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Interaction rate informs harvester ant task decisions

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Social insect colonies operate without central control, and colony organization results from the ways that individuals respond to local information. We investigated how temporal information, in particular the rate of interaction among workers, stimulates foraging activity in the red harvester ant (*Pogonomyrmex barbatus*). Patrollers scout the foraging area each morning. Previous work showed that the patrollers' safe return to the nest stimulates the foragers to leave the nest; if the patrollers do not return, the foragers do not emerge. Here, we tested whether contact with returning patrollers must occur at a particular rate to stimulate foraging. We varied the rates at which we introduced patroller mimics, glass beads coated with an extract of the cuticular hydrocarbons of patrollers. A return rate of 1 patroller mimic every 10 s stimulated the highest level of foraging activity. We found that the onset of foraging depends on the rate of patroller return. Adding beads coated with patroller hydrocarbons at a rate of 1 per 10 s caused otherwise undisturbed colonies to forage 17.9 ± 19.7 (standard deviation) min faster than colonies that received blank control beads. These results show that rate is a crucial source of information in the network of interactions among workers. *Key words:* cuticular hydrocarbons, foraging behavior, interaction rate, social insects, task allocation. *[Behav Ecol]*

Social insect colonies are organized without central control. Workers perform a variety of tasks, such as foraging, nest construction, and care of the brood. Task allocation is the process that adjusts the numbers of workers performing each task in a way appropriate to current colony needs (Gordon 1996). Each worker uses local information, including its interactions with other workers, to decide whether to perform a task. In the aggregate, individual decisions produce adjustments in colony behavior (Biesmeijer et al. 1998; Gordon and Mehdiabadi 1999; O'Donnell 2001; Fernandez et al. 2003; Pratt 2005).

Networks of interaction are important in many complex dynamical systems, from the Internet to neural systems (Barabasi and Albert 1999; Jeong et al. 2000; Albert and Barabasi 2002; Willinger et al. 2002; Victor et al. 2004; Butland et al. 2005; Litvinov et al. 2005). As with other systems, there is growing evidence of coordination in social insect colonies based on patterns of interactions among workers that act as modulated signals (Gordon 1996). For example, interactions between returning and inactive foragers stimulate foraging in honeybees (Fernandez et al. 2003). Honeybee workers respond with an increase in activity after receiving a vibration signal (Lewis et al. 2002). Biting interactions among paper wasp workers stimulate foraging (O'Donnell 2001).

In a network of interactions, temporal information may be crucial. The rate at which interactions occur, or the intervals elapsed between interactions, is an important signal in many complex dynamical systems (Selzer and Schrieber 1999; Jeong et al. 2000; Sima and Orponen 2003; Victor et al. 2004). In the nervous system of vertebrates, for example, an increased rate of action potential firing by sympathetic nerve fibers to pacemaker cells of the sinoatrial node of the heart leads to an increase in heart activity (Widmaier et al. 2005). In social insects as well, the rate of interaction appears to be important. The ant *Liasus fuliginosus* regulates encounter rate as density changes by avoiding interaction with other ants in crowded conditions (Gordon et al. 1993). A harvester ant worker is more likely to change tasks to do midden work when its rate of encounter with midden workers is high (Gordon and Mehdiabadi 1999). The ant *Temnothorax albipennis* uses encounter rate or number to assess nest-mate density and thus the suitability of new nest sites (Pratt 2005). The interval elapsed between 2 loads of water brought to the nest influences nest construction behavior in wasps (Jeanne and Nordheim 1996). The time elapsed before a honeybee can unload its nectar influences its decision whether to leave on another foraging trip (Seeley and Tovey 1994).

Here, we investigate whether the rate of interaction between members of different task groups influences the onset of foraging activity in the red harvester ant, *Pogonomyrmex barbatus*, a seed-eating species of the southwestern USA and central Mexico. A colony is not active every day (Gordon 1991), apparently in response to the shifting costs and benefits of foraging. The emergence of the foragers on a particular day is triggered by the return of a distinct group of ants that emerge early in the morning, before the foragers are active, called the "patrollers" (Gordon 1989, 2002). The patrollers search the area around the nest and then the foraging area. The foragers will not emerge if the patrollers do not return safely (Gordon 2002), and when the foragers do emerge, they take the directions chosen by the patrollers (Gordon 1991). Ants currently inactive, including the foragers, wait in the large chambers just inside the nest entrance until they are stimulated to leave the nest. The return of the patrollers determines whether the foragers will be active at all that day.

In previous work, we found that foragers recognize returning patrollers by detecting cues in the patrollers' cuticular hydrocarbon profile (Greene and Gordon 2003). Ants returning to the nest engage in brief antennal contact with the ants waiting inside the nest. The antennae are the organs of chemical perception, and during antennal contact, one ant can assess the cuticular hydrocarbon profile of another

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(Greene and Gordon 2003). A harvester ant's cuticular hydrocarbon profile depends on its task because, as an ant moves from work inside the nest to long foraging trips outside the nest, exposure to the warm, dry conditions outside causes an increase in the abundance of *n*-alkanes on its cuticle (Wagner et al. 2001). When patrollers were prevented from returning to the nest, we were able to stimulate foraging by dropping into the nest glass beads coated with an extract of patrollers' cuticular hydrocarbons. This work showed that the cuticular hydrocarbon profile was informative to the ants, but did not establish whether any other information was important, such as the amount of hydrocarbon a forager encounters, or the rate at which a forager encounters the hydrocarbons of patrollers. Temporal information, as well as olfactory cues, may be essential for interaction with patrollers to stimulate the onset of foraging activity.

Here, we examine the role of temporal information in interactions between foragers and patrollers. We investigate how the rate at which foragers encounter patroller hydrocarbons influences the onset of foraging activity. In field experiments, we varied the rate at which beads coated with patroller hydrocarbons were introduced to harvester ant nests, to ask 1) Must contact with patrollers occur at a particular rate to stimulate foraging? 2) Does the rate of encounter with patrollers determine when foraging begins? We first measured the rate of patroller return at the onset of foraging in undisturbed colonies (part A). We then tested the effect of artificially increasing the rate of patroller return, to determine if this would hasten the onset of foraging (part B).

MATERIALS AND METHODS

Patrollers emerge from the nest at about sunrise. A patroller shows characteristic behavior: it walks around the nest mound, frequently stopping to inspect the ground with its antennae, and often tucks its abdomen under its thorax (Gordon 1987). Foragers travel directly out on in a stream of foragers, which may extend 10–15 m from the nest (Gordon 1995).

Extraction and isolation of cuticular hydrocarbons

Patrollers were collected 4 days before the start of experiments. The ants were frozen after capture and stored at −20 °C until cuticular lipids were extracted. Cuticular lipids were extracted by soaking thawed ants in approximately 2 ml of 100% pentane for 10 min with periodic shaking. Cuticular hydrocarbons were separated from other surface lipid constituents by eluting the pentane extracts through a silica gel column with a 1-ml wash of 100% pentane (Nelson and Blomquist 1995). To create patroller mimics, patroller cuticular hydrocarbons in pentane were added to a tube containing 3-mm-diameter glass beads, and the solvent was allowed to evaporate (Greene and Gordon 2003). Each bead was coated with approximately one ant equivalent of hydrocarbon. Beads were stored at −20 °C until they were used in an experiment. Colonies were tested with beads coated with hydrocarbons extracted from patrollers of the same colony.

Experiment 1: Is a particular rate of encounters with patrollers necessary to elicit foraging?

To test whether encounters with patrollers must occur at a particular rate to elicit foraging, we mimicked patroller return at different rates, using glass beads treated with patroller hydrocarbons. On the day of a trial, foraging activity was inhibited by collecting patrollers as they emerged from the nest, preventing their return to the nest. After 30 min had elapsed since the last bout of patrolling, beads coated with patroller hydrocarbons were dropped into the nest. Ten patroller beads were added to the nest at 4 different rates, each presented to each of 5 colonies on different days: 1) 1 bead per 3 min, 2) 1 bead per 45 s, 3) 1 bead per 10 s, and 4) 1 bead every 1 s. Two days elapsed between trials, during which the colonies were left undisturbed. After the addition of the last bead, we measured foraging activity as the number of foragers active within 1 m of the nest entrance every 10 min for 60 min. An observer recorded the number of foragers on the trails at the same distance of 1 m from the nest entrance. The observer did not count ants within 5 cm of the nest entrance, where there were workers engaged in other tasks besides foraging, such as nest maintenance activities. The intensity of foraging varies among colonies and from 1 day to the next (Gordon 1991; Schafer et al. 2006), probably because of variation in colony needs, food availability, and climatic conditions. We rotated treatments among the same colonies on different days.

The mean number of foragers was calculated from all counts taken for each treatment in a trial. The raw data were normalized for variation among colonies in the absolute number of foragers by dividing the mean number foraging per trial by the largest number of foragers observed in any treatment for that colony. To meet the assumptions of normality of analysis of variance (ANOVA), the proportional data were transformed using an angular transformation (arcsine of the square root of the proportion). Repeated-measures ANOVA was used to test for significant differences in the means among treatments, and post hoc analyses were conducted using least significant difference (LSD) tests.

Experiment 2: Does the rate of encounter with patrollers influence when foraging begins?

A. Rate of patroller return at the onset of foraging in undisturbed colonies

To estimate the rate of patroller return that normally elicits the onset of foraging, we observed the behavior of 12 undisturbed colonies, from the beginning of the morning activity period when the first patrollers emerged from the nest. The number of patrollers returning to the nest entrance and the number of foragers exiting the nest were recorded for consecutive 5-min intervals until the onset of foraging. We recorded the onset of foraging as the time when the first 3 foragers exited the nest and moved out along foraging trails.

B. Foraging response to artificial boost in patroller return rate

We artificially increased patroller return rate to determine if this would hasten the onset of foraging. Whether foragers are ready to emerge probably depends on many factors, of which patroller return rate is only one. Other relevant factors might include temperature, because ants cannot move quickly when it is cold, and light intensity. We attempted to boost artificially the patroller return rate as late in the morning as possible, so as to approach the time and temperature at which the foragers were ready to emerge. We added beads coated with patroller hydrocarbons to otherwise undisturbed colonies in which patrollers were freely leaving and returning to the nest. The data collected in part A showed that the rate at which patrollers returned to the nest was relatively steady, fluctuating between a return rate of about 1 patroller per min and 3 patroller per min, for about the first 60 min of activity early in the morning. A steep increase in patroller return rate was observed approximately 30 min before foragers emerged (Figure 2). Using the same 12 colonies as in part A, and with each colony monitored by the same observer on all 3 days, we performed part B on days 2 and 3 of the experiment. We added glass beads coated with patroller cuticular hydrocarbons or

solvent-treated beads (as a control) to nest entrances. We chose to boost patroller return rate when the rate of patroller return began to increase sharply, but before a return rate of 1 patroller per 10 s was reached, so beads were added to nests when the rate of patroller return had approximately doubled, relative to the return rate 10 min before. A colony received either hydrocarbon or solvent-treated beads on a given day; the order of treatments for each colony was random. Ten beads were added to the nest at a rate of 1 bead every 10 s.

After the addition of the last bead, we measured foraging behavior. For consistency among observers, we defined the onset of foraging behavior as the return of 3 foragers carrying food back to the nest entrance within a 1-min interval. Thus, we defined the onset of foraging activity to be at a later point than the emergence of the first foragers. We compared the mean time elapsed from the addition of the first bead with the onset of foraging activity for each treatment using a paired-sample *t*-test, pairing data from the day the colony received solvent-treated beads as a control with data from the day the colony received beads treated with patroller hydrocarbons.

RESULTS

Experiment 1: Is a particular rate of encounters with patrollers necessary to elicit foraging?

The extent of foraging activity depended on the rate at which patroller mimics were introduced (repeated-measures ANOVA, $F_{1,4} = 11.94$, $P < 0.026$; Figure 1). A rate of 1 bead every 10 s elicited the greatest foraging response and elicited foraging from 4 out of 5 colonies. One of the 5 experimental colonies foraged actively when undisturbed but did not forage actively in any of the experimental conditions. Longer intervals of 45 s or 3 min elicited low levels of foraging. The shorter interval, less than 1 s between introductions, elicited foraging, but to a significantly lesser extent than the interval of 1 bead every 10 s (LSD, $P > 0.05$).

Experiment 2: Does the rate of encounter with patrollers influence when foraging begins?

A. In undisturbed colonies, foragers emerged from the nest after patrollers had achieved a mean (±standard deviation) patroller return rate of 4.09 ± 2.65 returning patrollers per min. This rate of return corresponds to a mean rate of 1 patroller returning every 14.6 s. Patroller return rates for 3 representative colonies are shown in Figure 2.

B. When the rate of patroller return was artificially boosted by adding beads coated with patroller hydrocarbons, foraging began sooner than when control beads were added to nests. Colonies receiving patroller hydrocarbons foraged 17.9 ± 19.7 min sooner than colonies receiving blank control beads (paired-samples *t*-test, $P < 0.01$; $n = 12$).

DISCUSSION

The rate of encounter with patrollers informs foragers' decisions to leave the nest to collect seeds. Our results confirm that harvester ants can distinguish the rate at which they encounter other ants. It appears that a forager decides to leave the nest on its first foraging trip of the day when it interacts with patrollers at a rate of about 1 per 10 s. Using patroller mimics, we showed that a return of 1 patroller or mimic about every 10 s elicited the highest level of foraging. When patroller return rates were artificially boosted, foraging began sooner than when blank control beads were added. Previous work showed that foraging activity was related to patrolling activity (Gordon 1987, 2002) and that this interaction depended on detection of patroller cuticular hydrocarbons (Greene and Gordon 2003). The results presented here show that the rate of contact with patrollers is crucial to stimulate foraging activity.

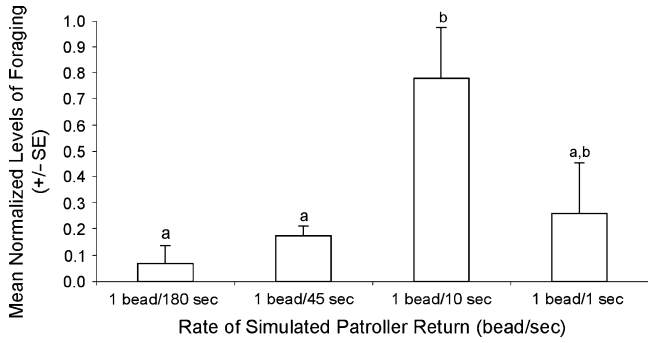

Figure 1
Rate of simulated patroller return affected foraging activity. Five colonies, each presented with all 4 return rates, were used in the experiment. Letters above bars denote differences in statistical significance among treatments (LSD, $P < 0.05$). Error bars denote standard error of the mean.

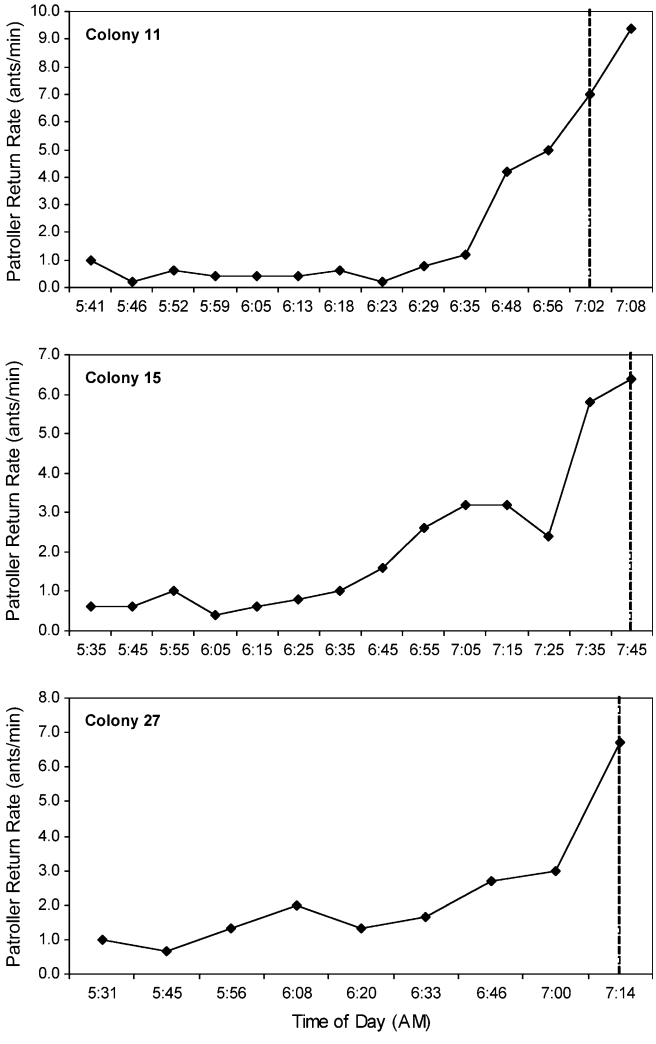

Figure 2
Patroller return rates in undisturbed harvester ant colonies over the course of the morning, from the first emergence of patrollers to the onset of foraging activity. The vertical dashed line indicates the onset of foraging activity.

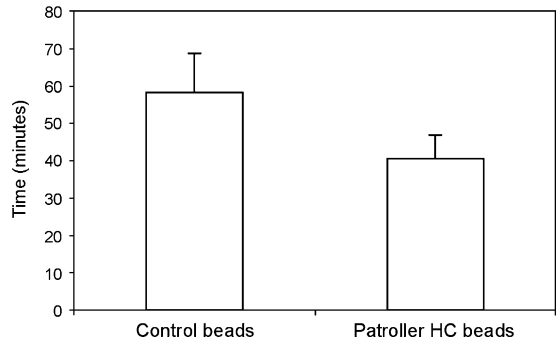

Figure 3
The time elapsed until the onset of foraging in colonies inwhich patroller return rate was boosted by the addition of patroller hydrocarbon beads or blank control beads. Error bars denote standard error of the mean.

Our results suggest that individual ants have a threshold response to multiple events. An interpretation consistent with the results is that each time a patroller interacts with a forager, the encounter triggers a response in the forager that decays over time. This response corresponds to the probability the forager will leave the nest. When encounters occur rapidly enough, so that one encounter occurs before the effect of the previous one has fully decayed, each successive encounter increases the probability of foraging. Eventually, if there are enough encounters per unit time, the ant exceeds some activation threshold and goes out to forage (Page et al. 1998; Gautraisw et al. 2002). The decay is apparently quite rapid, on the order of 10 s. Such a decay in response to multiple stimuli is found in other biological systems. For example, both the dose and duration of stimulation can lead to sensory adaptation in olfactory sensory systems (Stortkuhl et al. 1999).

The threshold response model, however, does not explain why the response to 1-s intervals was substantially lower than the response to 10-s intervals. Encounters that are too rapid apparently interfere with the foragers' response. There may be a physiological reason for this; as with action potentials, there may be a short refractory period during which subsequent interactions cannot trigger a response (Widmaier et al. 2005). In the nervous system, this ensures that action potentials are distinct and nonoverlapping as they are propagated down axons.

A very high rate of encounter with patrollers, corresponding to our experimental rate of 1 bead per second, occurs only in a dangerous situation when all the patrollers return almost simultaneously, and it is not appropriate for foragers to emerge. We have observed alarmed patrollers that rushed back into the nest. In response to high winds or predation, mainly by horned lizards (genus *Phrynosoma*), alarmed patrollers may return very quickly to the nest (Gordon 2002).

There is no evidence that the rate of patroller return is associated with the availability of food in a particular location. Foraging intensity is linked to food availability through a separate process that occurs later in the day. Once foraging begins, foragers are stimulated to make further trips by the return of successful foragers (Schafer et al. 2006). Thus, the more food that is available, the more quickly it is brought back to the nest and the more foragers leave the nest on another trip. The safe return of patrollers informs the foragers that it is safe to leave the nest; if patrollers can return safely, so can foragers. The rate at which patrollers return indicates that sufficient numbers of patrollers were able to withstand the weather and hazards of that day to return at a rate of 1 every 10 s.

There is some evidence that social insects can assess rate and duration. The ant *Ectatomma ruidum* uses ambush tactics that repeatedly involve waits of the same duration (Schatz and Wcislo 1999) and can learn to schedule its feeding behavior at a certain time of day (Beugnon et al. 1995). In honeybees, the duration of the waggle dance is related to the quality of the resource it refers to (Seeley et al. 2000). Whether a honeybee nectar forager leaves the nest depends on the time she waits for her nectar to be unloaded (von Frisch 1967; Seeley and Tovey 1994). Like the responses of harvester ant foragers to the interval between contacts with patrollers, these assessments of duration may depend on the rate at which the effect of a stimulus decays. Further research is needed to investigate the neurological processes involved in temporal cognition in social insects.

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