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FACULTATIVE MUTUALISM BETWEEN WEEDY VETCHES BEARING EXTRAFLORAL NECTARIES AND WEEDY ANTS IN CALIFORNIA[1]

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ABSTRACT

Vetches (*Vicia* spp.) were studied in the San Francisco Bay Area of California in the spring of 1978. The stipular nectaries of the vetches are visited by the Argentine ant, *Iridomyrmex humilis* Mayr. The nectaries were removed to exclude ants in controlled experiments to determine if these ants protect the vetches from herbivores or seed predators. Plants with excised nectaries suffered substantially greater damage to their foliage than control plants, indicating that ants protect the foliage. There was no indication that ants protect the vetches from seed predators, but fruit set was substantially lower in plants with excised nectaries. Analysis of sugar and amino acid composition of extrafloral nectar served as a basis for feeding tests with Argentine ants by using artificial nectar solutions. Ants preferred sucrose and glucose solutions over fructose. They showed no preference for any one sugar mixture over another, nor did they exhibit differential recruitment to artificial nectar solutions containing only sugars or sugars and amino acids.

VICIA SATIVA L. agg. (Fabaceae: Papilionoideae) comprises six or so specific segregates that vary nearly continuously (Hollings and Stace, 1978). At least two of the segregates, *V. sativa* sensu stricto and *V. angustifolia* L., have extrafloral nectaries. In vetches, these nectaries are on the stipules, and are often dark or reddish-purple. *V. angustifolia* also has very small, linear nectaries on the calyx teeth. Stipular nectaries secrete nectar from early in the ontogeny of a leaf until the leaf is mature; ants visit the nectaries even until the leaflets senesce and abscise. Ant visitation therefore accompanies flowering and fruit maturation at a given node.

Extrafloral nectar is secreted when the plants are in sunlight (first noted by Darwin, 1900). The nectaries are visited by bees, flies, small moths, and ants. In the San Francisco Bay Area, ants are by far the most abundant visitors to these nectaries, especially Argentine ants, *Iridomyrmex humilis* Mayr (Formicidae: Dolochoderinae) (Fig. 1). Other species of ants visit vetch nectaries in areas of California where *I. humilis* is not found.

The function of extrafloral nectaries has long been the subject of speculation and debate (as reviewed by Bentley, 1977). Most experimental studies of the ecological significance of extrafloral nectaries have been done with coevolved systems (i.e., with plants and ants native to the area in which they were studied). This experimental study involves two non-native, weedy species. *Vicia sativa* and *V. angustifolia* were introduced to California from Europe (USDA, 1960) as a forage crop for large mammalian herbivores, and have escaped from cultivation to become naturalized in grassy fields, along semidisturbed roadsides, and along residential streets in Berkeley and the Bay Area. *Iridomyrmex humilis* has been called a "tropicopolitan tramp" (Haskins and Haskins, 1965). It is native to Argentina and Brazil (Essig, 1926), and has spread successfully over a large portion of the southern United States, often displacing native ant species (Wheeler, 1910; Goetsch, 1957).

Few species of herbivores are found on the vetches in the Bay Area (occasional geometrid larvae; alfalfa weevils, *Hypera* sp.; and black bean aphids, *Aphis fabae*). Why are there so infrequently herbivores on the vetches? Plants frequently escape their coevolved herbivores in a new habitat. However, one generally finds other insects on a vetch when that particular plant has no ants on it. This suggests that the ants protect the plants against foliage-eating insects, or ovule and seed predators. The specific epithet "sativa" means "edible," and these vetches are known to be palatable to cows, sheep, horses, and deer; perhaps what the vetches lack in generalized chemical defense against insect herbivores is compensated by ant protection.

The preference of ants for various nectar constituents has received little attention. Ricks and Vinson (1970) did preference tests with the im-

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Fig. 1. Argentine ants visiting stipular nectaries of *Vicia sativa*. ×1.5.

ported fire ant, *Solenopsis saevissima*, by using crushed insect prey, solutions of sugar, vitamins, and amino acids. Taylor (1977) found that *Solenopsis geminata* was recruited at higher rates to sugar solutions when sugar concentration was increased, indicating the preference of ants for more concentrated sugar solutions. In my field experiments in the lowland wet forest of Panama, ants on *Inga* spp. (Mimosoideae) extrafloral nectaries preferred sucrose over hexose sugars at the same concentration, but preferred a more concentrated hexose solution over the weaker sucrose solution. This may correlate with the fact that extrafloral nectars are usually highly concentrated, and are frequently hexose dominated.

Baker and Baker (1977) suggested that nectar composition results from coevolutionary interactions between plants and nectar-feeding visitors. The preferences of groups of visitors have been characterized both by correlative means (analyzing many nectars and looking at the overall picture, e.g., Baker and Baker 1973a,b, 1975, 1977), and by experimental means (Wykes, 1952; Waller, 1972; Hainsworth and Wolf, 1977; Stiles, 1976). The preferences of ants in general have not been demonstrated. Extrafloral nectars vary dramatically in sugar composition, and in the array and concentration of amino acids they contain (Baker, Opler, and Baker, 1978). Carroll and Janzen (1973) state that extrafloral nectar as a food source is extremely generalized, and is eaten by "nearly any ant that encounters it." The preference of ants for different nectar constituents is most meaningfully investigated in a coevolved system; but it is also of interest to determine the preferences of a weedy scavenger, particularly if it occurs abundantly on a particular nectar resource.

MATERIALS AND METHODS—This work was conducted from April–June 1978, in Tilden Regional Park, Alameda Co., California. The experimental fields were approximately 2.4 km west of the Nature Center, in the southwest corner of the park. The flora of the study site consists predominantly of grasses interspersed with vetches, lupines, and mints. The field in which *Vicia angustifolia* was studied is well drained, on a slight slope, and is approximately 15 m × 20 m. The field in which *V. sativa* was studied is less well-drained, flatter, and measures 13 m × 18 m. The ant nectar preference tests were done in the first field.

The sprawling, climbing habit of *Vicia* did not allow straightforward exclusion of ants with tanglefoot. Instead, it was necessary to remove the nectaries. The stipules from all the leaves were excised on a number of plants (n = 30 for *V. angustifolia*, n = 20 for *V. sativa*). To control for the reduction in the amount of photosynthetic area (the stipules are green) and for any detriment caused by the cutting of leaf tissue (such as increased access for pathogens, or the release of an olfactory cue to herbivores), the lowest leaflets of each leaf on the same number of control plants were partially excised (about the same area as a stipule). Subsequent examinations of the plants revealed that ants were virtually absent from the plants with excised nectaries, save for an occasional random encounter as might be experienced by the other forbs and grasses in the field. The initial cutting coincided with initial blooming, and continued for a month (18 April–18 May). Plants were monitored every other day and the stipules or lowest leaflets of the new leaves were trimmed. After a month, I harvested the plants. I assessed folivory by tracing leaves on graph paper that had developed over the course of the experiment, shading the damaged areas, and expressing damage as a proportion of total leaf area. Leaves that had developed over the month were recognized because marker tags were initially placed three nodes below the apex.

To assess seed/ovule predation, the fruits from the same plants (*V. angustifolia* only, as *V. sativa* individuals were still largely vegetative) were collected and examined with a dissecting microscope for damage to maturing ovules and seeds. As with many weeds, these vetches are self pollinating. Virtually every flower will form a fruit without the aid of a pollen vector, as evidenced by both field and greenhouse-grown plants. The numbers of fruits, seeds, damaged fruits, and damaged seeds were recorded for each plant.

The extrafloral nectar of *V. angustifolia* was analyzed for sugars and amino acids by using techniques developed by I. Baker. Sugar concentration was measured with a pocket refractometer (Bellingham and Stanley brand, which reads weight of sugar/weight of solution). The sugars were separated and identified by descending paper chromatography, and their relative concentrations determined with fluorometry (Baker and Baker, 1979). Overall amino acid concentration was measured by a ninhydrin test, and the histidine scale was employed to translate the intensity of the ninhydrin response into approximate concentration of amino acids. Amino acids were separated by 2-dimensional thin-layered chromatography, and relative concentrations were determined with fluorometry (Baker and Baker, 1976). The results provided a recipe for artificial nectars used in feeding tests.

Feeding tests were performed in the field, by pairwise presentation of different solutions. In the first tests the preferences for individual sugar solutions of sucrose, glucose, and fructose were examined by comparisons of recruitment to the three 30% (weight/total weight) solutions of single sugars, n = 20 for each comparison. I placed a drop of each of two solutions on a small white card (3 cm × 3 cm) and set these cards on the ground. (The runs were short enough so that absorption of the solutions by the cards was not a problem.) Five cards were set out at one time, in a line, approximately 1 m apart. As an ant's first encounter may be random, I observed the cards for approximately one minute after discovery, and recorded which of the two solutions attracted more ants. Feeding tests also consisted of three different sugar mixtures (30% sugar) as described above. One solution was glucose dominated, as in nature, the second was a "balanced" nectar, and the third was sucrose dominated (see Table 4 for compositions). Finally, a comparison was performed of "complete" artificial nectar containing both sugars and amino acids in their natural concentrations and a solution containing only sugars.

RESULTS—*Ant exclusion experiments*—Leaf damage results—Damage proportions were used to generate means and variances for each experimental (with excised nectaries) and control group. The Wilcoxon rank sum test was applied to test the null hypothesis that there is no difference between experimental and control, versus the alternative that damage was greater in the experimental group (Table 1). For both species, the null hypothesis must be rejected. Plants with extrafloral nectaries removed suffered greater damage than control plants. (Statistical reference: Lehmann, 1975.)

The Spearman rank correlation test was used to examine the relationship between total leaf area and damage proportion. There was no correlation between total leaf area of a plant and the proportion of area damaged (a slight negative, though not significant, correlation was seen in both experimental groups; $P = .19$), indicating that plants were equally likely to receive a given amount of damage regardless of their size.

TABLE 1. *Leaf damage: effect of removal of extrafloral nectaries*

	Proportions of leaf area damaged		
	$\bar{x}$	s	n
Vicia angustifolia			
Plant with excised nectaries	0.091	0.045	30
Plants with intact nectaries	0.020	0.014	26

Wilcoxon statistic for controls: $w_c = 386$
Normal approximation: $z = -5.83$, $P < .001$

	Proportions of leaf area damaged		
	$\bar{x}$	s	n
Vicia sativa			
Plants with excised nectaries	0.052	0.039	18
Plants with intact nectaries	0.015	0.010	20

Wilcoxon statistic for controls: $w_c = 238$
Normal approximation: $z = -4.44$, $P < .001$

TABLE 2. *Fruit and seed set: effect of removal of extrafloral nectaries*

Vicia angustifolia	$\bar{x}$	s	n
Fruit set:			
Plants with excised nectaries	3.66 fruits/plant	1.63	29
Plants with intact nectaries	5.08	2.23	26

Wilcoxon statistic for controls = 885.5
Normal approximation: $z = 2.693$, $P \approx .004$
(This P-value is approximate as there were many observations of ties)

	$\bar{x}$	s	n
Seed set:			
Plants with excised nectaries	33.72 seeds/plant	18.69	29
Plants with intact nectaries	49.5	25.20	26

Wilcoxon statistic for controls = 888.5
Normal approximation: $z = 2.706$, $P \approx .004$

TABLE 3. *Sugar composition of V. angustifolia extrafloral nectar*

Overall sugar concentration ($\bar{x}$ of 5 samples)	30% by weight
Sucrose component	5.7%
Glucose	17.6%
Fructose	6.6%

Seed predation—The amount of seed and ovule predation on the vetches was extremely low. Of 228 fruits collected from the experimental and control plants, only two pods were damaged. The Wilcoxon rank sum test was used to compare the mean number of fruits and seeds set in the experimental and control groups (Table 2). Since vetch flowers are self pollinating, and all will set fruit under ideal greenhouse conditions, the proportion of flowers that set fruit (represented as the total number of seeds/plant) may be a measure of stress. Plants with excised nectaries had a substantially lower fruit set and seed set. Lower fruit and seed set may have resulted from lower total leaf area in the plants with excised nectaries, or from more damage to the plants.

The relationship between proportion of leaves lost and number of seeds produced was examined with Spearman rank correlation; no correlation was seen in either control ($r_s = -0.1775$, $P \gg .10$) or experimental ($r_s = -0.0920, P \gg .10$) groups. This may be the result of small sample sizes and high variance in the number of fruits per plant.

TABLE 4. *Tabulation of data and sign-test analysis of sugar preference tests*

Solutions (refer to list below)	n trials	# times one preferred	# times other preferred	Probability	Action
1 vs. 2	20	13	7	.132	accept H_0
1 vs. 3	20	17	3	.0017*	reject H_0
2 vs. 3	20	16	4	.006*	reject H_0
4 vs. 5	19	8	11	.324	accept H_0
4 vs. 6	20	8	12	.252	accept H_0
5 vs. 6	20	13	7	.132	accept H_0

*indicates significant differences between solutions

Solutions used in sugar preference tests:

1) 30% Sucrose
2) 30% Glucose
3) 30% Fructose
4) "Balanced" = 10% S:10% G:10% F
5) "Natural"(glucose-dominated) = 5.7% S:17.6% G:6.6%
6) Sucrose-dominated = 17.6% S:5.7% G:6.6% F

TABLE 5. *Amino acid composition of V. angustifolia extrafloral nectar*

Amino acids	Relative concentrations (all are ±)
Alanine	.003 μg/ml nectar
Arginine	.0275
Asparagine	.0124
Cysteine	.0137
Glutamine	.0851
Glycine	.0343
Lysine	.0645
Serine	.0192
Threonine	.0439
Tryptophan	.0206
TOTAL (overall amino acid concentration)	.3211 μg/ml nectar

Preferences of ants for artificial nectars with varying constituents—The sugar constitution of the extrafloral nectar of *Vicia angustifolia* appears in Table 3. The results of the sugar preference tests are presented in Table 4. In comparisons with solutions of single sugars, the sign test examined the null hypothesis that there is an equal probability of the ants being recruited to either sugar solution in a pair, versus the alternative that recruitment is greater to either one. (Ties were eliminated.) Sucrose and glucose were preferred over fructose ($P = .002$ and $P = .006$, respectively); no preference appears between sucrose and glucose. Furthermore, no mixture was preferred over the other.

The amino acid constitution of the extrafloral nectar of *V. angustifolia* is shown in Table 5. Ants showed no substantial preference for the "complete" artificial nectar over the one containing sugars only. In 25 trials there was a preference for the sugar solution, 34 trials showed preference for the amino acid and sugar solution, and 1 trial was a tie. (Normal approximation to sign statistic $z = -1.17$, $P = .121$.)

DISCUSSION—Argentine ants are aggressive towards other insects, being entomophagous as well as nectarivorous (Creighton, 1950, and pers. obser.). Although not coevolved with the weedy vetches, the ants utilize the extrafloral nectar and decrease the amount of herbivory by insects. It was not determined in this study whether insects are actively deterred by the Argentine ants, or whether the mere presence of the ants may discourage other insects from alighting on the "occupied" plants. Differences in the amount of leaf damage between plants with excised nectaries and control plants provide evidence that the aggressive display need not be flagrant, nor the system tightly coevolved, to afford some protection to a plant with extrafloral nectaries. B. Bentley

(1976) found that there are more plants with extrafloral nectaries growing in disturbed areas. Subsequent studies may demonstrate that facultative mutualism is common in colonizing and weedy extrafloral-nectaried plants. Vetches may be successful weeds, not only because of their ability to set lots of seed without outcrossing but also by their ability to attract a biotic defense in non-native locations.

The amount of damage experienced by plants with excised nectaries may not be enough to limit the establishment and success of the vetches in the Bay Area in the absence of ant protection. However, an individual plant may lose as much as 19% of its total leaf area to herbivores (although the average is 9% loss), which may result in lower seed set (a component of fitness) for that individual.

There is little predation upon seeds or ovules of *V. angustifolia* in the Bay Area; whether or not Argentine ants protect weedy vetches against seed predators can therefore not be ascertained. The nectaries function during the time of flower development, and the spatial location of the stipules (and nectaries) is very close to the bases of the sessile, axillary flowers, suggesting that ants may protect ovules and seeds in native habitat of the vetches.

Argentine ants find glucose as acceptable as sucrose. This may be advantageous from the plant's perspective, for glucose is metabolically less expensive than sucrose. If the ants will imbibe a glucose-dominated nectar as readily as a sucrose-dominated nectar, the former will presumably be selected. Amino acids are present in this extrafloral nectar in low concentrations compared with some extrafloral nectars (Baker, Opler, and Baker, 1978), whereas the sugars are highly concentrated. The fact that the ants did not distinguish between the artificial nectar with amino acids from that without amino acids is perhaps the result of these ants acting as opportunistic foragers, and the attractiveness of sugar outweighs any subtle nutritional differences.

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