesworth 1978, Charlesworth 1981). If inbreeding depression affects fecundity, the greatest fecundity differences between female and hermaphroditic plants will occur under pollination regimes that cause outcrossing in females and selfing in hermaphrodites (Lewis 1941, Lloyd 1974, Charlesworth and Charlesworth 1978, Charlesworth 1981, Maurice and Fleming 1995). For gynodioecious *P. pulvinata*, ants are the epitome of the small, unspecialized pollinator whose inefficient, but frequent, visits enable female plants to be cross-pollinated, but whose predominantly geitonogamous movements probably enhance selfing in hermaphrodites.

No effects of flower-visiting ants were detected for pollen receipt or seed production in *O. alpina*. This was somewhat surprising, given that ants are frequent visitors to the umbels, carry numerous pollen grains, and regularly contact the reproductive parts of the flowers (Puterbaugh 1997). Moreover, on average, ants move between umbels separated by more than 10 cm every 6 min, suggesting possible cross-pollination (Puterbaugh 1997). These attributes meet the basic prerequisites for ant pollination as proposed by Peakall et al. (1991). Nonetheless, two features of *O. alpina* could explain the negligible effect of ants on fecundity. First, for *O. alpina*, pollen receipt and seed-set were unaffected by the exclusion of all insects, suggesting efficient autogamy or apomixis. Second, the high loads of pollen observed on stigmas of the uniovulate carpels argue against pollen limitation in this species.

### Flower shape and the effects of flower-visiting ants

As predicted (Hickman 1974, Peakall et al. 1991), floral morphology appears to play a key role in determining

the effects of ants as flower visitors. Most ant-pollinated species have small, open flowers (see references in Ramsey 1995); ants are typically nectar thieves or floral herbivores of species with larger flowers (Galen 1983, Herrera et al. 1984, Norment 1988). My results indicate that flower shape, as well as size, is important. The tubular flower of *E. aretioides*, with lipid attractants at the surface of the flower, provides little opportunity for large ants to contact the reproductive parts within the flower tube (see also Petersen 1977). Conversely, ant pollination is facilitated by the small bowl-shaped flowers of *P. pulvinata*, such that ants frequently contact the reproductive parts while foraging for nectar.

### Ants and relative fecundity in female and hermaphroditic plants

Most theoretical models predict that the ratio of female seed-set to hermaphrodite seed-set depends on the mode of inheritance of sex but that the ratio must be  $\geq 1$  for female plants to compensate for their inability to make pollen and attain male fitness (Lewis 1941, Lloyd 1974, Charlesworth and Charlesworth 1978, Charlesworth 1981). For both *E. aretioides* and *P. pulvinata*, the ratio of mean female seed-set to average hermaphrodite seed-set increases when ants have access to plants (Fig. 5). An exciting aspect of this result is that the same ant species changes this ratio by negative effects in one plant species and positive effects in the other. In *E. aretioides*, ants, acting as herbivores, tend to cause a greater decrease in seed-set in the maphrodites than in the females, whereas in *P. pulvinata*, ants, acting as pollinators, tend to increase seed-set in females more than in hermaphrodites. In both species, the ratio was greater than one even in the absence of ants, suggesting that the ants alone do not explain fecundity differences between morphs. Other factors, such as resource reallocation, may help explain gynodioecy in these species. Nonetheless, ants apparently contribute to maintenance of the observed sex ratios, since the relative fecundity of females to hermaphrodites determines their frequency in a population at equilibrium (Lewis 1941, Lloyd 1974, Charlesworth and Charlesworth 1978, Charlesworth 1981).

Cox (1982) proposed that the evolution of sexual specialization could be driven by floral predators that were more attracted by the male floral parts but which caused damage to the female parts in perfect flowers. In several species, floral predators attack staminate inflorescences more frequently than pistillate inflorescences, e.g. *Freycinetia reinecki* (Cox 1982), *Simarouba glauca* (Bawa and Opler 1978), and *Siparuna* spp. (Feil 1992). In *E. aretioides*, ants have a negative effect on seed-set, and this tends to be magnified in hermaphrodites. The mechanism for differential ant damage is unknown. Like other animal-pollinated spe-

cies with unisexual flowers, *E. aretioides* flowers with male function are larger than flowers without male function (Delph 1996, Puterbaugh et al. 1997). Possibly, ants are more attracted to the larger perfect flowers or cause greater chewing damage to their larger corollas (Puterbaugh et al. 1997, Puterbaugh 1997). Either explanation could explain the slightly greater fecundity advantage of females in the presence of ants, and these data further support Cox (1982) proposal that biotic factors can be important in the evolution of breeding systems.

As explained above, ants probably affect the relative fecundity of females and hermaphrodites in *P. pulvinata* through their foraging behavior by promoting outcrossing in females and selfing in hermaphrodites. The reduced seed-set in females in the absence of ants suggests pollen limitation, since the exclusion of ants significantly reduced pollen receipt on flowers of female plants. Recently, Maurice and Fleming (1995) showed that the maintenance of polymorphic breeding systems is influenced by the degree of pollen limitation. Ants are pollinators of at least one other sexually polymorphic species, *Borderea pyrenaica* (García et al. 1995), and monoecy has been reported in ant-pollinated *Voandzeia subterranea* (Doku and Karikari 1971). The effect of

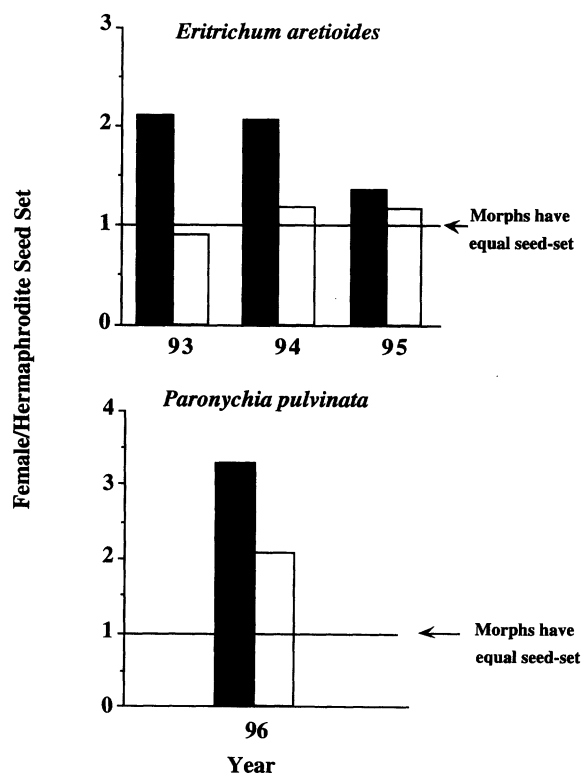


Fig. 5. Relative fecundity (mean seed-set) of pistillate plants compared to hermaphrodite seed-set in *Eritrichum aretioides* and *Paronychia pulvinata* for plants that were unmanipulated (solid bars) and plants from which ants were excluded (open bars). For *E. aretioides*, data are presented for three years.

ants on the breeding systems of these species is unknown.

## Indirect effects between plant species through flower-visiting ants

The outcomes of direct two-species interactions vary with other conditions, including the indirect effects of a third species (Bronstein 1994). Indirect effects are a frequently overlooked, but potentially important, evolutionary factor (Strauss 1991). Indirect effects of changing the abundances of *O. alpina* in the community could change ant-visitation rates to *E. aretioides* or *P. pulvinata*, such that individuals of *E. aretioides* might suffer greater or lesser ant-caused damage and females of *P. pulvinata* might suffer more severe pollen limitation compared to hermaphrodites. The consequences of a change in *O. alpina* abundance depends on whether *O. alpina* tends to compete with these other two species for ant-visitation or whether the presence of *O. alpina* promotes ant abundance and activity (Thomson 1981, 1982, Rathcke 1988). Alpine plant-ant interactions represent a system very amenable to investigation of indirect effects, and studies are currently planned to consider the impact of plant community composition on the pairwise species interactions.

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