

Maternal Effect on Female Caste Determination in a Social Insect

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Summary

Caste differentiation and division of labor are the hallmarks of social insect colonies [1, 2]. The current dogma for female caste differentiation is that female eggs are totipotent, with morphological and physiological differences between queens and workers stemming from a developmental switch during the larval stage controlled by nutritional and other environmental factors (e.g., [3–8]). In this study, we tested whether maternal effects influence caste differentiation in *Pogonomyrmex* harvester ants. By conducting crossfostering experiments we identified two key factors in the process of caste determination. New queens were produced only from eggs laid by queens exposed to cold. Moreover, there was a strong age effect, with development into queens occurring only in eggs laid by queens that were at least two years old. Biochemical analyses further revealed that the level of ecdysteroids was significantly lower in eggs developing into queens than workers. By contrast, we found no significant effect of colony size or worker exposure to cold, suggesting that the trigger for caste differentiation may be independent of the quantity and quality of resources provided to larvae. Altogether these data demonstrate that the developmental fate of female brood is strongly influenced by maternal effects in ants of the genus *Pogonomyrmex*.

Results and Discussion

Effect of Cold Exposure, Queen Age, and Colony Size on Queen Production in *Pogonomyrmex* Lineages

Some *Pogonomyrmex* lineages produce queens and workers that differ genetically [9–14]. In these lineages, queen-destined eggs abort at periods when only workers are raised [15, 16], whereas the same eggs hatch and develop into adult queens at periods of reproduction. We conducted crossfostering

experiments in the lineages labeled “F1-F2” and “H1-H2” [12, 14] to identify the factors affecting egg development and to test whether these factors acted on queens or on workers. Because field studies indicate that new queens are typically produced in large four- to five-year-old colonies [17, 18] and only once per year in spring [17–20], we investigated the role of age- and season-related maternal effects (i.e., queen age and their exposure to cold) as well as nutritional and other worker-mediated factors (i.e., colony size and worker exposure to cold). In our fully crossed design, eggs were taken from donor colonies containing a single queen ranging in age from a few months to four years and 10 or 180 workers. Half of these colonies were exposed to cold for 2.5 months (hereafter referred to as “overwintered” and “nonoverwintered” queens). Each of these 204 egg-source colonies was used as a donor for four different recipient colonies containing either 10 or 180 workers that had been exposed or not to cold (overwintered versus nonoverwintered workers, see Figure S1A, available online, for an illustration of the design). We used colonies of 180 workers because our preliminary experiments revealed that new queens were frequently produced in laboratory colonies of this size (see the Experimental Procedures for details).

These crossfostering experiments revealed no significant effect of worker overwintering on the production of new queens ($z = -9.03$, $p = 0.99$, with a logistic regression using one data point per recipient colony; queens were produced in 13.6% of both overwintered and nonoverwintered recipient colonies), but a strong effect of overwintering by queens ($z = 3.93$, $p = 0.008$, with a logistic regression using one data point per egg-source colony). The eggs of only one of the 101 nonoverwintered queens gave rise to female sexuals. By contrast, the eggs from a significant proportion of the 103 overwintered queens gave rise to female sexuals, but in this case there was also a strong effect of queen age on new queen production ($z = 4.92$, $p = 0.0001$, with a logistic regression using one data point per colony of egg origin; Figure 1A). Although no eggs from the 36 overwintered queens under 1 year of age developed into queens, the eggs from 18 of the 19 overwintered queens older than 2 years gave rise to female sexuals. The values were intermediate for queens that were 1–2 years old, with 7 out of 48 overwintered queens producing daughter queens.

The likelihood of female sexual production was not significantly associated with the size of the egg-source colony ($z = -1.10$, $p = 0.27$) nor the size of the recipient colony ($z = -0.70$, $p = 0.49$). However, in line with the idea that colony size affects the availability of food resources [1, 21], larger recipient colonies were able to produce significantly more females (queens and workers) than smaller recipient colonies (Kruskall-Wallis test; $X^2_1 = 177.5$, $p < 0.0001$; 26.2 ± 15.0 females in smaller versus 54.3 ± 35.3 females in larger recipient colonies). Queens produced in larger colonies were as large as the mother queens, whereas smaller colonies typically produced very small queens (data not shown).

The finding that small colonies readily produced new queens in the laboratory is in apparent contrast with the observation that new queens and males are produced only in colonies that exceed a given size in the field (e.g., [1, 17, 18, 20–22]).

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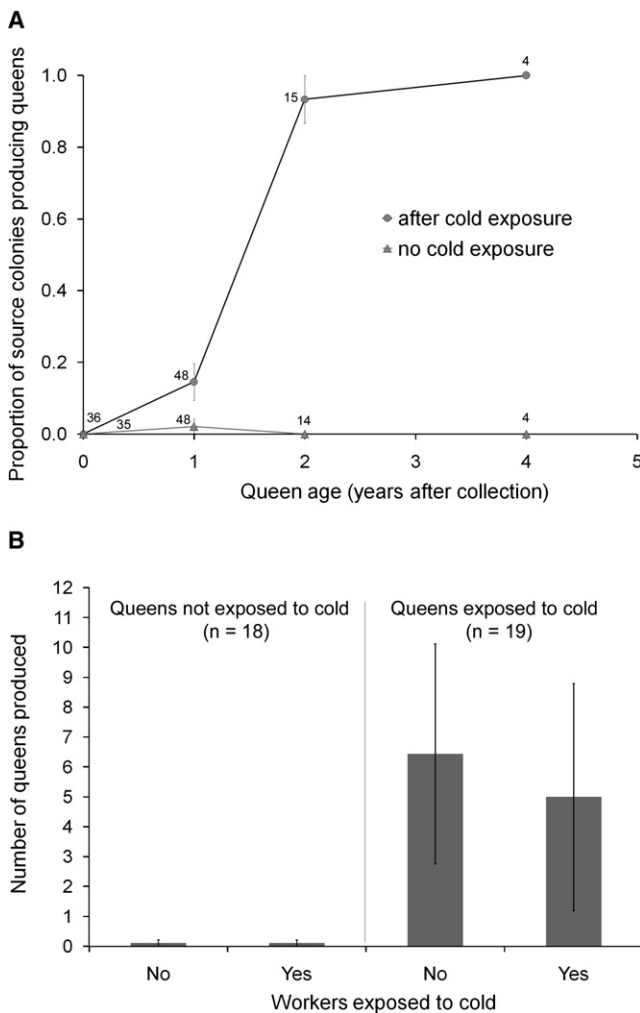


Figure 1. Maternal Effects Are an Important Trigger for Female Caste Fate
(A) Maternal effects triggered by queen age and cold exposure influence female caste in *Pogonomyrmex* lineages. Proportion (\pm binomial SD, where applicable) of queens of *Pogonomyrmex* lineages producing eggs that developed into female sexuals depending on the age of the queen and whether or not she was exposed to low temperatures. Numbers indicate the number of egg-source colonies per queen age group and treatment.
(B) Queen cold exposure also triggers the production of daughter queens in *P. rugosus*. Mean (\pm SD) number of queens produced in *P. rugosus* colonies composed of queens and workers that were exposed to low temperatures or maintained constantly at 30°C.

However, there is generally a tight correlation between colony size and queen age (e.g., [17, 18, 23, 24]). Thus, the fact that larger field colonies contain older queens may account for the observed association between colony size and whether or not colonies produce new queens.

Effect of Cold Exposure on Queen Production in *Pogonomyrmex rugosus*

To determine whether maternal effects are specific to the genetic caste determination system in the *Pogonomyrmex* lineages or might instead be a general feature of caste determination in the genus, we conducted a second set of similar experiments in *P. rugosus*, a species thought to have a typical environmentally based system of caste determination [9, 10, 12–14]. Because the previous experiment revealed no effect

of colony size, we used only colonies with 100 workers. We crossfostered queens between colonies rather than eggs to additionally confirm that any maternal effects on the process of caste determination occur in queenright colonies as well as in queenless fragments. In total there were ten colonies with overwintered queens and workers, nine colonies with overwintered queens and nonoverwintered workers, nine colonies with nonoverwintered queens and overwintered workers, and nine colonies with nonoverwintered queens and workers (see Figure S1B for an illustration of the design). The queens were 2–4 years old.

Only two out of the 18 (11%) colonies with a nonoverwintered queen produced daughter queens as compared to 17 out of the 19 (89%) colonies with an overwintered queen (Fisher's exact test, $p = 0.007$). A total of 108 daughter queens were produced by colonies with an overwintered queen, in contrast to only two in colonies with a nonoverwintered queen (permutation anova with 5,000 replicates; effect of queen overwintering, $p = 0.0002$; Figure 1B). As in the previous experiment, the developmental fate of female brood was not influenced by whether or not workers had overwintered (permutation anova; effect of worker overwintering, $p = 0.35$; interaction with queen overwintering, $p = 0.29$; Figure 1B).

Altogether these experiments reveal that the developmental fate of female brood depends on maternal effects in ants of the genus *Pogonomyrmex*. In both the genetic lineages and *P. rugosus*, the process of caste determination is strongly influenced by queen age and exposure to cold whereas there was no observable effect of colony size or exposure of workers to cold.

Hormone Titers of Eggs Laid by Overwintered and Nonoverwintered Queens and Maternal Factors in Other Ants

To investigate a potential mechanism by which queens' cold exposure influences the caste fate of their eggs, we compared ecdysteroid and juvenile hormone titers in batches of eggs laid by overwintered queens that had produced sexual offspring to those laid by nonoverwintered queens that had produced only workers. The per-egg ecdysteroid titer was significantly lower in eggs from overwintered queens than in eggs from nonoverwintered queens (Kruskal-Wallis test, $X^2_1 = 4.1$, $p = 0.04$; Figure 2A). In contrast, there was no significant difference in juvenile hormone titers between the two types of eggs (Kruskal-Wallis test, $X^2_1 = 0.51$, $p = 0.47$; Figure 2B). Interestingly, ecdysteroids also are associated with the developmental fate of eggs in *P. pallidula*, and the eggs laid just after overwintering and which usually develop into queens also have lower ecdysteroid levels than eggs laid during the summer [25].

The finding of maternal effects on female caste determination in *Pogonomyrmex* raises the question whether similar mechanisms may affect female caste determination in other ants. Although it is widely accepted that the developmental fate of female eggs only depends on the social environment and the amount of food provided to larvae, there is no strong experimental support for this view in ants. Many experiments have shown that changes in environmental conditions can alter the likelihood of new queens being produced (for a review, see [26]), but no studies have demonstrated that all eggs can develop into queens or workers at any time (i.e., that they are always fully totipotent). Data from at least four other ant species (*Pheidole pallidula* [25, 27], *Formica polyctena* (formerly *F. rufa rufa-pratensis minor*) [28, 29], *Linepithema humile* [30], and *Monomorium pharaonis* [31]) suggest that temperature or queen age also may influence the developmental fate

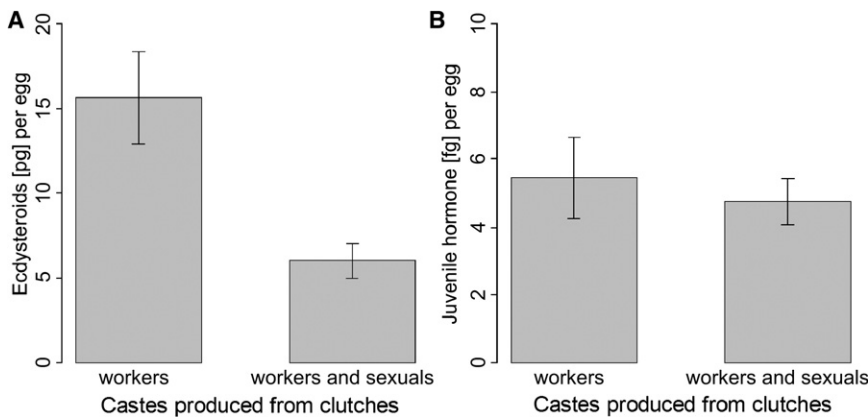


Figure 2. Maternal Effects on Female Caste Differentiation May Be Mediated by Hormone Contents of Eggs

(A) Ecdysteroid titers were significantly higher in egg batches yielding only workers than in batches yielding both workers and queens. Mean (\pm SD) ecdysteroid titers in egg batches yielding only workers ($n = 26$ colonies) or both workers and queens ($n = 15$ colonies). (B) The two groups of eggs did not differ significantly in their juvenile hormone content. Mean (\pm SD) juvenile hormone titers in egg batches yielding only workers ($n = 26$ colonies) or both workers and queens ($n = 15$ colonies).

of female eggs. These data come from different branches of the ant phylogeny [32], which shows that maternal factors affecting female caste determination are either ancestral or have evolved multiple times.

Conclusion

This study revealed that *Pogonomyrmex* queens can influence, via maternal effects, the developmental fate of their eggs. Although workers might respond differently to eggs and larvae developing into queens or workers, the initial trigger for female caste stems from maternal, rather than nutritional, factors. Data from other ants also suggest that queens may strongly influence caste determination of their female offspring. Maternal effects on female caste determination may have consequences for potential queen-worker conflicts over reproductive decisions and sex allocation, and worker-brood conflict over caste because conflict resolution critically depends on who has power over caste development [3, 4] and the traditional view is that queens and brood have relatively little influence over female caste determination [4, 5, 33, 34]. By allowing a precise timing of queen production, maternal effects on female caste determination may provide benefits in species in which queens and males are produced only during a short period of the year. Whether ecdysteroids and/or other hormones may directly affect the pattern of gene expression and be responsible for a developmental switch remains to be investigated. Regardless of the mechanism used by queens to affect the developmental fate of their eggs, our study, together with the growing evidence of nonenvironmental factors affecting caste determination in other species [35–40], calls for a re-evaluation of the idea that the ant caste system is based solely on nutritional and social effects during the larval stage on gene expression and the developmental pathway of females.

Experimental Procedures

Colonies were established in the laboratory from single queens collected from mating flights (June and July) in Arizona, New Mexico, and Texas from 2001–2005. The queens of the genetic lineages were collected at the sites “Hidalgo” and “Alpine” whereas *P. rugosus* queens were collected at the sites “D,” “B,” and “PC” (see [14] for a detailed description of the sites). Colonies were maintained in $15 \times 15 \times 5$ cm transparent boxes under natural light and a superposed 12 hr:12 hr artificial light:dark cycle at 30°C , 60% humidity, and fed twice per week with a mixture of mealworms, eggs, ground almond, and vitamins and once per week with a mixture of grass and birdseed.

To simulate overwintering, we placed colonies for 2.5 months in a dark climate chamber at $12\text{--}14^\circ\text{C}$ and 60% humidity. The transition to and out of overwintering was done over a period of 2 weeks by progressively decreasing or increasing temperatures and using an 8 hr light:16 hr dark

cycle. Previous to the experiment, none of the colonies had experienced overwintering.

To study the effect of colony size and overwintering of workers and queens in the lineages, eggs were transferred from queenright source colonies to queenless recipient colonies (see Figure S1A). Recipient colonies were queenless to prevent queens from interfering directly with the development of the larvae through pheromones and/or other mechanisms. We set up four different types of queenright and recipient colonies: small (10 workers) or large (180 workers) overwintered colonies and small or large nonoverwintered colonies. Colony sizes were chosen according to long-term observations in the laboratory, where colonies occasionally produce new queens at sizes of approximately 150 workers when kept in small boxes such as used in our experiment. Colonies reach larger sizes when kept in boxes of larger size but never reach the size of mature field colonies, which typically contain over 10,000 workers [17]. Eggs coming from each of 204 egg-source colonies (101 nonoverwintered and 103 overwintered) were transferred into four recipient colonies, one of each type (see Figure S1A). To prevent workers from eating eggs before their transfer, the queen was isolated for egg-laying in a glass vial containing food and closed with a wire-mesh inside her colony for 48 hr. After 48 hr, the queen was reintroduced into her colony and the eggs she had laid were counted and transferred into one out of the four recipient colonies. This process was repeated 10–14 times over a period of 5–7 weeks, with queens being left at least 48 hr with their workers between the isolation tests. Overall, the number of eggs transferred was 118 ± 46 eggs in large (180 workers) recipient colonies and 81 ± 27 in small (10 workers) recipient colonies.

Twelve weeks after the last egg transfer, we checked each recipient colony for the presence of new (winged) queens. For the statistical analyses, we first used each recipient colony as an independent data point in a logistic regression with queen production as the binary response and the following explicative variables: colony size (small versus large) of the egg-source and recipient colonies, overwintering versus nonoverwintering of queens and overwintering versus nonoverwintering of workers, and queen age. Because the only significant effects in this analysis were related to the egg-source colony we conducted a second analysis using only one point per egg-source colony. Again, we used queen production as the binary response and size of the egg-source colony, overwintering, and queen age as explicative variables. In this analysis, an egg-source colony was classified as giving rise to daughter queens if queens were produced in at least one out of the four recipient colonies. Using a classification threshold of two, three, or four, recipient colonies producing daughter queens did not qualitatively change the results as 21 out of the 25 overwintered egg-source colonies that produced eggs yielding queens in at least one recipient colony actually produced queens in all four recipient colonies. For the four remaining queenright colonies, daughter queens appeared in three out of the four recipient colonies.

To investigate whether a maternal effect on female caste determination also occurs in *P. rugosus*, we conducted crossfostering experiments with overwintered versus nonoverwintered queens and workers (see Figure S1B). We overwintered (as described before) 22 of 43 queenright colonies. We next removed all queens and transferred them across the 43 colonies. Six out of the 43 queens died during the transfer, so we obtained ten colonies with an overwintered queen and overwintered workers, nine colonies with an overwintered queen and nonoverwintered workers, nine colonies with a nonoverwintered queen and overwintered workers, and nine colonies with a nonoverwintered queen and nonoverwintered workers.

Because workers do not easily accept a foreign queen, we first separated the queen from her adoptive workers with a wire mesh and progressively allowed more and more workers to physically interact with her. Once the queens were accepted, colonies were maintained for 12 weeks as described before and the new queens produced were counted in each colony.

To investigate a potential mechanism by which queens could affect the caste fate of her female offspring, we isolated 15 overwintered *Pogonomyrmex* queens who had produced new queens and 26 nonoverwintered queens who had produced only workers for 34 hr and collected all of the eggs laid during this time (4–54 eggs per queen). We then quantified, for each batch of eggs, the levels of ecdysteroids and juvenile hormone. Ecdysteroid titers were determined by using a radioimmunoassay (RIA) developed by Warren et al. [41] (see the Supplemental Data for details). The JH titer was measured by using GC-MS as described in [42] (see the Supplemental Data for details). The titers were weighted by the number of eggs in each batch and compared between queens producing new queens and queens producing only workers.

Supplemental Data

Supplemental Experimental Procedures and one figure are available at <http://www.current-biology.com/cgi/content/full/18/4/265/DC1/>.

Acknowledgments

We thank and Florian Dessimoz for great help with raising ant colonies and two anonymous reviewers for useful comments on this manuscript. This study was supported by several grants from the Swiss National Science Foundation to L.K.

Received: November 27, 2007

Revised: December 24, 2007

Accepted: January 11, 2008

Published online: February 14, 2008

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