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Quantifying ant foraging preferences in the field using a slow-flow nectar pump

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ABSTRACT

Question: What aspects of nectar production affect ant recruitment in natural conditions?

Organisms: The most common ant species were *Formica pergandei*, *Monomorium minimum* and *Pheidole bicarinata*.

Field site: Old field in Duke Forest, Durham, North Carolina.

Method: I developed a slow-flow pump that allows for experimental manipulation of nectar characteristics, including flow rate, within a biologically reasonable range. This pump can be used in field conditions. I assayed ant preferences for sugar concentration, nectar flow rate, number of nectaries per plant, and spatial location of the nectar sources.

Conclusions: Ant nectar preferences varied across species. *Formica pergandei* recruitment changed when sugar concentration, flow rate and the number of nectaries did. However, the direction of change depended on the position of the nectar sources. *Monomorium minimum* and *P. bicarinata* recruitment patterns changed only if nectar flow rate changed. *Monomorium minimum* also preferred certain locations. *Pheidole bicarinata* consistently preferred intermediate nectar flow rates.

Keywords: ant–plant interactions, diet preferences, foraging, nectar.

INTRODUCTION

Nectar is the most common substance used by plants to reward mutualists (Kearns and Inouye, 1993). If plants that successfully attract mutualists have higher fitness than those that do not, then any preferences of mutualists for nectar quality or quantity may help to shape nectar characters. However, the very slow rate of nectar production for most extrafloral nectar bearing plants has made it difficult to mimic natural nectar production. Previous experiments have been limited to providing discrete nectar sources, either requiring frequent replenishment by the researchers or presenting nectar in greater volume than typically encountered in nature.

To understand how ants might be choosing between plants, and how ant species might vary in their preferences, I developed a slow-flow pump that could be used to continuously

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supply a nectar solution to many artificial plants simultaneously at biologically reasonable rates, and used this pump to manipulate several nectar characters, including rate of nectar production, in a field in Durham, North Carolina. I used the results of these experimental manipulations to assess the extent to which ant species in the field vary in their recruitment responses to extrafloral nectar sources.

MATERIALS AND METHODS

Rationale for the nectar pump

An obstacle to the manipulation and simulation of natural variation in nectar characters is the small volume and slow rate of nectar production of most plants. The nectaries of many plants secrete nectar on the order of a few microlitres per hour or even a few microlitres per day (Pierre *et al.*, 1999). Standing volumes may be very small or difficult to measure without damaging or altering nectary tissue (McKenna and Thomson, 1988). Insect visitors to natural nectar sources may completely remove all visible nectar, but may still be able to detect even extremely minute droplets of nectar. Ants still tend and feed from nectaries without any nectar visible to the eye (personal observation). On experimental plates, or flowers altered by experimenters, insects may completely exhaust the nectar source, forcing the experimenter to frequently replenish the nectar – potentially disturbing the interaction.

To alleviate these problems, I designed a pump that allows secretion at extremely low rates at artificial nectaries. With this pump, it is straightforward to manipulate the rate of production, concentrations of nectar constituents, and the number of nectaries per 'plant'. In addition, this pump delivers nectar at a constant rate regardless of temperature or humidity, unlike gravity-based pumps. The nectar sources associated with each pump can be arranged to test hypotheses about the effect of spatial arrangement on the behaviour of mutualists, such as ants responding to nectar (Barton, 1986; Nowak *et al.*, 1994; Ferriere and Michod, 1995). The nectar input is separate from the outputs (artificial nectaries) and thus the composition of nectar can be changed without directly interfering with the outputs and potentially disrupting feeding behaviours. Finally, this pump is easy to transport and to use in remote field locations.

Nectar pump design

The nectar pump is a type of syringe pump and can be made from readily available, inexpensive materials. As shown in Fig. 1, the pump uses a slow motor to propel a worm drive, which compresses a syringe filled with nectar into a reservoir, which leads into a set of capped plastic tubes with very small holes. In the configuration used in this study, the holes began 1 m from the reservoir and were spaced 10 cm apart over a second metre of tubing (5 cm apart for the 20 nectary plants described below). These holes function as artificial nectaries.

The motor, the size of the syringe and the number of nectaries will jointly determine the rate of nectar flow from the pump. Motors from speeds of 1/100 $rev \cdot min^{-1}$ to 10 $rev \cdot min^{-1}$ (available from Hankscraft Motors and other suppliers) will allow rates of nectar production similar to most natural nectaries. If a set-up similar to most of the experiments described here (100 nectaries total per pump and a 1 ml syringe) is used, these motor speeds will correspond to a rate of 0.01–10.0 $\mu l \cdot h^{-1}$ at each nectary.

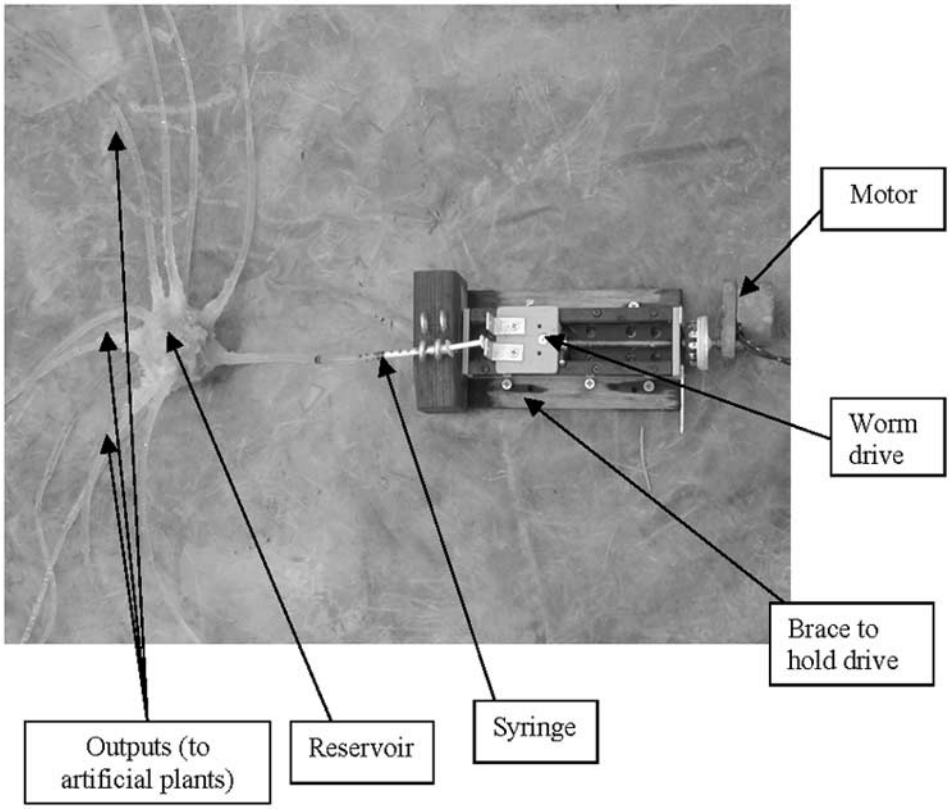

Fig. 1. Photograph illustrating the organization of the main elements of the nectar pump.

The worm drive used in this experiment was obtained from Edwards Optics and is listed as a '6.5 inch Stage Mechanism'. The worm drive is a threaded rod fixed in a steel rectangle. A steel square is attached to the rod by a threaded nut, and to the metal rectangle by fitted grooves in the frame. Turning a dial on the drive moves the square back and forth along a straight line. The motor can be connected to the worm drive using a drain plug (or other connector which grips the dial of the worm drive) that is connected by epoxy to the motor.

The metal square on the worm drive contains holes used to attach right-angle brackets to the worm drive. These brackets will then move linearly, and compress the syringe. The syringe is attached to a reservoir (a racquetball worked well) by plastic tubing. Spring clamps used to seal the plastic tubing served to maintain pressure in the system while nectar supplies were replenished. Syringe needles functioned as connectors to the reservoir: one for the input from the syringe, and 10 outputs to the artificial plants. The connections between the input/output lines and the reservoir were sealed with a silicon sealant to prevent leakage. The artificial plants, or nectar sources, were made of plastic tubing, capped with plastic snap-tight catheter fittings. Holes were punctured in the tubing with a needle to create artificial nectaries. As these holes are much smaller than the diameter of the tubing, flow rates at the nectar sources are only very slightly affected by position along the tubing and are nearly equal. The plastic tubes were wound around and attached to bamboo stakes stuck into the ground. Before use, the pump was primed (filled repeatedly by the syringe

until nectar began to leak from all of the nectaries) to pressurize the system. The total cost of the pump materials is approximately US$350, and a pump can be constructed in 10 h.

Variation in nectar production and ant recruitment

I used the nectar pump in the summer and fall of 1999 and the summer of 2000 to assess the impact of variation in: (1) the rate of nectar production, (2) the sugar concentration of nectar, (3) the number of nectaries on a plant, and (4) the spatial location of the nectar sources on ant recruitment. These experiments were carried out in an old field in Duke Forest in Durham, North Carolina. This field contained at least seven species of ants known to feed on extrafloral nectar of *Chamaecrista fasciculata* in the field.

In this experiment, two pumps were employed simultaneously. In every experiment, one pump delivered one quality or quantity of nectar to one set of artificial plants, while the other pump delivered a contrasting quality or quantity of nectar to another set of artificial plants. Each pump was connected to ten artificial plants – plastic tubes wrapped around bamboo stakes stuck through a plastic bag into the ground. The bag served to catch uncollected sugar droplets that fell to the ground, preventing a build-up of sugar at the base of each plant. The motor, syringe and reservoir were placed in a shaded plastic tub.

Each trial lasted approximately 70 min. I did not perform trials on rainy days, when nectar would have been washed off and most ant species were inactive. I measured ant recruitment simply as a snapshot count of the number of ants present at each artificial plant at 15 min intervals and at the end of the trial. The species of recruited ants were also noted and sample specimens were collected for identification. The artificial plants were arrayed in a semi-circle around the reservoir, so that the plants of a given treatment were clumped together, with individual plants being separated by approximately 1.5 m. The position of plants representing a particular treatment was shifted between trials, and individual artificial plants were frequently replaced with newly constructed units between trials. In the experiments below, the position of each type of nectar-producing pump was alternated between one of two locations approximately 5 m apart. These two locations served as the spatial component of the experiment and were identical for all experiments. Hereafter, they are referred to as position A and position B, respectively. The time of day for each experiment was also randomized, and generally one trial was performed in the morning and one trial was performed in the afternoon. Between trials, all ants were removed from the artificial plants, all residual sugar was removed from the plants and sandwich bags at the base of the plants, and ant recruitment was allowed to subside to zero. If two trials were performed on the same day, I varied a different nectar character in each trial (i.e. two trials examining the effects of nectar concentration would not be performed on the same day). The plastic tubing of the pump was changed frequently, because untended nectaries became coated with sugar and clogged. Also, nectar was pumped to the artificial plants for 3 days before the beginning of any experiments, allowing ants to discover the presence of a new food source in the area.

Nectar concentration

Three nectar sugar concentrations were compared: 1, 3 and 5 $mol \cdot l^{-1}$ sucrose solutions. This is representative of a range of extrafloral nectar producing plants: *Vicia faba* extrafloral nectar is approximately 1 $mol \cdot l^{-1}$ sugar (Engel *et al.*, 2001), postfloral nectaries tended by

ants in *Ruellia radicans* are near 3 mol·l^{-1} sugar (Gracie, 1991), and extrafloral nectar in *Anacardium occidentale* is higher than 5 mol·l^{-1} sugar (Wunnachit *et al.*, 1992). Sucrose was used as the sugar because variation in sucrose concentration has been previously demonstrated to affect nectar preferences in some ant species (Breed *et al.*, 1987; Cassill and Tschinkel, 1999). Two comparisons were made: 1 versus 3 mol·l^{-1} ($n = 10$ artificial plants per treatment per trial in all comparisons, four trials in this comparison) and 1 versus 5 mol·l^{-1} ($n = 10$ trials). Flow rates were held equal at 3 μl·h^{-1}, and there were 10 nectaries per plant. If ants are choosier when there is a large difference between nectar concentrations, then a larger treatment effect should be found in the 1 versus 5 mol·l^{-1} comparison than in the 1 versus 3 mol·l^{-1} comparison.

Rate of nectar production

Three rates of nectar production were contrasted: fast (approximately 5 μl·h^{-1} for each nectary), moderate (3 μl·h^{-1} per nectary) and slow (1 μl·h^{-1} per hour). A rate of 5 μl·h^{-1} is near the highest rates of extrafloral nectar production per nectary reported for plants (Raine *et al.*, 2002), and *Urena lobata* leaves have been reported to produce nectar at approximately 1 μl·h^{-1}(Dreisig, 2000). Two comparisons were made: fast versus moderate ($n = 4$ trials) and fast versus slow ($n = 10$ trials). Sugar concentrations were held equal at 3 mol·l^{-1} sucrose, and there were 10 nectaries per plant. If ants demonstrate stronger preferences with increasing differences between treatments in production rate, then there should be a stronger effect of treatment in the fast versus slow comparison.

Number of nectaries

I tested for an effect of overall nectary number on ant recruitment by comparing recruitment to plants with two different quantities of artificial nectaries per artificial plant: 10 nectaries per plant and 20 nectaries per plant ($n = 5$ trials). To keep the flow rate equal (at 3 μl·h^{-1}) for each nectary, the motor for the plants with 20 nectaries per plant was twice as fast as the motor for the plants with 10 nectaries per plant. The sugar concentrations were held equal at 3 mol·l^{-1} sucrose.

Data analysis

As is common for species count data in natural conditions, the most frequent count for any given species at any given time was zero (Scheiner, 1993). The data were approximately Poisson distributed. Data were pooled across snapshots within a trial (yielding a measure of total number of ants observed per plant per trial), but the data remained strongly skewed towards zero. Of the five species observed feeding from the nectaries (see below), two species (*Lasius umbratus* and *Crematogaster pilosa*) fed only very rarely from the artificial nectaries and so were excluded from most analyses.

To examine the effects of treatment and spatial positioning on the ant counts for the three remaining experiments, I used a multivariate extension of the Kruskal-Wallis test. This test was the equivalent of a multivariate analysis of variance (MANOVA), except on ranked data. Using a test with multiple response variables allows both for an uncorrected alpha level (as only a single test rather than multiple tests must be performed in each case) and for interrelatedness of the dependent variables (Scheiner, 1993). In every case, I tested for treatment,

position (representing one of the two spatial locations of the artificial plant), and treatment × position interaction effects including all ant species counts simultaneously as three (or, in one case, four) dependent variables. To determine if including the spatial effect in the model produced a qualitatively different result, I also performed an analysis including only treatment effects. Roy's greatest root was used to assess the significance of all effects. Furthermore, I examined the standardized canonical coefficients to determine the relative importance of each species in causing each effect. The magnitude of the canonical coefficient for each species indicates the extent to which a given species contributed to a statistical effect of treatment or position. The canonical coefficients were also used to look for possible differences in preferences between ant species (Scheiner, 1993). If the signs of the canonical coefficients differ between two species, this indicates that the two species had opposite preferences for that aspect of nectar production or that they were most abundant at different locations. When components of the MANOVA were significant, I performed follow-up analyses of variance on each individual species to evaluate the effects of treatment and position on each species' recruitment response.

RESULTS

Ant species data and general observations

The pump and artificial plants successfully attracted ants from the natural community. Ants in this field were readily attracted to nectar droplets emerging from the plastic tubing. Five ant species were observed to tend the nectaries over the course of this experiment: *Monomorium minimum* (Myrmicinae), *Lasius umbratus* (Formicinae), *Formica pergandei* (Formicinae), *Pheidole bicarinata* (Myrmicinae) and *Crematogaster pilosa* (Myrmicinae) (species are hereafter referred to by their genera). *Lasius* and *Crematogaster* were very rare, and I thus restrict my analyses to recruitment patterns of *Monomorium*, *Formica* and *Pheidole*. Although individuals of each of these three species were observed to emerge from multiple colony entrances, I did not excavate the colonies. Thus, species-level differences described below could also potentially be ascribed to differences between colonies.

I observed no obvious differences between handling and feeding behaviours of ants attending nectar droplets from the artificial pumps and ants feeding from nectaries of a nearby extrafloral nectar producing plant, *Chamaecrista fasciculata*. Ant recruitment to artificial plants was continuous throughout the day, although ant species differed in the time of day of peak activity. However, ant activity was also highly variable, and ants ignored many individual artificial plants during a given trial. Generally, ants tended lower nectaries on each artificial plant much more than higher nectaries. The nectar also attracted flies, beetles and wasps, but these visitors were at very low frequency. The pump functioned well at a variety of temperatures (the ambient temperature ranged from 21 to 37°C during the experiments), and nectar flowed easily from the tubes during the changing nectar viscosity associated with these temperatures. Thus, the nectar pump provides a method for comparing insect nectar preferences in the field.

Analyses of variance testing for the effect of treatment on the total number of ants recruiting (combining all ant species) did not detect significant effects of any treatment. However, multivariate analyses of variance accounting for species differences detected significant effects of treatments, described below.

Nectar concentration

The relationship between nectar concentration and ant recruitment depended on the ant species and the spatial position of the artificial plants (Table 1). For the 1 versus 5 $mol \cdot l^{-1}$ comparison, spatial position of the nectar source affected ant recruitment, and there was a significant interaction between treatment and the position of the plants of a given treatment. Subsequent analyses of variance of these effects on the response of each species indicated that the significant position effect resulted from biased recruiting by *Monomorium* and *Formica* favouring position B during these trials (Fig. 2A). The significant treatment × position effect results from a change in nectar preference by *Formica* across the two spatial positions. In position A, *Formica* demonstrated little preference. In position B, where *Formica* was more common, this species recruited much more to the nectar with the higher sugar concentration. Neither *Monomorium* nor *Pheidole* changed its recruiting patterns significantly in response to this concentration comparison.

In the MANOVA of the 1 versus 3 $mol \cdot l^{-1}$ sugar concentration comparison, both main effects of treatment and position were significant, as was the interaction effect (Table 1). In these trials, as in the 1 versus 5 $mol \cdot l^{-1}$ comparison, *Monomorium* and *Formica* were more common at position 2, while *Pheidole* was rare in both positions, and follow-up analyses of variance indicated that the varying abundance of *Monomorium* and *Formica* explained the significant position effect (Fig. 2B, Table 1). These analyses also indicate that *Formica* is the only species with a response to this sugar concentration treatment. However, the nature of this response was related to the spatial position of the plants, with ants exhibiting a complete preference for low concentration nectar in position A, and a complete preference for high concentration nectar in position B, during these experimental trials. Combining the results of both nectar sugar concentration comparisons, only *Formica* showed a significant recruitment preference in this range of nectar sugar concentration. *Formica* ants preferred nectar with higher sugar concentration at position B where they were more abundant, but did not express this preference at position A. Thus there is evidence that some ants in this field are discriminating between nectar sources based on the nectar sugar concentration, but the choice is influenced by the location of the nectar source, and not all species are influenced by this factor.

Rate of nectar production

Patterns of ant recruitment to nectar sources that varied in flow rate were similar to those found from varying nectar sugar concentration (Table 2). In a MANOVA testing for differences in ant recruitment to fast flowing nectar to nectar that flowed at a slow rate, the main treatment effect was not significant, while the effect of spatial position and the interaction between treatment and position effects were both significant. Once again, *Monomorium* individuals were found more often at position 2. Follow-up analyses of variance indicated that the significant treatment × position interaction effect results from preferences in all three species that change when spatial position of the nectar source is changed. In both *Formica* and *Pheidole*, slow-flowing nectar was preferred at position A, while fast-flowing nectar was preferred at position B (Fig. 3A). In *Monomorium*, there was a consistent preference for fast-flowing nectar, but this preference was much stronger in position B.

When the ants in this field had the opportunity to choose between fast-flowing nectar and nectar flowing at an intermediate rate, the flow rate did affect ant recruitment, both directly

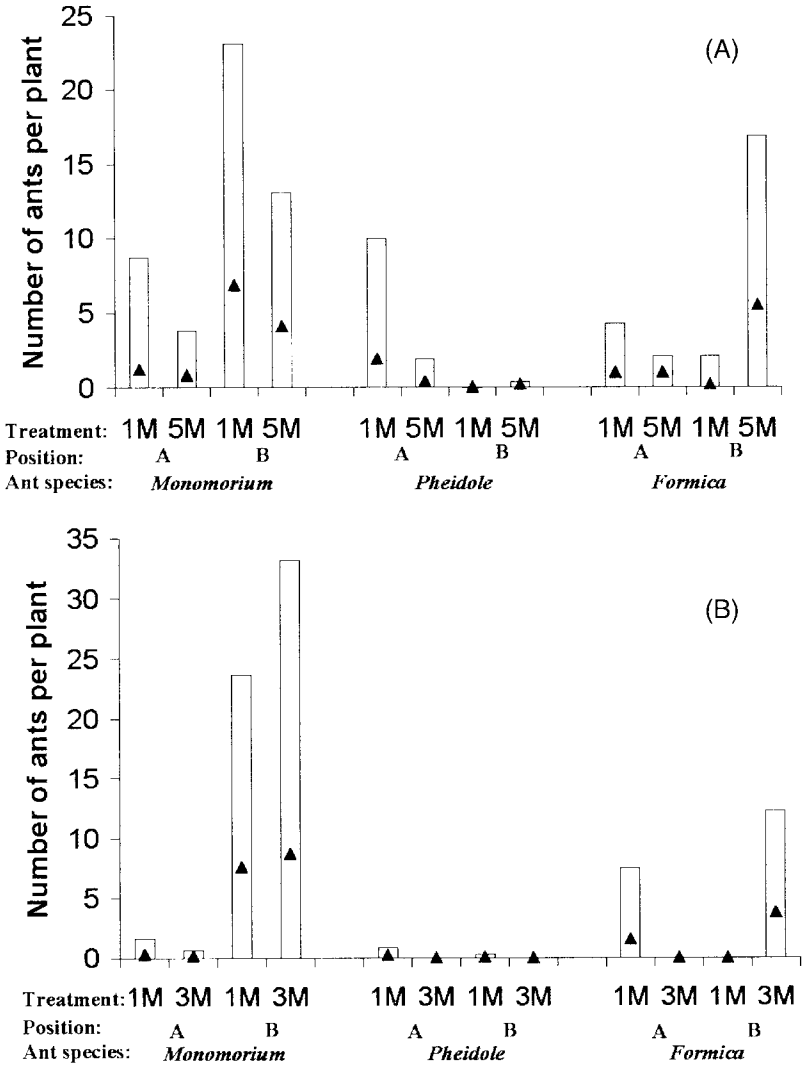

Fig. 2. Comparison of ant recruitment levels to different concentrations of sucrose sources located in two spatial positions. In Figs. 2–4, 'A' and 'B' represent two different spatial locations in which the artificial plants were located. In each plot, the mean is marked with a triangle and surrounded by a box denoting one standard deviation above and below the mean. Because most plants had zero ant recruitment, the box plots usually intersect with the x-axis 0 value. (A) Ant recruitment to 1 versus 5 mol·l^{-1} (1M *vs* 5M) sucrose nectar. (B) Ant recruitment to 1 versus 3 mol·l^{-1} (1M *vs* 3M) sucrose nectar.

and in interaction with the spatial position of the plants (Table 2). Again, a significant effect of spatial position on foraging was driven by a greater abundance of *Monomorium* at position B. Further exploration of the significant treatment effect with an analysis of variance on each species' recruitment response indicates that the direct effect of treatment is likely driven by a consistent preference by *Pheidole* for nectar flowing at an intermediate rate

Table 1. The significance levels and standardized canonical coefficients for the effect of nectar concentration on ant recruitment to artificial nectar sources

			Standardized canonical coefficients		
Experiment	Effect	Significance	*Monomorium*	*Pheidole*	*Formica*
1 *vs* 5	Treatment (T)	0.70			
mol·l^{-1}	Position (P)	<0.0001	**0.61**	−0.27	**0.85**
sucrose	T × P	0.01	−0.59	0.48	**0.77**
1 *vs* 3	Treatment (T)	0.02	−0.16	−0.53	**0.94**
mol·l^{-1}	Position (P)	0.0009	**0.81**	−0.53	**0.74**
sucrose	T × P	0.005	−0.19	0.17	**1.11**

Note: Canonical coefficients are displayed for significant effects in the MANOVA. Significant effects ($P < 0.05$) in follow-up univariate tests for each ant species are indicated by bold canonical coefficients.

Table 2. The significance levels and standardized canonical coefficients for the effect of the rate of nectar production on ant recruitment to artificial nectar sources

			Standardized canonical coefficients		
Experiment	Effect	Significance	*Monomorium*	*Pheidole*	*Formica*
Very fast *vs*	Treatment (T)	0.35			
fast	Position (P)	0.02	**0.97**	−0.43	0.10
	T × P	<0.0001	**−0.64**	**0.64**	**0.55**
Very fast *vs*	Treatment (T)	0.02	0.06	**0.80**	0.41
moderate	Position (P)	0.001	**0.85**	0.15	0.30
	T × P	<0.0001	**0.86**	−0.26	**0.85**

Note: Canonical coefficients are displayed for significant effects in the MANOVA. Significant effects ($P < 0.05$) in follow-up univariate tests for each ant species are indicated by bold canonical coefficients.

(Fig. 3B). Both *Monomorium* and *Formica* altered preferences across positions, contributing to the significant treatment × position interaction effect. In *Monomorium*, there was a preference for nectar of an intermediate rate when these ants were rare at position A, and a preference for fast-flowing nectar when they were more abundant at position B. In *Formica*, the opposite pattern was observed – a preference for rapid flowing nectar when this species was less common at position A, and a preference for intermediate rates of nectar flow at position B.

Together, the results from the two nectar flow rate comparisons indicate that recruitment by the three most common ant species in this field was sensitive to differences in the flow of nectar from each nectary. However, their responses to these different patterns were complex, varied between species, and often depended on the location of the nectar source. In only two cases were preferences for nectar flow rate unaffected by the spatial position of the nectar: a preference by *Monomorium* for fast-flowing nectar over slow-flowing nectar, and a preference by *Pheidole* for intermediate-flowing nectar over fast-flowing nectar.

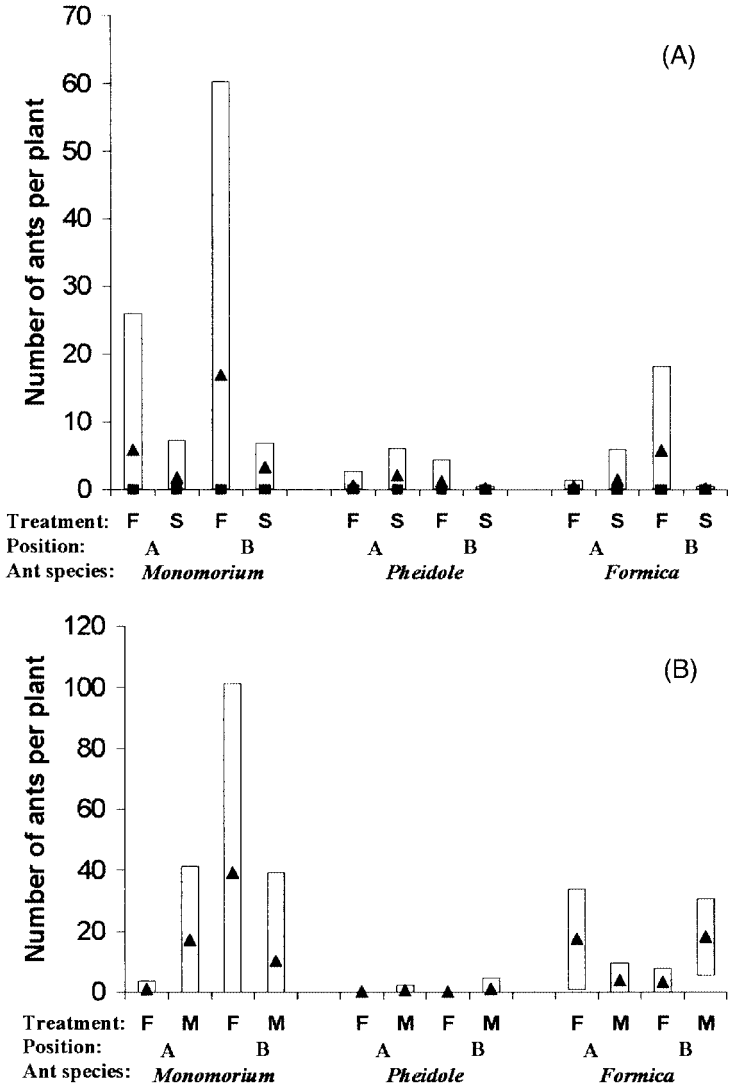

Fig. 3. Comparison of ant recruitment levels to each plant for two nectar flow rates in two spatial positions. (A) Ant recruitment to fast (abbreviated 'F', 5 $\mu l \cdot h^{-1}$) versus slow (abbreviated 'S', 1 $\mu l \cdot h^{-1}$) nectar flow rates. (B) Ant recruitment to fast (3 $\mu l \cdot h^{-1}$) versus moderate (abbreviated 'M', 3 $\mu l \cdot h^{-1}$) nectar flow rates.

Number of nectaries

When artificial plants were compared that differed in the number of nectaries per plant, there was no significant effect on ant recruitment to the plants, but both position and the interaction between position and treatment significantly affected ant foraging (Table 3). As in the other experiments, the position effect was shaped by spatially biased recruitment patterns in *Monomorium*, although in these trials they were found more frequently at

Table 3. The significance levels and standardized canonical coefficients for the effect of variation in the number of nectaries per artificial plant on ant recruitment to the artificial plants

			Standardized canonical coefficients		
Experiment	Effect	Significance	*Monomorium*	*Pheidole*	*Formica*
10 *vs* 20	Treatment (T)	0.11			
nectaries	Position (P)	0.03	**1.01**	0.22	0.34
	T × P	<0.0001	−0.18	0.23	**1.53**

Note: Canonical coefficients are displayed for significant effects in the MANOVA. Significant effects ($P < 0.05$) in follow-up univariate tests for each ant species are indicated by bold canonical coefficients.

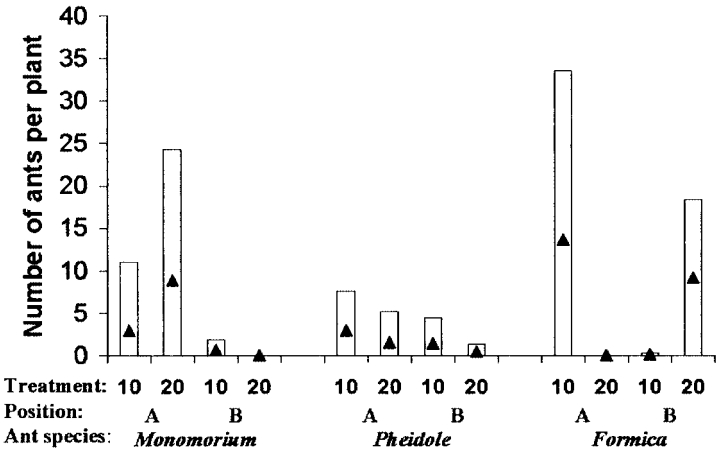

Fig. 4. Comparison of ant recruitment levels to each plant for two different numbers of nectaries per plant (10 nectaries per plant and 20 nectaries per plant) in two spatial positions.

position A. Analyses of variance on each individual species' response indicate that the significant interaction effect is derived from a strong switch of preferences by *Formica* ants. *Formica* had a complete preference for plants with fewer nectaries at position A, and a nearly complete preference for plants with more nectaries at position B (Fig. 4).

DISCUSSION

I have described the use of a slow-flow pump to simulate nectar production to successfully attract ants to artificial extrafloral nectar sources in the field. This technique could be used to investigate other systems and questions that involve manipulating nectar production in the field. Other attributes of nectar production could be altered. For example, concentrations of amino acids could be altered, or flow rates could be adjusted during an experiment to mimic induction of greater nectar flow rates (Agrawal and Rutter, 1998). In addition, the pump approach could be used in conjunction with artificial flowers to simulate floral nectar production for assessing pollinator nectar preferences when nectar is produced at biologically realistic rates. An approach combining artificial floral nectaries and artificial extrafloral

nectaries could be used to further examine the Kerner hypothesis that extrafloral nectaries distract ants from floral nectaries (Kerner, 1878; Rosenzweig, 2002; Wagner and Kay, 2002).

I employed the nectar pump to address several questions about the influence of nectar attributes on ant recruitment. The artificial nectaries provided a source of slowly replenishing, continuous nectar for foraging ants, and ants readily responded to these sources. I found that ant recruitment responded significantly to changes in nectar sugar concentration, the total number of nectaries and the rate of nectar production. The effects of sugar concentration on ant preferences have been the subject of most previous studies. The results reported here, that preferences for sugar concentration vary between ant species, are consistent with previous work (Lanza *et al.*, 1993; Cassill and Tschinkel, 1999; Wagner and Kay, 2002; Blüthgen and Fiedler, 2004). However, there had previously been no explicit examination of the relation of ant recruitment patterns to changing rates of nectar production, and only one study vatied the number of nectaries per plant (Wagner and Kay, 2002). The paucity of these types of studies probably reflects the lack of an adequate method for simulating plant nectar production. The nectar pump described here is capable of delivering continuously flowing nectar at a range of rates equivalent to those of plant nectaries. The results obtained using this pump to simulate the preferences of ants for extrafloral nectar suggest that plant traits other than sugar concentration, particularly the rate of nectar production and the number of nectaries per plant, influence patterns of ant recruitment, and should be incorporated into studies of the interaction of ants and extrafloral nectar bearing plants. In fact, manipulation of the rate of nectar production was the only alteration of nectar to which all three of the species studied here changed their recruitment response.

While I found significant effects on ant recruitment of the rate of nectar production, nectar sugar concentration and the number of nectaries per plant, it would not have been possible to achieve an accurate understanding of the effect of the treatments without considering differences between ant species or the impact of spatial location of the treatments. The differences between treatments were large relative to natural variation in local extrafloral nectar producing plants such as *Chamaecrista fasciculata* (Rutter and Rausher, 2004). Still, the treatment differences largely failed to affect total ant visitation rate when all ant species were pooled. Multivariate analyses partitioning ant recruitment by ant species suggested that ants did forage preferentially, but negatively correlated responses of different species obscured this in a pooled analysis. There was no clear-cut effect of the size of the differences between treatments on ant preferences (comparing Figs. 2A with 2B, or 3A with 3B). *Formica* was the most sensitive to the treatment, and responded to all tested changes in nectar sugar concentration, rate of nectar flow and total number of nectaries. *Monomorium* and *Pheidole* each responded only to differences in flow rate. It is important to note that these experiments cannot differentiate species-level preferences from colony-level preferences. Nonetheless, it is clear that in this field there were three distinct entities with differing nectar preferences.

The result that different species in the same field differ in their preferences for nectar is perhaps unsurprising, but it can potentially have a considerable impact on the evolution of traits such as nectar production. Many, if not most, extrafloral nectar producing plants are tended by more than one species of ant (Morellato and Oliveira, 1994). This fact complicates the selection pattern on nectar traits in a given field, especially if different ant species provide different quality benefits per ant (i.e. Fiala *et al.*, 1994). If a single ant species of the nectar-feeding community is the 'optimal' mutualist, there may be selection to match the preferences of that ant species, which may even lead to a decline in the number of ant visitors.

The physical location of the artificial plants in a given treatment consistently had an effect on ant recruitment. The importance of space throughout the experiments also suggests that this spatial scale was important to ants, even though the scale of the experiment was small, with the greatest distance between plants less than 10 m. This result is consistent with the idea that travel to distant plants may be hazardous or entail costs. Foraging ants may take these travel costs and the relative benefits provided by each plant into account when choosing among plants, thus optimizing their foraging strategy (Kay, 2002). This could explain the significant treatment × position interaction found in all comparisons. Only *Pheidole* exhibited a direct effect of treatment uncomplicated by spatial considerations – in one comparison they preferred slower flowing nectar. In all other cases, the effect of treatment was found to interact with the position of the artificial plants. The patterns of interaction may derive from the trade-off between distance (which decreases the value of a nectar reward) and higher reward through an increase in sugar concentration, rate of flow and number of nectaries. It is not possible to determine from these data whether the correlations between ant species derive from actual ant preferences or from competitive interactions between the ants (there may have been a single common preference, but the best competitors dominated the best nectar sources) (Blüthgen and Fiedler, 2004).

Generally, these results demonstrate that nectar sugar concentration, rate of nectar production and the number of nectaries per plant can affect ant recruitment. It is possible that a level of nectar production exists for the plant to maximize the benefits and minimize the costs of its mutualism with ants (Keeler, 1985; Rutter and Rausher, 2004). Nectar production may be selected to match ant preferences for nectar production traits. However, the pattern of selection generated by these preferences may not be simple – it is certainly not possible to make generalizations such as stating ants preferentially recruit to higher sugar concentrations of nectar. Competitive interactions between ant species and optimal foraging strategies that involve the distance of the plant from ant colonies can complicate description of ant preferences in the field. Furthermore, ant species may vary in their quality as plant bodyguards. Within a single field, the intricacies of the selection pressures on plant traits involved in attracting ants may be sizeable. By continuing to collect detailed information on ant–plant interactions in the field, we can assess these complexities and attain a more complete understanding of the role of ecological context in mutualisms (Bronstein, 1994, 1998).

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