

**LARVAL HEMOLYMPH FEEDING AND OOPHAGY :
BEHAVIOR OF QUEEN AND WORKERS
IN THE PRIMITIVE PONERINE ANT
PRIONOPELTA KRAEPELINI
(HYMENOPTERA, FORMICIDAE)**

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Abstract. The behavior of the queen and workers in the amblyoponine ant *Prionopelta kraepelini* was observed and compared with that of the queen and workers of *P. amabilis*. The queen fed mainly on larval hemolymph by pinching the larval body. Workers often laid trophic eggs, most of which were given to larvae. The foraging and recruitment behavior of workers were similar to *P. amabilis*.

Key words : ants, Ponerinae, *Prionopelta*, oophagy, recruitment, larval hemolymph feeding

INTRODUCTION

The ant tribe Amblyoponini represents an important group for understanding the diversity of ant behavior, since they are morphologically the most primitive of the Ponerinae (HÖLLDOBLER & WILSON, 1990). It remains controversial, however, whether they are a primitive or derived group (HASHIMOTO, 1996). Their bionomic information is still largely lacking, because most species are distributed in the tropics and are very rare (BROWN, 1960). *Prionopelta* Mayr, 1866 is a genus of small amblyoponine ants, the bionomics and foraging behavior of which are known only from the neotropical *P. amabilis* Borgmeier, 1949. This species has very special behavioral and morphological characteristics : queens feed only on trophic eggs laid by workers, the pupal chamber shows « wall papering » of the surface with cocoon fragments, and workers show recruitment of nestmates for prey retrieval by using a trail pheromone which originates from special basitarsal glands in the hindlegs (HÖLLDOBLER & WILSON, 1986 ; HÖLLDOBLER *et al.*, 1992). However, it is still unknown whether these characteristics are typical for this species only, or whether they also apply to other species in this genus.

We collected a colony of the very rare *Prionopelta kraepelini* Forel, 1905 in the type locality of this species, the Bogor Botanic Gardens (West Java, Indonesia). In the laboratory, we observed the behavior of queen and workers, and examined the morphology of

the leg glands of workers. In this paper, we describe our findings on *Prionopelta kraepelini*, and compare this species with the neotropical *P. amabilis*.

MATERIAL AND METHODS

The colony was collected in the Bogor Botanic Garden, Bogor (6°35'S, 106°47'E), West Java, Indonesia in December 1995. Judging from the revision of BROWN (1960), the specimens were identified as *Prionopelta kraepelini* (TERAYAMA, pers. comm.), which was originally described from the same locality. The voucher specimens were deposited in the Bogor Zoological Museum.

The colony was kept in an artificial nest box of 10 x 6.3 x 2.5 cm. The bottom of the nest was covered with plaster of Paris mixed with activated carbon powder. A brood chamber was excavated in the centre of the plaster floor, the top of the chamber was covered with clear glass. Small crickets were fed as prey. The nest was kept at 24 to 28° C. Water was often applied to the plaster floor to maintain high humidity. Because of the small body size of this species, all observations were carried out under a binocular dissecting microscope. During the observations, the number of workers ranged between 80 and 100, while the colony also contained a few males and all stages of brood. Queen behavior was observed for a total of 21h20 min during one month. Each observation period lasted 20 to 40 min. All behavioral acts performed by the queen were observed during this period. The foraging behavior of workers was observed four times. For each observation, we recorded the number of workers in the foraging arena every 5 min during 1 hour. Then, a small cricket was offered in the foraging arena. Counting the number of workers in the foraging arena and around the prey was continued for 1 hour. After these observations, the queen and several workers were dissected to check their reproductive condition.

Hindlegs of workers were fixed in 2% glutaraldehyde, buffered at pH 7.3 with 0.05 M Na-cacodylate and 0.15 M saccharose. After postfixation in 2% osmium tetroxide, tissues were dehydrated in acetone and embedded in Araldite. Thin sections were double stained with a LKB Ultrastainer, and examined with a Zeiss EM 900 transmission electron microscope. Worker hindlegs for scanning microscopy were coated with gold and viewed with a Philips SEM 515 microscope.

RESULTS

Nest and colony composition

The colony of *P. kraepelini* was collected from a moist, dead stump in the Bogor Botanic Gardens. The stump was inhabited by a colony of *Leptogenys diminuta* (F. Smith, 1857) but also contained two nest chambers of *P. kraepelini*, from which we collected all individuals. Chamber A contained a dealate queen and ca. 30 workers with larvae. The queen was immediately dissected: she was virgin without mature oocytes and yellow bodies. Chamber B contained a dealate, mated queen and ca. 60 workers with eggs, larvae and pupae. The wall of the chamber was covered with cocoon fragments. It was not sure

whether the two chambers may have been interconnected by a tunnel. Behavioral observations were carried out for the queen and workers in chamber B. Apart from this colony, we found only one other colony fragment of this species during our annual investigation of the ant fauna of the Bogor Botanic Gardens since 1990. This other colony fragment was equally found in a dead and moist stump. Behavioral observations were carried out for the queen and workers in chamber B.

Queen behavior

The behavioral repertory of the queen is given in Table 1. In all, 14 behavioral acts were recognized. Unlike queens of *P. amabilis* that feed only on trophic eggs laid by workers (HÖLLDOBLER & WILSON, 1986), the queen of *P. kraepelini* fed on prey insects, larval hemolymph, and trophic eggs laid by workers. Feeding on prey brought into the nest was observed only twice, and represented 3.0% of the feeding activity (total time budget to feeding activity was 88 min 36 s), whereas 14.8% occurred as oophagy and 87% through larval hemolymph feeding (LHF). The manner of LHF is very similar to that of *Amblyopone silvestrii* (Wheeler, 1928) (MASUKO, 1986). However, the queen of *P. kraepelini* pinched over the whole body of the larva unlike *A. silvestrii*, in which the queens mainly pinched on the 4th and 5th larval segments (MASUKO, 1986). As in *A. silvestrii*, larvae subjected to LHF did not die from their wounds.

TABLE 1

Behavioral catalogue of the P. kraepelini queen during 21h 20 min of observations

<i>Behavioral acts</i>	<i>Frequency</i>	<i>%</i>
self grooming	117	22.8
allogrooming		
received from workers	115	22.4
received from males	1	0.2
toward workers	11	2.1
antennation		
toward workers	132	25.7
toward males	1	0.2
from workers	87	16.9
from males	4	0.8
biting workers	20	3.9
licking larvae	15	2.9
larval hemolymph feeding	25	4.9
licking prey	2	0.4
oophagy	2	0.4
oviposition	2	0.4
Total	534	100

Oophagy was observed 11 times: two eggs were eaten by the queen, one by a male, and eight by larvae, indicating that trophic eggs are mainly used for larval nutrition. We confirmed by direct observation that four of the eggs were laid by workers. Their eggs were evidently smaller than those laid by the queen. After oviposition of the egg, a worker picked it up from the tip of her abdomen with the mandibles, and walked in the nest chamber for 1 to 3 min before presenting it to a nestmate. When giving the egg, the worker held it with her mandibles and the egg was put on the mouthparts of the nestmate until the end of feeding.

Queens had three ovarioles per ovary. Oviposition by the queen was observed twice. The queen never picked up eggs from the abdominal tip with her mandibles. After oviposition, eggs were laid on the nest floor, and subsequently a worker grasped the egg and brought it to the egg pile. Egg care by the queen was never observed, but was only performed by workers.

The queen frequently received grooming from workers. She sometimes groomed workers, however, the frequency of this behavior was low. Antennation towards and received from workers was often observed. After antennation to workers, the queen sometimes bit the worker's head or mandibles. In *P. amabilis*, the queen was surrounded most of the time by a retinue of ca. 10 workers and was frequently groomed by them (HÖLLDOBLER & WILSON, 1986). In *P. kraepelini*, such worker retinue was not observed.

Worker behavior

Workers foraged individually. Before prey was given, between 7 and 16 workers were found in the foraging arena (Fig. 1). When a worker found a prey insect in the foraging

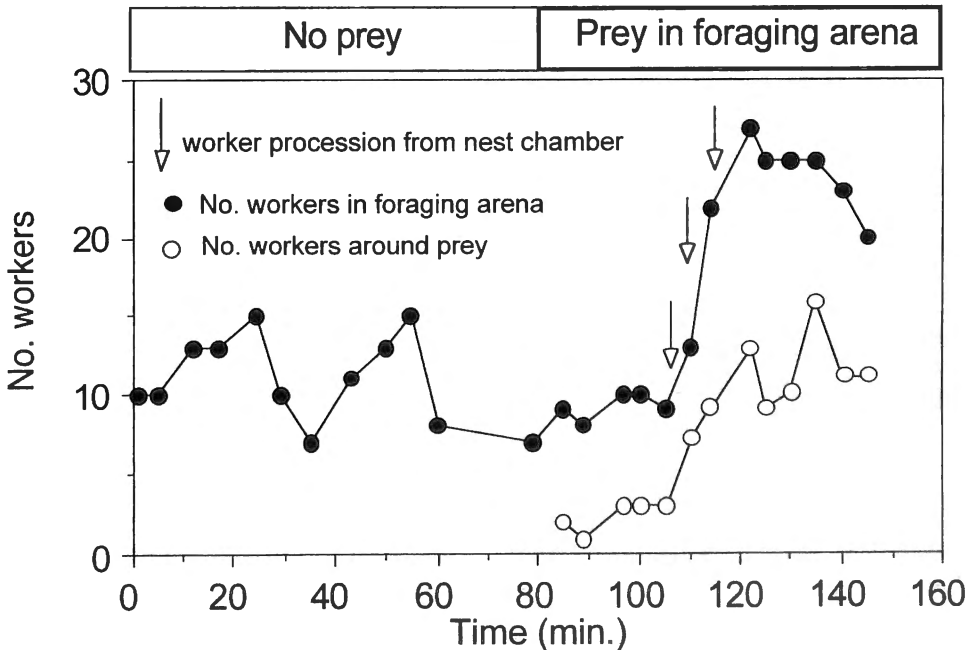


Fig. 1. - Number of foraging workers of *P. kraepelini* before and after prey was offered.

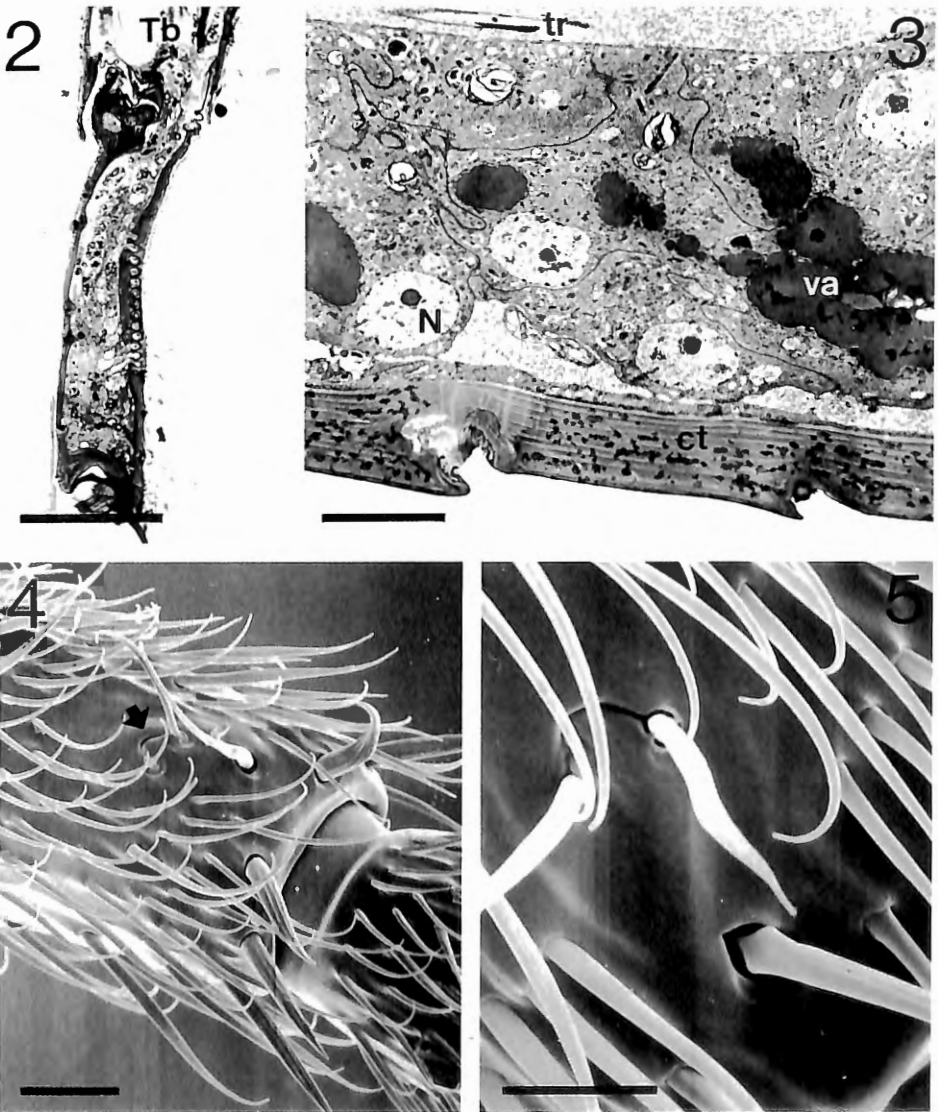


Fig. 2. – Semithin section through foreleg basitarsus, showing position of antenna cleaner gland. Cuticular pores correspond with nerve endings associated with gland. Tb = tibia (scale bar 50 μ m).

Fig. 3. – Electron micrograph of secretory cells in hindleg basitarsus. ct = cuticle, N = nuclei, tr = tracheoles, va = vacuolar areas (scale bar 5 μ m).

Fig. 4. – Scanning electron micrograph of ventrodistal portion of hindleg basitarsus indicating position of ventrally occurring slit (arrow) (scale bar 10 μ m).

Fig. 5. – Detail showing semi-circular slit in between two associated hairs (scale bar 5 μ m).

arena, she opened her mandibles, and slowly approached the prey. Then, she grasped its appendages and stung. During this interaction with prey, a few workers usually joined in the prey attack. After that, one or a few workers went to the nest chamber and recruited nestmates to the prey site. The number of workers recruited by one recruitment episode was small, up to seven workers made a single procession, and followed the route of scout workers. Such recruitment was repeated one to four times for retrieving one prey. Beside the procession, single workers also went out and followed the trail. Worker behavior during this process was very similar to *P. amabilis*: recruiting workers dragged their hind legs, and showed vertical body shaking when they entered the nest (see HÖLLDOBLER *et al.*, 1992, Fig. 1). Like *P. amabilis*, foraging workers after entering the nest often displayed self-grooming, especially by stroking the hindlegs with the forelegs. Preliminary morphological examination of the limited material available revealed the presence of glandular cells in the basitarsus of both the fore- and hindlegs. In the frontleg basitarsi, densely packed secretory cells occur (Fig. 2), that correspond with the antenna cleaner gland as described by SCHÖNITZER and co-workers (1996). In the hindlegs, polymorphic glandular cells with a rounded nucleus and large vacuolar areas occur (Fig. 3), although we were unable to trace their precise structural contact with the outside. In the scanning microscope, however, we observed on the ventral side the presence of a narrow semi-circular slit occurring between two hairs in the distal portion of the hindleg basitarsus (Figs 4, 5), similar to the situation reported for *P. amabilis* by HÖLLDOBLER *et al.* (1992), who found this to be the site where the basitarsal gland opens. These two hairs are shorter than the surrounding leg hairs, and are situated approx. 4 μm from each other (Fig. 5).

As already mentioned, some workers laid trophic eggs. After the observations, we examined ovarian development in 9 foraging and 10 domestic workers. Judging from their pigmentation, 6 of the latter were apparently young individuals. All workers had one ovariole per ovary. All foraging workers had no developing oocytes while all but one domestic worker had one or two developing oocytes, suggesting that trophic eggs were laid by the domestic workers.

Brood care was similar to the description by HÖLLDOBLER & WILSON (1986). In their artificial nest, workers sometimes put cocoon fragments on the glass ceiling cover, which looks like «wall-papering» behavior. The tips of cocoons of pupae was usually opened: workers may remove the meconium of larvae after they become prepupae. Workers sometimes removed cocoons of young white pupae. Such pupae were laid on the nest floor and emerged later. Adult transport was never observed, although the nest was disturbed by removing the covering glass cover. On such occasions, the queen walked by herself.

DISCUSSION

Comparison of the bionomics between *P. kraepelini* and *P. amabilis* based on the present study and studies by HÖLLDOBLER and co-workers (1986, 1992) remains fragmentary, because for both rare species a very limited number of colonies was available for study. However, the similarity in several aspects of bionomics, e.g. the «wall papering» of the nest chamber, the foraging and recruitment behavior, and the trophic egg production by workers, may indicate that these behaviors are common characteristics of the genus

Prionopelta. Besides in *Prionopelta*, the peculiar « wall papering » with cocoon fragments has only been found in *Harpegnathos saltator* Jerdon, 1851, where this behavior may contribute to stabilizing humidity inside the nest chambers (PEETERS *et al.*, 1994).

Both species also display apparently similar exocrine glands in the basitarsi of both their fore- and hindlegs. The glandular cells in the foreleg basitarsus are thought to be associated with the tibio-tarsal cleaning apparatus (HÖLLDOBLER *et al.*, 1992; SCHÖNITZER *et al.*, 1996). In *P. amabilis*, the hindleg basitarsal gland is reported to produce trail following substances during recruitment to food sources or new nest sites (HÖLLDOBLER *et al.*, 1992). In *P. kraepelini*, a similar function may be likely, as we could clearly demonstrate worker recruitment to prey in this species.

A remarkable difference between both species, however, is found in the queen behavior. HÖLLDOBLER & WILSON (1986) reported that the queen of *Prionopelta amabilis* exclusively fed on trophic eggs, which might be laid by workers. They observed oophagy three times, the three eggs being eaten by the reproductive queen, a virgin queen, and a larva. In *P. kraepelini*, we observed oophagy of 11 trophic eggs, only two of these were eaten by the queen while most eggs were fed to larvae, suggesting that the primary role of the trophic eggs in this species is food for larvae. Time budget data suggest that larval hemolymph is the most important food source for the queen of *P. kraepelini* as is also the case for *Amblyopone silvestrii* (MASUKO, 1986). Since the observation period was rather short for *P. amabilis* (5 hours, HÖLLDOBLER & WILSON, 1986), it is difficult to conclude whether this is a significant difference between the two *Prionopelta* species. In *P. amabilis*, presentation of trophic eggs by workers was very often observed, and up to 5 workers simultaneously present their eggs to the queen (HÖLLDOBLER & WILSON, 1986), while such high frequency of egg presenting was not found in *P. kraepelini*. The reason for this difference may be related to the number of workers: colony size is much smaller in *P. kraepelini* (ca. 100 workers) than in *P. amabilis* (more than 500 workers).

Larval hemolymph feeding (LHF) has been reported for two genera of ponerine ants (*Amblyopone silvestrii* and three species of *Proceratium* Roger, 1863) and the leptanilline ant *Leptanilla japonica* Baroni Urbani, 1977 (MASUKO, 1986, 1989). These species are all specialized predators in which no regurgitation nor trophic eggs are known. *A. silvestrii* and *L. japonica* hunt for geophilomorph centipedes, while *Proceratium* lives from arthropod eggs. Such prey items are often difficult to obtain (due to the large size of centipedes, and parental care of arthropod eggs), which may have an important bearing on the occurrence of LHF as an aberrant feeding mode (MASUKO, 1986, 1989). In contrast to these species, the queen of *Prionopelta kraepelini* predominantly feeds on larval hemolymph even though the workers can lay trophic eggs. Food specialization in *Prionopelta* is apparently less developed than in *A. silvestrii*, *L. japonica* and *Proceratium*. Under laboratory conditions, *P. kraepelini* workers attacked and brought termites, crickets and mealworm larvae to the nest chamber. For *P. amabilis*, a preferential diet of campodeid dipturans has been reported, although they also accept other small arthropods (HÖLLDOBLER & WILSON, 1986). The possibility has been mentioned that LHF may also have a function in control of caste differentiation, in which the queen controls the development of female larvae into workers by LHF (MASUKO, 1986). If this would also be the case for *P. kraepelini*, the occurrence of both LHF and trophic egg production would imply queen-worker conflict

for the production of workers and gynes: queens prefer more to invest in colony maintenance (production of workers) than in production of sexuals, while workers show no preference for these fractions of investment (BOURKE & FRANKS, 1996).

HÖLLDOBLER & WILSON (1986) listed the similarities and differences between *Amblyopone* and *Prionopelta*, based on observations of *A. pallipes* (Haldeman, 1844) (TRANIELLO, 1982) and *P. amabilis*. Subsequently, detailed studies of the behavior of the two amblyoponine genera have been published for *A. silvestrii* and *A. reclinata* (MASUKO, 1986, 1996; ITO, 1993a,b) and for *P. amabilis* (HÖLLDOBLER *et al.*, 1992). HÖLLDOBLER & WILSON (1986) stressed that *Prionopelta* is a more derived genus than *Amblyopone*, because of its large colony size, specialized morphology and behavior of queens and workers, and the occurrence of age polyethism. However, recent studies of *Amblyopone* revealed the occurrence of a clear age polyethism also in *A. silvestrii* (MASUKO, 1996) and *A. reclinata* Mayr, 1879 (ITO, unpubl.), an elegant recruitment system for prey retrieval and trophic egg laying by virgin workers in *A. reclinata* (ITO, 1993a, unpubl.). Our study showed that a queen retinue was not found in *P. kraepelini* and that the queen performed LHF as in *A. silvestrii*. Colony size and other life history characteristics may affect the evolution of behavioral elements found in the two amblyoponine genera, rather than phylogenetic constraints.

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