RECRUITMENT RESPONSE TO SIX CARBOHYDRATES IN THE ANT *PHEIDOLE DENTIGULA* SMITH, 1927 (HYMENOPTERA: FORMICIDAE)[1]

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ABSTRACT: We examined recruitment behavior of the ant *Pheidole dentigula* to six different carbohydrates each presented at two concentrations. In each case, the recruitment response was enhanced by raising the carbohydrate concentration from 0.5 to 1.0 M. However, glucose and sucrose were more effective than other sugars at inducing recruitment activity at 0.5 M. In the simultaneous presence of both sugar solutions, but moving away the more concentrated one, the colony opted in the case of glucose and sucrose, but not with maltose, for the food source of less energy content. These findings suggest that taste receptors of *Pheidole dentigula* are differentially sensitive to distinct carbohydrates. This species was also responsive to trehalose, which has no recruitment activity in other ants. We consider that functional and molecular taste receptor diversity may represent an evolutionary advantage for insect societies, since it allows them to organize efficiently the required effort to exploit the existing resources.

KEY WORDS: recruitment response, *Pheidole*, trehalose, taste receptor, ants, Tamaulipas

Recruitment is defined as the communication that attracts nestmates to some point in space where work is required (Wilson, 1971). Ants employ this strategy to recruit workers for joint efforts to obtain food, construct and defend nest sites, and immigrate to new nesting sites. Several species use particular combinations of contact and chemical signals to achieve these goals. A common form of recruitment behavior occurs through chemical trail communication. Carthy (1950) was one of the first to conduct an experimental study of trail laying in ants (Hölldobler and Wilson, 1990). When workers locate a food source, they consume it and return to the nest laying a chemical trail secreted by specialized glands (Hahn and Maschwitz, 1985; Wilson and Hölldobler, 1985; Hölldobler and Wilson, 1990). Eventually, differences in chemical concentrations among trails allow recruited ants to maximize efficiency by following the shortest route (Denny et al., 2001). Also, when ants locate two distinct food sources differing in energy content, they preferentially lead the recruitment toward the one with more nutritional quality (Beckers et al., 1993; Sumpter and Beekman, 2003) or over the one that allows a single ant to drink a larger volume of food (Mailleux et al., 2000). In the present study, we analyzed the recruitment response of *Pheidole dentigula* (Formicidae: Myrmicinae) to six different carbohydrates that are perceived as sweet in humans. Each compound was tested at two concentrations, 0.5 and 1.0 M. Our major aim was to determine whether differential sensitivity to carbohydrates could generate

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distinct recruitment activities toward two sources of food with low and high content of calories and adding the effect of the distance to the sugar.

METHODS

Experiments were conducted on a naive colony located in an urban zone in Victoria City, Tamaulipas, México, over a period of 18 consecutive months (from May 2007 to October 2008) before and after a winter season and several rainy seasons. All tests were carried out in an area of 3 m^2 during daytime at a temperature in shade of 30-36ºC. In each experiment a small piece of sausage (2 g) was put in three places at random to stimulate the activity of the ants; after one hour the test was effectuated in the place with higher or unique recruitment behavior. Experimental trials were performed with a T-shaped metal structure, 4 cm high and 12 cm of top length, placed about 10 cm from the entrance to the nest without any predetermined orientation. The carbohydrates used as chemical stimuli were D(-)-fructose, D(+)-glucose, D(+)-maltose, D(+)-melezitose, D(+)-sucrose, and D(+)-trehalose (obtained from Sigma Chemical Co.) and tested at a concentration of 0.5 and 1.0 M. The sugar-containing solutions were prepared immediately before the experiments and aliquots (3 ml) of them were placed in a circular container (feeder) at one or both top ends of the T-shaped structure, depending on the experimental protocol.

Kinetics of recruitment. To determine the intensity of recruitment, the activity of the colony was first induced with the bait. Subsequently, the sweet solution was put in place on the T-shaped structure and the ants (workers) on the edge of the feeder were then counted (taking a digital photograph) every 3 min since the arrival of the first individual. This allowed us to set a range of time in which a stable recruitment was established. The results obtained with a given chemical were always normalized relative to the activity level observed when using the respective 1.0 M solution.

Stimuli test. A complete typical experiment with each carbohydrate consisted of placing the 0.5 M solution at one end of the T-shaped structure and counting the ants feeding over the stable recruitment period mentioned above. Once the 0.5 M solution was withdrawn, the 1.0 M solution was placed at the opposite end and the registration of recruitment was then repeated in the same period of time. This phase of the experiment is called independent or individual concentrations exposure. In the second phase, both sugar concentrations were submitted simultaneously, one at each end of the T-shaped structure, and the recruitment was registered as above described. This phase is called simultaneous concentrations exposure.

Effort vs stimulus. In a series of experiments, we tested whether the colony was able to discriminate between two sources of food differing in quality, but with the better one placed at a longer distance from the nest. This procedure was carried out for three of the chemicals (D(+)-glucose, D(+)-maltose and D(+)-sucrose) using a modified T-shaped structure in which one of the arms was extended

50 cm. The distance between the nest and the 0.5 M solution had a fixed value of 20 cm (10 cm + 4 cm + 6 cm), whereas the maximum of three distances tested to reach the 1.0 M solution was 70 cm (10 cm + 4 cm + 6 cm + 50 cm).

Data analysis. Data are given as the mean (± S.E.) of number of workers for the number (n) of separate experiments indicated. Paired t-test was used for comparison of two means and P values < 0.05 were considered significant. Multiple comparison of means was conducted by analysis of variance across all sugars, followed by a Tukey test with 0.05 of significance level (Motulsky, 1995).

RESULTS

Activity level of the colony

The activity of the colony was variable and changed not only from one experiment to another, but also within the same session (see Fig. 1). It was thus important to consider in each experiment the maximal number of ants counted. This parameter, which was called activity level, had an average value of 37.7 ± 2.0 individuals throughout the study, with a minimum of 17 and a maximum of 70 ants. Differences in activity level among the experimental groups were found to be not significant ($F_{5,18} = 2.1574$, $P>F = 0.105$).

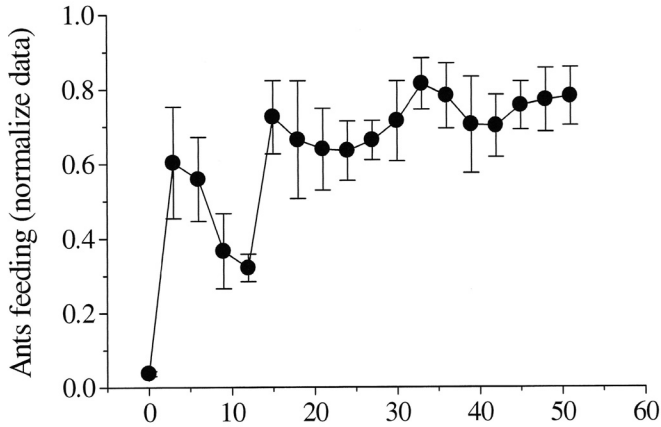
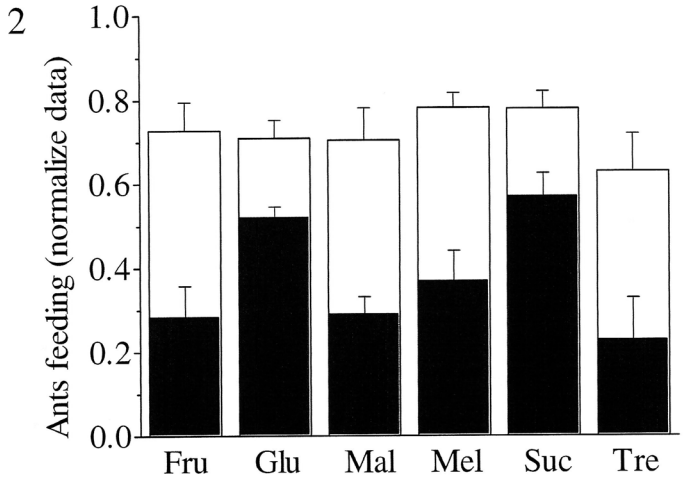


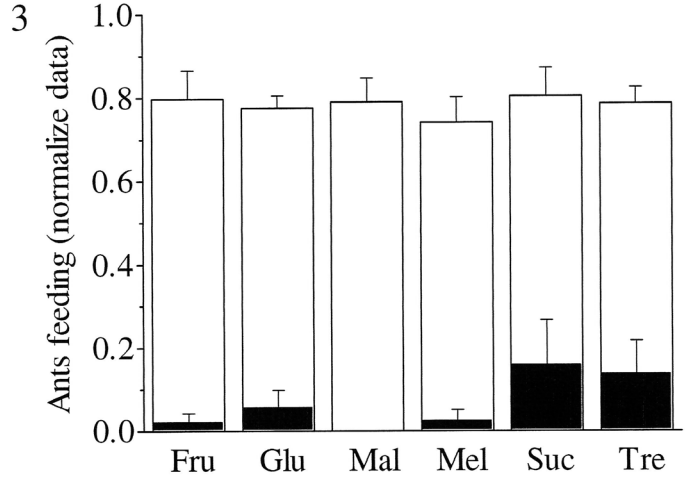
Fig. 1. Time course of the recruitment response with 1.0 M glucose (n = 5).

Time course of the recruitment response

Results of five experiments of recruitment kinetics using 1.0 M glucose are shown in Fig. 1. As can be seen, a sudden increase in the number of ants foraging at the feeder was detected in the first 5 min, which was followed by a transient decrease within the next 5-10 min. Subsequently, the number of feeding ants rose again, reaching a maximum by minute 20. This was the beginning of a plateau in which the number of individuals did not change significantly as a function of time for at least 30 min. As in previous works (Ferradas et al., 1986;

Sumpter and Beekman, 2003), we consider that during the plateau phase the trail of pheromone was already well established, inducing the continual recruitment of nestmates to the feeder at a nearly stable rate. Experiments with 1.0 M trehalose resulted in a similar recruitment kinetics (data not shown). Based on these results, the recruitment response to chemicals in the following experiments was estimated by calculating the mean number of ants at the food source from minute 27 to minute 42, counted after the arrival of the first individual to the feeder.





Figs. 2-3. Recruitment responses induced by the indicated sugars at 0.5 M (■) or 1.0 M (□). Fig. 2. Independent exposure experiments (n = 4 in each case). Fig. 3. Simultaneous exposure experiments (n = 4 in each case). (Abbreviations: Fru, Fructose; Glu, Glucose; Mal, Maltose; Mel, Melezitose; Suc, Sucrose; Tre, Trehalose)

Recruitment response in independent exposures to the two concentrations of carbohydrates

For each chemical investigated, the recruitment response was enhanced by raising the sugar concentration from 0.5 to 1.0 M (Fig. 2). The normalized mean response induced by the 1.0 M solution was found to be practically independent of the chemical used. However, when tested at 0.5 M, significant differences among sugars were noted ($F_{5,18}$ = 4.6802, $P>F$ = 0.007). From these latter results, the six chemicals studied could be divided into two main groups. In particular, glucose and sucrose elicited relatively strong responses when applied at 0.5 M. By contrast, the remaining sugars (trehalose, maltose, melezitose, and fructose) were less effective at inducing recruitment activity at that same concentration.

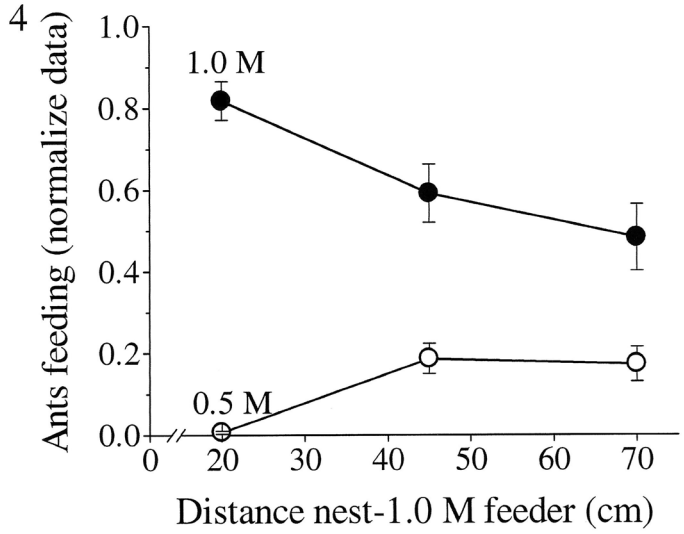
Recruitment response in simultaneous exposures to the two concentrations of carbohydrates

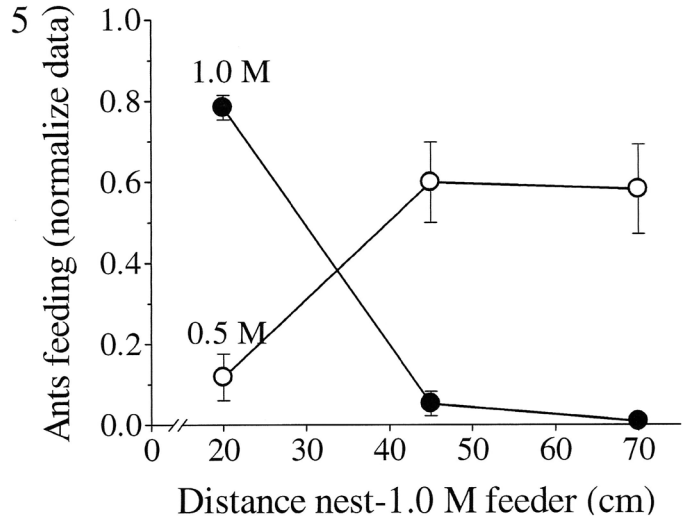
In the simultaneous presence of the two sugar-containing solutions, and with both feeders located at the same distance from the nest, the colony opted preferentially for the 1.0 M solution regardless of the chemical used (Fig. 3). This was the case even when the small response to 0.5 M sucrose was compared to the null response elicited by 0.5 M maltose under the present conditions. Overall, in these experiments the recruitment response to the 1.0 M solution was, on average, almost 12 times larger than that induced by the 0.5 M solution. For five of the chemicals tested, the consequent reduction of the response to the 0.5 M solution was significant and resulted equivalent, on average, to an approximately 82% decrease in effectiveness. The only exception was trehalose, which was almost equally effective in both experimental protocols. More precisely, the recruitment response induced by 0.5 M trehalose in the experiments of Fig. 3 was also smaller as compared to that observed in Fig. 2, but this difference turned out to be not significant ($F_{5,18}$ = 1.3427, $P>F$ = 0.291).

Effect of distance between the nest and the sources of energy

When the feeder containing the 1.0 M solution was placed at a distance progressively longer (20, 45 and 70 cm) from the nest, the corresponding recruitment response gradually decreased. At the same time, there was a clear, although moderate, increase in the number of ants recruited by the 0.5 M solution.

These changes in the response of the colony were amplified by using glucose instead of maltose as the chemical stimulus (Fig. 5). This was so to the extent that the recruitment response induced by the 1.0 M glucose solution fell to a very low level or practically to zero when the distance to the corresponding feeder was increased to 45 or 70 cm, respectively. Likewise, the concomitant increase in the number of ants recruited by the 0.5 M solution was marked and almost similar in magnitude to the decrease in the response to 1.0 M glucose. Similar results to those presented in Fig. 5 were obtained by using sucrose (data not shown).





Figs. 4-5. Recruitment responses in simultaneous exposure experiments (n = 5). In these determinations, the feeder containing the 1.0 M solution was placed at three different distances from the nest, as indicated, while the 0.5 M feeder remained at a fixed distance of 20 cm. Fig. 4. Maltose. Fig. 5. Glucose.

DISCUSSION

The maximal number of ants counted in our experiments was, on average, close to 40 individuals. Similar data have been previously reported for another species of *Pheidole* (Ferradas et al., 1986) as well as for other genera (Mailleux

et al., 2000; Tinti and Nofre, 2001; Sumpter and Beekman, 2003). The activity of the colony was variable both between and within tests, and this characteristic has also been described in previous studies of the recruitment behavior in ants (Ferradas et al., 1986; Mailleux et al., 2000; Sumpter and Beekman, 2003). In spite of such fluctuations, it was possible to register the recruitment response of *Pheidole dentigula* in a concentration dependent manner for each carbohydrate investigated. The relative response to 0.5 M sugar solutions in individual exposure experiments was greater with sucrose and glucose followed by melezitose. These three carbohydrates are also reported in the ant *Lasius niger* as forming part of the four more effective phagostimulating sugars (Tinti and Nofre, 2001).

It is widely known that when ants are forced to decide between two food sources of different nutritional quality, the colony focuses its foraging effort preferentially on the most profitable patch (Beckers et al., 1993; De Biseau and Pasteels, 1994; Denny et al., 2001; Sumpter and Beekman, 2003). Our present data in *Pheidole dentigula* are consistent with this ecological strategy, as we found that the recruitment activity induced by 0.5 M sugar solutions was generally lower in simultaneous exposure experiments than when they were submitted alone. Moreover, such a reduction in the effectiveness of the 0.5 M solutions, caused by the simultaneous presence of a 1.0 M solution of the sugar in turn, was not only significant, but also dramatic for five of the carbohydrates investigated.

A second ecological strategy of ants is that they tend to exploit food sources that involve a lower cost of expenditure energy (Goss et al., 1989). In line with this, the colony of *Pheidole dentigula* recruited more nestmates for exploiting food sources closest to the nest, even discriminating the one with higher calories which would require more workforces spent because of being located at a longer distance. However, this behavior was observed with sucrose and glucose but not with maltose, suggesting differences in the nutritional value of these carbohydrates for *Pheidole dentigula*.

The second strategy has clear ecological benefits, as it results in a more efficient organization of the inverted effort. Its use, therefore, may confer an evolutionary advantage to the insect societies. To achieve this, insects must be endowed with a chemosensory gustatory system that has the ability to detect the quality and quantity of available food sources. The existing information, including this work, suggests that different species of ants may differentially express taste receptors to sugars, which enables them to effectively exploit the resources present at their habitat. In this regard, it should be mentioned that *Pheidole dentigula* showed a concentration-dependent sensitivity to trehalose, which is in contrast with a previous study in another ant, *Lasius niger*, where this disaccharide did not have any phagostimulating action at 1.0 M (Tinti and Nofre, 2001). Strong evidence indicates that the gustatory receptor called Gr5a in the sensory cells of the fruit fly is selective for trehalose (Tanimura et al., 1988; Dahanukar et al., 2001; Chyb et al., 2003). Therefore, our data imply the presence of a similar, if not identical, specific taste receptor in *Pheidole dentigula*.

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