Research article

Caste specialization and differentiation in reproductive potential in the phylogenetically primitive ant *Myrmecia gulosa*

In the memory of Caryl P. Haskins who pioneered the sociobiological studies of phylogenetically primitive ants.

V. Dietemann [1], B. Hölldobler [1] and C. Peeters [2]

[1] *Lehrstuhl für Verhaltensphysiologie und Soziobiologie, Biozentrum der Universität, D-97074 Würzburg, Germany,
e-mail: dietemann@biozentrum.uni-wuerzburg.de*

[2] *Laboratoire d'Ecologie, CNRS UMR 7625, Université Pierre et Marie Curie, 7 quai Saint Bernard, F-75005 Paris, France,
e-mail: cpeeters@snv.jussieu.fr*

Summary. Division of labour is associated with morphological specialization in many species of social insects. Comparative data on phylogenetically "primitive" ants can help understand the selective forces promoting the production of multiple worker castes as well as the morphological divergence between queens and workers. We quantified size variations among workers and queen-worker dimorphism in the Myrmeciinae ant *Myrmecia gulosa*. Worker size varies over a wide range (14–23 mm), exhibiting a bimodal distribution. Growth is monophasic and only slightly allometric. Workers do not perform trophallaxis but lay trophic eggs, which constitute the main channel of food exchange in the colony. Large individuals have more ovarioles than small individuals and lay more trophic eggs. The difference in egg output persists when orphaned workers start to reproduce. Although small workers are less fecund, they reproduce more readily than large individuals following queen removal. Queens are not much larger than the largest workers, but have disproportionately more ovarioles and have a 10-fold higher egg-laying rate than workers. We examine size frequency distribution and bi-logarithmic plots of head and gaster measurements in order to infer the developmental processes that generate divergence in size and proportion among colony members. The appearance of two classes of workers in *M. gulosa* has not been accompanied by a loss in reproductive potential as in some "higher" ants. We review the morphometric studies available for *Myrmecia*, and discuss the evolution of morphological specialization in the genus.

Key words: Worker polymorphism, queen-worker dimorphism, reproduction, trophic eggs, allometry.

Introduction

A large proportion of hymenopteran societies are structured by important morphological differences among female members. This specialization increases the efficiency of division of labour (Wilson, 1971), and one aspect of its evolution is the capacity of larvae to initiate developmental changes in response to nutritional factors, combined with the social regulation of larval nutrition (Wheeler, 1986). The existence of divergent queen and worker castes is the clearest manifestation of physical specialization, but some ants exhibit also marked phenotypic diversity among the adult workers. This is restricted to 15% of ant genera (45/297) and includes both continuous size variation and polymorphism (Hölldobler and Wilson, 1990). Polymorphism, defined by Wilson (1953) as allometry occurring over a sufficient range of size variation within a normal mature colony to produce individuals of distinctly different proportions, occurs only in eight independent lineages (Hölldobler and Wilson, 1990). Comparative data are needed to understand the evolution of physical castes in ants, and phylogenetically "primitive" species are crucial for this, because they represent the early stages of increasing complexity of social organization (Peeters, 1997).

Ants in the subfamilies Myrmeciinae and Ponerinae are characterized by a number of ancestral morphological traits and are commonly believed to be less complex socially than "higher" ants (Brown, 1953; Wilson, 1971; Taylor, 1988; Ogata, 1991; Peeters, 1997). The Myrmeciinae includes a single living genus, *Myrmecia*, composed of 89 described species, which can be divided into 9 monophyletic groups (Ogata, 1991). *Myrmecia* is endemic to the Australian region. Its colonies vary from a few dozens ants to a few thousands (Haskins and Haskins, 1950; Gray, 1974; Higashi and Peeters, 1990; Ito et al., 1994). Variation in worker size has been reported in various species, and sometimes a bimodal

distribution of their size frequency (Haskins and Haskins, 1950; Gray, 1973). Wilson (1953) considered these features to be the first steps leading toward the evolution of worker polymorphism. The queens of *Myrmecia* are generally not much larger than the largest workers (Wheeler, 1932; Haskins and Haskins, 1955; Gray, 1971; Ito et al., 1994). Workers generally have functional ovaries and produce trophic eggs in the presence of the queen (Freeland, 1958; Barnett, 1974). They lay male-destined eggs once orphaned, as in many ants from other subfamilies (Bourke, 1988; Choe, 1988; Dietemann and Peeters, 2000).

We studied morphological specialization in *M. gulosa*, a member of the *gulosa* group (Ogata, 1991), which are the typical "bulldog ants". This group of 42 species (Ogata and Taylor, 1991) exhibits the largest colony sizes (several thousands of workers, Haskins and Haskins, 1950; Higashi and Peeters, 1990) as well as the largest individuals (reaching 36mm, Higashi and Peeters, 1990). We describe differences in body size and body proportions of queens, large and small workers, as well as in their ovaries and egg laying rates. We compare our results with the available data on morphologically "primitive" ants and discuss the evolution of queen-worker dimorphism and worker polymorphism in the Myrmeciinae.

Methods

Collection: Colonies of *M. gulosa* were excavated in sandstone areas close to Waterfall (n=13) and Glenorie (n=1), New South Wales, Australia, between September 1998 and October 2000. Adults and brood were collected and counted (Table 1). Given the complex structure of the nests, some workers may have been missed in unexplored tunnels. In order to minimise this problem, the walls of the excavation were checked on the following days for tunnels reopened by buried workers. Stray foragers were also collected. *M. gulosa* nests consist of mounds up to 1m in diameter, made of excavated soil and plant litter. The entrances are situated on top or at the sides of the mounds. Since colonies with the biggest mounds were not collected, the average colony size reported in our study is probably an underestimate. Given the large size of these ants, the number of individuals brought back for laboratory rearing was reduced in colonies larger than 1000 workers. Together with the queen and all the eggs found, 500 to 1000 workers, and up to 250 larvae and 100 cocoons were randomly selected. The remaining workers were frozen for later measurements and dissection.

Laboratory rearing: Ants were kept in plaster-of-Paris nests into which chambers had been moulded and covered with glass plates to allow observation. These nests were connected to foraging arenas where food (pieces of cockroaches or entire crickets and honeywater) was deposited every 1–2 days. The temperature was maintained at 24 ± 1 °C, the photoperiod was set at 10:14 h (light:darkness) cycles and a high humidity was maintained inside the nests by regularly moistening the plaster. These conditions proved to be favourable for the ants, which produced several hundreds of new individuals over the 24 months study period.

Queen/worker and worker/worker size variation: Workers and queens are distinct in *M. gulosa*, but dealate virgin queens are indistinguishable from mated queens without dissection. We therefore call queen any individual having the queen morphology, either mated or not (Peeters and Crozier, 1988). When the distinction has to be made, the terms virgin queen or mated queen are used. Queen-worker dimorphism and worker polymorphism were assessed by morphometric data and ovarian dissection.

Outerorbital distance is measured in full-face view through the points of highest convexity of the eyes. Head length is measured in full-face view from the midpoint of a transverse line connecting the anteriormost points of the clypeal anterior projections to the midpoint of the occipital margin of the head, excluding the occipital carina (Ogata and Taylor, 1991). We measured the outerorbital distance and head length of 157 workers, 10 mated queens and 17 virgin queens. In the Myrmeciinae, the tergites and sternites of the first gastral segment (abdominal segment IV) are fused to form a tubular structure (Taylor, 1978). As stated by Ito et al. (1994), gaster width can therefore be used as a morphometric feature as it is less or not subject to size variation due to trophic state or ovarian activity of the individuals. The maximal width of this segment, viewed dorsally, was measured in 49 of the above-mentioned workers, in 16 virgin queens and 6 mated queens. All mated queens were collected from the field, unlike virgin queens, which pupated in the laboratory. The workers were randomly chosen among individuals frozen just after field collection. They all belonged to the same colony. In contrast, virgin and mated queens originated from 2 and 10 colonies respectively. Data were plotted on log-log scales and regression lines calculated for workers and queens separately, as well as for small and large workers separately. Plotting both queens and workers on the same graph gives information about the caste differentiation process in a species with limited queen-worker dimorphism.

Ovarian morphology: Dissections were done in Ringer solution. The number of ovarioles per ovary and number of clearly visible oocytes (with and without yolk) per ovarioles was counted for 4 mated queens, 438 workers reared in the laboratory (from 10 colonies), and 153 workers (from 3 colonies) which were frozen immediately after field collection. The correlation between gaster width and number of ovarioles was calculated for 49 workers and 2 mated queens all born in the field, as well as for 10 virgin queens partially reared under laboratory conditions. Maturity of oocytes is defined by their basal position in the ovariole and their size. Oocyte and egg sizes were measured under a binocular microscope with an accuracy of 0.1 mm. Mature trophic as well as reproductive oocytes (n=385 and n=222 respectively) of large and small workers (from 5 queenright and 3 orphan colonies) were measured in the ovarioles. Presence of yellow bodies was checked in 476 and 234 workers from the queenright and orphan colonies respectively. Age classes of workers were known in 2 of the queenright colonies (n=234 workers), allowing to examine the correlation of yellow body load with age. Reproductive eggs of workers and queens (n=262 from 6 colonies and n=420 from 7 colonies respectively) were collected in the nests and measured. Normal distributions of oocytes and egg sizes were tested with the Shapiro-Wilks test and compared with an ANOVA, Tukey post hoc test for unequal sample sizes. The same test was used to compare the lengths of queen-laid eggs for interindividual differences among queens. Since trophic eggs lose shape once laid and have a thin membrane that is easily punctured during handling, it was impossible to measure them. Spermathecae of all workers were checked for presence of sperm. Additionally, in order to check if workers active outside of the nest produced trophic eggs, 16 individuals observed returning to their nest (n=3) after a foraging trip in the field and 110 foragers or guards picked up on the colony mounds (n=2) were dissected. Their ovarian development was classified in three groups: ovaries containing mature, sub-mature or no oocytes.

Egg-laying activity: Individuals laying reproductive eggs (queens or workers) did not systematically bend their gaster forward between their legs, as typically shown by ovipositing ants in ponerine species. In addition, queens could lay several eggs at a time. Reproductive egg-laying rates as well as the duration of the queens' oviposition cycles were therefore deduced from the daily counts of eggs present in the nests (8 colonies over 207 days) and not from direct observation of ovipositions. Egg production was monitored during the first days of oviposition activity, when egg number was small enough to ensure precise counting. As workers laying trophic eggs characteristically bend their gaster forward and seize the egg with their mandibles, trophic egg-laying rate was measured by direct observation. We monitored trophic egg laying as well as the fate of the eggs in colonies with brood (n=5), over a total of 83 hours. Furthermore, we compared the egg laying rates of large and

small workers in 4 groups of 200 workers (100 small with 100 large individuals) without brood, over a total of 44 hours. Trophic eggs laid after solicitation from the queen were not counted since there may be preferential solicitation of the small workers surrounding the queen.

In a total of 23 groups of 20 workers and in 5 larger groups containing at least 100 workers, we followed workers' egg-laying activity once they are separated from their queen. Their behaviour was regularly monitored. The reproductive egg-laying rate of workers was deduced from the eggs counted in 14 orphaned groups of 20 workers over periods ranging from 3 to 80 days of oviposition activity. At the end of the study, all workers in the groups were dissected to count the number of individuals possessing reproductive oocytes in their ovaries, hence the number of egg-layers. Six of these groups contained exclusively small workers, and the 8 other groups contained exclusively large workers. We could thus assess the fertility of each class of individuals.

Results

Colony size

Average size of the societies collected was 992 ± 551 workers (average $\pm$ standard deviation, n=14, range 134–1859), and usually one dealate queen (Table 1). A group of small workers constantly surrounded the queen. In 2 colonies out of 14, two dealate queens were found peacefully cohabiting (Table 1), and small workers gathered around both queens. One of these digynous colonies was split into two, with one queen in each part. Both queens laid eggs and produced new workers, revealing that both were mated. In the second colony, only one queen was dissected and her spermatheca was full.

Queen/worker and worker/worker polymorphism

External morphology

Worker size (from tip of mandibles to gaster) in *M. gulosa* varied from 14 to 23 mm. The measurements of outerorbital

Table 1. Demography of 14 colonies of *M. gulosa*

collection date	number of			
	dealate queens	workers	cocoons	larvae
14/09/98	2	761	69	120
19/09/98	1	209	3	88
15/10/99	1	1435	51	562
16/10/99	1	313	14	106
17/10/99	1	1318	36	40
18/10/99	1	411	41	169
19/10/99	1	1523	145	450
20/10/99	1	134	3	25
21/10/99	1	913	30	261
22/10/99	1	1361	33	256
03/11/99	2	1429	>112	> 353
04/11/99	1	1264	279	550
10/10/00	1	955	27	> 137
12/10/00	1	1859	46	> 260
average		991.8		
st. dev.		551.0		

> in front of a figure indicates that brood has not been totally collected.

distance of 157 workers showed a bimodal size distribution around 2.4–2.5 and 3.8–3.9mm (Fig. 1). We therefore differentiated 2 classes of workers, hereafter referred to as small and large workers. Individuals with an outerorbital distance of at least 3.4mm were considered as large workers. Although it was not systematically quantified, this distribution pattern was constant in all the colonies collected. Workers were sorted out according to their size and counted in 2 freshly collected colonies. These were composed half of large workers and half of small workers (51% and 49% respectively).

Mated (n=10) and virgin queens (n=17) differed neither in their outerorbital distances nor in their head lengths (U=79, p=0.76 and U=74, p=0.58 respectively) and were therefore pooled together. Queens' outerorbital distance overlapped with the largest workers measured (Fig. 1). The plot of outerorbital distance versus head length showed that workers (n=157) and queens (n=27) fit on a single regression line (r^2=0.99). The regression coefficient (0.93) was significantly different from 1 (t=9.54, p<0.01), the relative growth is thus slightly allometric (Fig. 2), with outerorbital distance increasing faster than head length. When regressions were calculated for workers and queens separately, the lines obtained had similar slopes (t=0.16, p=0.87) and intercepts (t=1.98, p=0.05). If only workers were considered and a regression equation calculated for small (outerorbital distance <3.4 mm) and large individuals (outerorbital distance >3.4mm) separately, neither their slope nor their intercepts differed significantly (t=0.45, p=0.65 and t=0.22, p=0.83 respectively).

Gaster widths of mated (n=6) and virgin queens (n=16) were similar (U=31, p=0.21) and pooled together. The comparison of the plots of gaster width vs. outerorbital distance between queens and workers (n=22 and n=49 respectively, Fig. 3) revealed a larger difference in their morphology. The regression coefficients were not significantly different from each other (t=0.38, p=0.70), but the intercepts were distinct (t=21.40, p<0.01). The regression coefficient for workers was significantly different from 1 (t=13.75, p<0.01), indicating allometric growth. For queens, the difference was not significant (t=1.10, p=0.28); this was most likely due to the low number of individuals available and to their small size range, and is probably not a real phenomenon. Outerorbital distance increased faster than gaster width, and with the same
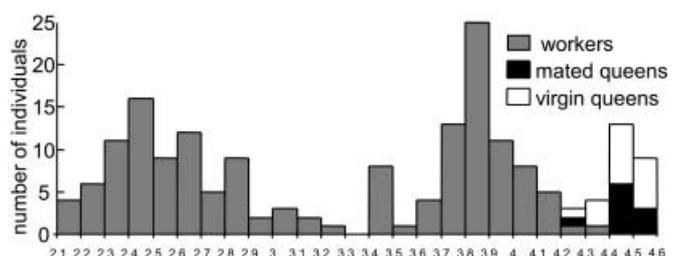

Figure 1. Size distribution of *M. gulosa* workers, virgin queens and mated queens using outerorbital distance. Workers (n=157) and queens (n=10) were collected in the field, whereas virgin queens (n=17) pupated in the laboratory
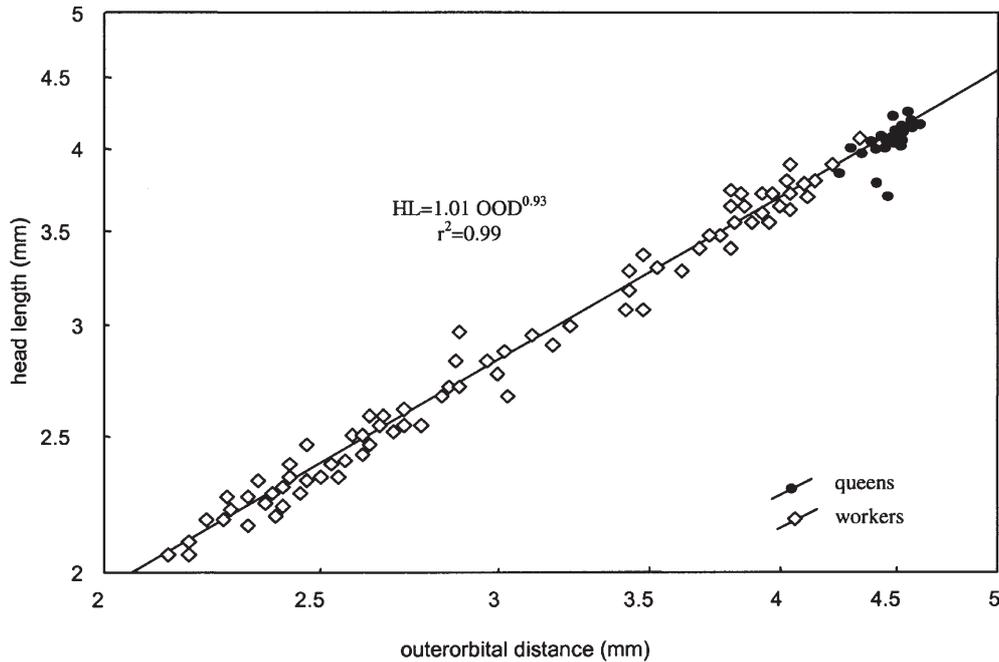
Figure 2. Log-log plot of outerorbital distance (OOD) vs. head length (HL) in workers (n=157), and queens (n=27, 10 mated + 17 virgin queens) of *M. gulosa*
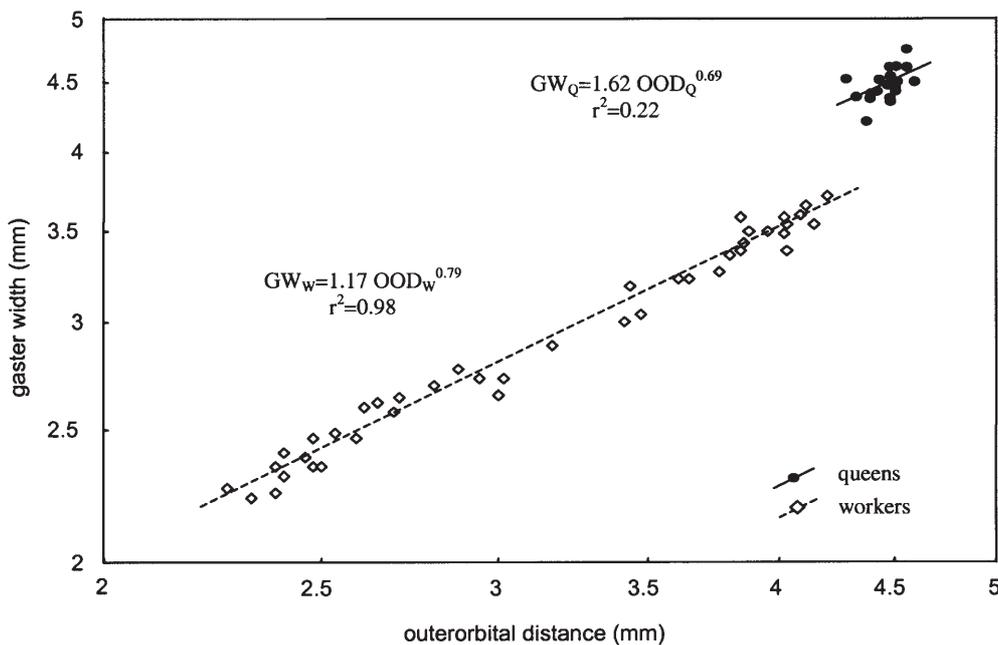
$$HL = 1.01\ OOD^{0.93}$$
$$r^2 = 0.99$$

Figure 3. Log-log plot of outerorbital distance (OOD) versus gaster width (GW) in workers (n=49) or queens (n=22, 6 mated + 16 virgin queens) of *M. gulosa*

$$GW_Q = 1.62\ OOD_Q^{0.69}$$
$$r^2 = 0.22$$

$$GW_w = 1.17\ OOD_w^{0.79}$$
$$r^2 = 0.98$$

rate in both queens and workers. However, queens had wider gasters than hypothetical workers of same head size. When small workers were compared with their larger nestmates and a regression was calculated for each of the size classes, again we found no differences between them (t=1.16, p=0.25, t=0.19, p=0.85 for slopes and intercepts respectively).

Ovarian morphology

Mated and virgin queens had the same number of ovarioles per ovary (n=4 and n=10 respectively, U=60.0, p=0.3). The

average number (±st. dev.) of ovarioles per ovary was 22.4±2.8 for queens (n=14). Large workers had more ovarioles per ovary (7.1±1.7, n=210) than small workers (4.3±0.9, n=228) (Fig. 4). The regression lines of gaster width against number of ovarioles were plotted for workers and queens separately (n=49 and n=13 respectively, Fig. 5). Although the correlation between gaster width and number of ovarioles in queens was not significant (p=0.08), the number of ovarioles in queens was about twice that of workers with comparable gaster width. Although no colony was completely dissected, all the workers (n=579 from 14 colonies) possessed
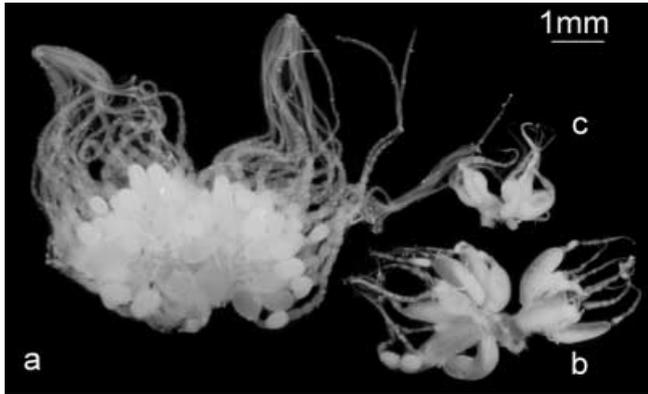
Figure 4. Dissection presenting typical ovaries of a) an egg-laying queen, b) a large and c) a small worker of *M. gulosa*

an empty spermatheca, whereas egg-laying queens' spermathecae (n=4) were full with sperm.

Egg-laying activity

Queens

The number of mature oocytes was significantly higher in mated queens than in workers (F=234, p<0.01, Fig. 6), each ovariole containing one mature oocyte. On average, large workers had more mature oocytes than small individuals, although not significantly so in all the colonies. Individual ovarioles of mated queens contain more developing oocytes compared to workers (20–25 compared to 3–15 for workers, F=55, p<0.01).

Queens of *M. gulosa* do not systematically bend their abdomen forward during oviposition. Indeed, eggs were sometimes ejected while the queen was in a resting position. She did not collect the eggs between her mandibles and they dropped onto the floor. They were then picked up by workers and deposited on the pile. Furthermore, queens were observed to lay up to 4 eggs at a time, these being glued together in a row and ejected in few seconds. The total number of eggs counted from one day to the next in the egg piles gave a median rate of 10 eggs laid per day (1st quartile=7, 3rd quartile=13, range 1–21, n=72 days). Eggs were present in the nests for periods of 31.7±11.4 days (mean±st.dev., range 22–63 days, n=22 periods assessed) and after they all hatched in larvae, it took another 20.2±9.5 days (range 8–39 days, n=22 periods assessed) before the new egg-laying period started and new eggs accumulated. The first larva appeared 19±2.4 days after the first egg was laid. This indicates that the active and inactive egg-laying periods of queens last approximately 12.7 and 39.2 days respectively. In a typical laboratory reared monogynous nest of *M. gulosa*, around 140 eggs could be counted on the pile before the first larva appeared. This gives a second estimate for the egg-laying rate of 11 eggs a day. This figure is close to the first rate calculated by daily egg counts. When the length of the eggs (n = 233) laid by different queens were compared, we found that each queen laid eggs of constant size, and this size varied among individuals (range: 1.08–1.15mm, F = 45.36, p<0.01).

Workers

Trophic eggs

In queenright colonies, both small and large workers laid exclusively trophic eggs (200 hours of observation on 13

$$ON_Q = 12.87 \, GW_Q - 12.69$$
$$r^2 = 0.26$$

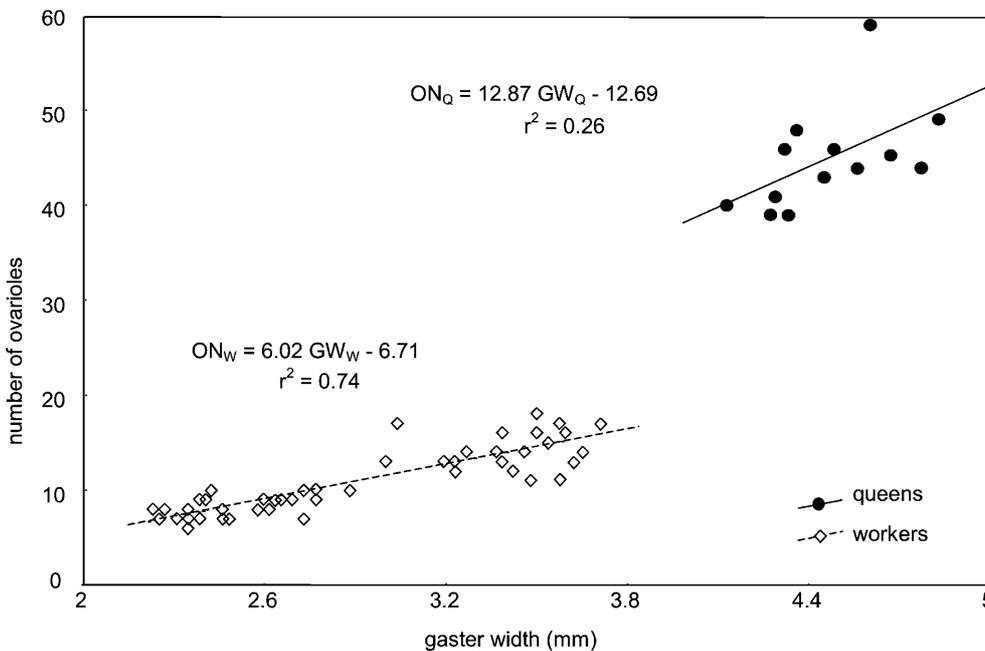$$ON_W = 6.02 \, GW_W - 6.71$$
$$r^2 = 0.74$$

Figure 5. Correlation between gaster width (GW) and ovariole number (ON) in workers (n=49) and queens (n=13, 3 mated + 10 virgin queens) of *M. gulosa*
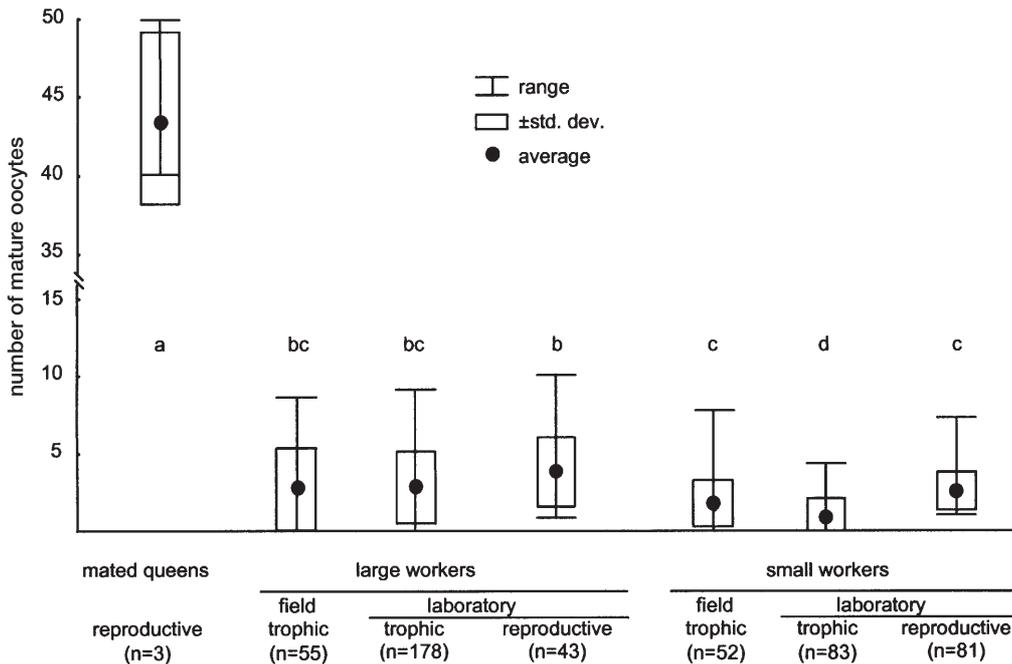
Figure 6. Number of mature oocytes found in mated queens, large and small workers of *M. gulosa*. Workers producing trophic or reproductive eggs were considered separately, as well as workers freshly collected from the field or laboratory reared. Sample size is given below group designation. Different letters indicate significant differences at the level 0.05 (Tukey HSD test for unequal sample sizes)

colonies, over 3 years of study). These eggs were ejected in seconds as the egg-layer bent the gaster forward between her legs. When it was still partly in the gaster, the ant seized it in her mandibles; the translucent egg then lost shape, appearing as a fragile yolk sac without a rigid chorion. In contrast, reproductive eggs were opaque, possessed a thick chorion and kept their shape after oviposition. Trophic eggs were fed to larvae (which received 66.9% of the eggs produced), to other workers (28.1%), to virgin queens (2.8%), or to the queen (0.9%). In addition, 1.4% were consumed by the egg-layers themselves. Large workers laid 2.4 times more eggs than small workers (n=44 hours of observation on 3 colonies). Trophallaxis does not occur in *M. gulosa* and trophic eggs constitute an important channel of food exchange in the colonies.

The characteristics of trophic eggs could already be detected in the ovaries, at the oocyte stage. They were easily distinguished from reproductive oocytes, as they were surrounded by a much thinner layer of follicular cells than reproductive oocytes and were longer than 1.25 mm (which corresponded to the maximum size of 95% of the reproductive oocytes measured, n=222). They were also distinguished by their partly translucent yolk. Yolk of reproductive oocytes appeared dense and homogeneous. Our dissections (n=681 large workers, 200 small workers) confirmed that queenright workers produce only trophic oocytes. Only some of their ovarioles contained mature or developing oocytes. At most, two yolky oocytes could be found in an ovariole, a basal mature one and a sub-mature one, followed by several non-yolky oocytes. The proportion of laboratory reared workers that were able to lay trophic eggs (i.e. which had mature oocytes) in a colony was 67%. A less conservative estimate was 92% of the individuals if we included workers with sub-mature, but without mature oocytes (Table 2). These data permitted us to

calculate an average trophic egg-laying rate of 1.0 egg/worker/day. A smaller proportion of field collected workers possessed yolky and mature oocytes. In laboratory reared colonies, more large workers had mature or yolky oocytes in their ovaries than small workers. For workers dissected soon after field collection, the trend was opposite (Table 2). The differences observed may be explained by the greater availability of food in the laboratory.

Trophic oocytes were significantly longer than reproductive oocytes and eggs produced by either the queens or the workers. Trophic oocytes produced by large workers are longer than those produced by small workers ($F=590$, $p<0.01$, Fig. 6). Yellow bodies were found in 89.5% of 476 laboratory reared workers, showing that trophic egg-laying activity produces yellow bodies. Similarly, 95.4% of 153 individuals frozen just after collection possessed yellow bodies. Yellow bodies load and age were correlated in laboratory reared individuals. They were found in 57, 80 and 99% of the workers of less than 1 month (n=7), 1–4 months (n=91) and more than 6 months old (n=136) respectively.

Seventy five percent of the field workers returning from a foraging trip or active on the nest mounds, possessed yolky oocytes (10/16 and 84/110 respectively). Thirteen and 63% of

Table 2. Ovarian development of laboratory reared and field collected workers of *M. gulosa*, expressed in percent of workers having sub-mature/mature trophic oocytes in their ovarioles

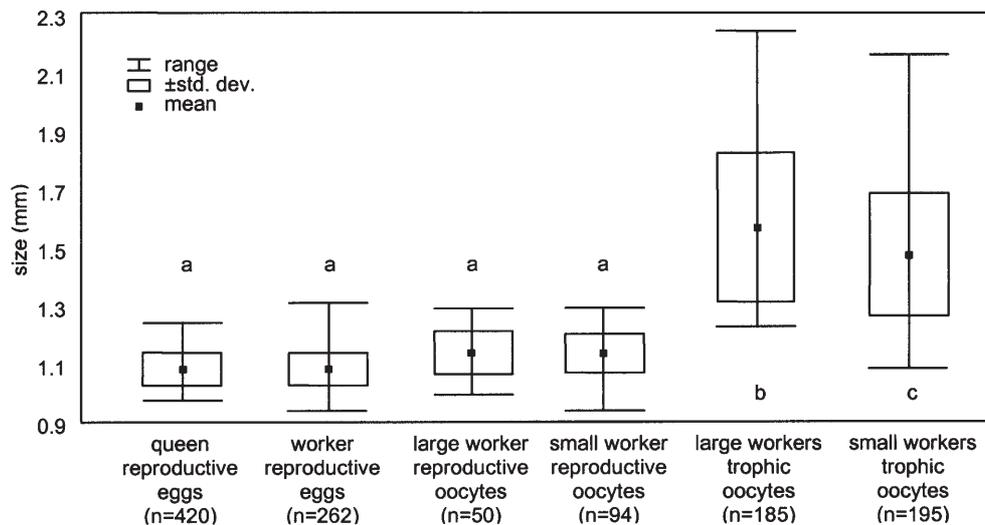|	laboratory	field
large workers	93/82 (n=108)	62/36 (n=58)
small workers	90/40 (n=60)	82/44 (n=50)
all workers	92/67 (n=168)	71/40 (n=108)

Figure 7. Egg and mature oocyte sizes in *M. gulosa*. Different letters indicate significant differences at the level 0.01 (Tukey HSD test for unequal sample sizes). Sample size is given below group designation

them respectively were able to oviposit (i.e. had mature oocytes, ready to be laid).

Reproductive eggs

In 91.3% (21 out of 23) of the orphaned worker groups (20 individuals per group), workers displayed infrequent agonistic behaviour (antennal boxing and biting) 18.1 ± 6.8 days (mean $\pm$ st.dev.) after they were separated from their queen. Worker-laid male eggs accumulated in all nests but two. The first eggs appeared after 25.0 ± 7.4 days. These reaction delays were shorter if larger colonies became orphaned. Indeed, in the latter situation, agonistic interactions already appeared after $3-6$ days and eggs were laid after $11-14$ days (n=3 colonies containing $200-300$ individuals).

Dissections of all the workers in the 23 groups showed that $0-8$ individuals shifted to the production of reproductive haploid eggs while the others continued to produce trophic eggs. Assuming that all workers having reproductive oocytes in their ovaries oviposited, the median egg-laying rate was estimated to be 1.0 eggs/worker/day for large workers (1st quartile=0.4, 3rd quartile=1.0, range $0-6$, n=78 days) and 0.7 eggs/worker/day for small workers (1st quartile=0.3, 3rd quartile=0.7, range $0-4$, n=73 days). Workers that shifted to the production of reproductive oocytes had the same number of ovarioles as trophic egg-layers (t=0.22, p=0.83 and t=0.92, p=0.36 for large and small workers respectively). Four large groups of orphan workers were reared long enough to follow brood maturation. All the groups reared a high number of worker-produced males. Complete dissection of these groups (total of 290 large and 190 small individuals) showed that the proportion of small workers (39.0%) which shifted to the production of reproductive eggs was higher than that of large workers (16.5%).

Not all the ovarioles of workers were fully active, some being without yolky oocytes. The ratio of mature oocytes to ovarioles fell from 1 in queens to 0.58 and 0.53 for small and large trophic egg-laying workers respectively. In small and large reproductive egg-laying orphaned workers, these ratios were 0.21 and 0.42 respectively. For small and large trophic egg-layers dissected soon after collection, we obtained the values 0.52 and 0.40 respectively. Mature reproductive oocytes measured in small and large orphaned workers' ovarioles had the same size (Tukey HSD test, p=1.00), unlike the trophic oocytes (Tukey HSD test, p<0.01). Reproductive eggs laid by workers had the same size as queen eggs (Tukey HSD test, p=1.00) (Fig. 7). A large proportion (90.4%) of the orphan workers having reproductive oocytes in their ovaries possessed yellow bodies.

When eggs and larvae produced by the queen before her death or removal were left in orphaned groups, an undetermined proportion of the diploid brood was reared into virgin queens.

Discussion

Queen-worker dimorphism

Queen-worker dimorphism in most morphologically "primitive" ant species is weak (Peeters, 1997), and the developmental basis of this divergence is poorly known. To represent both castes on the same plot of body measurements may allow the identification of some of the developmental parameters differentiating queens from workers. Queens of *M. gulosa* are not much larger than the largest workers. The size frequency distribution placed the queens as a third mode, but with an overlap with the largest workers. The log-log plot of outerorbital distance vs. head length showed similar regression lines for queens and workers. Likewise, regression lines of outerorbital distance vs. gaster width for queens and workers were parallel, but they were discontinuous and displaced. The number of ovarioles increases twice as fast with gaster size in queens compared to workers. This suggests that queen and worker developmental pathways diverge in some but not

all features, producing individuals of comparable sizes but nevertheless morphologically specialized. According to Wheeler's (1991) model of the evolution of worker castes in ants, the first step consists in the reprogramming of critical sizes (i.e. the size at which a larva initiates metamorphosis). Our data suggest that the queen and worker castes might have derived from the monomorphic ancestor by the same mechanism.

In queens each of the 43 ovarioles on average contained a mature oocyte, whereas in workers, only about half of their 8–14 ovarioles were active and contained oocytes. Queen fecundity was estimated to be approximately 10 times higher than that of workers, with 10–11 eggs laid per day. Queens monopolise reproduction in their colonies and workers only produce trophic eggs in their presence. Oviposition by *M. gulosa* queens is cyclical: active periods were 13 days long, during which approximately 140 eggs were laid. Active periods were separated by 39 days long intervals. Cyclical egg-laying activity seems to be common in *Myrmecia* as it occurs in several other species from different species-groups (Barnett, 1974; V. Dietemann, unpubl.).

Differences in external and ovarian morphology of queens and workers were studied by Ito et al. (1994) in *M. froggatti*, belonging to the *aberrans* group of species. This group is the most basal within the genus (Ogata, 1991). Worker length varies from approximately 10 to 15 mm (V. Dietemann, unpubl.) and the colonies contain 38 individuals on average (Ito et al., 1994). We reanalysed our data in order to compare them with those obtained by Ito et al. (1994). Using their regression equation, a *M. froggatti* queen of 3 mm gaster width has 13 ovarioles, whereas a worker of 2.5 mm gaster width (16.7% difference) has 9 ovarioles (31.2% difference). According to our results, for the same relative difference in size (16.7%), a *M. gulosa* queen has 31.8% more ovarioles than the corresponding worker. Therefore, the correlation between body size and number of ovarioles is similar in these species. The divergence in ovariole number between workers and queens (workers / queens: 4–15 / 15–18 [Ito et al. 1994]; 6–24 / 39–59, this study) occurs in at least two other species (*M. pilosula* and *M. simillima*, V. Dietemann, unpubl.) and suggests that it is an ancestral trait of the genus. This contrasts with the subfamily Ponerinae in which the two castes often have the same number of ovarioles (Peeters, 1993; Ito and Ohkawara, 1994). In *Harpegnathos saltator*, females have only eight ovarioles, but the queen's are longer than worker's (queens lay twice as many eggs, Peeters et al., 2000). A proportion of ponerine species nevertheless exhibit marked queen specialization in ovariole number (Peeters, 1993; Ito and Ohkawara, 1994).

In 2 colonies of *M. gulosa* out of 14, we found two dealated and most probably mated queens cohabiting. Although facultative polygyny was never reported in the *gulosa* group, our data suggest that two queens can reproduce in the same colony. Colonies were regularly spaced and their density was high, suggesting habitat saturation. Under such conditions, newly mated queens may found colonies pleometrotically or may be adopted in existing nests. The only other known example of polygyny in the genus is found in the *M. pilosula* complex of species (Craig and Crozier, 1979).

Worker size variation and egg-laying activity

Worker size frequency distribution of *M. gulosa* is bimodal (Haskins and Haskins, 1950; this study). Although we measured adults and not pupae, as recommended by Wheeler (1991), our two modes in worker size distribution are not consequences of different nutritional conditions for successive cohorts of brood: the differences in worker size were consistent among colonies (V. Dietemann, unpubl.) and not seasonal (Haskins and Haskins, 1950, p. 478). Allometry of workers is weak and monophasic.

Seventy-one percent of the 992±551 workers composing a colony had active ovaries and at least 40% of them could lay trophic eggs at a rate of approximately 1 egg/individual/day. Most of these trophic eggs were given to larvae, which also fed on the insect prey retrieved to the nest. Alternatively, trophic eggs were offered to workers or to the queen, or solicited by them. Although trophallaxis occurs in the genus (Haskins and Whelden, 1954), it is absent in *M. gulosa* and trophic eggs constitute the main channel of food exchange among colony members. In *M. froggatti*, large workers possess disproportionately wider gasters and more ovarioles than small workers (Ito et al., 1994). Similarly, *M. gulosa* large workers possess more ovarioles than small workers and indeed laid 2.4 times more eggs than their smaller nestmates. Large workers forage almost exclusively (Haskins and Haskins, 1950) and hence have direct access to nutrients. They collect nectar from flowers and hunt for insect prey. Our dissection of field collected individuals showed that although a larger proportion of foragers had oocytes in their ovaries compared to workers found in the nests, the oocytes of the latter were more often mature. Whether this indicates a higher egg-laying rate or a lower production rate is not known, hence whether foragers are the primary producers of trophic eggs remains to be verified. The importance of large workers in the nutrient intake and sharing is also supported by several other facts: the trophic eggs produced by large individuals are slightly larger than the ones produced by small workers, and they produce more of them per capita. Large workers are in charge of caring for larvae, which they feed with trophic eggs and prey, whereas small workers mostly care for eggs and cocoons, which are non-feeding stages. Some small workers form a retinue-like group around the queen, but she rarely solicits or obtains trophic eggs from them (V. Dietemann, unpubl.). The central role that large individuals play in the trophic balance of a colony has been highlighted in several species belonging to different subfamilies. In *Crematogaster smithi*, large workers or intercastes (intermediates between workers and queens) produced more unfertilized eggs than their smaller nestmates (Heinze et al., 1999). These eggs were then fed to the larvae or to the queen. In *Acanthomyrmex ferox*, a few major workers produce most of the trophic eggs that are fed to other workers or to larvae (Gobin and Ito, 2000).

As in many other ants, workers are able to lay male-destined haploid eggs when the queen dies or is removed. The shift between trophic and reproductive eggs takes at least 11 days. A proportion of the first eggs laid by orphaned workers is not viable (they do not hatch) and are often destroyed by small workers. In the case of reproductive egg-laying, large workers have a higher output. They laid 1.0 eggs/individual/day compared to 0.7 egg/individual/day for small workers. However, when a large group of workers becomes orphaned, a larger proportion of small workers shifts to the production of reproductive eggs, while most of the large workers continue to produce trophic eggs. Thus, although small workers are less fecund than their larger nestmates, they reproduce more readily. The divergence of worker sizes in *M. gulosa* has therefore not been accompanied by a reduction in reproductive potential of workers. Ovarian polymorphism was also reported in species with marked worker castes (e.g. *Atta*, *Acanthomyrmex*, *Camponotus*, *Crematogaster*, *Oecophylla*). In contrast, in other genera (*Eciton*, *Pheidole* and *Solenopsis)* exhibiting worker polymorphism (Hölldobler and Wilson, 1990, table 8–2), workers lack ovaries altogether (Bourke and Franks, 1995, p. 228).

Evolution of worker polymorphism

Morphometric studies of several *Myrmecia* species reveal variability in the degree of worker size variation. In the basal *aberrans* group of species, Gray (1973) described *M. froggatti* as a monomorphic species (isometric relative growth), which was confirmed by Ito et al. (1994). In the *pilosula* group, *M. dispar* (Gray, 1971) and *M. varians* (Gray, 1973) are monomorphic with little size variation of workers. However, size frequency distribution of *M. dispar* workers (n=37 colonies measured) varied between colonies from uni- to bimodality, indicating an unstable pattern among colonies, which might be due to environmental variation external to the colony. Species in the derived *gulosa* group show a bimodal size frequency distribution of workers over large size ranges (Haskins and Haskins, 1950; Gray, 1973; Higashi and Peeters, 1990; this study). The relative growth we measured deviated only slightly from isometry and is similar to that described for *M. brevinoda* (Higashi and Peeters, 1990). Thus *M. gulosa* and *M. brevinoda* cannot be considered as true cases of polymorphism. Differences in size, but not in shape represent the first step in the evolution of more complex worker caste proposed by Wheeler (1991). The various *Myrmecia* species studied therefore seem to represent different stages toward the evolution of morphological diversity within the worker form, but none show true polymorphism (sensu Wilson, 1953).

Comparable cases of worker size variation occur in some ponerine ants. In *Paraponera clavata*, workers show a wide range of size variation (though size frequency distribution is unimodal). The large workers are guards and foragers, whereas the small workers remain inside the nest (Breed and Harrison, 1988). In *Megaponera foetens,* worker size frequency distribution is bimodal (Crewe et al., 1984). The existence of small and large workers is here more closely associated with task partitioning during foraging raids on termite colonies. The small workers enter termite galleries and large workers bring bundles of immobilized termites back to the nest (Longhurst and Howse, 1979).

Behavioural flexibility was shown to be a more important mechanism of adaptation to short-term environmental variations than changes in physical caste distribution (Schmid-Hempel, 1992). Modifications in caste structure could be considered as a slowly reacting, hard-wired pattern characteristic for the species, which could reduce the scope of conditions favourable to the evolution of caste polyethism (Schmid-Hempel, 1992), thus explaining why worker polymorphism is not more widespread in ants. Wheeler (1991) suggested that morphological commitment is made at the expense of individual flexibility. The advantage of increased ergonomic efficiency provided by additional physical specialization may not offset the disadvantage of decreased flexibility in response to environmental and demographic variability. Wheeler (1986) also proposed that developmental factors constrain the evolution of worker caste complexity. Some system of gyne determination may not be compatible with further evolution of morphological complexity. As the form of each subsequent caste is dependent on the one preceding it, gyne determination may occur late in larval development and prevent extensive changes in worker development. Whether gyne differentiation occurs early in *M. gulosa* and leaves scope for diverging worker development requires a better knowledge of larval development and in particular the timing of gyne determination.

Beshers and Traniello (1994, 1996) proposed that size variations might have been pre-adaptations for division of labor. Among the correlates of worker body size that may affect colony fitness, Beshers and Traniello (1994) listed for large workers a general better resistance to stressful conditions, lower per unit energetic costs of activity, better performances in certain tasks, or greater food or water storage. These arguments can be applied to *M. gulosa* where large workers are the exclusive foragers (Haskins and Haskins, 1950) and are therefore exposed to environmental stress. Furthermore, our results show that large workers have an important food transfer function, as they have more ovarioles and produce more trophic eggs than small individuals. They could also act as buffer against periods of food shortage by delivering trophic eggs that can be considered as food stores. In addition, colonies could gain in efficiency by producing "cheaper" small workers, which can take care of brood and perform maintenance tasks inside the nest, where less resistance is needed because is buffered against harsh external abiotic conditions.

Although they belong to different clades of the Formicidae, the morphologically "primitive" subfamilies Myrmeciinae and Ponerinae are comparable in that they share a mixture of ancestral morphological traits (Ogata, 1991) and derived features (Peeters, 1997; this study). Among the latter are early forms of worker polymorphism (Wilson, 1953).

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