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Regulation of Diet in the Fire Ant, *Solenopsis invicta*

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In social insects, colony nutrition depends upon the volume and quality of food distributed, ingested, and assimilated by its members. The ability of Solenopsis invicta *workers and larvae to regulate the volume of food ingested individually has been well documented. In this paper, the ability of fire ant workers and larvae to regulate the quality and type of food ingested is demonstrated. Larvae displayed independent appetites for solid protein, amino acid solution, and sucrose solution. When larvae that had fed on one food type were switched to another, they fed on the second food type at rates characteristic of that food type, not of the volume of food previously ingested. Larvae preferred concentrated sucrose and amino acids solutions over dilute solutions. Larval "fullness" was thus a relative property, depending upon the nature of food as well as the volume ingested. The number of workers recruiting to food sites also depended upon food concentration and food type. Workers preferred sucrose to amino acids, concentrated to dilute solutions, and novel to accustomed food. The absence of protein in the worker diet rather than the presence of larvae caused workers to switch their preference from sugar to amino acids solutions. When the colony was offered sucrose and amino acids solutions simultaneously, individual workers ingested from one or the other site, but not both. Little mixing of crop contents occurred when workers solicited from one another inside the nest. Workers tended to regurgitate to larvae after ingesting amino acids and to other workers after ingesting sucrose. The mechanism regulating the distribution of protein pellets, which workers do not ingest, among larvae is unknown. In summary, colony nutrition was regulated by a chain of demand. Forager hunger determined the rate at which food flowed from the environment into the nest. Larval hunger and nest-worker hunger determined the rate and direction in which food moved within the nest.*

KEY WORDS: recruitment; larvae; feeding; trophallaxis; nutritional ecology.

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INTRODUCTION

The fitness of solitary insects depends in part on the ability of larvae to find, ingest, and assimilate the appropriate quantity and quality of nutrients (reviewed by Slansky and Rodriguez, 1987). Pioneering work on caterpillar nutrition, first published in 1902 by Pictet (in Slansky and Rodriguez, 1987), led to the founding of the field of nutritional ecology (Slansky, 1982). The premise of nutritional ecology is that adults rarely achieve their maximum mating success, reproductive output, dispersal ability, longevity, or energy reserves (Slansky, 1993) because of environmental constraints on feeding or food absorption during the juvenile phase of development. Caterpillars counteract environmental constraints by adjusting the amounts and rates of food eaten, assimilated, and converted into tissue by rejecting food items based on toughness, nutritional richness, food type, food state, or levels of noxious/toxic chemicals (reviewed by Slansky and Wheeler, 1992). Although longer feeding bouts can increase growth and fecundity, these gains can be offset by increased exposure to predation or desiccation (Slansky, 1982). Therefore, when food is ephemeral or patchy, a larva may adjust its feeding or development rates and metamorphose into a small adult rather than risk predation or desiccation by remaining a larva.

These threats to larvae of social insects are less intense because larvae are protected from desiccation by a physical nest and from predation by legions of adult defenders. Within this protected environment, variation in larval nutrition and development has diversified over evolutionary time, particularly in ants, to produce such extremes as sterile workers that vary 20- to 500-fold in size [*Solenopsis invicta* (Porter and Tschinkel, 1985) and leaf-cutter ants (Hölldobler and Wilson, 1990), respectively] and fertile queens that may be reproductively active for up to 20 years (Wilson, 1971; Hölldobler and Wilson, 1990; Wheeler, 1994).

How are diverse larval nutrition and development regulated? To date, the paradigm has been that workers control larval diet, thus controlling larval developmental fate (reviewed by Hunt and Nalepa, 1994). Recent work on the flow of food to larvae of the fire ant, *Solenopsis invicta*, suggests an alternative paradigm. Several decades ago, Howard and Tschinkel (1981a,b) and Sorensen *et al.* (1980, 1981, 1985) showed that three food types moved to larvae in different quantities, suggesting the possibility of diet regulation by the larvae themselves. Recently, we provided the first direct evidence that individual larva regulate food volume in relation to their size and level of hunger (Cassill and Tschinkel, 1995, 1996; Cassill *et al.*, 1999). In this paper, we demonstrate that larvae regulate food quality as well. Together, these findings are relevant to the mechanisms that shape individual morphology and function, colony growth and reproduction, and social organization.

MATERIALS AND METHODS

In a previous study (Cassill and Tschinkel, 1995), a standard mixture of 10% sucrose and 5% amino acids was used. In this study, the effects of food type, food concentration, and food state on the flow of food from worker to larva and worker to worker were quantified.

Source Colonies and Experimental Nest

For all experiments, mature field colonies were collected in Tallahasee during June 1994 and, again, in January 1995 and maintained on a constant diet (tenebrionid beetle larvae and 20% sugar water) at 28°C in constant light. Laboratory rearing and handling methods were similar to those described by Banks *et al.* (1981).

Experimental nests consisted of water-saturated, plaster bases (10 × 14 × 2 cm) with a 0.3-cm-high rim around the top edge that, when covered by glass, formed a brood chamber through which workers and larva could be viewed by eye, microscope, or videocamera. Nests were sealed on the outside with paraffin wax to reduce evaporation. These experimental nests were placed in plastic trays (13.5 × 12 × 2.5 cm) whose sides had been treated with Fluon to prevent worker escape. Two entrance tunnels were available at opposite ends of the brood chamber to allow workers to move freely to and from the brood chamber.

Standard Experimental Treatment

Workers were removed randomly from the arena and the brood chamber of source colonies, weighed, placed into an experimental nest, and deprived of food for 48 h to allow their crops to empty (Cassill and Tschinkel, 1999). Larvae were taken from several source colonies, separated from workers, mixed together, deprived of food for 24 h, then sampled, weighed, and placed into the experimental nest, usually 1 h before initiation of treatment. In this way, each colony fragment received a random subset of larvae from the same pool. Unless otherwise noted, source colony fragments in experimental nests contained 2 g workers (~3000–4000) and 1 g larvae (~2000).

Food Types and Dyes

Amino acid solutions were made from casamino acids powder [DIFCO Laboratories (Howard and Tschinkel, 1981b)] in distilled water (w/v). Sucrose solutions were made from granulated cane sugar in distilled water (w/v). Solid foods were obtained by feeding excess tenebrionid beetle larvae to a laboratory colony, collecting the masticated pellets from that colony a day later, and refrigerating them in a sealed plastic container. When required for an experiment, both pellets and solutions were dyed with over-the-counter food dyes (French's

or McCormick's), usually green, sometimes red. Pellets were dyed by soaking them in a 10% solution of dyed distilled water, then evaporating the water from the mixture in a drying oven. Nondyed pellets were treated in the same manner with distilled water to assure treatment consistency. The addition of dyes to food solutions at concentrations of 10–30% (v/v) had no effect on the percentage of larvae fed (Cassill and Tschinkel, 1995).

Videotaping Larval Feedings

Videotaping equipment consisted of a Sony color videocamera (WV D5100) with lens (Taylor, Taylor & Hobson, Ltd.; 2 in., F/1.4) and 1- to 6-cm extensoin tubes providing 20–80× magnification on the TV monitor, a JVC video cassette recorder (HR-D600U), a Sony Trinitron color monitor, and fiber-optic lights. On tape, the camera's field of view at 40× power sampled 50–100 larvae of the thousands placed in each experimental nest.

Data Analysis

Feeding behaviors were transcribed from the videotape to a spreadsheet using a computerized event reorder to quantify the lapsed time to the first feeding, the number of feedings, and the mean duration of these feedings for a larva for 1 h. The videotape was rewound and feeding data on a second larva were obtained. This was repeated until feeding data on a sample of 10 larvae for each treatment in each replicate were obtained (for transcription details, see Cassill and Tschinkel, 1995).

The mean volume of food ingested by fourth-instar larvae during one regurgitation event is about 1.5 nl (Cassill and Tschinkel, 1996), which was used as a conversion factor to estimate the volume of liquid food consumed by larvae per unit time (1.5 nl × number of trophallactic events).

Data were analyzed using analysis of variance (ANOVA), with colony as a blocking factor and larval size as a covariate (Statistica-W; Statsoft, 1994). Tukey's honest significant difference test (Tukey's HSD) was used for pairwise comparisons of cell means. When appropriate, regression or Student's *t* test was used (Ryan *et al.*, 1985). Residual and normal score analysis were routinely performed to check for outliers (SD >2.99) and to determine if assumptions of normality and uniform variance held. When needed, square root or log transformations were completed. For several experiments, food consumption rates were quantified over time. Because the experimental unit was the larva and because a different set of ~50 larvae was videotaped from the thousands of larvae in the brood chamber for each hour of videotaping, the independence of the experimental units was effectively 100%, thus analysis by standard ANOVA rather than repeated-measures ANOVA was appropriate. All statistics reported under Results are ANOVA unless otherwise noted.

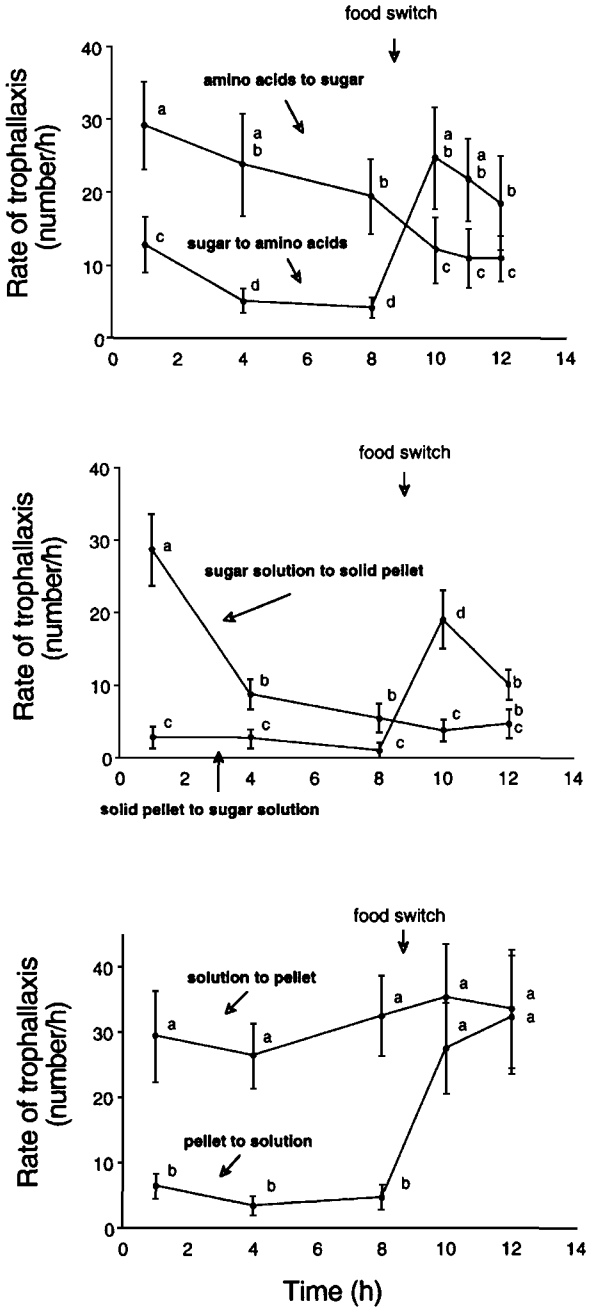
RESULTS

Food Switch

Several food-switching experiments were conducted to determine if larvae adjusted their rate of ingestion according to the type of food offered to them or simply according to the total volume ingested. Food was paired by type and by state (10% amino acids solution versus 10% sucrose solution; protein pellets versus 10% sucrose solution; protein pellets versus 10% amino acids solution; and finally, protein pellets with or without water. The food-switching procedure was the same in all experiments. One source colony was divided into two experimental nests of standard worker and larva number and was subjected to standard food-deprivation periods. Each experimental nest received one of the two food types being tested. For example, one nest received the undyed sucrose solution, and the other the undyed amino acids solution. After 8 h, the first food type was removed and the other food type was introduced (food was replenished every 2 h to reduce evaporative concentration as a cofounding factor). Larval feeding was videotaped for each fragment at 1, 4, 8, 10, 11, and 12 h. Food dyes were added to the second food types introduced at the beginning of the ninth hour to confirm ingestion by workers and larvae. Dyes were especially important in the experiments using pellets because the ingestion of pellets by larvae does not involve trophallaxis, which confirms ingestion. Each videotaping session lasted 1 h and care was taken to videotape a different section of the larval brood pile to ensure independence of the larva samples. The experiment was replicated using a total of three source colonies.

Sucrose Solution Versus Amino Acid Solution

Both the type of food ($F_{1,348} = 135.5$, $P < 0.0001$) and time ($F_{5,348} = 6.45$, $P < 0.0001$) affected the rate of worker-larva trophallaxis (Fig. 1A); the interaction was not significant ($F_{5,348} = 2.02$, P = n.s.). The rate of larval ingestion was significantly greater for amino acids solution than for sucrose solution ($F_{1,58} = 27.65$, $P < 0.0001$). Larvae satiated quickly on sucrose ($F_{2,87} = 41.21$, $P < 0.0001$); the rate of trophallaxis decreased dramatically between the first and the fourth hour (Tukey HSD, $P = 0.01$), then remained low through the eighth hour (Tukey HSD, $P = 0.999$). The rate of trophallaxis for sucrose during the first hour after it was introduced was the same whether larvae had been empty or full of amino acids (Tukey HSD, $P = 0.50$), suggesting independent appetites for sucrose and amino acids. Larvae were not as quick to satiate on amino acids. Although the rate of trophallaxis declined when larvae were fed amino acids ($F_{2,87} = 4.26$, $P < 0.02$), the decline was not measurable from the first through the fourth hour (Tukey HSD, $P = 0.72$) or from the fourth to the eighth hour (Tukey HSD, $P = 0.88$), but only when comparing the first and the eighth hour


food switch
amino acids to sugar
sugar to amino acids
Rate of trophallaxis (number/h)
food switch
sugar solution to solid pellet
solid pellet to sugar solution
Rate of trophallaxis (number/h)
food switch
solution to pellet
pellet to solution
Rate of trophallaxis (number/h)
Time (h)

(Tukey HSD, $P = 0.01$). The rate of trophallaxis for amino acids during the first hour after it was introduced was the same whether larvae were empty or full of sucrose (Tukey HSD, $P = 0.19$), again suggesting that larval appetites for amino acids and sucrose were independent. In other words, larvae changed their rate of ingestion in response to the novel food. When food was switched from amino acids to sucrose, the rate of trophallaxis declined to a level consistent with an appetite for sucrose. When food was switched from sucrose to amino acids, the rate of trophallaxis increased to a level consistent with an unsated appetite for amino acids. These results demonstrated that food type was a stronger regulator of larval satiation than was food volume.

The duration of individual trophallactic events did not differ significantly with a switch in food type ($F_{1,348} = 0.50$, P = n.s.) or with time ($F_{5,348} = 0.91$, P = n.s.). The interaction was not significant ($F_{5,348} = 0.13$, P = n.s.).

Solid Food Pellets Versus Sucrose Solution

Both the type of food ($F_{1,290} = 61.34$, $P < 0.0001$) and time ($F_{4,290} = 36.91$, $P < 0.0001$) affected the rate of worker-larva trophallaxis (Fig. 1B). The interaction was significant ($F_{4,290} = 56.45$, $P < 0.0001$) because protein pellets depressed the larval appetite for sucrose, but not vice versa. When larvae were fed sucrose, a significant decrease in the rate of trophallaxis occurred between the first and the fourth hour (Tukey HSD, $P = 0.0001$). As in the previous experiment, the rate of trophallaxis remained low from the 4th to the 12th hour of feeding (Tukey HSD, $P = 0.077$) even after the introduction of dyed pellets at the 8th hour (4th vs 9th Tukey HSD, $P = 0.52$). The presence of solid protein in the diet lowered the rate of trophallaxis of sucrose (Tukey HSD, $P = 0.00001$). We cannot rule out the possibility that the difference in feeding rates was because workers contained a mixture of sucrose and digested protein in their crops. When larvae were fed solid protein, the rate of trophallaxis was consistently low (Tukey HSD, 1st vs 4th, $P = 0.98$; Tukey HSD, 1st vs 8th, $P = 0.99$). The number of larvae that were fed protein pellets was affected by time ($F_{4,80} = 10.34$, $P < 0.0001$),

Fig. 1. Food-switch experiments. (A) Rates of trophallaxis per larva before and after a switch in food type from 10% amino acid solution to 10% sucrose solution, or vice versa, at the eighth hour of a 12-h observation period. Rates of trophallaxis depended upon food type as well as food volume, with larvae preferring amino acid solution to sucrose solution. (B) Rates of trophallaxis per larva before and after a switch in food type from protein pellets to 10% sucrose solution, or vice versa, at the eighth hour of a 12-h observation period. Satiation on solid pellets reduced the larval appetite for sucrose solution. (C) Rates of trophallaxis per larva before and after a switch in amino acid state from protein pellet to 10% amino acid solution, or vice versa, at the eighth hour of a 12-h observation period. Satiation on solid protein did not affect larval appetites for amino acids solution; however, satiation on amino acid solution preempted the delivery of solids to larvae. Bars = mean ± SE. Lowercase letters above bars denote significant differences among means.

but not by the presence or absence of sucrose in the larva's diet ($F_{1,80}$ = 24.26, $P < 0.0001$). There was no interaction ($F_{4,80}$ = 14.74, $P < 0.0001$). These findings suggest that larval appetites for protein solids and sucrose solutions were independent.

Durations of individual trophallaxis events did not vary significantly with time ($F_{4,142}$ = 1.19, P = n.s.) or food type ($F_{1,142}$ = 0.71, P = n.s.). There was no interaction ($F_{4,142}$ = 0.36, P = n.s.).

Protein Pellets Versus Amino Acid Solution

Both food state ($F_{1,190}$ = 75.32, $P < 0.0001$) and time ($F_{4,190}$ = 14.00, $P < 0.0001$) affected the rate of worker-larva trophallaxis (Fig. 1C). Interaction was significant ($F_{4,190}$ = 9.60, $P < 0.0001$) because, although the rate of trophallaxis increased dramatically when amino acid solution was introduced in the ninth hour to larvae feeding on pellets for the previous 8 (Tukey HSD, $P = 0.00001$), the reverse was not true. The rate of trophallaxis remained the same when pellets were introduced in the ninth hour to larvae feeding on amino acid solution for the previous 8 h (Tukey HSD, $P = 0.99$). Even though the high rate of trophallaxis for the entire 12 h suggested that larvae were not yet satiated on protein, not one dyed pellet was delivered to larvae from h 9 to h 12. Colonies were observed for an additional 12 h after dyed pellets were introduced, and even then, pellets were not distributed among larvae. Rather, they were collected and stored in a corner of the brood chamber. Perhaps because workers still carried liquid amino acids in their crops, they had no incentive to deliver solid protein to larvae. Larvae were equally hungry for amino acids solution whether they had just dined on protein pellets or had been starved for 24 h (Tukey HSD, 1st h after 24-h starvation vs 9th h after ingesting pellets, $P = 0.99$).

Both food state ($F_{1,20}$ = 686.32, $P < 0.0001$) and time ($F_{4,20}$ = 189.9, $P < 0.0001$) affected the number of larvae that received a pellet. Interaction was significant ($F_{4,20}$ = 192.9, $P < 0.0001$) because, although the majority of larvae received pellets when starved or when full of sucrose solution (see results from previous experiment), no larvae received pellets when full of amino acids solution (Tukey HSD, 1st h with amino acids solution vs 9th h with amino acids solution and pellets, $p = 0.99$). This finding suggests that protein in solution form is far more attractive to larvae than in solid form. Perhaps solids are energetically too expensive to process in large quantities.

Durations of trophallaxis did not vary significantly with time ($F_{4,178}$ = 0.16, P = n.s.) but did differ with food state ($F_{1,178}$ = 31.91, $P < 0.0001$), however, differences were small and may be a result of the greater viscosity of the liquefied pellets. Interaction was significant ($F_{4,178}$ = 3.03, $P < 0.02$).

Protein Pellets With or Without Water

The presence/absence of water did not affect the rate of trophallaxis (rates were low regardless; $F_{1,96} = 0.60$, P = n.s.), but time did ($F_{1,96} = 7.76$, $P < 0.006$). There was an interaction effect ($F_{1,96} = 13.42$, $P < 0.0004$) largely because, although the mean intake of water over the 2 h was the same, the initial and final rates varied. Without water, larvae ingested liquefied pellets at a low but steady rate for 2 h; with water, larvae ingested either water or liquefied pellets at relatively high rates initially, then at low rates by the second hour. Neither the presence of water ($F_{1,96} = 12.40$, $P < 0.0006$) nor time ($F_{1,96} = 1.78$, P = n.s.) significantly affected the number of larvae receiving pellets. There was no interaction ($F_{1,96} = 0.13$, P = n.s.). Water was not a limiting factor in the delivery or ingestion of solid pellets, suggesting that solid pellets are not a volumetrically abundant item in the diet of fourth-instar larvae.

Neither the presence/absence of water ($F_{1,96} = 2.20$, P = n.s.) nor time ($F_{1,96} = 3.88$, P = n.s.) affected the duration of trophallaxis. There was no interaction ($F_{1,96} = 0.00$, P = n.s.).

Food Concentration

To test whether colony members discriminated among food of different concentrations (1, 10, and 30% solutions), a source colony was fragmented into six experimental nests composed of workers and larvae at a standard number and ratio, which were then deprived of food for the standard time period. Three experimental nests were offered one of three concentrations of a sucrose solution, and the other three experimental nests were offered one of the three concentrations of amino acids solution. Videotaping of larval feedings was completed at 1 and 8 h after the initial introduction of food into the arena. Each session lasted 1 h. Recruited workers were counted 30 min and 4 h after the introduction of food into the arena. Eight replicates (eight source colonies) were completed, but because of a variety of technical problems associated with Murphy's Law (after playwright Arthur Murphy, 1727–1805), only two of the eight had complete cells.

Rate of Worker Recruitment

Worker recruitment to liquid food varied significantly with concentration ($F_{2,8} = 52.39$, $P < 0.0001$), with food type ($F_{1,8} = 11.42$, $P < 0.0001$), and with time ($F_{1,8} = 19.77$, $P < 0.0001$; Figs. 2A and B). The food type/concentration interaction was significant ($F_{2,8} = 23.01$, $P < 0.0001$), because although workers preferred sucrose to amino acids at 30% concentration, they preferred amino acids to sucrose at lower concentrations. When starved, workers preferred concentrated sucrose to concentrated amino acids (Tukey HSD, $P = 0.0001$). Worker

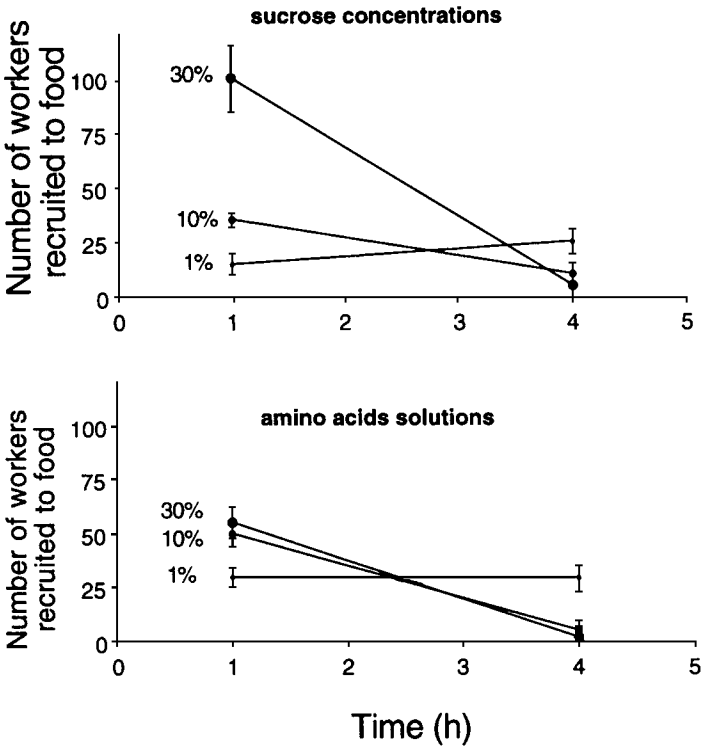


Fig. 2. Worker recruitment based upon food concentration. (A) Workers recruited to concentrated solution of sucrose to greater numbers than to diluted solutions. Worker recruitment decreased significantly with time for higher concentrations of sucrose, suggesting colony satiation, but remained stable for low concentrations of sucrose. (B) Although workers recruited to higher concentrations of amino acid solutions than the diluted solution, workers did not discriminate between the 10% and the 30% amino acid solutions. Worker recruitment decreased significantly with time for higher concentrations of amino acids, again suggesting colony satiation, but remained stable for low concentrations. Worker recruitment was far more variable among sucrose concentrations than among amino acid concentrations, suggesting a specific preference for concentrated sucrose and a general preference for amino acids regardless of its concentration. Bars = mean ± SE.

recruitment declined substantially over time when both sucrose and amino acids were concentrated (Tukey HSD pairwise comparisons, all p's < 0.0001), suggesting colony satiation. In contrast, worker recruitment remained steady during the 4-h period for the 1% solutions of sucrose and amio acids (Tukey HSD pairwise comparisons, all n.s.) suggesting that the colony had not become satiated. In total, these findings suggested that workers could discriminate among foods of differing quality and type.

Rate of Larval Trophallaxis

The rate of trophallaxis varied significantly with concentration ($F_{2,216}$ = 23.19, $P < 0.0001$), food type ($F_{1,216}$ = 54.45, $P < 0.0001$), and time ($F_{1,216}$ = 10.02, $P < 0.002$; Figs. 3A and B). Interactions were significant for food type and concentration ($F_{2,216}$ = 104.6, $P < 0.0001$) and for food concentration and time ($F_{2,216}$ = 37.64, $P < 0.0001$). In general, larvae preferred concentrated food to diluted food for both sucrose (1 vs 30%: Tukey HSD, $P = 0.00001$) and amino acid solutions (1 vs 30%; Tukey HSD, $P = 0.00001$). By 8 h, the rates of trophallaxis were reversed for amino acids, with diluted concentrations of amino acids showing higher rates of trophallaxis than concentrated solutions (1 vs 30%; Tukey HSD, $p = 0.00001$). At a 1% concentration, larvae ingested four times as much amino acids by weight as sucrose (Tukey HSD, $P = 0.0001$). In total, these facts demonstrated that larvae discriminated food type and concentration and could compensate somewhat for low-quality amino acids by ingesting more of them as time passed. The metabolic costs of processing dilute sucrose solutions may preclude ingesting them in sufficient volume to fuel growth.

Duration of Larval Trophallaxis

The mean duration of worker-larval trophallaxis was not affected by food concentration ($F_{2,206}$ = 1.90, P = n.s.), food type ($F_{1,206}$ = 1.07, P = n.s.), or time ($F_{1,206}$ = 1.39, P = n.s.). Interactions were not significant for any variables and are not reported.

Food Choice: Sucrose Solution and Amino Acid Solution

Do Workers Recruit Differentially?

An experimental nest containing the standard number of workers and larvae was starved for the standard time. Two food types, 10% sucrose solution and 10% amino acid solution, each dyed a different color, were introduced simultaneously into the arena of the colony fragment. The number of workers present at the food was counted 20 min after food introduction. The percentage of larvae fed each food type (determined by the color of their midguts) was determined at 1 h and, again, at 2 h after food introduction. The percentage of workers with either or both food types in their crops was determined 2 h after food introduction by randomly selecting 10 foragers from the arena and 20 reserves from the brood chamber and crushing their gasters on white paper. The area of the colored smear was proportional to their crop contents. Four replicates from four source colonies were completed. Amino acid and sucrose solutions were alternately dyed red or green between replicates.

Twice as many workers were present at the food site containing sucrose solution (~70 workers) as at the site containing amino acids solution (~30 work-

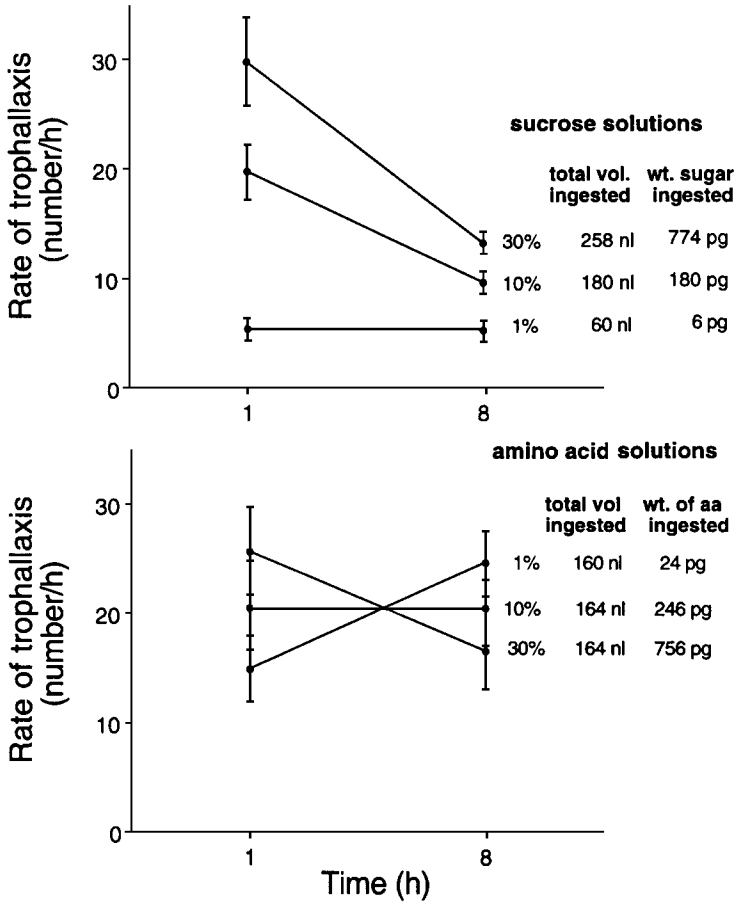


Fig. 3. Larval feeding based upon food concentration. (A) Larvae preferred concentrated sucrose solutions to diluted solutions. Larval ingestion rates decreased significantly with time for the concentrated solutions, suggesting larval satiation, but remained stable for low concentrations of sucrose, suggesting a general disinterest in sucrose at this concentration. (B) Larvae preferred concentrated amino acid solutions to diluted solutions. Larval ingestion rates decreased significantly with time for the most concentrated solution, suggesting larval satiation. Ingestion rates remained stable at the moderate concentration and increased at the low concentration of amino acids, suggesting a partial compensation for the diluted condition. Larval ingestion rates were more variable among sucrose concentrations than among amino acid concentrations, suggesting a specific preference for concentrated sucrose and a general preference for amino acid solution regardless of its concentration. Larvae ingested four times more 1% amino acids than 1% sucrose. Bars = mean ± SE.

ers; $t_{13} = -8.15$, $P < 0.0001$). In spite of fewer workers recruiting to amino acids, more larvae were fed amino acids than were fed sucrose ($F_{1,255} = 23.38$, $P < 0.001$). Initially, 85% of larvae had ingested amino acids, and 50% sugar (35% had received both nutrients). After 2 h, 97% of larvae had ingested amino acids, and 65% had received sugar (63% had received both nutrients).

Three hours after introducing both food types into the arena, 69% of workers still contained only one food type in their crops, whereas 27% of workers contained a mixture of food. Of those workers with a mixture of foods in their crops, most (96%) were reserves (those found inside the brood chamber) rather than foragers. In summary, the moderate occurrence of mixed food types in worker crops appeared to occur during food sharing within the rest rather than by foragers visiting multiple food sites.

Mixing of Food Types

Do workers who are satiated with one type of food solicit a novel food from other workers? Two grams of workers was randomly aspirated from a source colony and starved for 48 h. This group was divided into two 1-g groups and each group was fed a different food type for 2 h. Among replicates, amino acids and sucrose solutions were alternately dyed red or green. After feeding, 30 workers were randomly sampled from the brood chamber and their gasters crushed onto white paper; ~80% of workers contained sucrose, and ~65% contained amino acids, suggesting that workers donate sucrose more readily to other workers than they do amino acids. Workers from each group were combined with starved larvae into a single experimental nest. At three 1-h intervals, larvae were scanned under a microscope and the percentage of larvae of each color (or a blend of the two colors) was determined. Just before terminating the experiment, 30 workers were sampled from the brood chamber to determine if any mixing of worker crop contents had occurred. The experiment was replicated using a total of four source colonies.

After 3 h of larval feeding, ~ $60 \pm 2\%$ (SE) of workers contained sucrose in their crops, whereas only ~ $30 \pm 4\%$ of workers still contained amino acids. None of these workers contained a mixture of food types in their crops, suggesting that mixing of food occurs inside the nest only when empty workers solicit food from more than one donor. When compared to the original proportion of workers, 25% of workers carrying sucrose had donated their crop contents to larvae, whereas 54% of workers carrying amino acids had donated crop contents to larvae, suggesting preferential solicitation by larvae for amino acids.

In every replicate, workers with amino acids in their crops (food dyes could be seen through the translucent membranes between gaster tergites) dominated the brood pile during the first half-hour of feeding and amino acids were fed far more frequently to larvae than was sugar. Thereafter, the number of workers

with either food type in their crops was fairly equal and larvae ingested both food types. The timing of workers reaching the brood pile might explain the initial asymmetry of food delivery to hungry larvae, as some workers offered food to other workers rather than to larvae during the initial phase of feeding.

To explore further the possibility that workers decide which type of colony member to donate to depending upon their crop contents, a similar experiment was completed in which workers were sequestered and fed to satiation on either a dyed amino acids solution or a dyed sucrose solution (dye colors were alternated among replicates). Workers were mixed together at a 50 : 50 ratio by food type with starved larvae. This time, however, rather than counting the number of larvae with dyed food in their midguts, a sample of medium-sized fourth-instar larvae was selected for observation at 60× magnification using a dissecting microscope. Individual feedings to each larva were observed and recorded in sequence. Food type was verified by noting the crop color through the intersegmental membrane of the worker's gaster while it fed a larva and by observing the color of food ingested by the larva. This experiment was replicated using a total of three source colonies.

As in the previous experiment, workers with amino acids in their crops appeared on the brood pile first, delivering significantly more feedings to larvae (11 workers/larva) during the first 30 min than did workers with sucrose (4 workers/larva; $t_{12} = 3.68$, $P < 0.001$). Thereafter, feedings to larvae by workers carrying amino acids (10 workers/larva) were not statistically different than for sucrose (8 workers/larva; $t_{10} = -1.24$, P = n.s.). Occasionally, workers misaligned their glossae with the larva's mouthparts (pseudotrophallaxis; Cassill and Tschinkel, 1996) such that larvae could not ingest the offered food.

These results demonstrated that foragers filled up at only one food site and recruits at one donor site. Infrequently, reserves solicited food from more than one donor. During the initial phase of food sharing, workers with amino acids in their crops tended to donate their food to larvae, whereas workers with sucrose in their crops tended to donate to other workers. Generally, larvae received mixed diets because many workers carrying only one food type or the other donated to them.

Does Brood or Worker Hunger Influence Recruitment Rate?

A source colony was fragmented into six experimental nests containing the standard mass of workers and brood. Three of the fragments contained fourth-instar larvae and the other three contained pupae (nonfeeding brood). Colony members in these six experimental nests were fed ad libitum on 20% sugar water and beetle larvae for 1 week to standardize their pretreatment diet. Larval and pupal groups were paired into three sets. Then, following a 48-h starvation period to bring the colony to complete hunger (Cassill and Tschinkel, 1995),

one larval/pupal pair of nests was fed a 10% sucrose solution, another a 10% amino acid solution, and the third pair, the control, was not fed. This pretreatment lasted for 2 h and filled a colony fragment with one food type to determine if full workers would still recruit to a novel food type. Food was removed, and 2 h later, while workers were still satiated, both food types were introduced simultaneously to the three pairs (six nests). The number of workers recruiting to each food type was recorded 20 min after the treatment foods were placed into the arena. Three replicates were completed using three source colonies which had been pretested to confirm that their preference for both food types was similar and would not confound the experimental results. This experiment was carried out by Holly Baker, who kindly allowed us to use her results.

As in a previous experiment, workers recruited more strongly to sucrose than to amino acids ($F_{1,108} = 31.94$, $P < 0.0001$). In spite of this preference, workers recruited more strongly to novel food ($F_{2,108} = 3.11$, $P < 0.05$). The presence/absence of hungry larvae did not affect recruitment ($F_{1,108} = 0.36$, $P =$ n.s.). An interaction between food type and worker hunger was significant ($F_{2,108} = 6.58$, $P < 0.002$), demonstrating that workers change food preference based upon their own hunger rather than that of larvae (Fig. 4). Twenty-four hours after satiation on both food types (posttreatment), worker recruitment had recovered to its usual pattern (recruiting to sucrose more strongly than to amino acids) and in numbers no different from those of the control group. However, overall recruitment was still slightly lower than when colonies are deprived of food for 48 h, suggesting a graded recruitment response in relation to a graded colony hunger. These findings further demonstrated that workers discriminated between food types and that satiation on one food type would limit further recruitment to that food type, but not to novel food types. The rules of thumb appear to be, "When partially full, recruit only to novel food" and "When empty, recruit to any food."

Solid Food Handling

An experimental nest with standard numbers of workers and larvae deprived of food for standard periods of time was established. A whole, freshly killed beetle larva of about 1 g was placed into the arena 7 cm from the nest entrance. Workers chewing on two of the larva's body segments, one without legs and an adjacent segment with one pair of legs, were videotaped for 6 h. Findings are preliminary as the observations were not replicated.

The beetle larva was saturated with workers within 2 min [coverage: 92.5 ± 7% (mean ± SD)] and remained saturated for the entire 6-h period. Workers nipped at the smooth portions of the cuticle (<1 s) and chewed for extended periods at the intersections in which a leg joined the body (median = 80 s; range = 2 to 265 s). Workers gained access into the beetle larva at the jointed sites by hook-

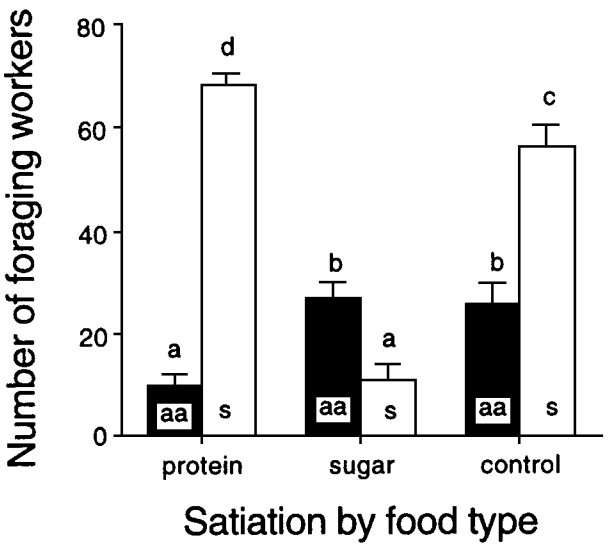


Fig. 4. Worker recruitment to novel food. Workers displayed independent appetites for amino acids and sucrose. Compared to the control group, prior satiation on amino acids repressed worker recruitment to amino acids but not to sucrose. Prior satiation on sucrose repressed recruitment to sucrose but not to amino acids.

ing one mandible onto the cuticular ridge formed at the joint and scraping with the other mandible long enough to wear a hole through the beetle larva's cuticle. The resulting hole was enlarged until workers were able to enter and mine the larva's flesh. Durations of chewing tended to increase as the initial hole was widened (regression: $y = 42.5 + 0.48$ s; $R^2 = 41\%$). In total, a colony's strategy for overcoming the hard, slippery cuticle of the beetle larva was to saturate the prey with nipping workers such that the probability of finding a deformation of the cuticle became a certainty. Workers locating an anchor point switched from brief nips to sustained scraping.

Tissue was torn from the larva's body and carried back to the nest either by the mining worker herself or by a passing recruit that retrieved it from the mining worker. Mastication of the tissue into a dry spherical pellet within the nest was not quantified.

Distribution of Solid Food Among Larvae. Dyed pellets were introduced into the arena of an experimental nest with workers and ant larvae of standard number and food-deprivation periods. The distribution of food to ant larvae was videotaped for 6 h to determine the number and duration of solid pellets ingested. A total of two replicates was completed.

The first pellets reached larvae 15–20 min after being placed into the forag-

ing arena, which is about the same time it takes for the first trophallactic feedings to occur between worker and larva after placement of liquid food into the arena (Cassill and Tschinkel, 1995). Larval pellets appeared to be of two basic geometries, either wafer-thin or spherical. Wafer-thin pellets were chewed and ingested in the solid state by larvae relatively quickly, like cows chewing grass. Spherical pellets were converted into a viscous mass with saliva on the larval food basket before ingestion. Ingestion time was highly variable, with a mean duration of ~10 min for wafers and ~30 min for pellets. Workers were observed ingesting a portion of this liquefied food from the larvae and distributing it among the other larvae. Of the several hundred larvae sampled, 100% had ingested protein within an hour, either by receiving a pellet directly (80.3%, fourth instar only) or via trophallaxis of the digested pellet (all instars). Most fourth-instar larvae were fed pellets during the first hour with very few receiving a pellet after the second hour. If a larva did not immediately spit on the pellet, the pellet was often removed and given to another larva. Even though an excess of pellets was available, most larvae (55%) were fed just one pellet, 15% were fed two pellets, and 10% were fed three pellets (20% were not fed). This frugal distribution of solid food suggested that pellet feedings are relatively rare events in the life span of a larva.

DISCUSSION

Food Flow

In *Solenopsis invicta*, colony nutrition is a decentralized homeostatic process, with both workers and larvae regulating their nutrition individually based upon their own crop or gut contents rather than the general nutritional needs of the colony. Liquid food was exchanged from individual to individual, not in a chain-of-transfer in which donors pressed food upon passive recipients, but in a chain-of-demand in which recipients solicited food from donors. The chain of demand began with larval hunger. This hunger is transmitted to workers feeding larvae, then to other workers, which donate to nurses, then to the forager, which then leaves the nest to still its hunger in the field. The major difference in food flow between workers and larvae is that workers move to food, whereas larvae must attract the source of food to them.

Regulation of Larval Diet

Larvae ingested food at different rates depending on the type, concentration, or state of food. Satiation on sucrose did not inhibit ingestion of protein in pellet or liquid state, and vice versa. Larvae preferred amino acids to sucrose. A simple explanation of dietary regulation would be that larvae signal their nutritional needs and that workers respond to these signals (though our experiments did not

directly address this issue). The nature of the larva's signal is probably chemical (Cassill and Tschinkel, 1995, 1996). Perhaps workers detect a larva's condition when they lick its cuticle or its saliva. In any case, social feeding is the result of interactions between workers and larvae, each contributing a modicum of regulation.

Amino Acids

When fed diluted amino acid solution, fire ant larvae increased their rate of ingestion, but not enough to compensate for the lower nutritional value. The response of fire ant larvae to food types differed from that of wood ant larvae [*Myrmica rubra* (Brian and Abbott, 1977)], which increased ingestion when starved of amino acids but not when starved of sucrose. Perhaps complete compensation in both species requires more time, as it does in many plant-feeding insects. When these are fed lower calorie food, it takes days before consumption rates increase significantly (Slansky and Rodriguez, 1987).

Sucrose

Sucrose solutions acted as a phagostimulant, with larvae ingesting six times the volume of concentrated sucrose relative to the diluted solution. Grasshoppers (Chapman, 1995) and rats (Smith *et al.*, 1992) demonstrate a similar feeding response, ingesting more sucrose solution when it was of a higher concentration. However, unlike rats, which ingested sucrose even when well-fed, fire ant larvae quickly satiated on moderate to high concentrations of sucrose. The fact that larvae satiate with respect to both food quantity (Cassill and Tschinkel, 1995) and food quality suggests that larval growth and final adult size/caste are regulated by other factors in addition to a simple nutritional switch (Wheeler, 1986, 1990, 1994).

Solids

Larvae seemed to have an insatiable appetite for dilute and intermediate solutions of amino acids but not for concentrated solutions or for solids. Over a 12-h period of time, protein pellets accounted for fewer than 1% of larval feedings. Likewise, in *Myrmica rubra*, only 4% of larval feeding events were pellet feedings (Kipiatkov and Lopatina, 1989). One likely explanation for the protein-hungry larvae not ingesting more pellets is that pellets represent a large number of calories compared to diluted protein. Alternatively, pellets may be detrimental to larval growth. In caterpillars, absorption of highly concentrated food increased the caterpillar's catabolic and excretory demands, leading to desiccation (Slansky and Wheeler, 1992). Diets that are highly concentrated and exclusively protein slow growth in ant larvae [*Myrmica rubra* (Weir, 1959; Brian,

1972); *M. scabrinodis, M. lobicornis, Formica polyctena, F. floridanus* (reviewed by Nonacs, 1992); *Solenopsis invicta* (Porter, 1989; Macon and Porter, 1994)], suggesting that ant larvae experience similar dietary constraints.

Regulation of Worker Diet

Workers regulated their diet, recruiting to food at different rates depending upon food type, concentration, and state. In general, workers recruited more strongly to sucrose than to protein and to novel food than to common food. Differential recruitment to food based upon individual need may explain the widely varying food preferences observed among fire ant colonies in the field (Glunn *et al.*, 1981) and among laboratory colonies maintained on the same diet (Lanza, 1991). Differential recruitment rates between carbohydrates and protein have been reported for other ant species [*Myrmica rubra* (Sudd, 1967, 1987), *Iridomyrmex humilis* (Markin, 1970), *Myrmica rubra* (Brian, 1972), and *Camponotus floridanus* (Nonacs and Dill, 1990)].

Crop Contents

Mixing of two food types in the fire ant worker crop occurred infrequently and only during food sharing in the nest rather than by foragers visiting multiple food sites. The lack of significant food mixing within the worker crop suggests that workers tended to fill up at one session and then to become donors themselves. Although Goetsch (1953, in Hölldobler and Wilson, 1990) also found that foragers passed food on to a clique of workers in a chain reaction that involved solicitation from a single donor, food source fidelity is not universal among ants (Markin, 1970). Maintaining separate food streams may allow fire ant larvae to fine-tune their nutritional requirements by signaling for different food types at different rates.

Workers displayed relatively complex decision-making when distributing food within the nest. Workers were more likely to donate to other workers away from the brood pile when they had ingested sucrose and to larvae when they had ingested amino acid solution. Whether nurses solicit for amino acids and nonnurses solicit for sugar or whether workers assort themselves according to their crop contents after ingestion is not clear. Extreme variation in crop volume [*Pogonomyrmex badius, Solenopsis saevissima, Crematogaster lineolata, Formica fusca* (Wilson and Eisner, 1958)] as well as variation in crop content [*Myrmica rubra* (Brian and Abbott, 1977), *Formica rufa* (Lange, 1967)] undoubtedly contributes to the distribution of labor above and beyond the influences of age or size (Cassill and Tschinkel, 1999).

Solids

An exception to individual hunger driving dietary decision is the retrieval and processing of solids by workers for consumption by larvae—an analogous behavior to the acquisition of pollen by bees. Ant scouts recruited other workers in sufficient numbers to assure continuous, complete coverage of the prey's body. The large number of gnawing workers overcame the low probability of any single worker gaining entrance to the prey's interior. Prey tissues were torn into transportable chunks on site, transported back to the nest, and masticated into dried pellets. Sudd (1967) similarly described the "skinning and butchering" of live insect prey by *Myrmica rubra.* The mechanisms regulating the acquisition and processing of solids by ant workers are unknown.

Summary

The larval diet was regulated by each larva's appetite for a particular food and its ability to communicate its hunger to workers. Likewise, the worker diet was regulated by the ability of each individual to assess food type and quality and to ingest that food based upon its own level of hunger. Why does individual hunger rather than some collective-level hunger regulate the flow of food into the colony? Most likely, this is a legacy of the solitary condition in which an empty midgut regulated ingestion in a more direct fashion. Whatever the origin, responsiveness to individual hunger makes it possible to meet the different dietary requirements of colony members at different life stages, especially when these life stages exist side by side.

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