Research article

A duration constant for worker-to-larva trophallaxis in fire ants

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Key words: Fire ant, larvae, feeding, trophallaxis.

Summary

The allocation of liquid food by workers to larvae, a central process in ant biology, could be regulated by the frequency of trophallaxis, its duration, or both. In 4th-instar fire ant larvae, the duration of trophallaxis, bolus size, and the rate at which boluses were swallowed were all constant, indicating that the volume of food ingested during each worker-larva trophallaxis was both small and constant. Neither larval size over a 20-fold volume range nor larval starvation had a significant effect on duration of trophallaxis (mean = 11 s, SD = 2 s), bolus swallowing rate (mean = 2/s, SD = 0.5/s), or bolus volume (mean = 0.0675 nl, SD = 0.0002 nl, based on the assumption that the stomodaeum's epithelial layer is not expandable). Larval body orientation and larval location within the brood pile also had no effect on duration. Durations of trophallaxis by workers of different sizes were similar. Durations of trophallaxis for 1st-, 2nd-, and 3rd-instar worker larvae were also constant but greater than that for 4th-instar worker larvae. Fourth-instar minim larvae (from founding colonies) and 4th-instar worker larvae (from mature colonies) were fed for the same duration by workers but for different durations by founding queens. Founding queens fed minim larvae longer than they fed worker larvae. The durations of feedings to 4th-instar sexual larvae were more variable than those to worker larvae. Altogether, these findings indicated that 4th-instar worker larvae ingested a small, nearly constant volume of food (mean = 1.50 nl, SD = 0.005 nl) during each trophallactic event. Consequently, the long-term allocation of liquid food by workers to these larvae is regulated by the frequency of trophallaxis. Several other ant species showed a similar brevity and constancy in the duration of worker-larva trophallaxis. This brevity of worker-larva trophallaxis is in contrast to the duration of worker-worker trophallaxis.

Although the duration of worker-larva trophallaxis appears to be determined by the worker, the data are not totally consistent with this interpretation.

Introduction

. . .with incredible affection and care the ants bring up their vermicules and omit not the least thing appertaining to their education and nurture (Swammerdam 1737–1738 cited by Wheeler, 1918).

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In Hymenoptera, the fecundity of adult females is determined largely by their nutrition as larvae (Hunt and Nalepa, 1994). Larval nutrition takes on special importance in eusocial Hymenoptera because it can influence not only the degree of a female's later reproductive success (through greater egg production) but her caste as well. Nutritional history can switch a larva's developmental pathway from fertile queen to sterile worker by means of trophic castration (Wheeler, 1986, 1990, 1994). Ants represent the most complex case of differential development in response to larval nutrition, creating not only queen-worker dimorphism but worker polymorphism and its associated division of labor.

The narrowness of the Hymenopteran petiole prohibits the passage of all but suspended microscopic particles (Hunt, 1994). Because adult ants are anatomically restricted to ingesting liquids, the primary mechanism for distributing nutrients among nestmates is trophallaxis, the sharing of regurgitated liquid food. Trophallaxis is a component of larval care in the majority of Myrmicinae, Dolichoderinae, and Formicinae (Wheeler, 1918; Hölldobler and Wilson, 1990) and may be a primary mechanism creating adult polymorphism by means of an unequal allocation of nutrition to larvae.

Approximately 75% of successful fire ant foragers collect food in a liquid state rather than as a solid (Tennant and Porter, 1991). Because liquids are the primary source of colony nutrition, trophallaxis is the essential mechanism of food allocation within fire ant colonies. Regulation of worker-larva trophallaxis must play a central role in shaping colony growth and reproduction.

Because fire ant larvae are fed individually by one worker at a time, food allocation to larvae could be regulated by four trophallactic mechanisms: frequency, duration, swallowing rate, and bolus volume. The frequency of worker-worker trophallaxis increases with worker hunger and colony hunger (Howard and Tschinkel, 1980, 1981a), whereas the frequency of worker-larva trophallaxis increases with larval size and larval hunger (Cassill and Tschinkel, 1995). In two ant species, worker-larva trophallaxis is reportedly brief, lasting between 5 and 15 s (*Iridomyrmex humilis*, Markin, 1970; *Monomorium pharaonis*, Borgesen, 1989). In contrast, worker-worker trophallaxis was lengthy and highly variable, lasting from 2 to 300 s in *Solenopsis invicta* (Cassill, unpublished data) and from 2 to 360 s in *Formica fusca* (Wallis, 1962) and averaging 120 s in *Camponotus vagus* (Bonavita-Cougourdan and Morel, 1986). Nothing has been reported on swallowing rates or bolus volumes in ants. Here we report that, unlike that of worker-worker trophallaxis, the duration of worker-larva trophallaxis is brief and constant in fire ants and in several other ant species.

Materials and methods

Stock colonies and artificial nests

Monogyne *Solenopsis invicta* colonies were reared in the laboratory from newly mated queens collected in Tallahassee, Florida, USA, during the spring of 1988, 1989, and 1990. Laboratory rearing and handling methods were similar to those described by Banks et al. (1981). Colonies were maintained at 28 °C in constant light.

Plaster observation nests ($10 \times 14 \times 2$ cm with a 0.3 cm high rim around the top edge to form a brood chamber) were covered with plates of glass, through which the ants were videotaped. During experiments, nests were kept moist because workers do not feed larvae in dry nests (Cassill and Tschinkel, unpublished data). Condensation forming on the glass cover of the moist artificial nest caused occasional, minor blurring of the recorded image.

Data collected with video recording

Once experimental treatments were established (e.g., larval size or food deprivation) and food was introduced, the frequency and duration of worker-larva trophallaxis was videotaped for $1-12$ h depending on experimental requirements. From the videotapes, feeding data for individual larvae were collected as follows: (1) The videotape was placed on pause and larval locations on the monitor were numbered in haphazard order with a water-soluble marker. (2) The first larva was selected for analysis. Experimental information for the larva (its size, food-deprivation state, location, body orientation, source colony, date) was recorded onto a computer event recorder. (3) The videotape and the computer event recorder were started simultaneously. The beginning and end of each trophallactic feeding to the larva was recorded for the entire observation period (usually 1 h, at most 12 h). (4) The tape was rewound, the computer event recorder was reset, and another larva was selected. (5) Procedures (3) and (4) above were repeated until data from a sufficient sample of individual larvae had been obtained. The computerized event recorder summarized, in table format, larval characteristics or conditions, the frequency (number of feeding/h) and the mean duration of trophallactic feedings (total duration of trophallactic feedings/h divided by number of feedings/h) for each larva in the sample.

Videotaping equipment consisted of a Sony color video camera (WV D5100) with lens (Taylor, Taylor and Hobson, LTD, 2 in, F/1.4) and $1-6$ cm extension tubes providing $20 \times - 80 \times$ magnification on the TV monitor, a JVC video cassette recorder (HR-D 600U), a Sony Trinition color monitor, and fiber-optic lights. On tape, the camera's field of view at $40 \times$ power encompassed $50-100$ larvae out of the thousands places in each treatment group.

Data reliability

Larval feedings were quantified from video tapes on the fast-forward mode ($5 \times$ real time). An automated conversion factor in the event recorder converted fast-forward time to real time. To determine agreement between fast-forward and real-time speeds, we compared the durations of trophallaxis on 26 consecutive feedings to one larva for each speed and found less than 2% difference between means (fast-forward mean = 11.4 s, SD = 1.3 s; real-time mean = 11.2 s, SD = 0.9 s).

Duration of trophallaxis to 4th-instar worker larvae

Larval food deprivation

Because larvae have nearly transparent body walls through which the color of the food they have ingested can be seen, food dyes were used to mark larvae exposed to different starvation periods. Dyed food consisted of solutions of distilled water, 6% (w/v) Casamino Acids powder (DIFCO Labs; Howard and Tschinkel, 1981b), 10% granulated sugar (w/v), and 1% (w/v) over-the-counter food dye (French's or McCormick's). The addition of food dyes to food solutions, at concentrations between 1 and 4% (w/v) had no effect on the percent of larvae fed (Cassill and Tschinkel, unpublished data).

For a test of the effects of larval hunger on the duration of worker-larva trophallaxis, larvae were isolated for 24 h from the source colony along with approximately 100 workers to attend them. Dyed food was introduced for half a day, then removed for the experimentally specified period. Red, green, and yellow food dyes were used to distinguish among larvae subjected to different food-deprivation periods. Larval food deprivation was tested in three experiments: 0 h or 48 h; 0 h, 12 h or 24 h; and at four ratios of 0 h and 48 h food-deprived larvae (100%, 20%, 5%, and 0% food-deprived) as detailed by Cassill and Tschinkel (1995). Differences in the duration of trophallaxis were analyzed with ANOVA.

Larval size

In each experiment, 1 or 2 g of workers (~ 1,500–3,000 individuals) were combined with 0.5 or 1 g of larvae (~ 700–1,500 individuals). Fourth-instar worker larvae were sifted into four size categories through standard testing sieves (mesh size that retained larvae: small = no. 35; medium = no. 30, large = no. 25, and extralarge = no. 20; Porter and Tschinkel, 1985). The effects of larval size were tested in four independent experiments. The first three are detailed by Cassill and Tschinkel (1995), so only summaries of these experiments are provided below; only the fourth is described in detail. (1) After sieving, each size group was subdivided, and the subgroups were fed to satiation with contrasting colors of dyed food. One subgroup was then starved for 48 h; the other subgroup was fed continuously to maintain satiation. Larvae were then combined (but remained distinguishable by dye color), and feedings were videotaped for 30 min. (2) After sifting, larvae were uniformly starved 48 h, then tested together in one experimental nest (adjacent larvae were of different sizes). Feedings were videotaped for 1 h. (3) After sifting, larvae were starved 48 h, then tested apart (adjacent larvae were of similar size). Feedings to each size group were videotaped for 1 h. (4) To determine whether the duration of trophallaxis changed as larvae of different sizes were brought to satiation over time, after sifting, we starved 1,000 4th-instar larvae of three size classes (small, medium, and large) 48 h, then placed them together in an experimental nest with 2 g workers. Each of four replicates from four source colonies was videotaped for 12 h *in situ*. Food was introduced 30 min before videotaping and was supplied *ad libitum* throughout the 12 h. In these four experiments, differences in the duration of trophallaxis were analyzed with ANOVA.

Larval location within the brood pile and larval body orientation

In experiments 1 and 2 above, each larva's location (top or bottom) within the brood pile and body orientation (mouth parts up or down) were recorded for analysis of their effects on the duration of trophallaxis (Cassill and Tschinkel, 1995).

Rate of bolus swallowing and bolus size by 4th-instar larvae during trophallaxis

An entire colony, producing worker larvae, was deprived of food for 24 h. Dyed food was then introduced, and 30 min later, the nest was placed under a microscope at $40 \times$ magnification for observation. Fourth-instar larvae were observed, one at a time, until a feeding occurred. An event-recorder key was depressed for every bolus swallowed during each observed trophallactic feeding. Rates of bolus swallowing were obtained for 10 larvae from each of four size classes. Differences among larval sizes were analyzed with ANOVA.

Bolus size was calculated from the circumference of the larval stomodaeum (pharynx and esophagus). Stomodaeal circumferences were measured from cross-sections of larvae. A sample of five larvae from each of eight size classes (1st-, 2nd-, 3rd-instar larvae; small, medium, large, and extralarge 4th-instar worker larvae; 4th-instar sexual larvae) were sectioned. Larvae were fixed in 10% Kahle's solution and aqueous Bouins, then postfixed in phosphate-buffered OsO_4. After embedding in Araldite 506, larvae were serially sectioned across the stomodaeum then stained with toluidine blue. Drawings of the cross-sections were made with a camera lucida. Because circumferences varied down the length of the stomodaeum of each larva (Fig. 1a), a mean circumference from four cross-sections of each larva's stomodaeum was calculated. Circumferences of the epithelial layer (Fig. 1b) were measured from the drawings with an opisometer.

From stomodaeal circumferences, bolus volume (V_b) was calculated as $4/3 \pi r^3$, where $r = c/2\pi$ (c = circumference), and r = radius. The mean volume of liquid ingested during each trophallactic event (V_t) was calculated as $V_b x_b t$, where V_b = mean bolus volume, x_b = mean number of boluses swallowed/s during trophallaxis, and t = the mean duration of trophallaxis in seconds.

Reliability of opisometer measurements from the camera lucida drawings was determined by measurement of two larval stomodaeal circumferences five times each. A mean was calculated for each circumference, and the difference between each value and its mean was compared. Values differed by 0.2 to 2.9% from the means; the average difference was 1.1%.

Other larval instars and castes

In the following set of experiments, worker size was constant while larval instar or caste varied. Mean durations of trophallaxis for 1st- and 2nd-instar larvae (combined), 3rd-instar larvae, 4th-instar minim larvae, and 4th-instar sexual larvae were compared to those of 4th-instar worker larvae by z-scores.
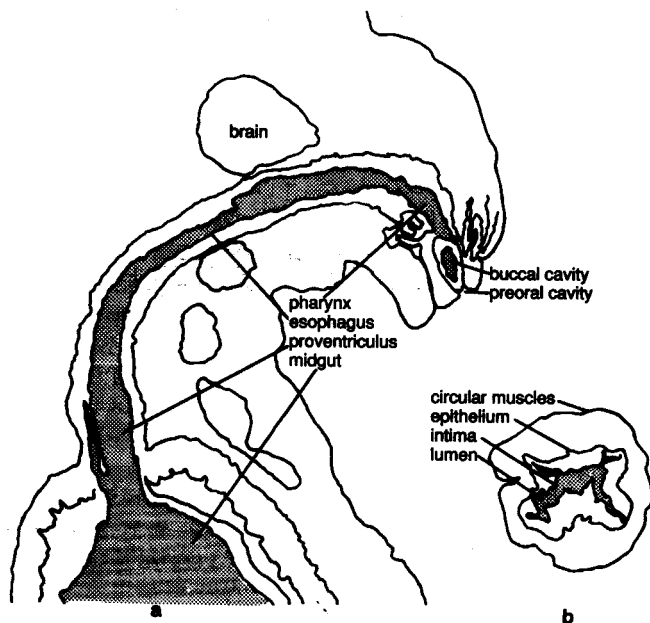
Figure 1. (a) Sagittal section of a 4th-instar worker larva showing variation in the diameter of the stomodaeum. External mouth parts are not shown. (b) Cross-section of a 4th-instar worker larva stomodaeum. The circumference of the epithelial layer was measured for calculation of bolus volume (on the assumption that boluses were limited in size by the nonexpandable epithelial layer)

First/second and third instars

One gram of workers and 0.05 g of microlarvae were aspirated from a source colony, placed in an experimental nest and starved for 24 h. Dyed food was placed in the arena, and feedings to 1st- and 2nd-instar larvae combined were video-taped for 12 h at 80 × magnification (these instars are clustered with the eggs). This procedure was repeated for 3rd-instar larvae from the same colony. Two replicates from each of two source colonies were completed. Differences in duration of trophallaxis were analyzed with ANOVA.

Fourth-instar minim larvae

Newly mated queens were collected in Tallahassee, Florida, USA, during May 1995. Within 24 h of collection, queens were sequestered in artificial nests made from test tubes (1 × 20 cm). The lower half of the test tube consisted of a water-filled chamber capped with a cotton ball to humidify the upper, dry chamber, in which the queen reared her first brood. The dry chamber was sealed at the top of the test tube with cotton to prevent the queen from escaping. When minim larvae had developed to the 4th-instar stage for 20 founding queens, larvae ($\cong 100$) were removed from the test tubes, combined, and placed in an experimental nest with 1 g workers from a mature laboratory colony that had been starved 48 h. Food was introduced and

worker-larva trophallaxis was videotaped for 2 h. Differences in durations of trophallaxis were analyzed with ANOVA.

Fourth-instar sexual larvae

Portions of mature field colonies containing sexual larvae were collected in March 1995 in Tallahassee, Florida, USA. Colony fragments were placed in experimental nests with glass covers and maintained for observation at 28 °C in constant light. Prior to videotaping, each colony was starved 48 h. Food was introduced into the arena and, 30 min later, feedings to 4th-instar sexual larvae were recorded on video-tape for 1 h at 20 × magnification. Four replicates were completed. One colony was again observed, but under a dissecting microscope at 80 × magnification, to verify larval ingestion and the anatomical juxtaposition of the worker glossae with the larva's mouth parts. A z-score was calculated for comparison of trophallactic durations between sexual and worker larvae.

Worker size

In the following experiment, larval instar was held constant (4th-instar worker larvae) while worker size varied. In December 1994, workers, brood, and queens were dug from field colonies in Tallahassee, Florida, USA, and transplanted into artificial nests in the laboratory. After one week's acclimation to constant light and temperature (28 °C), workers were aspirated from the brood chamber and arena and sifted into six size classes through standard testing sieves of mesh sizes No. 16, 18, 20, 25, 30, and 35. Large workers were collected from mesh sizes No. 16 and 18 (mean head width = 1.27 mm; mean weight = 4.1 mg), medium workers from mesh size No. 25 (mean head width = 0.86 mm; mean weight = 1.3 mg), and small workers from mesh size No. 35 (mean head width = 0.68 mm; mean weight = 0.6 mg). Each worker size class was tested independently in its own experimental nest with approximately 2,000 workers per size class with 0.5 g (1,000) larvae. Workers were food-deprived 48 h and larvae 12 h prior to food introduction and video-taping. A polymorphic control group consisted of workers from the three size classes mixed into one experimental nest (about 700 workers per size class) with 0.5 g larvae. Four replicates were completed. Data were analyzed with ANOVA.

Founding queen

Newly mated queens were used to test for differences between 4th-instar minim larvae and 4th-instar worker larvae in duration of trophallaxis from the queen. In May 1994, newly mated queens were collected in Tallahassee, Florida, USA. Within 24 h, queens were sequestered in artificial nests as described above (see Fourth-instar minim larvae) at 28 °C in constant light. When minim development had progressed to the fourth instar, the durations of queen-larva trophallaxis were videotaped. Nine incipient colonies were videotaped for 6 h each.

Newly mated queens were collected in Tallahassee, Florida, USA, during May 1995 and sequestered in artificial nests at 28 °C in constant light. Ten days later, five 4th-instar worker larvae were placed with each queen and her eggs. Feedings of worker larvae by queens were filmed for 6 h. Four replicates were completed. Data were analyzed with ANOVA.

Control of duration of worker-larva trophallaxis

If the duration of worker-larva trophallaxis is controlled by workers (e.g., according to an intrinsic timer), one would expect larvae fed by hand to ingest food for longer or more variable durations. If, on the other hand, duration is controlled by larvae, one would expect larvae to ingest for no longer when hand fed than when fed by workers. Interactive control shared by larva and worker is also possible. Fourth-instar worker larvae were deprived of food for 12 h while sequestered with a few (<50) adult workers to groom them. Larvae were selected by size, one at a time (n = 11 per size class), and placed on moist burlap under a dissecting microscope. A droplet of dyed food was delivered from a microcapillary tube onto the anteroventral region of the larva rather than being directly applied to the larval mouth parts as in true trophallaxis. An event-recorder key was depressed for every bolus swallowed during the duration of hand feeding. When no bolus was observed being swallowed for more than 5 s, hand feeding was terminated.

Other ant species

In the summer of 1993, several ant species (*Aphaenogaster rudis*, Emery; *Brachymyrmex obscurior*, Forel; *Camponotus floridanus*, Buckley; *Crematogaster ashmeadi*, Mayr; *Crematogaster minutissima*, Mayr; *Pheidole dentata*, Mayr; *Prenolepis imparis*, Say; *Solenopsis geminata*, Fabricius; *Solenopsis invicta*, Buren; and *Solenopsis picta*, Emery) were collected as mature colonies or reared from founding queens collected in Leon County, Florida, USA. Colonies were maintained in the insectary at 28 °C in constant light. Each species was filmed in its artificial source nest without fragmentation of the colony into experimental nests. Colonies were deprived of food for approximately 24 h, after which dyed food was introduced and larval feedings by workers were videotaped for 6 h. Videotapes were viewed only once, during which all trophallactic feedings were recorded regardless of larval identity. Data on duration of trophallaxis in *Iridomyrmex humilis* and *Monomorium pharaonis* were taken from Markin (1970) and Borgesen (1989).

Results

Description of worker-larva trophallaxis

In the eight species tested that fed their larvae by trophallaxis, nurse workers feeding larvae displayed a signature posture quite different from that displayed during
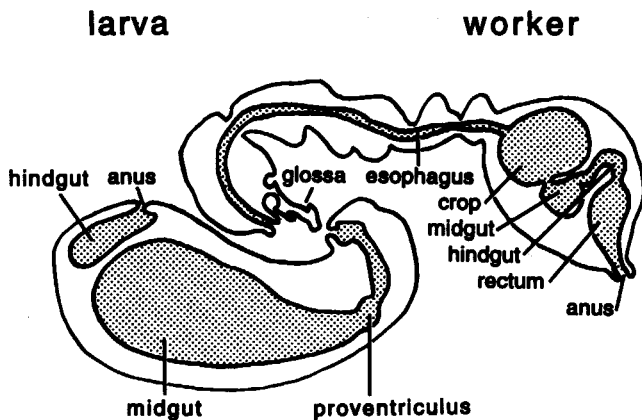
larva   worker

Figure 2. Sagittal section of a 4th-instar worker larva and an adult worker engaged in trophallaxis. External mouth parts are not shown. Their orientation is anterioposterior. The other, less-common worker orientation to a larva during trophallaxis is anterioanterior

larval grooming or worker-worker trophallaxis. During worker-larva trophallaxis, workers were uncharacteristically still, antennae were bent with the tips almost touching the larva's mouth, mandibles were wide, and the labium and glossae were fully extended. When a worker was oriented in an anterioposterior position to a larva (Fig. 2), the bulbous tips of its glossae were pressed onto (but not into) the larva's labium and associated mouth parts. Larval mandibles were more often closed than not. When a worker was oriented in an anterioanterior position to a larva, the sections of the glossae just above the bulbous tips were pressed onto the larva's mouth parts. Before ingestion, the worker dabbed its glossae about the larva's head until it located the larva's mouth parts – usually 1–3 brief glossal licks were required to do so. As soon as a worker's glossae found their mark, the larva would begin ingestion. During feeding, the larva's hyperextended labium (which often remained extended between feeding bouts) quivered at a steady rate suggesting active sucking. This glossal-labial contact between worker and larva may be the feedback signal for the worker's decision to continue or terminate larval feeding. Workers frequently signaled their intent to terminate trophallaxis by slightly raising and extending their antennae a second or two before retracting their glossae.

Duration of trophallaxis to fourth-instar larvae

Trophallaxis was both brief and nearly uniform in duration (mean = 11 s, SD = 2.0 s; range = 6–37 s) for 4th-instar worker larvae regardless of their level of starvation, size, location on the brood pile, or body orientation (Table 1).

Table 1. Durations in seconds of worker-larva trophallaxis in relation to larval attributes and conditions

Treatment	Level	Duration	
		(mean ± SD)	N/n
Larval food deprivation	0 h	10.5 ± 1.5	78/2000
	48 h	10.7 ± 1.2	
	0 h	10.8 ± 1.6	126/12000
	12 h	11.0 ± 1.4	
	24 h	10.4 ± 2.0	
Larval location	top	11.4 ± 0.8	
	bottom	11.2 ± 1.0	
Larval size (4th-instar)	small	11.3 ± 1.7	418/10000
	medium	10.8 ± 1.3	
	large	11.7 ± 1.6	
	extra large	10.9 ± 1.0	
Larval body orientation	mouth up	11.5 ± 1.0	80/1000
	mouth down	11.8 ± 1.2	
Over time (12 h)	1– 3 h	11.0 ± 2.4	766/4000
	4– 6 h	11.6 ± 2.8	
	7– 9 h	10.8 ± 2.2	
	10–12 h	11.0 ± 2.2	

N = sample size of larvae from which feeding data were recorded from the videotape; n = number of larvae in the experiment. The P-values are not reported because there were no significant differences in trophallactic durations between or among treatments.

### Rate of bolus swallowing and bolus size for fourth-instar larvae

For 4th-instar worker larvae, body size did not affect the rate of swallowing during trophallaxis (mean = 2.0/s, SD = 0.5/s; ANOVA: $F_{3,37} = 0.95$, $P = $ N.S.). With one exception, 4th-instar sexual larvae were too opaque or poorly oriented for boluses to be counted. During one 137-s feeding to a 4th-instar sexual larva, a mean 2.3 (SD = 0.9/s) boluses per second were swallowed; this rate was not significantly different from that of 4th-instar worker larvae ($t = 1.64$, $P = $ N.S.). Because of the limited feeding sample size (n = 1), this result should be considered preliminary.

The mean stomodaeal circumference increased significantly as larval size increased from the 1st to the 4th instar (ANOVA, $F_{3,9} = 21.78$, $P < 0.001$) but not as larval size increased within the 4th instar (ANOVA, $F_{4,34} = 2.26$, $P = $ N.S.; Fig. 3), even though most larval growth occurs during this molt. The constancy of stomodaeal circumference among larvae (including sexual larvae) within the 4th instar (mean = 0.159 mm, SD = 0.02 mm) suggested bolus volume increases between molts but not with growth within a molt (4th-instar bolus volume mean = 0.0675 nl, SD = 0.002 nl; see Materials and Methods for formulas) even though growth within the 4th instar can be 20-fold for worker larvae and 50-fold for sexual larvae (Tschinkel, 1988).

In summary, the constancy of duration, bolus size, and swallowing rate indicate that many feedings of a constant volume are delivered by adult workers to bring
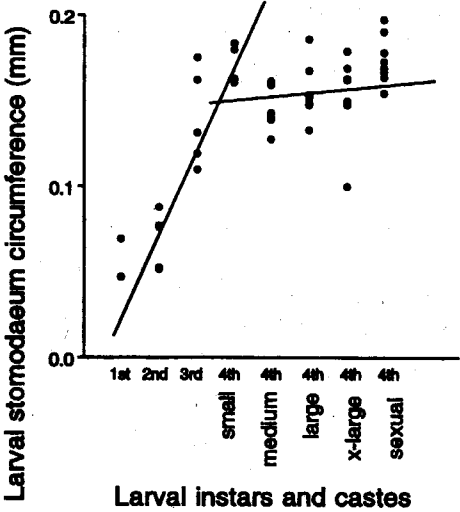
Figure 3. Circumferences of the larval stomodaeum (pharynx and esophagus) among larval instars. The size of the stomodaeum increased significantly between the 1st and 4th instars (REGRESSION: $y = -0.003 + 0.04 \times$, $R^2 = 0.82$) but did not increase with larval growth within the 4th instar (REGRESSION: $y = 0.16 - 0.008 \times$, $R^2 = 0.003$; not significantly different from zero)
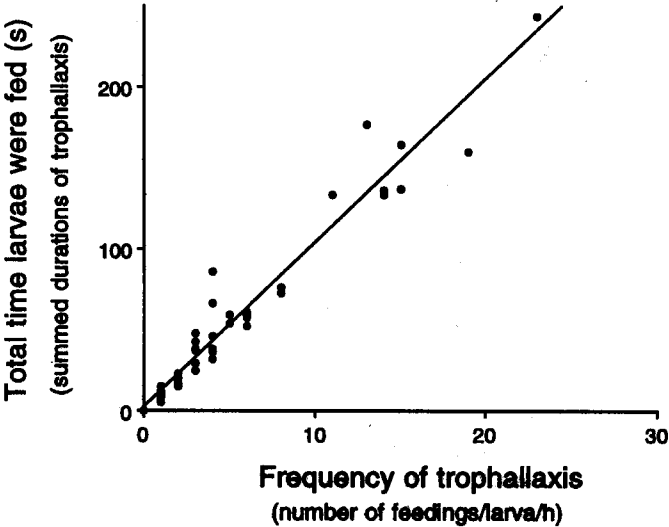
Figure 4. Total feeding duration per hour regressed on the number of feedings per hour (REGRESSION: $y = 1.8 + 10.1 \times$, $R^2 = 0.95$). The number of feedings was an excellent predictor of the total amount of food delivered to larvae ($N = 55$)

4th-instar larvae to satiation. This conclusion is supported by the fact that the number of feedings to larvae predicted 95 % of the observed variation in the total time larvae were fed (Fig. 4; data are from larval size experiment 1).

Other larval instars and castes

The 1st- and 2nd-instar larvae differed significantly from 3rd-instar larvae in duration of trophallaxis (ANOVA: $F_{1,229} = 16.98$; $P < 0.0001$) as did microlarvae and 4th-instar worker larvae (Fig. 5). The duration of trophallaxis for 4th-instar minim larvae fed by adult workers was not significantly different from that for 4th-instar worker larvae (Fig. 5). Trophallaxis for 4th-instar sexual larvae was more variable in duration (range $= 3 - 294$ s) than it was for 4th-instar worker larvae. Because of the large variabiality of trophallactic durations for 4th-instar sexual larvae, none of the durations for microlarvae was statistically different from those for sexual larvae (median test for 4th-instar sexual larvae and 3rd instar larve: $\chi^2_{0.05,1} = 1.04$, $P = N.S.$).

Because the anatomical nature of the feeding contact between worker and sexual larva could not be clearly resolved with the video equipment, feedings were observed under a microscope at $80 \times$ magnification. Of 103 feeding attempts to three 4th-instar sexual larvae, 80 resulted in ingestion of food by the larvae; 23 were pseudotrophallactic events in which ingestion did not occur. During pseudotrophallaxis, workers alligned with larvae but pressed their glossae above or to one side of the larval mouth parts. Worker crop contents were regurgitated during pseudotrophallaxis, as evidenced by a film of green-dyed food on the glossae, but were not ingested by the larva. After pseudotrophallaxis ended, a worker sometimes left behind a small residue of food on the head of the larva, subsequently attracting a few other workers to that spot for attempted trophallaxis. The duration of pseudotrophallaxis (median $= 19$ s, range $= 5 - 46$ s), although considerably less variable, was not significantly different from the duration of true trophallaxis (median $= 20$ s, range $= 3 - 294$ s; median test: $\chi^2_{0.05,1} = 1.58$, $P = N.S.$). Because of the small sample size, these findings are considered preliminary.

Worker size

The durations of trophallaxis to 4th-instar worker larvae were unaffected by differences in adult worker size (ANOVA: $F_{3,119} = 2.44$, $P = N.S.$; Fig. 6).

Founding queen

Duration of trophallaxis for 4th-instar worker larvae fed by the founding queen did not differ from that of similar larvae fed by adult workers (Fig. 7). Trophallaxis for 4th-instar minim larvae fed by the founding queen was significantly longer and more variable than that of similar larvae fed by adult workers (Fig. 7). Although adult workers fed 4th-instar worker larvae and 4th-instar minimum larvae for the same durations (Figs. 5 and 7), founding queens fed 4th-instar minim larvae for
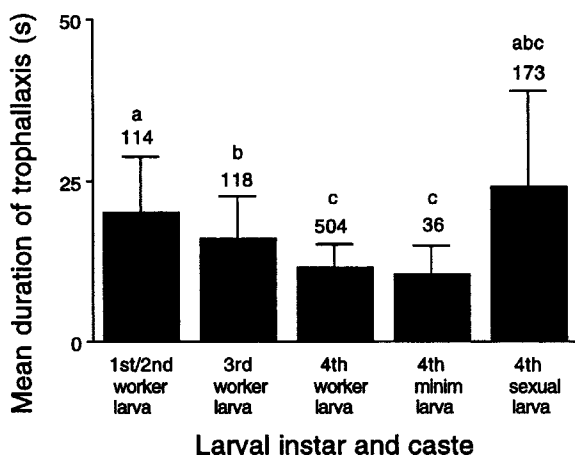
Figure 5. Duration of trophallaxis among worker instars, minim larvae, and sexual larvae. Lower-case letters indicate significant differences between means. Bars = mean ± 1 standard deviation. Numbers above the bars are sample sizes

Figure 6. Duration of trophallaxis by workers of various sizes and by founding queen. There were no significant differences. Bars = mean ± 1 standard deviation. Numbers above the bars are sample sizes

Figure 7. Ďuration of trophallaxis to 4th-instar worker larvae and 4th-instar minim larvae, each fed by either adult workers or founding queens. Different lower-case letters indicate significant differences between means. Bars = mean ± 1 standard deviation. Numbers above the bars are sample sizes

longer durations than they fed 4th-instar worker larvae (Fig. 7). Founding queens pierced and ingested trophic eggs during bouts of larval feeding, suggesting yolk as a primary nutrient for minim larvae.

Control of the duration of worker-larva trophallaxis

When fed by hand, 4th-instar worker larvae ingested twice as many boluses (t-test: $t_{54} = 4.87$, $P < 0.001$) over a period eight times as long (t-test: $t_{54} = 16.79$, $p < 0.0001$) as when fed by adult workers. The rate of larval bolus swallowing was relatively constant during the first 10 s of hand feeding (mean = 1.30/s, SD = 0.7/s; Fig. 8), then dropped significantly thereafter (ANOVA: $F_{10,428} = 67.7$; $P < 0.0001$). During the first 10 s, the rate of bolus swallowing was significantly lower for hand-fed larvae than it was for worker-fed larvae ($z_{1,0.01} = 3.80$, $P < 0.01$) suggesting that more than the taste or texture of food stimulates larvae to ingest. Possibly, workers apply some pressure with their gossae to the larval labium that triggers larval ingestion and was not a factor when hand-administered droplets were placed on the larval food basket.

Other ant species

Of the ten species that fed larvae via trophallaxis, eight fed larvae for relatively brief and uniform durations ranging from means of 3 s to 18 s (Fig. 9). Trophallaxis was longer and less uniform in duration for *Crematogaster minutissima* (55 s) and *Pheidole dentata* (47 s). Although *C. minutissima* foragers readily ingested dyed food and passed it on to adult nestmates by worker-worker trophallaxis, worker-
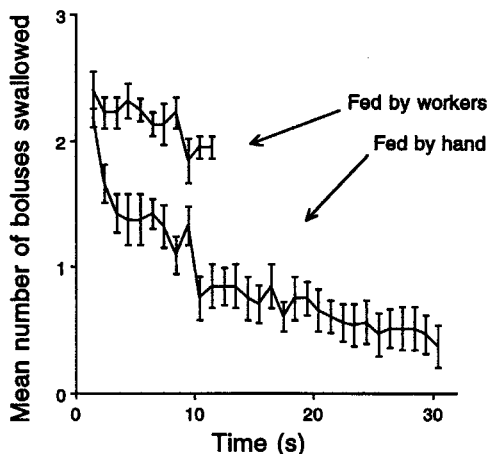
Figure 8. Rate of bolus swallowing (number/s) by larvae during the first 21 s of hand feeding. Larvae swallowed boluses at a fairly constant rate from seconds 2 to 11, after which the rate decreased significantly. Bars = mean ± 1 standard deviation; N = 22 hand-fed larvae
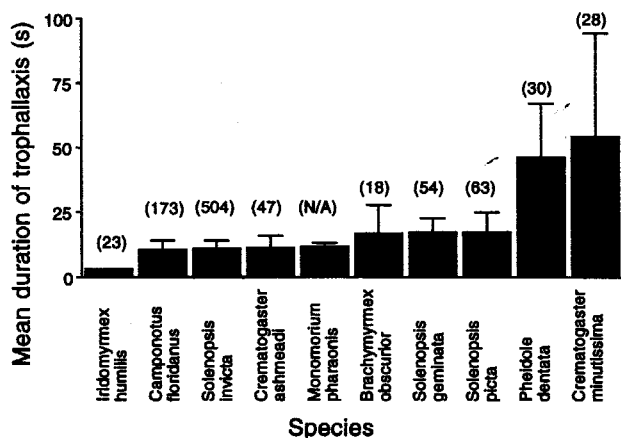
Figure 9. Durations of worker-larva trophallaxis in 10 ant species. Species are shown in ascending order by mean duration time: *Iridomyrmex humilis* (range = 2–10 s; Markin, 1970), *Camponotus floridanus* (range = 4–27 s), *Solenopsis invicta* (range = 6–31 s), *Crematogaster ashmeadi* (range = 6–28 s), *Monomorium pharaonis* (Borgesen, 1989), *Brachymyrmex obscurior* (range = 3–39 s), *Solenopsis geminata* (range = 6–33 s), *Solenopsis picta* (range = 7–41 s), *Pheidole dentata* (range = 20–94 s), *Crematogaster minutissima* (range = 14–200 s). Bars = mean ± 1 standard deviation. Numbers above the bars are sample sizes

larva trophallaxis was infrequent. When it did occur, workers would fed a larva, terminate feeding, lick the larva's mouth parts, and initiate another feeding to the same larva. Of the 18 larvae for which data were taken, six (33.3%) were fed multiple times by one worker. One worker fed the same larva six times (durations ranged from 33 to 62 s). The majority of larvae had been feeding on eggs, as evidenced by an egg or its empty shell surrounding a larva's mouth parts. Neither *Aphaenogaster rudis* nor *Prenolepis imparis* workers were observed feeding larvae by trophallaxis. *Aphaenogaster rudis* workers provisioned their long-necked larvae with large chunks of foraged mealworm. *Prenolepis imparis* workers placed worker-laid trophic eggs directly on the larval food basket, whereupon the larva pierced the egg's cuticle and ingested its contents.

Discussion

Because the duration of trophallaxis and the rate of food intake was constant for 4th-instar worker larvae, the food volume ingested during each trophallactic feeding was constant (mean = 1.50 nl, SD = 0.005 nl). The only variable feature of food allocation to fire ant worker larvae was the frequency of trophallaxis. Consequently, hundreds of feedings by hundreds of workers are required to bring larvae to satiation (Cassill and Tschinkel, 1995).

Two findings suggest that the duration of trophallaxis may be determined by a physiological timer intrinsic to the worker. First, true trophallaxis and pseudotrophallaxis were of similar duration, in spite of the absence of larval swallowing. Second, the duration of larval ingestion was eight times longer when larvae were fed by hand than when they were fed by workers. It appears that workers terminate trophallaxis much sooner than larvae might choose for them to do so. The dramatic drop in the rate of larval swallowing after 10 s of hand feeding suggested that larvae are conditioned by workers to cease feeding at about the time workers terminate regurgitation. However, because of the variable nature of trophallactic durations among larval instars, and between workers and newly mated queens, we cannot yet state conclusively whether the duration of trophallaxis is controlled by the worker, the larva, or interactions between the two.

Curiously, larvae ingested food only once (duration, approximately 11 s; approximately 22 boluses swallowed) during the course of hand feeding even though several attempts were made for each larva over a one-hour period (Cassill and Tschinkel, unpublished data; Wheeler, personal communication). In contrast, when fed by workers, larvae ingested 20–50 times per hour, some feedings following others within seconds (Cassill and Tschinkel, unpublished data). This lack of larval response to multiple attempts at hand feeding suggests that worker cues in addition to food cues are involved in stimulating larvae to ingest. Larvae may require a tactile cue to trigger swallowing such as pressure of the worker's glossae on the larva's labium. Support for this speculation is provided by the fact that larvae rapidly swallow air when their labia touch their ventral surfaces (Cassill, personal observation). Alternatively, larvae may have different ingestive responses for solid and liquid foods. The indirect application of liquid food to the larva's ventral surface may resemble the placement of solid food on the larva's "food basket" (Petralia and

Vinson, 1979), triggering the slower ingestive response typical of solid-food inges-
tion by fire ant larvae. During hand-feeding, larvae were observed to move the
mandibles in a scooping fashion while ingesting the applied liquid; this mandibular
motion has been observed during the ingestion of solid protein after it had been
liquified by the larva's salivary secretions (Cassill, personal observation).

Of the 10 ant species for which the duration of worker-larva trophallaxis has
been quantified (Fig. 9), eight species regurgitated liquid food to their larvae in
brief bouts of relatively uniform duration. This mechanism of food allocation may
be a general trait among ant species that feed their larvae a liquid diet. Because
many small meals would homogenize diverse food types across the larval popula-
tion, this feature of trophallaxis may have evolved in species that are diet generalists
(most ant species; Hölldobler and Wilson, 1990). Short duration may be a dominant
feature of worker-larva trophallaxiss in the ants.

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