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## Ant visitation of extrafloral nectaries of *Passiflora*: the effects of nectary attributes and ant behavior on patterns in facultative ant-plant mutualisms

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**Abstract** Extrafloral nectary (EFN) plants are widespread and can be quite species-rich in some communities. Thus, ants that utilize extrafloral nectar may have the opportunity to discriminate among a wide variety of nectar sources, resulting in variation in the ant attention EFN plants receive. In this study, we compare ant visitation rates of three *Passiflora* species that coexist in an early successional neotropical forest. These three vine species (*Passiflora auriculata*, *P. biflora*, and *P. oerstedii*) differ in their extrafloral nectary structure and placement, and thus may attract different numbers or species of ants. Through censuses of ants tending extrafloral nectaries, we found that *P. auriculata* received significantly higher numbers of ant visitors than *P. oerstedii*, but did not differ significantly from *P. biflora* in its attractiveness to ants. We also found that termite worker baits (simulating herbivores) placed on *P. auriculata* and *P. biflora* were discovered by ants significantly more quickly than baits placed on *P. oerstedii*. In both ant visitation censuses and in termite bait trials, we found no significant associations between *Passiflora* species and the species of ant visitors. We also performed experimental manipulations of several characteristics of *P. auriculata*, which resulted in changes in levels of ant visitation. When petiolar nectaries of *P. auriculata* were experimentally blocked, visitation by the common ant *Eciton ruidum* declined, even though nectaries on the leaf surfaces were still functional. Connections with other vegetation also had an effect on ant visitation. Though experimental creation of connections between growing *P. auriculata* shoots and other vegetation did not enhance ant visitation, eliminating connections resulted in a significant decline in the number of ant visitors. The results

of this study suggest factors that may contribute to variation in ant visitation of extrafloral nectary plants. In addition, this study demonstrates that extrafloral nectary plants co-occurring in a habitat and available to the same ants may differ in patterns of visitation by ants and perhaps in the quality of protection from herbivores that they receive.

**Keywords** Ants · Extrafloral nectaries · Facultative interactions · *Passiflora* · Mutualism

### Introduction

The prevalence and variety of interactions between ants and plants with extrafloral nectaries (EFNs) have been documented by researchers for over 20 years. The majority of these studies focus on the benefits conferred to the plant by the presence of ants attracted to its nectar-producing structures. These positive effects include a reduction in damage from folivores (Bentley 1976; Koptur 1979; Stephenson 1982; Koptur 1984; Kelly 1986; Koptur et al. 1998; de la Fuente and Marquis 1999) and increased seed or fruit set (Bentley 1977; Schemske 1980; Horvitz and Schemske 1984). However, much variation exists in the outcomes of these interactions. The magnitude of the effects of ants on EFN plants can depend on the intensity of herbivore pressure (Inouye and Taylor 1979; Barton 1986; de la Fuente and Marquis 1999), environmental conditions (Kelly 1986), the composition of the local ant fauna (Horvitz and Schemske 1990), and the abundance of ants (Bentley 1976; Inouye and Taylor 1979; Koptur 1985; Barton 1986). Most studies, however, have not considered the community-wide availability of EFN and other carbohydrate resources that may affect patterns in foraging among ants and the relative attractiveness to ants of different EFN plant species (but see Schupp and Feener 1991; Rico-Gray 1993; Rico-Gray et al. 1998).

A wide variety of EFN sources may be available to ants, giving them the opportunity to exhibit preferences

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among various nectar sources in their territories. In a survey of a dry tropical lowland site in Veracruz, Mexico, Rico-Gray (1993) found 30 ant species and 102 plant species in 312 associations, 40% of which involved EFNs on leaves, stems, or reproductive structures. Schupp and Feener (1991) surveyed 243 plant species on Barro Colorado Island in Panama and found that 32% had EFNs on non-reproductive structures. In the presence of such variety in carbohydrate sources for ants, EFN plants may compete for ant attention. Differences in plant attributes may determine which plants receive the most ant visitors, and possibly the best protection from herbivores.

In an early successional neotropical forest, we compare aspects of ant attendance of three common *Passiflora* vine species that differ in their extrafloral nectary structure and placement. These three species – *Passiflora auriculata*, *P. biflora*, and *P. oerstedii* – may vary in their ability to attract tending ants. In a study at La Selva Biological Station in Costa Rica, Smiley (1978) characterized the attraction of ants to *P. auriculata*, *P. biflora*, and *P. oerstedii* as high, intermediate, and low, respectively, based on the proportion of times he observed plants with ants and on differences in their extrafloral nectary structures. Plants that are visited by a greater number of ants, or that are associated with ants for a greater proportion of time, may receive superior protection against herbivores.

Ant numbers or the consistency of an ant presence are not the only factors determining the quality of protection that ants may provide: the identity of tending ants is important as well. Interactions between ants and extrafloral nectary plants are notoriously general in nature: for example, Longino (1984) counted 85 ant species among visitors to *P. pittieri*, and Smiley (1986) recorded 29 species of ants at nectaries of *P. vitifolia* and *P. quadrangularis*. However, ant species can differ in their effectiveness as defense against herbivores and seed predators (Schemske 1980; Horvitz and Schemske 1984; Rico-Gray and Thien 1989), and a plant potentially could benefit from adaptations to attract the most effective ants. Schemske (1980) found that while six ant species visited nectaries of *Costus woodsonii*, *Wasmannia auropunctata* was by far the most effective in defense against a dipteran seed predator because its small size allowed it to forage beneath the bracts of the inflorescence and feed on the fly larvae. An EFN plant that produces small but predictable rewards may be able to attract a reliable force of smaller, less aggressive ant species without risk of their exclusion by more competitive but perhaps less effective ants. Schemske (1980) suggested that the low nectar secretion rate of *C. woodsonii*, compared to other *Costus* species, may be such an adaptation to attract smaller ants.

Factors other than inherent plant attributes and heterogeneity in ant communities may also affect patterns in visitation. Bentley (1981) hypothesized that the vine life-form may enhance the success of ant-EFN plant interactions: connections with other vegetation provide more

paths for ant foraging trails and perhaps increase the frequency of ant visitation. Only by experimentally testing for such effects can we determine whether these other factors contribute to variation in ant visitation of EFN plants.

Most studies of ants and EFN plants focus on only one species of plant; thus, little information is available about the effects of differences in nectary structure or nectar production among EFN plants on the plants' relative abilities to attract various species of ants. A number of studies of extrafloral nectary plants have documented within-species variation in the species composition of ant visitors (Barton 1986; Rico-Gray and Thien 1989; Horvitz and Schemske 1990), but very few have attempted to quantify or explain patterns in such intraspecific variation or identify between-species differences. An exception is Schemske's (1982) investigation of ant assemblages on four *Costus* species in Panama. He found that the percent difference in species composition of ants visiting *Costus* ranged from 52% to 84% for pairs of plant species.

The assemblage of *Passiflora* species at La Selva, Costa Rica, constitutes an excellent system in which to compare species with different extrafloral nectary arrangements with respect to their abilities to attract different ant species. In this study we address the following questions: (1) Do *P. auriculata*, *P. biflora*, and *P. oerstedii* differ in the frequency with which they are attended by ants and in the species of ants they attract? (2) Do these plants differ in their potential to receive protection from herbivores by ants? (3) How do manipulations of extrafloral nectaries and connections with other vegetation affect visitation by ants?

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## Materials and methods

### Study site and species

We conducted this study at the La Selva Biological Station in Costa Rica from 1 to 29 September 1995. We worked in the successional strips, a 2.5-ha area maintained by the station to represent forested areas in the first 1–5 years of succession. Every year, the vegetation in one-fifth of the area is cut and left to regenerate naturally until 5 years later when it is cut again (Hartshorn and Hammel 1984). Our three study species, *P. auriculata*, *P. biflora*, and *P. oerstedii* all occur in this early successional habitat, primarily in the 1- to 3-year-old strips. These three species of vines differ substantially in their EFN structures and locations. *P. auriculata* has a pair of relatively large (1–1.5 mm diameter), cup-shaped nectaries on each petiole, as well as small, flat nectaries on the underside of each leaf. *P. biflora* has small (1 mm diameter), flat nectaries on the lower surfaces of its leaves. *P. oerstedii* has about six very small nectaries on 1- to 2-mm stalks on each petiole.

### Ant visitation censuses

To determine the frequency with which each of these *Passiflora* species is attended by ants, we conducted 2-day censuses of ant visitors on subsets of the plants. To help distinguish differences in ant visitation due to plant characteristics from differences due to spatial heterogeneity in habitat and ant communities, we grouped plants of different species into pairs (*P. auriculata* and *P. biflora*

or *P. auriculata* and *P. oerstedii*). The members of these pairs were never separated by more than 5 m. The proximity of the paired plants guaranteed that they were surrounded by similar vegetation and subjected to similar microclimatic conditions. We could not form *P. biflora*-*P. oerstedii* pairs or groups of all three species because *P. biflora* and *P. oerstedii* infrequently occurred within 5 m of each other. Ant visitation was examined only on the growing end of a single shoot on each plant, from the shoot tip to the fifth leaf from the end. Since observations were made on similarly sized units of each plant, and all of the plants were tangled with the surrounding vegetation, the absolute size of each plant should not have affected our results and thus was not considered in our selection of pairs.

We visited each pair of plants every 30 min from 0700 to 1700 hours over 2 days and recorded the numbers and identities of ants observed at the instant of our arrival at each plant. We discontinued observations during episodes of rain that caused ant visitation to slow or cease. Six to 11 pairs were observed during each 2-day census, but some pairs were eliminated from our study due to the poor condition of 1 or both members. In our analysis, we included a total of 14 *P. auriculata*-*P. biflora* pairs and 14 *P. auriculata*-*P. oerstedii* pairs; for each pair of plants we recorded a total of 26–31 observations, depending on the frequency and duration of rain. Because downpours often interrupted census periods, observations were missed at different times of days 1 and 2 of each census for different sets of pairs. (For example, *P. auriculata*-*P. oerstedii* pairs 2–4 censused on 6/7 September were observed over different time intervals than pairs 5–11 censused on 9/10 September.) Standard methods of repeated measures analysis of variance require that every subject be observed over the same number of time intervals (Milliken and Johnson 1984). To satisfy this condition, we excluded from our analysis all time intervals for which we did not have observations from all the pairs. This exclusion left 15 time intervals in which we had observations from all 28 pairs of plants; these measures were used in a repeated measures ANOVA to compare the numbers of ants visiting shoots of each of the *Passiflora* species, treating pairs (either *P. auriculata* and *P. biflora*, or *P. auriculata* and *P. oerstedii*) as blocks. To compare the constancy with which each species was attended by ants, we performed Wilcoxon signed ranks tests on the proportion of all observation periods in which ants were present on each plant. All statistical analyses, unless otherwise indicated, were performed using SYSTAT for Windows, version 6.0.1 (SYSTAT 1996).

#### Termite bait trials

To evaluate the extent to which each of these *Passiflora* species attract effective ant guards, we compared how quickly ants discovered baits of workers of the termite *Nasutitermes* placed on each plant to simulate potential herbivores. Researchers have used this method to compare the potential antiherbivore activity of ants on plants with and without extrafloral nectaries (Bentley 1981; Barton 1986; Oliveira et al. 1987; Oliveira 1997), and to describe the patrolling activity of ants in different locations within a plant (Bentley and Benson 1988; Freitas and Oliveira 1996). While this method cannot predict the effectiveness of ants in deterring particular herbivores, it does provide a way of evaluating whether the activity of ants on a plant's surface improves the likelihood that a herbivore on the plant will encounter ant aggression. Using white glue, we attached live termite workers at five different locations (tendrils, stem, leaf, petiole, and shoot tip) on each individual plant. We checked each plant every 10 min over a 1-h period and recorded when or if each termite was removed and, if possible, by what ant species. We performed bait trials on nine of each of the *P. auriculata*-*P. biflora* and *P. auriculata*-*P. oerstedii* pairs used in the ant visitation census and on an additional four individuals of both *P. biflora* and *P. oerstedii*. We tested the effects of *Passiflora* species and bait location on the time of bait discovery using the Cox proportional hazards model for failure-time analysis in JMP version 3 (SAS Institute 1994).

#### Patterns in ant associations

We used  $\chi^2$  contingency table analyses to test for associations between *Passiflora* species and particular ant species during censuses and termite bait trials; estimation of *P* values with Monte Carlo procedures in the StatXact-Turbo nonparametric statistics package permitted us to perform this analysis on our sparse data sets (Mehta and Patel 1992). To create the  $\chi^2$  contingency tables, we tallied the number of individuals of each *Passiflora* species with which each ant taxon was associated in the census or bait trials. We considered an ant species or genus to be associated with a particular plant individual in our ant visitation censuses if we recorded its presence in more than one-third of the time intervals in which the plant had any ant visitors. Though arbitrary, we considered this condition a reasonable way to define the principal ant visitors to each plant, and both restricting and relaxing this prerequisite yielded similar results. For the termite bait trials, the ant species that discovered the most termite baits on a plant was considered to be associated with that individual. Because samples of ants could be collected only at the end of the 2-day censuses or bait trials to avoid interference with ant activity, we were unable to get specimens of all ants and relied on identifications in the field to the genus level (if possible) for this analysis. Voucher specimens will be deposited at the Museum of Comparative Zoology at Harvard University in Cambridge, Mass.

#### Manipulation of extrafloral nectaries

*P. auriculata* differs in its nectary arrangement from other *Passiflora* species in this study in having larger, cup-shaped nectaries on its petioles in addition to small, flat nectaries on the lower surfaces of its leaves. To evaluate the role of the petiolar nectaries of *P. auriculata* in attracting ants, we compared visitation by *Ectatomma ruidum* on growing shoots with experimentally blocked nectaries to visitation on control shoots. We focused on the relatively large, ground-nesting ant *E. ruidum* in this experiment because it is one of the most common ants at this site and is a frequent visitor to *P. auriculata* shoots, especially those found near the ground. Though its effectiveness in deterring herbivores has not been demonstrated conclusively, *E. ruidum* was the second most common ant species found on an EFN plant, which was shown to suffer less damage from herbivores and pathogens in the presence of ants (de la Fuente and Marquis 1999). Prior to the manipulation, we censused 15 *P. auriculata* plants frequently observed with *E. ruidum*; for this experiment, we used only plants which were visited by *E. ruidum* in at least 75% of the observations. Petiolar nectaries on seven randomly selected plants were blocked by coating them with clear, quick-drying nail polish. On eight control plants, nail polish was applied to the petiole but not to the nectary itself, to control for any repellent effects. After the manipulation, we censused plants for *E. ruidum* visitors every 30 min between 0700 and 1700 hours (excluding rainy episodes) over a 2-day period. We used repeated measures ANOVAs to examine how the number of ants visiting each set of control and experimental plants varied over time, and compared time intervals before and after the manipulation with an a priori contrast.

#### Manipulation of vine connectedness

To assess the influence of connections with other vegetation on the amount of ant visitation experienced by *P. auriculata*, we experimentally eliminated or created connections between growing shoots of *P. auriculata* and other plants. We censused ant visitors on 23 experimental plants at 30-min intervals (excluding periods of rain) for several days prior to the manipulations. Plants with free, unconnected growing shoots ( $n=12$ ) were manipulated by bringing them into contact with other vegetation using twist-ties. Plants in contact with other vegetation ( $n=11$ ) were manipulated by using Tanglefoot to eliminate access to the shoots from other plants. We continued to census for ant visitors at 30-min intervals

(between 0700 and 1700 hours) for 2 days after the manipulations. Again, we used repeated measures ANOVAs to examine how the number of ants on each plant varied over time, and compared time intervals before and after the manipulations using a priori contrasts.

## Results

### Ant visitation censuses

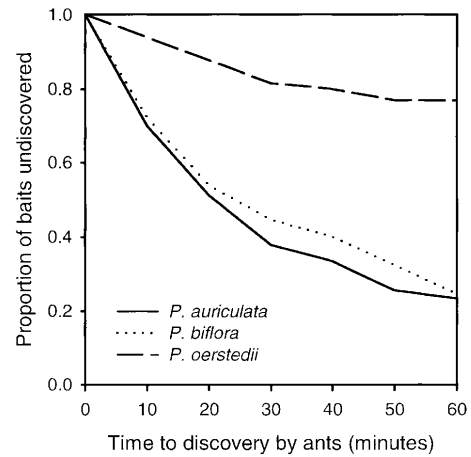
The number of ants on *P. auriculata* and *P. biflora* plants throughout census periods did not differ significantly (repeated measures ANOVA,  $F_{1,13}=0.155$ ,  $P=0.701$ ; Table 1). We also did not find a significant difference between *P. auriculata* and *P. biflora* in the proportion of observations in which ants were present on the shoots (Wilcoxon signed ranks test:  $Z=-1.29$ ,  $P=0.197$ ). *P. auriculata*, however, received more ant visitors over census periods than did *P. oerstedii* (repeated measures ANOVA,  $F_{1,13}=22.404$ ,  $P<0.001$ ). Also, the mean proportion of observations in which ants visited *P. auriculata* was significantly greater than that for *P. oerstedii* (Wilcoxon signed ranks test:  $Z=-3.297$ ,  $P<0.001$ ).

### Termite bait trials

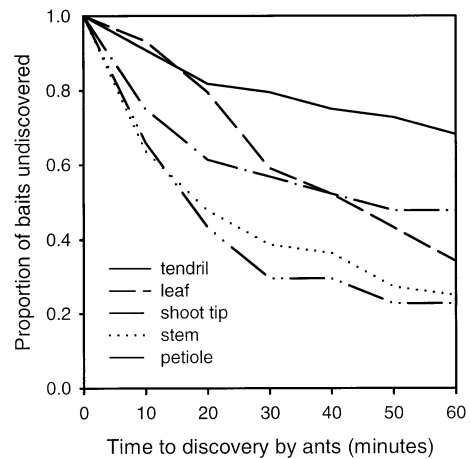
According to failure-time analysis, both *Passiflora* species and bait location were correlated with time to discovery of termite baits by ants (*Passiflora* species:  $\chi^2=53.89$ ,  $df=2$ ,  $P<0.001$ ; bait location:  $\chi^2=30.15$ ,  $df=4$ ,  $P<0.001$ ; Figs. 1, 2). Baits were discovered significantly faster on *P. auriculata* and *P. biflora* than on *P. oerstedii*. However, when *P. oerstedii* was excluded from the analysis, the species effect was no longer significant ( $\chi^2=0.0283$ ,  $df=1$ ,  $P=0.866$ ), indicating that discovery times did not differ for *P. auriculata* and *P. biflora*. Combining results for all three species, baits on the petioles and stems were discovered the fastest, while baits on tendrils tended to remain undiscovered at the end of the trials. There was no significant interaction between bait location and *Passiflora* species ( $\chi^2=5.58$ ,  $df=8$ ,  $P=0.694$ ).

### Patterns in ant associations

Visitors to plants in the ant visitation censuses and termite bait trials included the following ants: *E. ruidum*, *E. tuber-*



**Fig. 1** Relationship between time and discovery by ants of termite baits placed on shoots of three *Passiflora* species



**Fig. 2** Relationship between time and discovery by ants of termite baits placed at different locations on shoots of three *Passiflora* species. Solid line = tendril, dashed line = leaf, dash-dot-dash line = shoot tip, dotted line = stem, dash-dot-dot-line = petiole

*culatum* (Ponerinae), *Crematogaster*, *Pheidole*, *Solenopsis*, *W. auropunctata* (Myrmicinae), *Pseudomyrmex* (Pseudomyrmecinae), *Brachymyrmex*, *Camponotus*, *Paratrechina* (Formicinae), and *Tapinoma* (Dolichoderinae) (Table 2). Most ants could only be identified to genus level in the field. Collections of ants made when possible indicate that some genera were represented by more than one morphospecies, which were not distinguished in field observations (S.P. Cover, personal communication). We

**Table 1** Ant visitation of *Passiflora* shoots over census periods. Values are means $\pm$ 1 SEM

	<i>P. auriculata</i> - <i>P. biflora</i> pairs		<i>P. auriculata</i> - <i>P. oerstedii</i> pairs	
	<i>P. auriculata</i>	<i>P. biflora</i>	<i>P. auriculata</i>	<i>P. oerstedii</i>
Number of ants present per observation	7.58 $\pm$ 3.65	5.70 $\pm$ 2.69	4.66 $\pm$ 0.97**	0.28 $\pm$ 0.13
Proportion of observations in which ants were present	0.94 $\pm$ 0.02	0.91 $\pm$ 0.03	0.87 $\pm$ 0.05**	0.17 $\pm$ 0.04

\*\* Indicates significant difference between species within pairs at  $P<0.01$ , Wilcoxon signed ranks test

**Table 2** Number of plants of each *Passiflora* species visited by common ant species or genera identified in the field

	Ant visitation censuses			Termite bait trials		
	<i>auriculata</i>	<i>biflora</i>	<i>oerstedii</i>	<i>auriculata</i>	<i>biflora</i>	<i>oerstedii</i>
<i>Brachymyrmex</i> spp.	5	2	2	0	0	0
<i>Camponotus</i> spp.	2	0	2	0	1	0
<i>Crematogaster</i> spp.	10	5	6	3	3	4
<i>Ectatomma ruidum</i>	4	2	1	4	1	1
<i>Ectatomma tuberculatum</i>	4	0	0	2	1	0
<i>Pheidole</i> spp.	1	0	0	0	0	0
<i>Pseudomyrmex</i> spp.	1	1	0	0	0	1
<i>Solenopsis</i> spp.	2	1	1	0	2	0
<i>Wasmannia auropunctata</i>	11	5	1	7	3	0
Unidentified species, medium	7	6	3	1	2	0
Unidentified species, small	3	2	1	0	0	0

**Table 3** Visitation of *Passiflora auriculata* shoots by *Ectatomma ruidum* before and after blocking petiolar extrafloral nectaries. Values are means $\pm$ 1 SEM

	Control shoots		Experimental shoots	
	Before manipulation	After manipulation	Before manipulation	After manipulation
Number of ants present per observation	2.29 $\pm$ 0.34	2.16 $\pm$ 0.25	2.31 $\pm$ 0.55*	0.78 $\pm$ 0.18
Proportion of observations in which ants were present	0.92 $\pm$ 0.03	0.91 $\pm$ 0.03	0.88 $\pm$ 0.03*	0.53 $\pm$ 0.10

\* Indicates significant difference between values before and after manipulations at  $P < 0.05$ , Wilcoxon signed ranks test

**Table 4** Visitation of *Passiflora auriculata* shoots by ants before and after manipulations of connections with surrounding vegetation. Values are means $\pm$ 1 SEM

	Connections created		Connections eliminated	
	Before manipulation	After manipulation	Before manipulation	After manipulation
Number of ants present per observation	5.57 $\pm$ 1.49	8.48 $\pm$ 2.23	7.11 $\pm$ 2.35	5.36 $\pm$ 1.87
Proportion of observations in which ants were present	0.90 $\pm$ 0.03	0.93 $\pm$ 0.03	0.94 $\pm$ 0.03*	0.78 $\pm$ 0.07

\* Indicates significant difference between values before and after manipulations at  $P < 0.05$ , Wilcoxon signed ranks test

found no significant associations between *Passiflora* species and the identities of ant visitors (classified into the groups listed in Table 2) in our ant visitation censuses ( $\chi^2=12.86$ ,  $df=20$ ,  $P=0.916$ ). Also, the identities of ants discovering termite baits did not differ significantly across species ( $\chi^2=20.43$ ,  $df=14$ ,  $P=0.083$ ).

#### Nectary-blocking experiment

Visitation by *E. ruidum* dropped when petiolar nectaries of *P. auriculata* were blocked (Table 3). The number of *E. ruidum* visitors on blocked-nectary plants varied significantly with time interval (repeated measures ANOVA,  $F_{42,252}=3.910$ ,  $P < 0.001$ ); a priori contrasts confirmed that this significant time effect was largely due to differences in the numbers of visitors before and after the manipulation ( $F_{1,6}=6.264$ ,  $P=0.046$ ). While *E. ru-*

*idum* visitation of control plants varied significantly with time as well (repeated measures ANOVA,  $F_{48,336}=3.342$ ,  $P < 0.001$ ), this effect could not be traced to differences between the numbers of visitors before and after applying the control treatment ( $F_{1,7}=1.561$ ,  $P=0.252$ ). Control and blocked-nectary plants did not differ significantly in ant visitation before the manipulation (repeated measures ANOVA,  $F_{1,13}=0.001$ ,  $P=0.973$ ), but they did differ significantly after the manipulation (repeated measures ANOVA,  $F_{1,13}=17.839$ ,  $P=0.001$ ).

#### Connections experiment

Creating connections on free-hanging *P. auriculata* vines did not significantly enhance ant visitation (Table 4). Following a repeated measures ANOVA, a priori contrasts comparing time intervals before and after the ma-

nipulation suggested that creating connections had no significant effect on the numbers of ants on the plants ( $F_{1,11}=1.801$ ,  $P=0.207$ ). Elimination of connections resulted in at least a marginally significant drop in ant visitation (Table 4). On these experimental plants, the numbers of ant visitors varied significantly with time (repeated measures ANOVA,  $F_{54,540}=2.823$ ,  $P<0.001$ ). A priori contrasts comparing the number of ant visitors in time intervals in day 2 of the census before the manipulation and in day 2 of the census after the manipulation revealed a marginally significant effect of severing connections ( $F_{1,10}=4.305$ ,  $P=0.065$ ). In addition, the mean proportion of observations with ants dropped significantly after connections were eliminated (Wilcoxon signed ranks test:  $Z=-2.501$ ,  $P=0.012$ ).

## Discussion

The results of the ant visitation censuses and termite bait trials are generally consistent with Smiley's (1978) predictions based on EFN structure: *P. auriculata*, with its cup-like petiolar nectaries and scattered leaf surface nectaries, and *P. biflora*, with its numerous flat nectaries on the leaf surface, experienced similar levels of ant visitation and exhibited higher rates of termite bait removal than did *P. oerstedii*, which has fewer, smaller, and perhaps less accessible nectaries. Most likely because of its larger and more numerous EFNs, *P. auriculata* received many more ant visitors than *P. oerstedii*. In contrast to our findings, Smiley (1978) reported a lower frequency of ant visitation to *P. biflora* than to *P. auriculata*. His sampling of *P. biflora* included individuals from habitats other than the successional strips, however.

By comparing ant visitation within pairs of nearby individuals of each species, we minimized differences in ant visitation created by spatial heterogeneity in the ant community. Our results suggest that differences in plant characteristics may explain differential visitation of *P. auriculata* and *P. oerstedii*. Nectary structure is one plant attribute that might influence attractiveness to ants, but other more subtle differences, like nectar secretion rates, timing of secretion, or nectar composition, may also account for variation in ant visitation. These characteristics were not measured directly in this study. Nectar secretion rates per unit leaf area may be correlated with the sizes and abundances of EFNs: the smaller, less abundant EFNs of *P. oerstedii* may provide a smaller total reward per unit time than do the larger, more abundant EFNs of *P. auriculata*. Several studies have documented ant preferences for nectars of varying composition (Lanza 1988, 1991). Evaluating the relative importance of different plant characteristics in attracting ants will require more experiments and accurate means of measuring nectar quantity and quality in the field.

We were unable to identify any consistent differences in the identities of ant visitors to these three *Passiflora* species. Smiley (1978) also did not find any associations between particular ant species and eight *Passiflora* spe-

cies, although he did find differences among habitats in the ants visiting *Passiflora*. The ants we did observe on plants varied substantially in their behavior: some ants, like *Wasmannia* and *Crematogaster*, recruit heavily to termite baits, while others, like *Ectatomma* and *Pseudomyrmex*, are solitary foragers. Ants with such different foraging strategies may vary in effectiveness against different types of herbivores. Such differences also must be kept in mind when using the numbers of ants as a gauge of the degree of ant protection experienced by the plant: while the number of ant visitors may provide a rough index of the plant's attractiveness, knowing the identity of the ants is crucial to evaluate the value of their presence to the plant. Finding patterns in the identities of ants associated with different plant species, if such patterns indeed exist, will require much more extensive sampling than was possible in this short-term study.

The nectary-blocking experiment suggests a role for particular nectary structures in attracting particular types of ants. Visitation by *E. ruidum* decreased when petiolar nectaries were blocked, even though leaf surface nectaries continued to be functional. Perhaps such large conspicuous nectaries are more effective in attracting larger ants to the plants than are smaller less obvious EFNs: without them, constant visitation by such ants may not be maintained. The decline in visitation to these plants that continued to produce nectar suggests that these ants may be selecting the most rewarding nectar sources in their environment and will discriminate against plants with inferior levels of production.

*P. oerstedii*'s possession of extrafloral nectaries seems puzzling in light of infrequent visitation by ants and low rate of discovery of termite baits. Gilbert (1977) and Smiley (1978) suggested that such small nectaries in some *Passiflora* species may have the role of attracting parasitoid insects rather than ants. Indeed, parasitoid wasps can be frequent visitors of EFN plants (Hespenheide 1985; Pemberton and Lee 1996), especially in areas of low ant abundance (Koptur 1985). In fact, Smiley (1978) documented higher rates of egg parasitism of the *Passiflora*-specialist *Heliconius* (Lepidoptera) on *P. oerstedii* than on any other *Passiflora* species in his study at La Selva. We did not note substantial visitation by insects other than ants in our censuses, but detection of such visits might require more sustained periods of observation rather than brief checks of plants at regular time intervals.

The reduction in ant visitation following the elimination of connections to other plants suggests that factors extrinsic to characteristics of *Passiflora* individuals may also affect patterns in ant visitation. The decreased visitation we observed after eliminating connections with surrounding vegetation might disappear with time as ants discovered alternative routes to the nectaries (i.e., along the main shoot instead of from surrounding plants). Our failure to see increased visitation after creating connections may suggest that the number of bridges to plants was not limiting visitation; in fact, we observed ants on these plants in 90% of the time intervals before the ma-

nipulation. Alternatively, our censuses might not have allowed enough time for ants to discover the new bridges, or creation of connections in different locations may have been more effective.

The benefits that extrafloral nectaries may confer to *Passiflora* species have yet to be established clearly. Longino (1984) found that ants had a relatively small effect on growth and herbivore loads of *P. pittieri* in Costa Rica. However, the presence of dominant ant species, particularly *W. auropunctata* and *Camponotus sericeiventris*, was significantly associated with successful shoots in the field. Smiley (1985, 1986) found that ant presence was responsible for substantial mortality of *Heliconius melpomene* and *H. ismenius* caterpillars placed on *Passiflora* plants. McLain (1983) demonstrated benefits from ant visitation in a temperate *Passiflora* species: plants with nectaries removed experienced a lower fruit set and higher herbivory than did plants with intact nectaries. In this study, the removal of termite baits by a number of different ant species indicates that attendant ants can respond to foreign organisms that alight on their plants; whether they would exhibit the same aggressive response to actual herbivores (i.e., chrysomelid beetles, lepidopteran larvae, Orthoptera) that might not represent as attractive a prey item as a relatively immobile and vulnerable termite worker cannot be determined from this experiment.

The three ant genera most commonly observed on these *Passiflora* species – *Crematogaster*, *Wasmannia*, and *Ectatomma* – have been credited with the ability to deter herbivores in other studies. *W. auropunctata*, *E. ruidum*, and an unidentified *Wasmannia* species were the three most consistent visitors of the EFN plant *Stryphnodendron microstachyum* at La Selva, which experienced less herbivore damage and a higher growth rate in the presence of ants than when ants were excluded (de la Fuente and Marquis 1999). Altshuler (1999) found that the presence of *E. ruidum* and *E. tuberculatum* on inflorescences of *Psychotria limonensis* in Panama resulted in higher pollination success, presumably because harassment from ants forced pollinators to make more frequent, shorter trips between flowers; ant attendance also resulted in less damage to developing fruits by insect herbivores. Rico-Gray and Thien (1989) documented a decline in damage to inflorescence spikes from a curculionid beetle in the presence of *E. tuberculatum*. In Corcovado National Park, Costa Rica, Smiley (1986) found that *Heliconius* larvae placed on *Passiflora* occupied by *E. tuberculatum* or *Crematogaster erecta* had a >60% chance of mortality. Ant exclusion experiments are needed to evaluate the effects of ant visitation on the *Passiflora* examined in our study, but such exclusions may be especially difficult to maintain on these fast-growing vines in dense second-growth vegetation.

The results of this study demonstrate that EFN plants sharing the same habitat and accessible to the same members of the ant community may differ in patterns of visitation by ants. Differential ant visitation may be attributed to differences in nectary characteristics, but the

role of nectary structure, secretion rate, and nectar composition in determining attractiveness of these *Passiflora* species to ants has yet to be determined. The fact that consistent differences in visitation exist across plant species emphasizes the importance of placing the study of interactions between ants and EFN plants in a community context. Because ants may have a variety of nectar sources available to them in a given habitat, the quality of ant protection experienced by any one plant species may depend on the presence or absence of other EFN plants. The possibility that different ant species may exhibit different preferences for EFN plants with certain nectary attributes is intriguing and deserves further investigation with larger sample sizes of plants and more experimental manipulation of plant characteristics. Such information may provide insight into the different strategies employed by plants to establish and maintain these mutualistic interactions and the process by which more specialized ant-plant interactions may develop.

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