

The influence of social structure on brood survival and development in a socially polymorphic ant: insights from a cross-fostering experiment

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Abstract

Animal societies vary in the number of breeders per group, which affects many socially and ecologically relevant traits. In several social insect species, including our study species *Formica selysi*, the presence of either one or multiple reproducing females per colony is generally associated with differences in a suite of traits such as the body size of individuals. However, the proximate mechanisms and ontogenetic processes generating such differences between social structures are poorly known. Here, we cross-fostered eggs originating from single-queen (= monogynous) or multiple-queen (= polygynous) colonies into experimental groups of workers from each social structure to investigate whether differences in offspring survival, development time and body size are shaped by the genotype and/or pre-foster maternal effects present in the eggs, or by the social origin of the rearing workers. Eggs produced by polygynous queens were more likely to survive to adulthood than eggs from monogynous queens, regardless of the social origin of the rearing workers. However, brood from monogynous queens grew faster than brood from polygynous queens. The social origin of the rearing workers influenced the probability of brood survival, with workers from monogynous colonies rearing more brood to adulthood than workers from polygynous colonies. The social origin of eggs or rearing workers had no significant effect on the head size of the resulting workers in our standardized laboratory conditions. Overall, the social backgrounds of the parents and of the rearing workers appear to shape distinct survival and developmental traits of ant brood.

Introduction

Phenotypic traits can be shaped by a number of interacting factors, including genotype, maternal effects and both abiotic and biotic conditions, particularly those experienced during early development (reviewed by Mousseau & Fox, 1998; Lindstrom, 1999; Dufty *et al.*, 2002). Many brood traits that have important fitness consequences, including brood survival, development time and offspring size, can be influenced by all of these factors, although the relative influence of each

varies among different organisms. Development time and offspring size are at least partially heritable traits in many vertebrates (in birds: Alatalo & Lundberg, 1986; e.g. in amphibians: Newman, 1988; in mammals: Kruk *et al.*, 2000) and invertebrates (e.g. Bradshaw *et al.*, 1997; Kause *et al.*, 2001; Purcell *et al.*, 2012). Offspring survival *per se* is unlikely to be highly heritable across all conditions, but gene by environment interactions are likely (e.g. in stressful conditions: Peripato *et al.*, 2002; in the presence of pathogens: Poulin & Thomas, 2008; Gagliano & McCormick, 2009). Many factors indirectly influencing the proportion of brood that survive may also have a genetic component (e.g. maternal rearing effort: Peripato & Cheverud, 2002; trophic egg production rate: Perry & Roitberg, 2006; sex and caste ratios: Frohschammer & Heinze, 2009). Prefoster maternal effects are often difficult to separate from

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genetic effects in brood rearing experiments, but factors such as the size and hormone content of eggs in birds (reviewed by Williams, 1994), fish (Heath *et al.*, 1999) and insects (e.g. Mousseau & Dingle, 1991; Schwander *et al.*, 2008) can influence the probability of brood survival, offspring body size and other developmental characteristics.

Environmental or social conditions experienced during development can also influence brood traits. The amount and quality of parental or alloparental care, which may depend on the social system or breeding system of the parents (Royle *et al.*, 1999; Olson *et al.*, 2008), influences brood survival rates and other developmental traits in a diverse range of species (e.g. social spiders: Avilés & Tufino, 1998; mice: Gubernick & Teferi, 2000; earwigs: Kolliker, 2007; eiders: Ost *et al.*, 2008). For example, development time was shorter and growth rate was greater in the presence of maternal care in a burying beetle with facultative care (Rauter & Moore, 2002). The environment experienced during early development, which includes factors such as climate, food resources, parasitism and predation, is also well known to influence brood survival (e.g. Brian, 1973), development time (e.g. Abril *et al.*, 2010) and body size (reviewed by Angilletta *et al.*, 2004). Moreover, environmental and genetic factors often interact during the ontogeny of the organism, jointly shaping the phenotype of both individuals and social groups (Keller & Ross, 1993, 1995; review of human studies: Shanahan & Hofer, 2005). For example, multiple reciprocal interactions and feedback loops can link gene expression in brood and the social environment in which the brood develops (Robinson *et al.*, 2008; Laland *et al.*, 2011).

Eusocial organisms provide an excellent opportunity to investigate the relative influence of factors present in the eggs (genotype and/or prefooster maternal effects) vs. those gained from the social environment experienced by the brood on their development and phenotype. First, the hallmark of eusociality is that reproduction and brood care are naturally performed by different individuals. Hence, the two processes can be experimentally decoupled with relative ease (Linksvayer & Wade, 2005; Linksvayer, 2006). Second, many social insect species exhibit intraspecific variation in the number of reproductives per social group. The presence of either one or multiple reproducing females in the colony is often associated with a suite of important individual and colony characteristics, such as body size, dispersal strategy or colony size (reviewed by Keller, 1995; Rosset & Chapuisat, 2007). Socially polymorphic species thus provide ideal systems to investigate the proximate mechanisms linking social structure change to natural variation in individual and colony traits, and to study the ontogeny of social traits.

Previous studies of eusocial insects have shown that both genetic and environmental factors can influence caste determination and within-caste body size (Linksvayer, 2006; Fournier *et al.*, 2008; Kovacs *et al.*, 2010; Schwander *et al.*, 2010; Libbrecht *et al.*, 2011; Linksvayer *et al.*, 2011; Rajakumar *et al.*, 2012).

Across species, the basis of caste determination systems varies from complete genetic determination to total environmental determination, with many species showing a combination of both factors (Schwander *et al.*, 2010). Within castes, variation in body size can also be influenced by factors in the eggs (Schwander *et al.*, 2005; Meunier & Chapuisat, 2009) and by the environment of the developing brood (e.g. origin or number of individuals providing brood care: Linksvayer, 2007; Purcell *et al.*, 2012). Less is known about the mechanisms underlying other brood traits, such as brood survival and brood development time (but see Howard & Jeanne, 2004; Purcell *et al.*, 2012).

The socially polymorphic ant *Formica selysi* exhibits variation in queen number within populations (Chapuisat *et al.*, 2004). In the field, single-queen (= monogynous) and multiple-queen (= polygynous) colonies differ in a number of ecologically important traits, including queen and worker body size, colony size and colony lifespan (Schwander *et al.*, 2005; Rosset & Chapuisat, 2007; Meunier & Chapuisat, 2009). For example, workers and queens produced by monogynous colonies are significantly larger. We are interested in how these differences arise: are they primarily mediated by factors present in the eggs, which include genes inherited from the parents and prefooster maternal effects, or does the social origin of the workers that rear the brood influence brood development in their colony? In addition, do other components of the ants' social and physical environments play a major role in these phenotypic differences?

A previous experimental manipulation of the worker-to-brood ratio during brood rearing showed that both the maternal origin of the eggs and the rearing conditions influenced offspring survival, development time and size (Purcell *et al.*, 2012). However, in the previous study, we used only eggs and workers originating from polygynous colonies, so we were not able to infer whether the social structure of queens or workers influenced brood development. In another experiment, we documented that sexual female offspring of monogynous queens were larger than the offspring of polygynous queens, even when the offspring were cross-fostered with workers from the other social form, which indicates that a genetic or prefooster maternal effect influences queen size (Meunier & Chapuisat, 2009). We do not yet know the mechanism underlying other differences between social forms, in particular those related to worker development.

Here, we seek differences between the monogynous and polygynous social forms in brood survival, development time and worker body size and ask whether these differences are due to the genotype and prefooster maternal effects inherited by the eggs, or to

the social environment provided by the workers rearing the brood. To this end, we cross-fostered worker-destined eggs sampled from single- or multiple-queen colonies into groups of workers collected from unrelated single- or multiple-queen colonies, respectively. We first investigate the survival rate and development time of the brood until eclosion into adult workers. To our knowledge, this is the first time that these traits are compared between monogynous and polygynous social forms. Next, we investigate the body size of newly eclosed adult workers. This will allow us to evaluate whether the larger body size of workers from monogynous colonies in the field (Schwander *et al.*, 2005) is due to genotype or pre-foster maternal effects present in the eggs, to the social origin of the workers that rear the brood, or to interactions with other environmental or social differences that were not included in our laboratory experiment. The results of this experiment will help to illuminate the proximate and ontogenetic causes of variation in multiple offspring traits that influence fitness and differ between social forms.

Methods

Study system

We performed this experiment using the ant *F. selysi*, which is polymorphic in colony queen number. We collected the individuals from a well-studied population along the Rhône River between Sierre and Susten in Valais, Switzerland (7°36'30"E, 46°18'30"N, altitude 565 m). The habitat consists of flood plain and steppe, and the focal nests are distributed in an area about 1500 × 400 m. The source colonies were individually marked and their social structure was previously assessed by genotyping workers at nine polymorphic microsatellite markers (Chapuisat *et al.*, 2004; J. Purcell & M. Chapuisat, unpublished). Colonies in our study population occasionally exhibit shifts (bidirectional) in their social structure (about 2% of colonies shift per year), but social structure is routinely monitored to ensure that each colony is correctly assigned (J. Purcell & M. Chapuisat, unpublished). There was no genetic differentiation between the two social forms at the nine microsatellite loci that have been investigated (Chapuisat *et al.*, 2004), but we cannot rule out a genetic basis for social structure variation, because these loci represent only a tiny proportion of the genome.

Formica selysi queens lay eggs during the spring and summer. The first clutch, often produced in early April (depending on the early season weather), usually develops into queens and males, whereas eggs laid later in the season tend to become workers (Rosset & Chapuisat, 2006; Meunier & Chapuisat, 2009). We therefore collected workers and eggs during the first week of May, 2011, about 1 month after the first brood were laid, to ensure that the majority of eggs would

develop into workers. This collection date also ensures that the sampled workers were produced during the past growing season (i.e. during the summer of 2010), so the ages of workers should be similar across the different colonies and social forms.

Cross-fostering experiment

Following the experimental design used by Meunier & Chapuisat (2009), we cross-fostered eggs with workers from different colonies to obtain the following four treatments: (i) eggs from a monogynous colony reared by workers from a different monogynous colony, (ii) eggs from a polygynous colony reared by workers from a monogynous colony, (iii) eggs from a monogynous colony reared by workers from a polygynous colony and (iv) eggs from a polygynous colony reared by workers from a different polygynous colony (Fig. S1). We collected eggs and workers from 40 field colonies (20 monogynous and 20 polygynous ones), and these were organized into ten blocks with two monogynous and two polygynous nests each. Each block contained a total of eight experimental groups, two in each of the four rearing treatments. Each field colony contributed workers to rear eggs from one monogynous and one polygynous colony and eggs to be reared by workers from one monogynous and one polygynous colony (Fig. S1, Table S1), so that neither eggs nor workers from a single colony were used more than once in each treatment. Ultimately, we had 15 experimental groups with monogynous workers rearing monogynous brood and 22 groups with polygynous workers rearing polygynous brood, due to a paucity of eggs in three monogynous field colonies and a shift from monogyny to polygyny in one colony prior to the start of the experiment (J. Purcell & M. Chapuisat, unpublished). The other two treatments each had 20 experimental groups.

Each experimental rearing group consisted of 50 workers and 50 eggs; we used a 1 : 1 worker/brood ratio because this configuration resulted in the largest number of offspring in a previous experiment (Purcell *et al.*, 2012). We first placed 50 workers in an individual plastic box (15 × 13 × 6 cm) lined with Fluon GP 1 (Whitford Plastics, Diez, Germany) to prevent ants from escaping. We then counted out 50 eggs on a petri dish, verified that the eggs were undamaged and immediately added the petri dish to the box containing the rearing group. Each rearing group was provided with *ad libitum* access to standard ant food (Meunier & Chapuisat, 2009), and water was supplied in two glass tubes with moist cotton wool. These rearing groups were maintained on the same shelf in a climate-controlled room at 24 ± 2 °C with 50% humidity and in a 12 : 12-h light/dark cycle.

In our study species *F. selysi* and the congeneric *Formica fusca*, workers tend to discriminate unrelated

eggs when they collect them for the first time, a process that has been studied extensively (Helanterä & Sundström, 2007; Helanterä & Ratnieks, 2009; Meunier *et al.*, 2010, 2011a). In particular, *F. selysi* workers tend to collect a greater proportion of foreign eggs of polygynous origin than foreign eggs of monogynous origin (Meunier *et al.*, 2011a) and more queen-laid eggs than worker-laid eggs (Meunier *et al.*, 2010). Here, we focus on brood survival once the eggs have been accepted by workers. For each rearing group, we therefore assessed the number of eggs that had been actually collected by workers after a period of 3 days. We then removed the eggs that were left uncollected and unguarded on the petri dish. In a few cases, eggs on the petri dish were still being actively guarded after the initial 3-day period, and in these cases, we left the petri dish in the rearing groups until all of the eggs had been collected or were left unguarded.

We estimated brood survival as the proportion of accepted eggs that successfully developed until the emergence of the callow workers. During egg and larval development, we inspected the rearing groups at least every 2 days. We counted the number of live brood remaining and monitored their development. After the first brood pupated, we checked the rearing groups daily and removed newly emerged callow workers, which are easy to recognize by their pale colour and soft cuticle. The callows were placed in a separate box and kept for further measurements. In this species, brood pupate either with or without a cocoon, so we also noted the presence or absence of a cocoon for each pupa.

We measured brood development time from the first observation of egg hatching in each rearing group until each individual emerged as a callow worker. In all boxes, hatching was synchronized, so most eggs in a group hatched within 1 day of each other. Finally, we measured the head size of the newly eclosed workers using a Leica S8APO microscope (Leica Application Suite 2.8.1, Leica Microsystems, Wetzlar, Germany). We accurately measured 330 callows; 14 were omitted due to escape or degradation of the corpse prior to measurement. Head size is a good proxy for overall body size in this species (Schwander *et al.*, 2005).

Statistical analysis

We investigated brood survival with parametric survival analyses. We used the `survreg` function implemented in 'survival' package in the R project for statistical computing (version 2.12; R Development Core Team, 2011). In the first model, we examined the effect of the social structure (monogynous or polygynous) of the colony on brood survival, decoupling the effects due to genotype and pre-foster maternal effects inherited by the eggs from the ones due to the workers that reared the brood. For this aim, we included the 'social origin of the eggs' (= social structure of the colony from which the

eggs originated), the 'social origin of the rearing workers' (= social structure of the colony from which the rearing workers originated) and the interaction term between the two factors as fixed effects. To control for the non-independence of the brood reared by the same group of workers, we included the rearing group identity as a random effect (Table S1). In a second model, we investigated whether there were colony-specific differences in brood survival. We included the colony identities of the egg source and rearing worker source as fixed effects, and the block as a random effect to control for variation resulting from the specific combinations of egg and rearing worker source colonies.

To examine the factors affecting brood development time and callow head size, we used linear mixed-effects models implemented using the 'nlme' package in R 2.10 (R Development Core Team, 2009). We included the social origin of the eggs, the social origin of the rearing workers, the interaction between the two and the size of the brood cohort (the number of brood that reached adulthood from each rearing group) as fixed effects in our initial models; the rearing group identity nested within block was included as a random effect (Table S1). To test for colony-specific differences in development and head size, we performed a second analysis with the colony identities of the egg source and rearing worker source as fixed effects and the block as a random effect.

We additionally investigated whether the presence of a cocoon was associated with development time or callow worker head size, whether the development duration was associated with head size and whether the growth rate, given by the head size divided by development duration, varied according to the social origin of eggs or workers. Finally, to further evaluate the respective influence of the social origin and source colony identity of eggs and workers, we calculated Cohen's *d* effect sizes for the relevant comparisons (see Table S2). In all cases where one data set was used in multiple analyses, we applied the Bonferroni correction to control for multiple comparisons.

Results

Brood survival

Brood survival until adult emergence strongly depended on the social origin of the eggs. Among the eggs that were collected by workers, eggs originating from polygynous colonies were more likely to survive to adulthood than eggs originating from monogynous colonies, regardless of the social origin of the rearing workers (full parametric survival model: $\chi^2 = 1134$, d.f. = 6, $P < 0.0001$; egg social origin: $\chi^2 = 45.5$, d.f. = 1, $P < 0.0001$). In contrast, workers originating from monogynous colonies were more successful at rearing brood to adulthood than workers originating from polygynous colonies, regardless

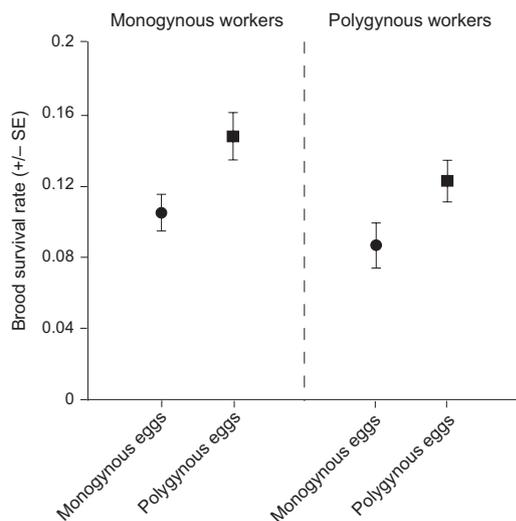


Fig. 1 Brood survival, measured as the proportion of collected eggs that developed to adulthood, in each of our four cross-fostering treatments. Brood survival varied significantly depending on the social background of both eggs and rearing workers: brood originating from polygynous colonies (squares) had higher survival rates than brood originating from monogynous colonies (circles) and workers originating from monogynous colonies (left panel) reared a higher proportion of brood than workers originating from polygynous colonies (right panel).

of the social origin of the brood (rearing worker social origin: $\chi^2 = 33.1$, d.f. = 1, $P < 0.0001$). There was no significant interaction between the social origin of the eggs and the social origin of the rearing workers ($\chi^2 = 0.015$, d.f. = 4, $P = 0.87$; Fig. 1). The survival of brood was significantly affected by the egg source colony identity and the rearing worker source colony identity (full parametric survival model: $\chi^2 = 1053$, d.f. = 77, $P < 0.0001$; egg source colony identity: $\chi^2 = 763$, d.f. = 38, $P < 0.0001$; rearing worker source colony identity: $\chi^2 = 291$, d.f. = 39, $P < 0.0001$). The mortality occurred primarily during the egg and early larval stages (Fig. S2).

Brood development duration

Eggs originating from polygynous colonies took longer to develop to adulthood than eggs originating from monogynous colonies, but neither the social origin of the rearing workers nor the interaction between the egg and rearing worker social origins influenced the brood development time (full linear mixed-effects model: $F_{7,337} = 8889$, $P < 0.0001$; egg social origin: $F_{1,343} = 8889$, $P = 0.017$; rearing worker social origin: $F_{1,342} = 0.011$, $P = 0.92$; interaction: $F_{4,337} = 0.869$, $P = 0.28$; Fig. 2). Cohort size was not significantly associated with development time ($F_{1,341} = 1.23$, $P = 0.36$). The identity of colonies supplying eggs and rearing workers was associated with brood development time (full model: $F_{39,304} = 2.60$, $P < 0.0001$; egg source colony identity:

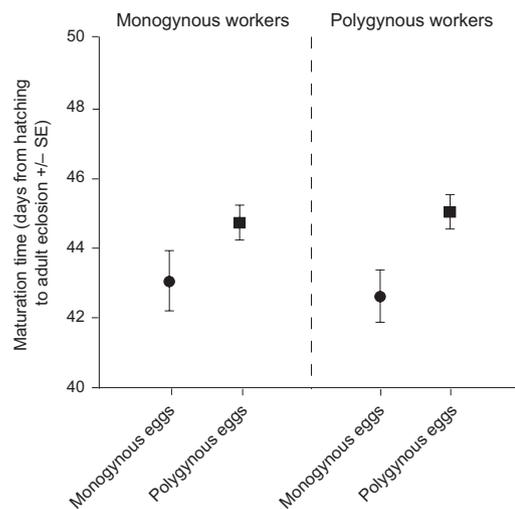


Fig. 2 Brood originating from monogynous colonies took less time to mature than brood originating from polygynous colonies, irrespective of the social background of the rearing workers.

$F_{27,316} = 2.81$, $P < 0.0001$; rearing worker source colony identity: $F_{12,304} = 2.11$, $P = 0.016$). The individuals that pupated in cocoons tended to take an average of 2.3 days longer to develop than individuals that pupated without forming cocoons ($t_{304} = 3.50$, $P = 0.0005$).

Offspring size

Neither the social origin of the eggs nor of the rearing workers had a significant effect on the head size of the emerging workers (full linear mixed-effects model: $F_{7,323} = 30514$, $P < 0.0001$; egg social origin: $F_{1,329} = 0$, $P = 0.99$; rearing worker social origin: $F_{1,328} = 1.09$, $P = 0.31$; interaction: $F_{4,323} = 0.78$, $P = 0.38$; cohort size: $F_{1,327} = 2.35$, $P = 0.14$; Fig. 3a). Offspring size varied significantly according to the identity of colonies supplying eggs, but not with the identity of colonies supplying rearing workers (full model: $F_{39,290} = 2.51$, $P < 0.0001$; egg source colony identity: $F_{27,302} = 3.12$, $P < 0.0001$; rearing worker source colony identity: $F_{12,290} = 1.15$, $P = 0.32$).

The individuals that experienced a longer development time were generally larger than those that developed more quickly (Pearson's correlation: $r = 0.17$, d.f. = 328, $P = 0.0022$). Pupation in a cocoon or naked, however, was not significantly associated with worker head size ($t_{291} = 1.33$, $P = 0.19$). The growth rate, given by the head size divided by development duration, was higher for brood originating from monogynous colonies than for brood originating from polygynous colonies (full linear mixed-effects model: $F_{7,323} = 4455$, $P < 0.0001$; egg social origin: $F_{1,329} = 4.68$, $P = 0.039$; rearing worker social origin: $F_{1,328} = 0$, $P = 0.99$; interaction: $F_{4,323} = 2.04$, $P = 0.16$; cohort size: $F_{1,327} = 2.43$, $P = 0.14$; Fig. 3b).

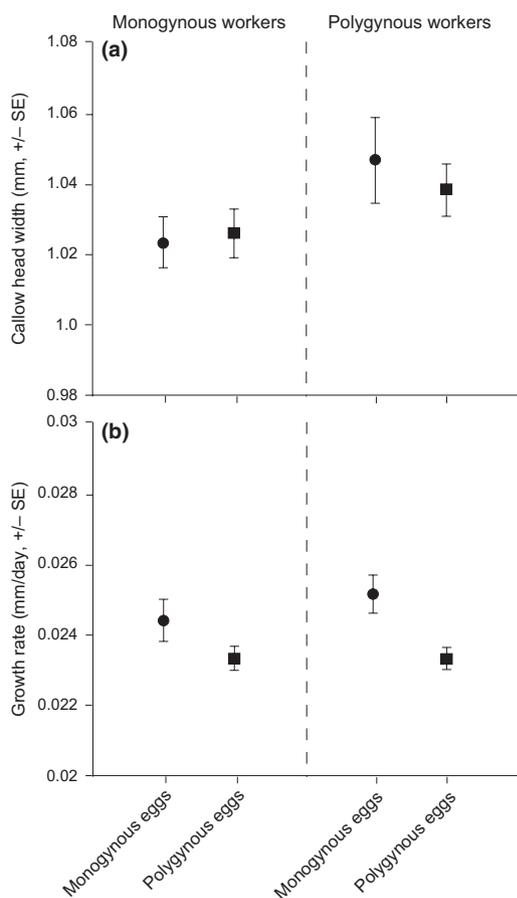


Fig. 3 The head size of newly eclosed adult workers did not differ significantly among the four treatments (a), but brood originating from monogynous colonies exhibited a significantly faster growth rate than brood originating from polygynous colonies (b).

For all three measures (offspring survival, development time and head size), the effect sizes of the source colony identity for eggs or workers were larger than the effect sizes of the social origin of eggs or workers (Table S2).

Discussion

Social animals show a great diversity in social structure and reproductive strategies, yet the factors contributing to the maintenance of this polymorphism remain poorly understood (Bourke & Franks, 1995; Chapuisat *et al.*, 2004). Gene by environment interactions and feedbacks from the social environment during brood development likely play a major role in shaping alternative social phenotypes (e.g. West-Eberhard, 1989; Chapuisat, 2010; Laland *et al.*, 2011). Here, we cross-fostered brood and workers coming from ant colonies with contrasted breeding systems, that is, headed by either one or multiple queens. To our knowledge, this

is the first study investigating the respective effects of the social backgrounds (i.e. whether individuals originate in monogynous or in polygynous colonies) of eggs and workers on the survival, development time and growth rate of brood.

Our cross-fostering experiment provides new insights into the proximate and ontogenic causes of the multiple differences that commonly occur between monogynous and polygynous ant colonies, which include queen and worker body size, queen fecundity and colony longevity (e.g. Hölldobler & Wilson, 1977; Ross & Keller, 1995; Rosset & Chapuisat, 2007). It shows that, in standardized laboratory conditions and in the absence of extrinsic mortality due to natural enemies, the social origin of the eggs, through the genotype or pre-foster maternal effects, had a strong impact on brood survival, development time and growth rate. Specifically, brood originating from polygynous colonies exhibited higher survival than brood originating from monogynous colonies, but took longer to develop and had a slower growth rate, regardless of the social origin of the rearing workers. The social origin of the workers caring for the brood influenced brood survival in the opposite direction, with workers originating from polygynous colonies being less successful at rearing brood than workers originating from monogynous colonies. The social origin of workers had no significant impact on brood development time and growth rate. Finally, the head size of the newly eclosed adult workers was not influenced by the social origin of the eggs or the rearing workers.

Our results indicate that genotypic differences or pre-foster maternal effects linked to social structure variation and transmitted to the eggs strongly influence brood survival and development. To control for differences among treatments due to egg discrimination by workers, we focused on the survival of eggs that were collected by workers (Helanterä & Sundström, 2007; Helanterä & Ratnieks, 2009; Meunier *et al.*, 2010, 2011a). Among these eggs, a larger proportion of brood of polygynous origin survived to adulthood, compared with brood of monogynous origin. Most of the mortality occurred during the egg and early larval stages, a pattern similar to the one observed when polygynous workers reared eggs originating from their own colony (Purcell *et al.*, 2012). Together, these results suggest that the social forms differ in egg viability.

This surprising difference in viability between brood of monogynous and polygynous origin might have several causes, in part related to the lower genetic relatedness and higher competition among queens in polygynous colonies (Rosset & Chapuisat, 2006). First, polygynous queens might produce stronger eggs. In line with this hypothesis, eggs laid by monogynous queens were significantly smaller than eggs from polygynous queens (Meunier & Chapuisat, 2009). Second, polygynous queens might lay on average more viable eggs if

polygynous queens tend to be younger and egg viability decreases with queen age, as has been shown in honeybees (Al-Lawati & Bienefeld, 2009). Third, monogynous queens might produce a higher proportion of trophic eggs, because these additional nutrient sources would benefit only their own offspring. Fourth, eggs from polygynous colonies may elicit more care from workers if the preference for polygynous eggs that we detected during egg collection (Meunier *et al.*, 2010) continues during later brood rearing, possibly triggered by the larger size of polygynous eggs or by quantitative differences in chemical cues (Meunier *et al.*, 2011a). Finally, monogynous queens or monogynous workers may lay a higher proportion of male eggs that would be discriminated against by workers (Rosset & Chapuisat, 2006). In *Formica exsecta* and *Solenopsis invicta*, for example, the primary sex ratio of eggs differs greatly from the secondary sex ratio at the pupal stage, because workers actively manipulate the sex ratio during brood development (Aron *et al.*, 1995; Sundström *et al.*, 1996). However, this seems unlikely in our case, because at the time we sampled eggs only worker brood, and no sexual brood, is being produced. Moreover, we have never detected worker reproduction in queenright conditions (Rosset & Chapuisat, 2006).

Brood of polygynous origin exhibited a longer development time and slower growth rate than brood of monogynous origin, regardless of the social background of rearing workers. This may again reflect a genetic difference, a difference in maternal investment or a differential treatment by workers of eggs originating from monogynous and polygynous queens. In birds, for instance, maternal investment in egg mass has been correlated with early chick growth and survival (reviewed by Williams, 1994). In our study species, eggs from monogynous queens tend to be smaller than eggs of polygynous queens (Meunier & Chapuisat, 2009), which suggests that egg mass *per se* does not explain the variation in growth rate between social forms. Other factors must be responsible for the faster development time and increased growth rate of brood from monogynous colonies. Prefoster maternal effects may also be mediated through variation in the hormonal contents transmitted to the eggs (Mousseau & Dingle, 1991; Russell & Lummaa, 2009). Several hormones, including juvenile hormone, have been implicated in brood development traits and caste differentiation in other ant species (e.g. de Menten *et al.*, 2005; Schwander *et al.*, 2008; Cahan *et al.*, 2011) as well as in bees (Rembold, 1987; Amdam *et al.*, 2004).

We also found evidence that the social origin of the rearing workers influences brood survival. Intriguingly, the social origin of the rearing workers exhibits the opposite pattern than the social origin of eggs with regard to brood survival. Rearing workers of monogynous origin reared a higher proportion of brood to adulthood than workers of polygynous origin, regard-

less of the social origin of the brood. This suggests that the ability to rear brood might have coevolved with the viability of eggs: indeed, workers of monogynous origin might compensate for the smaller and less viable eggs laid by monogynous queens. Alternatively, monogynous workers may be more efficient if they generally perform less discrimination than polygynous workers (Hannonen & Sundström, 2003). Finally, the performance of workers may also depend on conditions. For example, it is possible that monogynous workers do better in our small experimental colony fragments because they are accustomed to living in smaller colonies in the field (Rosset & Chapuisat, 2007).

Neither the social origin of the eggs nor the social origin of the rearing workers had a significant effect on the head size of the newly emerged adult workers. If anything, rearing workers from polygynous colonies showed a nonsignificant tendency to produce larger workers than rearing workers from monogynous colonies (Fig. 3). This result is surprising for two reasons. First, workers from monogynous colonies are significantly larger than workers from polygynous colonies in the field (Schwander *et al.*, 2005; Rosset & Chapuisat, 2007). The size variation observed in field colonies therefore cannot be entirely explained by genotype or maternal effect in eggs, nor by the social origin of rearing workers, as these factors had no effect on worker size in our standardized experimental rearing groups. Hence, other social or environment factors that differ between monogynous and polygynous colonies in the field, such as resource availability or provisioning rate per larva, must be affecting worker size, either alone or in interaction with the brood genotype. In line with this hypothesis, a previous manipulation of the worker/brood ratio showed that this component of the social environment had a relatively large effect on worker head size (Purcell *et al.*, 2012; Table S2).

Second, in a previous cross-fostering experiment with sexual brood, we found that eggs originating from monogynous colonies developed into significantly larger queens than those of polygynous origin, whereas the social background of the rearing workers had no effect (Meunier & Chapuisat, 2009; Table S2). This difference in the determinants of queen size vs. worker size is of interest. Worker size may be more plastic, and depend more on conditions, whereas the size of alate queens may be more critical for the fitness of the mother queen if queen size influences colony founding success (Keller & Passera, 1989; Reber *et al.*, 2010). Hence, queen size might be genetically determined, whereas worker size might be more variable and rather environmentally determined, as has been suggested in the ant *Formica truncorum* (Bargum *et al.*, 2004).

A substantial amount of natural variation in brood viability, development time and offspring head size was explained by the source colonies, independently of their social structure. This variation was due to the col-

ony of origin of both eggs and rearing workers, and the average effect size of these comparisons exceeded the effect sizes measured in our experimental treatments (Table S2). It is somewhat surprising to find such a large amount of variation among colonies in brood traits that are important for fitness (see also Purcell *et al.*, 2012 for similar results). Because selection should decrease genetic variance for these traits, the variation could result from differences in queen or worker age, or differences in the ecological conditions prevailing in the field colonies from which the brood and workers originated. In fact, variation in the performance of eggs and workers collected from different field colonies likely reflects a combination of many environmental and social factors experienced by the workers and the mother queens prior to sampling.

The presence of a mix of pupae with and without cocoons is also puzzling. Such polymorphism occurs in many ant species (Hölldobler & Wilson, 1990). Here, we show that pupae without cocoons develop significantly faster, which suggests that producing a cocoon is costly. A cocoon may offer protection against pathogens, desiccation, freezing or mechanical stress (reviewed by Danks, 2004). Hence, a mix of pupae with and without cocoons may be a bet hedging strategy: some brood will complete development faster, but with greater risk, whereas others are protected from some dangers but complete development more slowly.

The observed differences in the development of worker brood may have important implications for the relative success of the monogynous and polygynous social strategies. The brood originating from monogynous colonies had lower survival but higher growth rate than the brood originating from polygynous colonies. This might reflect different constraints in the two social forms. If queens from monogynous colonies initiate new colonies alone, their success relies on producing workers rapidly, before their energy reserves are exhausted, and they indeed produce very small workers (reviewed by Choe & Perlman, 1997). Thus, selection may maintain a faster absolute growth rate in the offspring of monogynous queens, even though workers produced by mature colonies may experience prolonged development to reach larger sizes. Polygynous queens, in contrast, often disperse by budding or by joining an established colony and therefore may experience less directional selection on the ability to produce brood very rapidly (in *Formica podzolica*: Deslippe & Savolainen, 1995; reviewed by Ross & Keller, 1995). Moreover, the reproductive competition among queens in polygynous colonies may result in the queens laying an excess of eggs and producing more viable offspring, even if this results in smaller workers that develop less rapidly than in monogynous colonies (e.g. Keller, 1995; Schwander *et al.*, 2005). These overall patterns are probably consistent across socially polymorphic ant species, because social structure variation is generally associated with

similar life history changes and morphological variation in queens and workers (Bourke & Franks, 1995; Heinze & Keller, 2000; Schwander *et al.*, 2005 and references herein). However, we do not yet have direct evidence that the social forms of *F. selysi* differ in their dispersal and colony founding strategies (Reber *et al.*, 2010; Meunier *et al.*, 2011b).

Natural variation in ant breeding systems continues to puzzle researchers, because colonies with multiple breeders are expected to experience more conflicts associated with decreased genetic relatedness of nestmates (Hamilton, 1964; Bourke, 2011). Previous research showed that different social forms exhibit substantial variation in a number of ecologically relevant traits (Ross & Keller, 1995; Rosset & Chapuisat, 2007). Here, we showed that the social background of the parents and, to a lesser extent, the workers that care for the brood contribute to shaping the survival and developmental trajectory of the brood. More generally, interactions between genetic and social factors during ontogeny are likely to play a major role in generating alternative social forms.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Schematic diagram showing the experimental design.

Figure S2 For each treatment, the survival of brood from the time that eggs were collected by rearing workers until the first pupae eclosed as adults.

Table S1 Explanation of terms describing our experimental design.

Table S2 Effect sizes (Cohen's *d*) for the rearing treatments (social origin of eggs and workers) and from the source colony identity (of eggs and workers) from the current experiment, and from previous experiments investigating the factors influencing offspring development and body size in *Formica selysi*.

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