Behaviour-mediated group size effect constrains reproductive decisions in a social insect

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Keywords: ant Aphaenogaster senilis colony size decision making oophagy queen production task allocation

Over the last 20 years, a growing field of research has emphasized the importance of cooperation as a pervasive evolutionary force explaining the major transitions of life from genome to animal societies (Maynard Smith & Szathmary 1995; Szathmary & Smith 1995; Queller 2000; Bourke 2011). Principles of social evolution are therefore useful to explain how and why the association of cooperating units leads to the formation of complex entities with enhanced morphological and behavioural specializations. Theoretical models have demonstrated that, apart from the degree of cooperation, the number of cooperative units determines group complexity (Bourke 1999; Kokko et al. 2001; Lehmann & Rousset 2010). Empirical studies have also shown that larger groups have a higher survival rate and/or per capita reproductive success in several cooperatively breeding vertebrates (e.g. Malcolm & Marten 1982; Balshine et al. 2001; Clutton-Brock et al. 2001; Woxxvold & Magrath 2005; Gusset & Macdonald 2010). However, it is in insect societies, which function as integrated biological entities, that group size effects are expected to be the most determinant for an individual’s inclusive fitness (Wilson 1971; Michener 1974; Bourke 2011). Yet, the mechanisms underlying group size effects in ants, bees, termites and wasps remain elusive.

Insects’ colony size varies greatly between species from a couple (e.g. Thaumatomyrmex, Jahyny et al. 2002) to several millions of individuals (e.g. Dorylus or Formica unicolonial species, Hölldobler & Wilson 1990; Dornhaus et al. 2012). Within a species, colony size may also vary by several orders of magnitude depending on colony age, queen number, food availability, etc. Typically, the number of workers increases logistically from colony foundation by the queen to colony maturity, when sexuals (males and virgin queens) are produced (Oster & Wilson 1979; Tschinkel 1988). Unpredicted events, such as predation, immune challenges or extreme weather conditions, are factors that may suddenly reduce the number of workers in the colony. Moreover, in species that disperse by colony fission, during the founding stage the queens are accompanied by workers, which by leaving the mother colony, greatly secure daughter colony foundation. However, mother colonies lose an important fraction of their worker population during each reproductive event (Seeley 2010; Chéron et al. 2011).

One consequence of colony size reduction is a severe constraining of collective decision making and task realization. For
instance, in the ant *Temnothorax albipennis*, the accuracy of new nest searching and colony relocation was shown to depend on the number of workers (Dornhaus & Franks 2006; Franks et al. 2006). The importance of colony size for the formation of self-assemblage *(Anderson et al. 2002)* is also well exemplified in *Oecophylla* smaragdina, a species in which the construction of chains of workers to bridge gaps requires a critical number of participants *(Lioni & Deneubourg 2004)*. Larger colonies were shown to create more complex collective foraging groups (Beckers et al. 1989), to forage over longer distances, to spend more time foraging, or to allow more foragers to visit each food source than small colonies (Beekman et al. 2004; Thomas & Framenau 2005). Within the nest, colony size enhances division of labour and task specialization in *Rhytidoponera metallica* and *Pogonomymex californicus* (Thomas & Elgar 2003; Holbrook et al. 2011) but not in *T. albipennis* or *Cataglyphis bursa* (Retana & Cerdá 1990; Dornhaus et al. 2009). However, how group size translates into offspring production is not well understood. Worker number has been shown to correlate positively with the production of sexuals in some ant species (Elmes & Wardlaw 1982; Savolainen & Deslippe 1996; Cole & Wiernasz 2000; Sorvari & Hakkarainen 2007; Shik 2008), but not in others (MacKay 1981). Similarly, large colony size may increase (Jeanne & Nordheim 1996), decrease (Michener 1964) or have no effect (Bouwma et al. 2006) on per capita productivity.

In the present study, we addressed the importance of worker number for the production of virgin queens in an ant that disperses by colony fission. In many species of ants, young queen-derived diploid larvae are more or less totipotent and can develop into either workers or queens depending on environmental conditions (Wheeler 1986; but see Julian et al. 2002). Workers were hypothesized to exercise great control over caste production through the amount of food they provide to larvae (Brian 1956). In stingless bees, larvae development into queens is triggered by the release of geraniol in their food (Jarau et al. 2010). In the honeybee, *Apis mellifera*, queen prospective larvae receive royalactin, a specific royal jelly protein (Kamakura 2011). In the ant *Pogonomymex badius* and in several Aphaenogaster species, queens have been shown to feed on a more protein-rich diet than workers, although this may not be the determinant for caste fate (Smith & Suarez 2010; S. Caut, M. Jowers, C. Ruel, X. Cerdá & R. Boulay, unpublished data).

Workers’ decision to rear workers or queens also seems to be related to their perception of pheromones emitted by the current fertile queen. This is particularly striking in our model organism, Aphaenogaster senilis, a common gipsy ant in the Western Mediterranean Basin. In this species, both field and laboratory data indicate that queen pheromones inhibit the development of diploid larvae into new queens (Boulay et al. 2007). However, if the current queen dies, emergency replacement queens are rapidly reared from the young totipotent diploid larvae, which would otherwise become workers (Ledoux & Dargagnon 1973; Chéron et al. 2009). In nature, such replacement queens would mate at the entrance of their nest and rapidly start laying eggs to allow the colony to proceed. Since colonies of this species are headed by a single queen (strict monogyny), the production of only one or a few new queens is sufficient to guarantee colony continuity. Moreover, any excess in the production of new queens inevitably reduces the production of workers necessary for colony maintenance. The exact mechanism allowing the colony to adjust caste production precisely to its needs is still unknown.

The number of workers in natural colonies of *A. senilis* recorded in more than 300 colonies ranges between 120 and 3900 individuals (Boulay et al. 2007). Here, we examined the effect of reduced group size on the production of replacement queens versus workers in a queenless (QL) situation. To that end, we tested whether queen rearing covaries with the number of workers, the number of larvae and their ratio in experimentally orphaned groups. If brood care is constrained by a high number of larvae and a low number of workers, we expected that the number of larvae would interact with the number of workers to determine the probability of queen replacement. We tested two nonexclusive hypothetical mechanisms by which worker number could affect larval development: through the care provided by workers and/or through oophagy of worker-laid eggs. First, according to the behavioural mechanism, the larvae would have to receive more care and food in order to develop into queens. Therefore, below a certain threshold number of workers, not enough care or food could be provided to each larva, thus forcing their development into workers. Group size might also constrain the capacity to retrieve food items, which is likely to be determinant for colony food intake and larval development. The faster food is retrieved and processed, the faster it becomes available to larvae. We thus tested whether large groups retrieved large food items more rapidly than smaller groups. Second, there was reason to expect that cannibalism of worker-laid eggs could also affect larval fate, as it would provide additional nutrition. Aphaenogaster senilis workers start laying haploid eggs within days following orphaning (Ichinohe & Lenoir 2009) and before new queens are produced. Preliminary observations indicated that only a small fraction of worker-laid eggs developed into males. It was therefore reasonable to hypothesize that the remaining were cannibalized, possibly by larvae, as observed in other species (Baroni Urbani 1991; Masuko 2003). In a species such as *A. senilis* in which trophallaxis does not exist (Delage & Jaisson 1969; Lenoir et al. 2001), oophagy could substitute for oral feeding by workers (Crespi 1992). If, as we hypothesized, eating worker-laid eggs triggers larval development into queens, a small worker group may contain fewer laying workers than a larger group and therefore may not produce enough eggs to modify larval development.

**METHODS**

Colony Collection and Maintenance

Stock colonies of *A. senilis* were collected between July 2008 and July 2011 in the National Park of Doñana (southwestern Spain). In the laboratory, they were housed in 2 × 20 cm test-tubes, the bottoms of which were filled with water retained by cotton plugs. These tubes were kept in containers measuring 28 × 18 cm and 11 cm high that served as foraging areas. The inner walls of the containers were lined with Fluon to prevent ants from escaping. Colonies were maintained in total darkness at 28 °C ± 1 °C and 50 10% air humidity, and were fed three times a week with mealworms, *Tenebrio molitor*, honey and fruits. All experiments comply with current Spanish legislation.

Experiment 1: Effect of Worker and Larvae Number on Queen Replacement

A total of 60 QL groups were formed from 13 stock colonies. Groups contained 50, 100 or 200 workers (2 replicates per size category), 50 and 100 being smaller than field colony size. Each group size was tested with every even-numbered increment of larvae from two to 40 (one replicate each: 2, 4, 6, ..., 40). The larvae originated from the respective queenright (QR) stock colony. Of the three identified instar larvae, the first instar has been shown to be totipotent (Boulay et al. 2009). Each group was installed in an artificial nest made from a test-tube as described for the stock colonies. Each tube was connected to a 10 × 10 cm diameter circular box the inner wall of which was lined with Fluon. All...
groups received the same nutrition comprising four sliced mealworms, provided every 3 days. Each group was monitored every day until pupation of all surviving brood in order to determine the number of groups producing at least one queen, the total number of queens per group and the first occurrence of worker-laid eggs.

All statistical analyses were performed with the R package software version 2.7.2 (R Development Core Team, Vienna, Austria). Two generalized linear mixed models (GLMM; lme4 package, Bolker et al. 2009) were fitted to test the effect of worker numbers (three-level factor), larvae numbers (continuous variable), their interaction and the ratio between worker and larvae numbers (continuous variable) on the production of at least one queen (binomial distribution), on the number of queens produced (Poisson distribution) and the occurrence of worker-laid eggs (binomial distribution). The stock colony was included as a random factor. For each model, the significance of explanatory variables was tested by analysis of variance based on the Akaike information criterion (AIC). The significance of each factor level was assessed by contrast analysis. Finally, linear and logarithmic regression models were fitted to test the relation between the number of larvae and the proportion developing into queen.

Experiment 2: Task Allocation and Group Size

Three colonies were each divided into one group of 50 and one group of 200 workers. The workers from the foraging area and the nest were mixed together and collected randomly. Each group was provided with 10 first- and 10 last-instar larvae. In each group 20 focal individuals were marked with a dot of colour oil-based paint (Mitsubishi Pencil UniPaint) on the head, thorax and gaster. They were housed in transparent plastic CD boxes (12 × 12 cm and 0.2 cm high) allowing behavioural observations. The CD boxes were kept in larger containers (28 × 18 cm and 11 cm high) that served as foraging areas. Water was provided by connecting the CD boxes to a small test-tube filled with water retained by a cotton plug.

A total of 5913 behavioural observations were then carried out over 6 days. Focal individuals’ behaviour was scanned by the instantaneous sampling method approximately 10 times per day on days 2, 3, 4, 7 (before the worker oviposition period), 14 and 15 (oviposition period). A principal component analysis whose first three components accounted for 90% of the variation in ant activities allowed all acts to be regrouped into four categories: (1) foraging (exploration outside the nest, prey retrieval), (2) brood care (making contact with a larva, grooming, antennation with brood), (3) inactivity and (4) other (social interactions, nest maintenance and guarding the nest entrance). Three GLMMs were fitted to test the effect of group size and period of observation on the proportion of foraging, brood care and inactivity per individual (all binomial distributions). Stock colony was included as a random factor. To extrapolate the amount of nursing and foraging activities performed not only by marked workers but also by the whole group, we multiplied the total number of acts observed in each group by 2.5 (because 20 marked workers were observed in a 50-worker group) or 10 (because 20 marked workers were observed in a 200-worker group) for 50- and 200-worker groups, respectively.

Since this experiment showed that 200-worker groups had a higher absolute number of foragers (see Results), we also tested whether this translated into faster prey retrieval at the group level. One mealworm was introduced every day for 1 week into the foraging area. After providing the mealworm, we observed each group for 30 min to determine the time taken to retrieve the prey. The effect of the number of workers on the time taken to retrieve the prey was tested by survival analysis, including the colony as a random (frailty) factor.

Experiment 3: Worker Ovarian Development

Twelve colonies were each divided into two groups of 300 workers. One group (nurses) was composed of workers collected inside the nest and performing internal activities (brood care, inactivity or other). The other group (foragers) was composed of workers collected in the foraging area. Six nurse and six forager groups contained the mother queen while the remaining groups were QL. Approximately 10 workers were collected from each group every 72 h for 21 days. They were dissected and their ovarian development assessed on a binary scale. The ovaries were considered developed when they contained growing oocytes (stage E1, E2, E3, V, R1v, R1 and R2 of Fénéron & Billen 1996) and undeveloped when no developing oocyte was observed (stage J and D).

A GLMM was fitted to test the effect of ant activity, day of collection (continuous variable) and queen presence/absence on the proportion of workers with developed ovaries (binomial distribution). Stock colony was considered a random factor.

Experiment 4: Effect of Worker-laid Eggs

Worker eggs were laid in 27 QL groups (hereafter Source) of 200 workers from 27 stock colonies. When at least 10 eggs were observed in a group, 35 sets of three QL groups were immediately prepared from the respective stock colonies. Seventeen sets were composed of three groups as follows: one group contained 200 workers (200control); the second group contained 50 workers (50control); the third group (50egg) also contained 50 workers and received the respective Source group’s egg production every day for 21 days. The remaining 18 sets of three QL groups were composed as follows: one group contained 200 workers (200control); the second group contained 100 workers (100control); the third group (100egg) also contained 100 workers and received the eggs produced by the respective Source group every day for 21 days. All the QL groups within the 35 sets, except the Source group, contained 20 first-instar larvae from the respective QR stock colonies. Eight of 27 stock colonies were large enough to create two sets. All brood reaching the pupa instar were counted and collected three times a week for 2 months. Two GLMMs were fitted to test the effect of group size and egg supplementation on the total number of diploid pupae (Poisson distribution) and on the proportion of queens among them (binomial distribution). The stock colony was included as a random factor.

RESULTS

Experiment 1: Effect of Worker and Larvae Number on Queen Replacement

One group containing 50 workers and 36 larvae was removed from the analysis because of high worker mortality during the course of the experiment. Group size strongly affected larval caste fate. The probability of producing at least one replacement queen in a QL situation depended on the interaction between the number of workers and the number of larvae provided (Table 1, Model 1). Hence, with 50 workers the probability of producing at least one queen increased significantly with the number of larvae (Fig. 1). With 100 and 200 workers it was significantly higher and did not depend on the number of larvae. However, there was no effect of the workers-to-larvae ratio on the probability of producing at least one queen (Table 1, Model 2).

The number of queens produced per group ranged between zero and six and increased significantly with both the number of larvae and the number of workers but did not depend on their interaction (Table 1, Model 2). Groups of 100 workers produced significantly
becoming queens (Fig. 2; linear

tion), number of queens produced (Model 2: Poisson distribution) and presence of
when the experiment began, the day they were collected and the presence/absence
hundred groups. One replicate of each even number of larvae from two to 40 was
tested for each group size. Circles represent groups in which at least one queen was
produced. The 50-worker and 36-larvae group was not included in the analysis
because of high mortality.

Figure 1. Effect of the number of larvae on the production of at least one queen (black
circles) and on the occurrence of worker-laid eggs (grey circles) in groups of 50, 100
and 200 workers. One replicate of each even number of larvae from two to 40 was
tested for each group size. Circles represent groups in which at least one queen was
produced. The 50-worker and 36-larvae group was not included in the analysis
because of high mortality.

more queens than those composed of 50 workers (mean T SE: 0.32 T 0.13 versus 0.95 T 0.29; Z ¼ 2.374, P ¼ 0.017) and fewer than those of 200 workers (2.35 T 0.36; Z ¼ 3.212, P ¼ 0.001). There
was no effect of the workers-to-larvae ratio on the number of queens produced (Table 1, Model 2).

In both 50- and 100-worker groups the number of larvae only explained a small amount of variation in the proportion of larvae
becoming queens (Fig. 2; linear fit: R² ¼ 0.063, P < 0.001; linear fit: log(x), R² ¼ 0.7, P ¼ 0.001).

Table 1
Results of the model selection (GLMM)

<table>
<thead>
<tr>
<th>Model</th>
<th>Models</th>
<th>ddf</th>
<th>AIC</th>
<th>C²</th>
<th>C²/df</th>
<th>P</th>
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<tbody>
<tr>
<td>Model 1</td>
<td>Production of at least one queen</td>
<td>Workers*Larvae 8 68.719 0.17 1 0.68</td>
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<td>Model 2</td>
<td>Number of queens produced</td>
<td>Workers*Larvae 7 66.89 7.02 2 0.03</td>
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<td>Model 3</td>
<td>Presence of worker-laid eggs</td>
<td>Workers*Larvae 7 75.448 3.419 2 0.18</td>
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<tr>
<td>Model 4</td>
<td>Presence of worker-laid eggs</td>
<td>Workers*Larvae 5 76.868 0.58 1 0.446</td>
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<tr>
<td>Model 5</td>
<td>Presence of worker-laid eggs</td>
<td>Workers*Larvae 5 74.867 6.741 1 0.009</td>
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<td>Model 6</td>
<td>Presence of worker-laid eggs</td>
<td>Workers*Larvae 5 71.409</td>
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<td>Model 7</td>
<td>Presence of worker-laid eggs</td>
<td>Workers*Larvae 7 70.509 0.639 1 0.424</td>
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<tr>
<td>Model 8</td>
<td>Presence of worker-laid eggs</td>
<td>Workers*Larvae 5 71.409</td>
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<tr>
<td>Model 9</td>
<td>Presence of worker-laid eggs</td>
<td>Workers*Larvae 5 71.409</td>
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The presence of eggs depended on the interaction between worker and larval numbers (Table 1, Model 3). While 65%

larvae, worker-laid eggs were found in only 26% and 30% of the
groups of 50 and 100 workers, respectively. In addition, in 50- and
number of larvae was small (Fig. 1). As for queen production, the
workers-to-larvae ratio did not affect the presence of worker-laid eggs (Table 1, Model 3).
Experiment 2: Task Allocation and Group Size

The number of workers significantly affected individual time allocated to inactivity compared to other tasks. The number of workers outside the nest was similar for the two group sizes (50 versus 200 workers: 15.3 \( \pm \) 1.9% versus 14.6 \( \pm \) 2.2%). In the groups of 50 workers, the observed individuals performed 4.6 times more brood care (20.4 \( \pm \) 2.8% versus 4.4 \( \pm \) 1%) and were 1.5 times less inactive (35.3 \( \pm \) 2.1% versus 53.4 \( \pm \) 2.3% of the observations) than in the groups of 200 workers. As a result, the activity rate of each focal worker was higher in 50-worker groups.

The likelihood of a focal individual performing brood care activities was significantly higher before oviposition than in the oviposition period, in both 50- and 200-worker groups (Fig. 3; Table 1, Model 4). In the 50-worker groups, a decrease in brood care was associated with an increase in inactivity in the oviposition period (Table 1, Model 5). However, inactivity in the 50-worker groups remained lower compared to the 200-worker groups during the two periods. By contrast, in 200-worker groups, brood care was replaced by increasing foraging activity (Table 1, Model 6). Foraging remained stable over the two periods in 50-worker groups. Therefore, 200-worker groups had more foraging activities than 50-worker groups during the oviposition period, while 50-worker groups had more before oviposition.

Only 40% and 10% of the individuals in the 50- and 200-worker groups were observed. To assess differences in brood care and foraging activities at the group level, the behavioural profiles of focal individuals were multiplied by 10 and 2.5 for 200- and 50-worker groups, respectively. The results indicated that throughout the period of observation the absolute number of brood care activities performed by all 50 workers would have ranged between 348 and 746 in the 50-worker groups. In the 200-worker groups, the 20 larvae would have received between 171 and 787 brood care activities. The absolute number of foraging activities would have ranged between 225 and 474 and between 707 and 1716 foraging acts, in the 50- and 200-worker groups, respectively. In other words, although each individual spent less time being inactive in a 50-worker group, this was not sufficient to compensate for the reduction of foraging activities. As the absolute number of foraging acts was higher in the three 200-worker groups than in the 50-worker groups, prey retrieval was significantly faster in the former than in the latter (survival analysis: \( G_3^2 = 18.8, \ p \leq 0.002 \)).

Experiment 3: Worker Ovarian Development

Dissections revealed that a high proportion of workers had developed ovaries, irrespective of the presence of the queen (Fig. 4; 44.7% versus 42.7% QR and QL situations, respectively; Table 1, Model 7). The proportion of workers with developed ovaries did not vary significantly during the 3 weeks of the experiment (Table 1, Model 7). However, nurses’ ovaries were significantly more developed than those of foragers (57.3% versus 30.3%; Table 1, Model 7).
Experiment 4: Effect of Worker-laid Eggs

Of 20 larvae that were initially provided to each group, 10.2 ± 0.5 reached the pupal stage. Overall, the number of diploid pupae (irrespective of the caste) did not differ significantly according to the combination of group size and the addition of worker-laid eggs (Fig. 5; Table 1, Model 8).

Each Source group produced on average 4.4 ± 0.4 eggs per day. The amount of eggs added to the 50_egg groups did not differ from that added to the 100_egg groups (Mann-Whitney test: U = 171, N1 = 17, N2 = 18, P = 0.563). The presence of worker-laid eggs did not have any effect on the proportion of each caste produced (Table 1, Model 9).

The proportion of larvae that developed into workers was significantly higher in the 50_control than in 100_control or 200_control groups (Fig. 5). Therefore, as group size increased, the proportion of workers decreased while that of queens increased. However, when all the 200_control groups were compared separately, there was no significant correlation between the number of queens and workers produced (Pearson correlation: r = 0.11, t = 0.648, P = 0.522).

On average only 0.53 ± 0.14 males were produced during the 60 days of the experiment in the control groups. Significantly more males were produced in the 50_egg and 100_egg groups (2.14 ± 0.57; Mann-Whitney test: U = 879, N1 = 52, N2 = 35, P = 0.003). This difference was due to an increase in male production in both groups from day 47. The few males produced before this date derived from some of the 20 larvae initially provided to each group (haploid larvae occurred in small numbers in laboratory colonies and are not distinguishable from diploid larvae). The excess of males produced after day 47 in the 50_egg and 100_egg groups derived in all likelihood from the added eggs. Yet the average number of eggs that eventually reached the pupal stage was extremely low compared to the number of eggs that were added during the first 3 weeks.

DISCUSSION

Elucidating how group size effects operate at the different levels of social organization is important for understanding the evolution and maintenance of cooperation. So far, however, the mechanisms underlying the effect of group size on reproductive decisions have not been investigated in depth. The ant A. senilis is a good model system for such studies because the production of queens is highly predictable even in the laboratory (Boulay et al. 2007). Hence, in the QR condition, all diploid brood develops into sterile workers while queen removal immediately triggers the production of a new queen from the totipotent larvae. Our results show that the workers-to-larva ratio did not affect the production of queens and that there were a critical number of workers below which queen replacement was less likely. The numbers of workers and larvae had a nonadditive effect on the success of queen replacement (e.g. the probability that at least one larva would develop into a queen; experiment 1). Hence, the number of larvae limited queen replacement in 50- but not in 200-worker groups. From 20 initially provided larvae, the 50-worker groups were able to rear the same number to the pupal stage as groups of 200 workers. But the 200-worker groups had a higher reproductive potential since the proportion of larvae developing into queens was higher (experiment 4). However, behavioural observations also indicated that a group of 50 workers was capable of enough plasticity to maintain a similar amount of brood care per larva as a group of 200 workers (experiment 2). Workers laying eggs could explain the high level of inactivity observed in the 200-worker groups. Although our results support the hypothesis that many worker-laid eggs are cannibalized, lower egg provisioning in 50-worker groups did not limit queen production (experiments 3 and 4).

Ant larvae are completely dependent on adults for feeding. The latter must forage, process the food and distribute it to the larvae in addition to other types of care (e.g. cleaning). The entire process from foraging to larval feeding is probably a determinant for larval development and is constrained by the number of workers. Our results (experiment 2) indicate that task allocation among colony members was plastic and varied with group size and time. The percentage of inactive individuals decreased from 55% in 200-worker groups to 35% in 50-worker groups. In small groups, inactive workers were therefore reallocated to nursing. During the worker groups. Multiple factors could explain these changes in colonial behaviour. Such behavioural plasticity has been observed in several other social insect species (Gordon 1996). The degree of worker specialization in various tasks has also been shown to increase with worker number (Thomas & Elgar 2003; proportion of apparently inactive individuals may constitute a reserve allowing the colony to cope with unpredictable worker loss (Anderson & McShea 2001; Cassill 2002; Jeanson et al. 2007). It has also been suggested that large colonies could afford a high number of inactive individuals because higher inactivity of some individuals might be compensated in large colonies by a higher degree of specialization of other individuals, enhancing overall group efficiency (Jeanson et al. 2007).

The result of experiment 2 showed that individual behavioural plasticity allowed a group of 50 individuals to maintain a colonial level of brood care similar to that of a group of 200 workers. In this experiment, the number of larvae was fixed to 20 in all the groups, which corresponds to the lower limit at which 50 workers were able to produce a new queen. The change from inactivity to other activities may be stimulated by the brood. Larvae are known to beg for food in several ant species (Creemers et al. 2003; Kaptein et al. 2005). Moreover, in the honeybee, the number of workers engaged in nursing behaviour was shown to increase with the number of...
larvae in the hive requiring food (Schmickl & Crailsheim 2002). It
can therefore be hypothesized that, in A. senilis, inactive individuals
must be stimulated by a small workers-to-larvae ratio or a critical
number of larvae to shift to brood care activities. This contrasts
with the behavioural hypothesis prediction that more queens
would be produced in groups with fewer larvae because high brood
care per larvae would be provided. Moreover, this could explain
why queen replacement failed in groups of 50 workers containing
fewer than 20 larvae. In addition, behavioural plasticity did not
allow the group to maintain the same level of foraging and food
retrieval in 50-worker as in 200-worker groups. As a consequence,
workers required significantly more time to retrieve prey to the
nest, which may have affected the entire food-processing chain up
to the larvae, also reducing brood care quality.

In groups of 200 workers, the probability of becoming a queen
decreased logarithmically with the number of larvae (experiment 1).
This suggests that in the QL condition, a negative feedback operates at
the colony level to limit the production of queens to a few individuals.
The mechanism underlying this feedback is unknown. The first larvae
developing into queens might inhibit the development of other
larvae into new queens either directly, by chemical signals, compe-
tition for food or aggressive interactions, or through worker behav-
ior. Hence, the lack of a significant negative correlation between the
number of workers and queens produced across group sizes
(experiment 4) suggests that several larvae that did not develop into
workers did not develop into queens either. Workers may be able to
detect and kill larvae developing into extra queens, as occurs in
Linepithema humile and Solenopsis invicta (Passera et al. 1995;
Klobuchar & Deslippe 2002). Independently of the mechanism, the
limitation of queen production in A. senilis is important because this
species is strictly monogynous and only one queen is necessary for
the colony to proceed. Moreover, during fission, the new queen leaves
her nest with workers protecting her, which favours high queen
survival. As a consequence, colonies are expected to limit their
investment in sexuals. Extra individuals may serve as ‘life insurance’
in case the first new queen should accidentally die (Chéron et al.
2009). However, a massive investment in new queens that would
compete among themselves would be useless and would probably
jeopardize colony growth and maintenance.

Although the number of queens increased with group size, the
total number of diploid pupae (workers ♀ queens) produced from
the 20 larvae provided in 50-, 100- and 200-worker groups did not
differ significantly (experiment 4). This suggests that the capacity
to rear the brood is maintained irrespective of group size, but
rearing queens requires an overhead that small worker groups may
not afford. Hence, our results show that in small groups, workers
did not sacrifice worker-destined larvae in order to produce more
costly queens. The specific cost of producing a queen instead of a
worker is difficult to estimate because we still lack information on
the physiological mechanisms triggering larval development into one
or the other caste. In species with worker−queen dimorphism such as A. senilis, queens need to receive an excess of food
compared to workers. Individual dry weight measurements of 196
workers and 60 queen pupae revealed the latter were only 1.83
times heavier than the former (R. Boulay, unpublished data). This
difference seems relatively small but may limit the production of
queens. Variation in food quality in the larval diet may also trigger
larval development into one or the other caste, especially in the
field where the colonies have access to diverse resources (Smith &
Suarez 2010). In our experiment, only one food source was provided
to the groups. This suggests that either variation in food quality was
not a determinant of larval development or the larvae had access to
another resource such as worker-laid eggs.

The first pile of worker-laid eggs appeared after 10 days under
QL conditions (Ichinose & Lenoir 2009). Several lines of evidence
suggest that many worker-laid eggs were cannibalized. First, very
few males were produced, even when four eggs were added daily to
100- and 50-worker groups. Second, worker ovarian development
did not depend on the presence of a queen but was related to their
activity, which most probably also reflected their age (experiment
3). Whether workers lay eggs in a QR situation remains unknown.
The delay between queen removal and the appearance of a pile of
worker-laid eggs may reflect the time for switching from trophic to
reproductive eggs in a QL situation. The presence of a chorian
suggested that the eggs laid by QL workers in experiment 4 were all
viable, but the presence of trophic eggs in a QR situation has not
been investigated in this species. Although workers may keep
developed ovaries in anticipation of the mother queen’s death
(Bourke 1988), this hypothesis is unlikely given the relatively long
queen life span (> 5 years, C. Ruel & R. Boulay, personal observa-
tion). Therefore, workers developing ovaries in both QR and QL
situations would be selected for by larval nutritional needs rather
than for potential worker reproduction. Oophagy has been shown
in other species such as Mesor semirufus (Baroni Urbani 1991). In
the ponerine ant Amblyopone silvestrii (Masuko 2003) small larvae
mostly feed on queen’s eggs, while large larvae are transported on
the prey. Similarly, the large larvae of A. senilis feed directly on the
prey (Agbogba 1986) while the diet of the small larva is unknown.
It is therefore reasonable to expect that, as in A. silvestrii, young
A. senilis cannibalized eggs too. This hypothesis is supported by the
finding that worker-laid eggs appeared when few larvae were
present (experiment 1). However, consumption of worker-laid eggs,
if confirmed, did not affect larval caste fate.

In insect societies, colony fitness can be constrained by group
size. Larger groups differ from small groups in their task distribu-
tion (Thomas & Elgar 2003; Holbrook et al. 2011). They outcompete
small groups in foraging (Beckers et al. 1989; Beekman et al. 2004;
Thomas & Framenau 2005) and invest their resources in repro-
ductive individuals (Jeanne & Nordheim 1996; Cassil 2002). Colony
fitness could also be limited by very large group sizes. The indi-
vidual and group efficiencies may peak and then decline because of
group dynamics constraints (Bruce & Bud 2012). The larval fate
does not solely depend on direct interactions between larvae and
workers, but rather relies on collective cooperative performance at
the colony level. The evolution of sociality leads to a dependency
not only on other members in the group, but also on the global
performance of the group as a whole.

Acknowledgments

We are grateful to Ana Carvajal for collection of colonies and
laboratory assistance, to Alain Lenoir and Abraham Hefetz for useful
comments on the manuscript and to Jacqueline Minett Wilkinson for
English language editing. We also thank the two anonymous referees for very helpful comments on the manuscript.
The authority of the Doñana National Park approved this research. This article forms part of C.R.’s Ph.D. thesis funded by the CSIC (JAE Predoctoral fellowship), which will be defended at the Universitat Autònoma de Barcelona. This work was funded by MICINN and
FEDER (projects CGL2009-12472 to R.B. and CGL2009-09690 to
X.C.).

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