Pollination by Ants: A Low-Energy System

Abstract. Polygonum cascadense, *a small, apparently self-incompatible, annual plant, is regularly cross-pollinated by the ant* Formica argentea. *Comparison of other purported ant-pollinated plants with traits favoring such pollination suggests that some, but not all, may be ant pollinated. Ant-pollination interactions are characterized by low expenditure of energy by both ant and plant.*

Ants are common flower visitors, but recent attention to the mutualistic interactions of ants and plants has emphasized nonfloral features (*1*). Almost invariably ants are considered the villains in floral interactions. The conception of ants as "the prototype of nectar thieves" (*2*) originated with the classical pollination biologists of the late 19th century (*3*) and has persisted effectively unchallenged (*2, 4, 5*).

I report here field and greenhouse observations that demonstrate ant pollination as a specialized mutualistic system in the small annual plant *Polygonum cascadense* Baker (Polygonaceae). Isolation of a set of plant traits favoring ant pollination in *P. cascadense* provides a basis for evaluating earlier (generally discounted) reports of ant-pollinated plants and may help predict ant pollination in species yet to be studied. Unequivocal demonstration of cross-pollination by ants is significant not only because of its controversiality but more importantly because all previously accepted pollinating animals fly from plant to plant. Since walking may reasonably be expected to require as much as an order of magnitude less energy per unit distance than flying (*6*), the results add another facet to the growing field of pollination energetics (*7*).

Polygonum cascadense is an annual of the open, hot, dry slopes of the Western Cascades of Oregon at an elevation of about 1500 m. Plants are erect and very small, ranging from 1 to 20 cm in height. Flowers are produced in nearly sessile axillary clusters along the length of the stem, with one flower per axil blooming at a time. Open flowers are held perpendicular to the main axis and are readily accessible to small insects clinging to leaves or stems. The perianth tube is funnelform, whitish, and 1.5 to 2 mm deep. All eight stamens (three alternate with the styles) shed their sticky pollen, which adheres readily to smooth surfaces, before elongation and maturation of the stigmatic surfaces. Small quantities of nectar are produced in both "male" and "female" stages by five minute glandular spots at the base of the perianth funnel, even during seasons when internal moisture stress is severe (xylem pressure potential below −70 atm) (*8*). No odor is detectable from the flowers.

On clear afternoons when soil surface temperatures approach 60°C, many workers of the silky ant *Formica argentea* Wheeler forage actively for nectar in dense, nearly monotypic stands of *P. cascadense*. The perianth shape brings the ant's head in contact with sticky pollen on the anthers if the protandrous flower is in the "male" stage, and with the receptive stigmas if the flower is more mature. Transfer of pollen grains from anther to ant and from ant to stigma has been observed in the field. Most pollen is carried on the mouthparts and lower parts of the head (especially labrum and mandibles) but occasionally grains adhere to antennae, legs, and gaster (Fig. 1). No ant was observed to engage in grooming behavior while foraging in a *Polygonum* population, and all *F. argentea* workers that were collected carried pollen. At high temperatures the ants move rapidly, usually visiting only one flower per plant (one ant visited three flowers on a single plant sequentially). Flower visits last about 1 second and interplant trips are rapid and frequent, often requiring less than 3 seconds. The ant in Fig. 1b visited one flower on each of six *Polygonum* plants within the minute prior to being photographed. Individual ants in the field show marked fidelity to *P. cascadense* flowers. It is difficult to follow individual, rapidly moving ants for long periods when many are present, but it is certain that some *F. argentea* workers visited at least 20 flowers on nearly as many plants before leaving the stand.

Other insects which have been observed visiting *P. cascadense* flowers include other ants (occasional workers of the smaller *F. lasioides* Emery and a single large male in the *F. rufa* group); several wasps of the families Braconidae, Chrysididae, and Ichneumonidae; and at least two species of syrphid flies. It is possible that ant species other than *F. argentea* also pollinate *P. cascadense*, but their observed frequency of visits is markedly lower, and no pollen was found on collected individuals. The behavior of the flying visitors contrasts strikingly with that of the ants. Visits are equally brief but, with only one exception, the approximately 50 flying insect visitors approached only one *P. cascadense* flower before leaving the population. It was not always possible to follow them to subsequent destinations, but in the few cases where data were obtained, the next visits were to species in neighboring habitats that attract a diversity of flying insects. Moreover, all the flying visitors have longer tongues than do the ants, and examination of the captured specimens revealed no *Polygonum* pollen.

Greenhouse specimens of *P. cascadense* growing in the absence of ants are healthy and flower profusely. Flying insects (chrysidids, halictids, and syrphids) may visit their flowers in nearly the same frequency as that observed in the field. Some of the pollen not removed by insect visitors almost always falls onto receptive stigmas of the same flower later in its development. Nevertheless, these plants set very few seeds (0 to 7 percent). Field populations occur only in habitats where ants are abundant and show 85 to 100 percent of the seeds set.

Demonstration of absolute dependence on ants for pollination would require excluding only ants from plants in the field without changing other environmental variables. Although this has not been done, the lines of evidence cited mandate three conclusions. (i) These lines of evidence firmly demonstrate that cross-pollination mediated by ants occurs in *P. cascadense*. Furthermore, they strongly indicate (ii) that this species, unlike its generally autogamous close relatives, is self-incompatible and (iii) that ants are essential for normal seed set.

Ten traits adapt *P. cascadense* for pollination by ants. I enumerate them below to allow predictive inferences concerning ant pollination in other species. Traits are given in generalized terms, with some functionally equivalent variations.

Two obvious but nontrivial traits are necessary for ant-flower interaction.

1) Plants must grow in those hot and dry habitats where the frequency and activity of ants is high.

2) Nectaries must be readily accessible to a small, short-tongued insect.

Four traits allow ready interplant access to flowers for a nonflying insect.

3) Of utmost importance is the height above the ground at which the flowers are borne. Plants must be short or prostrate (they may be tall only if flowers are borne at the base). In some large

populations of *P. cascadense*, all flowers are within 2 cm of the soil surface, minimizing vertical distances to be traveled.

4) Populations must be dense or stands must contain few species. Plants so dense as to have interdigitating branches, eliminating the necessity to return to the ground between plants, have lessened strictures on height.

5) Synchronously blooming flowers must be few per plant, since many attractive flowers will maximize intraplant rather than interplant foraging and pollen transport. Many-flowered species have increased strictures on plant height or density, or both, but, in dense populations, genetic self-incompatibility or dioecy may allow more flowers per plant.

6) If plants are erect, flowers must be sessile or nearly so; if plants are matted, flowers must be on the mat surfaces. Flowers on long peduncles or scapes will be exposed to flying pollinators and will require greater foraging distances by ants.

Two traits are related to inappropriateness of ant integument and ant behavior for transporting large quantities of pollen.

7) Pollen volume per flower must be small to avoid stimulation of self-grooming. This can be achieved by reducing the number of pollen grains, their size, or both.

8) Seeds must be few per flower, since each seed requires at least one pollen transfer. *Polygonum* fruits are uniovulate and only one pollen grain is required per flower for full seed set.

Two traits deter flying "thieves" without disadvantaging ants.

9) Flowers must be small and of minimal visual attraction. Ants see well, but use primarily exploratory behavior, tactile and olfactory clues, and learned behavior patterns to locate sugars (*9*). Visually attractive flowers will increase the rate of nectar theft by flying insects not necessarily capable of mediating cross-pollination. Small flowers also facilitate pollen exchange with a small vector.

10) Nectar quantity must be small enough to support ants while discouraging visits by insects whose energy demands while they are foraging exceed the available reward. Since ants are inferred to incur much smaller energetic debts while moving from plant to plant (if the vertical displacement is small) than flying insects do when covering the same horizontal distances (*6*), considerably less caloric output by a plant is necessary to support ant pollinators. Indeed, offering a larger nectar reward would increase the likelihood that generalist flying insects not structured to effect cross-pollination would steal the nectar. Ant-pollinated plants have one of the most effective devices for inhibiting nectar thieving—offering too little reward to support the continued visits of any insect but the proper pollinator. Low nectar volume may also be of advantage to plants that are limited in productivity by water supply, as plants of hot dry areas are likely to be.

These traits are all interrelated and when taken together minimize the interaction energy outputs by both plant and pollinator. Foraging distances are minimized for the ant, pollen and nectar rich in energy are conserved by the plant, and theft by nonpollinators is greatly reduced.

The ant-pollination syndrome should have predictive value for other plants. I have attempted to corroborate this by semiquantitatively comparing the syndrome traits with six species for which ant pollination has been proposed previously (*10*), two typical hummingbird-pollinated species (*11*), and two typical bumblebee-pollinated species (*12*).

Four of the six purported ant-pollinated species have 90 to 100 percent positive matches with the syndrome traits. Thus these proposals may be correct, but additional field data would be necessary to demonstrate them. *Trinia glauca* has 20 to 80 percent positive matches (*13*). This species may have a mixed pollination strategy, but is probably not pollinated solely by ants. *Seseli libanotis* has only 10 to 50 percent positive matches (*13*), corroborating Hagerup's implication (*10*) that ant pollination is highly unlikely as a regular phenomenon.

The two hummingbird-pollinated species both have 10 percent positive matches, while the bee-pollinated species both have 10 to 30 percent positive matches, depending on the state of variable characters.

From these limited data it appears that the ant-pollination syndrome has some value in distinguishing ant-pollinated plants from those adapted to

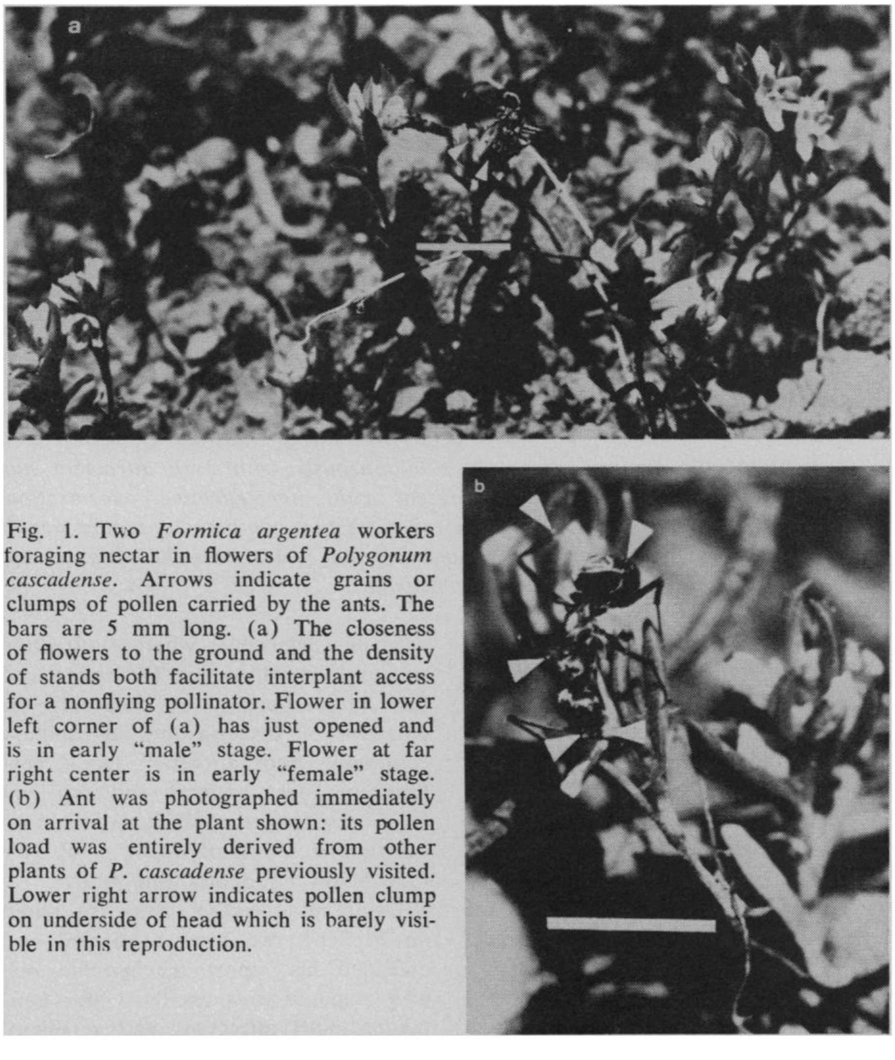

Fig. 1. Two *Formica argentea* workers foraging nectar in flowers of *Polygonum cascadense*. Arrows indicate grains or clumps of pollen carried by the ants. The bars are 5 mm long. (a) The closeness of flowers to the ground and the density of stands both facilitate interplant access for a nonflying pollinator. Flower in lower left corner of (a) has just opened and is in early "male" stage. Flower at far right center is in early "female" stage. (b) Ant was photographed immediately on arrival at the plant shown: its pollen load was entirely derived from other plants of *P. cascadense* previously visited. Lower right arrow indicates pollen clump on underside of head which is barely visible in this reproduction.

larger, flying pollinators, and also from those adapted to small, generalist, short-tongued pollinators that differ from ants primarily in their ability to fly. Its ultimate value can be determined only through broader application and refinement.

The wet or seasonally rainy tropics, where studies of other ant-plant mutualisms have concentrated, do not support many plant species exhibiting the characters of the ant-pollination syndrome. Hagerup has suggested (*10*) that the best habitats for ant pollination are extreme deserts, and these results offer some support for that hypothesis. Three of the four demonstrated and likely ant-pollinated species which grow consistently in hot dry areas are annuals. This suggests that more ant-pollinated species are to be found among the inconspicuous-flowered desert and mediterranean-climate annuals of the world.

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12. *Helianthus annuus* L.; *Mimulus guttatus* DC.
13. Range depends primarily on whether individual flowers or many-flowered compact umbels are considered the visual and functional pollination units, and secondarily on variable traits.
14. I thank S. A. Cook, P. R. Ehrlich, L. Gass, D. E. Gill, C. S. Hickman, R. W. Holm, D. H. Janzen, P. H. Raven, J. Roughgarden, and C. D. White for helpful comments on the manuscript; C. S. Hickman for assistance in the field; A. Francoeur for identification of the ants; and G. Savage for translations. The work was supported in part by a Sloan Foundation grant to Swarthmore College.

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"Decision"-Making in Bacteria: Chemotactic Response of Escherichia coli to Conflicting Stimuli

Abstract. *Motile bacteria presented simultaneously with both attractant and repellent respond to whichever one is present in the more effective concentration. Apparently bacteria have a processing mechanism that compares opposing signals from the chemoreceptors for positive and negative taxis, sums these signals up, and then communicates the sum to the flagella.*

What will a motile bacterium do if confronted simultaneously with a gradient of attractant (let us say increasing concentrations of attractant to the right) and a gradient of repellent (let us say increasing concentrations of repellent to the right)? In this "conflict" situation a bacterium must "decide" whether to pursue the attractant, ordinarily a nutritious chemical (*1, 2*), or flee from the repellent, ordinarily a harmful chemical (*3*).

Already in 1888 Pfeffer (*4*) reported that the relative strength of the two gradients determines whether attraction or repulsion will occur. He determined this microscopically by observing the entrance of bacteria into a capillary containing both attractant (KCl, peptone, or meat extract) and repellent (acid, base, or ethanol) at various concentrations. Work of Tsang *et al.* (*5*) (see below) supports this study.

Using an objective assay (*6*) based on Pfeffer's, we have confirmed and extended his report. *Escherichia coli* were exposed to a capillary tube containing both attractant and repellent, and then after an hour the number of bacteria that had entered the capillary tube was determined. For attractant we used L-aspartate, a chemical that is beneficial because it can be readily metabolized (*2*), and for repellent we used L-valine, a chemical that is harmful because it inhibits the growth of *E. coli* (*7*).

At a relatively low concentration ($10^{-6}M$) of aspartate, the bacteria fail to be attracted at relatively high concentrations ($10^{-1}M$) of valine (Fig. 1A). The repelling effect of $10^{-1}M$ valine can be largely overcome by increasing the concentration of aspartate to 10^{-4} to $10^{-3}M$ (Fig. 1B, left). However, at yet higher concentrations of aspartate (Fig. 1B, right) the repelling effect of valine reappears; this can be explained by saturation of the aspartate chemoreceptor, the sensing mechanism, making aspartate poorly detectable at these high concentrations (*8, 9*).

Similar results have been obtained with DL-α-methylaspartate, an attractant that is a nonoxidizable analog of aspartate (*2*), and with L-leucine, a repellent found to be harmless (*3*); hence the phenomena under study are not mechanistically related to benefit or harm. We have obtained such results also with other attractants, L-serine and D-galactose (*10*), and another repellent, acetate.

With valine present in both the bacterial suspension and the capillary tube at $10^{-2}M$ [the concentration found just inside the mouth of the capillary when the capillary is filled with $10^{-1}M$ valine and the pond contains none (*6*)], there was no inhibition of aspartate taxis; hence a gradient of repellent is required for inhibition of attraction, and the mere presence of repellent does not inhibit. A mutant that is not repelled by valine (*3*) failed to show the inhibition of aspartate taxis that is illustrated in Fig. 1A, even when the capillary contained $10^{-1}M$ valine; hence the inhibition cannot be ascribed to some nonspecific effect, but rather it is specific for repellents.

It appears that half-inhibition occurs when the concentrations of repellent and attractant both exceed their respective thresholds by the same factor. In Fig. 1A, half-inhibition occurs at $10^{-2}M$ valine and $10^{-6}M$ aspartate, both roughly 30-fold above threshold. Thresholds determined by this method are $2.5 \times 10^{-4}M$ and $6 \times 10^{-8}M$, respectively. In Fig. 1B (left), half-inhibition occurs at $10^{-1}M$ valine and $2 \times 10^{-5}M$ aspartate, roughly 350-fold