

# Larval hemolymph feeding in the ant *Leptanilla japonica* by use of a specialized duct organ, the “larval hemolymph tap” (Hymenoptera: Formicidae)

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**Summary.** The larvae of the migratory Japanese ant *Leptanilla japonica* Baroni Urbani have a specialized duct organ on each side of the 4th abdominal segment. Behavioral and histological studies have shown that the adult ants are able to imbibe hemolymph directly from the larval body cavity through these organs, each of which is referred to here as a “larval hemolymph tap.” Laboratory observations further confirm that larval hemolymph feeding (LHF) is the sole source of nutrient for the queens. *L. japonica* is cyclical in its brood production. All larvae in a colony develop in concert, and when they are mature, the queen performs much active LHF on them. Nourished in this way, she achieves full physogastry within a period of only a few days and then lays a batch of 100–200 eggs. LHF thus facilitates synchronization of brood maturation and concentrated production of eggs by the queen. The larval hemolymph tap has probably evolved *de novo* in *Leptanilla*, in relation to cyclical brood production by species with small colony populations. Obviously, the function of this larval organ is non-selfish, contributing to overall colony-level functioning. The larvae of *Leptanilla* are thus properly qualified as a distinct class of caste in ant societies.

## Introduction

Although the larvae of social Hymenoptera are apodous and nearly immobile, their service to the colony as a distinct functional caste has often been suggested, and there is some evidence supporting this role (e.g., Wilson 1971; Oster and Wilson

1978). In weaver ants (*Oecophylla*) and some species of *Camponotus*, *Dendromyrmex*, and *Polyrhachis*, the larvae contribute silk for nest construction (Hölldobler and Wilson 1983). Also, and more generally in social insects, individuals of all immature stages serve as a food reservoir during hard times. Finally, the larvae of some ants and social wasps produce proctodeal or stomodeal secretions that are ingested by adults. This last “labor” by ant larvae as media in food exchange has long been discussed in connection with special larval organs presumed to serve this function. Thus, the larvae of *Tetraponera* and *Crematogaster* have remarkably developed structures on the body (Wheeler 1918; Eidmann 1926; Menozzi 1930; Casevitz-Weulersse 1983) that Wheeler (1918) suggested were likely to be exocrine glands that produce secretions attractive to their adults. He named such organs “exudatoria.” Despite some anatomical features noted by previous authors, the function of these structures in ants as exudatoria remains largely speculative. On the other hand, Masuko (1986) found that in *Amblyopone silvestrii* Wheeler, larval hemolymph feeding is a normal means of nutrition for the queen in a larger colony even when prey feeding is possible, or in a founding colony when other food is in short supply. In this case no specialized organ is involved; the queens injure the larval integument and take the hemolymph from the resulting puncture.

I report here that the larvae of the ant *Leptanilla japonica* have a pair of duct organs specialized for hemolymph feeding by adults. These are termed the “larval hemolymph taps.” This is the first case among social insects where the presence of exudatoria serving nutrient transfer from immatures to adults has been confirmed. It is suggested that to serve this function the larval hemolymph tap probably evolved *de novo*.

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## Methods

*Leptanilla japonica* is a tiny species. The worker and queen are about 1.2 mm and 1.8 mm long, respectively. Between July 1981 and August 1983, eleven colonies were collected in the evergreen broadleaf forest at Cape Manazuru, Kanagawa Prefecture. At that time details of the biology of the Leptanillinae were unknown and subject to some speculation. The colony reproductive cycle of *L. japonica* is strictly cyclical, like that of the army ants of the subfamilies Dorylinae and Ecitoninae. Egg production by the queens is synchronized with larval development. Only when all larvae in a colony have matured in concert, does the queen simultaneously achieve physogastry. She then lays a batch of eggs within a few days. From these eggs new larvae hatch and develop in synchrony throughout their larval and pupal lives.

Of the 11 colonies collected, 6 were reared in the laboratory (at 20–25° C). Colonies were housed in polystyrene observation nests (10 × 11 × 2 cm); the floors were covered with a mixture of gypsum and activated carbon powder. No brood chambers were provided. The tops of the nests were covered with clear glass, permitting observation of the ants by use of a swing-arm-stereomicroscope (magnifications 10–80 ×). Although various prey were provided to avoid food shortage, the ants accepted only small-sized geophilomorph centipedes. For analysis of time budgets, activities were monitored using an audio tape recorder or a portable microcomputer (Epson HC-20). In the most successful culture (83-14), the ants survived 103 days. Quantitative behavioral data were recorded for the queen of this colony, and that of another (82-33), which survived 43 days in the laboratory.

For histological examination, semi-thin sections (1 µm) were made from material fixed in Kahle's solution, embedded in Spurr's (1969) medium, cut with a LKB 4800A Ultratome, and stained with methylene blue. The scanning electron micrograph was taken using a J.E.O.L. JSM-25 microscope. Larvae were critical-point-dried with liquid CO<sub>2</sub> or treated with hexamethyldisilazane (Nation 1983). In the latter procedure, the material was punctured or partly incised to facilitate penetration of the hexamethyldisilazane.

## Results

### *Larval hemolymph feeding in Leptanilla*

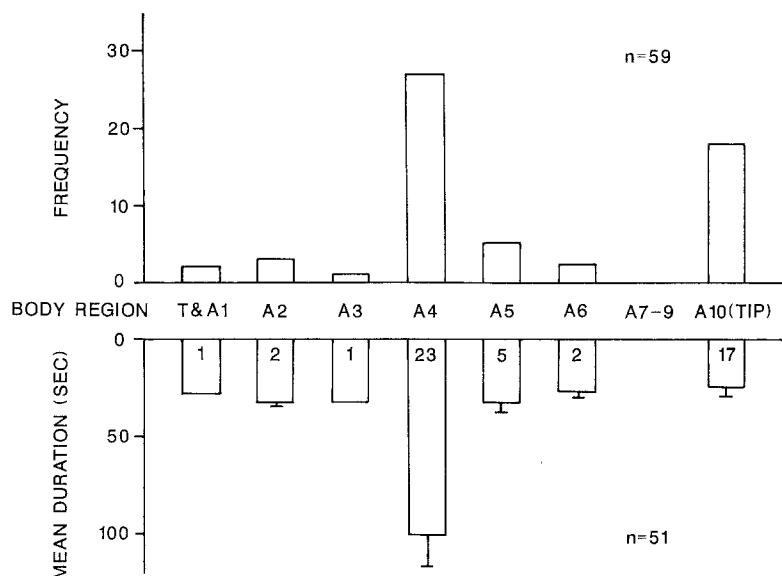
This behavior was first noted during observation of the queen of colony 82-33 (Fig. 1). After briefly but strongly stroking a final-instar larva with her antennae, she held it with her legs and attached her lower mouthparts to its lateral surface. She then maintained oral contact with the lateral body surface while moving the larva with her forelegs, as if searching for some specific spot on its body. During this exploration, the queen frequently nipped the larva lightly with her mandibles, concentrating especially on the thin lateral borders of the abdominal segments. The queen grasped the larva with all three leg pairs, usually lying on her side during this act. Finally, she ceased exploration at the 4th abdominal segment and attached her mouthparts to it for a period of time. During culture of this colony, the queen was never observed



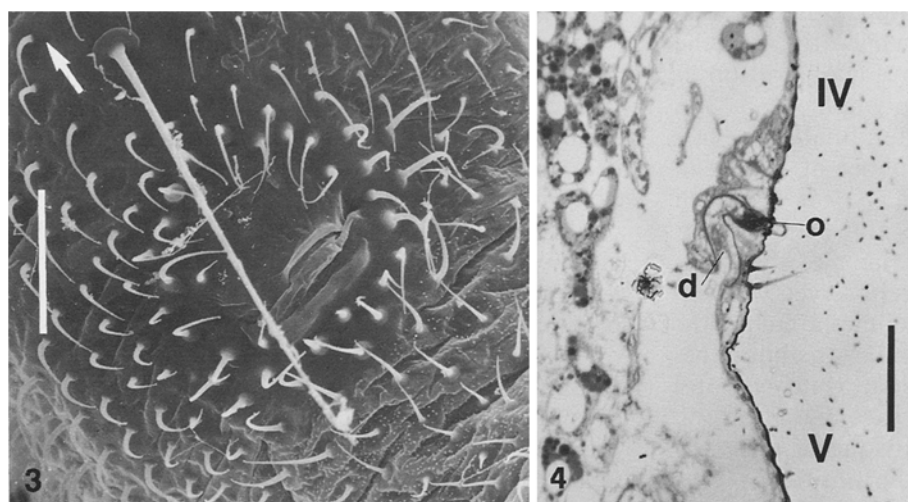
Fig. 1. *Leptanilla japonica* queen, partly physogastric, drinking hemolymph of a final-instar larva. Drawn from a photograph

to feed on prey, even though the larvae and workers were actively feeding on prey at this time. The frequency of this behavior of the *L. japonica* queen increased greatly when she became physogastric. Shortly thereafter she laid a batch of about 50 eggs within a period of only 3 days (3–6 April 1982). This colony had a biased composition due to damage caused by field collection; the colony consisted of only 12 larvae and approximately 120 workers. It perished 43 days after the onset of laboratory rearing. Further behavioral details were studied using colony 83-14, which had a more normal composition (1 queen, approx. 130 workers, and 100 larvae). During the 103-day life of this colony, feeding on prey by the queen was never observed, although the ants were provided with 25 prey items (intact geophilomorph centipedes or their chopped bodies), most of which were consumed avidly by larvae and workers. These observations were similar to the larval hemolymph feeding previously discovered by the author in *Amblyopone silvestrii* (Masuko 1986).

To determine whether the 4th abdominal segment is a region involved in LHF behavior and to analyze the behavioral characteristics of *Leptanilla japonica* queens, a behavioral time budget was developed for this queen. Her activities were assayed for a total of 745 min, comprising eight 90–100 min observation periods, conducted from 12 January to 13 March 1983. This period covered both her nonphysogastric and fully physogastric conditions. During these observations, the queen performed a total of 67 acts of "freezing", i.e. the mouthparts were attached to different larval regions for varying lengths of time. On 59 occasions the region involved was recorded; on 51 occasions time spent at that region was accurately measured (Fig. 2). The results show clearly that the 4th abdominal segment is a region of special emphasis for attention activity, in terms of both



**Fig. 2.** Distribution of frequency and mean duration of stationary mouthpart-attaching by a *Leptanilla japonica* queen at various body regions of the final-instar larvae. Abbreviations for body regions are: *T*=thorax; *A*=abdominal segment. The figures in lower columns are the numbers of this performance of which the time duration was accurately measured. Mean durations and standard errors are based on these data. Total observation time=745 min



**Fig. 3.** SEM photograph of the larval hemolymph tap on the 4th abdominal segment of a final-instar larva of *Leptanilla japonica*. Right side view. Arrows points anteriorly. Intersegmental grooves between the 4th and 5th abdominal segments are seen at lower right. Scale: 20  $\mu$ m

**Fig. 4.** Longitudinal section (1  $\mu$ m) through the region of the larval hemolymph tap at the posterior end of the 4th abdominal segment. The duct (d), which is U- or S-shaped, is sectioned along the full length. o=position of the outlet on the integument. IV=4th abdominal segment; V=5th abdominal segment. Scale=40  $\mu$ m

its frequency and duration. The frequency of attention to the abdominal tip, where the larval hindgut opens, was also high, but the mean durations observed indicate no persistence by the queen in this region (see also Larval duct organs).

#### Larval duct organs

Close observation indicated that whenever the queen concentrated attention on the 4th abdominal segment, her mouthparts were placed near its posterior lateral border. A pair of unusual bilateral structures were found in this position (Fig. 3). Each of these consists externally of a naked circular area bordered by a fringe of curved, stiff hairs (10  $\mu$ m long), overhung by a single long hair

(about 60  $\mu$ m in length). In the center of this naked area, there is a slit-like opening that extends dorso-ventrally (10  $\mu$ m long and 1–2  $\mu$ m wide) and is adjacent posteriorly to two smaller grooves (Fig. 3). Histological sections of this region revealed that the opening is attached internally to a short duct (7–8  $\mu$ m in diameter) that is strongly bent and opens internally directly into the larval body cavity (Fig. 4). In some cross sections, particles of trophocytes remained in the duct. It can be concluded that hemolymph passes through this duct to be discharged outside the larval body, and that the slit-like structure on the integument is the outlet of the duct (although in the preparations, its fine structure was unclear because of its small size). Thus, both behavioral and histological evidence

was obtained supporting the initial hypothesis that this organ is an "exudatorium" (to use the terminology of Wheeler, 1918), secreting nutrients for adult feeding. Each organ is, therefore, termed a "larval hemolymph tap."

Lapping movements of the lower mouthparts of queens were absent during the behavior described above, suggesting that the pharyngeal pump was used for imbibing hemolymph. Since no larval muscles were found internally attached to the duct organ, it seems likely that the queen induces the flow of hemolymph by her actions and that the flow is stopped simply by passive, spontaneous closure of the outlet when she ceases feeding. Alternatively, flow can be stopped by coagulation of contained hemolymph. The mandibular nipping observed during early stages of LHF possibly effects the opening of the outlet. When larvae were lean, contracted, and, thus, apparently had low hemolymph volume, queens sometimes repeatedly pressed them by strongly closing the middle and hind legs around their bodies while attaching the mouthparts to a duct organ, presumably in order to enhance the flow of the hemolymph.

Because immatures, other than final-instar larvae, were not available, the number of instars in *L. japonica* larvae is not known. Nor is it known whether the final-instar larvae alone are subject to LHF. In any case, every member of the final-instar population in the colonies observed was utilized for LHF. The queens sometimes attached the mouthparts at points other than the 4th abdominal segment (Fig. 2). Since an exudatorium was not found by SEM investigations except on the 4th abdominal segment, these acts by queens probably involved misidentification of the position of the hemolymph taps. The larval abdominal tip might be secondarily significant for nutrition of the queen. This was suspected following observations on queen 83-14. When physogastric, she frequently attached her mouthparts to the abdominal tips of larvae while simultaneously nipping them with her mandibles. Such behavior was not observed during the time-budget studies that were conducted during the term of her nonphysogastric condition. A larva being held by this queen for LHF was observed to secrete a droplet of clear fluid from the anus, and this was immediately consumed by the queen. On the other hand, queens showed strong aversion to droplets of whitish, translucent anal fluid emitted by larvae. This opaque fluid presumably contains excretory material, whereas the clear fluid most likely contains nutrients, or, at least, substances that stimulate feeding by queens.

Oral trophallaxis is evidently absent in this spe-

cies, since it was never observed. LHF is, thus, probably the only way in which nutrient is provided to queens. Unlike *Amblyopone silvestrii*, in which the queens suppress LHF by workers (Masuko 1986), *L. japonica* workers ordinarily performed LHF, even when close to the queen, in addition to feeding on prey.

## Discussion

Two points in reference to evolution of LHF in *Leptanilla* will be discussed. Although the presence or absence of LHF has been barely investigated in ants (Masuko 1986), I believe that it will prove ultimately to be uncommon. This poses the question as to why LHF has evolved in *Leptanilla*, and in *Amblyopone* and *Proceratium* in which the larvae are not provided with hemolymph taps. This leads to the second question: why have the specialized exudatoria developed in *Leptanilla* and not elsewhere?

*Evolution of LHF in Leptanilla.* LHF in *Amblyopone silvestrii* has been suggested to be a nutritive adaptation related to the absence of other modes of social food transfer and to the specialized predation on large sporadic prey (centipedes) in this species (Masuko 1986). Only mature or near-mature larvae of the final (5th) instar are employed for LHF by *A. silvestrii* queens. Since these larvae are present in colonies throughout the active season of the year, through LHF the queen's fecundity can be sustained during unstable food conditions, thus avoiding destruction of the larvae by the only possible alternative, cannibalism. Laboratory and field data strongly suggest that *Leptanilla* is also a specialized predator of geophilomorph centipedes (Masuko et al., unpublished data). *Leptanilla* also lacks the habit of oral trophallaxis, and feeding the queens with trophic eggs produced by workers is not possible because due to the absence of ovaries workers cannot produce eggs. Prey is returned to the nest in *Amblyopone* by solitary foragers; in contrast, *Leptanilla* workers are, apparently, obligate group-predators using trail systems, though on a far smaller scale than is known among the army ants (members of the Dorylinae and Ecitoninae). Field data on hunting success are lacking for both *Leptanilla* and *Amblyopone*. Nevertheless, considering the much smaller body size of *Leptanilla* workers (1.2 mm vs 4–5 mm in *Amblyopone*), and that group foraging is supported by a very small worker force (the total observed worker population in *L. japonica* colonies is only 100–200), it is possible that prey acquisition in *Leptanilla* is

more limited than in *Amblyopone*. Furthermore, the nest emigration observed in *Leptanilla* could be related to local food depletion, which has been invoked to account for the nomadism of army ants (Wilson 1971; Topoff 1984). The selective advantage of LHF previously suggested for *Amblyopone* could, therefore, hold also for *Leptanilla*. Efficient and opportune intranidal distribution of food is especially important in ant species that produce a large amount of brood cyclically and in which brood development is synchronized. Typical of this are *Leptanilla*, the army ants and some ponerine species belonging to *Cerapachys* (Hölldobler 1982), *Simopelta* (Gotwald and Brown 1966), and *Onychomyrmex* (Taylor, cited in Schneirla 1971, p. 167). As previously suggested (Masuko 1987), LHF is an expedient way for the queen to synchronize mass egg-production with the maturation of larvae in her colony. Nothing is known in regard to the nutrition of the queens in army ants (Rettenmeyer 1963; Schneirla 1971; Gotwald 1982). Cannibalism of mature brood, either destructively or in a nondestructive manner like LHF, are likely modes of queen nutrition in these ants. Worker trophic eggs are another potential source of nourishment for the queens, but their production has not been confirmed in army ants.

According to the descriptions of ant larvae by Wheeler and Wheeler (e.g., 1976 and 1984), structures like the duct organs of *Leptanilla* are not known for dorylines or ecitonines. Hence, I argue the development of duct organs in *Leptanilla* as a feature related to small colony size.

*Evolution of larval duct organs in Leptanilla.* The fewer the available larvae in colonies, the greater the potential cost of LHF. This could explain why *A. silvestrii* foundresses suppress LHF when prey is available, whereas the queens of developed colonies continue LHF even when feeding on prey is possible (Masuko 1986). Cyclical brood production in small *Leptanilla* colonies could necessitate some special device for queen nutrition by LHF in order to secure efficient hemolymph transfer enabling rapid egg production, and concurrently preventing the larvae from damage due to intensive LHF. For this purpose, simple duct-like organs could be more suitable than cellular exocrine glands. It seems likely that LHF involving damage to the larval integument, as in *Amblyopone*, formerly existed also in the ancestors of *Leptanilla* prior to the evolution of the hemolymph tap.

*Leptanilla* has an exceptional mode of life involving specialized predation of centipedes, very small colony size, and evolution of LHF utilizing

special hemolymph taps. All of these specializations must have occurred after ancestral species had acquired migratory behavior and cyclical production of brood. The evolutionary precursor of the hemolymph tap is, however, totally unknown. This organ was considered by Wheeler and Wheeler (1965 and 1976) to be a spiracle, and they suggested that a single pair alone was present in *Leptanilla* larvae. I have examined the body surfaces of 30 final-instar larvae of *L. japonica* with an SEM and was, thus, able to identify the true spiracles. They were found on the meso- and metathoraces and the anterior eight abdominal segments, including the 4th (Masuko, unpublished data). Hence the larval hemolymph tap is not a structure derived from a respiratory organ. It could have evolved *de novo* in *Leptanilla*. Evolution of novel structures is accompanied by morphogenetic costs. In the case of *Leptanilla*, this new organ might not have evolved until the larval populations in colonies had become generally subjected to LHF. This could account for the absence of specialized larval organs related to LHF in doryline and ecitonine ants, since colonies of these ants are enormously large, usually containing 60,000 to 20,000,000 workers, depending on the species (Schneirla 1971). Thus, if nutrition of the queen involved cannibalism or LHF by wounding as in *Amblyopone* then only a very small fraction of the brood would be required.

The function of the larval hemolymph tap of *Leptanilla* is totally directed towards colony maintenance. *Leptanilla* larvae are thus regarded here as composing a distinct "caste" category in insect sociality (e.g., Wilson 1980).

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