Antennal Drumming, Trophallaxis, and Colony Development in the Social Wasp *Polistes fuscatus* (Hymenoptera: Vespidae)

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Abstract

Parental care is an important component of social behavior in both vertebrates and invertebrates. Social wasps are a useful system for investigating the interplay between behaviors associated with the feeding of larvae by adults and their role in the evolution and maintenance of sociality. Females of the primitively eusocial wasp genus *Polistes* perform conspicuous vibratory behaviors closely associated with adult–larva feeding interactions. Prior research strongly indicates that these signals are directed toward the larvae, but their function(s) remain unclear. Existing hypotheses on the function(s) have posited releaser effects on larvae, either stimulating or inhibiting release of larval saliva, a nutrient-rich glandular secretion attractive to adults. *Polistes fuscatus* queens perform antennal drumming (AD), a behavior in which they rapidly beat their antennae synchronously on the rims of the nest cells during the feeding of larvae. We used radiolabeled prey to show that adults extract juice from the prey, which they subsequently regurgitate to larvae immediately following each AD burst. We also show that no saliva is imbibed by the queen during the contact. These results are consistent with the inhibition hypothesis on the function of AD, but not the stimulation hypothesis. We further demonstrate that AD is first performed on nests when the oldest larvae reach the third instar, and that the third instar is the first to produce measurable volumes of larval saliva. Removal of third-, fourth-, and/or fifth-instar larvae from single-foundress, pre-pupal-stage colonies did not cause a reduction in the queen's AD rates compared with controls, suggesting that later-stage larvae do not maintain AD behavior via an immediate releaser effect. We propose instead that third-instar larvae, possibly via chemical components of the salivary secretion itself, modulate the physiology of queens so as to indirectly cause the onset and maintenance of AD behavior.

Introduction

Parental care is widespread among both vertebrates and invertebrates. In some species, the parents provide the young only with protection from predators and/or parasites, but in most others the adults provide nutrients for their immature offspring. The latter context has led to the evolution of social behavior in a few groups, notably in some of the mammals and in the social Hymenoptera (Alexander 1974; Wilson 1975). In all social species, signals have evolved that coordinate parent–offspring interactions. It is common, for example, for the offspring to communicate their hunger with a variety of acoustic and/or visual signals such as cries in mammals, gaping behavior and begging sounds in altricial birds (Wilson 1975), and scraping behavior by larvae of hornets (Ishay & Landau 1972).

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The social wasps (Hymenoptera: Vespidae) have well-developed parent–offspring feeding interactions, but with a twist. Unlike in vertebrates and honey bees, the flow of nutrients goes both ways: the larvae, as well as being fed by the adults, produce from their labial glands copious amounts of salivary secretion that is rich in sugars and amino acids and is avidly solicited and imbibed by the adults (Morimoto 1960; Hunt et al. 1982). Furthermore, in some of the social wasps, contacts with larvae are accompanied by some form of vibratory behavior by the adults (Reeve 1991). In the North American paper wasp *Polistes fuscatus*, for example, the queen drums her antennae ('antennal drumming', AD) synchronously on the rim of a brood cell in the nest before entering the cell to contact the larva inside. In the European species *P. dominulus*, the queen vibrates the gaster vigorously from side-to-side against the nest in much the same context ('abdominal wagging', AW) (Brillet et al. 1999; Brennan 2007). In both cases, the bursts of vibration last for approx. a second, occur at frequencies of 15–30 strikes per second, produce an audible buzz or rattle, and often cause the nest to shake visibly. There can be little question but that these behavior patterns constitute signals: they are repeated regularly, must be costly to produce, and the sound produced could potentially attract predators. The contexts in which the behaviors are performed make it highly likely that they are signals directed at colony members, rather than, for example, at potential enemies (Jeanne in press). In recent years, several studies have addressed the question of the functions of these bizarre vibratory behavior patterns in *Polistes* (Gamboa & Dew 1981; Pratte & Jeanne 1984; Brillet et al. 1999; Brennan 2005).

The context in which AD is performed in *P. fuscatus* begins when the queen returns to her young nest with a caterpillar or other prey item that she captured. She malaxates (macerates with the mandibles) the material extensively, during which the prey liquids are ingested into her crop. She then feeds the solid remains piece by piece to the older/larger larvae in the nest. After grooming herself, she again goes from cell to cell on the nest, now drumming her antennae on each cell rim for approx. a second. After making the rounds of the cells several times, she begins to follow each burst of drumming on a cell with regurgitation to its larva of some of the prey liquid from her crop (Pratte & Jeanne 1984). Larvae of all sizes are fed this liquid. The entire 'bout' of feeding typically ends with grooming by the adult. Pratte & Jeanne (1984) reported that queens begin to perform AD with the hatching of eggs into 'the first' larvae. However, precisely which larval instars (of five) comprise this category is not known.

In *P. dominulus* the sequence is similar, except that the female begins AW as she distributes solid food to the larvae. The behavior ends when the solid is completely distributed, but then resumes with lower intensity as the queen proceeds to distribute regurgitated prey liquid to the larvae (Brillet et al. 1999). Brillet et al. (1999) also noted that the onset of AW behavior coincided with the first appearance of third-instar larvae in the nest.

Two hypotheses have been proposed on the function of vibrations performed in the context of feeding larvae. Both link the behavior to the control of release of larval trophallactic saliva, but with opposite effects. One, developed for *P. fuscatus*, states that AD inhibits the release of saliva by larvae as they are about to receive liquid crop contents from a female (Pratte & Jeanne 1984). The effect is that the incoming prey liquid is not diluted by larval saliva, which would reduce the amount of food the larva can ingest. Pratte & Jeanne (1984) found that AD in *P. fuscatus* reduced larval saliva release by 23% over controls. The second hypothesis, based on work with *P. dominulus*, proposes that vibrational signals stimulate the release of saliva (Brennan 2005). Brennan (2005) found that applying simulated AW-like vibrations to nests caused a 51% increase in larval saliva release compared with non-vibrated controls.

When *P. fuscatus* adults on the nest are fed prey items macerated with vegetable dye, the dye-colored liquid appears on the mouthparts of larvae during feeding sequences accompanied by AD, indicating that the direction of liquid flow is toward larvae (Pratte & Jeanne 1984). Radioactive tracers were used to reach the same conclusion for *P. metricus* (Hunt 1984). However, these earlier studies have not ruled out the possibility that larvae may be releasing saliva after being fed. That is, vibrating adults could be feeding each larva first, and then imbibing saliva from it.

Some of the lack of agreement over the direction of nutrient flow and the function(s) of vibrational signaling in the context of adult–larva interactions may be due to actual behavioral differences among species. Alternatively, the nature of nutrient flow and the functions of the vibrations may be the same across these species, despite the differences in detail, and the different conclusions reached may be due to different methods used in the various studies.

The purpose of this study is to investigate the nature of the relationship between vibrational signaling,

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larval saliva production and distribution, and larval feeding. We focus on one species, *P. fuscatus*, and address four questions. First, with a radioisotope, we directly test whether nutrient flow accompanying AD occurs in one or two directions. Radioisotopes have been used to track both nutrient flow (Hunt 1984) and larval saliva release (Morimoto 1960) in *Polistes* sp. Second, we determine whether the onset of AD in *P. fuscatus* coincides with the appearance of the first third-instar larvae in the young nest, as does the onset of AW in *P. dominulus* (Brillet et al. 1999). Third, we ask whether all five larval instars produce saliva in amounts proportional to their weight. Finally, we ask whether the third and the later instars are necessary to maintain AD behavior.

Methods

Laboratory Rearing

In May 2007, 40 single-foundress, pre-emergence-stage nests of *P. fuscatus* with no larvae older than second instar were either founded in the laboratory with field-caught gynes or were brought into the laboratory from the field. Each colony was housed in a Lucite® cage (20 × 20 × 20 cm^3) (Lucite International, Beaumont, TX, USA) under controlled temperature (27°C) and humidity (65–75%). The photoperiod was started at L12:D12 and the light phase was increased by 15 min/wk for 8 wk (Downing & Jeanne 1985). Queens were fed a diet of late-instar wax-moth larvae, crickets, meal-worms, water, and honey. Stage of development of brood in each cell was recorded twice per week.

Do Queens Feed Prey Liquid to Larvae During a Bout of Antennal Drumming?

Tritiated water (Perkin-Elmer, Waltham, Massachusetts, USA; 2.43×10^6 dpm/gm) was used to trace the direction of trophallactic transfer during AD. We first determined the time-point at which maximum radioactivity could be detected in the saliva of larvae that had been fed tritiated water. Fifth-instar larvae were used because they most reliably yielded saliva in detectable volumes. Working with 14 larvae on six queenless laboratory nests, we sampled saliva from each for a measure of background radioactivity. We then used a 2- to 20-μl pipette to feed them to satiation with a mixture of tritiated water and blue vegetable dye (99.5:0.5 v/v). The blue dye allowed us to visually detect and remove with a bit of filter paper any tritiated water adhering to the larvae's mouthparts. Each larva was subsequently solicited for saliva by gently touching its mouthparts with a calibrated 5-μl glass micropipette (Pratte & Jeanne 1984) at 5-min intervals for the first 30 min, at 1-h intervals for the next 7 h, then at 22 and 23 h. Radioactivity in collected larval samples was measured using liquid scintillation counting (Packard TriCarb model 2500TR, Packard Instrument Co., Meriden, CT, USA) as mean disintegrations per minute (dpm) per μl of saliva. The scintillation fluid was Ultima Gold (Packard). Change in radioactivity in saliva for each larva was computed as the dpm per μl at given time-point minus dpm per μl in saliva collected before feeding tritiated water.

Queens on five laboratory colonies were fed a 50:50 mixture of prey liquid and tritiated water + blue-dye, using a 2–20 μl pipette. After each queen made the rounds of larvae while performing AD, she was removed and kept at 4°C. At approximately the time of peak radioactivity in larval saliva, 19 fifth-instar larvae on the five colonies were solicited for larval saliva with calibrated 5-μl glass micropipettes. Saliva samples were subjected to liquid scintillation counts. Mean radioactivity levels of larval saliva samples from treated colonies were compared with those of 19 fifth-instar larvae from five control colonies that were fed non-radioactive prey liquid by queens, using the non-parametric Mann–Whitney test. All wasps, larvae, and nests exposed to tritiated water treatments were disposed of as appropriate for radioactive waste.

Do Queens Receive Saliva from Larvae During a Bout of Antennal Drumming?

The queens on 10 laboratory nests were carefully removed and each was given a prey load to malaxate. Meanwhile, all fifth-instar larvae on each nest were fed a mixture (99.5:0.5 v/v) of tritiated water + blue dye. Each queen with her malaxated prey load was then allowed back on the nest to perform the feeding sequence with AD. For five colonies, once queens started AD and made their first three contacts with the radioactive fifth-instar larvae, they were carefully grasped by the thorax with forceps, removed from the nest, and non-lethally restrained with pins onto a dissection pan kept on ice. The gaster was gently squeezed until a drop of ingluvial fluid from the crop appeared on the mouthparts (Hunt et al. 1987). The droplet was collected in a calibrated 10-μl glass micropipette and subjected to liquid scintillation counting. Queens on the other five colonies were allowed to finish the

entire feeding sequence before being removed and assayed in the same way for radioactivity in their crops. Each of the 10 colonies had similar numbers of fifth-instar larvae that had been loaded with the tritiated water mix. Mean radioactivity levels of queens from each of the two treatment colonies were compared with radioactivity in the crop fluids of queens from five non-radioactive colonies, and with scintillation fluid alone, using a mixed-model analysis of variation (PROC MIXED) with colony as a co-variate and Tukey–Kramer adjustment for multiple comparisons (SAS Institute 2006).

Does Antennal Drumming Begin with the Appearance of Third-Instar Larvae?

Fourteen unmanipulated single-foundress colonies nesting under eaves of buildings at the University of Wisconsin Arboretum in Madison (43.1°N, 89.4°W) were observed in situ from nest initiation in May 2005 until the appearance of pupae. Cell visits and AD bursts were recorded for each adult during a bout of feeding. As AD occurs only in the context of feeding prey (Pratte & Jeanne 1984), observations were opportunistically focused on colonies with incoming prey loads, as detected by frequent checks of all nests in one location. Observations were made between 09:00 h and 16:00 h daily, for a total of 96.3 h. The number of AD bursts and number of accompanying feed-liquid (FL) events were recorded for each feeding bout. A feeding bout comprises the entire round of feeding the solid and liquid portion of a prey load. An FL event is a single contact between a wasp and a larva, during which the adult brushes the sides of the larva's body with the tips of her antennae and regurgitates a droplet while slowly moving her head into the cell to bring the droplet into contact with the larva's mouthparts. The mouthparts of the adult remain still during this behavior and an inward telescoping of the gaster can sometimes be seen (see Pratte & Jeanne 1984 for behavioral descriptions of AD and FL). Only larval contacts with these distinguishing characteristics were recorded as FL events. The number of larvae of each instar and their cell locations on nests were recorded on brood maps once each week (Grechka & Kipyatkov 1984). Larvae were assigned to instar on the basis of head–capsule width. The pre-emergence stage (Reeve 1991) was divided into five sub-stages, based on the oldest stage of brood present on a nest: (1) eggs, (2) instars 1 and 2, (3) instar 3, (4) instars 4 and 5, and (5) pupa. (Instars 1 and 2, and 4 and 5 were lumped because our weekly brood censuses were unable to resolve them temporally). Least square means of the ratio of the number of bursts of AD to the number of FL events (AD-to-FL ratio) were calculated for each ontogenetic sub-stage. Normalizing the number of AD by dividing by the number of FL controlled for the effect of variance in size of prey load on FL, and therefore potentially on AD. Sub-stages were compared using a mixed-model analysis of variation (PROC MIXED) with colony as a co-variate and Tukey–Kramer adjustment for multiple comparisons (SAS Institute 2006).

Do All Instars Produce Saliva in Proportion to Their Weight?

Larvae were allowed to generate saliva overnight away from soliciting adults. Adults were removed from six single-foundress, pre-emergence-stage laboratory colonies at lights-off at night. At lights-on the next morning, nests were inverted so that larval mouthparts were facing upward. Each larva was induced to yield saliva by means of a standard stimulus: the weight of a calibrated 5-μl glass micropipette. The pipette was lowered via a copper wire loop onto the mouthparts of the larva being sampled and held in place until the volume of saliva in the pipette ceased to increase. Saliva volumes for each instar were divided by the mean body weight for that instar. Mean fresh wet weights of larval instars were obtained by destructive sampling of 95 larvae from 21 colonies. Mean microliter of saliva produced per mg of larva weight was calculated for each instar. Values were arcsine transformed to meet normality assumptions and compared using a mixed-model analysis of variation (PROC MIXED) with colony as a co-variate and Tukey–Kramer adjustment for multiple comparisons (SAS Institute 2006).

How Does Removal of Larval Instars Affect Antennal Drumming?

To investigate whether the presence of larvae of certain instars is necessary to maintain AD behavior in adults, we assessed AD performance in response to removal of various combinations of larval instars. Eighteen single-foundress, pre-emergence laboratory colonies containing all five instars but no pupae were assigned to six treatments of three colonies each: (1) unmanipulated, (2) instars 1 and 2 removed, (3) instars 4 and 5 removed, (4) instar 3 removed, (5) instars 3, 4 and 5 removed, and (6) instars 1, 2 and 3 removed. At the beginning of the experiment, prey, but not water and honey, was

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withheld from all colonies for 1 d (Brennan 2005). On the following day, all larvae of the designated instar(s) in each treatment group were gently removed using blunt-end forceps. Colonies were then allowed at least 2 h to recover, during which time queens were held off their nests at 4°C. Each queen was then returned to her nest and hand-fed a prey item, which she proceeded to malaxate and feed to larvae. The numbers of AD bursts and accompanying FL events were recorded for each feeding bout. Mean AD-to-FL ratios (see above), AD bursts and FL events were compared separately among treatments using a mixed-model analysis of variation (PROC MIXED) with colony as a co-variate and Tukey–Kramer adjustment for multiple comparisons (SAS Institute 2006). Mean ratios of the number of empty cells to the number of cells with larvae were also calculated.

All wasps belonging to colonies that did not undergo radioisotope treatments were released from the laboratory back to their environment following the conclusion of all experiments (see above for disposal of wasps that underwent radioactive treatment).

Results

Do Queens Feed Prey Liquid to Larvae During a Bout of Antennal Drumming?

Radioactivity levels in larval saliva peaked 15 min after feeding, and then slowly decayed over the next 23 h (Fig. 1). Queens accepted 49.2 ± 4.1 μl of the tritiated water + prey liquid mixture. Saliva collected (collected volumes: 0.5–2.0 μl) from the larvae 15 min after FL with AD by these queens showed significantly higher radioactivity levels (316.2 ± 51.3 dpm/μl, n = 19 fifth-instar larvae, 5 colonies) than did saliva from control larvae fed non-radioactive prey liquid (110.8 ± 14.8 dpm/μl, n = 5 colonies) ($p < 0.001$, $n_1 = n_2 = 19$, Mann–Whitney test; Fig. 2).

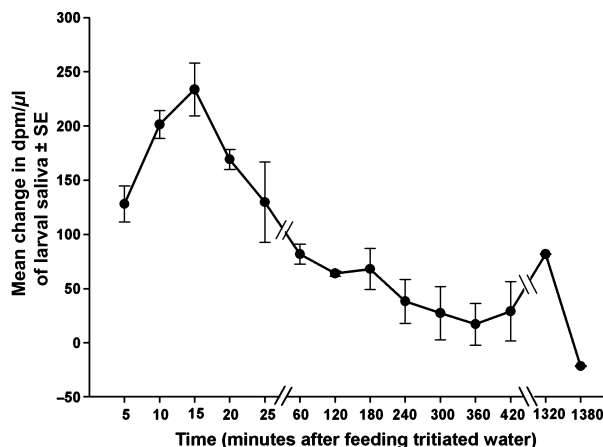

Fig. 1: Time course of detection of radioactivity in larval saliva. Each point indicates the difference in radioactivity (dpm/μl) in larval saliva for the time-point compared with radioactivity in larval saliva before feeding tritiated water. Error bars show SE. Hatch-marks indicate breaks in the time scale.

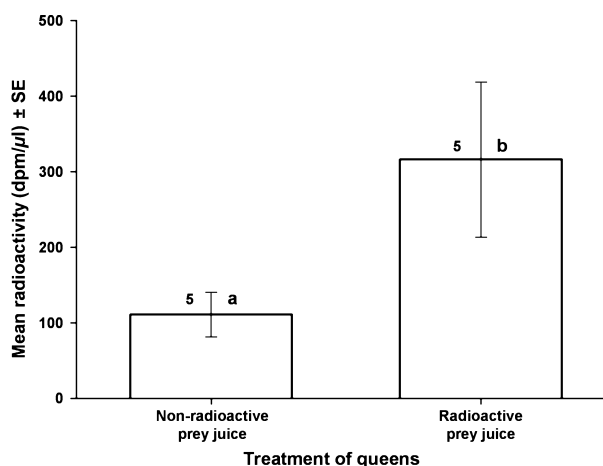

Fig. 2: Radioactivity in larval saliva 15 min after being fed by queens. 'Non-radioactive prey juice' = larvae fed by queens that were given non-radioactive prey juice; 'Radioactive prey juice' = larvae fed by queens that were given radioactive prey-juice. Error bars show SE. Numbers above bars give sample sizes (colonies). Means with the same letter are not significantly different at p > 0.05.

Do Queens Receive Saliva from Larvae During a Bout of Antennal Drumming?

Fifth-instar larvae accepted 13.3 ± 1.5 μl of the tritiated water + blue dye mixture. Because radioactivity in larval saliva peaked 15 min after feeding, we sampled queen crop fluids 15 min after larvae were hand-fed tritiated water and during which time queens were allowed to perform FL with AD. Queens yielded 0.5–2.5 μl crop fluid. Queens that engaged in trophallaxis with tritiated fifth-instar larvae showed no increase in radioactivity over those visiting non-radioactive control colonies or over blank scintillation fluid ($F_{2,11} = 0.86$, $p = 0.45$, n = 15 colonies; Fig. 3).

Does Antennal Drumming Begin with the Appearance of Third-Instar Larvae?

Frequency of AD with FL rose significantly with the appearance of third-instar larvae in the nest

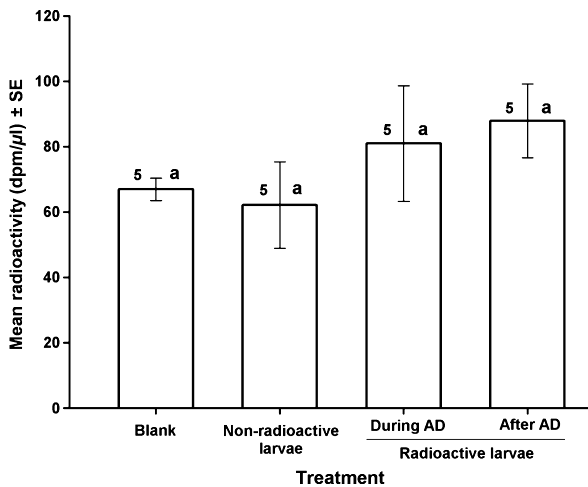

Fig. 3: Radioactivity in queen crop fluid after contact with larvae fed tritiated or non-tritiated water. 'Blank' = background radioactivity in vials containing scintillation fluid alone; 'Non-radioactive larvae' = larvae fed non-tritiated water; 'During AD' = queens sampled after their first three contacts with radioactive larvae; 'After AD' = queens sampled at the end of their feeding sequence. Error bars show SE. Numbers above bars give sample sizes (colonies). Means with the same letter are not significantly different at p > 0.05.

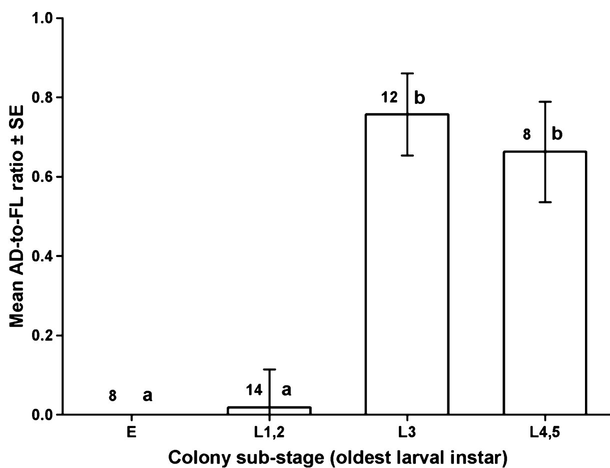

Fig. 4: Onset of AD coincides with the appearance of third-instar larvae. Colony stages indicate the oldest brood stage present in the nest: E = eggs, L1,2 = 1st and 2nd instars, L3 = 3rd instar, L4,5 = 4th and 5th instars. Colony-wide AD rate was measured as the mean AD-to-FL ratio. Numbers above bar indicate sample sizes (colonies). Error bars show SE. Means with the same letter are not significantly different at p > 0.0005.

($F_{3,25} = 13.72$, $p < 0.001$; 14 colonies, 42 feeding bouts; Fig. 4). Queens did not feed solid to first- and second-instar larvae, dropping the malaxated solid before initiating FL events. AD-to-FL ratios did not differ between the third-instar colony sub-stage and

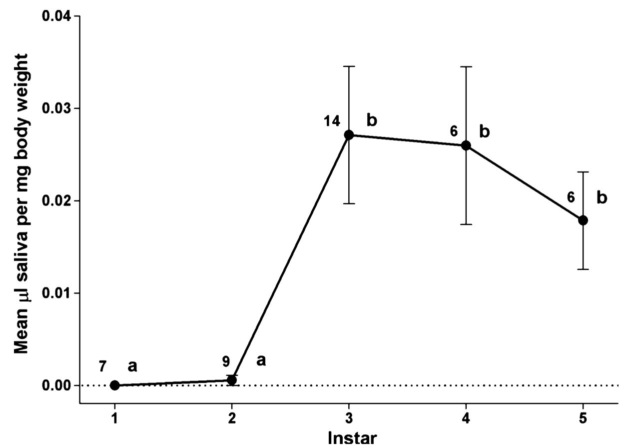

Fig. 5: Mean volume of larval saliva produced per mg of instar weight. Salivary secretions were obtained from 42 larvae belonging to six colonies of *Polistes fuscatus*. Numbers above line indicate sample sizes (individual larvae). Error bars are SE. Means with the same letter are not significantly different at p > 0.05.

the fourth-fifth-instar sub-stage ($p = 0.93$), or between the egg and the first-second-instar sub-stages ($p = 0.99$).

Do All Instars Produce Saliva in Proportion to Their Weight?

The volume of saliva yielded per mg of larval weight was significantly influenced by instar (Fig. 5). First-instar larvae produced no saliva. Although one of the nine second-instar larva sampled gave a small amount (0.01 μl), the remaining eight did not; instars 1 and 2 did not differ from each other statistically ($p = 0.92$). Mean volume of larval saliva per mg of larval weight was significantly lower for instars 1 and 2 than for the other instars ($p = 0.0005$), whereas the third, fourth, and fifth instars did not differ from one another ($p > 0.5$). Instar weights are reported in Table 1.

How Does Removal of Larval Instars Affect Antennal Drumming?

Instar-specific removal of larvae did not reduce the AD-to-FL ratio (Fig. 6). Least square means of AD-to-FL ratio of larva-removal treatments did not differ from the control (no instars removed) ($p > 0.05$). However, least square means of AD-to-FL ratio was significantly higher following the removal of instars 3–5 than that following the removal of third-instars alone ($p = 0.026$), but did not differ from other larva-removal treatments ($p > 0.05$) (p-values

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Table 1: Fresh weights of larval instars

Instar	$\bar{x} \pm$ SE (mg)	Sample size (larvae)
1	0.9 ± 0.2	5
2	2.9 ± 0.2	22
3	9.8 ± 1.0	29
4	37.5 ± 3.4	21
5	119.1 ± 14.5	18

Numbers indicate instar, body weight mean in mg, SE, and sample size for each instar, obtained by destructive sampling of 95 larvae from 21 colonies.

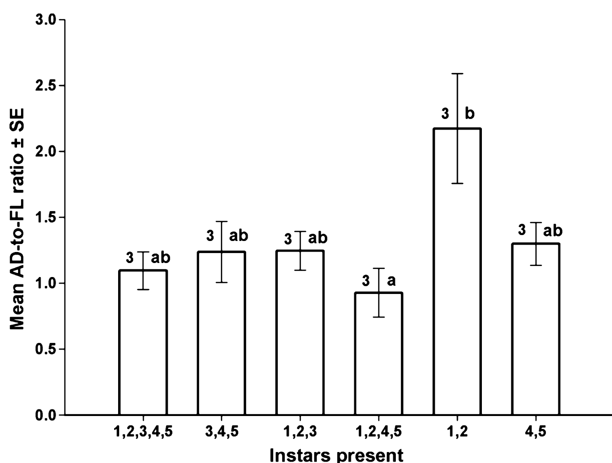

Fig. 6: Effect of stage-specific removal of larval instars on queen AD-to-FL ratio. Error bars show SE. Numbers above line indicate sample sizes (colonies). Means with the same letter are not significantly different at p > 0.05.

Table 2: Effect of removal of instars on queen AD bursts and FL events per bout

Instars present							
1	2	3	4	5	AD bursts	FL events	Empty:larval cells
X	X	X	X	X	37.7 ± 18.9	31.3 ± 12.0	0.36 ± 0.12
–	–	X	X	X	19.3 ± 3.28	17.0 ± 4.7	0.83 ± 0.26
X	X	X	–	–	21.7 ± 3.84	17.7 ± 3.5	1.68 ± 0.17
X	X	–	X	X	28.7 ± 1.45	34.3 ± 8.6	0.68 ± 0.09
X	X	–	–	–	29.7 ± 4.3	14.7 ± 3.7	1.83 ± 0.44
–	–	–	X	X	27.0 ± 7.6	22.3 ± 8.4	1.18 ± 0.14

Empty:larval cells = ratio of empty cells to cells containing larvae as a result of each combination of removals. Values are $\bar{x} \pm$ SE. Differences in least square means of AD bursts among treatments: $F_{5,12} = 0.55$; $p = 0.74$. Differences in least square means of FL events among treatments: $F_{5,12} = 1.18$; $p = 0.37$.

adjusted for multiple comparisons). Removals also did not cause significant differences in raw numbers of AD bursts or of FL events (Table 2). The ratio of the number of empty cells to cells with larvae was nearly three times higher when instars 3–5 were removed than when third-instars alone were removed (Table 2).

Discussion

The results of our radiotracer experiments show that when queens engage in a bout of cell visits accompanied by AD, they are feeding crop fluid to larvae, confirming for *P. fuscatus* what Hunt (1984) demonstrated for *P. metricus*. Saliva-collection times in this experiment may not have coincided exactly with peak radioactivity, as determined using tritiated water alone, because of the variations introduced by potential binding of tritiated water with components of the prey liquid. However, the clear difference between the test and control colonies in this experiment makes this a non-issue.

On the contrary, we found no evidence that adults receive larval saliva during these bouts. In other words, trophallaxis in this context is one-way, from adult to larva. This result refutes for *P. fuscatus* the hypothesis that AD is a signal that stimulates larvae to release saliva (Rau 1928; Owen 1962). It is of course possible that in other *Polistes* species vibrational signaling in this context has this function (see Brennan 2005).

Our results clearly show that queens do not perform AD until the nest contains third-instar larvae. However, while third instars appear to cause the initiation of AD, they are not required to maintain it. Removal of any combination of instars 3–5 failed to cause a reduction in rate of AD, at least during the 2 h of the experiment. This is consistent with similar observations on *P. dominulus* by Brillet et al. (1999) (but see Brennan 2005). In contrast, Brennan (2005) found AW rates to change as a function of the queen's food supply and the numbers of third–fifth larval instars on the nest.

Although removing instars 3–5 resulted in an increase in AD-to-FL ratio, this increase was significant only in comparison with the treatment in which third instars had been removed. We interpret the increase to be an artifact of our normalizing our measure of AD rate by dividing by the FL rate. Removal of instars 3–5 left large numbers of empty cells. The queens on these nests continued to drum on all open cells, but had few larvae that she could feed; the low denominator inflated the value of the AD-to-FL ratio, although overall AD rate showed little change. In fact, in comparing the two treatments – removal of instar 3 only vs. removal of 3–5 – the

mean numbers of AD bursts are very similar, while mean FL events are much lower in the latter treatment.

It is interesting that there is a step-increase in per-mg production of saliva in the transition from instar 2–3. Instars 3–5 produce at rates proportional to their weight. Toward the end of the fifth instar, the labial glands switch from producing larval saliva to producing silk (Chao & Hermann 1983). Thus, our measure of mean amount of saliva produced per mg of larval weight could have been biased downward somewhat for fifth-instars if our sample included individuals that were making this switch. However, this possibility does not affect the main conclusion of this experiment that larvae appear to undergo a significant physiological change in developing from the second to the third instar.

The fact that the onset of AD coincides with the appearance of third instars, and therefore with the appearance of larval saliva in the colony, suggests a link between the two. On the one hand, our results are consistent with the hypothesis that AD is simply a signal that inhibits the release of larval saliva during feeding (Pratte & Jeanne 1984). On the contrary, they suggest that there is more to it than that. The finding that removing instars 3–5 does not arrest the queen's AD behavior suggests that the signal or cue that third-instar larvae emit does not have a direct behavioral, or releaser, effect on AD performance, but instead modulates the queen's behavior (e.g., endocrine physiology) via some kind of primer effect (Le Conte & Hefetz 2008). Citing evidence for a similar effect in *P. dominulus*, Brillet et al. (1999) suggested that the stimulus may derive from cuticular compounds on third-instar larvae. An alternative possibility raised by our findings is that the larval saliva itself may contain a primer pheromone. Larval salivary glands in the honey bee (*Apis mellifera*) produce a blend of fatty acid esters that modulate the behavioral development of adults (Le Conte et al. 2006). *Polistes* larval saliva is a rich mixture of free amino acids and sugars (Hunt et al. 1982). Amino acids, in their ester forms, constitute the major components of pheromones in many holometabolous insects (Nojima et al. 2003; Moto et al. 2004). This hypothesis leads to the testable prediction that foundresses on early-stage colonies – those with only first- or second-instar larvae – will begin performing AD if they are experimentally provided with larval saliva collected from third- and higher instars.

Our results answer some questions about the nature of the interaction between AD and larval saliva, but raise others. The parent–offspring interaction in *Polistes* societies appears to be more complex than once thought. It is almost certainly integral to the maintenance, and perhaps the origin, of eusocial behavior in the genus, if not in the social vespids as a group.

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Literature Cited

Alexander, R. D. 1974: The evolution of social behavior. Ann. Rev. Ecol. Syst. **5**, 325—383.

Brennan, B. J. 2005: Vibratory communication in the social paper wasp *Polistes dominulus* (Hymenoptera: Vespidae). PhD Dissertation, Cornell Univ., Ithaca.

Brennan, B. J. 2007: Abdominal wagging in the social paper wasp *Polistes dominulus*: behavior and substrate vibrations. Ethology **113**, 692—702.

Brillet, C., Tian Chansky, S. S. & Le Conte, Y. 1999: Abdominal waggings and variation of their rate of occurrence in the social wasp, *Polistes dominulus* Christ. I. Quantitative analysis. J. Insect Behav. **12**, 665—686.

Chao, J. T. & Hermann, H. R. 1983: Spinning and external ontogenetic changes in the pupae of *Polistes annularis* (Hymenoptera: Vespidae: Polistinae). Insectes Soc. **30**, 496—507.

Downing, H. A. & Jeanne, R. L. 1985: Communication of status in the social wasp *Polistes fuscatus* (Hymenoptera: Vespidae). Z. Tierpsychol. **67**, 78—96.

Gamboa, G. J. & Dew, H. 1981: Intracolonial communication by body oscillations in the paper wasp, *Polistes metricus*. Insectes Soc. **28**, 13—26.

Grechka, E. O. & Kipyatkov, V. E. 1984: The seasonal cycle and caste determination in *Polistes gallicus* (Hymenoptera: Vespidae) 2. Dynamics of growth and productivity of colonies. Zool. Zhurnal **63**, 81—94.

Hunt, J. H. 1984: Adult nourishment during larval provisioning in a primitively eusocial wasp, *Polistes metricus* Say. Insectes Soc. **31**, 452—460.

Hunt, J. H., Baker, I. & Baker, H. G. 1982: Similarity of amino acids in nectar and larval saliva: the nutritional

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basis for trophallaxis in social wasps. Evolution **36**, 1318—1322.

Hunt, J. H., Jeanne, R. L., Baker, I. & Grogan, D. E. 1987: Nutrient dynamics of a swarm-founding social wasp species, *Polybia occidentalis* (Hymenoptera: Vespidae). Ethology **75**, 291—305.

Ishay, J. & Landau, E. M. 1972: *Vespa* larvae send out rhythmic hunger signals. Nature **237**, 286—287.

Jeanne, R. L. (in press): Vibrational signals in social wasps: a role in caste determination? In: Organization of Insect Societies (Gadau, J. & Fewell, J., eds). Harvard Univ. Press, Cambridge.

Le Conte, Y. & Hefetz, A. 2008: Primer pheromones in social Hymenoptera. Annu. Rev. Entomol. **53**, 523—542.

Le Conte, Y., Bècard, J., Costagliola, G., Vaublanc, G., Maâtaoui, M., Crauser, D., Plettner, E. & Slessor, K. N. 2006: Larval salivary glands are a source of primer and releaser pheromone in honey bee (*Apis mellifera* L.). Naturwissenschaften **93**, 237—241.

Morimoto, R. 1960: Experimental study on the trophallactic behavior in *Polistes* (Hymenoptera, Vespidae). Acta Hymenopterol. **1**, 99—103.

Moto, K., Suzuki, M. G., Hull, J. J., Kurata, R., Takahashi, S., Yamamoto, M., Okano, K., Imai, K., Ando, T. & Matsumoto, S. 2004: Involvement of a bifunctional fatty-acyl desaturase in the biosynthesis of the silkmoth, *Bombyx mori*, sex pheromone. PNAS **101**, 8631—8636.

Nojima, S., Robbins, P. S., Salsbury, G. A., Morris, B. D., Roelofs, W. L. & Villani, M. G. 2003: L-leucine methyl ester: the female-produced sex pheromone of the scarab beetle, *Phyllophaga lanceolata*. J. Chem. Ecol. **29**, 2439—2446.

Owen, J. 1962: The behavior of a social wasp *Polistes fuscatus* (Vespidae) at the nest, with special reference to differences between individuals. PhD Dissertation, Univ. of Michigan, Ann Arbor.

Pratte, M. & Jeanne, R. L. 1984: Antennal drumming behavior in *Polistes* wasps. Z. Tierpsychol. **66**, 177—188.

Rau, P. 1928: Trophallaxis in *Polistes pallipes*. Psyche **35**, 153—156.

Reeve, H. K. 1991: *Polistes*. In: The Social Biology of Wasps (Ross, K.G. & Matthews, R.W., eds). Cornell Univ. Press, Ithaca, pp. 99—148.

SAS Institute 2006: SAS version 9.1.3 software. SAS Institute, Cary.

Wilson, E. O. 1975: Sociobiology. Harvard Univ. Press, Cambridge.