Larval nourishment in *Leptothorax acervorum* (Fabricius, 1793) (Hymenoptera: Formicidae), with description of a larval mensarium as a trophophoretic structure for the handling of food particles

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Abstract

Larvae of ants are fed either with regurgitated material from the worker's crops, including glandular secretions, or with solid food particles, or both. Feeding of larvae of *Leptothorax acervorum* (Fabricius, 1793) was studied with behavioral observations, with dyed food, and with SEM pictures of larvae. Young larvae of 1st and 2nd instars receive only regurgitated food, whereas 4th, and rarely also 3rd instar larvae also ingest solid food particles. The 4th instar larvae have specialized rough cuticular structures on the ventral side of four anterior segments, i.e., the meso- and metathorax and the abdominal segments I and II. Evidently these structures help to keep food particles in place while manipulated and ingested by the larva. The entirety of the structures may be homologous to other specializations of the anteroventral region such as food basket, or the praesaeipium, but probably not the trophothylax, found in larvae of other ants, though various differences in shape and localization justify a new term, the "mensarium".

Key words: Formicidae, *Leptothorax acervorum*, larval development, larval nutrition, solid food, mensarium.

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Introduction

Ant larvae in general are fed by the workers and in numerous species during colony foundation by the young queens. Liquid food stored in the crops of workers is frequently distributed to larvae as well as to other workers via trophallaxis. This may be honeydew, nectar, hemolymph and other liquid substances, but may also contain secretions of certain glands of the workers that were swallowed before (GÖSSWALD & KLOFT 1960a, b, NAARMANN 1963).

In many ants, though, larvae can be observed either feasting on larger pieces of prey (e.g., *Myrmica*, *Aphaenogaster*; LE MASNE 1953, BUSCHINGER 1973), or chewing smaller particles of prey with their mandibles that workers have placed on the mouthparts or on the anteroventral side of the larvae. Recently, CASSILL & al. (2005) have found evidence for cooperative prey digestion by larvae and workers in a *Pheidole* species: larvae dissolve prey particles placed on their mouthparts by workers who then ingest and distribute the liquid material among other larvae and workers. Further sources of larval food can be trophic eggs (numerous ant genera, TOROSSIAN 1979, HOLLIDYER & WILSON 1990, WHEELER 1994). Finally the larvae in their first instar seem to devour neighboring sister eggs or trophic eggs lying within the egg pile (e.g., *Myrmica*; WEIR 1959).

Despite a lot of data that have been published on larval nourishment in ants of numerous genera and species, more detailed information is needed in many cases. When studying the life cycle of a gregarine species (Protozoa) parasitizing ant larvae (BUSCHINGER & al. 1995, KLEESPIES & al. 1997) we needed information on how and when the parasite is transmitted to the host specimens. We therefore investigated feeding and food uptake of larvae of *Leptothorax acervorum* (Fabricius, 1793), a non-natural but suitable host species in which the gregarine could be successfully reared. We directly observed the behaviour of larvae and workers, and also offered dyed insect tissue and carbohydrate food. In addition nourishment of larvae was studied in relation to the annual cycle and larval development. SEM micrographs revealed an as yet unknown structure in the anteroventral region of larvae evidently related to food uptake.

Materials and methods

Colonies of *Leptothorax acervorum*, a facultatively polygynous, widespread European myrmicine species, were collected in pine forests near Nuremberg, Bavaria. For laboratory culture the colonies were kept according to BUSCHINGER (1974), in artificial daily and annual temperature cycles.

The experimental colonies were living in artificial nests made of microscopic slides (BUSCHINGER 1974). They were watched under dim illumination with a dissecting microscope (WILD M5) at magnifications of ×12 or ×25.

Small pieces of insect tissue (muscles of *Periplaneta americana* (LINNAEUS, 1758) or the content of pupae of *Tenebrio molitor* LINNAEUS, 1758) and diluted honey (c. 1:1) were offered ad libitum. The food was replaced every other day.

Experimental colonies with one queen each, 5 - 10 workers and 10 - 20 larvae belonging to one or two instars were formed out of larger stock colonies. For a couple of experiments we also used complete natural colonies.
Diluted honey was mixed 1:1 with a 1 % solution of Neutral Red. For preparing dyed protein food pieces of freshly killed mealworm pupae were placed for 1.5 hrs into a 0.5 % aqueous solution of Neutral Red, then dried with paper tissue and put into the experimental colonies. Preliminary experiments had revealed that Trypan Blue, Nile blue sulfate, and Methylene Blue were less appropriate. For studying larval feeding in spring conditions, the experimental colonies were taken out of the hibernation temperature regime of 0 / 10 °C (where they had not been fed) and placed into a spring temperature regime of 10 / 20 °C where food was provided from the first day on. Other colonies were deprived of food during the last two weeks of "summer" (at 15 / 25 °C). Then larval food uptake was studied during the two weeks of fall conditions (10 / 20 °C) prior to hibernation (at 0 / 10 °C). Particularly in order to achieve sufficient consumption of dyed materials the colonies had to be deprived of honey or protein food for no less than 14 days. After only two to six days of food deprivation very few larvae took up the dyed food substances.

For SEM microscopy, larvae of all instars were fixed for 2 hrs in 2.5 % formalin, then dehydrated in a series of increasing concentrations (ethanol 60 %, 75 %, 85 %, 90 %, isopropanol 100 %, xylol 100 %, for 0.5 hrs each). The objects then were dried at 40 °C over night, and glued to SEM specimen mounts (Mikrotechnik München) with nail varnish. Photos were taken with a JEOL JSM-K 3 on Agfa pan APX 100 at magnifications of ×80 to ×820.

Results

1. Larval development in *Leptothorax acervorum*

Larval development of *L. acervorum* comprises four larval instars (MARGA 1975, FRENZ 1977) (Fig. 1). The instars can be identified with measurements of head capsules and mandibles. Number, size and shape of hairs and the degree of sclerotization of the mandibles also are characteristic for the different instars. For the purposes of this study living larvae had to be classified under a dissecting microscope at magnifications up to ×50. 4th instar larvae were identified by their dark brown mandibles and the long body hairs. 3rd instar larvae have smaller and yellowish mandibles. The mandibles of 2nd and 1st instar larvae are not visible with a dissecting microscope. So they had to be distinguished by size and the naked appearance of the 1st instar.

As Figure 2 reveals, most of larval growth occurs in the 4th instar. Therefore we subdivided this instar into three overlapping size classes (4-1 to 4-3).

2. Feeding of larvae with prey particles

*Leptothorax acervorum* is one among the numerous Myrmicinae species in which older larvae are provided with solid food: The larvae, often lying on the back, masticate small prey particles with their mandibles, bending forth and back the head and anterior segments.

In no case, however, did we observe such a behavior in larvae belonging to the 1st and 2nd instar. 3rd instar larvae exhibited this behavior only exceptionally, whereas all three size classes of 4th instar were seen regularly and frequently ingesting protein food in this way.

Under a dissecting microscope it can be seen how workers bite off small particles from the pieces of mealworm pupae we offered, and carry them into the nest. Such workers themselves, or indoor workers taking over the food load, offer the particles to larvae. Hungry larvae react to antennation by a worker with a kind of begging behavior, rocking the head back and forth, and simultaneously opening and closing the mandibles.
The worker then deposits the food particle directly between the mandibles or onto the ventral surface of the larval thorax. With chewing mandible movements the larva eats the food (Figs. 3, 4). Due to the translucent head capsule tiny food particles sometimes can be seen gliding down the oesophagus.

When dyed food particles were provided, the gut content quickly turned red (Fig. 5).

Larvae not always are lying on the back while eating. Within the narrow nest chambers they also can lie laterally, or, due to humidity, hang head down from the ceiling, stick to the lateral nest walls and even "stand upright" from the floor.

Quite frequently a worker remains close to an eating larva. From time to time the worker may lick the food particle, the larva’s mandibles or its belly. She also may take away the particle, lick it, then give it back to the same, or to another larva nearby. The duration of an eating sequence in a larva is extremely variable. It may last from a few seconds up to 90 minutes but commonly is finished after about 15 minutes. Food particles carried into the nest may be deposited also on the nest floor, and then fed to larvae up to three days later.

3. Feeding of larvae with worker crop content

**Dyed protein food:** Fourteen groups of hungry (two weeks deprived of food) workers and brood were fed with dyed insect particles for 24 hrs. Ten workers of each group not having visible meat particles between the mandibles then were placed to other groups of hungry 4th instar larvae. After another 24 hrs the larvae were checked for dyed gut content. Only one or two (once five) larvae out of 25 - 80 in each group had received any dyed protein. Hence, feeding liquid protein to larvae seems not to be common.

**Dyed carbohydrate food:** Twelve complete colonies received dyed honey-water for four up to 12 hrs, after which time all larvae of the four instars were checked for presence of dye in the gut. The total number of larvae varied between 56 and 495, and also the proportions of the various larval instars varied, as is always the case with naturally composed colonies. The results in short are: All larval instars received dyed honey, even after a short experimental time of only four hours. The proportions of dyed larvae varied considerably within the instars and among the colonies, though in general between 35 and 84 % of the larvae had received dyed honey. Similarly variable was the proportion of dyed larvae within the various instars, with proportions of 15 to 100 %. Only in a couple of colonies none of the L1 and / or L2 had received dyed honey, but in one colony with 38 1st instar larvae 23 (= 61 %) were dyed.

4. Larval feeding in spring and fall conditions

Larval food ingestion might be different in spring (after hibernation) and in fall (prior to hibernation). In order to address this question we made a couple of feeding experiments within the two weeks just after the end of a hibernation, and after the summer brood period, when the colonies prepared for their next hibernation.

**Larval feeding in spring conditions:** A total of 38 spring colonies were provided with natural or dyed protein food beginning with the first day in spring conditions. The food was changed every other day. For the following 14 days we checked whether larvae could be seen eating prey particles, or had ingested dyed food. Three colonies already began feeding larvae on day two, others followed up to 10 days later, and in eight out of the 38 colonies no feeding larvae were seen until day 14.

Four colonies were provided with dyed honey-water from the first day on after the end of hibernation. Dyed larvae appeared already in the first day in two colonies, the others followed up to 10 days later, and in eight out of the 38 colonies no feeding larvae were seen until day 14.

Four colonies were provided with dyed honey-water from the first day on after the end of hibernation. Dyed larvae appeared already in the first day in two colonies, the others followed on days two and four. Larvae of all instars were fed with honey-water in these first days of "spring".

In summary, feeding carbohydrate food seems to begin somewhat earlier than feeding on prey particles.

**Larval feeding in fall conditions:** After the summer period, 31 colonies were watched during the last two weeks before the (artificial) onset of hibernation, when pupae and eggs were no longer present. We daily recorded the number of colonies in which larvae could be seen still ingesting prey particles. In about one half of the colonies (16 out of 31) no larvae were found to eat prey particles within
the two weeks. In a few colonies feeding larvae were seen until 7 days before the onset of hibernation, and in four colonies feeding had not ended at the last day.

Seven other colonies received dyed protein food. In five colonies newly dyed larvae were recorded until days 4 to 7, and two colonies apparently had stopped larval feeding already at or before the beginning of the experiment, 2 weeks before hibernation began.

Seven colonies that were kept in fall conditions were checked for ingestion of honey-water by larvae during the last two weeks before the scheduled onset of hibernation. Three colonies received dyed honey-water on day 1 of this period, one colony on day 5, and three colonies on day 8. In all colonies dyed larvae appeared the very day when dyed food had been presented. Newly dyed larvae were found until the last day before the onset of hibernation.

In summary, carbohydrates were fed to larvae throughout the warm period from the first to the last days, whereas feeding of prey particles apparently began a few days later in spring. In a considerable number of colonies protein feeding ended well before the onset of hibernation.

5. Description of a larval "mensarium"

SEM pictures of 4th instar larvae revealed a distinct structure on their ventral side. An oval field with a rough cuticular structure extends across several segments, beginning with the mesothorax and ending with the second abdominal segment (Figs. 6, 7). We term this structure a "mensarium" since it bears food particles like a tiny table (Latin mensa = table).

In living larvae of L. acervorum the mensarium is usually hidden because the head and anterior part of the body are bent ventrally so that the mouthparts touch the second abdominal segment. When slightly squeezed with a forceps, the larva stretches its body and the mensarium becomes visible at ×50 magnification. The surface appears as slightly shrivelled, wavy cushions that look similar to the SEM pictures (Fig. 7).

Discussion

Larval feeding behavior of Leptothorax acervorum is described in detail for the first time in this paper. Though feeding of solid (insect) particles and / or liquid crop content containing mainly honeydew, oily substances or / and glandular secretions of the workers has been studied quite frequently (e.g., LE MASNE 1953, NAARMANN 1963, HÖLD-DÖBLER & WILSON 1990), it is rare to find exact data on which instars will receive what kind of food. Such data unfortunately are lacking for most ant species or genera, and so this paper may stimulate further research. In particular, it is evidently necessary to know such details for species to be kept in laboratory culture, or, e.g., for developing poison baits (see also PETRALIA & VINSON 1978).

We report that in Leptothorax acervorum all four larval instars receive liquid food (worker crop content). This material may contain pre-digested protein material, honey-water, glandular secretions or simply drinking water, which we were unable to distinguish. However, in the experiments with dyed protein food, dye was transferred to younger larvae only rarely, whereas with dyed honey-water all larval instars got a dyed midgut, indicating that worker crops mainly contained carbohydrate food.

Protein food apparently is distributed rarely to 3rd instar larvae and regularly to 4th instar larvae, in the form of prey particles that the larvae macerate and ingest by themselves. It remains open whether or not a partial extra-intestinal predigestion by the workers is involved, and it is unclear as yet whether some extra-intestinal digestion by the larvae themselves can occur. Dissections of older larvae (A. Buschinger, unpubl.), however, revealed that the larval midgut often contains tiny pieces of insect cuticle. This corresponds to our direct observation of small particles gliding down the oesophagus of larvae. Thus, not only liquid, predigested material is swallowed, but also small, solid food particles. The observations are in line with the finding of gregarine spores of about 8.5 × 12.5 µm size being swallowed by L. acervorum larvae (BUSCHINGER & al. 1995).
The existence of such a process in workers. Our experiments, however, gave no indication of then regurgitate it to other colony members, larvae and gested by the larvae but is swallowed by workers, who

<table>
<thead>
<tr>
<th>Name</th>
<th>Localization</th>
<th>Shape</th>
<th>Described in</th>
<th>Author(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food platter</td>
<td>Ventral side of abdomen</td>
<td>Ventrally flattened segments, trough-like</td>
<td>Ponerinae: Odontomachus, Pachycondyla</td>
<td>Wheeler (1918)</td>
</tr>
<tr>
<td>Food basket</td>
<td>&quot;Median portion of anteroventral region&quot; (= Simple type of praesaepium sensu Petralia &amp; Vinson 1978)</td>
<td>Smooth hairless surface, though with minute spines and surrounded by long straight hairs</td>
<td>Myrmicinae: Solenopsis</td>
<td>Petralia &amp; Vinson (1978)</td>
</tr>
<tr>
<td>Praesaepium</td>
<td>Shallow depression on ventral surface of anterior abdominal segments</td>
<td>Abdominal segment II raised to form a transverse welt, over-hanging the depressed ventral surface of segment I; &quot;floor always conspicuously spinulose&quot;</td>
<td>Formicinae: Camponotus (Colobopsis)</td>
<td>Wheeler &amp; Wheeler (1953)</td>
</tr>
<tr>
<td>Mensarium</td>
<td>Median portions of ventral surface of mesothorax, metathorax, abdominal segments I and II</td>
<td>Wrinkled hairless &quot;cushions&quot;</td>
<td>Myrmicinae: Leptothorax, Harpagoxenus</td>
<td>Buschinger &amp; Schaefer (this paper)</td>
</tr>
<tr>
<td>Trophothylax</td>
<td>Abdominal segment I, medioventral</td>
<td>Deep pouch opening forward</td>
<td>Pseudomyrmecinae</td>
<td>Wheeler &amp; Bailey (1920)</td>
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Cassill & al. (2005) reported on extra-intestinal pre-digestion of prey particles by 4th instar larvae of Pheidole spadonia Wheeler, 1915. The liquefied material is not ingested by the larvae but is swallowed by workers, who then regurgitate it to other colony members, larvae and workers. Our experiments, however, gave no indication of the existence of such a process in Leptothorax acervorum.

The experiments also aimed at revealing some information on the annual cycle of larval feeding (Results, section 4). The results have been presented in a semi-quantitative manner because numerous factors influencing the outcome of the experiments could not be standardized: Composition of colonies with respect to numerical relations of workers to larvae, of larval instars within a colony, of sex ratio among larvae, of colony age, and others. The main results are not surprising, though. The shirvelled hibernating larvae in spring usually quickly grow and become "fat", which is due to provisioning with carbohydrates and water. In natural conditions it may be more difficult for the workers to capture prey insects in early spring. Hence, the larvae of 4th instar may not directly get protein food. In the experiments, however, where protein food was provided from the first spring day on, they received solid protein particles only slightly later than honey-water. After the summer period, when the queens have stopped egg-laying and the last young larvae have hatched, the brood may receive liquid food until low temperature stops all activities. Feeding insect particles apparently stops already a couple of days before, or better to say: the preparation of larvae for hibernation includes a kind of early feeding stop. The date when protein feeding stopped varied considerably among our colonies. The reason may be that the single colonies individually differed in their preparedness for hibernation. Larvae of L. acervorum in this pre-hibernation period and during the first weeks of hibernation are known to shrink considerably, losing a lot of water (Buschinger 1973, Knoth 1978).

As a conclusion from this study we wish to point out here that in Leptothorax, and probably in other ant genera, too, it is normal that the workers provide liquid crop content and prey particles separately to the larvae. With artificial diets, e.g., that described by Bhakar & Whitcomb (1970), in which proteins and carbohydrates are homogeneously mixed, such a separate feeding is precluded. In addition, that diet contains by far not enough protein. It may be inappropriate therefore for rearing such species over long time (Buschinger & Pfeifer 1988). Wheeler & Wheeler (1955) meticulously described the shape and morphological characters including mouthparts, hairs and sensilla of a number of Formicoxenini larvae, among them Harpagoxenus sublaevis and its slave, Leptothorax acervorum. They also cited all reports on larval behaviour and nutrition as known at that time. Larger larvae feeding on insect particles or the exuviae of sister larvae were well known, and for L. canadensis (a close relative of L. acervorum) they mentioned "prothorax with a ventral spinulose swelling and a pair of ventrolateral bosses". For "half-grown larvae" they stated a "more or less prominent welt across the ventral surface of each thoracic somite and the abdominal somites I and II". These wels may represent what we term "mensarium", though the latter does not include the prothorax, is found in fully grown
lariae as well, and the authors apparently have overlooked the characteristic rough structures depicted in Figs. 6 and 7. The wrinkled cuticular surface of this structure, however, is partly an artefact due to desiccation of the larvae during preparation for the SEM pictures. In living larvae it is more vaulted and less sharply wrinkled. Perhaps it is underlain by a glandular epithelium secreting some sticky substance for gluing the food particles to the larval surface, though this has not been investigated. In any case, in the SEM pictures it is an easily detectable, particular structure which may be suggested to have a function in keeping the food particles in place. The name "mensarium" is derived from this function as kind of a small table (Latin: mensa).

PETRALIA & VINSON (1978) described a similar, probably homologous structure in 4th instar larvae of Solenopsis invicta: A slightly vaulted, bare anteroventral portion of the larva is surrounded by lateral rows of straight simple hairs, called a "food basket". However, the smooth surface here is bearing transversely arranged groups of very small (1-2 \( \mu \)m long) stiff spines suggested to pierce the food particles that are kept in place by the lateral rows of hairs. In L. acervorum neither these lateral hairs nor the tiny setae were found. PETRALIA & VINSON (1978) discuss possible homologies of their "food basket" as a simple type of praeaspium, a term used by WHEELER & WHEELER (1953).

BARONI URBANI & al. (1992) consider the "conspicuous food pocket (trophothylax)" of the larvae as an autopomorph of the subfamily Pseudomyrmecinae. Emphasis should be laid on the word "conspicuous", this trophothylax forming a comparatively deep pouch in the ventral side of the larval thorax (cf. WILSON 1971: fig. 14-5). It is more conspicuous than the mensarium found in Leptothorax larvae, or any other such structure though both are comparable in their function. KERRMAREC & FEBVAY (1985) described a glandular epithelium within a so-called "trophothylax" in 4th instar larvae of the myrmicine ant, Acromyrmex octospinosus (REICH, 1793). This "trophothylax", however, is situated directly behind the trophorhiniun (WHEELER & WHEELER 1976) surrounding the mouth and opening of the salivary gland.

Table 1 provides an overview of the various described structures.

In conclusion, the "mensarium" of Leptothorax larvae, situated on the mesothoracic through second abdominal segments, and protruding instead of forming a depression, probably is not homologous to the trophothylax of the Pseudomyrmecinae. Larvae of other ant genera and subfamilies other than the Myrmicinae should be considered, probably is not homologous to the trophothylax of the Pseudomyrmecinae. Larvae of other ant genera and subfamilies other than the Myrmicinae should be checked for the presence of comparable structures.

Acknowledgements

We thank A. Maiazza for taking the SEM-pictures and H. Pohl for valuable help with the preparation of the SEM samples. We are grateful to two anonymous referees for helpful suggestions to improve the manuscript. This paper summarizes the diploma thesis by SCHAEFFER (1996). Unfortunately the first author was unable to contact her to obtain the agreement for this publication under her name. The first author takes over all responsibility for the correct representation and translation of her results. The term "larval mensarium" had not been suggested in the thesis.

Zusammenfassung


References


