

Larval begging behaviour in the ant *Myrmica rubra*

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Female ant larvae develop either into sterile workers or, when they receive extra nutrition, into fertile queens. Larvae are thus expected to maximize their fitness by acquiring as much food as possible. However, immobile ant larvae are totally dependent on worker care and have only one option for the manipulation of their food intake, begging. Until now it was unclear whether larvae show begging behaviour at all and how workers respond to this. We identified begging behaviour by quantitative correlation of larval behaviours with the frequency of feeding received from workers in experimental groups of the ant *Myrmica rubra*. Hungry larvae can attract feeding from workers by keeping their head bent upwards, in a stretch position. This position is assumed without previous contact with workers (non-induced). Although head movements of the larvae co-vary with adoption of the stretch position, we argue that they do not play a role in begging, as larvae that attract more feedings also prolong their stay in the stretch position. Furthermore, in those experimental groups where workers fed the larvae frequently, larvae showed this begging behaviour less often.

KEY WORDS: caste, larval development, conflict, trophallaxis, Formicidae.

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INTRODUCTION

Ant queens are morphologically specialized for reproduction, whereas workers perform helping tasks such as foraging for food, nest-building and nursing (HÖLL-

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DOBLER & WILSON 1990). Immature brood (eggs, immobile larvae and pupae) is strongly dependent on workers for survival and development. Female brood can develop into either workers or into queens (caste determination), which represent different phenotypic expressions of the female genotype (EVANS & WHEELER 1999). Except for a single case (ASHE & OLDROYD 2002), the development towards either caste in ants is dependent on only environmental factors such as social influence, temperature and nourishment (WHEELER 1986). The amount of nutrition a bipotent larva receives is the proximate trigger for queen determination (WHEELER 1994).

As female larvae are more closely related to their own would-be offspring than to those of other females, each larva would maximize its fitness by developing into a queen (BOURKE & RATNIEKS 1999). It is thus in the interest of a developing female to influence its own development given that it has this ability for self-determination (BOURKE & RATNIEKS 1999). The idea that larvae influence their development is controversial because, ever since WHEELER (1918) legless larvae have been considered passive and dependent on the whims of workers for their nourishment.

Deception of workers through begging for extra food is a possible mechanism that larvae could exploit to achieve self-determination (NONACS & TOBIN 1992, BOURKE & FRANKS 1995, BOURKE & RATNIEKS 1999, REUTER & KELLER 2001). To date, begging behaviour in social Hymenoptera has only been identified in hornet larvae that signal hunger by scraping their mandibles against the cell wall (MATSURA & YAMANE 1984).

The occurrence of begging in ant larvae is unclear. Workers feed ant larvae by regurgitating liquid food (larval trophallaxis, WILSON 1971) or by giving larvae pieces of prey (LE MASNE 1953). LE MASNE (1953) proposed that workers randomly check larvae for hunger prior to feeding ('test' feeding). Hungry larvae lift their head as response to antennal stroking by workers, so that mouth-to-mouth transfer can occur (LE MASNE 1953). BRIAN (1956) stressed that this test system differs from a signaling system. On the other hand, signaling is widespread in ants in many social contexts (HÖLLDOBLER & WILSON 1990, KELLER & NONACS 1993), thus we can expect larvae to signal hunger themselves independent of worker testing. Larval head movements are likely candidates for this (LE MASNE 1953, BRIAN 1977) and some authors suggest that saliva secretion could attract workers (WHEELER 1918, LE MASNE 1953, BRIAN 1977). In ant literature, ample anecdotal descriptions of larval begging behaviours can be found (HÖLLDOBLER 1971, O'NEAL & MARKIN 1973, WHEELER & WHEELER 1974, BRIAN 1977, HÖLLDOBLER & WILSON 1990), but unfortunately, these observations of supposed begging behaviour were never correlated with feeding data. This lack of information does not permit the identification of true begging behaviour and as a consequence limits further research on potential manipulation of caste fate in ants (BOURKE & RATNIEKS 1999). There are, however, strong indications that larvae of social Hymenoptera can signal their hunger, because workers apparently respond to greater needs of larvae after larval starvation or increases in the number of larvae (HUANG & OTIS 1991, CASSILL & TSCHINKEL 1995, BROWN & TRANIELLO 1998, RIBEIRO et al. 1999). Some authors suggest that larvae regulate their food intake with a non-volatile chemical cue (WILSON 1971; HUANG & OTIS 1991; CASSILL & TSCHINKEL 1995, 1999). Only recently, this hypothesis was empirically validated in bumblebees (DEN BOER & DUCHATEAU 2002).

Here we report the results of an experiment intended to study the existence of a behavioural begging cue by which larvae of *Myrmica rubra* may influence their development. We searched for behaviours that correlated with larval trophallaxis and inferred causation from a correlation analysis between replicates. In particular, we tested the hypothesis that head movements of the larvae signal hunger to feeding workers (the 'head movement hypothesis').

MATERIAL AND METHODS

Colony collection and maintenance

Six queenright colonies and one queenless colony of *Myrmica rubra* were collected in August, September and October 2001, in Heverlee (Belgium, $n = 5$) and near Tongeren (Belgium, $n = 2$). The stock colonies were held at room temperature (19–24 °C) in 20 × 20 × 7 cm boxes with a lid and a plaster bottom, in which a nest chamber of 10 × 5 × 0.5 cm was excavated and covered with a glass of 12 × 9 cm. The colonies were fed 3 times a week with sugar solution, Bhatkar diet (BHATKAR & WHITCOMB 1970) and pieces of crickets or mealworms. The plaster was regularly moistened with water.

Experimental setup

We used two kinds of boxes for our experiments: small isolation boxes (23 for each replicate) to starve larvae and bigger observation boxes (one for each replicate) for the actual experiment. The isolation boxes were 2.8 × 2.8 × 2.1 cm with a plaster bottom. The observation boxes were 6 × 12 × 4.7 cm with a bottom layer of plaster mixed with charcoal powder. This colored the plaster gray, so the white larvae were easier to observe. A nest cavity of 1.5 × 0.7 cm and 0.5 cm deep had been pressed in the plaster before drying and was covered with a thin piece of glass that allowed observation. Experiments were done in a climate room under a fluorescent light and videotaped with a JVC 3-CCD colour video camera and an 18-108/2.5 zoom lens on S-VHS videotapes. We transferred larvae between boxes with a moist brush.

Experiments were carried out in September, October and November. To create the optimal setting to identify begging behaviour and its correlation to feeding, we used:

(1) *Food-deprived larvae*, because we expect larval begging behaviour to be expressed more frequently when larvae are hungry, as in other animals (KILNER & JOHNSTONE 1997, RAUTER & MOORE 1999, KAPTEIN et al. submitted). Food deprivation also eliminates the possibility that larvae give food to workers during trophallaxis. We used larval sizes of 1 to 3 mm. Queen determination has yet to occur at these larval sizes (BRIAN 1954, 1955a) and they were large enough to facilitate observation.

(2) *Low worker/larvae ratio* (6 workers and 15 larvae), because this limits the resources available to the larvae and might enhance competition between larvae. A low worker/larvae ratio has also the advantage that larval-worker interactions are easier to observe. We used workers and larvae from different colonies (naive workers), to exclude the possibility that workers recognize and preferentially treat specific larvae. Ant workers readily adopt and nurse conspecific larvae (HÖLLDOBLER & WILSON 1990). In addition, we sampled the workers from the queenless colony, because we expected these workers to nurse the large larvae better (BRIAN & HIBBLE 1963) (e.g. to produce a more optimal reaction to begging behaviour).

A total of seven replicates were used in the analysis. The larvae of five replicates originated from different colonies and two replicates contained larvae from a sixth colony. As a different batch of larvae and workers were used with 7 weeks delay, we feel safe to assume that these last two replicates can be considered independent.

The larvae were starved separately in isolation boxes to avoid larval cannibalisation during the starvation period. Each isolation box contained water, but no food and two workers from the natal colony to groom the larvae, keeping them free from infections. To ensure that these workers could not feed the larvae with food reserves from the workers crop during the larval food-deprivation period (CASSILL & TSCHINKEL 1995) we placed these workers in the isolation boxes 24 hr beforehand. Twenty-three larvae were starved in this way for 15 hr before 15 of them were introduced to the naive workers in the observation box.

Eight paint-marked naive workers were placed in the observation box 12 to 24 hr before the food-deprived larvae were introduced, to acclimatize to the new environment. Ample food (crickets and sugar solution) was provided. Fifteen starved larvae were introduced into the observation box just after two naive workers were removed so that six workers were in the box

at time the larvae were introduced. The excess number of larvae and workers were needed to keep the experimental setup equal, buffering any deaths during the pretreatment period.

Videotaping began 1 hr after the larvae were introduced, thus allowing the workers to retrieve the larvae into the nest chamber. We videotaped the experiment for 3 hr.

After the experiment, the experimental groups were held in the same laboratory conditions as the stock colonies. This way, we could rear the larvae to the pupal stage, at which point sex and caste could be assessed, so that we could investigate if queen development was possible under these conditions.

Analysis

Videotapes were viewed and analyzed using playback. First, we analyzed feedings by larval trophallaxis for the entire 3 hr. The larvae that were fed with solid food (2 out of 200 feedings) were not used in the analysis.

When workers began feeding they heavily antennated the head of the larvae. When a worker found a good position to place her half-opened mandibles, she locked on to the larval mouthparts and, typically, held her own head motionless. At this moment the antennal tips lost contact with the larval head, but remained in a sharp angle with the scape, facing backwards towards the abdomen. At the moment when the antennae lost the contact, we recorded the start of feeding. A feeding lasted until the worker broke the connection by, again, making heavy strokings on the larval head with the tips of the antennae.

We identified the most and least fed larvae according to the frequency and total time of feeding, and used them to analyze larval behaviours by observing the videotape in three blocks of 5 min. The unfed larvae were thus excluded from the analysis. Each time block was as close as possible to the middle of each recorded hour, depending on the visibility of the larva (larvae could not be observed in periods where a worker stood above it, when larvae were hidden in the brood pile or when they had their mouthparts downwards). Larval behaviours were described and the frequencies and durations of these behaviours were noted. We also noted whether behaviours were preceded by a worker inspecting the larval body with her antennae and mouthparts (I = induced behaviours) or not (NI = non-induced behaviours). This allowed us to determine if the most fed larvae were fed more because workers interacted more with those larvae or because they showed a particular behaviour that was not induced by workers more often or longer.

Data were analyzed with Statistica 5.5. Within replicates, the data were analyzed using t-tests for dependent samples with the most and the least fed larvae of each replicate as pairs. Normality assumptions on the paired differences were checked with Shapiro-Wilk's W test and consequent transformations were performed if deviations of normality were detected. Between replicates total frequencies and total duration of feeding by workers were correlated with average larval behaviours within each replicate using the Spearman rank order correlation. For the average larval behaviours of each replicate we used as a rough measure the average of the behaviours of the least and the most fed larva. A correction for multiple testing within the same hypothesis for the variance as well as the correlation analysis was made using the Bonferroni method.

RESULTS

Production of males and new queens

Two queens were reared in our experimental groups, while 4 queens emerged in stock colonies during the same period. Males were regularly produced in the stock colonies. However, only 3 males appeared out of 65 larvae reared in the

groups to the pupal stage. As a consequence, interference of male larvae with our experiment was negligible.

Feeding activity

All but two feedings occurred by larval trophallaxis, though feeding activity was low in general. In two groups, there were more feedings (46 and 89 feedings) than in the other groups (0-16 feedings), but even here not all larvae were fed. We observed a total of 198 feedings.

*Behavioural repertoire of the larvae of *Myrmica rubra**

We were able to discriminate two main larval positions, three kinds of head movements and contractions of the body. We also considered “feeding” as a separate behaviour. In the “relax position” (see Fig. 1A) the mouthparts were parallel with the larval body and the larvae showed no active behaviour. In the “stretch position” (see Fig. 1B) a larva had its mouthparts bent upwards, at an angle to its body. Most of the time (65-100% of observed time in this study) larvae were in the relax position. A larva could stay in the stretch position for a period of from less than a second up to a few minutes, before it made a head movement called “relaxing” to go back into the relax position. We referred to “stretching” when a larva made a head movement from the relax position to the stretch position. A “swirling” occurred occasionally, when a larva made a head movement in the stretch position without going back to the relax position. Contrary to stretching and relaxing, this head movement was not always in the plane of the larval body.

Most versus least fed larvae

We divided the observed time into behavioural time blocks. During such a time block a larva showed one of the following behaviours: being in the relax position, in an induced (I) stretch position or non-induced (NI) stretch position or doing other behaviours. Other behaviours included I and NI forms of feeding events, contractions and swirling movements. These were pooled because they were too infrequent to be used as individual variables in the analysis. We used this division of the observed time to calculate frequencies and total time for three variables (I stretch position, NI stretch position and other behaviours) and used this in a general analysis, to search for behaviours that differed between the most and the least fed larvae. A Bonferroni correction for multiple testing (critical value $\alpha = 0.00625$) did not change our results.

The most fed larvae assumed the NI stretch position more frequently (Fig. 2A) than the least fed larvae (dependent t-test, $t_6 = 4.2$, $P = 0.0057$) in six out of seven replicates. In one replicate, both larvae showed this behaviour once. There were no significant differences between the most and the least fed larvae for the frequencies spent in the I (induced) stretch position ($t_6 = -0.43$, $P = 0.68$) and for the frequencies spent on other behaviours ($t_6 = 0.25$, $P = 0.81$).

The most fed larvae spent significantly more time in the NI stretch position (Fig. 2B) than the least fed larvae (dependent t-test on log-transformed data, $t_6 =$

5.6, $P = 0.0014$). This was true for all seven replicates. There were no significant differences between the most and the least fed larvae for the cumulative time spent in the I (induced) stretch position ($t_6 = 0.42$, $P = 0.69$) and that spent on other behaviours (log-transformed data, $t_6 = 0.28$, $P = 0.79$).

The general analysis did not allow for testing of the “head movement” hypothesis. However, we were able to test this hypothesis with an alternative specific

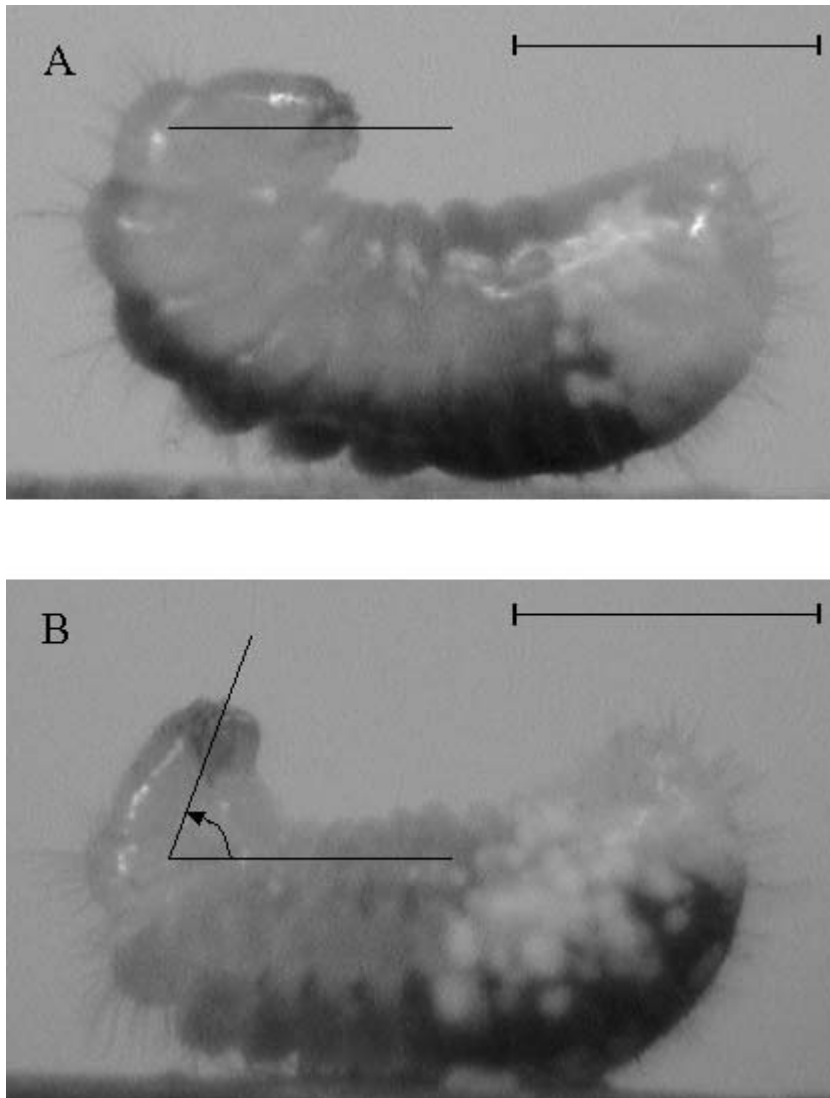


Fig. 1. — Larval positions. Scale bars = 1 mm. Lines indicate the angle that the mouthparts make with the axis of the body. This angle is approximately 0° in the relax position (A) and 45° to 180° for the stretch position (B).

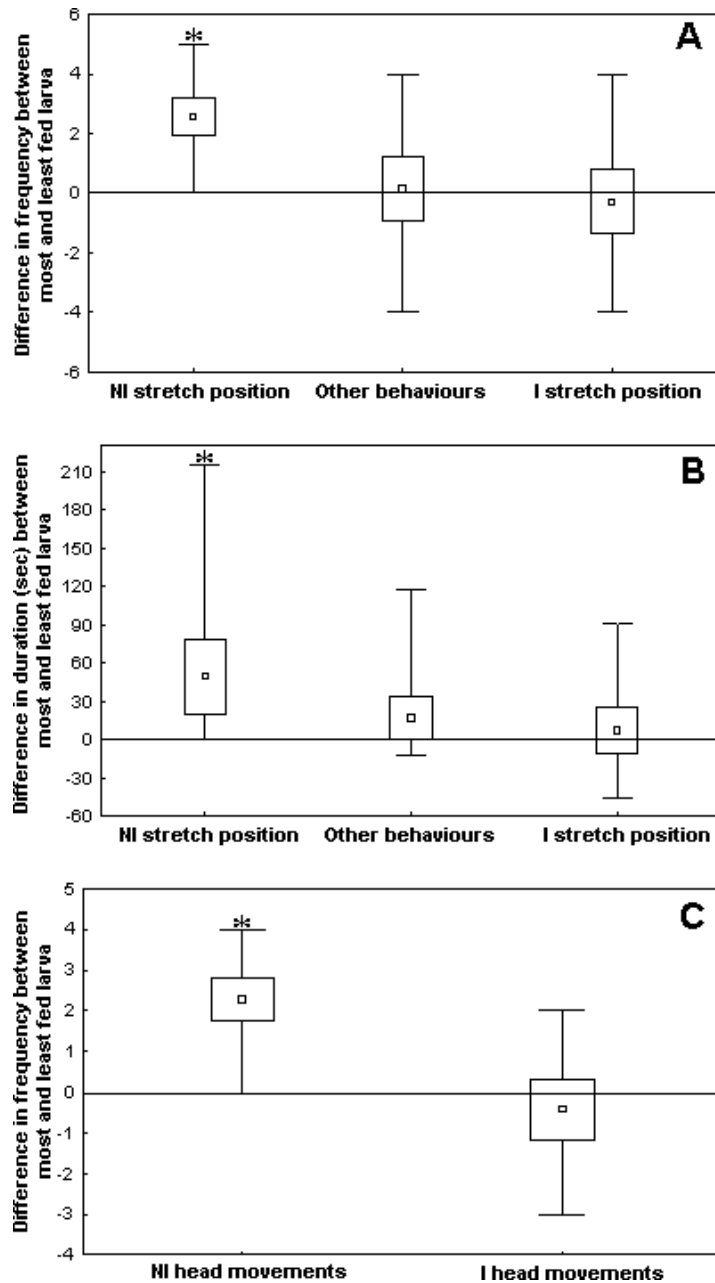


Fig. 2. — Boxplots of differences in the frequencies of the behaviours (A for the general analysis, C for the specific “head movement” hypothesis) and for the duration of the behaviours (B, general analysis) between the most and the least fed larva of each replicate during the 15 min observation period. Points are means, boxes are standard errors of the means and whiskers denote the range of the differences. If the mean is above zero, the behaviour is greater in the most fed larvae. Asterisks show the behaviours where there is a significant difference between the two groups.

Table 1.

Spearman rank correlation coefficients between the averages of the behaviours of the most and the least fed larva seen in each replicate (frequency and total duration, except for the head movements, see text) and the total feeding intensity in each replicate (total frequency and duration by workers). The P -value for each correlation is shown in brackets.

Behaviour	Measure	Feeding frequency	Feeding duration
NI stretch position	Frequency	- 0.7 (0.08)	- 0.78 (0.039)*
	Duration	- 0.93 (0.0025)**	- 0.89 (0.007)*
I stretch position	Frequency	0.35 (0.43)	0.3 (0.52)
	Duration	0.07 (0.88)	0.18 (0.7)
Other behaviours	Frequency	0.11 (0.82)	0.16 (0.72)
	Duration	0.07 (0.88)	0.14 (0.76)
NI head movements	Frequency	- 0.75 (0.053)	- 0.8 (0.029)*
I head movements	Frequency	- 0.4 (0.37)	- 0.4 (0.37)

* Significant without Bonferroni correction ($\alpha = 0.05$); ** significant after Bonferroni correction ($\alpha = 0.0031$).

analysis. In this analysis we calculated the frequencies of head movements of the least and the most fed larvae and treated the data in the same way as in the general analysis. We pooled the frequency of each time block that we had characterized as (I or NI) swirling movements with the frequencies of transitions between a time block characterized as relax position and a time block characterized as a (I or NI) stretch position or feeding. The frequencies of these transitions gave the frequencies of (I or NI) stretching and the pooled variable was called the frequency of (I or NI) head movements. Although a relaxing movement is a head movement too, we did not include this in the variables I and NI head movements, because relaxing movements have no relevant extra information (for each stretching movement, there is a relaxing movement).

There was a difference for the frequencies of the NI head movements (dependent t-test, $t_6 = 4.38$, $P = 0.0047$) in favour of the most fed larvae (Fig. 2C). There was, however, no difference for the I head movements ($t_6 = -0.42$, $P = 0.69$). The differences in NI head movements could have caused the differences in the frequency and total duration of the NI stretch position, because all head movements were followed by time spent in the stretch position, except when head movements were followed by feeding. The significant difference for the total time spent in the NI stretch position was, however, not only determined by the frequency of the NI stretch position, but also by the average length of time a larva stayed in this position. In all seven replicates the most fed larvae stayed longer in the NI stretch position than the least fed larvae. In two replicates, the least fed larvae did not even show this behaviour during the observed period. A t-test for dependent samples on the log-transformed average duration in the NI stretch position from the other five replicates (average duration in the NI stretch position for the most fed larvae = 12.55 ± 13.8 sec, for the least fed larvae = 4.8 ± 5.23 sec) gave a significant result ($t_4 = 4.33$, $P = 0.012$). This indicates that the most fed larvae attract more feedings by spending more time in the NI stretch position rather than by performing more NI head movements. They do this by placing themselves more in this position as well as by prolonging their stay in this position.

Correlations between replicates (see Table 1)

We used the same variables that we defined for the general and specific variance analysis in this regression analysis. We found negative correlation coefficients between the feeding activity (frequency and duration) and the NI stretch position and NI head movements. After correcting for multiple testing, only the negative correlation between the average (between the most and the least fed larva per replicate) duration spent in the NI stretch position and the total feeding frequency per replicate was significant. This suggests a higher begging frequency when the feeding rate was low. The I stretch position, the I head movements and other behaviours showed no association with feeding activity.

DISCUSSION

We found a strict correlation within experimental groups between the frequency of workers feeding a larva and the behaviours performed by that larva, suggesting the behaviours play a role as begging signals. Behaviours that followed an inspection by a worker (induced behaviours, I) did not show a correlation with feeding frequency, but non-induced (NI) behaviours did. These were (i) the frequencies of NI head movements (stretching and swirling behaviours), (ii) the frequencies of NI stretch position and (iii) the total time spent in the NI stretch position. But which behaviour, NI head movements or NI stretch position, is the proximate mechanism that enhances the probability that larvae will be fed? It is difficult to answer this question because almost every head movement is followed by a time spent in the stretch position. Head movements were indeed traditionally considered likely to be hunger signals in ant larvae (LE MASNE 1953, O'NEAL & MARKIN 1973, BRIAN 1977). However, a forward projection of the mouth without movement, could also possibly elicit feedings because the mouth is made more accessible to workers (BRIAN & ABBOTT 1977). Higher frequencies of NI head movements were not the only factor that augmented the total time spent in the NI stretch position. The most fed larva of an experimental group remained 2.7 times longer in the NI stretch position than least fed larvae. This suggests that the time spent in the NI stretch position is the actual, or at least a part of the actual begging behaviour, rather than the NI head movements. In this hypothesis, a larva could enhance its chances of being fed by spending a maximal amount of time in the NI stretch position by placing itself more often in the NI stretch position (with the covariation of the NI head movements as a consequence) and by staying longer in this position.

An additional argument for this hypothesis is that the strongest correlation between experimental groups is the one between the average length of time a larva spends in the NI stretch position and the measure for feeding intensity (total feeding frequency for each replicate). This correlation is negative which rules out the alternative explanation for the correlation within the groups that the behaviour was caused by the feedings. If larvae of a certain group spent more time in the NI stretch position because they were fed more than other larvae in that group, we would expect this behaviour to be seen more frequently in groups that received more feedings. The contrary was true, however, suggesting that the larvae of different experimental groups begged more when workers performed less feedings. It is unclear why workers in some groups were "bad feeders", but possi-

bly a sampling effect of using low worker numbers could result in the absence of proper foragers or nurses. Differences in the quantity of food received by larvae (feeding frequencies) in the experimental groups were not caused by differences in the rate of larval inspections by workers, because there were no differences in the frequencies of I (induced) head movements. Consequently there were also no differences between the most and the least fed larva for each replicate in the frequencies and the total duration of the I stretch position. Other behaviours such as swirling and contractions could also have a signaling function, but they were too infrequent to assess their possible relation to feeding. The infrequency of these behaviours indicates, however, that they must be less important than the (NI) stretch position. The main function of a contraction seems to be connected to urination (BRIAN 1977).

New queens emerged from female larvae in our experimental groups as well as in the stock colonies that were collected in late summer. Vernalisation, in which female larvae have to endure cold temperatures before queen development (BRIAN 1955b) is thus not absolutely necessary in *Myrmica rubra*. The number of males that emerged in the experimental groups was negligible, which excludes their potential interference in the experiment.

To conclude we can say that food partitioning (which larva gets more and which gets less) among larvae within the experimental groups was governed by means of relative intensities of begging cues and that the level of begging cues (level of competition) between groups increased when resources (e.g. feeding nurses with food) decreased.

The relative importance of signaling and test feeding have to be investigated. In natural colonies food is often scarce and resource competition occurs among larvae. Test feeding is possibly a consequence of experimental conditions where plenty of food is given (SUDD 1967), but cannot be ruled out as a functional mechanism for food partitioning. However begging cues can still be important as the labour intensive test system is inefficient (BRIAN 1956, CASSILL et al. 1998). By chance, some larvae will get less feeding inspections than needed for basic survival and normal growth. We suggest that this inefficiency can be partially compensated by a complementary begging system, in which hungry larvae can efficiently communicate their need for food to the workers. Begging is thus likely to be selected for, even when some larvae might try to abuse the signal.

CASSILL & TSCHINKEL (1995) pointed out that hunger signals cannot be visual, auditory or volatile chemical cues because the ant brood is piled in one great mass and tended in the dark. They believed that the hunger cue in *Solenopsis invicta* was likely to be non-volatile rather than behavioural or tactile. Non-volatile hunger cues have recently been shown for the first time by DEN BOER & DUCHATEAU (2002) in the bumblebee *Bombus terrestris*. Our study on *Myrmica rubra* is the first to give quantified evidence for a behavioural cue, although, from our observations, we cannot exclude the possibility that chemical cues reinforce the behavioural signal. For example, larvae can exchange saliva during feeding (OHLY-WÜST 1977, cited in HÖLDOBLER & WILSON 1990).

Independent of more detailed mechanistic studies, the duration of the NI stretch position can be a useful tool to quantify the begging intensities of larvae. It is expected that female larvae manipulate begging mechanisms for self-determination (BOURKE & FRANKS 1995, BOURKE & RATNIEKS 1999). The duration of the NI stretch position can thus be used as a measure of larval self-interest. This makes it not only possible to test begging models in general (e.g. GODFRAY 1995, RODRIGUEZ-

GIRONÉS & ENQUIST 2001, PARKER et al. 2002), but also to test specific predictions about larval interest in caste determination. This interest includes a larger interest in developing into a queen in bigger colonies (NONACS & TOBIN 1992, KELLER & NONACS 1993, KELLER & REEVE 1994), in colonies with lower relatedness between larvae as a result of the higher mating frequencies of queens or higher queen numbers (BOURKE & FRANKS 1995, REUTER & KELLER 2001, WENSELEERS et al. 2003) and when chances are higher for a queen to head a colony (BOURKE & FRANKS 1995, REUTER & KELLER 2001).

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REFERENCES

- ASHE A. & OLDROYD B.P. 2002. Genetic determination of caste in harvester ants. *Trends in Ecology & Evolution* 17: 448-449.
- BHATKAR A.P. & WHITCOMB W.H. 1970. Artificial diet for rearing various species of ants. *Florida Entomologist* 53: 229-232.
- BOURKE A.F.G. & FRANKS N.R. 1995. Social evolution in ants. *Princeton: Princeton University Press*.
- BOURKE A.F.G. & RATNIEKS F.L.W. 1999. Kin conflict over caste determination in social Hymenoptera. *Behavioral Ecology and Sociobiology* 46: 287-297.
- BRIAN M.V. 1954. Studies of caste differentiation in *Myrmica rubra*. 1. The growth of queen and males. *Insectes Sociaux* 1: 101-122.
- BRIAN M.V. 1955a. Studies of caste differentiation in *Myrmica rubra* L. 2. The growth of workers and intercastes. *Insectes Sociaux* 2: 1-34.
- BRIAN M.V. 1955b. Studies of caste differentiation in *Myrmica rubra* L. 3. Larval dormancy, winter size and vernalisation. *Insectes Sociaux* 2: 85-114.
- BRIAN M.V. 1956. Group form and causes of working inefficiency in the ant *Myrmica rubra*. *Physiological Zoology* 29: 173-194.
- BRIAN M.V. 1977. *Ants*. London: Collins.
- BRIAN M.V. & ABBOTT A. 1977. The control of food flow in a society of the ant *Myrmica rubra* L. *Animal Behaviour* 25: 1047-1055.
- BRIAN M.V. & HIBBLE J. 1963. Larval size and the influence of the queen on growth in *Myrmica*. *Insectes Sociaux* 10: 71-81.
- BROWN J.J. & TRANIELLO J.F.A. 1998. Regulation of brood-care behavior in the dimorphic castes of the ant *Pheidole morrisi* (Hymenoptera: Formicidae): effects of caste ratio, colony size, and colony needs. *Journal of Insect Behavior* 11: 209-219.
- CASSILL D.L., STUY A. & BUCK R.G. 1998. Emergent properties of food distribution among fire ant larvae. *Journal of Theoretical Biology* 195: 371-381.
- CASSILL D.L. & TSCHINKEL W.R. 1995. Allocation of liquid food to larvae via trophallaxis in colonies of the fire ant, *Solenopsis invicta*. *Animal Behaviour* 50: 801-813.
- CASSILL D.L. & TSCHINKEL W.R. 1999. Regulation of diet in the fire ant, *Solenopsis invicta*. *Journal of Insect Behavior* 12: 307-328.

- DEN BOER S. & DUCHATEAU M.J. 2002. The smelly side of feeding behaviour in the bumble bee *Bombus terrestris*, pp. 85. In: Adaptations and constraint. Abstract book 9th BENELUX Congress of Zoology 2002. *Antwerp*.
- EVANS J.D. & WHEELER D.E. 1999. Differential gene expression between developing queens and workers in the honey bee, *Apis mellifera*. *Proceedings of the National Academy of Sciences USA* 96: 5575-5580.
- GODFRAY H.C.J. 1995. Signalling of need between parents and young: parent-offspring conflict and sibling rivalry. *The American Naturalist* 146: 1-24.
- HÖLLDOBLER B. 1971. Communication between ants and their guests. *Scientific American* 224 (3): 86-93, 124.
- HÖLLDOBLER B. & WILSON E.O. 1990. The ants. *Cambridge, Mass.: Harvard University Press*.
- HUANG Z.-Y. & OTIS G.W. 1991. Inspection and feeding of larvae by worker honey bees (Hymenoptera: Apidae): effect of starvation and food quantity. *Journal of Insect Behavior* 4: 305-317.
- KAPTEIN N., BILLEN J. & GOBIN B. (submitted). Larval begging behaviour in the ponerine ant *Gnamptogenys striatula*. *Animal Behaviour*.
- KELLER L. & NONACS P. 1993. The role of queen pheromones in social insects: queen control or queen signal? *Animal Behaviour* 45: 787-794.
- KELLER L. & REEVE H.K. 1994. Partitioning of reproduction in animal societies. *Trends in Ecology & Evolution* 9: 98-102.
- KILNER R. & JOHNSTONE R.A. 1997. Begging the question: are offspring solicitation behaviours signals of need? *Trends in Ecology & Evolution* 12: 11-15.
- LE MASNE G. 1953. Observations sur les relations entre le couvain et les adultes chez les fourmis. *Annales des Sciences Naturelles (Sér. 1, Zoologie)* 15: 1-56.
- MATSURA M. & YAMANE S. 1984. Biology of the vespine wasps. *Berlin: Springer*.
- NONACS P. & TOBIN J.E. 1992. Selfish larvae: development and the evolution of parasitic behavior in the Hymenoptera. *Evolution* 46: 1605-1620.
- O'NEAL J. & MARKIN G.P. 1973. Brood nutrition and parental relationships of the imported fire ant *Solenopsis invicta*. *Journal of the Georgia Entomological Society* 8: 294-303.
- PARKER G.A., ROYLE N.J. & HARTLEY I.R. 2002. Begging scrambles with unequal chicks: interactions between need and competitive ability. *Ecology Letters* 5: 206-215.
- RAUTER C.M. & MOORE A.J. 1999. Do honest signalling models of offspring solicitation apply to insects? *Proceedings of the Royal Society of London (Biology)* 266: 1691-1696.
- REUTER C.M. & KELLER L. 2001. Sex ratio conflict and worker production in eusocial Hymenoptera. *The American Naturalist* 158: 166-177.
- RIBEIRO M.F., VELTHUIS H.H.W., DUCHATEAU M.J. & VAN DER TWEEL I. 1999. Feeding frequency and caste differentiation in *Bombus terrestris* larvae. *Insectes Sociaux* 46: 306-314.
- RODRIGUEZ-GIRONÉS M.A. & ENQUIST M. 2001. Role of begging and sibling competition in foraging strategies of nestlings. *Animal Behaviour* 61: 733-745.
- SUDD J.H. 1967. An introduction to the behaviour of ants. *London: E. Arnold*.
- WENSELEERS T., RATNIEKS F.L.W. & BILLEN J. 2003. Caste fate conflict in swarm-founding social Hymenoptera: an inclusive fitness analysis. *Journal of Evolutionary Biology*, 16: 647-658.
- WHEELER D.E. 1986. Developmental and physiological determinants of caste in social Hymenoptera: evolutionary implications. *The American Naturalist* 128: 13-34.
- WHEELER D.E. 1994. Nourishment in ants: patterns in individuals and societies, pp. 245-278. In: Hunt J.H. & Nalepa C.A., Edits. Nourishment and evolution in insect societies. *Boulder: Westview Press*.
- WHEELER G.C. & WHEELER J. 1974. Ant larvae of the myrmicine tribe Attini: second supplement (Hymenoptera: Formicidae). *Proceedings of the Entomological Society of Washington* 76: 76-81.
- WHEELER W.M. 1918. A study of some ant larvae, with a consideration of the origin and meaning of the social habit among insects. *Proceedings of the American Philosophical Society* 57: 293-343.
- WILSON E.O. 1971. The insect societies. *Cambridge, Mass.: Harvard University Press*.