Sociobiology 64(4): 417-422 (December, 2017) DOI: 10.13102/sociobiology.v64i4.2092

Sociobiology
An international journal on social insects

RESEARCH ARTICLE - ANTS

Independent colony foundation in *Paraponera clavata* (Hymenoptera: Formicidae): First workers lay trophic eggs to feed queen's larvae

C Peeters

Institute of Ecology and Environmental Sciences (iEES), Sorbonne Université UPMC, Paris, France

Article History

Edited by
Gilberto M. M. Santos, UEFS, Brazil
Received 25 September 2017
Initial acceptance 02 November 2017
Final acceptance 12 November 2017
Publication date 27 December 2017

Keywords
Caste, founding behavior, prothorax, neck muscles, functional morphology.

Corresponding author
Christian Peeters
Sorbonne Université UPMC
Institute of Ecology and
Environmental Sciences (iEES)
4 Place Jussieu, Paris 75005, France
E-Mail: christian.peeters@upmc.fr

Abstract

Paraponera clavata Smith is a large, notorious, and widely distributed ant, yet its colony founding behavior is poorly known. In the laboratory, a dealate queen collected from Peru reared a first generation of ten adult workers over 18 months; eight cocoons and several larvae failed. Food was obtained outside the nest and given to larvae. It took five and six months before the first two workers emerged, and they were smaller than average (i.e.'nanitic'). At Q+4, trophic eggs were laid by workers and given directly to medium and mature larvae on three occasions. Six workers were dissected immediately after the queen's death, and five had yolky oocytes in their ovaries. Queen foraging is known from anecdotal field observations, despite the prothorax (and corresponding neck muscles) being smaller than in other poneroid queens.

Introduction

Paraponera clavata Smith is a conspicuous, 18-30 mm long inhabitant of wet forests in the Neotropics. Its notoriously painful sting underlies a variety of common names ('24 hour' ant, 'bullet ant') and even native initiation rites. It nests in soil next to the base of trees and forages in the canopy. Such arboreal habits are found in only few poneroid ants (Gobin et al., 1998a). Colonies of *P. clavata* are monogynous with a few hundred workers, reaching up to 2326 workers (Breed & Harrison, 1988).

Paraponera is the only genus in the poneroid subfamily Paraponerinae (Bolton, 2003). Its morphology includes a mixture of primitive traits shared with poneroid subfamilies and derived traits found among formicoids (Keller, 2011). The latter include the presence of well-developed frontal lobes and antennal scrobes, and a clypeus broadly inserted between the antennal sockets. Also, unusual for a poneroid is the fusion of the promesonotal junction, as well as the tergosternal plates of the petiole (Keller, 2011). The second gaster segment shows a well-marked constriction anteriorly, but without forming a true postpetiole. This combination of traits was long considered evidence of *Paraponera*'s affinities with Ectatomminae (now considered as formicoids), although *Paraponera* was always kept separate. Molecular studies support the view that *Paraponera* is a unique lineage that evolved in isolation from other ant groups (e.g. Moreau et al., 2006).

Independent colony foundation (ICF) implies risks as the queen is not assisted by workers. Mortality is high in species with queens that forage outside the nest (termed 'non-claustral' ICF) (Peeters, 1997). Two factors determine ICF in poneroids (Peeters & Molet, 2010): (1) queens and workers exhibit a very limited dimorphism in body size, so a foundress must provide a lot of food to each larva; (2) founding queens have small wing muscles (compared to formicoid queens; Keller et al., 2014) as well as limited fat and protein reserves in the gaster, hence additional food is needed for the first larvae. When foundresses hunt outside, more foraging trips

Open access journal: http://periodicos.uefs.br/ojs/index.php/sociobiology
ISSN: 0361-6525

means more risks. It is crucial that the first workers quickly forage outside instead of the foundress, and new data in *P. Clavata* show they also feed larvae with trophic eggs.

Material and Methods

A few winged queens of *P. clavata* were walking on the ground during Ant Course 2013, held in Estación Biológica Villa Carmen (12°53’25” S, 71°24’39” W, 545m altitude), Madre de Dios province, Peru. On 13 August, I collected a lone dealate queen close to the base of a small tree. It was brought back to Paris and kept in a plaster nest with 2 small chambers (7 x 7 x 1 cm) under a glass roof, with a separate foraging arena. Temperature was controlled at 25°C, and plaster was moistened irregularly. Freshly cut-up mealworms and small crickets were put in the arena at regular intervals, as well as pieces of fruits and honey diluted in water. Mites were intermittently removed from inside the nest.

Most first workers were color-marked to keep approximate age records and monitor individual activities; to keep disturbance to a minimum, marking was usually done when an individual was active in the foraging arena. Gaps between successive emergence of workers were sufficiently long to facilitate keeping track of the order of emergence. Records of behavior (photos and videos) were compiled using an iPhone 4. Freshly dead workers were dissected to determine ovarian activity, and all six surviving individuals were dissected at the end. Prior to this, these six workers were weighed with an electronic balance. Head width (HW; broadest point of the head capsule) was measured with a binocular eyepiece.

Results

Queen behavior

The foundress was able to rear the first generation of workers, and this includes foraging and cutting up prey for larvae (Fig 1). Eggs were laid regularly in small numbers (Table 1); only towards the end were there more than 10 eggs. Counts of queen eggs over short periods (e.g. 8-18 Nov. 2014) suggest some were eaten by larvae. Queen-laid eggs acquired a stiff golden chorion after a few days (Figs 1 and 2), thus oophagy must occur before. Mature larvae were buried with debris by the queen to help them spin a cocoon. Spinning took several hours and several larvae frequently died at this stage. The meconium (from voiding of the gut; ejected at one extremity of the cocoon) appeared after 4-5 days. The first two cocoons were cut open by the queen, and the pupae discarded outside. Slightly over 5 months elapsed before the first adult worker emerged (Fig 2).

Noteworthy is an eccentric behavior of the foundress: it remained highly alert inside the brood chamber, darting around at the slightest disturbance. I have never observed such wary behavior in the workers of this or other poneroid species. After a few workers had emerged, the queen stopped foraging and became very quiet (Fig 3).

Fig 1. Founding queen together with first brood. A medium larva is busy feeding on a piece of mealworm (arrow). One yellowish egg can be seen.

Colony growth

Another five pupae were discarded outside and a few larvae failed to produce a cocoon, consequently only ten workers reached the adult stage over 18 months (Table 1). One reason for this mortality may have been infestation by mites, which settled on the neck region of larvae and were included in cocoons. Newly emerged (‘callow’) workers retained lightly pigmented leg extremities for a few days (Fig 3). Two workers (one was callow) were taken from inside the nest for color-marking and then released in the arena, and older workers carried them back inside (grabbing their head induced a pupal posture). The first two workers were nanitic (see below), and died five months (worker #1, denoting order of emergence) and seven months (#2) after emergence (Table 1). Workers #2 and #3 were marked outside when five and three months-old, respectively. Worker #4 was also three months-old when first seen outside.

Fig 2. Founding queen together with worker #1 and brood. Two large larvae are feeding. Yellowish eggs lie singly on the ground next to a small larva (arrow).

Small and large larvae were kept alive for two days after the queen died and all workers had been dissected; a small larva molted its skin (head capsule and hind parts) which remained uneaten. This larval skin was seen to be eaten by one worker before, indicating that the adults benefit from this recyclable resource.

Production of trophic eggs

At stage Q+4, three larvae (medium as well as large) fed on white eggs that were smaller than queen eggs. Worker #2 was observed with the sting extruded, an egg was laid and immediately placed on a nearby larva who quickly consumed it. The whole sequence lasted less than one minute.

Table 1. Demographic data for an incipient colony of *Paraponera clavata* monitored in the laboratory for 18 months.

date	first workers	cocoons	larvae	eggs
26-9-**2013**				7
29-10			4	9
8-11			5	4
15-11			5	2
18-11			5	1
20-12			3	3
4-1-**2014**		2	1	4
20-01		1 + 2 dead	1	4
21-02		2	4	2
7-03	worker #1	2	3	4
31-03	Q + 1w	1 + 1 dead	3	7
9-04	worker #2	1	1 +1spin §	8
16-04	Q + 2	1 dead	3	7
9-05	Q + 2	0	2	9
28-05	Q + 2	1	3	4
10-06	Q + 2	1	3	5
23-06	#3	1	3	8
?-07	#4			
4-08	#5; dead#1	1 dead	N/A	N/A
4-09	Q + 4	1	5	6
15-09	Q + 4	4	2	9
25-09	Q + 4*	3 + 1 dead	2 +1spin §	8
29-09	Q + 4	3	1 +1spin §	8
15-10	#6	3	3	7
27-10	#7	1 +1 dead	3	N/A
4-11	#8; dead#2	0	2 +1spin	4
7-11	Q + 6	0	3	5
12-12	dead #6 + #7	2	2	5
30-12	Q + 4	2	1	8
16-2-**2015**	#9 + #10	0	4	17
24-02	Q + 6 Queen dies	1	6	15

* 3 workers active outside § failed

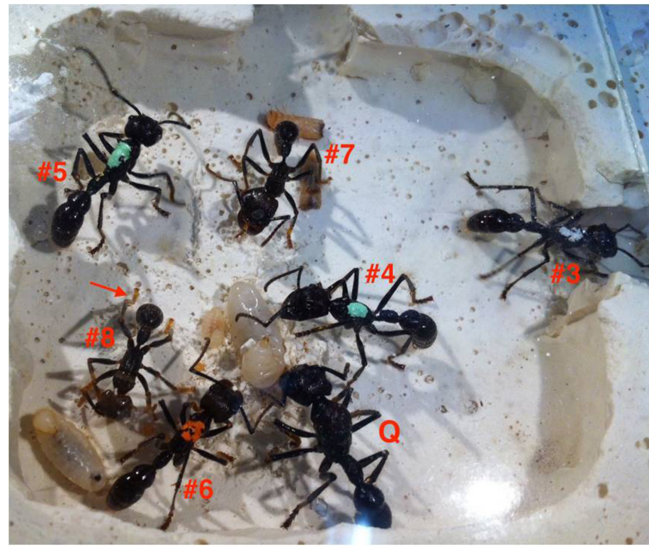

Fig 3. Founding queen with workers and brood on 4 November 2014. The oldest worker (#3) is guarding the entrance. One mature larva (next to worker #4) has reached final size and started spinning. Individuals #4 and #5 have the same colour dot with different shapes. The two recently emerged workers were not marked, but the youngest (#8) can be recognized by its lightly pigmented hind tarsi (arrow).

Worker #2 was six months-old at this time, and had been foraging three weeks earlier. Another worker (yet unmarked, thus young) also laid a trophic egg.

Upon the queen's death, all six remaining workers were promptly dissected, revealing that all but the oldest (8 months-old) had large yolky oocytes. This suggests that trophic eggs could be laid regularly, but because they are directly consumed by larvae, this behavior was only rarely observed. Ovaries consisted of 14 ovarioles in both queens and workers.

Workers #1 and #2 were distinctly smaller (HW= 3.7 mm and 3.9 mm, respectively) and thus nanitic. Other workers had head widths 4.2-4.7 mm, with size roughly increasing with order of emergence. For comparison, the queen's head was 4.8 mm wide. Fresh weights of workers ranged 120-160mg, while the queen weighed 185mg (she lacked yolky oocytes).

Discussion

The incipient colony of *P. clavata* studied here took about one year to produce the first five workers, even though food was provided *ad libitum* in the foraging arena. During independent colony foundation (ICF) in ants, the success rate of first larvae to develop as adult workers depends almost entirely on the queen's ability to provide them with adequate food. This is harder in poneroid species because workers are almost as big as their mother, hence more food is needed before each larva can pupate (as opposed to most formicoids where workers are much smaller than queens; Peeters & Ito, 2015). Not only foundresses must take big risks to forage outside, but they need to continue this for a few months, which presumably causes a very high failure rate. The prolonged development of poneroid brood contributes to the difficulty of ICF. Brood development times are difficult to determine in ants because larvae do not grow in fixed cells, unlike social wasps and bees. Thus, individuals cannot be monitored over time, and estimates must be based on demographic data. Although complicated by oophagy and larval mortality, data in this colony of *P. clavata* suggest the following: egg-larva 4 weeks; larva-cocoon = 6-8 weeks (n=2): cocoon-adult 6-7 weeks (n=3). These very long development times are somewhat higher than those recorded for other poneroids: 2-3 months in total (Peeters & Ito, 2015). This may be linked to the large size of *Paraponera* workers. In sharp contrast, many formicoids develop considerably quicker, e.g. 28 days for major workers of *Solenopsis invicta* (Tschinkel, 2006), 40 days for soldiers of *Pheidole bicarinata* (Wheeler, 1982), 54 days for *Camponotus kiusiuensis* (Ito et al., 1988). Shorter developmental times seem mostly due to a striking reduction in the body size of formicoid workers.

Producing nanitic workers is an adaptation to reduce the time taken before the first workers can forage in replacement of the founding queen. Nanitics are common in the formicoid subfamilies Myrmicinae and Formicinae, but there are only very few anecdotal reports in Ponerinae (Peeters & Molet, 2010). In *P. clavata*, only two workers were conspicuously nanitic, however the first two cocoons that failed may have enclosed nanitics also. Workers emerging later were larger, although below the upper range of head widths (5.4 mm) recorded in a mature colony by Breed and Harrison (1988). Nanitic workers result from a novel modification of the endocrine regulation of larval development: although the queen controls access to pieces of prey (and she was regularly observed to take prey away from larvae busy feeding), the spinning behavior is initiated by the larvae. This prompts the queen or workers to bury pupating larvae with debris, thus providing a scaffold that is crucial for the attachment of silk strands.

Once a few adult offspring were present in the colony, the foundress stopped going outside as workers started to forage. The latter also groomed the brood and distributed prey pieces. Limited observations indicated that first workers help to feed the queen's larvae with trophic eggs. Larval oophagy is known during ICF in Attini (Fernández-Marín et al., 2004) and some other lineages (e.g. *Rhytidoponera confusa;* C. Peeters, unpublished data) but involves eggs laid by founding queens only. Production of trophic eggs by first workers has never been reported. In many ant species, trophic eggs cannot develop as they derive from unviable oocytes (e.g. Gobin et al., 1998b). Such yolk packets are a valuable protein source that facilitates the development of larvae, and fills the gaps while hunting is unsuccessful. Breed and Harrison (1988) found that many young workers collected inside established colonies of *P. clavata* had large yolky oocytes; these could be laid as trophic eggs.

P. clavata collects both nectar and insect prey (Tillberg & Breed, 2004), however nectar is likely to be less important for larval development. Pseudotrophallaxis (i.e. carrying a drop of liquid between open mandibles) was observed infrequently, but there was also intimate contact between the mouths of some workers and larvae, suggesting trophallaxis. This may function to distribute nectar, while trophic eggs increase the flow of proteins to larvae.

The founding behavior of *Paraponera* queens has not been investigated in the field, but the following anecdotal observations suggest ICF is non-claustral. In the field, dealate queens hunt arthropod preys and collect nectar, moreover nest entrances of newly founded colonies remained open (M. Breed, personal communication, April 3, 2012). Hölldobler and Wilson (1990) collected queens foraging on the lower trunks of *Pentaclethra* trees; most *Paraponera* colonies nest at the base of such trees in Costa Rica. Haskins and Enzmann (1937) stated that founding queens need to forage. Similarly, in the laboratory context described here, a foundress regularly went outside to get food. Non-claustral ICF is generally associated with small differences in body size between queens and workers, together with limited reserves accumulated by queens before aerial dispersal (Peeters & Molet, 2010). Queen foraging also depends on worker-like neck muscles, and the thorax of *P. clavata* queens can give information about this (see below).

All ant workers have a large prothorax (T1) that houses powerful muscles connecting with the head, essential to use the mandibles as tools (Keller et al., 2014). In flying insects, the second thoracic segment (T2) is typically large with a predominant mesonotum for the attachment of wing muscles. Formicoid queens generally have a large mesonotum that overhangs a much smaller prothorax, hence the neck muscles are reduced in size and this is consistent with lack of foraging during claustral ICF. In Ponerinae, flying queens have a pronotum intermediate in size to workers', reflecting a distinct trade-off between the size of muscles that power the head and wing muscles, thus allowing queens to hunt and carry prey during colony foundation (Keller et al., 2014). Surprisingly among poneroids, *P. clavata* queens have a small prothorax (Fig 4), more similar to that of queens in Myrmicinae. In *Tatuidris tatusia,* belonging to a poneroid subfamily (Agroecomyrmecinae) closely related to Paraponerinae, winged queens also have a small prothorax (Donoso, 2012). This derived trait shared by *Paraponera* and *Tatuidris* distinguish them from queens in Ponerinae, and suggests an evolutionary trend towards larger wing muscles that are used as metabolic reserves. In attine (fungus-growing) ants, most genera are non-claustral except *Atta* (Fernández-Marín et al., 2004). Attini queens were not included in the comparative study of Keller et al. (2014), but visual examination of the thorax in different attine genera (AntWeb.org) points a trend to a small prothorax even in non-claustral genera. Observations of ICF are generally scarce in the ant literature, and there are no published records for many widespread genera. Thorax morphology can be used to predict the founding behavior of queens (Keller et al., 2014), but predictions need to be tested empirically.

Besides various morphological peculiarities (see Introduction), what other differences separate Ponerinae from Paraponerinae? *Paraponera* exhibits an omnivorous diet, the beginnings of trophallaxis, and a reduced prothorax reflecting modifications in flight morphology. The latter may be necessary to carry a heavier abdomen filled with fat and protein reserves (see Helms & Kaspari, 2014), which is characteristic of most formicoids, i.e. over 80% of ant species. *Paraponera* is one of several scattered experiments in gigantism because the majority of ants evolved dwarf workers and larger colonies (Peeters & Ito, 2015).

Fig 4. Dealate queen and worker of *Paraponera clavata,* showing differences in thorax segmentation. In queens (top), pronotum (dorsum of prothorax, T1) is small relative to the mesonotum (T2), reflecting a trade-off between neck muscles and wing muscles. Posterior segments of queen gaster are missing because of ovarian dissection.
Photos by Mônica Ulysséa

Acknowledgments

This research was made possible through funding from Ant Course 2013, and a collecting permit to B.L. Fisher. I thank Romain Péronnet for help with caring for live ants (and monitoring them during my absences), Roberto Keller for comments on the systematics of *Paraponera,* Mônica Antunes Ulysséa for automontage photos (Fig 4), and Thibaud Monnin for useful comments on a draft. Funded by the French National Research Agency (ANTEVO ANR-12-JSV7-0003-01).

References

Bolton, B. (2003). Synopsis and classification of Formicidae. Memoirs of the American Entomological Institute, 71: 1-370.

Breed, M. & Harrison, J.F. (1988). Worker size, ovary development and division of labor in the giant tropical ant, *Paraponera clavata* (Hymenoptera: Formicidae). Journal of the Kansas Entomological Society, 61: 285-290.

Donoso, D.A. (2012). Additions to the taxonomy of the armadillo ants (Hymenoptera, Formicidae, *Tatuidris*). Zootaxa, 3503: 61-81.

Fernández-Marín, H., Zimmerman, J. K. & Wcislo, W. T. (2004). Ecological traits and evolutionary sequence of nest establishment in fungus-growing ants (Hymenoptera, Formicidae, Attini). Biological Journal of the Linnean Society, 81: 39-48. doi: 10.1111/j.1095-8312.2004.00268.x

Gobin, B., Peeters, C. & Billen, J. (1998a). Colony reproduction and arboreal life in the ponerine ant *Gnamptogenys menadensis* (Hymenoptera: Formicidae). Netherlands Journal of Zoology, 48: 53-63. doi: 10.1046/j.1365-3032.1998.234102.x

Gobin, B., Peeters, C. & Billen, J. (1998b). Production of trophic eggs by virgin workers in the ponerine ant *Gnamptogenys menadensis*. Physiological Entomology, 23: 329-336.

Haskins, C.P. & Enzmann, E.V. (1937). Studies of certain sociological and physiological features in the Formicidae. Annals of the New York Academy of Sciences, 37: 97-162.

Helms, J.A. & Kaspari, M. (2014). Found or Fly: Nutrient loading of dispersing ant queens decreases metrics of flight ability (Hymenoptera: Formicidae). Myrmecological News, 19: 85-91.

Hölldobler, B. & Wilson, E.O. (1990). Host tree selection by the Neotropical ant *Paraponera clavata* (Hymenoptera: Formicidae). Biotropica, 22: 213-214.

Ito, F., Higashi, S. & Maeta, Y. (1988). Growth and development of *Camponotus (Paramyrmamblys) kiusiuensis* Santschi colonies (Hym. Formicidae). Insectes Sociaux, 35: 251-261.

Keller R.A. (2011). A phylogenetic analysis of ant morphology (Hymenoptera: Formicidae) with special reference to the poneromorph subfamilies. Bulletin of the American Museum of Natural History, 355: 1-90. doi: 10.1206/355.1

Keller, R.A., Peeters, C. & Beldade, P. (2014). Evolution of thorax architecture in ant castes highlights trade-off between flight and ground behaviors. eLife, 3:e01539. doi: 10.7554/eLife. 01539.001

Moreau, C.S., Bell, C.D., Vila, R., Archibald, S.B. & Pierce, N.E. (2006). Phylogeny of the ants: diversification in the age of angiosperms. Science, 312: 101-104. doi: 10.1126/ science.1124891

Peeters, C. (1997). Morphologically "primitive" ants: comparative review of social characters, and the importance of queen-worker dimorphism. In J. Choe & B. Crespi (Eds.), The Evolution of Social Behavior in Insects and Arachnids (pp. 372–391). Cambridge University Press. doi: 10.13140/RG.2. 1.2001.8646

Peeters, C. & Ito, F. (2015). Wingless and dwarf workers underlie the ecological success of ants (Hymenoptera: Formicidae). Myrmecological News, 21: 117-130. doi: 10.5281/zenodo.845751

Peeters, C. & Molet, M. (2010). Colonial reproduction and life histories. In: L. Lach, C. Parr & K. Abbott (Eds.), Ant Ecology (pp. 159–176). Oxford University Press. doi: 10.1093/acprof:oso/9780199544639.003.0009

Tillberg, C.V. & Breed, M. (2004). Placing an omnivore in a complex food web: Dietary contributions to adult biomass of an ant. Biotropica, 36: 266-271. doi: 10.1111/j.1744-7429.2004.tb00318.x

Tschinkel, W.R. (2006). The Fire ants. The Belknap Press of Harvard University Press, Cambridge, MA, 723 pp.

Wheeler, D.E. (1982). Soldier determination in the ant, *Pheidole bicarinata.* Ph.D. dissertation, Duke University USA.