Original Article

Regulation of queen development through worker aggression in a predatory ant

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Extreme developmental plasticity within the eusocial insects defines the separation between the queen and worker castes. The switch between these 2 developmental pathways is thought to be under tight social control. Although a large emphasis has been placed on the effect of larval nutrition on caste determination, workers of many species have no direct control over larval feeding. This may be particularly relevant to the early evolution of ants, when behaviors that allow fine control over larval nutrition, such as mouth-to-mouth food exchange between larvae and workers, were probably not yet present. We investigated larval-directed aggression as an alternative means to regulate queen development in the ant *Harpegnathos saltator*, a species that retains ancestral characteristics. We tested worker response toward natural queen-destined larvae and larvae induced to develop as queens using a juvenile hormone analog (JHA). Workers from colonies that were not rearing queens bit queen-destined larvae, whereas worker-destined larvae were not attacked. When colonies were naturally rearing queens, workers did not bite JHA-treated larvae, and a larger proportion of these larvae developed into queens compared with larvae that received biting. This supports the hypothesis that workers of *H. saltator* use biting to inhibit queen development during periods when the conditions for queen rearing are not met. We propose that mechanical stress, in addition to nutrition, could serve as a mechanism to regulate queen development in species that lack fine control over larval food consumption, and this may have played a role during the early evolution of eusociality in ants. *Key words:* aggression, developmental plasticity, juvenile hormone, queen determination, social regulation, stress. *[Behav Ecol]*

INTRODUCTION

In nature, some organisms display a striking degree of developmental plasticity where a single genotype can produce multiple discrete forms in response to environmental variation. These alternative forms are termed "polyphenisms" (Mayr 1963; West-Eberhard 1989; Nijhout 2003), and species where polyphenisms occur have traditionally served as models for understanding basic principles of developmental regulation (Nijhout 1999; West-Eberhard 2003; Hartfelder and Emlen 2005; Simpson et al. 2011). In solitary species, the switch between these alternative forms is determined by environmental factors, but development in social species may be regulated via behavioral interactions. This is particularly relevant to the production of the queen and worker castes in social insects, where alternative morphologies define a reproductive division of labor (Wilson 1971). The behaviors that workers use to regulate caste development, therefore, represent a fundamental aspect of the evolution of social insect castes and should have played an important role during the early evolution of eusociality.

With few exceptions (Cahan et al. 2002; Smith, Toth, et al. 2008; Schwander et al. 2010), social insect larvae are totipotent and their caste fate is regulated through interactions with adult workers. Worker control over larval nutrition has been considered the major factor used to regulate caste in the eusocial Hymenoptera (ants, wasps, and bees) (Wheeler 1986; Hunt 1991). This is exemplified by the honeybee, *Apis mellifera*, where queens are fed a special diet of royal jelly. Components of this diet initiate a cascade of transcription and endocrine changes that leads to queen development (Winston 1991; Kamakura 2011; Mutti et al. 2011). In other social insect species, workers and larvae share food directly through mouth-to-mouth food exchange (trophallaxis); workers may inhibit queen development through "nutritional castration" by restricting larval access to food or by forcing larvae to regurgitate excess food (Wheeler 1986; Hunt 1991).

There are species, however, where workers lack behaviors that allow for direct control over larval food consumption (Hölldobler and Wilson 1990) or where differences in larval nutrition do not completely explain differences in caste (Wenseleers et al. 2004; Suryanarayanan et al. 2011). This may be especially pertinent for the early evolution of ants, where distinct queen and worker castes are already present in the earliest known ant fossils (Wilson 1987). In most ants, larval development is thought to be regulated via trophallaxis between larvae and adult workers, but trophallaxis between larvae and adults is considered a derived trait (Peeters 1997). In species that display ancestral characteristics—an absence of trophallaxis, low queen-worker dimorphism, predatory behavior, etc.—larvae feed more or less independently on insect prey that is carried into the nest by foragers. In these species, it is unlikely that workers could control larval caste development exclusively through differential feeding. If regulation of caste is required, then other means should exist.

In addition to nutritional factors, there is evidence that mechanical stress may affect larval development. Direct evidence for this has been found in the flour beetle *Tribolium castaneum*, where mechanical stress caused a 54% reduction in larval growth and 55% reduction in weight gain (Hirashima et al. 1992, 1993). With respect to caste development in the social insects, there has been previous speculation that mechanical

stress may be used to inhibit queen development in wasps (Jeanne 2009), and observations of antennal drumming in *Polistes fuscatus* have been linked to regulation of caste development (Suryanarayanan et al. 2011). In the ant *Myrmica*, workers have been observed biting queen-destined larvae at the end of the breeding season, piercing the larval cuticle, and a portion of these larvae revert to worker development (Brian 1973). In the context of these previous studies, we hypothesized that mechanical stress may serve as a mechanism to regulate queen development in ants, particularly species from the relatively basal subfamily Ponerinae whose members share a number of ancestral characters in morphology and behavior that may limit worker control over larval feeding (Schmidt 2009).

We chose the Ponerine ant *Harpegnathos saltator* as a model to investigate caste regulation in species where adult workers have limited control over larval feeding. Adult workers of this species do not engage in trophallaxis with larvae, a trait shared by other Ponerine species (Hölldobler and Wilson 1990; Liebig et al. 1997). Foragers of *H. saltator* are predatory hunters and larvae feed more or less independently on whole insect prey carried into the nest. Queen-worker dimorphism is low (adult queens are 1.7 times larger than workers by dry weight [Peeters et al. 2000]), and larvae remain capable of developing into either queens or workers until the final stages of larval development (results presented in this paper). Additionally, larvae of *H. saltator* are mobile and capable of self-organizing around insect prey without assistance from adult workers (Supplementary Figure S1). The combination of these factors makes *H. saltator* a prime candidate for investigation of the regulation of caste development in a species that lacks fine control over larval nutrition.

In laboratory colonies of *H. saltator*, we observed workers biting larvae, but unlike other ant species where larval-directed biting has been observed (Brian 1973; Masuko 1986; Ito and Billen 1998), workers of *H. saltator* did not penetrate the larval cuticle (Penick CA, Jürgen L, personal observation). Based on our observations, we predicted that larval-directed biting may be used by workers to inhibit queen development during periods when the colony is not rearing new queens (i.e., outside of the breeding season or during early stages of colony growth). To investigate this behavior, we developed a method to induce larval queen development in colonies that were not normally rearing queens using the juvenile hormone analogue (JHA) methoprene. Juvenile hormone (JH) has been previously shown to play a role in queen development in ants, honeybees, and bumblebees (Wheeler 1986; Rachinsky et al. 1990; Cnaani et al. 1997) as well as soldier development in ants and termites (Lüscher and Springhetti 1960; Wheeler and Nijhout 1981; Wheeler 1990; Rajakumar et al. 2012). Using this method, we predicted that workers of *H. saltator* would preferentially bite queen-destined larvae compared with worker-destined larvae and that biting would cause a significant portion of these larvae to revert toward worker development. Although previous research in the eusocial Hymenoptera has focused on nutrition as a means to regulate queen development, here we provide insight into how mechanical stress may serve this function in ants. Factors aside from control over larval nutrition may have been important during the early evolution of morphological castes, and we predict that mechanical stress may be used to regulate caste development in other social insect species.

MATERIALS AND METHODS

Study species and lab conditions

Colonies of *H. saltator* were originally collected from Karnataka State in southern India between 1994 and 1999. In the laboratory, colonies were maintained under constant conditions (25 °C, 12:12 light/day) and fed live crickets (*Acheta domesticus*) twice per week, which workers paralyze in the foraging arena and bring into the nest. All colonies used in this experiment were headed by gamergates (mated reproductive workers).

JH application and induction of queen development

To confirm that JHA application could induce queen development in *H. saltator*, we tested the effect of topical application of JHA on final instar larvae (fourth instar). Twenty to thirty fourth instar larvae (4.1–6.5 mm in length) were taken from a single mature colony and divided evenly between 2 groups containing 20 workers (we could not distinguish larval sex until after pupation, so both male and female larvae were included in this study). This was repeated for a total of 10 independent colonies, and the groups were fed crickets ad libitum. In the treatment group, the JHA compound methoprene (Chem Service, West Chester, PA) was suspended in acetone and applied topically. Individual larvae received 0.25–0.5 µg JHA per mg larval weight. Control larvae were treated with acetone only. Caste was determined during the pupal stage by opening pupal cocoons and identifying queens based on the clear presence of wing buds.

Biting response

We predicted that biting may be used to inhibit larval queen development in *H. saltator* based on previous observations of workers biting larvae in this species (Penick CA, Jürgen L, personal observation). To quantify the level of biting received by larvae induced to develop as queens we established colonies as described above and introduced JHA-treated larvae and control larvae (worker destined). Larvae received a 1 µl topical application of either the JHA solution (0.25–0.5 µg JHA per mg larval weight) or an equivalent amount of the acetone solvent for the control. Behavioral observations were made between 18 and 24 h after treatment and consisted of three 10-min observation sessions per colony evenly spaced over a 6-h period. An aggressive interaction was distinguished from larval grooming by observation of workers repeatedly biting larvae using the 2 protrusions at the base of each mandible (Supplementary Figure S2). This often caused larvae to react with a reflexive head jerk (Supplementary Figure S3), which has not been observed during grooming. Prolonged encounters between the same larva and worker were counted as a single aggressive interaction. After 24 h, larvae were exchanged between treatment and control groups to control for the possibility that exposure of adult workers to JHA would influence their behavior. Behavioral observations were continued 18–24 h after larvae were exchanged.

To control for any effects of JHA not related to queen development, we conducted a second experiment where we provided naturally produced queen larvae and worker-destined larvae (fourth instar) to nonqueen producing colonies and observed the worker response. Queen-destined larvae were initially identified based on their larger size compared with worker-destined larvae, and a portion of these larvae were reared to pupation to confirm that they developed into queens. To ensure that worker-destined larvae were not males, we reared all larvae to pupation after the experiment and excluded male larvae from our analysis. The number of biting events was quantified over 5 min after the larva was introduced, and the tests were conducted blind. A total of 51 larvae were taken from 10 colonies producing queens, and an equivalent number of larvae were tested from colonies that were not producing queens. To ensure that the larvae used in the experiment were truly queen destined or worker destined, a group of larvae (1–7 individuals) from each colony used in this

Table 1

Queen induction by application of JHA to fourth instar larvae

	Colonies with queen development (N = 10)	% Female brood developed as queens	Totals for each caste		
			Queen	Worker	Male
JHA treatment	7	57	12	9	4
Control	0	0	0	36	25

experiment was isolated with 10 workers. Additional larvae of all size classes (~25) were also included in these groups to assist with the breakdown of insect prey to prevent workers from cannibalizing larvae. After 8–10 days, we opened all pupal cases to confirm that queens were produced.

To investigate whether workers from colonies naturally rearing queens would not bite JHA-treated larvae, we removed all brood from these colonies and provided them with JHA-treated larvae from a foreign colony. These were fourth instar larvae treated with 0.25–0.5 μg JHA per mg larval weight. Half of these JHA-treated larvae were also provided to a colony that had not been rearing queens. Larval-directed biting was compared between both groups based on three 10-min observation sessions separated by at least 2 h, and these began 24 h after larval treatment. After all treated larvae had pupated, we determined the caste of larvae in each group (worker or queen) to assess the effect of worker biting on the inhibition of queen development.

Larval feeding

Although biting may serve as a mechanism to regulate queen development in *H. saltator*, this does not rule out worker control over larval feeding as another potential mechanism. We conducted 2 experiments to examine the effects of worker control over larval feeding. In the first experiment, we examined whether differences in larval feeding occur between JHA-treated larvae that received biting and worker larvae. We fed JHA-treated larvae crickets ad libitum and compared larval weight gain in these groups with control larvae that were either fed ad libitum or were starved over a 2-day period. Ten groups from separate colonies were established for each of 3 treatments. Boxes contained 5 larvae and 10 workers, and controls were treated with acetone. The combined weight of all 5 larvae was taken at the start of the experiment, at 24 h, and at 48 h after treatment.

In the second experiment, we compared food consumption during the first 12 h after JHA treatment (before workers began to bite larvae) and from 12–24 h after treatment when worker biting occurred. This experiment was designed to examine changes in larval feeding that occurred before and after workers began biting larvae to test whether workers actively restricted larval feeding when they perceived larvae as queen destined. Groups from 10 separate colonies were established with 5 larvae and 10 workers. In one group, larvae received 5 pre-stung crickets immediately after JHA or control treatment. Cricket mass was measured at the beginning of the experiment and after 12 h to estimate the cricket mass consumed. In the second group, larvae did not receive crickets until 12 h after treatment (i.e., at the onset of worker biting), and we measured the cricket mass consumed during the period 12–24 h after JHA treatment.

Statistical analysis

To test for the effect of JHA on queen development, we used the Cochran's test for binary data to compare the number of

colonies producing queens in both treatments. Data from behavioral experiments were not normally distributed, and differences among groups were tested using nonparametric Kruskal–Wallis and multiple comparison tests. For experiments on larval feeding the data were normally distributed and had equal variance (Bartlett's test, $P > 0.05$: larval weight gain experiment; initial, $P = 0.94$; 24 h, $P = 0.28$; 48 h, $P = 0.15$; cricket consumption experiment, $P = 0.19$). In these cases, data were analyzed by parametric tests including analysis of variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) for multiple comparisons. Tests were performed using Statistica version 9 (StatSoft, Tulsa, OK).

RESULTS

Induction of queen development

To test the effect of JH on larval queen development, we treated larvae of *H. saltator* with either methoprene (JH analog) or acetone as a control. Topical treatment of JHA induced queen development in 70% of treatment colonies, whereas no queens developed in the control (Cochran's test, $N = 10$, $P = 0.008$) (Table 1). For this experiment, caste and sex were determined at the pupal stage. Previous trials revealed that JHA-treated larvae rarely eclosed from the pupal stage, a result consistent with the use of methoprene as an insecticide to disrupt pupal development (Dhadialla et al. 1998). In these previous trials, we observed workers eating queen, worker, and male pupae, and this indicated that workers were not specifically culling one caste or sex.

Biting response

We investigated worker response to larvae induced to develop as queens by providing colonies with either JHA-treated larvae or control-treated larvae. Workers responded toward JHA-treated larvae with a high frequency of biting on day 1 (median: 17 bites per 30 min, $N = 10$) with little biting directed toward control larvae (median: 0 bites per 30 min, $N = 10$) (Figure 1a). On day 2, larvae were exchanged between worker groups to control for possible effects of residual JHA on worker behavior. The frequency of biting showed a similar pattern to day 1, with a higher frequency of biting directed toward JHA-treated larvae (median: 11.5 bites per 30 min, $N = 10$) compared with controls (median: 0.5 bites per 30 min, $N = 10$) (Figure 1a). We did not find evidence that JHA was influencing adult behavior, and workers that had been previously biting JHA-treated larvae switched to normal brood care when they were given control larvae. Examination of larvae that received biting did not reveal signs that workers penetrated the cuticle or caused physical damage.

Time-lapse video surveillance showed that worker biting began, on average, 11 h and 8 min after JHA treatment (range: 8.4–15 h, $N = 10$). During the interval before biting occurred, workers retrieved larvae, brought them into the nest, and arranged them in a group. When larvae were bitten by workers, they responded with a characteristic head jerk. This
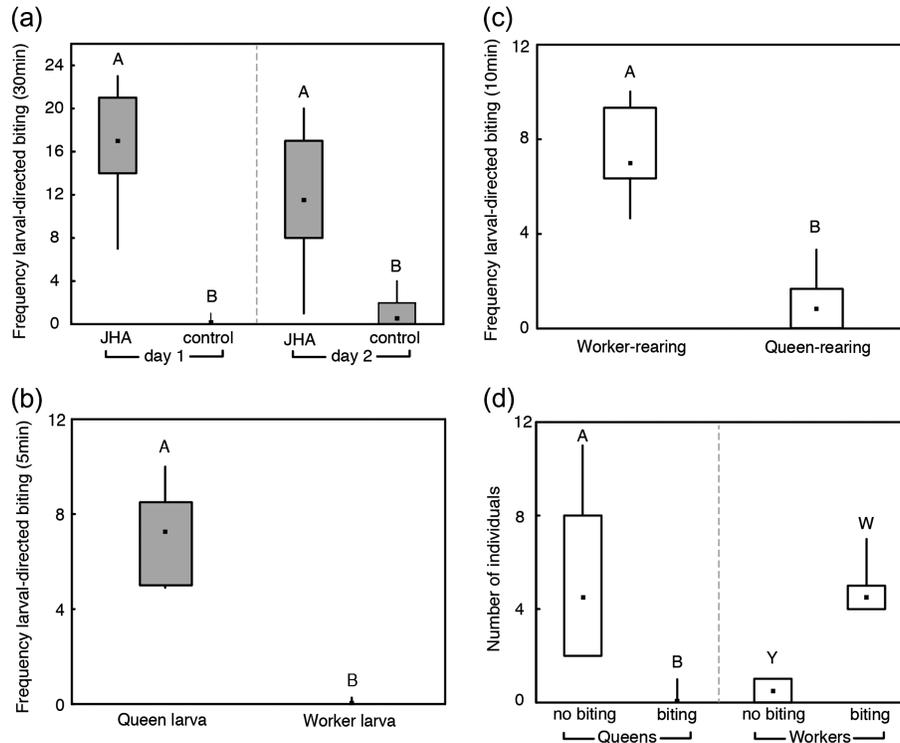
Figure 1

Worker response to queen larvae and the effect of biting. Box plots represent median, 25–75%, and range. (a) Frequency of biting directed toward JHA-treated larvae and control larvae (treated with acetone) ($N = 10$ colonies). Workers preferentially bit JHA-treated larvae (Kruskal–Wallis, degrees of freedom [df] = 3, $P < 0.0001$; multiple comparisons, $P < 0.0001$ [JHA day 1 vs. control day 1], $P < 0.001$ [JHA day 1 vs. control day 2], $P = 0.002$ [JHA day 2 vs. control day 1], $P = 0.025$ [JHA day 2 vs. control day 2]). (b) Frequency of biting directed toward natural queen larvae or worker larvae. Queen larvae were preferentially attacked, whereas worker larvae received little to no biting (Wilcoxon signed-rank test, $N = 10$, $Z = 2.80$, $P = 0.005$). (c) Frequency of larval-directed biting exhibit toward JHA-treated larvae by workers from colonies rearing queens or colonies rearing workers. Workers from colonies rearing queens bit JHA-treated larvae at a lower frequency than workers from colonies rearing workers (Wilcoxon matched-pairs test, $N = 6$, $Z = 2.20$, $P = 0.028$). (d) Queens and workers produced from JHA-treated larvae that received or did not receive biting. A larger proportion of female larvae developed as workers in response to biting, whereas a greater proportion of queens were produced in colonies that showed reduced biting (Wilcoxon matched-pairs test, $N = 6$; queens produced in biting vs. nonbiting colonies, $P = 0.028$; workers produced in biting vs. nonbiting colonies, $P = 0.028$). Letters indicate significant differences.

behavior could be artificially induced by pinching larvae with forceps (Supplementary Figure S3). Pinching at the base of the neck triggered the head jerk in all cases, whereas pinching the main body did not lead to any visible response (Cochran's test, $N = 20$, $P < 0.001$). In each trial, the head jerk was accompanied by the expulsion of clear liquid from the anal pore, but there was no evidence that workers consumed this liquid.

Naturally produced queen larvae were also used to test worker response to control for effects of JHA treatment not associated with queen development (e.g., presence of a foreign chemical on the larval cuticle). When naturally produced queen larvae were presented to worker groups, workers responded with a high frequency of biting (median: 7.3 biting events over 5 min), whereas worker-destined larvae were rarely bitten by workers (median: 0 biting events over 5 min) (Figure 1b). In all cases, worker-destined larvae were groomed and placed with other larvae inside the nest. We confirmed that the colonies used as the source for queen-destined larvae were in fact rearing queens by allowing worker groups to rear a portion of these larvae to the pupal stage where caste could be assessed; larvae from colonies used for the source of queen-destined larvae produced queens in all cases ($N = 10$), whereas larvae from control colonies produced no queens ($N = 10$).

Workers from colonies that were previously rearing queens were rarely observed biting JHA-treated larvae (median: 0.8

bites per 10 min) compared with controls (median: 7 bites per 10 min) (Figure 1c). Larval mortality determined at the pupal stage did not differ between groups (Wilcoxon matched-pairs test, $N = 6$, $Z = 0.41$, $P = 0.69$); however, a greater proportion of queens were produced in colonies where larvae did not receive high levels of biting, whereas colonies where biting did occur produced a higher frequency of workers (Figure 1d).

Larval food consumption

In addition to biting, we found effects of JHA treatment on larval feeding behavior. First, JHA treatment was associated with a reduction in larval weight gain. JHA-treated larvae gained 82% less weight than control larvae over 48 h, even with excess food provided to both groups (Figure 2a). The final mass of JHA-treated larvae 48 h after treatment was 12.6 ± 2.5 mg (mean ± standard deviation [SD]) compared with 19.0 ± 3.0 mg for control larvae ($N = 10$). Despite a reduction in weight gain, JHA-treated larvae still weighed more than larvae that were completely starved (mean ± SD: 9.2 ± 1.7 mg, $N = 10$).

Second, differences in larval feeding did not occur until after workers perceived larvae as queen destined (12 h after treatment) and began targeting larvae with biting. When larvae were allowed to feed during the first 12 h after treatment

(before worker biting occurred), the mean cricket mass consumed by JHA-treated larvae (14.0 ± 6.1 mg; mean ± SD) was slightly less than controls (20.9 ± 9.4 mg; mean ± SD), although this difference was not significant (Figure 2b). In larvae that were fed after worker biting began (12–24 h after treatment), the cricket mass consumed by JHA-treated larvae (7.8 ± 5.9 mg; mean ± SD) was less than half the amount consumed by controls (18.4 ± 10.8 mg; mean ± SD), and this difference was significant.

DISCUSSION

We identified worker aggression toward larvae as a potential factor in regulating caste development in the early evolution of ant eusociality. The regulation of caste in social insect species cannot be completely explained by differences in larval nutrition. We provide evidence for an additional mechanism to regulate caste in ants: mechanical stress. Workers of *H. saltator* preferentially bit queen-destined larvae whether they were induced to develop as queens using JHA or if natural queen-destined larvae were introduced to a nonqueen producing colony. Our results indicate that workers from nonqueen producing colonies specifically targeted queen-destined larvae to receive biting and were not responding to other factors. Such aggressive behavior toward queen-destined larvae may be expected in other ant species with ancestral characteristics.

Application of JHA induced queen development as late as the final larval instar, showing that female larvae of *H. saltator* remain bipotential until near the end of the larval period. Queens and workers of this species have relatively small size dimorphism (queens of *H. saltator* weigh 1.7 times more than workers by dry weight [Peeters et al. 2000] compared with queens of the fire ant *Solenopsis invicta*, which are 25 times heavier than the average worker [Tschinkel 2006]). Queen development in species that display high queen-worker dimorphism is often fixed early in development, even inside the embryo (Passera and Suzzoni 1979; Schwander et al. 2008). In contrast, *H. saltator* larvae may be capable of crossing the threshold between queen and worker development late in the larval period. Under these conditions, restriction of larval feeding alone may not be effective to inhibit queen development if a larva has already consumed enough food to develop into a queen.

The observation that workers from nonqueen producing colonies bite queen-destined larvae suggests that they try to inhibit queen development during periods when queen production is not beneficial to the colony—for example, outside of the normal season for sexual production or when the colony does not have sufficient resources. This hypothesis was supported, as workers from colonies that were naturally rearing queens did not bite JHA-treated larvae. Furthermore, these larvae developed into queens at a higher proportion than larvae that received biting, which suggests that biting successfully reverted JHA-treated larvae to worker development.

We propose that biting may be necessary to regulate caste in this species because workers do not have fine control over larval feeding, and larvae remain bipotential until the last stages of larval development. We found some evidence, however, that workers do restrict larval feeding in *H. saltator* when queen development is induced using JHA. JHA-treated larvae showed reduced weight gain during the first days after treatment and they consumed less food than controls, especially after the onset of biting. This suggests that workers restricted larval food consumption to some degree. However, workers may not be able to fully suppress queen development if larvae have already consumed food in excess. In this case, biting may be the most effective mechanism to suppress queen develop-
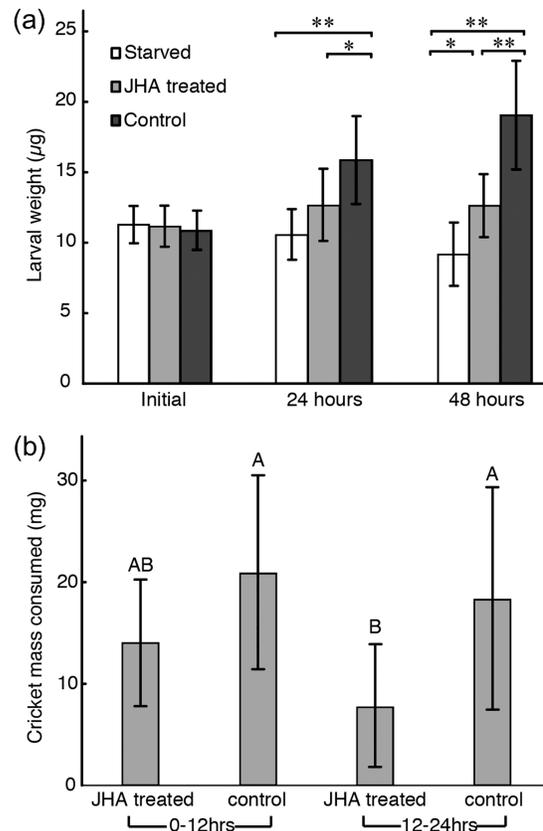

Figure 2
Larval feeding response. Plots represent mean ± SD. (a) Change in larval weight (*N* = 10 groups) over 48 h in groups that were starved, treated with JHA, or treated with the acetone control (initial: ANOVA, df = 2, *N* = 30, *P* = 0.92; 24 h: ANOVA, df = 2, *N* = 30, *P* = 0.0014; Tukey's HSD multiple comparisons, *P* = 0.17 [starved vs. JHA treated], *P* < 0.001 [starved vs. control], *P* = 0.02 [JHA treated vs. control]; 48 h: ANOVA, df = 2, *N* = 30, *P* < 0.0001; Tukey's HSD multiple comparisons, *P* = 0.028 [starved vs. JHA treated], *P* < 0.001 [starved vs. control], *P* < 0.001 [JHA treated vs. control]). (b) Effect of worker biting on larval food consumption showing cricket mass consumed (mean, *N* = 10) by JHA treated and control larvae before (0–12 h) and after (12–24 h) larval-directed biting occurred. JHA-treated larvae consumed less cricket mass than controls, especially after the onset of biting (ANOVA, *F* = 4.73, df = 3, *P* = 0.007; Tukey's HSD multiple comparisons, *P* = 0.006 [JHA treated 12–24 h vs. control 0–12 h], *P* = 0.036 [JHA treated 12–24 h vs. control 12–24 h], *P* = 0.27 [JHA treated 0–12 h vs. control 0–12 h], *P* = 0.34 [JHA treated 12–4 h vs. JHA treated 0–12 h], *P* = 0.65 [JHA treated 0–12 h vs. control 12–24 h], *P* = 0.90 [control 0–12 h vs. control 12–24 h]). Letters or asterisks indicate significant differences.

ment. In species where larvae and adults share food through trophallaxis, workers can induce larvae to regurgitate food that they have already consumed (Hunt 1991).

Without trophallaxis, some ant species use alternative means to sequester resources from larvae, such as the so-called "dracula ants" (subfamily Amblyoponinae) whose workers bite larvae and feed on larval hemolymph directly (Masuko 1986) or the ant *Leptanilla japonica* whose larvae possess specialized "hemolymph taps" that allow workers to feed on hemolymph without puncturing the larva (Masuko 1989). In the ant *Myrmica*, workers bite queen-destined larvae at the end of the breeding season to inhibit queen development (Brian 1973). Although there is no evidence that workers of *Myrmica* feed on larval hemolymph, they do rupture the outer cuticle and produce

visible scars on larvae (although pinching larvae with forceps induced the same response). In contrast, workers of *H. saltator* do not penetrate the cuticle when they bite sexual larvae, and no visible scars appear on larvae after they have been attacked. This implies that the stress induced by biting affects larval development directly and is not related to loss of hemolymph or physical damage. A second difference between the behavior in *Myrmica* and *H. saltator* is that *Myrmica* species represent the derived condition where workers and larvae both share food through trophallaxis (Creemers et al. 2003). The biting response in *Myrmica* appears to be used as a last resort when larvae have already passed the threshold toward queen development. Nevertheless, the example in *Myrmica* illustrates that biting may serve as a mechanism to regulate caste under some circumstances even when a species has developed mechanisms for controlling larval feeding.

Predatory behavior, a lack of trophallaxis and low queen-worker dimorphism are considered ancestral characteristics in ant evolution (Peeters 1997; Hölldobler and Wilson 2009), and all 3 reduce the efficacy of nutritional control as a means to regulate caste. This suggests that factors in addition to larval nutrition may have been important during the early evolution of ants for the production of reproductive and sterile castes. Similar to *H. saltator*, mechanical stress may be an important mechanism for caste regulation in other species where fine control over larval nutrition is not possible, such as other predatory ants and wasps. This is supported by observations of larval-directed biting and hemolymph feeding in other species of predatory ants (Masuko 1986; Masuko 1989; Ito and Billen 1998), and the description of antennal drumming in the paper wasp *P. fuscatus* as a mechanism to inhibit queen development (Suryanarayanan et al. 2011). In this species, larvae are prevented from developing as queens when they receive a specific frequency of vibration (17 Hz), whereas other frequencies are not effective. This indicates that antennal drumming may serve as a mechanical signal rather than a general stressor. There is also evidence that pheromones, rather than nutritional factors, directly influence larval fate in ants of the genera *Odontomachus* and *Tetraponera*, both members of relatively basal subfamilies (Ponerinae and Pseudomyrmecinae) (Terron 1977; Colombel 1978) as well as in the stingless bee *Melipona beecheii*, where differences in larval nutrition alone do not explain differences in caste (Jarau et al. 2010).

These examples emphasize the importance of mechanisms aside from nutrition to regulate caste in species that have low queen-worker dimorphism or other limitations on regulation of larval provisioning. In more derived species, where workers have greater control over larval feeding, nutritional regulation of caste may be the most reliable mechanism. There is evidence in bumblebees and some ants that queens produce a pheromone that causes workers to underfeed larvae, and removal of the queen can induce workers to rear larvae into new queens (Wheeler 1986). In the harvester ant *Pogonomyrmex badius*, Smith, Anderson, et al. (2008) found that queen-destined larvae had higher N content and fed at a higher trophic level compared with worker-destined larvae suggesting that food quality may play a role in caste development in this species similar to the honeybee. In some cases queen determination may be triggered by maternal effects incorporated into the egg (Passera and Suzzoni 1979; Schwander et al. 2008) or, in some highly derived instances, caste may be determined genetically (Cahan et al. 2002; Schwander et al. 2010).

In *H. saltator*, queen development results from interactions between larval nutrition, hormones, and behavioral regulation. From this information, we can create a model for how queen production is regulated in this species. Larvae remain bipotential until late in larval development (the fourth in-

star). If the environmental conditions for queen production are met, adult workers may allow larvae to feed in excess, triggering a rise in JH production that ultimately leads to queen development. However, if a larva approaches the threshold between queen and worker development and conditions for queen production are not met, workers may restrict larval feeding and use biting to revert larvae to the worker trajectory. The physiological response to biting is not yet known, but it may inhibit JH production via differences in biogenic amine release, and this in turn may affect gene transcription associated with queen development (Barchuk et al. 2007; Patel et al. 2007; Smith, Toth, et al. 2008). Vibration and mechanical stress have been shown to affect biogenic amine levels in other insects (Davenport and Evans 1984; Woodring et al. 1988; Hirashima et al. 1992, 1993), and biogenic amines, particularly octopamine, have been shown to affect JH synthesis or release (Thompson et al. 1990; Kaatz et al. 1994; Woodring and Hoffmann 1994).

Finally, larval nutrition and stress are each known to affect insect growth and development (Hirashima et al. 1992; Mirth and Riddiford 2007), and the interaction between these 2 factors may be required for regulation of caste. With increased interests in the proximate genetic factors that influence developmental polyphenisms (Emlen et al. 2006; Smith, Toth, et al. 2008), the effects of mechanical stress may provide a novel system to investigate the pathways that underlie developmental plasticity. Studies on caste determination in social insects have been used as models for understanding developmental plasticity more generally, and the integration of alternative factors that regulate development with nutritional cues should provide insight into this field.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/.

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