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SHORT COMMUNICATION

Bruno Gobin · Fuminori Ito

Queens and major workers of *Acanthomyrmex ferox* redistribute nutrients with trophic eggs

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Abstract In *Acanthomyrmex ferox*, two distinct egg-types are produced: reproductive eggs that give rise to offspring and trophic eggs that serve to distribute nutrients. Queens lay both reproductive and trophic eggs, while major and minor workers lay only trophic eggs in the presence of the queen. The queen lays on average 17% of the trophic eggs in a colony, while majors and minors produce 42% and 41%, respectively. The large proportion of trophic egg production by the queen and soldiers is quite remarkable, since ant queens are expected to focus entirely on reproduction and majors, which have a defensive function in many species, to be sterile.

Food distribution is one of the most important aspects of colonial life in social insects. Since only a part of the workforce forages, the collected resources have to be directed towards interior workers, reproductives and brood. Food can be carried to the nest or be ingested at the source and distributed via trophallaxis, the exchange of liquids from the crop. In ants, retrieved food items generally deteriorate quickly and the long-term storage capacity of the crop is limited in most species. When food sources are unpredictable, or colony sizes are small, there can be a necessity to store food for longer periods (Crespi 1992; Hölldobler and Wilson 1990). In many ant and stingless bee species, workers store nutrients in their fat tissue, and redistribute them by laying specialized trophic eggs (Hölldobler and Wilson 1990; Sakagami 1982). These eggs are non-viable and morphologically distinct from worker-laid haploid reproductive eggs (Gobin et al. 1998; Passera et al. 1968). Trophic eggs are generally given voluntarily to nestmates and larvae. This is a process distinct from cannibalism of reproductive eggs, in which eggs that can develop into larvae are destroyed.

Acanthomyrmex ferox is a myrmicine species with very small colonies, containing 24.8 ± 10.8 minor workers and 2.6 ± 1.5 major workers ($n=49$, ± SD). The genus *Acanthomyrmex* is known for the spectacular head size of the majors (Moffet 1986; Terayama et al. 1998), which was proposed to serve for defensive and seed milling purposes (Moffet 1985; Oster and Wilson 1978). Indeed, fig seeds were found in several colonies of *A. ferox*. Colonies either had many (more than 40) seeds, or none at all, suggesting that seeds are an unpredictable resource (F. Ito and B. Gobin, unpublished work). In *Acanthomyrmex*, all colony members have ovaries, but only the queen has a sperm receptacle for mating. Majors surprisingly have an equal number of ovarioles to the queen (Terayama et al. 1998). Very little is known about the biology and social interactions of this genus. We report the occurrence of trophic eggs in *A. ferox,* and the prominent role both queen and majors play in nutrient storage and redistribution.

A. ferox colonies were collected from rotten twigs near the Ulu Gombak Field Station, Malaysia, housed in plaster nests covered with a glass plate and fed every 2 days with small pieces of mealworm and sugar water. Majors were colour-marked for individual discrimination. Ten colonies were observed directly under a binocular microscope for 120 h and four colonies were videotaped using 24-h time-lapse recording for 5–6 days to observe egg-laying rates and egg types. After observations ended, all colony members were dissected to check ovary development and the presence of yellow bodies (14 queens, 35 majors and 150 minor workers). We measured head width (HW), thorax width (TW) and width of the third abdominal segment (AW) as an indication of storage capacity.

In queenright colonies of *A. ferox*, all female classes lay eggs. Before laying an egg, they assume a typical

B. Gobin (✉) · F. Ito
Faculty of Agriculture, Kagawa University,
Takamatsu 760–8522, Japan

Present address: B. Gobin, Zoologisches Institut I, Universität Erlangen-Nürnberg, Staudtstrasse 5, 91058 Erlangen, Germany
e-mail: bgobin@biologie.uni-erlangen.de
Tel.: +49-9131-8528073
Fax: +49-9131-8528040

Table 1 Egg-laying in four queenright *Acanthomyrmex ferox* colonies. Oviposition and egg-laying rates per day of each caste and subcaste are given, with extrusion times (the time between assuming an egg-laying position and the extrusion of an egg in min:s) according to the egg-types they lay (*R* reproductive, *T* trophic, *n* the number of individuals investigated). Minor workers were not identified individually so the total number of eggs laid by minors was divided by the number of workers for four colonies

	n	Oviposition rate	Eggs extruded per oviposition	Egg-laying rate	Egg type	Extrusion time
Queen	4	2.83±0.76	1	2.83±0.76	R (51%)	5:56±0:46, *n*=30
					T (49%)	1:09±0:23, *n*=30
Major	10	0.39±0.20	3.51±0.96	1.36±0.76	T	1:57±0:24, *n*=15
Minor	(4)	0.16±0.04	1.02±0.04	0.17±0.07	T	1:11±0:23, *n*=16

egg-laying posture, with the abdomen bent forward between their legs and the sting protruded. We observed a total of 85 ovipositions by queens, 39 by major and 120 by minor workers. All eggs laid were classified into two categories based on their distinct morphological appearance. Reproductive eggs are slightly bean-shaped with a regular size (0.55±0.03×0.33±0.02 mm, *n*=10) and sturdy appearance. Trophic eggs are variable in size and generally round. When held between mandibles or forceps they dent easily, suggesting that the chorion is less rigid than that of reproductive eggs. This flexibility might account for their lower extrusion time (Table 1). Queens lay about three eggs per day, extruding a single egg at each oviposition event. In four colonies, 49.25±4.35% of the queen's eggs were trophic. Major and minor workers lay only trophic eggs in the presence of the queen. Minors lay a single trophic egg, while majors lay a package of 3–11 trophic eggs. Detailed observation of four colonies shows that the queen lays 17±4% of the total amount of trophic eggs, while major and minor workers contribute 42±6% and 41±7%, respectively. Given that only a single queen and two or three majors were present against 18 to 29 minors, queens lay significantly more trophic eggs than majors (χ^2=33.64; *df*=3; $P<0.01$) and minors (χ^2=693.67; *df*=3; $P<0.01$), while majors lay significantly more trophic eggs than minors (χ^2=612.21; *df*=3; $P<0.01$).

When the queen lays reproductive eggs, none of the workers respond. After extrusion, the egg will fall on the ground, and subsequently be deposited on the egg-pile by a minor or the queen. When soldiers or the queen lay trophic eggs, nearby minor workers respond as soon as the eggs appear at the abdomen tip. They will approach the egg-layer and pull the egg from the oviduct. When minors lay a trophic egg, it may be removed either by another minor worker or by the egg-layer. Trophic eggs will be passed on among all nestmates for feeding, before being given to larvae.

Trophallaxis also occurs in *A. ferox*. However, this appears to serve only for quick distribution of collected nutrients. Indeed, trophallaxis occurs 1.26±0.62 times per individual per hour on days when sugar water and prey are available to the colony, but falls significantly to 0.03±0.01 times per individual per hour on days when no food is given (t=4.06, $P<0.05$, n=4 colonies).

Queens of *A. ferox* have three ovarioles in each ovary (length: 3.96±0.31 mm, *n*=13). Each ovariole contains several yolky and non-yolky oocytes and large dark-yellow bodies. Large, mature yolky oocytes show two distinct types. Slightly elongate to bean-shaped oocytes containing uniform yolk, surrounded by a thick layer of follicle cells, correspond to oocytes developing into reproductive eggs (Fig. 1). Roundish oocytes with yolk arranged in large globules and a very thin layer of follicle cells correspond to trophic eggs. A single ovariole can contain both reproductive and trophic oocytes, which is in concordance with the more or less alternate egg-laying of trophic and reproductive eggs.

Major workers also have six ovarioles, with a few exceptions having only four or five (length: 2.23±0.21 mm, *n*=13). Their ovarioles contain one basal mature oocyte with granulated yolk and a thin layer of follicle cells (Fig. 1) and a small second devel-

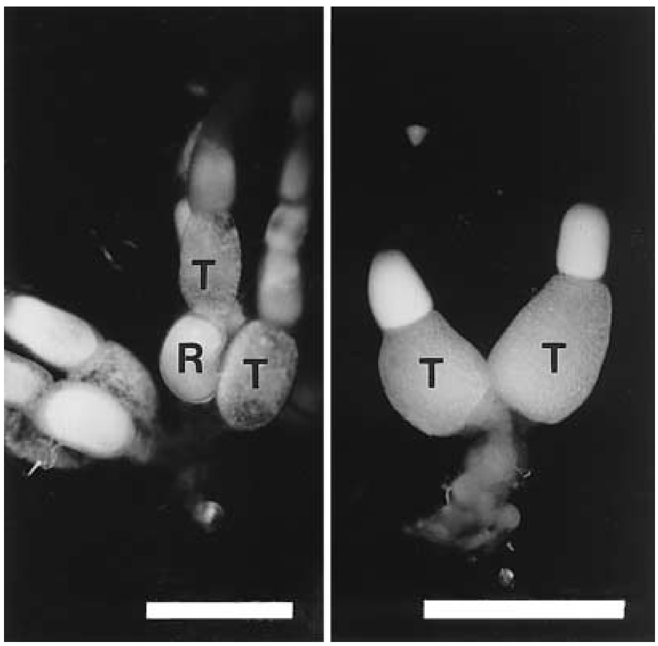

Fig. 1 Photo of dissected ovaries of queen (*left*) and major (*right*). Four of the major's ovarioles were removed for clarity (*R* oocytes with a clear ring of follicle cells and homogenous yolk give rise to reproductive eggs, *T* oocytes with thin follicle cells and granulated yolk give rise to trophic eggs). Note that the queen has a *bean-shaped mature reproductive oocyte* and a *trophic oocyte* in the same ovariole (*scale bar* 1 mm)

oping yolky oocyte. In two majors we found two mature oocytes in a single ovariole. Majors dissected just after egg-laying ($n=6$) had empty basal regions in four to six of their ovarioles, proving that several trophic eggs are extruded simultaneously in one oviposition. In contrast, minor workers only have one ovariole per ovary. Minors have single basal yolky oocytes, have significantly shorter ovaries (1.01±0.19 mm, $n=19$; ANOVA $F_{2,42}=593.1$, Tukey post hoc comparison with queens and majors $P<0.01$), and lay trophic eggs at a lower rate than majors (Table 1). Only once did a worker lay two trophic eggs simultaneously.

Queens (HW 2.44±0.06 mm; TW 1.88±0.1 mm; AW 2.21±0.09 mm; $n=10$) and major workers (HW 2.62±0.13 mm; TW 0.96±0.06 mm; AW 1.87±0.1 mm; $n=10$) have larger bodies than minor workers (HW 1.25±0.04 mm; TW 0.69±0.03 mm; AW 1.06±0.05 mm $n=10$; ANOVA $F_{2,27}$ HW=749.22, TW=744.75, AW=526.34; Tukey post hoc comparison $P<0.01$), suggesting that they have a larger storage capacity. Indeed, their abdomens are filled with fat cells, while it is possible that tissues in head or thorax also have a storage function.

The distinct appearance of yolk and follicle cells, as well as the different shape and fate of laid eggs allows us to clearly distinguish between trophic and reproductive eggs in *A. ferox*. Both worker subcastes (minors and majors) as well as the queen caste produce trophic eggs. Trophic eggs contain concentrated protein, which are especially needed for larval growth (Wheeler 1994). The ability to store food in a colony has advantages if food sources are unpredictable (Crespi 1992), which is more likely in species with small colony sizes. Apart from previously suggested task specialization such as defence and seed milling, majors clearly play a very important role in food storage, since they produce a large proportion of trophic eggs. Their ovary morphology allows for elevated egg-laying, while their large body size allows for higher food storage capacities. Indeed, Hasegawa (1993) found that majors of *Colobopsis nipponicus* store more than twice as much fat than minor workers do. In only one other ant, *Crematogaster smithii*, is a group of larger workers known to be the main unfertilized egg layers (Heinze et al. 1995, 1999).

Majors as food storers are also known in *Pheidole ryukyuensis* (Tsuji 1990). Although these repletes are completely sterile, they store food in their crop for distribution via trophallaxis during periods of food shortage. In *A. ferox*, trophallaxis is only used for short-term food exchange, and nutrients are ingested and stored in the fat tissue for later redistribution via trophic eggs.

Majors have often been granted the status of the ultimate altruistic caste, specialized as soldiers for colony defense (Oster and Wilson 1978; Passera et al. 1996). Because of this high-risk task, soldiers are expected to be non-reproductive (Bourke 1999). Sterile soldiers are found in several species, but many exist where soldiers have a higher ovariole number than minor workers (Baroni Urbani and Passera 1997; Ward 1997). Although large body size is likely to be a specialization for defense, it could also provide an opportunity to store larger amounts of nutrients. If these nutrients can be distributed via trophic eggs, soldiers need not evolve towards complete sterility. Especially when intrinsic storage capacity is limited, as in *A. ferox* with its small colony size, we expect that storage will be optimized, including the use of major workers and queens.

Trophic egg-laying by queens in mature colonies is a remarkable trait known in only one other ant (Heinze et al. 1995, 1999). Many independent founding queens remetabolise nutrients stored in fat tissue and wing muscles to feed the first larvae with immature or trophic eggs (Crespi 1992; Wheeler 1994). It is striking that queens can develop trophic and reproductive eggs simultaneously in their ovaries. Our findings in *A. ferox* confirm that the switch from trophic to reproductive egg-laying is not regulated at a general level, but can be fine-tuned to specific oocytes. Nevertheless, once a workforce is bringing food into the colony, we would expect queens to concentrate solely on reproductive egg-laying. The large fraction of trophic eggs laid by the queen of *A. ferox* gives further evidence for the importance of trophic eggs for colony survival.

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References

Baroni Urbani C, Passera L (1997) Origin of ant soldiers. Nature 383:223

Bourke AFG (1999) Colony size, social complexity and reproductive conflict in social insects. J Evol Biol 12:245–257

Crespi BJ (1992) Cannibalism and trophic eggs in subsocial and eusocial insects. In: Elgar B, Crespi BJ (eds) Cannibalism: ecology and evolution among diverse taxa. Oxford University Press, Oxford, pp 176–213

Gobin B, Peeters C, Billen J (1998) Production of trophic eggs by virgin workers in the ponerine ant *Gnamptogenys menadensis*. Physiol Entomol 23:329–336

Hasegawa E (1993) Caste specialization in food storage in the dimorphic ant *Colobopsis nipponicus* (Wheeler). Insectes Soc 40:261–271

Heinze J, Cover SP, Hölldobler B (1995) Neither worker, nor queen: an ant caste specialized in the production of unfertilized eggs. Psyche 102:173–185

Heinze J, Foitzik S, Oberstadt B, Rüppell O, Hölldobler B (1999) A female caste specialized for the production of unfertilized eggs in the ant *Crematogaster smithi*. Naturwissenschaften 86:93–95

Hölldobler B, Wilson EO (1990) The ants. Springer, Berlin Heidelberg New York

Moffet MW (1985) Behavioral notes on the Asiatic harvesting ants *Acanthomyrmex notabilis* and *A. ferox*. Psyche 92:165–179

Moffet MW (1986) Revision of the myrmicine genus *Acanthomyrmex* (Hymenoptera: Formicidae). Bull Mus Comp Zool 151:55–89

Oster GF, Wilson EO (1978) Caste and ecology in the social insects. Princeton University Press, Princeton

Passera L, Bitsch J, Bressac C (1968) Observations histologiques sur la formation des oeufs alimentaires et des oeufs reproducteurs chez les ouvrières de *Plagiolepis pygmea* Latr. (Hymenoptera Formicidae). C R Acad Sci Paris D 266:2270–2272

Passera L, Roncin E, Kaufmann B, Keller L (1996) Increased soldier production in ant colonies exposed to intraspecific competition. Nature 379:630–631

Sakagami SF (1982) Stingless bees. In: Hermann HR (ed) Social insects, vol 3. Academic Press, New York, pp 361–423

Terayama M, Ito F, Gobin B (1998) Three new species of the genus *Acanthomyrmex* Emery (Hymenoptera: Formicidae) from Indonesia, with notes on the reproductive caste and colony composition. Entomol Sci 1:257–264

Tsuji K (1990) Nutrient storage in the major workers of *Pheidole ryukyuensis* (Hymenoptera: Fomicidae). Appl Entomol Zool 25:283–287

Ward PS (1997) Ant soldiers are not modified queens. Nature 385:494–495

Wheeler DE (1994) Nourishment in ants: patterns in individuals and societies. In: Hunt JH, Napela CA (eds) Nourishment and evolution in insect societies. Westview, Boulder, pp 245–278