ASIAN MYRMECOLOGY — Volume 16, e016004, 2023
ISSN 1985-1944 | eISSN: 2462-2362 DOI: 10.20362/am.016004

Use of trophic eggs at colony founding in the queen-foraging ant, *Manica yessensis*

Keiichi Masuko

Biological Laboratory, Senshu University, 2-1-1 Higashimita, Tama-ku, Kawasaki, 214-8580 Japan

Corresponding author: kmasuko@isc.senshu-u.ac.jp

ABSTRACT. Colony founding queens of the ant, *Manica yessensis* Azuma, 1955, forage outside the nest to retrieve insect prey. During colony foundation, they also lay trophic eggs in addition to reproductive eggs that give rise to larvae. Thus, colony foundation to produce the first workers in this species is based concurrently on retrieved prey and trophic eggs. Laboratory experiments were conducted to examine how these two types of nutrition function for maturation of the first workers. Thirty foundresses were divided into three groups that had different food regimens, and that were compared in regard to outcomes of brood production. The results suggest that trophic eggs are sufficient for larval cohorts to reach the final instar stage (4th instar), but further maturation requires additional food, which is supplied from outside the nest. Therefore, new queens of *M. yessensis* use a blended strategy with trophic egg production and prey retrieval.

Keywords	foundress, semi-clausral, Mt. Fuji, Japan
Citation	Keiichi Masuko (2023). Use of trophic eggs at colony founding in the queen-foraging ant, *Manica yessensis*. Asian Myrmecology 16: e016004
Copyright	This article is distributed under a Creative Commons Attribution License CCBY4.0
Communicating Editor	Fuminori Ito
ESM	Electronic supplementary information for this article can be downloaded from the Asian Myrmecology website

INTRODUCTION

In the development of social insect colonies, the founding stage is the most critical period, in which the highest mortality occurs (Hölldobler & Wilson 1990; Brown & Bonhoeffer 2003; Johnson 2006). This is because during this period, single gynes (haplometrosis) or multiple gynes (pleometrosis) must perform all colony functions without assistance from worker ants. Such totipotency of new queens includes construction and defense of the nest, acquisition of food, food processing and distribution to the immature brood, and hygienic activities, including self-body cleaning. Therefore, this kind of founding is called independent colony foundation (ICF) (Peeters & Molet 2010). In ICF, food acquisition outside the nest is especially risky for founding gynes (foundresses), not only because of predation, but also infection. Therefore, circumvention of these risks has evolved in many social insect taxa (Cronin et al. 2013). Especially in ants, two strategies have become widespread. One is an independent, but "claustral" mode of foundation, and the other is a worker-assisted, (dependent) foundation. In the former, prior to dispersion from their natal nests, new queens accumulate fat reserves, and after copulation, they transform it into trophic eggs or excretory mate-

rial for brood nutrition. Therefore, before the first workers emerge, foundresses shelter continuously inside the nest, or claustrally, without obtaining food from outside. In the latter case, some workers from the foundresses' natal nests accompany them when they disperse. This type of worker-associated foundation is called budding, swarming, or fission, or dependent colony foundation (DCF) (Peeters & Molet 2010; Cronin et al. 2013). Despite the presumed advantages of these two strategies, there are still several ant groups in which foundresses forage externally. This form of foundation, termed semi-claustral, was previously assumed to be typical of so-called "primitive ants" belonging to the former subfamilies, Myrmeciinae and Ponerinae (Haskins & Haskins 1951; Haskins & Haskins 1955). In the most updated ant classification, the Myrmeciinae remains, but the Ponerinae has been divided into 6 subfamilies (Bolton 2022). However, beside these subfamilies, foundress foraging was observed first in the European ant, *Manica rubida* (Latreille, 1802), of the subfamily, Myrmicinae (Le Masne & Bonavita 1969), and since then, according to Brown and Bonhoeffer (2003), it has also been confirmed in other myrmicine species such as fungus-growing ants, dacetine ants, seed-harvesting ants in the genera, *Pogonomyrmex* and *Messor,* and in *Myrmica*. Semi-claustral foundation is quite rare in the subfamily Formicinae, where full-claustrality prevails, but only a few genera are reported to be semi-claustral, i.e., *Cataglyphis, Polyrhachis, and Myrmoteras* (Fridman & Avital 1983; Lenoir & Dejean 1994; Ito et al. 2017). Therefore, this mode of colony foundation is presently not considered "primitive", but the question has shifted to ecological factors that necessitate or permit foraging in these ants.

Manica is a small ant genus of the Myrmicinae, containing one species each in Japan and Europe and four species in North America (Bolton 2022). Recent molecular phylogenetic analyses confirm that *Manica* and *Myrmica* are sister groups (Ward et al. 2015). When cultured in the laboratory, foundresses of the Japanese species, *Manica yessensis* Azuma, 1955*,* regularly forage and bring captured insects into their nests for larval food. In addition, I have recently confirmed that *M. yessensis* foundresses lay two kinds of eggs, reproductive eggs and trophic eggs (Masuko 2000). In other words, nutrition for production of the first worker offspring in this ant is based both on trophic eggs and retrieved prey. Therefore, in the present study, consequences for brood development were experimentally compared in laboratory colonies when foundresses depended only on their initial body reserves (trophic eggs) and when they also obtained prey from outside the nest.

MATERIALS AND METHODS

Field collection

For laboratory experiments, 66 foundresses of *M. yessensis* were collected from the vicinity of Gotenba Trail New 5th Station on the east slope of Mt. Fuji (35.3352° N, 138.7833° E, alt. 1700 m), Shizuoka Prefecture, on 4 October 2002. Although nuptial flights have not been witnessed in the field, dispersion of *M. yessensis* sexuals is assumed to occur from mid-September to early October, because a number of eclosed adult males and winged gynes were found in shallow nest chambers in August and September, followed by discovery of many single, dealated foundresses under rocks in early October. By this time, most established colonies of this species have ceased above ground activity. Collected gynes were carried to the laboratory, where they were weighed to the nearest mg on 5 October 2002. Wet masses of 66 gynes ranged 16–24 mg (mean ± SD = 20.4 ± 1.8 mg) and their distribution did not conform to a normal distribution (Shapiro-Wink Normality Test, $W = 0.9585$, $P = 0.0269$). Thereafter, they were kept separately in vials at 3 °C in a refrigerator until December 7, in imitation of low temperatures that gynes would have experienced during hibernation in the field.

Larval instars

Apart from the present study, external morphology of *M. yessensis* larvae was examined by light and scanning electron microscopy, and morphometrics were gathered, including measurements of head widths, diameters of spiracles, and setal counts (Masuko 2017). These studies revealed that the larval stage of *M. yessensis* consists of four instars. Based on morphological characteristics confirmed in this study, especially instar differences in chaetotaxy and head morphology, instars of larvae used in these experiments were easily distinguished using a stereomicroscope.

Laboratory experiments

Excluding two gynes that died in the refrigerator and another gyne used for dissection to check insemination, 63 gynes were each transferred to experimental nests from 7–21 December 2002 and kept in a room without heating (the room temperature ranged from 16–22 ˚C, mean = 18.2 ˚C). Polystyrene boxes (30 × 60 × 18 mm) were used as brood chambers. These were placed in plastic containers (205 × 280 × 35 mm) that functioned as foraging arenas. Brood chambers were floored with plaster of Paris mixed with activated charcoal. This floor was kept moistened by spraying it with water, as necessary, using a washing bottle. Through an entrance hole that opened on one side of the brood chamber, foundresses went out freely into the foraging arenas. Ants were kept under these conditions without food until 23 March 2003. During this period, seven gynes died, two gynes were dissected, and 24 gynes started oviposition. Another 30 gynes remained to lay eggs, and they were used in the following experiments. First, they were randomly assigned to one of the three experimental groups, prey-fed, liquid-fed, and unfed groups, each with 10 gynes. There were no significant differences in body mass among the three groups (Kruskal-Wallis one-way ANOVA, $P = 0.9894$). In the prey-fed group, gynes were able to access live, small (5–7 mm) mealworms, which were provided in foraging arenas ad libitum (see below). In the liquid-fed group, gynes could access only undiluted cultured milk solution (®CALPIS; see Electronic Supplementary Material (Table S1) for nutrient composition) that was given in a small watch glass (diameter 45 mm), placed in a corner of the foraging arena. The unfed group consisted of gynes that were not able to access any food. The liquid-fed group was included in this experiment because, in the field, workers of *M. yessensis* ordinarily visit floral nectaries and also attend aphids on plant leaves, or root coccids underground (Masuko, unpublished observations). Since it is reported that nectars and honeydew of aphids and mealybugs contain significant concentrations of amino acids and proteins (Blüthgen et al. 2004; Sabri et al. 2013; Levin et al. 2017), cultured milk drink containing proteins (Table S1) was used instead of a honey solution.

In the prey-fed group, the food supply began on the survey day when trophic eggs were exhausted. On that day, an intact mealworm or cut pieces were placed in the foraging area. When a prey item was retrieved and consumed in the brood chamber, another one was added, or when the previous one was abandoned in the terrarium, it was replaced with a fresh one. For the liquid-fed group, cultured milk drink in a watch glass was supplied in the terrarium continuously when trophic eggs disappeared in the brood chamber.

On 25 March 2003, these 30 cultures were warmed to 23 °C. Light conditions were not controlled. Ants were kept mostly in darkness, except when the brood was censused. At the time of this census, foundresses were gently removed from the brood chamber using forceps and the brood chamber was placed on the stage of a stereomicroscope. Then, under a microscope, an egg pile was carefully loosened using dissecting needles, and the numbers of both types of eggs and larvae of each instar were counted. Trophic and reproductive eggs could easily be differentiated by size and shape (see Results). In addition, the behavior of larvae (oophagy, prey feeding, and cannibalism on larva) was recorded whenever observed. These censuses were conducted approximately every 4 days (mean = 3.96 days, SD = 0.84, range = 2–6, $N = 24$) for 3 months until 21 June 2003. To investigate the loss of body mass of the 30 foundresses during the experiments, they were again weighed on 23–24 June 2003.

Measurements of eggs

Lengths of live (15 reproductive and 16 trophic) eggs were measured at 40× magnification using a stereomicroscope equipped with an ocular micrometer accurate to 0.025 mm. These measurements were conducted in October and November 1990 and the results were briefly described in Masuko (2000). These eggs were taken out from the egg mass laid by each of 16 foundresses, which were collected from the field in the autumn of 1990 and cultured separately in the laboratory.

Wet masses of eggs were determined as follows. In foundress nest #2-14, a lump of nine reproductive and 21 trophic eggs was found on 22 April 2003. Total wet masses of nine reproductive

and 21 trophic eggs were 1.63 mg and 1.97 mg, respectively, yielding means of 0.1811 mg for reproductive eggs and 0.0938 mg for trophic eggs. Thus, the mass of reproductive eggs was twice that of trophic eggs.

Statistical analyses

Statistical tests were carried out using Statistix 10 (2013, Analytical Software, Tallahassee, FL, USA) and "Online Web Statistical Calculators" (https://astatsa.com, accessed on 11 December 2022).

RESULTS

Reproductive eggs and trophic eggs

These two kinds of eggs differ in shape and size (Fig. 1a). Reproductive eggs are bean-shaped and larger. Their lengths (mean ± SD) were 0.983 ± 0.083 mm (range = 0.800–1.100, $N = 15$). On the other hand, trophic eggs are ovoid and smaller. Their lengths (mean ± SD) were 0.816 ± 0.072 mm (range = 0.700–0.925, $N = 16$).

With appropriate illumination, reproductive eggs appear transparent when embryos have developed inside, while trophic eggs, having no embryos, remain totally opaque (Fig. 1a). Whenever they coexisted, the two kinds of eggs were not separated, but clumped into a single heap.

The mean observed maximum numbers of reproductive eggs (R_{max}) and trophic eggs (T_{max}) of 29 gynes (one of the 30 turned out to be uninseminated, see below) were 8.2 ± SD 1.7 (range 4–11) and 18.2 ± SD 5.7 (range 7–36), respectively. Here, the maximum numbers were the largest numbers that were recorded without (in unfed-group) or before (in prey-fed and liquid fed groups) being given prey or cultured milk. Total egg numbers ($R_{max} + T_{max}$) were similar among the three groups (one-way ANOVA, $F = 0.32$, $P = 0.7315$), but a negative correlation, or a trade-off, is expected between these maximum numbers because each foundress used her limited physiological reserve to produce these eggs. As predicted, the two maximum numbers were negatively correlated ($r = -0.404$, $P = 0.0298$) (Fig. 2). Moreover, following Brian & Rigby (1978), the production equivalence of the two egg types was calculated. This was obtained from the regression between R_{max} and T_{max} : $R_{max} = 10.514 - 0.121\ T_{max}$ ($P = 0.0298$)
Therefore, production of one trophic egg reduces reproductive egg production by 0.121 eggs.

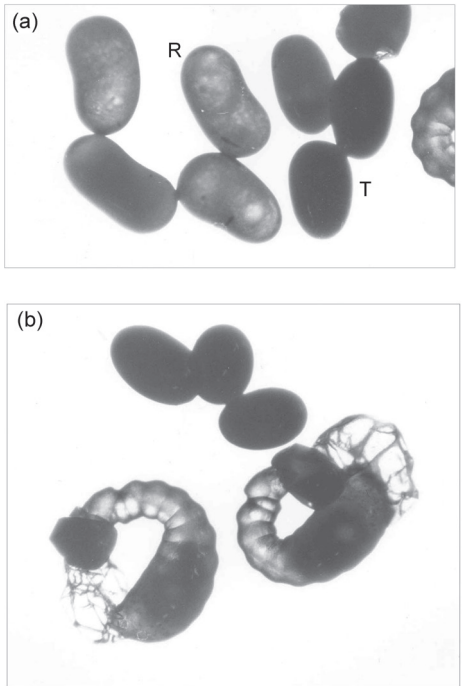

Fig. 1. Reproductive eggs (R) and trophic eggs (T) laid by an *M. yessensis* foundress (a) and feeding on trophic eggs by two first instar larvae, which have shed egg membranes on the posterior end (b).

Table 1. Maximum numbers (mean ± SD) of trophic eggs, reproductive eggs, and larvae, and survival rates (mean ± SD) of reproduction eggs until hatch. The maximum number is the largest number recorded before (prey-fed and liquid-fed groups) or without (unfed group) being given prey or cultured milk.

Treatment	N	A			B	
		Maximum number of trophic eggs (TE)	Maximum number of reproductive eggs (RE)	Ratio (TE/RE)	Maximum number of larvae	Survival rate of RE until hatch
Prey-fed	9	18.8 ± 7.2	7.9 ± 2.4	2.4	6.0 ± 1.4	0.79 ± 0.16
Liquid-fed	10	18.9 ± 5.4	8.6 ± 1.5	2.2	6.7 ± 1.5	0.78 ± 0.20
Unfed	10	17.2 ± 4.8	8.4 ± 1.2	2.0	6.3 ± 1.3	0.76 ± 0.15
ANOVA		NS	NS	–	NS	NS

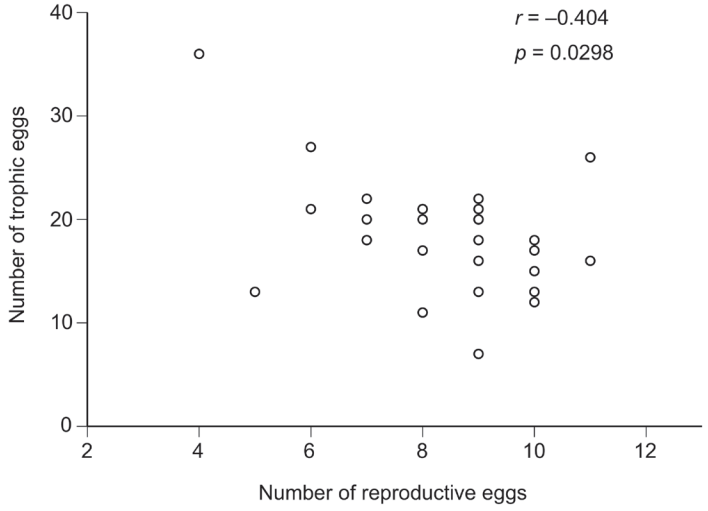

Fig. 2. Correlation between numbers of reproductive eggs and trophic eggs. Egg numbers are the maximum numbers recorded before or without feeding for 29 *M. yessensis* foundresses.

Temporal pattern of egg production

Frequencies of the two types of eggs and hatched immatures change during colony foundation. To visually capture these changes, a typical example is shown for each of the three experimental groups (Fig. 3a–c). Gyne #02-51 (Fig. 3a) belonged to the prey-fed treatment. Mealworms were given after trophic eggs disappeared. She laid trophic eggs first (14 April) and the first reproductive egg was confirmed at the next survey (18 April), when trophic eggs numbered seven. This pattern of laying trophic eggs before reproductive eggs was confirmed in 14 of the 30 replicates, whereas the opposite pattern occurred in only two (Fisher's exact test, $P = 0.0042$). For the other 14 replicates, it was unclear which occurred first, because of the interval between surveys (Fig. 3b). Of the 30 gynes assigned to different treatments, one gyne of the prey-fed group (#02-46) laid eggs of both types, but no larvae hatched during the 3-month study period. This gyne was most likely uninseminated, but this could not be confirmed because of the gyne's accidental death before dissection.

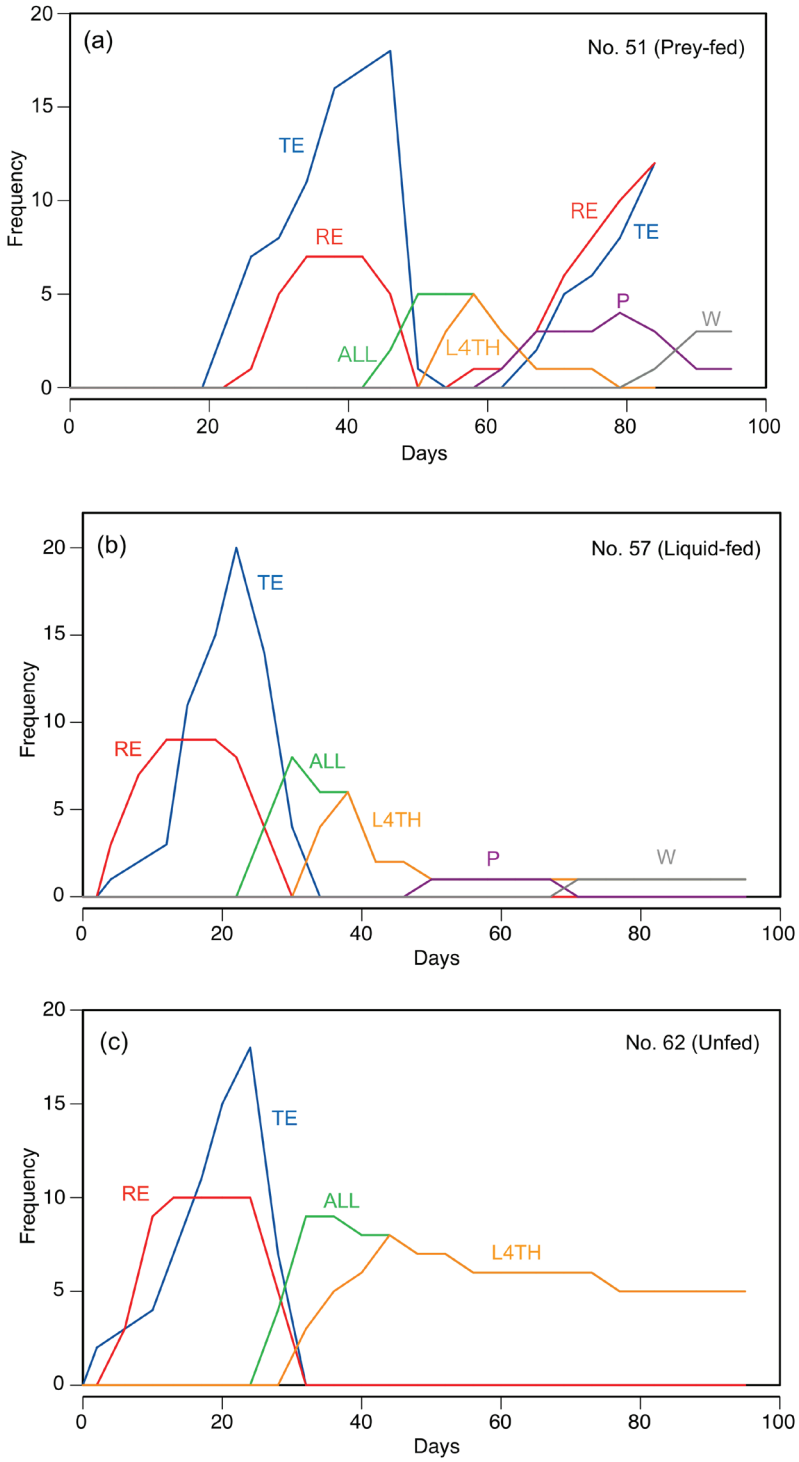

Fig. 3. Examples of the frequency change in each immature stage for three nutritional treatments. Censuses were usually made every 4 days from 23 March 2003 (Day 0). TE: trophic egg; RE: reproductive egg; ALL: larvae of all instars; L4TH: 4th instar larva; P: prepupa and pupa; W: adult worker.

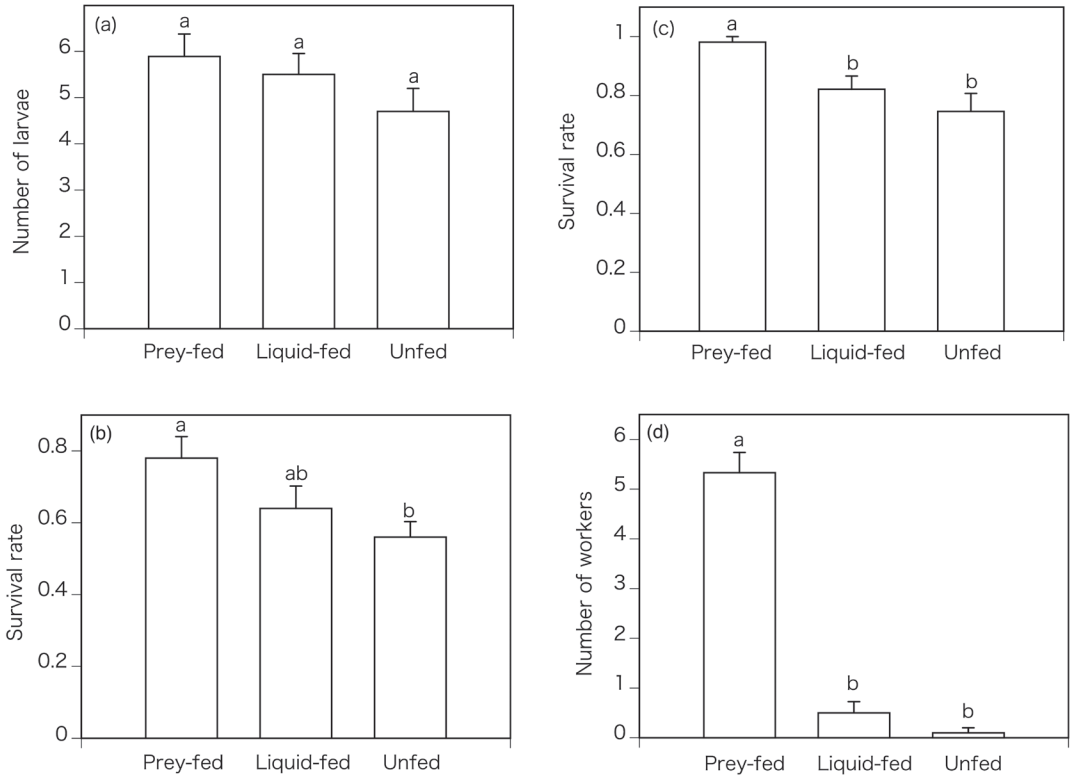

Fig. 4. Comparisons (mean ± SE) between prey-fed ($N = 9$), liquid-fed ($N = 10$), and unfed ($N = 10$) experimental groups of foundresses. Means denoted by different letters indicate significant differences between groups ($P < 0.05$) as determined with one-way ANOVA followed by Tukey's honestly significant difference comparisons. (a) The largest number of 4th instar larvae recorded before or without feeding. (b) Proportion of reproductive eggs surviving until the 4th larval instar. (c) Proportion of hatched larvae surviving until the 4th larval instar. (d) Number of workers produced until the last census of experiments.

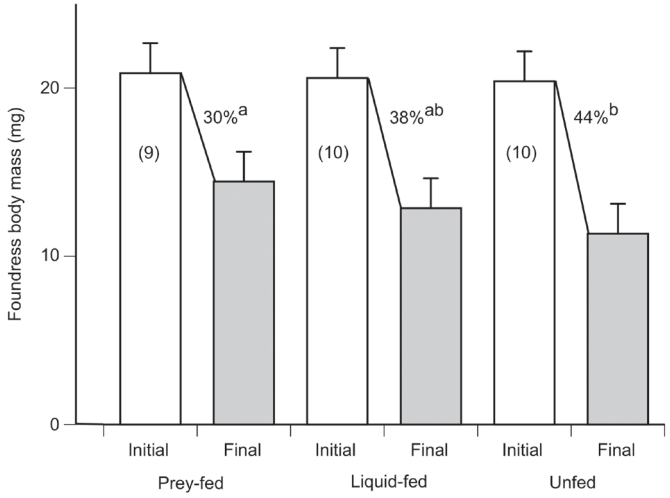

Fig. 5. Loss of body mass (mean ± SE) in *M. yessensis* foundresses during culture experiments. Values of % lost (indicated with different letters) were significantly different as determined with the Wilcoxon rank sum test. Numbers in parentheses denote sample sizes.

Survivorship of eggs and larvae

Observed maximum numbers of reproductive and trophic eggs did not differ among the three groups (one-way ANOVA, $F = 0.42$, $P = 0.6606$ for reproducive eggs and $F = 0.26$, $P = 0.7720$ for trophic eggs; Table 1A), and their ratios did not differ either. To know whether larger foundresses produced larger egg masses, the correlation between foundress masses and total egg masses was investigated. For this purpose, the total mass of eggs that each foundress produced was calculated by observed maximum numbers of reproductive eggs and trophic eggs (before or without food supplements) multiplied by the single egg wet mass, 0.181 mg and 0.094 mg, respectively. The correlation ($r = 0.260$) was not significant ($N = 29$, $P = 0.173$).

Next, survival rates of eggs and larvae were investigated. Here, the maximum number of larvae, irrespective of their instars, and that of only 4th instar larvae recorded before the first pupation are used. Means of this number of all instars ranged from 6.0 to 6.7 in the three groups with no significant difference among them (one-way ANOVA, $F = 0.61$, $P = 0.5515$; Table 1B), so that data from the three groups were combined, and the mean ratio of the number of all larvae to the number of reproductive eggs was 0.782 (SD = 0.163, $N = 29$). That is, about 80% of reproductive eggs laid by foundresses survived to hatch. Again, the observed largest number of 4th instar larvae did not differ significantly among the three groups (Fig. 4a). However, when the proportion of these larval numbers to the reproductive egg numbers was taken, survival of reproductive eggs until 4th instar larvae differed significantly between the prey-fed and unfed groups (one-way ANOVA followed by Tukey's HSD (honestly significant difference) comparisons, $F = 4.0582$, pooled $N = 29$, $P = 0.0293$) (Fig. 4b). Moreover, the survival rate of larvae from hatch to ecdysis into the final instar was approximately 75% in the unfed group (Fig. 4c), while it increased to 82% in the liquid-fed group and reached 98% in the prey-fed group (Fig. 4c). The difference was significant between the prey-fed group and the other two groups (one-way ANOVA followed by Tukey's HSD comparisons, $F = 6.3665$, pooled $N = 29$, $P = 0.0056$).

In the prey-fed group, immediately after the census that confirmed consumption of all trophic eggs, prey insects were provided in the enclosures and trophic eggs again appeared in the nests of this group 12 to 25 days after their disappearance (mean ± SD = 17.6 ± 4.5, $N = 9$, this elapsed number of days was based on census dates) (Fig. 3a). In contrast, foundresses of the unfed group ($N = 10$) never produced either type of eggs after trophic egg disappearance for 53–65 days until the last census day. In the liquid-fed group, one foundress laid only one trophic egg 28 days after consumption of the other trophic eggs. That egg was not observed on the next census day. Therefore, with this single exception, no additional trophic or reproductive eggs were laid in the two groups that were deprived of insect prey (Fig. 3b, c). In the prey-fed group, production of reproductive eggs was also confirmed 4 to 20 days (mean ± SD = 14.2 ± 6.4, $N = 9$) after the first offering of prey (Fig. 3a).

Number and body mass of the first-brood workers

The number of larvae reaching the 4th instar did not differ among the three groups (Fig. 4a), but maturation during this final larval stage differed greatly between them. Table 2 details the fate of these larvae confirmed until the last census. In the prey-fed group, four to seven worker pupae appeared, from which three to six adults emerged by the last census, and none of the pupated immatures were lost during this period (Table 2). In the liquid-fed group, each of four colonies produced only one or two worker pupae, all of which later eclosed as adults. In the unfed group, two colonies produced only one worker pupa each, which successfully eclosed as an adult in one colony, but was lost, perhaps due to cannibalism, in the other. Correlation coefficients were calculated between the initial body mass of foundresses and the maximum number of pupae for each of the three groups. Only in the liquid-fed group, a statistically significant correlation was found ($r = 0.7813$, $N = 10$, $P = 0.0076$).

As a consequence of these differences in maturation during the final larval and pupal stages, the number of emerged workers differed greatly among the groups (Fig. 4d). Mean worker numbers (± SD) confirmed at the last census were 5.3 ± 1.2 in the prey-fed ($N = 9$), 0.5 ± 0.7 in the liquid-fed ($N = 10$), and 0.1 ± 0.3 in the unfed ($N = 10$) groups.

Table 2. Numbers of pupae produced in each culture and their fate confirmed at last census. Only in the liquid-fed group, the correlation between the initial body mass of foundresses and the maximum number of pupae was statistically significant ($r = 0.7813$, $N = 10$, $P = 0.0076$). Mean numbers of pupae denoted by different letters indicate significant differences between groups ($P < 0.005$) as determined with one-way ANOVA followed by Tukey's honestly significant difference comparisons.

Treatment	Culture #	Initial body mass of foundresses (mg)	Maximum number of pupae	Mean ± SE	Numbers at last census	
					Adult workers	Pupae
Prey-fed	39	22	7	$5.44^{a} \pm 0.41$	6	1
	42	20	6		6	
	35	19	6		6	
	37	21	7		5	2
	33	22	6		5	1
	26	24	5		5	
	58	20	4		4	
	51	21	4		3	1
	48	19	4		3	1
Liquid-fed	45	24	2	$0.50^{b} \pm 0.22$	2	
	59	23	1		1	
	57	21	1		1	
	61	21	1		1	
	11	21	0			
	15	21	0			
	44	20	0			
	30	19	0			
	53	19	0			
	49	17	0			
Unfed	54	21	1	$0.20^{b} \pm 0.13$	1	
	27	19	1		0	0
	62	24	0			
	13	23	0			
	47	22	0			
	50	21	0			
	14	20	0			
	66	20	0			
	41	18	0			
	38	16	0			

Table 3. Wet mass (mg) of first-brood workers from three food treatments. Means of prey-fed and liquid-fed groups are significantly different ($P = 0.0003$) as determined by the Wilcoxon rank sum test.

Treatment	N of workers (N of cultures)	Mass (mean ± SD)
Prey-fed	43 (9)	2.61 ± 0.48
Liquid-fed	5 (4)	1.35 ± 0.15
Unfed	1 (1)	1.26

Table 4. Comparison of results (mean ± SD) from culture studies in 1990 and 2003.

	1990	2003	*P* (*t*-test)
Foundress number	9	29	
Initial wet mass of foundresses (mg)	20.2 ± 1.76	20.6 ± 1.97	NS
Maximum number of trophic eggs	29.1 ± 9.82	18.3 ± 5.67	< 0.001
Maximum number of reproductive eggs	8.2 ± 2.86	8.3 ± 1.69	NS
Maximum number of 4th instar larvae	6.1 ± 1.62	5.3 ± 1.52	NS

Table 5. Census of three founding colonies of *M. yessensis* collected on the east slope of Mt. Fuji, Gotenba, Japan.

Date of collection	Nesting site	Queen	Worker	Egg	Larva	Prepupa	Worker pupa
7 July 1990	Under stone	1	0	0	1st–3rd instars: 7	0	0
12 July 1991	Under stone	1	0	0	4th instar: 4	0	3
15 August 1995	Under ceramic tile	1	0	Reproductive: 3	4th instar: 1	1	3

In addition, the body masses of emerged workers differed greatly between the prey-fed group and the other two groups (Table 3). They were measured on 23–26 June 2003 (after the last census). Workers of the prey-fed group were nearly twice as heavy as those of the other groups (Table 3). Combining the number and mass of emerged workers, mean total biomasses of first-brood workers produced per foundress was 13.83 (= 2.61 × 5.3) mg in the prey-fed, 0.68 (= 1.35 × 0.5) mg in the liquid-fed, and 0.13 (= 1.16 × 0.1) mg in the unfed groups.

Also as predicted, the body mass loss of foundresses during these experiments differed among the three groups (Fig. 5). Only the difference between the prey-fed and unfed groups was statistically significant (Wilcoxon rank sum test, $P = 0.0028$). Those between the prey-fed and liquid-fed groups and between the liquid-fed and unfed groups were not significant (Wilcoxon rank sum test, $P = 0.1495$ and $P = 0.1076$, respectively).

Feeding by each larval instar

Even though mealworms were given to the prey-fed group, it was rare to observe larvae feeding on prey. This is because observations were conducted only on census days, four days on average after the previous census when prey was given, so that the prey had already disappeared from the brood chamber. However, prey feeding by the 3rd and 4th instars and that by the 4th instar were observed in nests #02-37 and #02-39, respectively, and in the prey-fed group, feeding on trophic eggs by 4th instars was never observed. In contrast, feeding by younger instars was generally observed. When trophic eggs were present, 3rd instar larvae, in addition to 1st and 2nd instars, were often observed feeding on these eggs. In the liquid-fed and unfed cultures, having consumed trophic eggs, cannibalism of larvae was often witnessed, and larval feeding on ecdysial skins was also observed.

Comparison of two rearing experiments and census of field-collected colonies

A preliminary study using nine foundresses was conducted in 1990 (Masuko 2000). These foundresses were collected in September and October 1990 from Mt. Fuji and reared using similar containers in the laboratory at room temperature (16–28 ˚C) immediately after collection. In this 1990 study, results similar to those of the present study (conducted in 2003) were obtained (Masuko, unpublished data), with one interesting difference. Between the two studies, there was no difference in foundress body masses, the maximum numbers of reproductive eggs, or the number of 4th instar larvae; however, the foundresses of the 1990 experiments produced many more trophic eggs (Table 4).

For founding colonies where the first workers had not yet emerged, three such colonies were collected in the field (Table 5). Considering the results of the current laboratory studies, brood numbers in these colonies are interesting. The total number of brood, whether larvae, prepupae, or pupae, in these field colonies was five to seven, which corresponds well with the number of brood arising from the first batch of eggs in laboratory experiments (4–7 pupae, Table 2).

DISCUSSION

The present study demonstrated that *M. yessensis* foundresses are obligate foragers because their initial physiological reserve is just enough to produce trophic eggs, with which approximately eight of their first larvae can only reach the final instar. For further larval maturation, additional food must be provided, and if foraging succeeds, some five to six adult workers may emerge. When only cultured milk was available to the foundresses, they could not lay additional trophic eggs (except for one egg laid by one foundress). The cultured milk used contains protein, but its concentration is less than one tenth that of live mealworms (Tables S1 and S2). In addition, despite the fact that workers promptly gathered around and imbibed cultured milk in laboratory colonies when it was given on a watch glass, no obvious oral trophallaxis has been observed between adults or between adults and larvae (Masuko, unpublished observations). Therefore, direct transfer of cultured milk from the foundress to her larvae is unlikely. After all, without prey, worker production, both in number and size, was so poor that colony founding failed.

These findings raise the question of why two nutritional sources, i.e., trophic eggs and retrieved prey, are both needed to rear the initial brood, because together these two food sources comprise the regular feeding regime that has evolved in *M. yessensis*. In other words, nutrition is different for younger and older larvae. But why?

As a first remark, trophic eggs for larval nutrition are widely known among ants (Hölldobler & Wilson 1990; Passera & Aron 2005; Meurville & LeBoeuf 2021; Aupanun et al. 2022), but information remains scattered and there has been no comprehensive examination of this phenomenon in ants. In addition, the nutrition of earlier instar larvae, especially the first instar of ants, has not been studied well.

Although it is not an observation about the founding stage, in another ant, *Stigmatomma silvestrii* Wheeler, 1928, it is easily understood why oophagy is necessary for micro-larvae. Eggs to be eaten are not trophic, but reproductive eggs in this ant (Masuko 2003). Larvae of *S. silvestrii* pass through five instars (Masuko 1990), and neither trophic eggs nor oral trophallaxis have been observed in this ant. Instead, 1st and 2nd instar larvae obligatorily cannibalize reproductive eggs. Each larva consumes two or three eggs before molting to the 3rd instar; thus, 66–75% of fertile eggs laid by the queens are lost due to oophagy. At the 3rd instar, the larvae start feeding on arthropods, which are mostly geophilomorph centipedes (Masuko 1993). Centipede prey are paralysed by ant venom, but workers do not dismember the prey and only place the larvae upon such whole prey. Therefore, conceivably, oophagy is the sole way of obtaining nutrients for small, less sclerotized larvae during first and second instars. Use of reproductive eggs for larval oophagy in the founding stage has also been reported for *Neoponera apicalis* (Latreille, 1802) (Fresneau (1994) cited in Passera and Aron (2005), p.48).

Such a dietary shift between younger and older larvae is inevitable in ant species that provision their larvae by foraging for arthropods or other invertebrates. Regardless of whether the ant is a specialist (*Stigmatomma*) or a generalist (*Manica*) predator of such prey, oophagy is convenient nutrition for early instar larvae, as their shells

are not hard and they are present next to the larvae. Therefore, the use of eggs, whether trophic or reproductive, may well be much more widespread among ants, especially, in predaceous species with semi-claustral foundation.

Despite this prediction, however, nutrition for early micro-larvae has been largely overlooked in ant nutritional biology. Accordingly, observations of the use of eggs to provision early instar larvae are rather few, and quantitative studies are even fewer, with the exception of a study of the fire ant, *Solenopsis invicta* Buren, 1972. In this fully claustral species, founding queens lay trophic eggs (Glancey et al. 1973; Voss and Blum 1987; Tschinkel 2006), and an average of thirteen first workers finally emerge with nutrition only from the queen's physiological reserve (Cassill 2002). Use of trophic eggs at colony founding has also been reported for five species of *Lasius* and *Nylanderia flavipes* (Smith, F., 1874) (Taki 1987). In all of these fully claustral ants, reproductive eggs were laid prior to trophic eggs and all (four) larval instars consumed trophic eggs (Taki 1987).

Trophic eggs are also known from *Myrmica* ants, a sister group of *Manica*. These eggs have a different shape than reproductive eggs (Weir 1959a; Brian & Rigby 1978; Wardlaw & Elmes 1995; Wardlaw & Elmes 1998). First instar larvae cannibalizes one egg apiece in the egg mass and second instar larvae are removed from the egg mass by workers (Weir 1959b). Consequently, a similar phenomenon has been confirmed in both *Manica* (founding colonies) and *Myrmica* (developed colonies). The production cost equivalence between the two egg types was also calculated for *Myrmica rubra* (Linnaeus, 1758) (Brian & Rigby 1978). It was 2.61 (the reciprocal of the regression coefficient of the number of reproductive eggs on that of trophic eggs, –0.384), much smaller than the value (8.26) obtained for *M. yessensis*. Species of *Myrmica* also employ semi-claustral colony founding (Brown & Bonhoeffer 2003), but the use of trophic eggs has not been studied.

In regard to the adaptability of claustral founding, Johnson (2006) questioned the rather anecdotal argument that it has evolved simply because foundress foraging is risky. He emphasized the need to measure both benefits and costs of the two founding strategies, i.e., fully claustral and semi-claustral founding, under the paradigm of capital and income breeding, respectively (Drent & Daan 1980; Jönsson 1997). He also discussed many plausible benefits from queen foraging. The present study indicated that, in addition to trophic eggs (capital), foraging (income) by foundresses is obligatory for first brood maturation. Thus, colony founding in *M. yessensis* is neither pure capital nor pure income breeding, but is a mixed strategy (Drent & Daan 1980; Johnson 2006).

As a physiological reserve used in colony founding, Hahn et al. (2004) compared amounts of queen storage proteins among five *Pogonomyrmex* species with different colony-founding strategies, and confirmed that foundresses or alate gynes of *Pogonomyrmex californicus* (Buckley, 1867), a semi-claustral species, maintain much smaller quantities of storage proteins compared to gynes of fully-claustral species, such as *Pogonomyrmex rugosus* Emery, 1895 and *Pogonomyrmex maricopa* Wheeler, 1914. Alate gynes of *M. yessensis* also have these storage proteins (Hidetoshi Inagaki, personal communication), which are likely sources for trophic eggs used at colony founding.

Finally, an important question remains after the present study. When does the foundress start foraging? If foraging starts before depletion of trophic eggs, the foundress can feed on prey and begin again to lay eggs (as occurred in the prey-fed group), which nourish larvae, even including 4th instars. In the current study, however, foundresses were removed from the brood chamber repeatedly (every 4 days) for the exact census of broods. Consequently, more details of their behavior could not be investigated in order to avoid increasing interference. Other experimental designs are needed in future studies.

ACKNOWLEDGEMENTS

I thank Fuminori Ito for valuable comments on an early version of the manuscript. This research was supported by KAKENHI Grant Number 10640621 from the Japan Society for the Promotion of Science and a Senshu University Research Grant in 2002. Two referees are thanked for their many valuable comments.

REFERENCES

Aupanun S, Obika M, Mizuno R, Jaitrong W, Suttiprapan P, Hashim R and Ito F, 2022. Trophic eggs in three ponerine ant species: *Harpegnathos venator, Odontomachus simillimus* and *Odontoponera denticulata*. *Asian Myrmecology* 15: e015001 (1-10). Doi.org/10.20362/am.015001

Blüthgen N, Gottsberger G and Fiedler K, 2004. Sugar and amino acid composition of ant-attended nectar and honeydew sources from an Australian rainforest. *Austral Ecology* 29: 418–429.

Bolton B, 2022. An online catalog of the ants of the world. https://antcat.org. (Accessed 8 March 2022)

Brian MV, Rigby C, 1978. The tropic eggs of *Myrmica rubura* L. *Insectes Sociaux* 25: 89–110.

Brown MJF, Bonhoeffer S, 2003. On the evolution of claustral colony founding in ants. *Evolutionary Ecology Research* 5: 305–313.

Cassill D, 2002. Brood care strategies by newly mated mononyne *Solenopsis invicta* (Hymenoptera: Formicidae) queens during colony founding. *Annals of the Entomological Society of America* 95: 208–212.

Cronin A, Molet M, Doums C, Monnin T and Peeters C, 2013. Recurrent evolution of dependent colony foundation across eusocial insects. *Annual Review of Entomology* 58: 37–55.

Drent RH, Daan S, 1980. The prudent parent: Energetic adjustments in avian breeding. *Ardea* 68: 225–252.

Fridman S, Avital E, 1983. Foraging by queens of *Cataglyphis bicolor nigra* (Hymenoptera: Formicidae): an unusual phenomenon among the Formicinae. *Israel Journal of Zoology* 32: 229–230.

Glancey BM, Stringer CE and Bishop PM, 1973. Trophic egg production in the imported fire ant, *Solenopsis invicta*. *Journal of the Georgia Entomological Society* 8: 217–220.

Hahn DA, Johnson RA, Buck NA and Wheeler DE, 2004. Storage protein content as a functional marker for coony-founding strategies: a comparative study within the harvester ant genus *Pogonomyrmex*. *Physiological Biochemistry and Zoology* 77:100–108.

Haskins CP, Haskins EF, 1951. Note on the method of colony foundation of the ponerine ant *Amblyopone australis* Erichson. *American Midland Naturalist* 45:432–445.

Haskins CP, Haskins EF, 1955. The pattern of colony foundation in the archaic ant *Myrmecia regularis*. *Insectes Sociaux* 2:115–126.

Hölldobler B, Wilson EO, 1990. *The Ants*. Belknap Press of Harvard University Press, Cambridge, MA, 732 pp.

Ito F, Miyazaki S, Hashim R and Billen J, 2017. Colony composition and behavioral characteristics of *Myrmoteras iriodum* and *M. jaitrongi* in Ulu Gombak, Peninsular Malaysia (Hymenoptera: Formicidae). *Asian Myrmecology* 9: e009010 (1-9). DOI:10.20362/am.009010

Johnson RA, 2006. Captal and income breeding and the evolution of colony founding strategies in ants. *Insectes Sociaux* 53: 316–322.

Jönsson KI, 1997. Capital and income breeding as alternative tactics of resource use in reproduction. *Oikos* 78: 57–66.

Le Masne G, Bonavita A, 1969. La fondation des sociétés selon un type archaïque par une Fourmi appartenant à une sous-famille évoluée. *Comptes Rendus (Hebdomadaires) des Séances de l'Academie des Sciences. Série D. Sciences Naturelles* 269: 2373–2376.

Lenoir A, Dejean A, 1994. Semi-claustral colony foundation in the formicine ants of the genus *Polyrhachis* (Hymenoptera: Formicidae). *Insectes Sociaux* 41: 225–234.

Levin E, McCue MD and Davidowitz G, 2017. More than just sugar: allocation of nectar amino acids and fatty acids in a Lepidopteran. *Proceedings of the Royal Society B* 284: 20162126. Doi.org/10.1098/rspb.2016.2126

Masuko K, 1990. The instars of the ant *Amblyopone silvestrii* (Hymenoptera: Formicidae). *Sociobiology* 17: 221–244.

Masuko K, 1993. Predation of centipedes by the primitive ant *Amblyopone silvestrii*. *Bulletin of the Association for Natural Sciences, Senshu University* No. 24: 35–43.

Masuko K, 2000. Brood producion by colony founding queens in the ant *Manica yessensis*: a preliminary report. *Transactions of the Association for Natural Sciences, Senshu University* No. 70: 13–23. (Japanese)

Masuko K, 2003. Larval oophagy in the ant *Amblyopone silvestrii* (Hymenoptera: Formicidae). *Insectes Sociaux* 50: 317–322.

Masuko K, 2017. The larval instars of the myrmicine ant *Manica yessensis* Azuma (Hymenoptera: Formicidae). *ARI (Journal of the Myrmecological Society of Japan)* No. 38: 1–11.

Meurville M-P, LeBoeuf AC, 2021. Trophallaxis: the functions and evolution of social fluid exchange in ant colonies (Hymenoptera: Formicidae). *Myrmecological News* 31: 1–30.

Passera L, Aron S, 2005. *Les Fourmis: Comportement, Organization Sociale et Évolution*. Counseil national de recherches du Canada, Ottawa. 480 pp.

Peeters C, Molet M, 2010. Colonial reproduction and life histories. In: Lach L, Parr CL, Abbott KL (eds) *Ant Ecology*. Oxford University Press, Oxford, pp 159–176.

Sabri A, Vandermoten S, Leroy PD, Haubruge E, Hance T, et al, 2013. Proteomic investigation of aphid honeydew reveals an unexpected diversity of proteins. *PLoS ONE* 8(9): e74656. Doi:10.1371/journal.pone.0074656

Taki A, 1987. The trophic eggs of colony founding ant queens. In: Eder J, Rembold H (eds) *Chemistry and Biology of Social Insects*: Proceedings of the 10th Intenational Congress of IUSSI. Verlag J. Peperny, München, p 268.

Tschinkel WR, 2006. The Fire Ants. Belknap Press of Harvard University Press, Cambridge, MA. 723 pp.

Voss SH, Blum MS, 1987. Trophic and embryonated egg production in founding colonies of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). *Sociobiology* 13: 271–278.

Ward PS, Brady S, Fisher BL and Schultz T, 2015. The evolution of myrmicine ants: phylogeny and biogeography of a hyperdiverse ant clade (Hymenoptera: Formicidae). *Systematic Entomology* 40: 61–81.

Wardlaw JC, Elmes GW, 1995. Trophic eggs laid by fertile *Myrmica* queens (Hymenoptera: Formicidae). *Insectes Sociaux* 42: 303–308.

Wardlaw JC, Elmes GW, 1998. Variability in oviposition by workers of six species of *Myrmica* (Hymenoptera, Formicidae). *Insectes Sociaux* 45: 369–384.

Weir JS, 1959a. Interrelation of queen and worker oviposition in *Myrmica*. *Physiological Zoology* 32: 63–77.

Weir JS, 1959b. Egg masses and early larval growth in *Myrmica*. *Insectes Sociaux* 6: 187–201.