

The Effects of Water, Season, and Colony Composition on Foraging Preferences of *Pheidole ceres* [Hymenoptera: Formicidae]

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*Field colonies of the ant *Pheidole ceres* were presented with a choice between a protein source and a carbohydrate source, under wet and dry conditions, at three different times in the year. These time periods corresponded with different reproductive (the production of sexuals) and growth (the production of workers) stages of the colony. Moisture had no effect on the foraging behavior of *P. ceres* but the colonies did change their foraging preferences during different times of the year. This behavior correlated with the amount of larvae in the colony. However, lab experiments demonstrated that larvae did not directly influence the foraging decisions of the workers but that adult reproductives did.*

KEY WORDS: *Pheidole ceres*; food preference; foraging; seasonal effects; decision-making; ants.

INTRODUCTION

Abiotic conditions, such as temperature and moisture, fluctuate in many natural environments. These changes may impact food availability. Animals adapted to these environmental fluctuations evolved mechanisms that cue in on these changes, allowing them to adjust their physiology and behavior

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in order to maximize reproduction and growth (Morse, 1980; Stephens and Krebs, 1986).

In temperate climates, the seasons cycle between warm months with high food availability and cold months with low food availability. Animals adapted to this cycle reproduce during seasons in which conditions are favorable. Once less favorable seasons are imminent, these animals switch strategies and store food in order to survive. As animals cycle between reproductive and survival strategies their foraging behavior changes as well (Pohl, 1976; Lucas, 1989). This pattern has been well documented in solitary animals. When birds (Levey and Stiles, 1992) and mammals (Davis, 1976; Hill and Florant, 1997) reproduce, they increase their protein intake, in order to feed growing young. These same animals switch to high-energy compounds such as carbohydrates and lipids when storing food for winter.

In addition to the long-term seasonal changes, short-term, less predictable changes can also occur. In arid climates, moisture potentially affects foraging choices. Kangaroo rats, for example, prefer seeds high in lipids to those high in protein in a normal dry environment (Frank, 1988). However, when the same choice is presented in a humid environment the kangaroo rat chooses the seeds high in protein. The kangaroo rat's decisions are driven by the fact that protein digestion costs metabolic water and lipid digestion produces metabolic water (Frank, 1988). Thus, a simple rainstorm could alter the foraging behavior of the animal.

Eusocial insect colonies that inhabit temperate zones face the same nutritional challenges as solitary animals. However, few studies have documented changes in colony level nutritional intake in response to changing abiotic conditions. *Polistes* workers forage for protein-rich caterpillars when the larvae are in the colony and switch to nectar once the reproductive adults emerge (West-Eberhard, 1969; Hoshikawa, 1981; Tsuchida, 1991; O'Donnell, 1998). In *Polistes*, only the gynes of rather than the colony as a whole survive to the following year (Reeve and Mathews, 1991). Thus, the change in foraging behavior represents a change in colony composition not a response to abiotic conditions. In perennial colonies, such as ants, the adults and larvae are maintained throughout the winter requiring colonies to store enough nutrients to sustain the entire colony. Thus, changes in abiotic conditions should affect changes in colony level food intake. Stein *et al.* (1990) showed a population of the imported fire ant, *Solenopsis invicta* foraged for protein during warmer months when the colonies were reproducing and foraged for carbohydrates during colder months when the colony was in its growth stage (Oster and Wilson, 1978). However, the imported fire ant inhabits areas in which the winter is not cold enough to completely stop the colony from foraging. A better test of the effects of seasonal changes is to examine a eusocial animal that over-winters as a colony in a climate in which winter conditions prevent foraging.

Mechanisms should be in place to allow foragers to adjust their behavior to match the needs of other colony members as the colony's needs are changing. One possible mechanism is other colony members communicate their needs to the workers. Hunger levels of nurse bees (Camazine, 1993) and nurse ants (Sorenson *et al.*, 1985; Cassill and Tschinkel, 1995; Cassill *et al.*, 1998; Cassill and Tschinkel, 1999a) have been shown to affect foraging decisions. Larval hunger has also shown to influence adult behavior. *Polistes fuscatus* adults ascertain the nutritional needs of the larvae through the use of nest vibrations (Savoyard *et al.*, 1998). Brian and Abbott (1977) found evidence that larvae of *Myrmica rubra* can cause workers to forage for protein. In the army ant, *Neivamyrmex nigrescens*, larval hunger will increase the likelihood a colony to emigrate (Topoff and Miranda, 1980). Thus, it is possible for a worker's behavior to be influenced by fellow workers as well as nonworkers.

In this study, I examined how external cues such as season and water and the internal cues such as the colony composition affect foraging behavior of the ant *Pheidole ceres* over the course of the year. *P. ceres* is found in an arid climate that has harsh winters. This provided a natural situation in which water stress, an immediate problem, is coupled with longer term climate change. As with all ants in this environment, colonies of *P. ceres* stop foraging during the winter (personal observation) so they have to store enough food to survive and produce new reproductives when conditions are favorable.

I presented field colonies of *P. ceres* with a choice between proteins and carbohydrates under both wet and dry conditions at different times of the year. This experiment allowed me to look for seasonal and short-term effects on *P. ceres*'s foraging behavior. The ants' foraging choices were also compared with the colony composition at each time interval. Additional laboratory experiments were performed to determine if worker foraging behavior is influenced by other castes.

METHODS

General Methods

Study Site

The study site was located in the Rocky Mountains (Larimer County, Colorado) at about 2285 m above sea level. The ant colonies were located on a south-facing slope covered with patchy vegetation (predominantly small shrubs, cacti, and grass). The slope received a lot of direct sunlight. Owing to the heat, very few ant species foraged during the day.

Study Organisms

Pheidole ceres (Gregg, 1963) colonies typically nest under flat rocks in open areas with multiple entrances. Multiple entrances enable the colony to exploit multiple food sources simultaneously. Foragers show a strong aversion to direct sunlight and are most active during evenings, mornings, and at night when the temperature is above 10°C.

Colonies of *P. ceres* are monogynous. There are 700–1000 individuals in a typical mature colony. As with all species in the genus *Pheidole*, there are two distinct worker castes, majors and minors. A total of 88.5% ($\pm 15\%$) of the workers are minors in *P. ceres* colonies. Minors and majors forage for the same food sources but only minors appear able to recruit more foragers. Although workers harvest seeds, *P. ceres* also consume dead insects and even milk aphids for honeydew (personal observation).

Feeders

I created feeders modeled after the bee feeders of von Frisch (1967) that allowed me to control the food sources and to easily assess the numbers of recruits. Each feeder consisted of a small round (6 cm) disk cut from translucent red plastic with eight grooves (about 2 cm long) evenly spaced radiating from the center. A small PVC pipe cap (2.5 cm in diameter) was inverted onto the disk so that the liquid placed in the cap flowed into the troughs (the ants appeared to be able to get to the liquid in the cap from any point where the cap rested on the disc). A vacuum created in the pipe cap prevented overflow. To prevent the *P. ceres* foragers from being disturbed while they foraged, I illuminated the feeders from underneath with a 1.5 V bulb placed in an inverted petri dish of the same diameter as the feeder and buried under the soil. Any light from above, such as a flashlight beam, disturbed the ants, even if it was filtered for red wavelengths. However, when the feeder was lit from underneath, the ants foraged readily on it.

Field Experiments

Part 1: Surveys—Methods

I collected baseline data on the same 10 colonies once a month from April 2000 until October 2000. During the surveys, I categorized the number of larvae and pupae of minors, majors, and sexuals as, absent, few, some, many. This circumvented destructive sampling. I also noted the presence or absence of adult males and female sexuals.

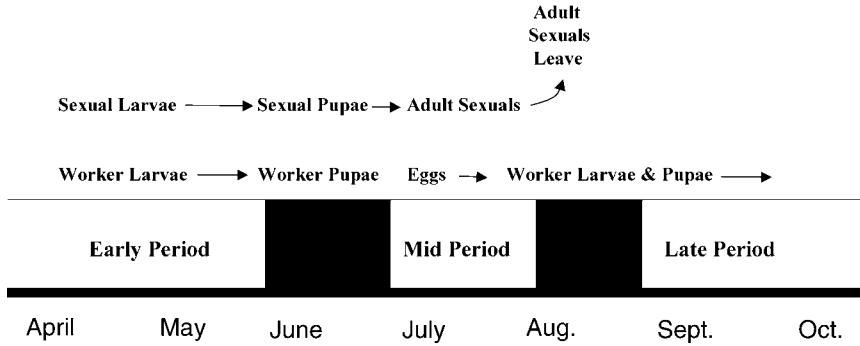


Fig. 1. Diagram depicting the change in colony composition of a typical colony of *Pheidole ceres* over the course of their active season. The early, mid, and late periods represent the three time periods the field experiments were conducted in.

Part 1—Results

Composition of the colonies of *P. ceres* I surveyed was synchronized throughout their active season (Fig. 1). During the spring (April–May), the brood consisted of both worker (minors and majors) larvae, and reproductive larvae. These larvae appeared to pupate during the early summer (June), and at this time there were very few larvae present in the colonies. The reproductive adults emerged during mid-summer (early July) and remained in the nest for about a month. During this time, there were many eggs and pupae but very few larvae. The reproductives departed at the beginning of August. From that point on, there were both larvae and pupae present for the rest of the ants’ active season (until late October).

Part 2: The Effects of Time of Year and Soil Moisture on Protein vs. Carbohydrate Preference—Methods

Colonies were tested during three time periods, representing different events during the colony cycle. The three periods (Fig. 1) were as follows:

Early Period. Spring: May—Both worker and reproductive larvae present,

Mid Period. Mid Summer: July—Adult reproductives and few worker larvae present,

Late Period. Early Fall: September–early October—Worker larvae only.

Within each period, the same 10 colonies (different from the surveyed colonies above) were tested for their preference between carbohydrates and proteins under wet soil and dry soil conditions. All trials were conducted when the soil was naturally dry (a minimum of 2 days after a daylong rain).

The wet treatment consisted of sprinkling 10 L of water on the colony and the surrounding soil (about a 50 cm radius) 2 h before a trial. This simulated a heavy rainfall. The dry treatment was not manipulated.

A pair of colonies, one wet, one dry, was tested for protein vs. carbohydrate preference on the same night. Five nights later the same pair was tested again except the conditions were reversed for the colonies. Thus, all 10 colonies received both treatments, and a wet and a dry colony were tested on the same day. All colonies were tested at roughly the same time of the evening. This experiment was repeated for all three time periods however, an early snowfall in the late period prevented completion of the second half of the trial. Thus colonies in the late period were only tested once.

Trials. Two feeders were both placed equidistant (30 cm) from the colony. One feeder contained 20% sucrose solution with 50 μ L/L solution of anise extract (to attract the initial scouts to the feeder: see Seeley *et al.* (1991); anise has no effect on the food preference in *P. ceres* (personal observation) as recruits follow chemical trails not the food scent). The other contained 20% egg white solution (egg white has a fairly strong smell so a scent was not necessary). The two feeders were placed on the left and right (relative to the slope) of the colony at random (flipping a coin) in the clear patches of soil such that there were no clumps of vegetation between the feeder and colony entrance.

Once the ants discovered one of the two feeders, I recorded the number of ants on each feeder every 5 min until 1 h after the second feeder was discovered. If the second feeder was not located within 40 min the trial was ended to prevent any satiation effects.

Part 2—Results

Behavior of Foragers. The recruitment behavior of *Pheidole ceres* was similar to that found in *Pheidole morrisi* (Johnson, 1988). A single minor (scout) found the food source and fed for approximately 3 min, usually stopping midway for a few seconds. The scout then headed back to the colony. Less than 8 min later, a stream of foragers, usually a few majors and many minors, headed to the feeder from a colony entrance. Once the recruitment to the feeder began, continuous streams of workers left and arrived from it.

Scouts showed the same recruitment behavior for both protein and carbohydrate sources.

The two foraging trails emerged from two separate entrances from the colony. These entrances usually appeared near the edge of the same rock, indicating they are from the same colony.

I have occasionally observed majors foraging in the absence of minors. In a few cases, a solitary major arrived at a feeder before any other ant, fed, and then returned to her colony. The major returned to the feeder several times but never recruited other foragers. Thus, only minors appear to recruit other individuals.

Numbers of Foragers. The absolute numbers of foragers were compared for the 10 colonies in all three periods. There was no significant difference in the total number of minors ($N = 10$, $p = 0.68$, Friedman's test) or majors ($N = 10$, $p = 0.39$, Friedman's test) outside each colony in all three periods. Thus, each colony was sending out the same number of recruits for each time period.

Index of Food Preference. The number of foragers at the feeder leveled out about 30 min into the trials. For each trial, I took the last four measurements and used the mean of those measurements to get a final score or index for the foraging preferences of each colony. For inter-colony comparisons, these measurements were converted to the percent of foragers at the carbohydrate feeder to control for colony size. Because the absolute number of foragers did not change from period to period, any change in percent of ants at a feeder represents a shift in food preference. This index was created for all 10 colonies in all three foraging periods. This index was used in the four analyses that follow.

Effects of Water. There were no significant effects from adding water to the soil around the colonies on foraging behavior in the Early or the Mid period (Fig. 2). This was true for both minors (Early: $N = 10$, $p = 0.575$; Mid: $N = 9$, $p = 0.260$, Wilcoxon Matched Pairs Test) and majors (Early: $N = 10$, $p = 0.374$; Mid: $N = 9$, $p = 0.999$, Wilcoxon Matched Pairs Test). Results from the Early and Mid periods suggested there would not have been a significant effect during the Late period either.

Effects of Time of Year. In all 10 colonies, minors showed a significant change in foraging decisions across the three periods ($N = 8$, $p = 0.030$, Friedman's test; Fig. 3). There was a significant increase from the Early period to the Mid period (Multiple Comparisons Procedure, MCP; Daniel, 1990). The minors showed a nonsignificant drop in preference for carbohydrates from the Mid period to the Late period (MCP).

The majors of all 10 colonies showed an even more dramatic change from the Early period to the Mid period ($N = 6$, $p = 0.0045$, Friedman's Test; MCP; Fig. 3). In fact 100% of the majors went to the carbohydrate

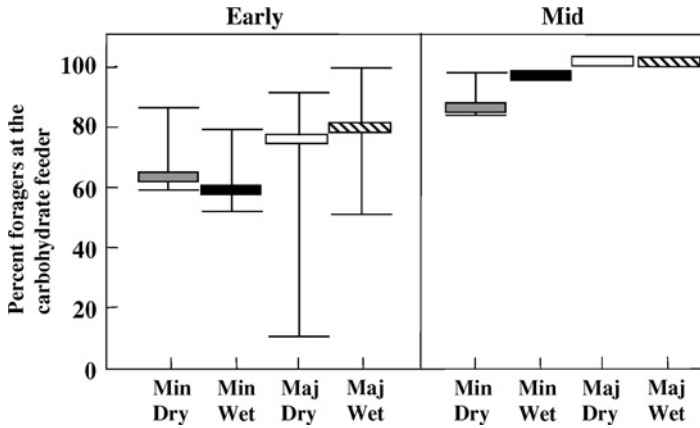


Fig. 2. The medians and quartiles of the percent number of *Pheidole ceres* minors and majors at the carbohydrate feeders during the wet and dry treatments. There were no significant differences between wet and dry trials.

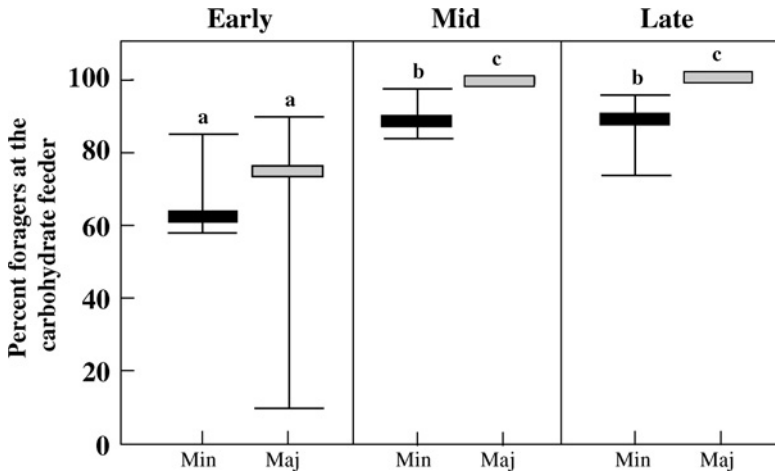


Fig. 3. The medians and quartiles depicting the percent foragers at the carbohydrate feeder for 10 *Pheidole ceres* colonies during the three time periods. The minors (black) and majors (gray) showed a significant change in preference over the three time periods. The letters summarize the significant differences for within-caste comparisons (between time periods; Friedman's test and Multiple Comparison Procedure) and between-caste comparisons (within time periods; Wilcoxon Matched Pairs Test).

feeder in the Mid period. This food choice was maintained in the Late period.

Food Preference. Food preference for both minors and majors during all three periods was tested using a Friedman's test followed by a Multiple Comparisons Procedure (MCP) (Fig. 3). Both minors and majors showed no significant preference during the Early period. During both the Mid and Late periods the minors ($p < 0.00001$; Friedman's test; MCP) and majors ($p = 0.0001$; Friedman's test; MCP) had a highly significant preference for carbohydrates.

Majors vs. Minors. Majors and minors were compared for each time period (Fig. 3) using the Wilcoxon Matched Pairs Test followed by a Bonferroni table wide correction (Rice, 1989). Majors and minors showed no significant differences in foraging choices in the Early period ($N = 10$, $p = 0.65$). However, majors showed a significantly higher preference for carbohydrates in both the Mid and Late period ($N = 9$, $p = 0.0077$; $N = 6$, $p = 0.028$). This suggests that majors and minors are not making the same foraging decisions.

Lab Experiments

I conducted three follow-up experiments in the laboratory to investigate the effects of larvae, adult reproductives, and humidity on the foraging behavior of adult workers.

General Methods

Collections. For each experiment, 10 mature colonies were dug up in the field and transported back to the lab. The colonies were then sorted from the soil and moved into the experimental setup described below. For experiment 1, 20 colonies were used, 10 in May and 10 in August. An additional 10 colonies were used for experiments 2 and 3 in that order.

Nestboxes. Nestboxes were made from Gladware[®] plastic salad containers (709 mL). The boxes were half-filled with dental plaster. A square indentation in the middle of the plaster with a plastic cover provided the actual living quarters for the colony. Two holes were drilled through the opposite sides of the nestbox into the nest cavity so that the ants could readily move from the nest cavity into the arena (see later).

Arenas. The occupied nestboxes were kept in arenas made from Sterilite[®] 11.4 L boxes (42 cm × 29.5 cm × 16.5 cm). A layer of plaster with an indentation for the nestbox was placed on the arena's bottom to control the moisture levels. The walls of the arena were covered with Fluon[®] to prevent escape. In order for the ants to forage normally, a light layer of dirt was placed in the arena.

Trials. Two feeders were placed in opposite corners of the arena. One feeder contained 10% egg white solution (protein) and the other 10% sucrose solution (carbohydrate). Once a feeder was discovered the number of recruits at both feeders was counted every 5 min for 1 h. The second feeder was generally discovered within 5 min of the first.

Use of the colonies was staggered across the week such that the experiment was initiated for only two colonies each night. This allowed me to test each colony at the same time each night.

Data Analysis. I analyzed the data from the laboratory experiments in a similar manner as the field experiments. In these analyses, I used the numbers of ants instead of percent ants because all of the subunits were the same size. There was no need to adjust for colony size.

Experiment 1: Brood Switching Experiment—Methods

In this experiment, I tested whether or not larvae could directly affect the foraging decisions of foragers. I created a situation in which larvae and workers were fed on different nutrients and then observed how this affected the behavior of the foragers.

Ten colonies were divided into four equal subunits consisting of 150 minors and one-fourth the available larvae placed in the experimental arenas described earlier. Two of the units (Fig. 4) were fed carbohydrates (10% sucrose solution) *ad lib* and the other two were fed protein (10% egg white solution) *ad lib*. The subunits were housed in a room with 12/12 h light/dark cycle for 5 days. The food was changed every 2 days. After the 5-day period, the food was removed from the colonies in the morning. That night (at 22:30), the subunits were tested for their food preference.

After the trial, the subunits were put back on their diets for another 5 days. The food was removed in the morning after day 10. That evening, the brood from a carbohydrate fed subunit and the brood from a protein fed subunit were switched (Fig. 4). To control for disturbance, the larvae from the controls on each diet were removed and returned the same subunit. In all cases, the larvae were quickly moved into the nestbox by the ants. After the larvae were switched, I had subunits with the following conditions for each of the 10 colonies:

- (A) protein-deprived workers and carbohydrate-deprived larvae,
- (B) carbohydrate-deprived workers and protein-deprived larvae,
- (C) protein-deprived workers and larvae,
- (D) carbohydrate-deprived workers and larvae.

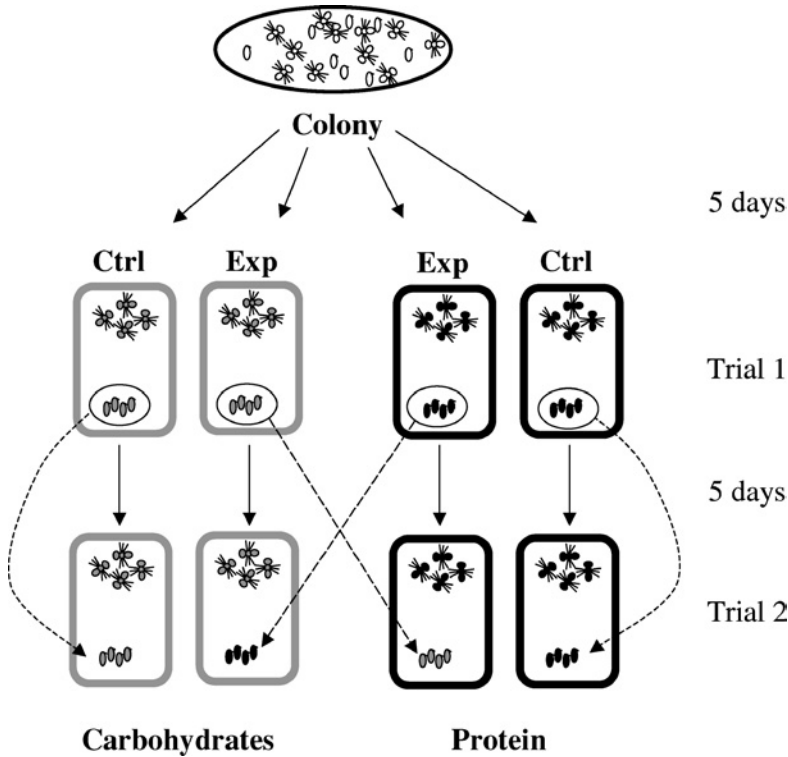


Fig. 4. The brood switching experiment. A single colony was divided into four subunits. Each subunit was given either carbohydrates (gray) or proteins (black). Trial 1 was performed without larval switching. Trial 2 was done after the larvae were switched between the two experimental subunits. The larvae were removed and then returned in subunits in the control subunits to control for disturbance.

The colonies were allowed to sit for at least 4 h after the larvae were moved before I began the second feeding trial. At the end of the final trial, numbers of larvae were counted again to insure that larval death did not influence the results.

This experiment was performed in August 2000 and repeated in May 2001 to control for possible seasonality effects on worker-larvae interactions.

Experiment 1 – Results

The results for August 2000 and May, 2001 were not significantly different (χ^2 Test of Homogeneity (Daniel, 1990), $\chi^2 = 2.4, p > 0.05$) and

were combined for the following analysis. I used the Wilcoxin Signed Rank Test followed by a Sequential Bonferroni Table wide test (Rice, 1989) for inter-subunit comparisons. Each subunit in which the brood was exchanged (experimental) was compared for both carbohydrate and protein feeders with (1) its result from the first feeding trial (control 1), (2) the control subunit fed on the same diet (control 2), and (3) the other experimental subunit (Fig. 4).

There was no significant effect of the type of larvae present in the subunit on worker behavior. Each brood-switched subunit showed no significant difference in food preference when compared to both controls (Fig. 5). Thus, workers with carbohydrate starved larvae behaved the same as workers with protein starved larvae regardless of their own diet.

However, workers in protein fed subunits foraged significantly more for carbohydrates than the workers in carbohydrate fed subunits (Wilcoxin Signed Ranked Test, $p = 0.0014$, $T = 3$, $Z = 3.8$; Fig. 5A). There were no significant differences between any of the subunits at the protein feeder (Fig. 5B). These results suggest that workers were responding to their own needs and not directly responding to larval needs.

Larval mortality was high ($80\% \pm 2\%$), but there was no difference in mortality between the carbohydrate and protein fed subunits. Thus, larvae were probably not being sacrificed in the protein-deprived subunits.

Experiment 2: Effect of Different Castes on the Foraging Behavior of Minors—Methods

In this experiment, I tested the ability of the brood, adult sexuals, and majors to affect the food preference of minors. Ten colonies were collected in late July 2001 when both larvae (represented by several different instars) and adult sexuals were present. Each colony was subdivided into four subunits that contained the following:

- (A) 150 minors and all of the adult sexuals (mean = 30 sexuals),
- (B) 150 minors and all of the larvae (mean = 25 larvae),
- (C) 150 minors (control),
- (D) 150 minors and all of the majors (mean = 35 majors).

The colonies were kept in a 12/12 light/dark cycle for 3 days without food. During the night after the last day, I tested the colonies for their food preference (see Trials in General Methods section). I used the Friedman's Test followed by a Multiple Comparisons Procedure (MCP) to compare the results of all four subunits (Daniel, 1990).

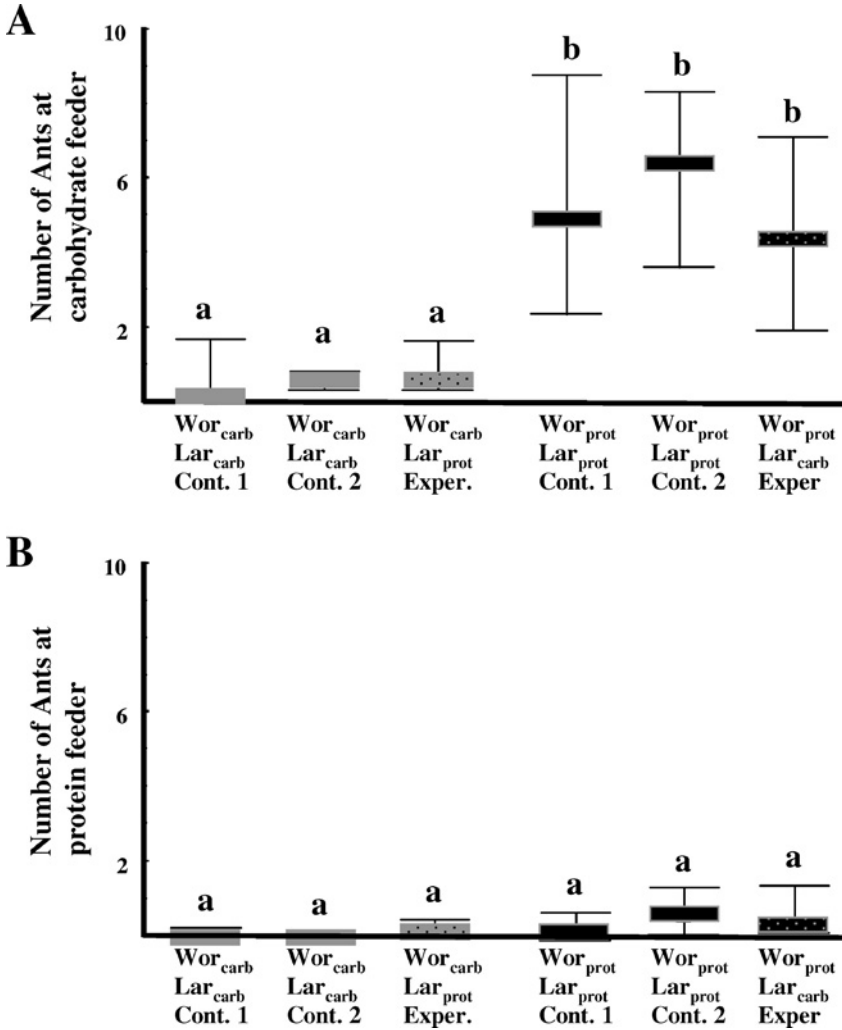


Fig. 5. The medians and quartiles of the protein fed subunits and carbohydrate fed subunits from the brood switching experiment for both (A) carbohydrate and (B) protein feeder. In each graph, control 1 = the result from the experimental subunit from trial 1, control 2 = the results from the control subunit from trial 2, and experimental = the result from the experimental subunit after it received larvae fed on the opposite diet. The gray bars indicate the carbohydrate fed subunits and the black indicated protein fed sub units. The different letters indicate significant differences based on the Wilcoxin Signed Rank Test ($p = 0.0014$, $N = 20$) corrected for using the Bonferroni Sequential Table Wide Correction (Rice, 1989). Each feeder type was analyzed separately.

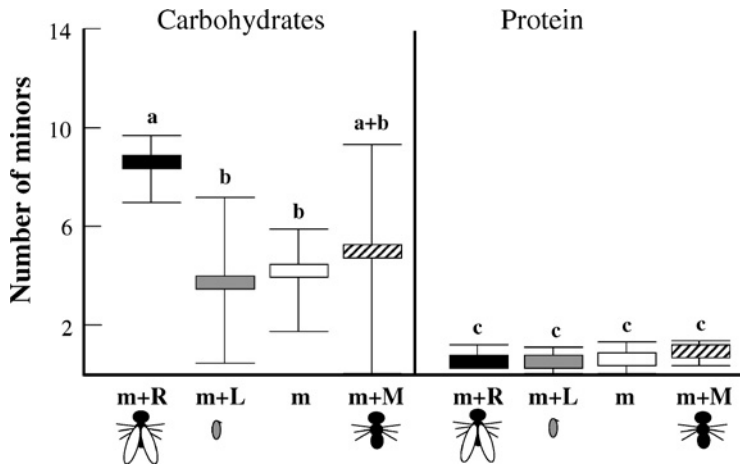


Fig. 6. The medians and quartiles depicting the effects of different castes on food preference of *P. ceres* minors in laboratory colonies. The four different subunits are represented as follows: m + R, minors and reproductives; m + L, minors and larvae; m, just minors; m + M, minors and majors. The different letters indicate significant differences based on the Friedman's test ($p = 0.056$, $N = 10$) and the Multiple Comparisons Procedure (Daniel, 1990). Each feeder type was analyzed separately.

Experiment 2—Results

The subunits that contained adult reproductives showed higher recruitment to carbohydrates than did the control subunits or the brood subunits (Friedman's test & MCP: $p = 0.056$, $\chi^2 = 7.56$, $N = 10$; Fig. 6). The subunit containing majors showed no significant differences from all three subunits (MCP) probably due to the considerable variation. There were no significant differences in the recruitment of workers to the protein source in four subunits (Friedman's test: $p = 0.31$, $\chi^2 = 3.53$, $N = 10$; Fig. 6). This suggests that the reproductives cause an increase in the amount of carbohydrates taken in by the colonies but the larvae have no effect on the behavior of the foragers.

Experiment 3: Effect of Moisture on Food Preference—Methods

This experiment tested the effects of humidity on the foraging behavior of *Pheidole ceres*. Ten colonies were divided into two subunits, humid and dry. The arenas and nestboxes used for the humid subunits were soaked in water overnight prior to adding ants. This insured the plaster was saturated

with water. After the bin was soaked, a small layer of dirt was placed in the arena. Once the ants were added, the arena was sealed using clear plastic wrap and tape.

The arenas and nestboxes used for the dry subunits had their moisture removed by placing a petri dish, containing Drierite[®] in them, overnight prior to adding ants. Once the ants were added, the petri dish with Drierite[®] was placed in the arena on top of the nestbox to prevent any humidity from building up in the box. A screen prevented ants from getting into the dish. The arena was then covered with clear plastic wrap and tape to maintain the low humidity.

I let the colonies stay in 12/12 light/dark room for 48 h without food. The colonies were not allowed to sit more than 48 h to prevent larvae in the dry bin from desiccating. After 48 h elapsed, the humidity within each subunit's arena was measured using a VWR Traceable[®] hygrometer/thermometer. Each subunit was tested for their food preference (see Trials in General Methods section). I used the Wilcoxin Signed Rank Test for inter-subunit and intra-subunit comparisons. These analyses were corrected using the Sequential Bonferroni Table Wide Test (Rice, 1989).

Experiment 3—Results

The dry bins had a mean humidity of 38.28 ± 9.04 RH% (range: 23.0–50.0 RH%) and the wet bins had a mean humidity of 95.7 ± 1.18 RH% (range: 94.4–98.2 RH%).

Colonies in the dry subunits showed a significantly higher recruitment to carbohydrates (Wilcoxin Signed Ranked Test: $p = 0.0077$, $T = 0$, $Z = 2.67$) but not to proteins (Wilcoxin Signed Ranked Test: $p = 0.17$, $T = 6$, $Z = 1.35$) than the humid subunits (Fig. 7). There was a significant preference for carbohydrates in the dry subunits (Wilcoxin Signed Ranked Test: $p = 0.012$, $T = 0$, $Z = 2.52$) but not in the humid subunits (Wilcoxin Signed Ranked Test: $p = 0.29$, $T = 10.5$, $Z = 1.05$). This difference could be explained by the lower numbers of individuals foraging for carbohydrates in the humid subunits (Fig. 7) and not by an increase in forager numbers at protein feeders in the wet subunits.

DISCUSSION

The results of this study demonstrate that (1) moisture levels had no effect on the foraging behavior of *Pheidole ceres* colonies tested in the field and laboratory; (2) the foraging behavior of *P. ceres* was influenced

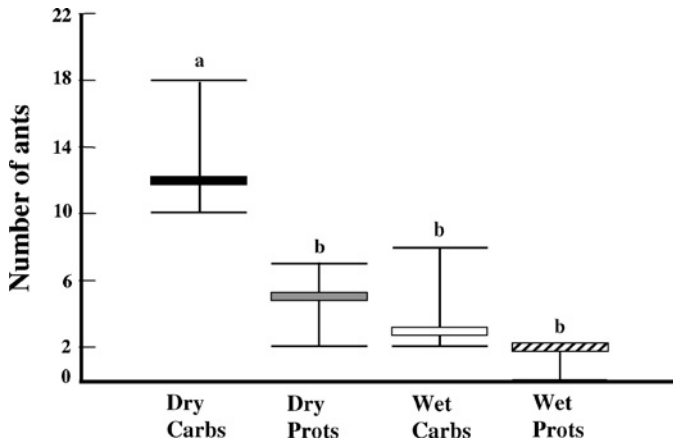


Fig. 7. The medians and quartiles depicting the effects of moisture on the foraging preferences of laboratory colonies of *P. ceres*. There was a significantly larger number of ants foraging for carbohydrates in the dry subunits than in of the other three feeders (Wilcoxin Signed Ranked Test: $p = 0.0077$, $N = 10$).

by season and colony composition. Field colonies responded to a choice between carbohydrates and protein differently at different times of their active season; (3) although the foraging behavior corresponded to the amount of larvae and adult reproductives in field trials, only the adult reproductives directly influenced the foraging behavior of the workers in colonies tested in the laboratory.

Moisture Effects

The absence of an effect of moisture in this study suggests that metabolic water is not a factor influencing the foraging preferences of *P. ceres*. Although insects living in arid climates are generally concerned with water loss, metabolic water loss may not be an important component (Edney, 1977). Most of the water loss experienced by arthropods is through respiration, excretion (Edney, 1977) or loss of the protective wax coating due to digging in soil (Johnson, 2000). All of these factors, although important for *P. ceres*, are not affected by diet.

Most ground dwelling ant colonies are able to adjust their moisture levels through nest architecture (Hölldobler and Wilson, 1990). Scherba (1959) found that nests of *Formica ulkei* were maintained at 30% humidity. He demonstrated that the larvae of *F. ulkei*, which consumed most of

the protein collected, were moved to moisture levels ideal for digesting proteins. These colony level adaptations would negate the need for individuals to adjust their foraging decisions based on metabolic water.

Seasonal Effects

In the spring and summer the food preference correlated with the types of individuals being reared. The highest preference for protein occurred during the spring when large numbers of larvae were present. Larvae are normally the chief consumers of protein in a colony (Vinson, 1968; Stradling, 1987; Weeks *et al.*, 2004) and their presence would increase the need for protein. In contrast, when the reproductive adults were present (Mid period), the colony increased its intake of carbohydrates. The reproductives needed to acquire energy-rich foods to fatten up for their mating flight and to start a new colony (Peakin, 1985; Hölldobler and Wilson, 1990; Passera and Keller, 1990). Although some protein has been shown to be stored at this time by adult sexuals (Wheeler and Buck, 1992; Wheeler and Martinez, 1995), they seem to acquire these stores during the larval stage (Peakin, 1985; Passera and Keller, 1990). At the time the adult reproductives were present, there were small numbers of larvae in the colony. Thus, the need for protein dropped from the spring to the summer as did the preference for protein. A similar pattern was seen in *Solenopsis invicta*. Colonies were shown to increase their intake of protein in warm months, when larvae were feeding, and collected more carbohydrates in cold months, when larvae were not feeding (Stein *et al.*, 1990). Both *S. invicta* and *P. ceres* foragers appear to be adjusting their behavior according to the nutritional needs of the colony.

In the fall, the colonies of *P. ceres* retained their preference for carbohydrates despite the presence of larvae. In this case, the colonies may have switched from reproductive strategies to survival strategies. Unlike *S. invicta* studied by Stein *et al.* (1990), *P. ceres* is unable to forage during the winter, and colonies must gather enough resources to survive the winter. *P. ceres* stores food by harvesting seeds (Wheeler and Rissing, 1975; Hölldobler and Wilson, 1990; Wilson, 2003) and physically storing food internally such as in the fat bodies (Nation, 2002). Several studies (Wilson, 1974; Lachaud *et al.*, 1992; Tschinkel, 1993a) found that majors store proportionally more lipids than minors. In this study, the majors showed a significantly higher preference for carbohydrates than the minors during the last two trial periods. It is possible that the majors of *P. ceres* are acting as fat stores. Majors rarely take on responsibilities such as feeding larvae or queens in natural colonies (Wilson, 1984; Wilson, 1985; Brown and

Traniello, 1998), and thus may be able to initiate food stores earlier than the minors who are feeding other colony members. Somehow foragers change their foraging strategy from gathering nutrients for reproduction to gathering nutrients for storage. The seasonal shift is clear in *P. ceres* but what drives this shift is still unclear.

The Effects of Colony Composition

The results of the brood switching experiment (lab experiment 1) and the caste effect experiment (lab experiment 2) show that larval hunger does not directly affect the foraging behavior of the workers, which matches the findings of Cassill and Tschinkel (1995) for *Solenopsis invicta*. However, the presence of adult reproductives causes workers to forage for more carbohydrates.

In the brood experiment, the larval mortality was high and may have affected the results. However, larval mortality was minimal in the caste effects experiment and the larvae still had no effect on worker behavior. Cassill and Tschinkel (1999b) found that *Solenopsis invicta* larvae directly affected worker behavior when the larvae to worker ratio was 1:1. This ratio never occurs naturally in *P. ceres* colonies (personal observation, $N = 40$). Thus, it is unlikely that *P. ceres* larvae ever directly influence worker behavior under natural conditions.

Why are the adult reproductives but not the larvae able to directly influence worker foraging behavior? On a proximate level, this influence might be due to the mobility of the individual in question. Larvae are immobile and depend on the nurse ants. It is the nurse ants that actually interact with the foragers, not the larvae themselves (Sorenson *et al.*, 1985; Cassill and Tschinkel, 1999c). Hence any larval effects on foragers would be indirect. The reproductives, on the other hand, are mobile and interact with foragers directly. Adult reproductives are possibly intercepting returning foragers before another worker unloads them. During many lab and field experiments, I saw reproductives come out to artificial food sources. Although the reproductives did not feed from the feeders, I occasionally observed trophallaxis between a worker and an adult sexual on the feeder. This suggests that returning foragers can attend adult reproductives.

Whether or not time to unload influences recruitment rate in *P. ceres*, as it does with honeybee foragers (Seeley and Tovey, 1994), is still unknown. If the workers are unloaded faster when reproductives are present in the colony this could help to increase the turnaround time to the carbohydrate feeder. This faster unloading time, in turn, could increase

recruitment rate as it does in honeybees (Seeley and Town, 1992; Seeley, 1993).

On an ultimate level, the adult reproductives are not expendable but one could argue that larvae, at least in part, are. If a colony is food-stressed, it benefits from the workers feeding the adults prior to larvae. If the larvae die, the colony fails to grow but it survives until food becomes more abundant. Indeed, in some cases larvae have been cannibalized (Tschinkel, 1993b). In addition, the wealth of sex ratio literature (which is beyond the scope of this paper to review) suggests that life as a larva is not 100% guaranteed. Workers sometimes cannibalize larvae differentially according to sex (Passera and Aron, 1993; Aron *et al.*, 1994; Passera and Aron, 1996; Sundstrom *et al.*, 1996; Chapuisat *et al.*, 1997; Aron *et al.*, 2001). By contrast, the adult reproductives are the embodiment of the reproductive investment of a colony. The colony should go to great lengths to insure their investment survives until the mating flight. Since adult reproductive survival is a much higher priority than larval survival in a colony, it might be beneficial to the colony if adult reproductives directly influence workers' foraging decisions.

Eusocial colonies cannot afford to have larvae controlling worker foraging decisions. In wasps (Hunt *et al.*, 1996, 1998), bees (Winston, 1987), and ants (Wheeler and Nijhout, 1981; Wheeler, 1986), larval nutrition has a direct effect on caste determination. In *Pheidole*, the fate of an individual is decided at the larval stage (Wheeler and Nijhout, 1981). If the larvae had control over the foraging decisions, they could determine their own fates regardless of the benefit to the colony (Reuter and Keller, 2001). This could easily result in the breakdown of a eusocial colony's caste system. As long as larvae can be deprived of certain nutrients, control of caste development can be maintained (Hunt *et al.*, 1998).

P. ceres must be using an indicator other than larval needs to decide what nutrients are needed by the colony. One possibility is that ants are using the nutrients contained internally as a cue. Sorenson *et al.* (1985) found that colonies of *Solenopsis invicta* containing hungry nurse ants are more likely to forage than colonies with satiated nurse ants. Hungry nurse ants could be depleting the reserves of the foragers and thus stimulating them to forage. In *Leptothorax albipennis*, lipid content in foragers has been shown to correlate with the likelihood of an individual to forage (Blanchard *et al.*, 2000). The authors suggested that corpulence is a possible cue for workers to forage. In my study, there was additional supporting evidence that workers self-assess. In the brood switching experiment, workers in the subunits fed on carbohydrates foraged significantly less than those fed proteins. Workers might be using their internal stores of carbohydrates to determine whether or not to forage.

General Conclusions

Workers of the ant *Pheidole ceres* appear to adjust their foraging preferences according to the colony's needs. This allows the colony as a whole to behave in a similar manner as a larger temperate animal, and to cycle between reproduction and survival strategies from year to year. During the spring, the colony is in a reproductive phase. Food availability is high and the temperatures are favorable for *P. ceres* foragers. In the fall, there is a switch to a survival strategy. The preference for carbohydrates stays high despite the presence of larvae. The colony as a whole seems to be responding to long-term predictable changes but not adjusting to smaller less predictable changes.

The results of this study clearly show that foragers are not responding directly to the nutritional needs of the larvae. However, adult sexuals are able to increase the colony's intake of carbohydrates. It would be advantageous to the colony to allow adult reproductives some control, to insure the survival of the colony's reproductive investment. When the adult sexuals are not present, the workers are apparently using a cue other than larval needs to determine whether or not to forage for a particular nutrient, possibly, what they are storing in themselves. Thus, larval nutritional needs are only met by an indirect mechanism mediated by nurse ants.

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REFERENCES

- Aron, S., Keller, L., and Passera, L. (2001). Role of resource availability on sex, caste and reproductive allocation ratios in the Argentine ant *Linepithema humile*. *J. Anim. Ecol.* **70**: 831–839.

- Aron, S., Passera, L., and Keller, L. (1994). Queen-worker conflict over sex-ratio—A comparison of primary and secondary sex-ratios in the Argentine ant, *Iridomyrmex humilis*. *J. Evol. Biol.* **7**: 403–418.
- Blanchard, G. B., Orledge, G. M., Reynolds, S. E., and Franks, N. R. (2000). Division of labour and seasonality in the ant *Leptothorax albipennis*: Worker corpulence and its influence on behaviour. *Anim. Behav.* **59**: 723–738.
- Brian, M. V., and Abbott, A. (1977). The control of food flow in a society of the ant *Myrmica rubra* L. *Anim. Behav.* **25**: 1047–1055.
- Brown, J. J., and Traniello, J. F. A. (1998). Regulation of brood-care behavior in the dimorphic castes of the ant *Pheidole morrisi* (Hymenoptera: Formicidae): Effects of caste ratio, colony size, and colony needs. *J. Insect Behav.* **11**: 209–219.
- Camazine, S. (1993). The regulation of pollen foraging by honeybees: How foragers assess the colony's need for pollen. *Behav. Ecol. Sociobiol.* **32**: 265–272.
- Cassill, D. L., Stuy, A., and Buck, R. G. (1998). Emergent properties of food distribution among fire ant larvae. *J. Theor. Biol.* **195**: 371–381.
- Cassill, D. L., and Tschinkel, W. R. (1995). Allocation of liquid food to larvae via trophallaxis in colonies of the fire ant, *Solenopsis invicta*. *Anim. Behav.* **50**: 801–813.
- Cassill, D. L., and Tschinkel, W. R. (1999a). Regulation of diet in the fire ant, *Solenopsis invicta*. *J. Insect Behav.* **12**: 307–328.
- Cassill, D. L., and Tschinkel, W. R. (1999b). Information flow during social feeding in ant societies. In Detrain, C., Deneubourg, J.-L., and Pasteels, J. M. (eds.), *Information Processing in Social Insects*, Birkhäuser-Verlag, Basel.
- Cassill, D. L., and Tschinkel, W. R. (1999c). Effects of colony-level attributes on larval feeding in the fire ant, *Solenopsis invicta*. *Insect. Soc.* **46**: 261–266.
- Chapuisat, M., Sundstrom, L., and Keller, L. (1997). Sex-ratio regulation: The economics of fratricide in ants. *Proc. R. Soc. Lond. Ser. B-Biol. Sci.* **264**: 1255–1260.
- Daniel, W. W. (1990). *Applied Nonparametric Statistics*, PWS-Kent, Boston.
- Davis, D. E. (1976). Hibernation and circannual rhythms of food-consumption in marmots and ground squirrels. *Q. Rev. Biol.* **51**: 477–514.
- Edney, E. B. (1977). *Water Balance in Land Arthropods*, Springer-Verlag, Berlin.
- Frank, C. L. (1988). Diet selection by a heteromyid rodent: Role of net metabolic water production. *Ecology* **69**: 1943–1951.
- Gregg, R. E. (1963). *The Ants of Colorado*, University of Colorado Press, Boulder, CO.
- Hill, V., and Florant, G. (1997). Essential fatty acids in free-ranging yellow bellied marmots (*Marmota flaviventris*). *FASEB J.* **11**: 3055–3055.
- Hölldobler, B., and Wilson, E. O. (1990). *The Ants*, Harvard University Press, Cambridge, MA.
- Hoshikawa, T. (1981). Some colony factors influencing the hunting activity of *Polistes chinensis antennalis* Perez (Hymenoptera, Vespidae). *Appl. Entom. Zool.* **16**: 395–405.
- Hunt, J. H., Rossi, A. M., Holmberg, N. J., Smith, S. R., and Sherman, W. R. (1998). Nutrients in social wasp (Hymenoptera: Vespidae, Polistinae) honey. *Ann. Entomol. Soc. Am.* **91**: 466–472.
- Hunt, J. H., Schmidt, D. K., Mulkey, S. S., and Williams, M. A. (1996). Caste dimorphism in the wasp *Epipona guerini* (Hymenoptera: Vespidae; Polistinae, Epiponini): Further evidence for larval determination. *J. Kans. Entomol. Soc.* **69**: 362–369.
- Johnson, C. (1988). Colony structure and behavioral observations in *Pheidole morrisi* (Hymenoptera: Formicidae). In Trager, J. C. (ed.), *Advances in Myrmecology*, E. J. Brill, Leiden, pp. 371–383.
- Johnson, R. A. (2000). Water loss in desert ants: Caste variation and the effect of cuticle abrasion. *Physiol. Entomol.* **25**: 48–53.
- Lachaud, J.-P., Passera, L., Grimal, A., Detrain, C., and Beugnon, G. (1992). Lipid storage by major workers and starvation resistance in the ant *Pheidole pallidula* (Hymenoptera, Formicidae). In Billen, J. (ed.), *Biology and Evolution of Social Insects*, Leuven University Press, Leuven, pp. 153–160.

- Levey, D. J., and Stiles, F. G. (1992). Evolutionary precursors of long-distance migration resource availability and movement patterns in neotropical landbirds. *Am. Nat.* **140**: 447–476.
- Lucas, J. R. (1989). Time scale and diet choice decisions. In Hughes, R. N. (ed.), *Behavioral Mechanisms of Food Selection*, Springer-Verlag, London, pp. 165–186.
- Morse, D. H. (1980). *Behavioral Mechanisms in Ecology*, Harvard University Press, London.
- Nation, J. L. (2002). *Insect Physiology and Biochemistry*, CRC Press, Boca Raton, FL.
- O'Donnell, S. (1998). Genetic effects on task performance, but not on age polyethism, in a swarm-founding eusocial wasp. *Anim. Behav.* **55**: 417–426.
- Oster, G. F., and Wilson, E. O. (1978). *Caste and Ecology in the Social Insects*, Princeton University Press, Princeton, NJ.
- Passera, L., and Aron, S. (1993). Social-control over the survival and selection of winged virgin queens in an ant without nuptial flight—*Iridomyrmex humilis*. *Ethology* **93**: 225–235.
- Passera, L., and Aron, S. (1996). Early sex discrimination and male brood elimination by workers of the Argentine ant. *Proc. R. Soc. Lond. Ser. B-Biol. Sci.* **263**: 1041–1046.
- Passera, L., and Keller, L. (1990). Loss of mating flight and shift in the pattern of carbohydrate storage in sexuals of ants (Hymenoptera; Formicidae). *J. Comp. Physiol. B* **160**: 207–211.
- Peakin, G. J. (1985). The growth and development of the overwintering larvae in the ant *Lasius flavus* (Hymenoptera, Formicidae). *J. Zool.* **205**: 179–189.
- Pohl, H. (1976). Seasonal changes in the whole animal. In Bligh, J., Cloudsley-Thompson, J. L., and Macdonald, A. G. (eds.), *Environmental Physiology of Animals*, Wiley, New York, pp. 309–338.
- Reeve, H. (1991). *Polistes*. In Ross, K., and Mathews, R. (eds.), *The Social Biology of Wasps*, Comstock, Ithaca, NY, pp. 99–148.
- Reuter, M., and Keller, L. (2001). Sex ratio conflict and worker production in eusocial hymenoptera. *Am. Nat.* **158**: 166–177.
- Rice, W. R. (1989). Analyzing tables of statistical tests. *Evolution* **43**: 223–225.
- Savoyard, J. L., Gamboa, G. J., Cummings, D. L. D., and Foster, R. L. (1998). The communicative meaning of body oscillations in the social wasp, *Polistes fuscatus* (Hymenoptera, Vespidae). *Insect. Soc.* **45**: 215–230.
- Scherba, G. (1959). Moisture regulation in mound nests of the ant, *Formica ulkei* Emery. *Am. Mid. Nat.* **61**: 499–508.
- Seeley, T. D. (1993). The tremble dance of the honey bee: Message and meaning. *Behav. Ecol. Sociobiol.* **31**: 375–383.
- Seeley, T. D., Camazine, S., and Sneyd, J. (1991). Collective decision-making in honey bees: How colonies choose among nectar sources. *Behav. Ecol. Sociobiol.* **28**: 277–290.
- Seeley, T. D., and Tovey, C. A. (1994). Why search time to find a food-storer bee accurately indicates the relative rates of nectar collecting and nectar processing in honey bee colonies. *Anim. Behav.* **47**: 311–316.
- Seeley, T. D., and Town, W. F. (1992). Tactics of dance choice in honey bees: Do foragers compare dances? *Behav. Ecol. Sociobiol.* **30**: 59–69.
- Sorenson, A. A., Busch, T. M., and Vinson, S. B. (1985). Control of food influx by temporal subcastes in the fire ant *Solenopsis invicta*. *Behav. Ecol. Sociobiol.* **17**: 191–198.
- Stein, M. B., Thorvilson, H. G., and Johnson, J. W. (1990). Seasonal-changes in bait preference by red imported fire ant, *Solenopsis invicta* (Hymenoptera, Formicidae). *Florida Entomol.* **73**: 117–123.
- Stephens, D. W., and Krebs, J. R. (1986). *Foraging Theory*, Princeton University Press, Princeton, NJ.
- Stradling, D. J. (1987). Nutritional ecology of ants. In Slansky, F., Jr., and Rodriguez, J. G. (eds.), *Nutritional Ecology of Insects, Mites, Spiders and Related Invertebrates*, Wiley, New York, pp. 927–970.
- Sundstrom, L., Chapuisat, M., and Keller, L. (1996). Conditional manipulation of sex ratios by ant workers: A test of kin selection theory. *Science* **274**: 993–995.
- Topoff, H., and Miranda, J. (1980). Army ants on the move: Relation between food supply and emigration frequency. *Science* **202**: 1099–1100.

- Tschinkel, W. R. (1993a). Sociometry and sociogenesis of colonies of the fire ant *Solenopsis invicta* during one annual cycle. *Ecol. Monogr.* **63**: 425–457.
- Tschinkel, W. R. (1993b). Resource allocation, brood production and cannibalism during colony founding in the fire ant, *Solenopsis invicta*. *Behav. Ecol. Sociobiol.* **33**: 209–223.
- Tsuchida, K. (1991). Temporal behavioral variation and division-of-labor among workers in the primitively eusocial wasp, *Polistes jadvigae* Dalla Torre. *J. Ethol.* **9**: 129–134.
- Vinson, S. B. (1968). Distribution of an oil, carbohydrate and protein food source to members of imported fire ant colony. *J. Econ. Entomol.* **61**: 712–714.
- von Frisch, K. (1967). *The Dance Language and Orientation of Bees*, Harvard University Press, Cambridge.
- Weeks, R. D. J., Wilson, J. T., Vinson, S. B., and James, W. D. (2004). Flow of carbohydrates, lipids, and protein among colonies of polygyne red imported fire ants, *Solenopsis invicta* (Hymenoptera: Formicidae). *Ann. Entomol. Soc. Am.* **97**: 105–110.
- West-Eberhard, M. (1969). The social biology of Polistine wasps. *Miscellaneous Public. Museum Zool. Mich.* **140**: 1–101.
- Wheeler, D. E. (1986). Developmental, and physiological determinants of caste in social Hymenoptera: Evolutionary implications. *Am. Nat.* **128**: 13–34.
- Wheeler, D. E., and Buck, N. A. (1992). Protein, lipid and carbohydrate use during metamorphosis in the fire ant, *Solenopsis xyloni*. *Physiol. Entomol.* **17**: 397–403.
- Wheeler, D. E., and Martinez, T. (1995). Storage proteins in ants (Hymenoptera, Formicidae). *Comp. Biochem. Physiol. B* **112**: 15–19.
- Wheeler, D. E., and Nijhout, H. F. (1981). Soldier determination in the ant *Pheidole bicarinata*—Effects of a juvenile-hormone analog on caste and size within castes. *Am. Zool.* **21**: 1028–1028.
- Wheeler, J., and Rissing, S. W. (1975). Natural history of *Veromessor pergandei*. 1. Nest—(Hymenoptera-Formicidae). *Pan-Pacific Entomol.* **51**: 205–216.
- Wilson, E. O. (1974). The soldier of the ant *Camponotus (Colobopsis) fraxinicola* as a trophic caste. *Psyche* **81**: 182–188.
- Wilson, E. O. (1984). The relation between caste ratios and division of labor in the ant genus *Pheidole* (Hymenoptera: Formicidae). *Behav. Ecol. Sociobiol.* **16**: 89–98.
- Wilson, E. O. (2003). *Pheidole in the New World*, Harvard University Press, Cambridge, MA.
- Wilson, E. O., and Hölldobler, B. (1985). Caste-specific techniques of defense in the polymorphic and *Pheidole embolopyx* (Hymenoptera: Formicidae). *Insect. Soc.* **32**: 3–22.
- Winston, M. L. (1987). *The Biology of the Honey Bee*, Harvard University Press, Cambridge, MA.