J Comp Physiol A (2011) 197:1063–1071
DOI 10.1007/s00359-011-0667-0

ORIGINAL PAPER

# Reproduction, dominance, and caste: endocrine profiles of queens and workers of the ant *Harpegnathos saltator*

**Clint A. Penick · Jürgen Liebig · Colin S. Brent**

Received: 11 May 2011 / Revised: 24 June 2011 / Accepted: 30 June 2011 / Published online: 20 July 2011


**Abstract** The regulation of reproduction within insect societies is a key component of the evolution of eusociality. Differential patterns of hormone levels often underlie the reproductive division of labor observed among colony members, and further task partitioning among workers is also often correlated with differences in juvenile hormone (JH) and ecdysteroid content. We measured JH and ecdysteroid content of workers and queens of the ant *Harpegnathos saltator*. In this species, new colonies are founded by a single queen, but after she dies workers compete in an elaborate dominance tournament to decide a new group of reproductives termed "gamergates." Our comparisons revealed that queens, gamergates, and inside workers (non-reproductive) did not differ in levels of JH or ecdysteroids. However, increased JH and decreased ecdysteroid content was observed in outside workers exhibiting foraging behavior. Application of a JH analog to virgin queens of *H. saltator*, although effective at inducing dealation, failed to promote egg production. Together, these results support the hypothesis that JH has lost its reproductive function in *H. saltator* to regulate foraging among the worker caste.

**Keywords** Juvenile hormone · Ecdysone · Dominance · Division of labor · Colony growth-rate

C. A. Penick (✉) · J. Liebig
School of Life Sciences, Arizona State University,
Tempe, AZ 85287-4501, USA
e-mail: Clint.Penick@asu.edu

C. S. Brent
US Department of Agriculture, Arid-Land Agricultural Research Center, Maricopa, AZ 85138, USA

## Introduction

At the heart of all eusocial societies is a reproductive division of labor, where only one or a few individuals reproduce while the rest serve as a functionally sterile workforce (Wilson 1971). In simple societies, this division of labor emerges as a result of dominance interactions among adults (West-Eberhard 1978; Fletcher and Ross 1985; Röseler 1991; Heinze et al. 1994), while in highly eusocial species this occurs as a consequence of the reproductive specialization of morphologically distinct queen and worker castes (Oster and Wilson 1978; Hölldobler and Wilson 2009). Workers have reduced fecundity, although they often retain functional ovaries and will begin to lay eggs in the absence of the queen. In order for a reproductive division of labor to be maintained, activation of worker ovaries must be inhibited so that they do not begin to lay eggs in the presence of an active reproductive. Differences in reproductive activity observed among castes are thought to be under endocrine control. Specifically, juvenile hormone (JH) and ecdysteroids have been implicated as regulators of reproductive physiology and behavior in social insects (Robinson 1987; Robinson and Vargo 1997; Hartfelder 2000) although other factors, such as biogenic amines (Sasaki and Nagao 2001; Sasaki et al. 2007, 2009), have been increasingly shown to be involved in these processes.

In solitary insects, JH and ecdysteroids generally function as gonadotropins and have been shown to affect the synthesis and uptake of vitellogenin, a precursor of yolk protein (Nijhout 1994; Klowden 1997; Hartfelder 2000; Raikhel et al. 2005 Dong et al. 2008). This gonadotropic function has been conserved in the social bumble bee, *Bombus terrestris*, where increased JH and ecdysteroid levels are correlated with reproduction by dominant workers and queens (Bloch et al. 2000; Geva et al. 2005). In contrast, JH appears

to be dissociated from reproductive activity in adult queens of the highly eusocial honey bee, *Apis mellifera* (Robinson et al. 1991; Robinson et al. 1992). Instead, JH serves as a behavioral pacemaker within the worker caste, promoting the onset of foraging behavior (Jaycox et al. 1974; Robinson 1987; Sullivan et al. 2000). This alternative role of JH as a regulator of foraging is thought to have evolved through co-option of the endocrine signaling system associated with reproduction in a solitary ancestor (West-Eberhard 1996; Amdam et al. 2004). Some evidence suggests a similar behavioral role for JH in other social insects (Robinson and Vargo 1997; Giray et al. 2005), including ants, where increased JH levels correlate with foraging by workers of the ant *Myrmicaria eumenoides* (Lengyel et al. 2007) as well as co-founding queens of the harvester ant *Pogonomyrmex californicus* (Dolezal et al. 2009).

In species of social insects where colony members compete over reproduction, differences in JH and ecdysteroids have been correlated with dominance. Examples in both bumble bees and wasps indicate that JH retains its gonadotropic function, and dominant individuals are characterized by having higher levels of circulating JH (Röseler et al. 1984; Bloch et al. 2000; Tibbetts and Izzo 2009; Tibbetts and Huang 2010). This pattern is reversed in two species of queenless ants—*Diacamma sp.* and *Streblognathus peetersi*—where JH levels are lowest in reproductive workers and elevated in subordinates (Sommer et al. 1993; Brent et al. 2006). These two Ponerine ant species are atypical in that they lack true queens, and a single dominant worker is responsible for reproduction. The role of JH in these worker reproductives contrasts with the role of JH found in true queens of the fire ant, *Solenopsis invicta*, where JH still has a gonadotropic function (Barker 1978; Brent and Vargo 2003; Burns et al. 2007).

Brent et al. (2006) proposed that queenless species evolved a separate gonadotropic regulatory mechanism when faced with the loss of the queen phenotype and the physiological constraints of the worker phenotype. To test this hypothesis we measured hormone content in the ant *Harpegnathos saltator*, another species of Ponerine ant in which both queens and fully reproductive workers occur. Colonies are founded by a single queen, but after the queen dies, workers compete in a dominance tournament to establish a new group of reproductives (Heinze et al. 1994; Peeters et al. 2000), and these are called "gamergates" (Peeters and Crewe 1984). Gamergates produce eggs at roughly half the rate of true queens (Peeters et al. 2000) and maintain their status by expressing a chemical fertility signal similar to that of queens (Liebig et al. 2000). Because there is a low queen–worker dimorphism in this species and they have similar behavioral repertoires (Peeters et al. 2000), a rare direct comparison of hormone levels and function can be made between castes.

For this study mature queens, gamergates, and workers performing in-nest tasks or foraging were sampled from mature colonies of *H. saltator* to determine whole-body JH and ecdysteroid content. In order to procure queen samples, we established new, queenright colonies in the laboratory. Finally, we investigated the role of JH during the period following colony foundation by treating virgin queens with a juvenile hormone analog (JHA). We monitored the effect of JHA treatment on the timing of dealation and the initiation of egg laying by virgin queens. Comparisons among groups allowed us to make inferences about the function of JH and ecdysteroids with respect to caste, dominance, and reproduction in *H. saltator* as well as to make comparisons of hormonal patterns across species.

## Methods

### Rearing conditions

Laboratory colonies of *H. saltator* were maintained at a constant temperature of 25°C and 12:12 light/dark cycle, and they were fed live crickets (*Acheta domesticus*) twice per week. The nests consisted of plastic boxes (19 × 27 cm) with a dental plaster floor, and each nest contained a preformed chamber covered with a glass plate (12 × 15 cm). Over 75 colonies of *H. saltator* were originally collected from Karnataka State in southern India between 1994 and 1999, and colonies have been crossbred in the laboratory to maintain genetic diversity.

### Determination of worker castes

Gamergates were identified in large colonies (200–300 workers) based on behavioral characteristics and direct observation of egg laying. Gamergates display a dominant stance characterized by an elevated posture where the gaster is raised above the level of the thorax, and subordinate workers approach dominants with their head lowered (Liebig et al. 1998). Workers identified as gamergates were paint-marked (Testors Pactra® enamel, Rockford, IL, USA) with an individual code and observed over several days to confirm reproductive status. In all cases, colonies contained multiple gamergates. Gamergates used in this study had been established at least 6 months prior to sampling, and there was little or no aggression observed among workers in these colonies.

Workers were selected from among 20 separate colonies. Individuals classified as foragers were taken directly from the foraging arena outside the glass-covered nest chamber. These workers had a darker cuticle than in-nest workers and responded defensively when provoked with forceps (defensive or nest-guarding behavior), which are

typical characteristics of foragers in other species (Hölldobler and Wilson 1990). Before selecting inside workers, all workers were removed from the foraging arena and held separately. One hour after their removal, any additional workers that entered the foraging arena were removed; inside workers were then chosen from those that remained in the nest chamber. Fully callow workers (newly emerged) were excluded from this study.

### Queen rearing and colony foundation

Queen samples were derived from new colonies founded in the laboratory, which is relatively easy in this species (Liebig et al. 1998). Young, unmated females were taken from their parent colonies and isolated with male pupae from a different colony. A single queen was used to found each colony except for one instance where a colony was founded with eight queens grouped together. Observation of egg laying was used to confirm that each queen was reproductive. Over 2 years, 100 queen-founded colonies were established, though not all produced workers. In order to confirm that queen colonies had reached a mature size at the time queens were sampled for JH and ecdysteroid content, we censused successful foundations at several points over 3 years to approximate colony growth rate. Based on the colony growth rate, it was determined that queens would be sampled for hormone analysis 8–15 months after colonies were established and contained at least 100 workers.

There is a limited queen-worker dimorphism in *H. saltator* relative to other species (Fig. 1). Peeters et al. (2000) found that both queens and gamergates have ovaries with eight ovarioles, although these are more developed and active in queens than workers. While queens are capable of ovipositing approximately two eggs per day, gamergates usually produce half that or less. These are minor differences relative to what is observed in most ant species (Hölldobler and Wilson 1990). Because these differences are minor, we report values that represent the total hormone content per ant. In order to account for any differences based on size with respect to caste (queen vs. worker), we analyzed all hormone data using both non-adjusted values as well as values standardized by dry-weight.

### Hormone quantification

For each sample, two individual ants of the same caste were placed in a vial containing 1 ml of methanol and crushed with a glass tissue grinder. Hexane was mixed into the vial with methanol (90% HPLC grade), centrifuged, and the hexane layer was pulled off to extract JH dissolved in this layer while ecdysteroid remained in the methanol portion. The methanol layer was centrifuged at 3,500 rpm for 5 min,

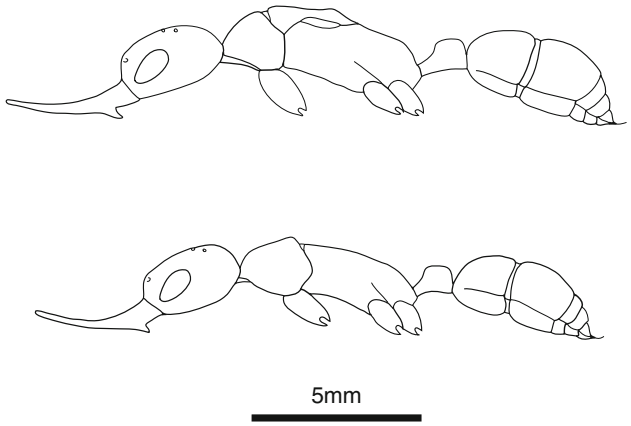


**Fig. 1** Illustration of queen (*top*) and worker castes of *H. saltator*. There is limited size dimorphism between both castes, with queens displaying increased thoracic development associated with wing musculature necessary for a mating flight

and the supernatant was removed. This extract was dried down, resuspended in 500 µl of 90% methanol, and stored at −80°C until analysis for ecdysteroid content.

The hexane portion was used to quantify JH using the gas chromatography/mass spectrometry (GC–MS) method of Bergot et al. (1981) as modified by Shu et al. (1997) and Brent and Vargo (2003). In order to filter-out contaminants, the hexane phase of the partitioned samples was eluted through aluminum oxide columns successively with hexane, 10% ethyl ether–hexane, and 30% ethyl ether–hexane. After drying, samples were derivatized by heating at 60°C for 20 min in a solution of methyl-D alcohol (Sigma-Aldrich, St Louis, MO, USA) and trifluoroacetic acid (Sigma-Aldrich, St Louis, MO, USA). Samples were dried down then resuspended in hexane and again eluted through aluminum oxide columns; 30% ethyl ether was used to remove non-derivatized components and 50% ethyl-acetate–hexane was used to collect the JH derivative. After drying, the sample was resuspended in hexane. The purified and derivatized JH was then analyzed using an HP 7890A Series GC (Agilent Technologies, Santa Clara, CA, USA) equipped with a 30 m × 0.25 mm Zebron ZB-WAX column (Phenomenex, Torrence, CA, USA) coupled to an HP 5975C inert mass selective detector. Helium was used as a carrier gas. JH form was confirmed by first running test samples in SCAN mode for known signatures of JH 0, JH I, JH II, JH III, and JH III ethyl; JH III was confirmed as the primary endogenous form in this species. Subsequent samples were analyzed using the MS SIM mode, monitoring at *m*/*z* 76 and 225 to ensure specificity for the $d_3$-methoxyhydrin derivative of JH III. Total abundance was quantified against a standard curve of derivatized JH III. The detection limit of the assay is approximately 1 pg.

Ecdysteroid content was determined by a competition radioimmunoassay originally developed by Warren et al. (1984) as modified by Brent et al. (2006). Duplicate 10 µl aliquots of the methanol phase of each partitioned sample were incubated overnight with 100 µl of (3H)-20-hydroxyecdysone stock (85.2 µCi/mmol, Perkin-Elmer, Waltham, MA, USA) in borate buffer, and 100 µl of a polyclonal ecdysteroid antiserum (H-22 antibody, L. Gilbert, UNC-CH) at 4°C on an orbital shaker. The specific ecdysteroid is unknown for *H. saltator*, but the H-22 antibody cross-reacts with ecdysone, ecdysterone, 20-hydroxyecdysone and makisterone A (Warren and Gilbert 1986). Intra- and inter-assay variability was minimized by generating standard competition curves for each set of samples, using 20-hydroxyecdysone stock (Sigma-Aldrich, St Louis, MO, USA) over a range of 15.6–2,000 pg. After incubating ~18 h, 20 µl of cleaned protein A solution (Pansorbin; CalBiochem, San Diego, CA, USA) was added to each tube to precipitate the antibody–antigen complex. Tubes were incubated for 1 h at 27°C and then centrifuged at 5,000*g*. Supernatant was removed and the remaining pellet was washed twice with 100 µl of borate buffer. The incorporation of microlabel was determined by a 2450 MicroBeta2 scintillation counter (Perkin-Elmer, Waltham, MA, USA) and ecdysteroid concentrations were estimated by nonlinear regression (Brent et al. 2006).

### JHA treatment of virgin alates

Virgin alate females were separated from their parent colony 3–6 weeks after eclosing. Individuals were treated topically (dorsal surface of the thorax) with 10µg of JHA (pyriproxyfen, technical grade, 98.9%) dissolved in 1 µl of acetone. Controls were treated with 1 µl of the acetone solvent alone. Female alates were kept in an individual box with a dental plaster floor and were fed small crickets twice per week. Observations were made daily to determine when individuals dropped their wings and when they began egg laying. An individual was considered to have begun egg laying when an egg was observed in their nest on each of two consecutive days to help exclude the possibility of counting non-viable, trophic eggs.

### Statistical analysis

Differences in hormone content among groups were compared using a Kruskal–Wallis ANOVA and post-hoc analyses in Statistica version 9 (StatSoft, Tulsa, OK, USA). When outliers were excluded (points that fell more than 1.5 times outside of the interquartile range), this did not change significant differences, and outliers are included in figures where they occurred. For queen samples, statistical comparisons were made using concentrations standardized for size differences (dry-weight) between queens and workers as well as non-adjusted values. Both analyses converged on the same pattern and did not affect the outcome of our statistical comparisons, so we report p-values based on non-adjusted measurements and report these in all figures. Additionally, the non-parametric Mann–Whitney *U* test was used to compare the timing of dealation and the initiation of egg laying among females treated with JHA or the control. Non-parametric tests were used in all cases due to heteroscedasticity (Brown-Forsythe test, $p < 0.05$) and a general lack of normality.

## Results

### Queen foundations and colony growth

To confirm that the queens used for the hormone bioassays were mature and reproductively active, we tracked colony growth of our queen-founded laboratory colonies. Colony growth during the first year after foundation was exponential (Fig. 2a), represented by the function $N_t = 1.94e^{0.0113t}$. Over this first year, colony age explained 75% of the variation in colony size. Over 3 years, growth-rate was best approximated by the logarithmic function $N_t = 117 \times \ln(t) - 595$, where $N_t$ represents the colony size at time $t$ (Fig. 2b). During this period, colony age explained 69% of the variation in colony size. The first workers did not eclose until 100 days after queens were isolated with male pupae in new nests. This was due in part to the time required for females to mate, drop their wings, and begin egg laying. In addition to workers and males, 5% of successful colonies were observed to produce female sexuals during their first year, 10% of colonies by the second year, and a total of 29% of colonies by the third year. These data confirmed that most colonies had reached a mature size at the end of the first year, which corresponded to the time when queens were sampled for hormone analysis.

### JH and ecdysteroid hormone content

JH content was elevated in foragers of *H. saltator*, but did not differ among inside workers, gamergates, or queens (Kruskal–Wallis, $df = 3$, $N = 57$, $H = 26.24$, $p < 0.0001$; multiple comparisons test: gamergate vs. forager, $p = 0.0002$; queen vs. forager, $p = 0.0004$; inside worker vs. forager, $p = 0.009$; gamergate vs. inside worker, $p = 0.94$; gamergate vs. queen, $p = 1.0$; inside worker vs. queen, $p = 0.79$) (Fig. 3a). The opposite pattern was observed with respect to ecdysteroids, where whole-body ecdysteroid content was depressed in foragers but did not differ among inside workers, gamergates, or queens (Kruskal–Wallis, $df = 3$, $N = 60$, $H = 27.5$, $p < 0.0001$; multiple comparisons

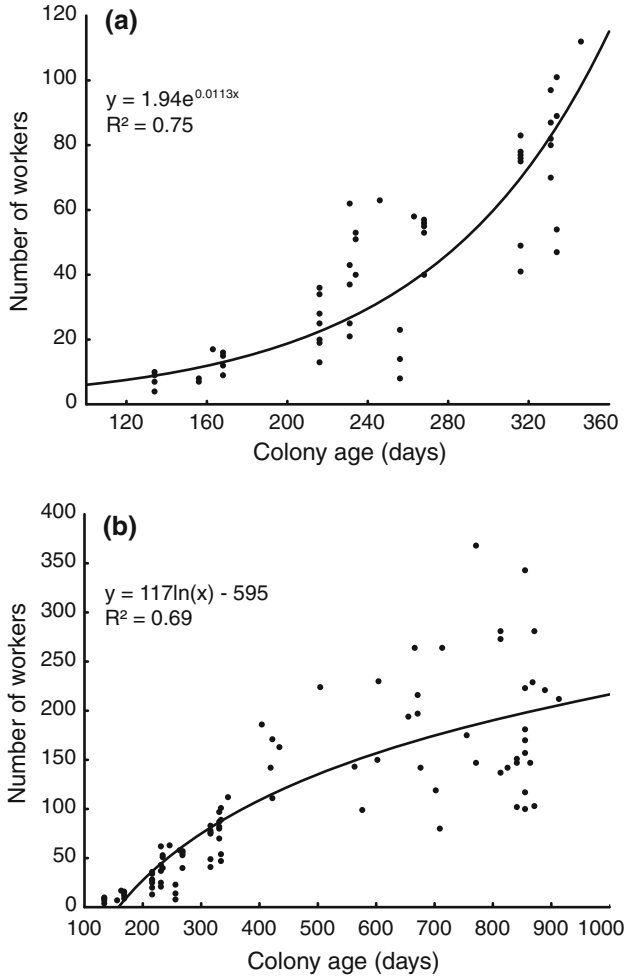


**Fig. 2** Total worker number as a function of colony age (days since colonies were founded in the laboratory). **a** Represents growth during the first year, which is best approximated by an exponential function, and **b** represents growth over 3 years, which is best approximated by a logarithmic function

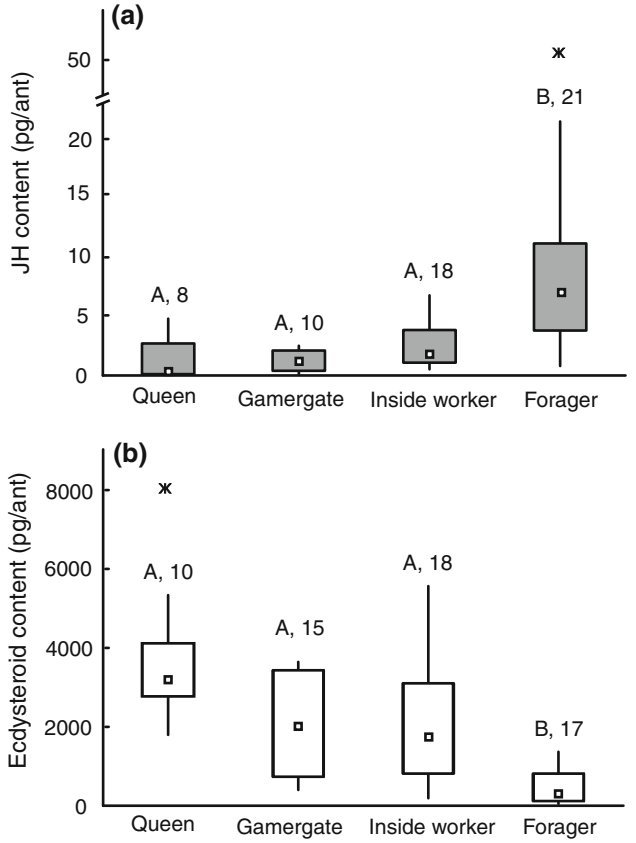


**Fig. 3** Median, 25–75% confidence interval, and non-outlier range of **a** JH and **b** ecdysteroid content of individual queens, gamergates, inside workers, and foragers. Sample size is noted for each group, and *letters* indicate significant differences ($p < 0.05$)

test: gamergate vs. forager, $p = 0.0032$; queen vs. forager, $p < 0.00001$; inside worker vs. forager, $p = 0.0036$; gamergate vs. inside worker, $p = 1.00$; gamergate vs. queen, $p = 0.436$; inside worker vs. queen, $p = 0.253$) (Fig. 3b).

JHA treatment of virgin alates

To better understand the function of JH in adult queens, we treated virgin queens with JHA and measured the effects on the timing of both dealation and oviposition. Virgin alates treated with JHA dropped their wings earlier (median 1 day) compared with females that received the control treatment (median 5 days) (Mann–Whitney *U* test; $N = 12,\ 11$; $Z = 2.89$; $p = 0.0034$) (Fig. 4a); however, treatment did not affect the time required for the initiation of egg laying (Mann–Whitney *U* test; $N = 9,\ 11$; $Z = -0.84$; $p = 0.401$) (Fig. 4b). In order to initiate egg laying, dealation was not required; 3 of 11 females in the control group began egg laying up to 13 days before they dropped their wings. Of the 12 individuals treated with JHA, 3 died prior to egg laying, and these were excluded from our analysis.

## Discussion

This study represents the first time that JH and ecdysteroid content of queens and reproductive workers have been compared within the same ant species. Although JH is normally associated with egg production in solitary insects, as well as the highly eusocial ant *Solenopsis invicta* (Brent and Vargo 2003), it appears to have lost this function in *H. saltator*. Queens and gamergates had low JH content, similar to that observed with inside workers. Instead, increased JH content was associated with foraging behavior by non-reproductive workers. This pattern resembles what has been observed in the honey bee, *Apis mellifera*, where JH no longer drives oogenesis in adult queens, but has been implicated in modulating foraging behavior in workers (Robinson and Vargo 1997).

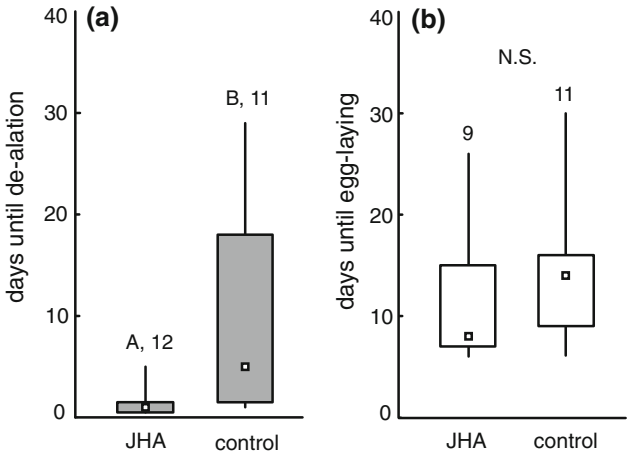


**Fig. 4** Median, 25–75% confidence interval, and range of time (in days) until **a** dealation and **b** first oviposition after virgin queens were separated from their natal nest and either treated with JHA or an acetone control. Sample size is noted for each group, and *letters* indicate significant differences ($p < 0.05$)

## Colony foundation and growth rate

Prior to examining the hormonal dynamics within *H. saltator* colonies, it was first necessary to determine an optimal time to sample queens and to establish that dynamics within laboratory colonies closely mimicked that of field colonies. After following the development of colonies founded by single queens for 3 years, it was determined that mature colony size was reached after 1 year. At this time the number of workers present (Fig. 2) fell within the range of 58–124 workers observed in mature field colonies (Peeters et al. 2000).

Similar to colony growth patterns observed in *Solenopsis invicta* (Tschinkel 1988), early colony growth in *H. saltator* was exponential during the first year but was best described by a logarithmic function when recorded over multiple years. Several colonies in our study produced female sexuals during the first year they were established. This corresponds to data from the field where relatively small colonies were found to produce female sexuals (Peeters et al. 2000). Many social insect species pass through an ergonomic phase of colony growth where sexual production is deferred several years until colonies reach a threshold size (Oster and Wilson 1978). From our data on colony growth and queen production it appears that colonies of *H. saltator* are capable of producing sexuals during their first year and may not pass through an extended ergonomic phase. This rapid development may have evolved to counter random factors that affect colony survival (Liebig and Poethke 2004). The similar development patterns of laboratory and field colonies also indicate that the hormone profiles determined in this experiment are likely to be comparable to what would occur under natural conditions. Therefore, hormone samples were collected roughly 1 year after colonies were established, when queens would be fully mature and regularly producing eggs.

## Endocrine differences among reproductive and behavioral castes

The subsequent hormonal analyses compared JH and ecdysteroid content between different castes to determine if there were endocrine associations with reproductive and dominance status. JH synthesis is inversely correlated with ovarian activity in the queenless ants *Diacamma* (Sommer et al. 1993) and *Streblognathus peetersi* (Brent et al. 2006). This is the reverse pattern found in *Solenopsis invicta*, a species that has a true queen caste (Brent and Vargo 2003). A proposed explanation for this difference was that queenless species had to develop an alternative means for controlling reproductive activity under constraints created by their worker phenotype (Brent et al. 2006). Because *H. saltator* has both queens and reproductive workers that regularly replace the queen upon her death, we were able to test this hypothesis, but we found no support for reproductive workers relying on an alternative regulatory mechanism. In this species, both queens and reproductive workers exhibited the same hormonal pattern—low JH and high ecdysteroid content (Fig. 3)—as previously observed in reproductive workers of queenless ants (Sommer et al. 1993; Brent et al. 2006). *H. saltator* belongs to the relatively basal subfamily Ponerinae, which also includes the queenless species *Diacamma* and *S. peetersi*. This suggests that the loss of JH's typical gonadotropic function (Raikhel et al. 2005) may be a common feature of Ponerine ants, not just the queenless species.

*H. saltator* colony dynamics are structured upon a stable dominance hierarchy with clear rank differences in behavior, ovarian activity, and the expression of a chemical fertility signal (Liebig et al. 2000; Peeters et al. 2000). In *S. peetersi*, elevated JH is associated with lower rank (Brent et al. 2006) and decreased expression of a cuticular fertility signal (Cuvillier-Hot et al. 2002). In *H. saltator*, there was no endocrine difference between high-ranking gamergates and the subordinate inside workers. Although we cannot exclude the possibility of small hormonal differences that were undetectable with our assay, it is unlikely that a subtle disparity could produce such divergent reproductive activity or fertility signaling.

The only significant difference among castes was for workers venturing outside the nest to forage, which, relative to the other groups, had elevated JH and depressed ecdysteroid content. This pattern resembles what has been observed in the highly eusocial honey bee, *Apis mellifera*, where JH has lost its reproductive function in adult queens and has a role in modulating foraging in the worker caste

(Robinson and Vargo 1997). JH has also been implicated as a releaser of foraging behavior in the young founding queens (Dolezal et al. 2009) and workers (Dolezal et al., submitted) of the ant *Pogonomyrmex californicus*, and in workers of *Myrmicaria eumenoides* (Lengyel et al. 2007). Our results fit this model, so it appears that JH may regulate foraging in *H. saltator* as well. With respect to ecdysteroids, the fall in ecdysteroid content in foragers may be related to the degradation of the ovaries, which occurs as workers age and begin foraging (Hölldobler and Wilson 1990). Ecdysteroids are primarily produced by the ovaries in adult female insects (Nijhout 1994), including honey bee workers (Amdam et al. 2010). In bumble bees, hemolymph ecdysteroid titers are low while ovarian ecdysteroid content is high (Geva et al. 2005). Similar to bumble bees, the pattern of ecdysteroid content we observed in *H. saltator* may indicate that ecdysteroids serve an intra-ovarian function (paracrine) rather than having an endocrine role.

### JH during the founding stage

While we did not find differences in JH or ecdysteroid content between mature reproductives and young workers, we did find evidence that JH is involved in changes that occur during the founding stage, when queens first activate their ovaries and begin egg laying (Fig. 4). Application of JHA caused alate queens to drop their wings earlier than untreated females. A similar effect was found in the fire ant, *Solenopsis invicta*, where JHA induced early dealation and oviposition in virgin queens (Vargo and Laurel 1994). Likewise, Brent and Vargo (2003) found that dealation and the initiation of oviposition in queens of this species corresponded with peak rates of JH biosynthesis. Dealation is known to trigger flight muscle histolysis and oocyte activation in other insect species (Tanaka 1986). Treatment with JHA did not, however, affect the timing of oviposition in *H. saltator*. This result provides further evidence that JH has lost its reproductive function in *H. saltator*. The role of JH during the founding stage may not correlate with reproductive activation directly, but because it affects the timing of dealation it may still be an important regulator of behavior and other physiological processes associated with colony founding. While JHA treatment of reproductive workers of *S. peetersi* inhibited ovarian activity, we found no such effect in virgin queens of *H. saltator*. This may reflect a difference in the function of JH in founding queens compared with mature reproductives.

## Conclusion

Overall, our results support the hypothesis that JH has lost a gonadotropic function in the basal Ponerine ants and that increased JH is more strongly correlated with foraging behavior among subordinate workers. This contrasts with other societies that maintain dominance hierarchies, such as wasps and bumble bees, where dominance and reproduction are associated with increased levels of JH and ecdysteroids (Bloch et al. 2000; Geva et al. 2005; Giray et al. 2005; Tibbetts and Huang 2010). Instead, *H. saltator* resembles the pattern observed in other highly eusocial species, where JH has lost its reproductive function and regulates a strong temporal polyethism among the worker caste (Hartfelder 2000; Lengyel et al. 2007). Our results show that JH may be involved in worker division-of-labor even in species where age-based polyethism is weak and where individuals still compete directly over reproduction. Within the eusocial Hymenoptera, regulation of foraging in wasps, bees, and ants shows convergence in the pattern of JH associated with division-of-labor among workers despite different levels of caste specialization (Hartfelder 2000).

Finally, we are able to show that the loss of a reproductive function for JH in Ponerine ants is not a consequence of specialization in worker reproduction, since queens show the same relationship between JH and reproduction in *H. saltator*. While increased JH production is associated with reproduction by queens of the fire ant *Solenopsis invicta* (Brent and Vargo 2003), our results clearly indicate that this pattern is not a general feature of all ant species. *S. invicta* displays advanced characteristics among ants, including large colony size and high queen-worker dimorphism, in contrast to Ponerine species which more closely resemble the ancestral form. Therefore, it is possible that the association of increased JH with reproduction in *S. invicta* is a derived characteristic. The reproductive function of JH in *S. invicta* may be related to the extreme fecundity of *S. invicta* queens, which have a dramatically increased egg-laying rate compared with queens or gamergates of Ponerine species. Also, reproductive conflict between adult workers and queens in *S. invicta* is almost completely absent because workers are sterile, and this may allow JH to regain a reproductive function in queens without affecting the role of JH in workers. Future investigations across other subfamilies may help define a general pattern of caste-associated differences in JH and ecdysteroid levels within the eusocial Hymenoptera and help to elucidate the physiological changes that occurred during the transition from solitary to social life.

**Acknowledgments** We thank Adam Dolezal, Charity Bashore, and Dan Langhorst for help with sample processing, and Kevin Haight, Adrian Smith, and Dani Moore for comments and advice. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

## References


Adkins-Regan E (2005) Hormones and animal social behavior. Princeton University Press, Princeton

Amdam GV, Norberg K, Fondrk MK, Page RE (2004) Reproductive ground plan may mediate colony-level selection effects on individual foraging behavior in honey bees. Proc Nat Acad Sci USA 101:11350–11355

Amdam GV, Page RE, Fondrk MK, Brent CS (2010) Hormone response to bidirectional selection on social behavior. Evol Dev 12:428–436

Barker JF (1978) Neuroendocrine regulation of oocyte maturation in imported fire ant *Solenopsis invicta*. Gen Comp Endocrinol 35:234–237

Bergot B, Ratcliff M, Schooley D (1981) Method for quantitative determination of the four known juvenile hormones in insect tissue using gas chromatography—mass spectroscopy. J Chromatogr A 204:231–244

Bloch G, Borst D, Huang Z, Robinson G, Cnaani J, Hefetz A (2000) Juvenile hormone titers, juvenile hormone biosynthesis, ovarian development and social environment in *Bombus terrestris*. J Insect Physiol 46:47–57

Brent CS, Vargo EL (2003) Changes in juvenile hormone biosynthetic rate and whole body content in maturing virgin queens of *Solenopsis invicta*. J Insect Physiol 49:967–974

Brent C, Peeters C, Dietmann V, Crewe R, Vargo E (2006) Hormonal correlates of reproductive status in the queenless ponerine ant, *Streblognathus peetersi*. J Comp Physiol A 192:315–320

Burns SN, Vander Meer R, Teal PEA (2007) Juvenile hormone III concentrations in female reproductives of *Solenopsis invicta* Buren. J Entomol Sci 42:306–309

Cuvillier-Hot V, Gadagkar R, Peeters C, Cobb M (2002) Regulation of reproduction in a queenless ant: aggression, pheromones and reduction in conflict. Proc Roy Soc Lond B 269:1295–1300

Dolezal AG, Brent CS, Gadau J, Hölldobler B, Amdam GV (2009) Endocrine physiology of the division of labour in *Pogonomyrmex californicus* founding queens. Anim Behav 77:1005–1010

Dong S-Z, Ye G-Y, Guo J-Y, Hu C (2008) Role of ecdysteroid and juvenile hormone in vitellogenesis in an endoparasitic wasp, *Pteromalus puparum* (Hymenoptera: Pteromalidae). Gen Comp Endocr 160:102–108

Fletcher D, Ross K (1985) Regulation of reproduction in eusocial Hymenoptera. Ann Rev Entomol 30:319–343

Geva S, Hartfelder K, Bloch G (2005) Reproductive division of labor, dominance, and ecdysteroid levels in hemolymph and ovary of the bumble bee *Bombus terrestris*. J Insect Physiol 51:811–823

Giray T, Giovanetti M, West-Eberhard M (2005) Juvenile hormone, reproduction, and worker behavior in the neotropical social wasp *Polistes canadensis*. Proc Natl Acad Sci USA 102:3330

Hartfelder K (2000) Insect juvenile hormone: from "status quo" to high society. Braz J Med Biol Res 33:157–177

Heinze J, Hölldobler B, Peeters C (1994) Conflict and cooperation in ant societies. Naturwissenschaften 81:489–497

Hölldobler B, Wilson EO (1990) The ants. Harvard University Press, Cambridge

Hölldobler B, Wilson EO (2009) The superorganism: the beauty elegance and strangeness of insect societies. WW Norton & Company, New York

Jaycox ER, Skowrone W, Guynn G (1974) Behavioral changes in worker honey bees (*Apis mellifera*) induced by injections of a juvenile hormone mimic. Ann Entomol Soc Am 67:529–535

Klowden MJ (1997) Endocrine aspects of mosquito reproduction. Arch Insect Biochem Physiol 35:491–512

Lengyel F, Westerlund SA, Kaib M (2007) Juvenile hormone III influences task-specific cuticular hydrocarbon profile changes in the ant *Myrmicaria eumenoides*. J Chem Ecol 33:167–181

Liebig J, Poethke HJ (2004) Queen lifespan and colony longevity in the ant *Harpegnathos saltator*. Ecol Entomol 29:203–207

Liebig J, Hölldobler B, Peeters C (1998) Are ant workers capable of colony foundation? Naturwissenschaften 85:133–135

Liebig J, Peeters C, Oldham NJ, Markstadter C, Hölldobler B (2000) Are variations in cuticular hydrocarbons of queens and workers a reliable signal of fertility in the ant *Harpegnathos saltator*? Proc Natl Acad Sci USA 97:4124–4131

Nijhout HF (1994) Insect hormones. Princeton University Press, Princeton

Oster GF, Wilson EO (1978) Caste and ecology in the social insects. Princeton University Press, Princeton

Peeters C, Crewe R (1984) Insemination controls the reproductive division of labour in a ponerine ant. Naturwissenschaften 71:50–51

Peeters C, Liebig J, Hölldobler B (2000) Sexual reproduction by both queens and workers in the ponerine ant *Harpegnathos saltator*. Insect Soc 47:325–332

Raikhel AS, Brown M, Belles X (2005) Hormonal control of reproductive processes. In: Gilbert L, Gill S, Iatrou K (eds) Comprehensive molecular insect science, vol 3, Elsevier Press, Amsterdam pp 433–491

Robinson GE (1987) Regulation of honey bee age polyethism by juvenile hormone. Behav Ecol Sociobiol 20:329–338

Robinson GE, Vargo EL (1997) Juvenile hormone in adult eusocial Hymenoptera: gonadotropin and behavioral pacemaker. Arch Insect Biochem Physiol 35:559–583

Robinson GE, Strambi C, Strambi A, Feldlaufer MF (1991) Comparison of juvenile hormone and ecdysteroid haemolymph titres in adult worker and queen honey bees (*Apis mellifera*). J Insect Physiol 37:929–935

Robinson GE, Strambi C, Strambi A, Huang ZY (1992) Reproduction in worker honey bees is associated with low juvenile hormone titers and rates of biosynthesis. Gen Comp Endocrinol 87:471–480

Röseler P (1991) Reproductive competition during colony establishment. In: Ross KG, Matthews RW (eds) The social biology of wasps. Cornell University Press, Ithaca, pp 309–335

Röseler P, Röseler I, Strambi A, Augier R (1984) Influence of insect hormones on the establishment of dominance hierarchies among foundresses of the paper wasp, *Polistes gallicus*. Behav Ecol Sociobiol 15:133–142

Sasaki K, Nagao T (2001) Distribution and levels of dopamine and its metabolites in brains of reproductive workers in honeybees. J Insect Physiol 47:1205–1216

Sasaki K, Yamasaki K, Nagao T (2007) Neuro-endocrine correlates of ovarian development and egg-laying behaviors in the primitively eusocial wasp (*Polistes chinensis*). J Insect Physiol 53:940–949

Sasaki K, Yamasaki K, Tsuchida K, Nagao T (2009) Gonadotropic effects of dopamine in isolated workers of the primitively eusocial wasp, *Polistes chinensis*. Naturwissenschaften 96:625–629

Shu S, Ihl Park Y, Ramaswamy S, Srinivasan A (1997) Hemolymph juvenile hormone titers in pupal and adult stages of southwestern corn borer [*Diatraea grandiosella* (Pyralidae)] and relationship with egg development. J Insect Physiol 43:719–726

Sommer K, Hölldobler B, Rembold H (1993) Behavioral and physiological aspects of reproductive control in a *Diacamma* species from Malaysia (Formicidae, Ponerinae). Ethology 94:162–170

Sullivan JP, Jassim O, Fahrbach SE, Robinson GE (2000) Juvenile hormone paces behavioral development in the adult worker honey bee. Hormon Behav 37:1–14

Tanaka S (1986) De-alation, flight muscle histolysis, and oocyte development in the striped ground cricket, *Allonemobius fasciatus*. Physiol Entomol 11:453–458

Tibbetts EA, Huang ZY (2010) The challenge hypothesis in an insect: juvenile hormone increases during reproductive conflict following queen loss in *Polistes* wasps. Am Nat 176:123–130

Tibbetts EA, Izzo AS (2009) Endocrine mediated phenotypic plasticity: condition-dependent effects of juvenile hormone on dominance and fertility of wasp queens. Hormon Behav 56:527–531

Tschinkel WR (1988) Colony growth and the ontogeny of worker polymorphism in the fire ant, *Solenopsis invicta*. Behav Ecol Sociobiol 22:103–115

Vargo E, Laurel M (1994) Studies on the mode of action of a queen primer pheromone of the fire ant *Solenopsis invicta*. J Insect Physiol 40:601–610

Warren J, Gilbert L (1986) Ecdysone metabolism and distribution during the pupal-adult development of *Manduca sexta*. Insect Biochem 16:65–82

Warren J, Smith W, Gilbert L (1984) Simplification of the ecdysteroid radioimmunoassay by the use of protein A from *Staphylococcus aureus*. Experientia 40:393–394

West-Eberhard MJ (1978) Polygyny and the evolution of social behavior in wasps. J Kansas Entomol Soc 51:832–856

West-Eberhard MJ (1996) Wasp societies as microcosms for the study of development and evolution. In: Turillazzi S, West-Eberhard MJ (eds) Natural history and evolution of paper wasps. Oxford University Press, New York, pp 290–317

Wilson EO (1971) The insect societies. Belknap Press of Harvard University Press, Cambridge