

Early developmental processes limit socially mediated phenotypic plasticity in an ant

Irene Villalta^{1,2} · Olivier Blight^{1,3} · Elena Angulo¹ · Xim Cerdá¹ · Raphaël Boulay^{1,2}

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Abstract Caste determination in social insects has long been considered to exemplify socially mediated phenotypic plasticity: young larvae can develop into queens or workers depending on the social environment. However, recent studies have challenged this view by showing that, in some species, larval development can be biased early by factors such as larval genotype. We analyzed this issue in the ant

species *Aphaenogaster senilis*. First, we found that the probability that a larva develops into a queen or a worker varies consistently among colonies. Next, we conducted a cross-fostering experiment in which larvae from colonies with relatively low queen production were transferred to colonies with relatively high queen production and vice versa. The results show a strong significant interaction between early determination and worker control of larval caste fate. Therefore, our study shows that socially mediated phenotypic plasticity is limited by processes occurring at an early developmental stage that possibly include direct or indirect genetic effects or non-genetic maternal effects.

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Significance statement Colonies of ants are composed of two castes of females with different morphologies and tasks. The queens specialize in reproduction whereas the workers are sterile and contribute to domestic tasks such as food gathering and colony defense. For decades, the difference between workers and queens has been thought to stem from nutritional and other environmental differences during the larval stage. We indeed show marked differences between colonies in the capacity of workers to rear new queens. However, worker control on the destiny of diploid larvae is constrained by precocious larval pre-determination. These precocious effects possibly include direct or indirect genetic effects or non-genetic maternal effects. Overall, our study shows that colony-level resource allocation to the production of new queens is the result of complex interactions between adults and larvae.

Keywords Social behavior · Caste development · Phenotypic plasticity · Colony level selection

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Introduction

Phenotypic plasticity is an important phenomenon with broad implications for organismal ecology and evolution (West-Eberhard 1989; Pigliucci 2001). In many plants and animals, the biotic or abiotic environment in which an individual develops conditions its phenotype. A particular case occurs in social animals, in which phenotypic plasticity can be mediated by the social environment (Rodd et al. 1997; Ramm and Stockley 2009; Dingemanse and Araya-Ajoy 2015). This is well exemplified by caste determination in social insects. Hence, in many species, like the honeybee *Apis mellifera*, bumblebees, and various ant species including *Aphaenogaster senilis*, diploid larvae are totipotent up until a certain stage and will then develop into queens or workers depending on social cues. Several lines of evidence suggest that larval development is partly controlled by adult workers that provide a different nutrition (particularly proteins) to the

✉ Irene Villalta
irenevillaltaalonso@gmail.com

¹ Estación Biológica de Doñana – CSIC, Av. Americo Vesputio, 41092 Sevilla, Spain

² Institut de Recherches sur la Biologie de l’Insecte, CNRS UMR 7261, Université François Rabelais de Tours, Tours, France

³ Institut Méditerranéen de Biodiversité et d’Ecologie, Université d’Avignon et des Pays de Vaucluse, UMR CNRS IRD Aix Marseille Université, Avignon, France

larvae that develop into queens (Pereboom et al. 2003; Kamakura 2011; Caut et al. 2014—see also Linksvayer et al. 2011). Moreover, workers are often inhibited from raising new queens whenever they perceive the pheromones produced by their fertile queen (Goulson 2003; Boulay et al. 2007, 2009; Seeley 2009).

In contrast with this view, recent studies have shown that, in some species, larval caste fate is determined very early on, thus limiting phenotypic plasticity and worker control of larval development (Schwander et al. 2010). Queen-laid eggs may be predisposed to follow certain developmental pathways due to hormonal effects (e.g., Passera 1980; Libbrecht et al. 2013). In *Pogonomyrmex*, the eggs laid by queens after a period of hibernation have a greater chance of yielding queens (Schwander et al. 2008). This phenomenon is mediated by an increase of queen juvenile hormone that enhances the accumulation of vitellogenin in the eggs, thus increasing the probability of yielding queens (Libbrecht et al. 2013). In addition, direct genetic influences that range from plastic genotypes that are biased toward queen or worker development (Hughes and Boomsma 2008) to strict genetic determination (Julian et al. 2002; Leniaud et al. 2012; Schwander and Keller 2012) may constrain caste fate. In *Pogonomyrmex* ants and Argentine ants, some combinations of maternal and paternal genotypes give rise to larvae that have a greater probability of becoming queens than do other combinations (Schwander and Keller 2008; Libbrecht et al. 2011). Finally, both direct and indirect genetic effects (i.e., effects of maternal and worker genotypes) may influence larval development. Using a cross-fostering experiment, Linksvayer (2006) reached the conclusion that larval caste fate depends on larval genotypes (i.e., direct genetic effect) as well as on maternal and worker genotypes (i.e., indirect genetic effects) in *Temnothorax* species. Interactions between brood and worker genotypes also induce differences in adult worker size in *Temnothorax* species (Linksvayer 2007) and in the clonal ant *Carapachys biroi* (Teseo et al. 2014).

In spite of the above-mentioned results, our understanding of caste determination in ants and other social hymenopterans is still limited by the reduced number of model systems on which these studies have been conducted. Here, we analyzed the relative importance of worker control and early determination on colony-level queen production in the ant *A. senilis*. This strictly monogynous species is well suited for such study because queen production is highly predictable in captive condition. Most diploid brood develops into workers when a queen is present in the colony. However, the first and second instar larvae can develop into queens if the current queen is removed or dies (Ledoux 1976, 1981; Boulay et al. 2009). This phenomenon, which relies on pheromonal communication among workers, larvae, and queens, allows the adjustment of resource allocation to queen production to be fine-tuned (Ruel et al. 2012, 2013). Because of monogyny, producing a few queens from the current brood guarantees that

one of them will succeed her mother; however, a massive allocation of resources to queen production would just increase local resource competition. Indeed, queenless colonies rarely produce more than 3–4 queens (Chéron et al. 2009), irrespective of the number of totipotent larvae that are present (Villalta et al. 2015).

In order to analyze the early developmental processes that limit caste differentiation, we first determined whether the proportion of larvae that develop into queens is a phenotypic trait that is expressed at the colony level. If so, it should vary more among colonies than among replicates within colonies. We also tested whether it co-varied with demographic variables and the season (late winter vs. late spring). These variables were shown to affect queen production in several species (e.g., Passera 1980; Tschinkel 1993; Schwander et al. 2008). Second, we conducted a cross-fostering experiment in order to determine whether the production of new queens was a trait deriving from worker state, larval state, or the interaction between both. To that end, workers and larvae were swapped between colonies that demonstrated the highest and lowest levels of queen production. The results provide clear evidence that early caste determination limits worker control and larval developmental plasticity.

Materials and methods

A. senilis colonies are composed of 200–3000 workers (Boulay et al. 2007). In 2012, we collected 16 large colonies from a single population near Aznalcázar (south-western Spain) in late winter (March) and then again in late spring (June). We immediately counted the number of workers per colony (hereafter colony size) and weighed the queens (± 0.1 mg). Three experimental groups of 200 workers and 20 recently hatched first instar larvae (Fig. 1a) were then taken from each colony (total: 96 experimental groups). In a colony, the first instar larvae are kept with the eggs and receive similar treatment (pers. obs.). Workers have been hypothesized to feed the larvae through proctodeal food (sensu Torossian 1958; Villalta et al. 2015). In the experiments, the first instar larvae were preferred over eggs because the caste is not yet determined at this stage (Boulay et al. 2009), and egg incubation lasts longer than the first larval stage (30 vs. 10 days, respectively). Hence, starting the experiment with the egg stage would have induced considerable asynchrony in the development of the larvae. Moreover, eggs die at a much higher rate than do larvae—larvae frequently cannibalize fertile eggs, although this phenomenon does not affect their development (Ruel et al. 2012). Over a period of seven weeks, we quantified the total number of female larvae (i.e., workers + queens, FEM, hereafter) and then calculated the proportion of queens among the female pupae (P(Q), hereafter). The groups were fed sliced mealworms twice a week, given water ad libitum,

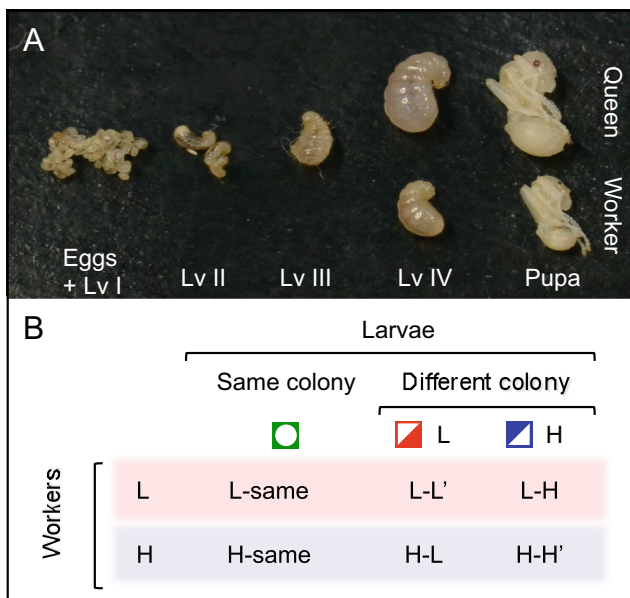


Fig. 1 **a** Larval development in *A. senilis*—larvae are bipotent until larva stage II. **b** Experimental design used in the cross-fostering experiment. *H* and *L* refer to colonies with high and low levels of queen production in experiment 1, respectively

and kept at 25 °C. At the end of the experiment, we counted the number of remaining workers to estimate the survival rate.

The eight late winter and late spring colonies with the highest average $P(Q)$ were classified as H_{Winter} and H_{Spring} , respectively. The remaining colonies were classified as L_{Winter} and L_{Spring} (for “low” queen production). We then conducted a cross-fostering experiment (Fig. 1b) in which batches of 20 newly hatched first instar larvae from each H_{Winter} colony were transferred into groups of 200 workers from a different H_{Winter} colony (hereafter H-H') or from an L_{Winter} colony (H-L). Conversely, larvae from each L_{Winter} colony were transferred into groups of workers from a different L_{Winter} colony (L-L') or from an H_{Winter} colony (L-H). In addition, the groups of workers from each colony received larvae from their own colonies (H-same and L-same). The same experiment was carried out using the late spring colonies. The cross-fostering experiments were conducted in two sets that took place approximately 10 weeks after the colonies had been collected. Overall, there were 14 replicates for H-control, H-H, and H-L conditions and 15 replicates for L-control, L-H, and L-L conditions. Three colonies were eliminated either because the queen had died or the number of workers was too small. As above, we monitored larval development for seven weeks. To minimize observer bias, a blinded method was used so that the person who monitored larval development had no knowledge of the treatment.

All data were analyzed using *R* (R Core Team 2012). Within-colony consistency in FEM and $P(Q)$ was tested by calculating the intraclass correlation coefficient (ICC) using the irr package (Gamer et al. 2012). Moreover, variation in

FEM and $P(Q)$ was analyzed by fitting general linear mixed effects (glm) models using the nlme package. For the two response variables, the variance components were extracted by including the colony of origin as a random factor. The explanatory variables were collection season (late winter vs. late spring), the size of the mother colony, queen mass, and worker mortality rate. FEM was log-transformed (Bartlett 1947) while $P(Q)$ was arc-sin transformed (Anscombe 1948; Zar 1999). Models were simplified by removing non-significant parameters (Crawley 2007). We then compared the number of workers in H and L colonies by means of a glm.

The data from the cross-fostering experiment were analyzed by fitting glms using the results from the H-H', H-L, L-L', and L-H groups only. We tested the effects of worker type (H or L), larva type (H or L), and their interaction on FEM (log-transformed) and $P(Q)$ (arc-sin transformed) using the lme4 package which allows including more than one random factor in the models. The season, worker colony, and larva colony were each included as random factors, and worker survival rate was included as a covariate. Finally, we ran glms to compare $P(Q)$ (arc-sin transformed) among the H-H', H-same, L-L', and L-same experimental groups to test the effect of larval adoption by foreign workers on queen production.

Results

In the first experiment, the groups composed of 200 workers and 20 first instar larvae produced, on average, 11.75 ± 1.04 (mean \pm SE) female pupae (FEM). A mean of just 0.875 ± 0.155 males was produced per group, which indicates that $37 \pm 2\%$ of the larvae that were initially provided died before the pupa stage (no larva remained at the end of the seven weeks of monitoring). Queens represented $8.16 \pm 1.02\%$ of the females produced. The ICC analysis indicated that $P(Q)$ was consistent within colonies (Fig. 2: $F_{31, 64} = 4.63$, $P < 0.0001$) while FEM was not (Fig. 2: $F_{15, 32} = 1.37$, $P = 0.146$). This result was confirmed by the variance components extracted from the linear mixed effects models. Hence, while variation among colonies explained 51 % of the total variance for $P(Q)$, it explained only 12 % of the total variance for FEM (Fig. 2). The remaining amount of variance was explained by differences among the groups within colonies. Of the fixed effects that we were able to test, only the size of the mother colony was significantly correlated with $P(Q)$ (Figure S1; Table 1; $F_{1, 30} = 4.67$, $P = 0.039$ after removing non-significant parameters). None of the factors were significantly correlated with FEM (Table 1). Based on the previous results, we identified H_{Winter} and H_{Spring} as the colonies with the highest $P(Q)$ in the winter and spring sessions, respectively (range: 0.07–0.15 and 0.10–0.19, respectively). H colonies contained significantly more workers than L colonies (F_1 ,

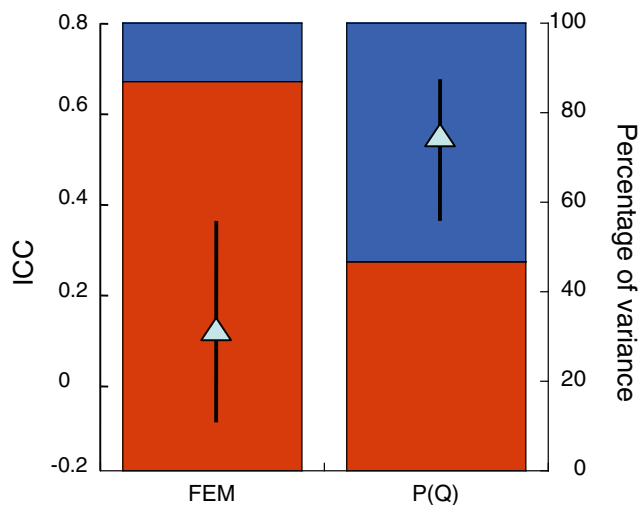


Fig. 2 Within-colony consistency in queen production. Intraclass correlation coefficient (*triangles* \pm 95 % confidence intervals) and percentage of variance explained by differences among colonies (*blue*) and among groups within colonies (*red*) for the number of female pupae (FEM) and the proportion of queens among the females produced (P(Q))

$_{30}=4.79$, $P=0.037$) but there was no significant difference between the seasons ($F_{1, 29}=0.61$, $P=0.612$); the interaction between the colony type and season was not significant either ($F_{1, 28}=2.69$, $P=0.112$).

In the cross-fostering experiment, FEM was significantly greater in the groups composed of H larvae than in those composed of L larvae (Table 2; 12.52 ± 0.67 vs. 9.86 ± 0.75 , respectively; $F_{1, 15}=8.788$, $P=0.009$). However, FEM depended neither on worker type, on the interaction between the worker, and larvae type nor on worker survival (Table 2). P(Q) was significantly affected by the worker type, larvae type, and their interaction (Fig. 3; Table 2). Hence, H workers produced a higher proportion of queens than did L workers only when they were provided with H larvae (Fig. 3; $F_{1, 15}=5.122$, $P=0.038$). When they were provided with L and H, workers produced a similar proportion of queens. P(Q) did not vary significantly with worker survival ($F_{1, 52}=0.884$, $P=0.351$) and was not significantly different between the H-H' and H-same groups (Fig. 3; $t=-0.098$, $df=2.42$, $P=0.93$) or the L-L' and L-same groups ($t=-3.411$, $df=2.36$, $P=0.060$), which indicates that being reared by foreign workers did not significantly bias larval fate.

Table 1 Result of the linear mixed effects models analyzing the main effects of colony size, queen mass, worker survival, and the season of collection on the number of female produced (FEM) and the proportion of queens among females (P(Q))

	FEM			P(Q)		
	Df (num, den)	<i>F</i>	<i>P</i>	Df (num, den)	<i>F</i>	<i>P</i>
Colony size	1, 28	0.005	0.943	1, 28	4.237	0.049
Queen mass	1, 28	0.533	0.471	1, 28	0.185	0.671
Worker survival	1, 63	0.585	0.448	1, 63	0.105	0.747
Season	1, 28	0.005	0.449	1, 28	0.108	0.745

Discussion

Our results show that the groups of workers and larvae taken from some colonies consistently produced a higher proportion of queens than did others. The propensity to produce new queens may therefore be considered a phenotypic trait that is expressed at the colony level. What could explain colony-level variation in queen production? The cross-fostering experiment reveals that it likely results from a significant interaction between the larvae and the adult workers that nurse them.

In the first experiment, in which we analyzed the consistency in larval development among colonies, P(Q) was correlated with the size of the mother colony. Large colonies are known to be more likely to produce new queens than small colonies in many species of social insects (i.e., Brian 1957; Torossian 1958; Passera 1980; Tschinkel 1993; Iwanishi et al. 2007; Boulay et al. 2007; Alaux et al. 2005; Smith and Suarez 2010). However, in our experiment, the group size was identical in all the groups so that queen production was not directly affected by the number of workers. Large colonies differ from small colonies in many other ways that may affect queen production. For example, the correlation between P(Q) and colony size may be due to large colonies being headed by relatively older queens as in bumblebees (Alaux et al. 2005). Even though colony fission dissociates colony growth from queen aging compared to species with independent colony-founding, newly formed colonies are likely composed of a young queen and a smaller number of workers than mature colonies (Chéron et al. 2011; Amor et al. 2011). Another hypothesis may be that queen production is fuelled by worker physiological condition (fat content), which increases with colony size as in *Solenopsis invicta* and *Pogonomyrmex badius* (Tschinkel 1993, 1998). We might thus expect the propensity to produce new queens to be a plastic trait that tends to increase as a colony ages. The fact that there was no difference between colonies collected in late winter and late spring contrasts with results obtained for *Pogonomyrmex* (Schwander et al. 2008) but fits with field observations showing that, in the study population, new queens can be produced from April to November (Boulay et al. 2007).

Previous studies have shown that requeening in *A. senilis* is possible only if first or second instar larvae are present in the

Table 2 Result of the linear mixed effects models analyzing the main effects of worker type, larvae type, their interaction, and worker survival on the number of female produced (FEM) and the proportion of queens among females (P(Q))

	FEM			P(Q)		
	Df (num, den)	<i>F</i>	<i>P</i>	Df (num, den)	<i>F</i>	<i>P</i>
Worker type	1, 25	0.585	0.461	1, 22	7.724	0.011
Larvae type	1, 16	8.788	0.009	1, 23	22.303	0.001
Worker survival	1, 53	2.430	0.125	1, 52	0.884	0.351
Worker × larvae type	1, 14	3.138	0.098	1, 15	5.122	0.038

colony when the mother queen is removed or dies (Boulay et al. 2009). However, the cross-fostering experiment suggests that the development of the first instar larvae into queens is constrained by the social environment. While the probability that H colonies produced new queens depended on the type of workers that cared for them, the probability that L-colony produced queens was low, irrespective of worker type. This important result is similar to what has been shown in *Temnothorax* ants (Linksvayer 2006). Several mechanisms can explain it. First, given the elevated mortality rate of the larvae, we cannot exclude the possibility that the low production of queens in L colonies resulted from higher mortality of queen oriented larvae than in H colonies. However, the result of previous studies showing that queen production is independent of larval number and survival do not support this hypothesis (Ruel et al. 2012; Villalta et al. 2015). Second, phenomena such as direct and indirect genetic effects and non-genetic maternal effects can account for early developmental determination. These effects are not mutually exclusive and may be additive or non-additive. A genetic effect, whereby some larval genotypes have a higher probability of becoming a queen, was suggested in *A. rudis* based on isozyme data (Crozier

1973). Although more and more examples are being discovered (Schwander and Keller 2012), we still have no evidence that such genetic effect operates in *A. senilis*. Queens may also control the development of their offspring through non-genetic maternal effects. For example, in *Pheidole pallidula*, queen hormones are known to determine brood sexualization (Passera 1980; Suzzoni et al. 1980). In *Pogonomyrmex*, queen hormones determine larval caste fate by affecting the accumulation of vitellogenin in the egg (Libbrecht et al. 2013). The role of queen hormones on brood sexualization may prevail in *A. senilis*. Hence, the queen's endocrine titre, which is likely to vary with age (and thus co-vary with colony size), may have a major effect on larval caste fate. The treatment received at the egg and early larval developmental stages may further constrain caste fate. Workers care for eggs and the first instar larvae by maintaining them together in a specific part of the nest and in close contact with the queen. The quality and quantity of care at this very early stage may therefore affect further development.

Even though the probability of becoming a queen is partly determined at an early stage, workers exercise a great deal of control over larval caste fate at later developmental stages. Larvae that become queens are bigger than those that become workers and must therefore receive more protein-enriched food (Smith and Suarez 2010; Caut et al. 2014). The rate at which workers nourish larvae may also influence caste differentiation, as is the case in some bumblebee species (Pereboom et al. 2003). More generally, the amount and quality of care a larva receives shapes its development. Interestingly, behavioral variation in care among colonies has been reported in several species of ants (Chapman et al. 2011; Jandt et al. 2014; Blight et al. 2015). So far, the fitness consequences thereof are poorly understood (Modlmeier et al. 2012), but they may be related to worker capacity to rear new queens.

To conclude, although the social environment influences many aspects of animals' phenotypes, including behavior, morphology, and physiology, our results clearly show that phenomena occurring at very early stages can constrain socially mediated phenotypic plasticity. These phenomena may include direct genetic effects (i.e., directly caused by the larval genotype) and indirect genetic effects (i.e., caused from the maternal or sib-social genotypes) as well as non-genetic effects (Linksvayer 2006). Ants constitute important model

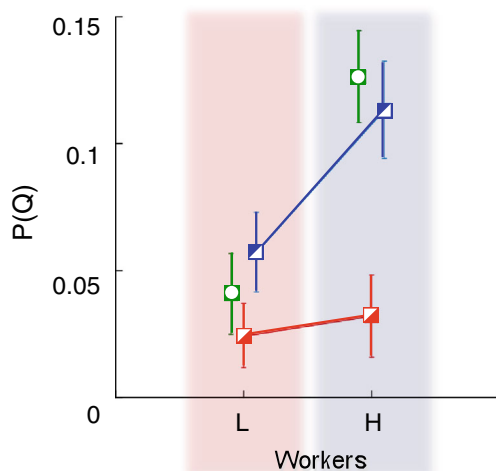


Fig. 3 Proportion of queens produced (P(Q); mean ± SE) by groups of L and H workers provided with L (red symbols) or H (blue symbols) larvae from different colonies. The green symbols stand for groups of L and H workers provided with larvae from their own colonies. H and L refer to individuals from colonies with high and low levels of queen production, respectively

systems for the study of socially mediated phenotypic plasticity but further studies are needed to disentangle the physiological and behavioral factors affecting caste determination.

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Conflict of interest The authors declare that they have no competing interests.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

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