# The determinants of queen size in a socially polymorphic ant

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## Abstract

In social animals, body size can be shaped by multiple factors, such as direct genetic effects, maternal effects, or the social environment. In ants, the body size of queens correlates with the social structure of the colony: colonies headed by a single queen (monogyne) generally produce larger queens that are able to found colonies independently, whereas colonies headed by multiple queens (polygyne) tend to produce smaller queens that stay in their natal colony or disperse with workers. We performed a cross-fostering experiment to investigate the proximate causes of queen size variation in the socially polymorphic ant *Formica selysi*. As expected if genetic or maternal effects influence queen size, eggs originating from monogyne colonies developed into larger queens than eggs collected from polygyne colonies, be they raised by monogyne or polygyne workers. In contrast, eggs sampled in monogyne colonies were smaller than eggs sampled in polygyne colonies. Hence, eggs from monogyne colonies are smaller but develop into larger queens than eggs from polygyne colonies, independently of the social structure of the workers caring for the brood. These results demonstrate that a genetic polymorphism or maternal effect transmitted to the eggs influences queen size, which probably affects the social structure of new colonies.

## Introduction

Body size is a key life-history trait in plants and animals (Stearns, 1992). In a great diversity of species, body size is positively correlated with fertility or mating success (Vander Meer *et al.*, 1992; Honek, 1993; Jamieson, 2002; Kovacs *et al.*, 2008), survival (Roff, 1992; Wiernasz & Cole, 2003; Schorr *et al.*, 2009) and dispersal abilities (Sundström, 1995; Paradis *et al.*, 1998; Rüppell & Heinze, 1999; Byers, 2000; Gonzaga & Vasconcellos-Neto, 2001). In social animals, body size affects social interactions and often correlates with variation in mating system and social structure (Bourke & Franks, 1995; Lindenfors & Tullberg, 1998).

Because of their modularity and complex social organization, eusocial insects (all species of ants and termites, as well as some bee and wasp species) are particularly interesting with respect to the causes and consequences

of body size variation (Oster & Wilson, 1978; Bourke & Franks, 1995; Burd, 2000; Emlen & Nijhout, 2000). Body size may be influenced by genes, maternal effects, environmental factors or social conditions, which can all affect brood development (Wheeler, 1986; Bargum *et al.*, 2004; Fjerdingstad, 2005; Schwander *et al.*, 2005). An important component of the social environment is constituted by the workers, which care for the brood and can manipulate offspring number or resource allocation (Chapuisat & Keller, 1999; Meunier *et al.*, 2008). Hence, intracolony conflicts (Chapuisat & Keller, 1999; Meunier *et al.*, 2008), social constraints (Rosenheim *et al.*, 1996) and indirect genetic effects (Linksvayer, 2006) may affect the body size of colony members.

In eusocial insects with physical castes, such as ants, the body size of young queens seems to play a key role in the evolution of social structure by mediating a feedback loop between the social structure of the natal colony, differential dispersal and mode of colony founding (Keller & Passera, 1989; Bourke & Franks, 1995). Typically, species with a single queen per colony tend to produce queens of relatively large size (Keller & Passera, 1989). These winged females have large flight

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muscles and elevated fat stores, so that they can fly away from their natal nest, excavate their own nest and raise their first brood independently, without the help of workers (Hölldobler & Wilson, 1990). In the mono-gynous ant species *Pogonomyrmex occidentalis*, the size of young queens correlated with their probability of survival through the initial steps of independent colony founding (Wiernasz & Cole, 2003).

In contrast, species with multiple queens per colony tend to produce smaller queens that generally stay within their natal nest or disperse by budding, a process whereby queens and workers depart from a colony to jointly establish a new colony in the vicinity (Keller & Passera, 1989; Hölldobler & Wilson, 1990; Keller, 1991; Ross & Keller, 1995). Hence, variation in the body size of young queens seems to depend on queen number in the mother colony, and in turn may constrain the mode of colony founding and the number of queens in future colonies.

The link between social structure, queen size and dispersal has been mostly studied across species. A comparative survey of 24 European ant species showed that 12 of 13 species with single-queen colonies (mono-gyne species) exclusively used independent colony founding, whereas eight of 11 multiple-queen colonies (polygyne species) used dependent colony founding (Keller, 1991). Moreover, ant species using independent colony founding produced queens with a significantly higher relative fat content than species using dependent colony founding, with no overlap in relative fat content between the two types of queens (Keller & Passera, 1989).

Several ant species exhibit intraspecific social poly-morphism, and in some cases monogyne and polygyne colonies coexist within the same population (Chapuisat *et al.*, 2004; Gyllenstrand *et al.*, 2005; Bargum *et al.*, 2007). These systems are interesting to assess the link between queen number and queen size independently of phylogeographical or ecological factors that correlate with queen number variation across species or popula-tions (Chapuisat *et al.*, 2004; Rosset & Chapuisat, 2007). So far, the relationship between colony queen number and queen size has been studied in only one species, *Leptothorax rugatulus*. Consistent with the evidence from the comparative surveys, large queens were more frequent in monogyne colonies, whereas small queens were mostly found in polygyne colonies (Rüppell *et al.*, 1998; Rüppell & Heinze, 1999). In polygynous colonies, the situation is however complicated by the fact that small queens might at least partly be intraspecific parasites exploiting unrelated colonies.

The proximate factors influencing queen size remain poorly understood. The size of young queens may be affected by their mother queen, through genes or maternal effects transmitted to the eggs (Bernardo, 1996), or by their social environment, i.e. the workers who raise the brood. Studies in four species of ants have suggested a genetic component to queen size. In the fire ant *Solenopsis invicta*, an allelic difference at the gene *Gp-9* correlated with both social structure and queen size (Ross, 1997; Ross & Keller, 1998; Keller & Ross, 1999). Reproductive queens in monogyne colonies invariably carried the $Gp-9^{BB}$ genotype, whereas the ones in polygyne colonies generally carried the $Gp-9^{Bb}$ genotype. At the end of the rearing period, young queens with the $Gp-9^{BB}$ were larger than young queens with the $Gp-9^{Bb}$ genotype. Young queens with the $Gp-9^{bb}$ genotype had lower survival, and the few that survived in polygyne colonies were the smallest queens. In *L. rugatulus*, the head widths of the mother and daughter queens were positively correlated, and there was no association with egg size (Rüppell *et al.*, 2001). In colonies of *Lasius niger* headed by one doubly-mated queen, the size of the queens produced differed significantly between the two patrilines, strongly suggesting a genetic component to size (Fjerdingstad, 2005). Finally, in the ant *Formica truncorum*, queen size was heritable in one of the two study years but not in the other, suggesting that the relative importance of environmental factors may vary over years (Bargum *et al.*, 2004). A genetic polymor-phism for body size and social structure may be main-tained by variability in ecological and social conditions causing disruptive selection on these traits (Ross & Keller, 1995).

An effect of the social environment on queen, male and worker body size has also been documented. Work-ers can control the amount of food that is allocated to brood (Wheeler, 1991). They can manipulate the size of the individuals produced in their colony in response to variation in food availability, colony age, or colony composition (Oster & Wilson, 1978; Wheeler & Nijhout, 1981; Wheeler, 1991). For example, the size of worker pupae increases with colony size and decreases with larval density in experimental colonies of *S. invicta* (Porter & Tschinkel, 1985). In *Pheidole pallidula*, the production of large workers specialized in colony-defence increases when resident workers perceive the presence of foreign workers close to their nest (Passera *et al.*, 1996). Finally, workers may preferentially eliminate or under-feed one type of brood (e.g. males) to funnel resources towards their preferred sex (Chapuisat *et al.*, 1997; Starks & Poe, 1997; Bourke, 2005; Meunier *et al.*, 2008).

We here investigate the proximate causes of queen size variation in the socially polymorphic ant *Formica selysi*. In our study population, monogyne and polygyne colonies coexist in the same habitat (Chapuisat *et al.*, 2004). In the field, colony social structure is associated with a signif-icant difference in the body size of the new queens (Rosset & Chapuisat, 2007). Specifically, monogyne colonies produce queens that have a 59% higher dry weight and a 2.1% larger head width than queens originating from polygyne colonies, whereas the males produced in the two types of colonies have the same size (Rosset & Chapuisat, 2007). In addition to this divergence

in the size of queens produced, monogyne and polygyne colonies differ in multiple life-history traits such as colony size, nest density, colony lifespan and worker size (Schwander *et al.*, 2005; Rosset & Chapuisat, 2007).

One interesting aspect of our study population is that there is no genetic differentiation at neutral markers between the monogyne and polygyne social forms and no sign of assortative mating with respect to social form (Chapuisat *et al.*, 2004; A. Reber, J. Meunier & M. Chapuisat, unpublished). Because sex and recombination tend to break allelic associations between multiple loci, the maintenance of two social forms in sympatry suggests that the primary switch towards the development of alternative social structure is triggered by a single polymorphic gene or tightly linked group of genes, as in the fire ant *S. invicta* (Keller & Ross, 1993b; Ross & Keller, 1998). Alternatively, the social forms may result from phenotypic plasticity depending on maternal, environmental or social factors affecting the developmental trajectory of the colony (West-Eberhard, 1989; Chapuisat *et al.*, 2004).

The aim of this study was to test if the size variation in young queens of *F. selysi* was caused by a genetic polymorphism or maternal effect present in the eggs, or by the social structure of the workers raising the brood. We cross-fostered eggs among experimental colonies, so that eggs collected from either monogyne or polygyne colonies were raised by workers originating from either monogyne or polygyne colonies. If the size of new queens is influenced by genes or maternal effects transmitted to the eggs, we expect that eggs from monogyne colonies will develop into larger queens than eggs from polygyne colonies, independently of the origin of the workers caring for the brood. In contrast, if the social environment influences the size of new queens, we expect that monogyne workers will produce larger queens than polygyne workers, independently of the origin of eggs. Understanding the causes of queen size variation will help to understand how the feedback loop linking queen number, queen size and colony founding may shape and stabilize social structure variation.

## Materials and methods

### Study species

Our study population of the ant *F. selysi* is located between Sierre and Susten along the river Rhône in central Valais, Switzerland (7°36′30′E, 46°18′30′N, altitude 565 m). The social structure (monogyne or polygyne) of each colony used in this experiment had been previously determined by genotyping eight to 100 workers per colony at nine microsatellite markers (Chapuisat *et al.*, 2004; Schwander *et al.*, 2005; Rosset & Chapuisat, 2006). Repeated sampling and genotyping of individuals in the same colonies over several years confirmed that the colonies have stable social structures (see Reber *et al.*, 2008).

In *F. selysi* queens lay eggs in spring and summer and no brood overwinters (Forel, 1920; Gösswald, 1989). Eggs laid early in the season (April) develop into queens and males, whereas eggs laid in late spring and summer become workers. For the cross-fostering experiment, we collected eggs and workers during the first week of April 2006. For the comparison of egg volumes, we sampled eggs on 5 April and 15 May 2007.

### Cross-fostering experiment

We cross-fostered eggs and workers to obtain the four following combinations: (1) workers from monogyne colonies with eggs from monogyne colonies, (2) workers from monogyne colonies with eggs from polygyne colonies, (3) workers from polygyne colonies with eggs from monogyne colonies and (4) workers from polygyne colonies with eggs from polygyne colonies (Fig. 1). In total, we used eggs and workers originating from 56 independent field colonies (28 monogyne and 28
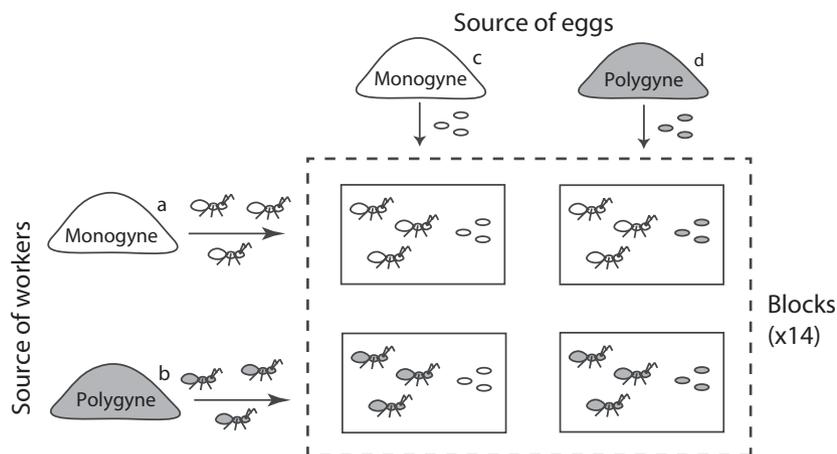


**Fig. 1** Cross-fostering design. The dashed line represents one 'block', which includes four experimental colonies. The experiment had a total of 14 such blocks. Colonies *a*, *b*, *c* and *d* are independent field colonies that were used in only one block of the experiment.

polygyne ones respectively). The cross-fostering was organized in 14 blocks, each block corresponding to the crossing of eggs and workers among four independent field colonies (Fig. 1). Hence, each block comprised four experimental colonies that had received workers from one polygyne or one monogyne field colony, and eggs from another monogyne or polygyne field colony respectively (Fig. 1).

Each experimental colony started with 290 ± 30 workers and 55 ± 6 eggs. They were established in plastic boxes (27 × 43 × 16 cm) side-lined with fluon and covered by a net to prevent ants from escaping. Each box contained ground material from the field nest of the workers and two test tubes with wet cotton wool that served as shelter for the brood and workers. Ants in experimental colonies had access to *ad libitum* water, 10% sugar water, and a protein-rich jelly provided on a daily basis (mix of beef meat, chicken eggs, mealworms, Oranol© (Bayer AG, Zürich, Switzerland) Vitamins for baby, sugar, salt, gelatine and water). They were kept at 25 ± 2 °C under a 14 : 10 h light : dark cycle.

### Size measurement

We measured the fresh weight of queens and males produced in the experimental colonies at two points in their development, namely at the callow and mature stages. The size of the males will serve as an internal control to check that the cross-fostering experiment is not affected by confounded factors. Callow individuals are very young adults that have just finished metamorphosis and are recognizable by their pale colour and soft cuticles. Queen and male callows appeared 51 ± 4 days (mean ± SD) and 56 ± 8 days after the start of the experiment respectively. Mature individuals were measured when they were ready to fly away from the nests. Mature males started to fly 10 ± 7 days after they had reached the callow stage and were weighed at this time. Because queens rarely fly under laboratory conditions, we weighed queens 30 days after the callow stage, which corresponds to the time period after which queens take flight in natural conditions. Mature males and queens were then stored at −20 °C until further analyses.

We measured the dry weight of queens ($n = 102$) and males ($n = 409$) to the nearest 0.001 mg, after having desiccated them for 24 h at 55 °C (Rosset & Chapuisat, 2007). We measured their head width (minimum width across the eyes) to the nearest $10^{-3}$ mm using a stereomicroscope Nikon V-12 (Nikon AG, Kusnächt, Switzerland) at a magnification of 50× (see Schwander *et al.*, 2005). Finally, we extracted lipids from the thorax and abdomen of queens by placing them in petroleum ether at 52 °C for 48 h. After this extraction, the queens were dried again for 24 h, re-weighed, and the lipid content was estimated as dry weight loss between the two measures divided by the dry weight before extraction (Keller & Passera, 1989).

We examined whether egg size differed between the two social forms, which would suggest a maternal effect based on the amount of resources packed in the eggs. We estimated the volume and shape of queen- and male-destined eggs sampled from 19 monogyne and 19 polygyne field colonies on 5 April 2007. We also measured worker-destined eggs collected from a subsample of nine monogyne and 15 polygyne field colonies on 15 May 2007. We used the IMAGEJ© v1.40 Software (http://rsbweb.nih.gov/ij/download.html) to measure the width and length of 15 eggs per colony to the nearest 0.001 mm. We estimated egg volume with the ovoid formula $4\pi/3 \times (\text{width}/2)^2 \times (\text{length}/2)$, and egg shape with the ratio of length on width.

### Statistical analysis

We analysed the fresh weight, dry weight, head width and lipid content of queens and males using two-way analyses of variance (ANOVA) in which the social structure of origin of eggs and workers (monogyne or polygyne) were used as fixed factors. We also tested the influence of these factors on the gain of fresh weight between the callow and mature stages. In all ANOVA, we used one mean value per experimental colony to avoid pseudo-replication because of the nonindependence of individuals that matured in the same colony. Moreover, we used 'blocks' as a random factor (mixed models), because some of the experimental colonies did not produce queens or males (Table 1) and each set of four field colonies might have had specific characteristics influencing the morphological traits of the individuals produced in a block (Fig. 1). We controlled that there was no significant correlation between the number of queens or males produced in each colony and their mean fresh weight, dry weight, head width and lipid content (Spearman's rank correlation tests, all *P*-values > 0.20).

To compare the production of queens and males in each type of experimental cross-fostering, we computed a two-way ANOVA on the odds of males and queens produced, using the social origin of eggs and workers as fixed factors. Finally, we compared egg volumes using a two-way ANOVA in which the social origin of eggs and date of sampling (5 April or 15 May) were entered as fixed factors. Since in some cases eggs were collected in the same field colonies on the two sampling dates, we entered the colony of origin as a random factor (mixed model). To achieve residual normality, dry weights, head widths and egg volumes were log-transformed.

## Results

### Queen and male size

Eggs originating from monogyne colonies developed into larger queens than eggs originating from polygyne colonies, independently of the social structure of the

**Table 1** Queens and males produced in the cross-fostering experiment.

| | Types of cross-fosterings in experimental colonies | | | |
| | Monogyne eggs | | Polygyne eggs | |
| | Monogyne workers | Polygyne workers | Monogyne workers | Polygyne workers |
| --- | --- | --- | --- | --- |
| Queens | | | | |
| Fresh weight of callows (mg) | 27.71 ± 1.16 | 28.37 ± 1.61 | 25.53 ± 0.94 | 25.41 ± 0.67 |
| Fresh weight of mature individuals (mg) | 32.15 ± 1.98 | 30.18 ± 1.99 | 28.27 ± 1.41 | 25.81 ± 1.15 |
| Dry weight (mg) | 14.71 ± 1.10 | 12.98 ± 1.40 | 12.27 ± 1.0 | 10.35 ± 0.59 |
| Head width (mm) | 1.24 ± 0.02 | 1.23 ± 0.03 | 1.21 ± 0.03 | 1.21 ± 0.01 |
| Lipid content (ratio) | 0.23 ± 0.01 | 0.20 ± 0.01 | 0.20 ± 0.02 | 0.19 ± 0.01 |
| No. queen-producing colonies | 7 | 4 | 10 | 6 |
| No. queens produced per colony | 3.86 ± 1.20 | 4.75 ± 2.05 | 3.20 ± 0.89 | 4.00 ± 1.37 |
| Survival rate of queens from emergence to day 30 | 0.95 ± 0.05 | 0.85 ± 0.12 | 0.85 ± 0.07 | 0.92 ± 0.06 |
| Males | | | | |
| Fresh weight of callows (mg) | 20.33 ± 0.38 | 19.97 ± 0.44 | 20.03 ± 0.38 | 19.64 ± 0.33 |
| Fresh weight of mature individuals (mg) | 15.86 ± 0.22 | 15.98 ± 0.28 | 15.89 ± 0.30 | 15.63 ± 0.33 |
| Dry weight (mg) | 4.89 ± 0.09 | 4.91 ± 0.11 | 4.89 ± 0.09 | 4.70 ± 0.14 |
| Head width (mm) | 0.93 ± 0.01 | 0.94 ± 0.01 | 0.93 ± 0.01 | 0.93 ± 0.01 |
| No. male-producing colonies | 13 | 13 | 13 | 10 |
| No. males produced per colony | 10.38 ± 0.51 | 4.85 ± 0.32 | 11.77 ± 0.63 | 6.40 ± 0.37 |
| Survival rate of males from emergence to flight | 0.86 ± 0.06 | 0.91 ± 0.04 | 0.91 ± 0.11 | 0.89 ± 0.05 |

Dry weight, head width and lipid content were measured on mature individuals. Mean measures are provided with standard errors.

workers caring for them. Specifically, queens originating from eggs sampled in monogyne colonies reached a significantly higher fresh weight (Table 1; callow stage: $F_{1,10} = 9.19$, $P = 0.013$; mature stage: $F_{1,10} = 7.94$, $P = 0.018$), higher dry weight (Table 1; $F_{1,10} = 6.79$, $P = 0.026$) and larger head width (Table 1; $F_{1,10} = 6.53$, $P = 0.029$) than queens coming from eggs sampled in polygyne colonies. Moreover, they tended to have more lipids, even if this last difference was not statistically significant (Table 1; $F_{1,10} = 2.84$, $P = 0.12$).

In contrast, the monogyne or polygyne origin of the workers caring for the brood had little effect on queen size. Specifically, the social structure of origin of workers had no significant effect on queen fresh weight (Table 1; callow stage: $F_{1,10} = 0.001$, $P = 0.97$, mature stage: $F_{1,10} = 1.77$, $P = 0.21$), dry weight (Table 1; $F_{1,10} = 2.17$, $P = 0.17$), head width (Table 1; $F_{1,10} = 0.17$, $P = 0.69$) and lipid content (Table 1; $F_{1,10} = 1.57$, $P = 0.24$). The only significant difference was that queens raised by workers from monogyne colonies gained more fresh weight between the callow and mature stages than queens reared by workers from polygyne colonies (Table 1; $F_{1,11} = 4.84$, $P = 0.050$). This gain of fresh weight did not differ significantly between queens originating from eggs sampled in monogyne or polygyne colonies (Table 1; $F_{1,11} = 2.18$, $P = 0.17$). Finally, there was no significant interaction between the social origin of workers and the social origin of eggs with respect to queen fresh weight (callow stage: $F_{1,10} = 0.50$, $P = 0.50$; mature stage: $F_{1,10} = 0.063$, $P = 0.81$), dry weight ($F_{1,10} = 0.08$, $P = 0.78$), head width ($F_{1,10} = 0.28$, $P = 0.61$) and lipid content ($F_{1,10} = 0.080$, $P = 0.78$).

The size of males produced in experimental colonies was independent of the social structure of origin of either eggs or workers. Specifically, the fresh weight of males was not significantly affected by the origin of eggs (Table 1; callow stage, $F_{1,32} = 0.81$, $P = 0.37$; mature stage, $F_{1,32} = 0.15$, $P = 0.70$), nor by the origin of the workers caring for these eggs (Table 1; callow stage: $F_{1,32} = 0.76$, $P = 0.39$; mature stage: $F_{1,32} = 0.01$, $P = 0.92$). We also found no significant effect of the origin of eggs or workers on male head width (Table 1; eggs: $F_{1,32} = 0.02$, $P = 0.89$; workers: $F_{1,32} = 2.17$, $P = 0.15$), on male dry weight (Table 1; eggs: $F_{1,32} = 0.91$, $P = 0.35$; workers: $F_{1,32} = 0.43$, $P = 0.52$), nor on the variation of male fresh weight between the callow and mature stages (Table 1; eggs: $F_{1,33} = 0.30$, $P = 0.59$; workers: $F_{1,33} = 1.42$, $P = 0.24$). Finally, there was no significant interaction between the social origin of workers and the social origin of eggs with respect to male fresh weight (callow stage: $F_{1,32} = 0.06$, $P = 0.94$; mature stage: $F_{1,32} = 0.39$, $P = 0.54$), dry weight ($F_{1,32} = 0.96$, $P = 0.33$) and head width ($F_{1,32} < 0.001$, $P = 0.99$).

The number of queens and males produced in experimental colonies varied from 0 to 10 and from 0 to 22 respectively. The number of queens produced was not significantly affected by the origin of eggs or workers (Table 1; $\chi^2 = 1.63$, $P = 0.65$). In contrast, experimental colonies containing workers originating from monogyne colonies produced significantly more males than experimental colonies containing workers originating from polygyne colonies (Table 1; $\chi^2 = 20.92$, $P < 0.001$). Experimental colonies with monogyne workers also produced a significantly higher proportion of males than colonies with polygyne workers (ANOVA on odds ratios, $F_{2,44} = 5.64$, $P = 0.007$), whereas this proportion was not significantly

influenced by the social origin of eggs ($F_{2,44} = 0.44$, $P = 0.65$) or the interaction between the social origin of eggs and the social origin of workers ($F_{2,44} = 0.55$, $P = 0.58$). Finally, the proportion of queens and males that died between the callow and mature stages did not differ significantly among the types of experimental cross-fosterings (Table 1; queens: Fisher's exact test, $P = 0.46$; males: chi-square test, $\chi^2_3 = 1.92$, $P = 0.59$).

### Egg size

Eggs sampled in monogyne field colonies were 25% smaller than eggs sampled in polygyne field colonies (Fig. 2; $F_{1,37} = 14.68$, $P < 0.001$). The worker-destined eggs sampled on 15 May were 28.5% smaller than the queen- and male-destined eggs sampled on 5 April (Fig. 2; $F_{1,21} = 81.12$, $P < 0.001$), but there was no significant interaction between colony social structure and date of sampling ($F_{1,21} = 1.67$, $P = 0.21$). The difference in egg volume was not associated with a difference in egg shape, as the ratio of length on width was not significantly different between eggs sampled in monogyne and polygyne colonies [5 April 2007: monogyne colonies: $2.05 \pm 0.06$ (mean $\pm$ SD), polygyne colonies: $2.02 \pm 0.08$, $t$-test, $t = 0.90$, d.f. $= 36$, $P = 0.38$; 15 May 2007: monogyne colonies: $1.95 \pm 0.07$, polygyne colonies: $1.94 \pm 0.07$, $t$-test, $t = 0.33$, d.f. $= 22$, $P = 0.74$].

### Discussion

The determination of queen size in ants is potentially complex and can be affected by genes, maternal effects and/or the social environment. Moreover, the size of



**Fig. 2** Volume of the eggs sampled in monogyne (M) and polygyne colonies (P). Eggs collected on 5 April 2007 mainly develop into queens and males, whereas eggs collected on 15 May 2007 only develop into workers. Sample sizes are provided at the bottom of each bar (number of colonies, average egg size based on 15 eggs measured per colony); ***$P < 0.001$.

young queens can play a central role in the transmission of social structure if it influences dispersal, colony founding and queen number in future colonies (Bourke & Franks, 1995; Wiernasz & Cole, 2003). Alternative social structures (monogyne or polygyne) indeed correlate with queen size across and within species, but the proximate factors at the basis of this relationship remain poorly understood (Keller & Passera, 1989; Bourke & Franks, 1995; Rosset & Chapuisat, 2007).

Our cross-fostering experiment in the socially polymorphic ant *F. selysi* demonstrates that the association between the social structure of the colony and the size of the new queens produced results from genetic or maternal effects transmitted to the eggs. Specifically, queens originating from eggs sampled in monogyne colonies became larger than queens developing from eggs sampled in polygyne colonies, independently of the origin of the workers that cared for the brood.

It is generally difficult to disentangle genetic and maternal effects in cross-fostering experiments. In social Hymenoptera, several studies have indicated a positive association between the sizes of old and new queens (Rüppell *et al.*, 2001) or a genetic component to queen size (Ross & Keller, 1998; Bargum *et al.*, 2004; Fjerdingstad, 2005). One important potential source of maternal effect is egg size, which in Hymenoptera is generally correlated with the body weight of the laying female (Berrigan, 1991). Hence, large queens might simply pack more resources into larger eggs that would develop into larger queens. In *F. selysi*, we found the opposite pattern, as eggs collected in monogyne colonies were significantly smaller but developed into significantly larger queens than eggs collected in polygyne colonies. Our results therefore suggest that the variation in queen size between monogyne and polygyne colonies is not caused by a maternal effect linked to the amount of resources packed into the eggs, and is probably affected by a genetic polymorphism, even if other forms of maternal effects are also possible. Similar conclusions had been reached for the ant *L. rugatulus*, in which macrogyne queens produced larger queens, but eggs of similar size, than microgyne queens (Rüppell *et al.*, 2001). To further distinguish between genetic and maternal effects in *F. selysi* would require us mating the queens with one male of each social structure and looking at queen size differences between patrilines.

Why do queens from monogyne colonies produce smaller eggs? One simple explanation would be that there is a trade-off between egg size and egg number (Kayaalp & Schwarz, 2007). Queens in monogyne colonies have to supply all the eggs for their colony, and have therefore a much higher egg-laying rate than queens from polygyne colonies (Vander Meer *et al.*, 1992). It is also possible that queens from polygyne colonies have to lay larger eggs because they compete with each other and workers preferentially rear large eggs. In line with this hypothesis, eggs originating from polygyne colonies had a higher
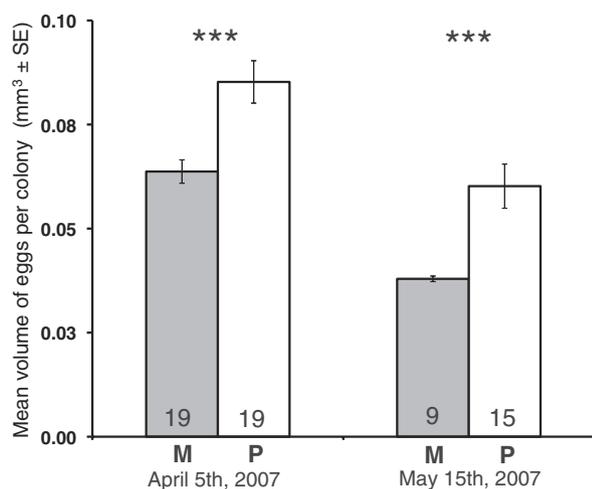
acceptance rate than eggs originating from monogyne colonies when they were introduced into groups of unrelated workers from each social structure (J. Meunier & M. Chapuisat, unpublished).

One major component of the social environment, namely the social structure of the workers caring for the brood, had no significant impact on the size of young queens at the end of the rearing period. However, we detected a small difference in the developmental trajectory of queens. Specifically, queens raised by workers originating from monogyne colonies gained slightly more fresh weight between the callow and mature stages than queens reared by polygyne workers, and this independently of the origin of eggs. A similar pattern has been documented in *S. invicta* (Keller & Ross, 1993a). Workers probably influence this variation in fresh weight by controlling the amount and quality of food given to young queens. In *F. selysi*, monogyne workers are larger than polygyne ones (Schwander *et al.*, 2005), and might thus be more efficient at feeding adult queens.

In contrast to queens, we found no significant difference in the size of males produced in the four types of experimental cross-fosterings. This result is in line with the observation that monogyne and polygyne colonies produce males of similar weight in the field (Rosset & Chapuisat, 2007). It is also an important internal control demonstrating that the differences in queen size were not because of some uncontrolled factor affecting brood development and varying between the four types of experimental cross-fosterings.

Interestingly, the origin of the workers did not significantly affect the number of queens produced but had a significant impact on the number of males produced. Experimental colonies with workers originating from monogyne colonies produced twice as many males as colonies with workers originating from polygyne colonies. As in many other species of the genus *Formica* (Helanterä & Sundström, 2007), young workers of *F. selysi* start to lay unfertilized haploid eggs shortly after being orphaned. Hence, part of the males produced in our experimental queenless colonies probably came from worker-laid eggs. Differences in male production between workers originating from monogyne or polygyne workers might thus be because of (i) monogyne workers laying more eggs than polygyne workers; (ii) polygyne workers destroying more worker-laid eggs than monogyne workers (policing, reviewed in Wenseleers & Ratnieks, 2006); and/or (iii) eggs laid by polygyne workers being less viable than eggs laid by monogyne workers. Further experiments will be needed to sort among these hypotheses.

To conclude, our cross-fostering experiment indicates that queen size in the socially polymorphic ant *F. selysi* is primarily influenced by a genetic polymorphism or possibly a maternal effect transmitted to the eggs independently of egg size. The fact that queen size is inherited may play a role in determining the social structure of future colonies, because queen size is likely to affect the success of independent colony founding by single queens (Wiernasz & Cole, 2003), and small queens may be constrained to found new colonies by budding. Further experiments will be needed to evaluate if the size and social origin of queens affect their colony founding success or acceptance rate by workers. These experiments will reveal if there is a strong and causal relationship between queen size and colony queen number, or if this association is just one component of a broader behavioural syndrome determining social structure variation.

## References

Bargum, K., Boomsma, J.J. & Sundström, L. 2004. A genetic component to size in queens of the ant, *Formica truncorum*. *Behav. Ecol. Soc.* **57**: 9–16.

Bargum, K., Helanterä, H. & Sundström, L. 2007. Genetic population structure, queen supersedure and social polymorphism in a social Hymenoptera. *J. Evol. Biol.* **20**: 1351–1360.

Bernardo, J. 1996. Maternal effects in animal ecology. *Am. Zool.* **36**: 83–105.

Berrigan, D. 1991. The allometry of egg size and number in insects. *Oikos* **60**: 313–321.

Bourke, A.F.G. 2005. Genetics, relatedness and social behaviour in insect societies. In: *Insect Evolutionary Ecology* (M.D.E. Fellowes, G.J. Holloway & J. Rolff, eds), pp. 1–30. CABI Publishing, Wallingford.

Bourke, A.F.G. & Franks, N.R. 1995. *Social Evolution in Ants*. Princeton University Press, Princeton, NJ.

Burd, M. 2000. Body size effects on locomotion and load carriage in the highly polymorphic leaf-cutting ants *Atta colombica* and *Atta cephalotes*. *Behav. Ecol.* **11**: 125–131.

Byers, J.E. 2000. Effects of body size and resource availability on dispersal in a native and a non-native estuarine snail. *J. Exp. Mar. Biol. Ecol.* **248**: 133–150.

Chapuisat, M. & Keller, L. 1999. Testing kin selection with sex allocation data in eusocial Hymenoptera. *Heredity* **82**: 473–478.

Chapuisat, M., Sundström, L. & Keller, L. 1997. Sex ratio regulation: the economics of fratricide in ants. *Proc. R. Soc. Lond. Ser. B* **264**: 1255–1260.

Chapuisat, M., Bocherens, S. & Rosset, H. 2004. Variable queen number in ant colonies: no impact on queen turnover, inbreeding, and population genetic differentiation in the ant *Formica selysi*. *Evolution* **58**: 1064–1072.

Emlen, D.J. & Nijhout, H.F. 2000. The development and evolution of exaggerated morphologies in insects. *Ann. Rev. Ent.* **45**: 661–708.

Fjerdingstad, E.J. 2005. Control of body size of *Lasius niger* ant sexuals: worker interests, genes and environment. *Mol. Ecol.* **14**: 3123–3132.

Forel, A. 1920. *Les fourmis de la Suisse*, 2ème edn. Imprimerie coopérative, La Chaux-de-Fond.

Gonzaga, M.O. & Vasconcellos-Neto, J. 2001. Female body size, fecundity parameters and foundation of new colonies in *Anelosimus jabaquara* (Araneae, Theridiidae). *Ins. Soc.* **48**: 94–100.

Gösswald, K. 1989. *Die Waldameise. Band 1. Biologische Grundlagen, Ökologie und Verhalten*. Aula-Verlag, Wiesbaden.

Gyllenstrand, N., Seppä, P. & Pamilo, P. 2005. Restricted gene flow between two social forms in the ant *Formica truncorum*. *J. Evol. Biol.* **18**: 978–984.

Helanterä, H. & Sundström, L. 2007. Worker reproduction in Formica ants. *Am. Nat.* **170**: E14–E25.

Hölldobler, B. & Wilson, E.O. 1990. *The Ants*. Springer-Verlag, Berlin.

Honek, A. 1993. Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos* **66**: 483–492.

Jamieson, I.G. 2002. The relationship between male head size and harem size in the sexually dimorphic mountain stone *weta Hemideina maori*. *Ecol. Ent.* **27**: 41–48.

Kayaalp, P. & Schwarz, M.P. 2007. Egg size and number is influenced by both environmental and social factors in a facultatively social bee. *Aust. J. Zool.* **55**: 357–362.

Keller, L. 1991. Queen number, mode of colony founding and queen reproductive success in ants (Hymenoptera, Formicidae). *Ethol. Ecol. Evol.* **3**: 307–316.

Keller, L. & Passera, L. 1989. Size and fat content of gynes in relation to the mode of colony founding in ants (Hymenoptera; Formicidae). *Oecologia* **80**: 236–240.

Keller, L. & Ross, K. 1993a. Phenotypic plasticity and ''cultural transmission'' of alternative social organizations in the fire ant *Solenopsis invicta*. *Behav. Ecol. Soc.* **33**: 121–129.

Keller, L. & Ross, K.G. 1993b. Phenotypic basis of reproductive success in a social insect: genetic and social determinants. *Science* **260**: 1107–1110.

Keller, L. & Ross, K.G. 1999. Major gene effects on phenotype and fitness: the relative roles of Pgm-3 and Gp-9 in introduced populations of the fire ant *Solenopsis invicta*. *J. Evol. Biol.* **12**: 672–680.

Kovacs, J.L., Hoffman, E.A. & Goodisman, M.A.D. 2008. Mating success in the polyandrous social wasp *Vespula maculifrons*. *Ethology* **114**: 340–350.

Lindenfors, P. & Tullberg, B.S. 1998. Phylogenetic analyses of primate size evolution: the consequences of sexual selection. *Biol. J. Linn. Soc.* **64**: 413–447.

Linksvayer, T.A. 2006. Direct, maternal, and sibsocial genetic effects on individual and colony traits in an ant. *Evolution* **60**: 2552–2561.

Meunier, J., West, S.A. & Chapuisat, M. 2008. Split sex ratios in the social Hymenoptera: a meta-analysis. *Behav. Ecol.* **19**: 382–390.

Oster, G.F. & Wilson, E.O. 1978. *Caste and Ecology in the Social Insects*. Princeton University Press, Princeton, NJ.

Paradis, E., Baillie, S.R., Sutherland, W.J. & Gregory, R.D. 1998. Patterns of natal and breeding dispersal in birds. *J. Anim. Ecol.* **67**: 518–536.

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

Porter, S.D. & Tschinkel, W.R. 1985. Fire ant polymorphism (Hymenoptera: Formicidae): factors affecting worker size. *Ann. Ent. Soc. Am.* **78**: 381–386.

Reber, A., Castella, G., Christe, P. & Chapuisat, M. 2008. Experimentally increased group diversity improves disease resistance in an ant species. *Ecol. Lett.* **11**: 682–689.

Roff, D.A. 1992. *The Evolution of Life Histories*. Chapman & Hall, New York.

Rosenheim, J.A., Nonacs, P. & Mangel, M. 1996. Sex ratios and multifaceted parental investment. *Am. Nat.* **148**: 501–535.

Ross, K.G. 1997. Multilocus evolution in fire ants: effects of selection, gene flow and recombination. *Genetics* **145**: 961–974.

Ross, K.G. & Keller, L. 1995. Ecology and evolution of social organization: insights from fire ants and other highly eusocial insects. *Ann. Rev. Ecol. Syst.* **26**: 631–656.

Ross, K.G. & Keller, L. 1998. Genetic control of social organization in an ant. *Proc. Natl Acad. Sci. USA* **95**: 14232–14237.

Rosset, H. & Chapuisat, M. 2006. Sex allocation conflict in ants: when the queen rules. *Curr. Biol.* **16**: 328–331.

Rosset, H. & Chapuisat, M. 2007. Alternative life-histories in a socially polymorphic ant. *Evol. Ecol.* **21**: 577–588.

Rüppell, O. & Heinze, J. 1999. Alternative reproductive tactics in females: the case of size polymorphism in winged ant queens. *Ins. Soc.* **46**: 6–17.

Rüppell, O., Heinze, J. & Hölldobler, B. 1998. Size-dimorphism in the queens of the north American ant *Leptothorax rugatulus* (Emery). *Ins. Soc.* **45**: 67–77.

Rüppell, O., Heinze, J. & Holldobler, B. 2001. Complex determination of queen body size in the queen size dimorphic ant *Leptothorax rugatulus* (Formicidae : Hymenoptera). *Heredity* **87**: 33–40.

Schorr, R.A., Lukacs, P.M. & Florant, G.L. 2009. Body mass and winter severity as predictors of overwinter survival in preble's meadow jumping mouse. *J. Mammal.* **90**: 17–24.

Schwander, T., Rosset, H. & Chapuisat, M. 2005. Division of labour and worker size polymorphism in ant colonies: the impact of social and genetic factors. *Behav. Ecol. Soc.* **59**: 215–221.

Starks, P.T. & Poe, E.S. 1997. ''Male-stuffing'' in waps societies. *Nature* **389**: 450.

Stearns, S.C. 1992. *The Evolution of Life Histories*. Oxford University Press, Oxford.

Sundström, L. 1995. Dispersal polymorphism and physiological condition of males and females in the ant *Formica truncorum*. *Behav. Ecol.* **6**: 132–139.

Vander Meer, R.K., Morel, L. & Lofgren, C.S. 1992. A comparison of queen oviposition rates from monogyne and polygyne fire ant, *Solenopsis invicta*, colonies. *Phys. Ent.* **17**: 384–390.

Wenseleers, T. & Ratnieks, F.L.W. 2006. Comparative analysis of worker reproduction and policing in eusocial hymenoptera supports relatedness theory. *Am. Nat.* **168**: E163–E179.

West-Eberhard, M.J. 1989. Phenotypic plasticity and the origins of diversity. *Ann. Rev. Ecol. Syst.* **20**: 249–278.

Wheeler, D.E. 1986. Developmental and physiological determinants of caste in social Hymenoptera: evolutionary implications. *Am. Nat.* **128**: 13–34.

Wheeler, D.E. 1991. The developmental basis of worker caste polymorphism in ants. *Am. Nat.* **138**: 1218–1238.

Wheeler, D.E. & Nijhout, H.F. 1981. Soldier determination in ants: new role for juvenile hormone. *Science* **213**: 361–363.

Wiernasz, D.C. & Cole, B.J. 2003. Queen size mediates queen survival and colony fitness in harvester ants. *Evolution* **57**: 2179–2183.