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

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The formation of cooperative entities between lower-level units is characterized by increasing complexity. Apart from the degree of cooperation, social complexity is determined by the number of cooperative individuals. Previous studies have considered the relationships between group size and traits affecting inclusive fitness, such as reproductive efficiency. In social insects, little is known about the conversion of resources into offspring relative to colony size. In the present study, we addressed the importance of worker numbers for the production of queens and workers, and investigated the mechanisms that could affect larval development in the ant *Aphaenogaster senilis*. In this species, if the current queen dies, replacement queens are reared from the totipotent diploid larvae. We found that the number of workers constrained reproductive decisions, since the production of queens was lower in small than in large groups. The number of larvae also limited the success of queen replacement when associated with a small group size. Rearing queens requires an overhead that small worker groups cannot afford. These effects derived from a limitation in the realization of tasks at the group level. The investment in foraging rather than nursing behaviour predicted the production of queens. We tested whether egg-laying workers in queenless nests increased queen production. Although oophagy was likely to occur, the eggs did not affect larval development. Our results suggest that the larval fate does not depend on direct interactions between larvae and workers, but rather relies on collective cooperative performance at the colony level.

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; Woxvold & 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

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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 *Pogonomyrmex californicus* (Thomas & Elgar 2003; Holbrook et al. 2011) but not in *T. albipennis* or *Cataglyphis cursor* (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 *Pogonomyrmex 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 (Ichinose & 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 $28 \pm 1\,^{\circ}C$ and $50 \pm 10\%$ 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 (20 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 ($200_{control}$); the second group contained 50 workers ($50_{control}$); the third group (50_{egg}) 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 ($200_{control}$); the second group contained 100 workers ($100_{control}$); the third group (100_{egg}) 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 1).

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

Table 1
Results of the model selection (GLMM)

	Models	*df*	AIC	χ^2	$\chi^2 df$	*P*
Model 1	**Production of at least one queen**					
	Workers*Larvae+ratio	8	68.719	0.17	1	0.68
	Workers*Larvae	7	66.89	7.02	2	**0.03**
	Workers+Larvae	5	69.909			
Model 2	**Number of queens produced**					
	Workers*Larvae+ratio	8	76.868	0.58	1	0.446
	Workers*Larvae	7	75.448	3.419	2	0.18
	Workers+Larvae	5	74.867	6.741	1	**0.009**
	Workers	4	79.609			
Model 3	**Presence of worker-laid eggs**					
	Workers*Larvae+ratio	8	70.509	0.639	1	0.424
	Workers*Larvae	7	69.148	6.261	2	**0.044**
	Workers+Larvae	5	71.409			
Model 4	**Nursing**					
	Workers*Time	5	1311.2	1.1822	1	0.277
	Workers+Time	4	1310.4	286.17	1	**<0.001**
	Workers	3	1594.6			
Model 5	**Inactivity**					
	Workers*Time	5	536.55	17.629	1	**<0.001**
	Workers+Time	4	552.18			
Model 6	**Foraging**					
	Workers*Time	5	1188.7	40.932	1	**<0.001**
	Workers+Time	4	1227.7			
Model 7	**Workers with developed ovaries**					
	Task+Time+Queen	5	1986.8	0.591	1	0.442
	Task+Time	4	1985.4	2.541	1	0.111
	Task	3	1985.9	118.67	1	**<0.001**
	Intercept	2	2102.6			
Model 8	**Number of diploids**					
	Eggs+Workers	5	299.24	3.184	2	0.204
	Eggs	3	298.42	0.135	1	0.714
	Intercept	2	296.42			
Model 9	**Caste proportion**					
	Workers+Eggs	5	134.71	0.078	1	0.780
	Workers	4	132.79	40.344	2	**<0.001**
	Intercept	2	169.13			

Models test (1) the effect of the number of workers, the initial number of larvae and their interaction on production of at least one queen (Model 1: binomial distribution), number of queens produced (Model 2: Poisson distribution) and presence of worker-laid eggs (Model 3: binomial distribution); (2) the effect of the number of workers and the period of observation on nursing activity (Model 4: binomial distribution), inactivity (Model 5: binomial distribution) and foraging activity (Model 6: binomial distribution); (3) the effect of the task each individual performed when the experiment began, the day they were collected and the presence/absence of the queen on the proportion of workers with developed ovaries (Model 7: binomial distribution); (4) the effect of the number of workers and presence of eggs on number of diploid pupae produced (Model 8: Poisson distribution) and proportion of each caste (Model 9: binomial distribution). Significant results are shown in bold.

more queens than those composed of 50 workers (mean ± SE: 0.32 ± 0.13 versus 0.95 ± 0.29; $Z = 2.374$, $P = 0.017$) and fewer than those of 200 workers (2.35 ± 0.36; $Z = -3.212$, $P = 0.001$). 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: $R^2 = 0.24$, $P = 0.03$, $R^2 = 0.07$, $P = 0.26$, respectively; log fit: $R^2 = 0.19$, $P = 0.06$, $R^2 = 0.07$, $P = 0.263$). However, in the 200-worker groups the proportion of larvae developing into queens decreased logarithmically with the number of larvae initially provided (log fit: $y = 0.528 - 0.301 \log(x)$, $R^2 = 0.63$, $P < 0.001$; linear fit: $R^2 = 0.47$, $P < 0.001$).

Twenty-four of 59 groups produced eggs. The first piles of worker-laid eggs appeared on average 13.5 ± 0.4 days after orphaning. The presence of eggs depended on the interaction between worker and larval numbers (Table 1, Model 3). While 65% of 200-worker groups laid eggs irrespective of the number of 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 100-worker groups egg piles were more likely to occur when the 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).

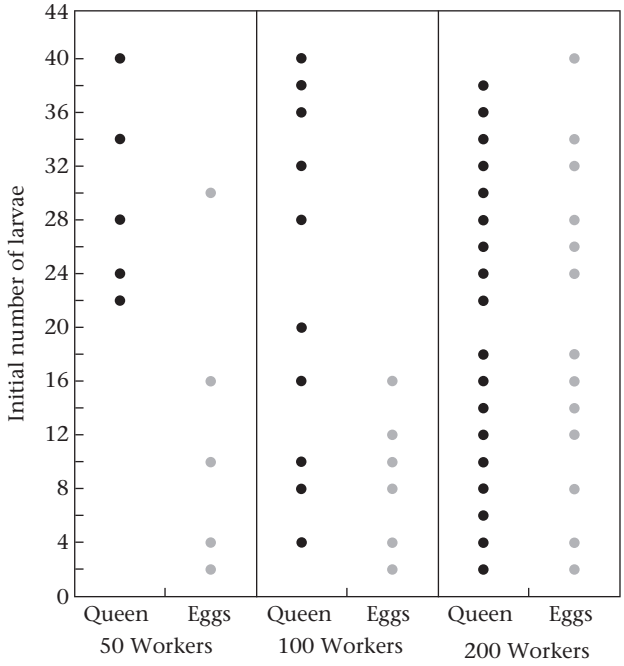

Figure 1. Effect of the number of larvae on the production of at least one queen (black circles) and on the occurrence of worker-laid eggs (grey circles) in groups of 50, 100 and 200 workers. One replicate of each even number of larvae from two to 40 was tested for each group size. Circles represent 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.

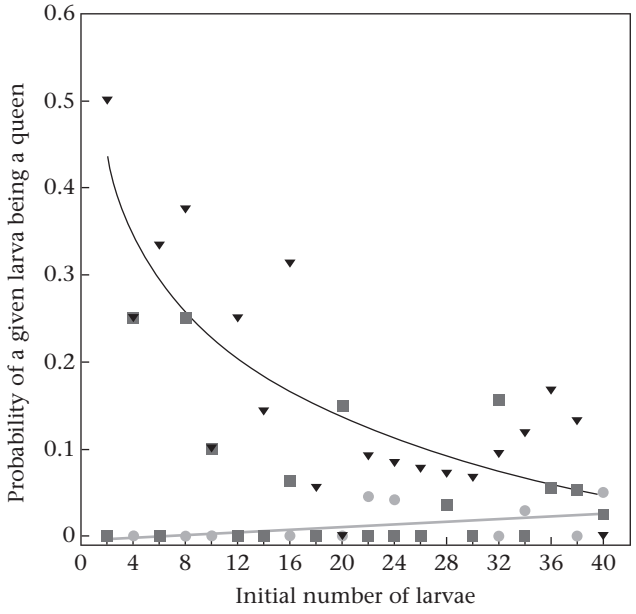

Figure 2. Probability of developing as a queen as a function of the number of larvae in groups of 50 (grey circle), 100 (grey square) and 200 (black triangle) workers. Black line: best fit for 200 workers; grey line: linear fit for 50 workers. See text for details.

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 ± 1.9% versus 14.6 ± 2.2%). In the groups of 50 workers, the observed individuals performed 4.6 times more brood care (20.4 ± 2.8% versus 4.4 ± 1%) and were 1.5 times less inactive (35.3 ± 2.1% versus 53.4 ± 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: $\chi^2_5 = 18.8$, $P = 0.002$).

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).

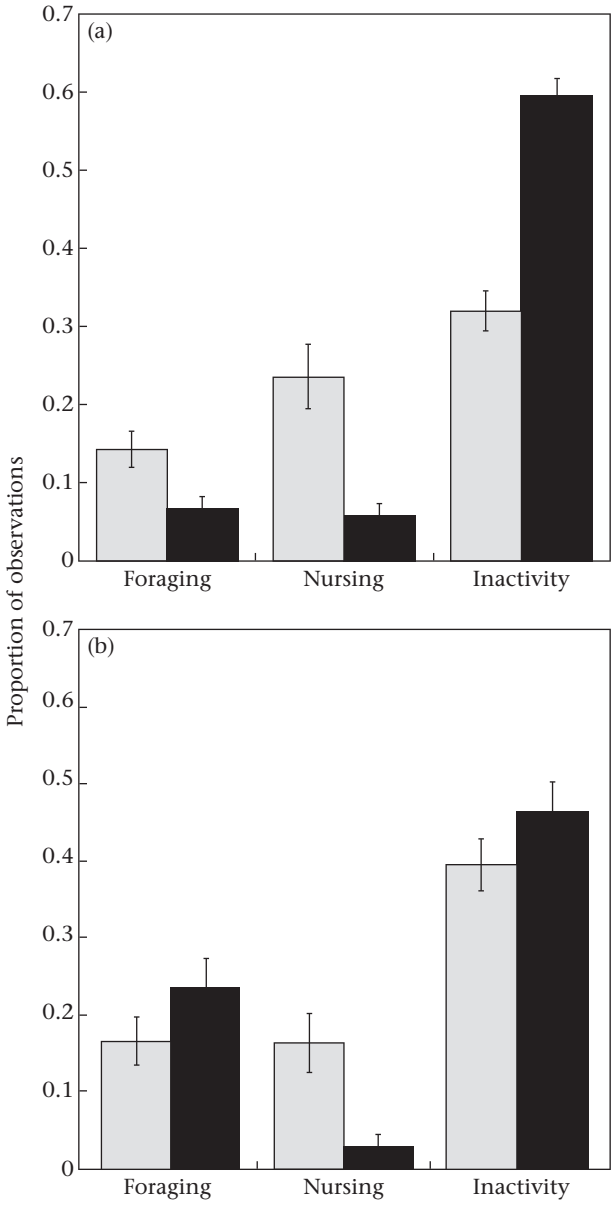

Figure 3. Proportion (mean ± SE) of foraging, nursing and resting activities by 20 focal workers in groups of 50 (grey bars) and 200 workers (black bars) during the two periods of observation: (a) days 2, 3, 4 and 7 (before worker oviposition); (b) days 14 and 15 (after oviposition).

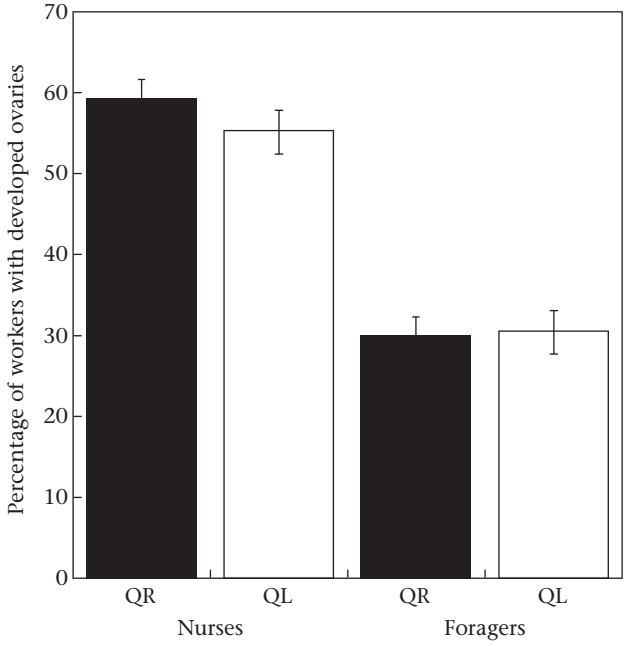

Figure 4. Development of workers' ovaries (% mean ± SE) in queenright (QR, black bars) and queenless (QL, grey bars) conditions and according to their task when the experiment started.

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_{eggs} groups did not differ from that added to the 100_{eggs} groups (Mann–Whitney test: $U = 171$, $N_1 = 17$, $N_2 = 18$, $P = 0.563$). 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: $r = -0.11$, $t_{34} = -0.648$, $P = 0.522$).

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: $U = 879$, $N_1 = 72$, $N_2 = 35$, $P = 0.003$). 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.

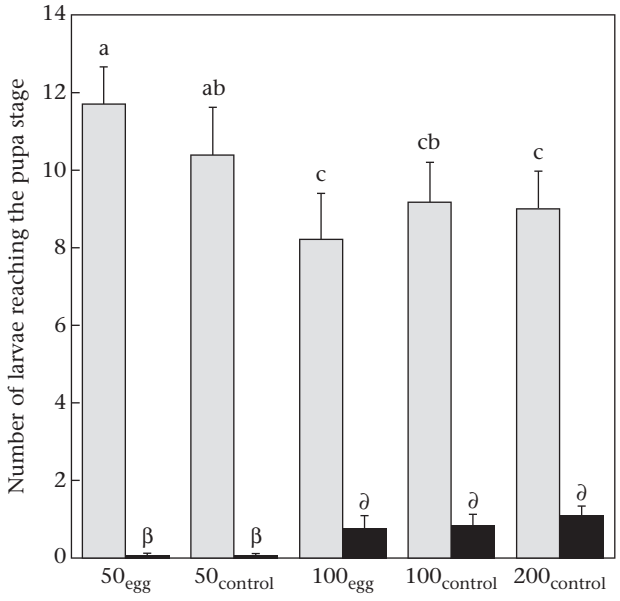

Figure 5. Number (mean ± SE) of worker (grey bars) and queen (black bars) pupae produced as a function of the number of workers and the addition of eggs from the source colonies. The letters a, b and c denote significant differences between groups in worker production ($50_{control}$ versus 100_{eggs} and $50_{control}$ versus $200_{control}$: $P \leq 0.05$; 50_{eggs} versus $100_{control}$: $P < 0.01$; 50_{eggs} versus 100_{eggs} and 50_{eggs} versus $200_{control}$: $P < 0.001$). The letters ß and ∂ denote significant differences between groups in queen production (50_{eggs} versus 100_{eggs}, 50_{eggs} versus $100_{control}$, $50_{control}$ versus 100_{eggs} and $50_{control}$ versus $100_{control}$: $P < 0.01$; 50_{eggs} versus $200_{control}$ and $50_{control}$ versus $200_{control}$: $P < 0.001$).

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-larvae 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 larvae 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 oviposition period, 200-worker groups became more similar to 50-worker groups. Multiple factors could explain these changes in colonial behavioural profile. 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; Holbrook et al. 2011; but see Dornhaus et al. 2009). The high 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, competition for food or aggressive interactions, or through worker behaviour. 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 chorion 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 observation). 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 *Messor semirufus* (Baroni Urbani 1991). In the ponerine ant *Amblyopone silvestrii* (Masuko 2003) small larvae mostly feed on queens' 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 larvae 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 distribution (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 reproductive individuals (Jeanne & Nordheim 1996; Cassill 2002). Colony fitness could also be limited by very large group sizes. The individual and group efficiencies may peak and then decline because of group dynamics constraints (Bruce & Burd 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.

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