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ORIGINAL PAPER

Intergenerational effect of juvenile hormone on offspring in *Pogonomyrmex* harvester ants

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Abstract Parents can influence the phenotypes of their offspring via a number of mechanisms. In harvester ants, whether female progeny develop into workers or daughter queens is strongly influenced by the age and temperature conditions experienced by their mother, which is associated with variation in maternal ecdysteroid deposition in fertilized eggs. In many insects, juvenile hormone (JH) is antagonistic to ecdysteroid release, suggesting that seasonal and age-based variation in maternal JH titers may explain maternal effects on offspring size and reproductive caste. To test this hypothesis, we artificially increased maternal JH titers with methoprene, a JH analog, in laboratory colonies of two *Pogonomyrmex* populations exhibiting genetic caste determination. Increasing maternal JH resulted in a 50% increase in worker body size, as well as a sharp reduction in total number of progeny reared, but did not alter the genotype of progeny reared to adulthood. The intergenerational effect of JH manipulation was not mediated by a reduction in ecdysteroid deposition into eggs; instead, changes in egg size, trophic egg availability or brood/worker ratio may have altered the nutritional environment of developing larvae. Egg ecdysteroid content was significantly negatively correlated with natural variation in worker body size, however, suggesting that there are multiple independent routes by which queens can modify offspring phenotypes.

Keywords Juvenile hormone · Social insect · Caste · Ecdysteroids · Harvester ant · Maternal effect

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Introduction

The process of development requires both resiliency in the face of environmental stressors and sufficient flexibility to meet varying environmental demands. Optimal size, morphology, and life history characteristics are often context-dependent, selecting for phenotypic plasticity in response to environmental cues experienced during offspring development (Via et al. 1995), and intergenerational effects in which parents respond to environmental cues by manipulating these traits in their offspring (Mousseau and Fox 1998).

Among the traits most commonly associated with intergenerational effects in insects are diapause, size, and polyphenisms (Steel and Lees 1977; Sano-Fujii 1979; Mousseau and Dingle 1991). A polyphenism recently demonstrated to be influenced by maternal environment is queen/worker caste dimorphism in eusocial Hymenoptera. It has long been assumed that reproductive caste is determined by environmental cues experienced directly by the developing larva, such as temperature and nutrition (Brian 1957; Hölldobler and Wilson 1990; Smith et al. 2008). Although rearing environment is important in many species, maternal effects on reproductive caste and size have also been described in some ants (Goetsch1938; Passera and Suzzoni 1978). In the harvester ant genus *Pogonomyrmex*, maternal cues associated with queen age and overwintering status may be the primary mechanism

influencing caste of female offspring (Schwander et al. 2008).

Maternal effects on progeny phenotype are often mediated by circulating levels of juvenile hormone (JH) and ecdysteroids in females and the eggs they produce (Nijhout and Wheeler 1982; Hardie 1987). Maternally synthesized ecdysteroids are produced in follicle cells of the ovary and deposited into developing oocytes (Lagueux et al. 1977; Bullière et al. 1979; Legay et al. 1976; Ito et al. 2008). Differences in ecdysteroid levels in eggs appear to be the physiological basis for maternal effects on diapause in locusts (Gregg et al. 1987); studies in two ant genera, *Pheidole* and *Pogonomyrmex*, both found significantly lower ecdysteroid levels in queen-destined than in worker-destined eggs (Suzzoni et al. 1980; Schwander et al. 2008), suggesting that ecdysteroids may also influence the caste determination process.

One mechanism by which reduced ecdysteroid deposition into oocytes could promote development to the larger reproductive form is by increasing the critical size at which molting occurs. Given that a peak of ecdysone is required for molting to occur (Nijhout 1994, 2003), reduced ecdysteroid levels may increase the duration of larval feeding and thus the size at molt. Increasing the body size of offspring is a likely mechanism by which reproductive caste could be affected, as size thresholds during larval development are believed to be a key factor influencing the switching of developmental pathways that lead to insect polyphenisms (Nijhout and Wheeler 1982), and both correlative and experimental studies suggest a strong link between body size regulation and reproductive caste in ants (Robeau and Vinson 1976; Hughes and Boomsma 2008; Smith et al. 2008). Individuals may also vary in their responsiveness, such that maternal hormonal cues or other environmental factors differentially impact offspring development and lead to within-cohort variation in body size, caste, or developmental potential (Schwander et al. 2005; Hughes et al. 2003; Hughes and Boomsma 2007). An interaction between maternal cues and differential offspring response may explain the genotype-specific pattern of development in ants with genetic caste determination (GCD), in which non-hybrid genotypes fail to develop when workers are being produced but develop successfully when the colony switches to producing reproductives (Helms Cahan et al. 2004; Schwander et al. 2008).

JH and ecdysteroid levels are inversely correlated in the adult ant queens that have been studied (Brent et al. 2006; Dolezal et al. 2009; C. S. Brent, unpubl. data), suggesting that JH may inhibit the release of ecdysteroids from ovarian follicle cells. If there is such an antagonistic relationship, then increased maternal JH levels could account for the reduced ecdysteroid titers observed in queen-biased eggs (Suzzoni et al. 1980; Schwander et al. 2008). Such a mechanism has been suggested in *Pheidole pallidula*, in which naturally monogynous queens, who produce eggs less likely to develop into queens, have lower JH titers than polygynous queens (de Menten et al. 2005), and experimentally increasing maternal JH levels increased the production of daughter queens (Passera and Suzzoni 1979). However, application of JH to queens of the fire ant *Solenopsis invicta* did not impact offspring size or caste, raising questions about the generality of this pathway in mediating maternal effects in ants (Robeau and Vinson 1976).

In this study, we tested whether maternal JH influences the body size and reproductive caste of female offspring in two GCD populations of *Pogonomyrmex* harvester ants. We experimentally increased JH titers in queens over a period of 8 weeks in laboratory colonies and measured the effect on body size, reproductive caste and genotypes of developing offspring. To identify the mechanism of any maternal effects, we tested whether the eggs of treated and untreated queens differed in number, size, viability, and titers of ecdysteroids and JH.

Methods

Study organism

Twenty-eight queens of the GCD *Pogonomyrmex* J lineage pair were collected after a mating flight in July 2006 and July 2007 at site J (Helms Cahan and Keller 2003) and eight queens of the GCD H lineage pair were collected after a flight at site AN (Schwander et al. 2007) in July 2007 and allowed to establish colonies in the lab. Colonies were housed in a Percival incubator at 30°C under constant light in transparent 17 × 12 × 6 cm boxes with two (16 × 150 mm) culture tubes wrapped in aluminum foil and filled 1/3 with water stoppered with a cotton ball for moisture and shelter. Ants were fed a diet of two mealworms (*T. molitor*) per week and a mixture of oat bran, wheat germ, millet, thistle seeds, and quinoa ad libitum. The number of adult workers per colony was censused just prior to the beginning of hormone manipulations.

Hormone treatments

In order to determine the effects of maternal JH levels on offspring development, we compared the characteristics of offspring of control queens to those in which JH levels were experimentally increased. Methoprene is a JH analog and has been used in ants to elevate the effective titer (Wheeler and Nijhout 1981; Vargo and Laurel 1994; Lim and Lee 2005; Schrempf and Heinze 2006; Lengyel et al. 2007). Each lineage was allocated evenly across the two

treatments; within each lineage, colonies were ranked by the number of workers and divided across treatments such that treatments spanned the entire range of initial colony sizes (range 10–275 workers).

Experimental queens received a weekly topical application of 2 µl of 5 µg/µl methoprene dissolved in acetone. Methoprene is more stable than JH (Henrick et al. 1976), and weekly applications were considered adequate for sustaining an elevated JH activity in the queens. Control queens received 2 µl of acetone. All solutions were placed on the thorax of the queen and the acetone was allowed to evaporate prior to reintroducing the queen to her colony. The concentration of methoprene was based on mass-specific quantities used previously in ants (Wheeler and Nijhout 1981; Burns et al. 2007). Queens were treated for a total of 8 weeks.

Offspring body size and caste

On the day of the first hormonal treatment (week 0) and each week thereafter for 8 weeks, all pupae were permanently collected from the colony. On the last collection day (week 8), larvae were also collected from those colonies that did not contain pupae. Pupae of similar caste were weighed to the nearest 0.01 mg as a batch and divided by the number of pupae to obtain the mean body mass. The caste of pupae could be easily distinguished based on the presence of wing buds indicative of reproductives. The relative size and shape of the head and gaster were used to distinguish male from female reproductives (Robeau and Vinson 1976). Direct comparison of pupal measurements to those of pupae from natural field colonies is difficult, as colonies can extend to a depth of 2 m or more (MacKay 1981). Instead, we used worker live mass as a proxy for pupal live mass in field colonies. To assess the accuracy of worker live mass in predicting pupal mass, we first calculated the ratio of adult worker dry mass to pupal live mass in laboratory colonies ($n = 34$ colonies), as well as the ratio of worker dry mass to worker live mass in field-collected specimens from site J ($n = 10$ colonies) and a nearby H site (site H, $n = 41$ colonies; Helms Cahan and Keller 2003). The laboratory and field ratios were virtually identical (0.366 laboratory, 0.364 and 0.362 field), suggesting that pupation does not appreciably alter adult live mass and thus worker live mass is a suitable proxy for comparison.

Under the GCD mechanism of caste determination, queens mate with both same-lineage and alternate-lineage males. Females produced from intra-lineage crosses fail to survive to adulthood when workers are being reared but develop successfully into queens during a colony's reproductive phase. In contrast, females produced from inter-lineage (hybrid) crosses become workers (Helms Cahan and Keller 2003; Helms Cahan et al. 2004; Clark et al. 2006; Volny et al. 2006). One possible effect of JH manipulation may therefore be a shift in the proportions of intra- and inter-lineage female progeny reared to adulthood. We assessed this at a gross level by subsampling a single worker pupa, and a reproductive pupa if present, from each colony at week eight (methoprene: $n = 14$, control: $n = 18$). A single larva was sampled if no pupa was available. We determined ancestry by genotyping all individuals at the microsatellite loci L18, Myrt3, P12-1 and Pb7 by homogenizing the pupa in 250 µl 5% chelex 100 resin and heating for 20 min at 90°C and amplifying and visualizing the loci following Helms Cahan and Julian (2010). Genotypes were compared to allele frequencies of the two lineages at these collection sites from previous studies (Helms Cahan and Keller 2003; Schwander et al. 2007) to identify ancestry.

Egg characteristics

To determine whether the hormone treatments altered egg size, the eggs laid by queens were collected on weeks 0, 4, and 8 of the experiment. Eggs were counted and batches of over five eggs were weighed collectively to the nearest 0.01 mg and divided by the number of eggs to yield mean egg mass per colony.

To determine if JH manipulation in queens affected hormone deposition into eggs, egg JH and ecdysteroid content were measured at week 8 of the experiment. All eggs were sampled and stored in 1 ml of 90% methanol at −80° C. JH was measured using GC–MS as described by Brent and Vargo (2003), and ecdysteroid content was quantified using a radioimmunoassay (Warren et al. 1984; Brent and Dolezal 2009). Each egg batch was homogenized in 90% methanol, centrifuged, and resuspended a total of three times to separate the hormones from the tissue. JH and ecdysteroids were separated using a hexane extraction with a 3:2 solution of hexane in 90% methanol. The hexane layer, containing JH, was purified in glass columns filled with aluminum oxide and derivatized by incubation at 60°C in 5% trifluoroacetic acid/95% methanol-d4. Derivatized JH was then analyzed using an 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 5975C inert mass selective detector. Helium was used as a carrier gas. 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 JHIII, the known JH isomer for *P. barbatus* (Schwander et al. 2008). Total abundance was quantified against a standard curve of derivatized JH III. The detection limit of the assay is approximately 1 pg.

The specific ecdysteroids utilized by *P. barbatus* are unknown, but the H-22 antibody cross-reacts with ecdysone, ecdysterone, 20-hydroxyecdysone and makisterone A, and low polarity conjugated forms (Warren and Gilbert 1986). Although the antibody does not cross-react as well with the medium and high polarity conjugated ecdysteroids that are typically found in eggs, we have successfully used H-22 to demonstrate differential hormone sequestration in *Pogonomyrmex* eggs using the same analytical approach (Schwander et al. 2008). Intra- and interassay 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). All samples were run twice and the results averaged to increase accuracy.

Dissection of treated queens

To examine the effects of JH addition on reproductive physiology, queens were sampled after week 8 and stored in 50% ethanol for dissection. Queens were dissected in PBS solution and the total number of large vitellogenic oocytes (>50 mm), the number of follicular remnants (yellow bodies), and the number of active ovarioles per ovary was counted.

Quantification of trophic egg production

It became apparent early in the experiment that fewer brood were reared to the pupal stage in the methoprene treatment (see results). This may have resulted from a shift toward producing trophic rather than viable eggs. In order to quantify trophic egg production, queens were treated one last time after the removal of eggs at week 8 and their egg piles were resampled 3 days after treatment. Trophic eggs were identified by their characteristic large, round shape and grainy texture.

Statistical methods

All variables measured over multiple time periods were analyzed with repeated measures ANOVA with GCD lineage (J or H) and JH treatment as between-subject factors and week as a within-subject variable. Differences between treatments in their response over time were indicated by a significant time by treatment interaction. Initial egg mass and final egg ecdysteroid titers were analyzed with ANOVA that included treatment as a factor and initial colony pupal mass as a covariate. Ecdysteroid titers were log-transformed to normalize the data. The proportion of trophic eggs produced by queens could not be normalized by transformation and were compared across treatments with a nonparametric Kruskal–Wallis test.

Results

Offspring body size and caste

Initial pupal body mass in the experimental colonies was positively correlated with the number of workers in the colony up until approximately 50 workers (linear regression on subset of colonies under 50 workers, $T = 4.10$, $P = 0.001$, $R^2 = 0.50$), after which mass plateaued at 12.44 mg ± 1.58 SD (Fig. 1). This was approximately 75% of the typical live mass of workers in natural adult field colonies (J worker average = 16.56 mg ± 0.76 SE; H worker average = 15.90 mg ± 0.43 SE). Over the course of the hormone treatments, pupae in the methoprene treatment grew significantly larger in size than the control pupae, reaching the field-typical mass range by week 6 and remaining in that range through week 8 (repeated measures ANOVA for weeks 0–5, $F_{5,110} = 6.81$, $P < 0.001$; Fig. 2). After week 5, the number of colonies producing pupae in the methoprene treatment group dropped below 10 (Figs. 2, 3), precluding statistical comparisons including these weeks because the degrees of freedom became too low to test for within-subject interaction effects.

All pupae produced were workers, with the exception of a single female reproductive sampled on week 8 of the

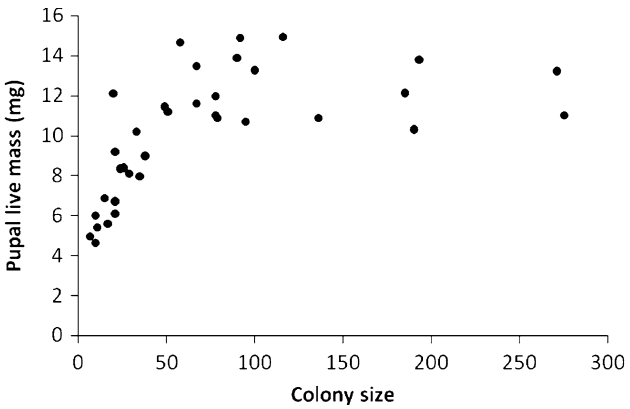

Fig. 1 Relationship between the number of workers and mean pupal body mass prior to the experiment ($n = 36$ colonies)

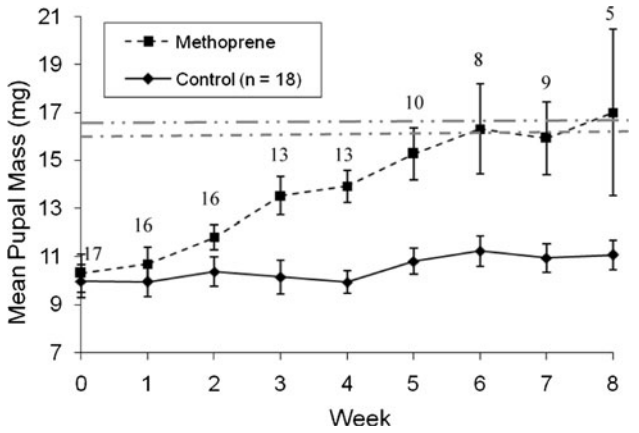

Fig. 2 Mean pupal body mass (±SE) over time in colonies in which queens received control and methoprene treatments. *Numbers above the top line* indicate the number of colonies in which pupae were available for measurement in the methoprene treatment, caused by a combination of mortality (3 of 17 colonies) and absence of pupae. The *gray horizontal lines* indicate mean worker live masses in adult field colonies of the J (*top line*) and H (*bottom line*) lineages

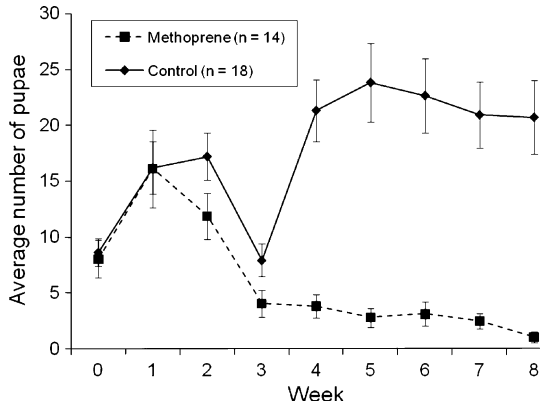

Fig. 3 Weekly average number of pupae (±SE) observed in colonies in which queens received control and methoprene treatment. Pupae from weeks 0–2 were already present as larvae prior to the first queen hormonal treatment. The uniform drop in number of pupae at week 3 corresponds with when the cohort of eggs removed on day 0 of the experiment would have reached this developmental stage; pupae censused at this time are presumed to be a mix of pre- and post-treatment progeny. Pupae from weeks 4–8 were reared from eggs laid following the first treatment. Sample sizes indicate the number of surviving colonies at week 8 in each treatment

experiment that was produced by a methoprene-treated queen. All the subsampled larvae and pupae genotyped from the week 8 collection were of inter-lineage hybrid ancestry, including the reproductive pupa.

Egg characteristics

Initial egg mass prior to experimental manipulation did not vary with initial pupal mass nor with worker number, and the treatment groups did not initially differ in mean egg mass (Student's t test, $t_{33} = 0.17$, $P = 0.66$). Over the course of the experiment, however, the direction of change in egg mass over time differed between treatments; egg mass increased for methoprene-treated queens and decreased for control queens (repeated measures ANOVA, week × treatment interaction, $F_{2,50} = 4.80$, $P < 0.02$; Fig. 4a).

JH manipulation also affected the number of eggs maintained in the egg pile (Fig. 4b). While egg number in the control treatment increased over time, it decreased with methoprene treatment (week × treatment interaction, $F_{4,88} = 4.50$, $P < 0.003$).

Hormone content analysis

Only trace amounts of JH were detected in the egg batches collected at the end of the treatment period, with an overall mean of 6.61 pg/egg ± 0.82 SE. There was no difference between treatment groups (Mann–Whitney test, $T_{13,10} = 132.0$, $P = 0.476$). Ecdysteroids were present at a higher level, with an overall mean of 24.51 pg/egg ± 4.54 SE. Ecdysteroid content did not differ across hormone treatments (ANCOVA on log-transformed data, $F_{2,34} = 2.10$, $P = 0.14$) but ecdysteroid content was significantly negatively related to pupal mass prior to treatment, both for the total dataset ($F_{1,26} = 5.40$, $P < 0.03$) and when an outlier

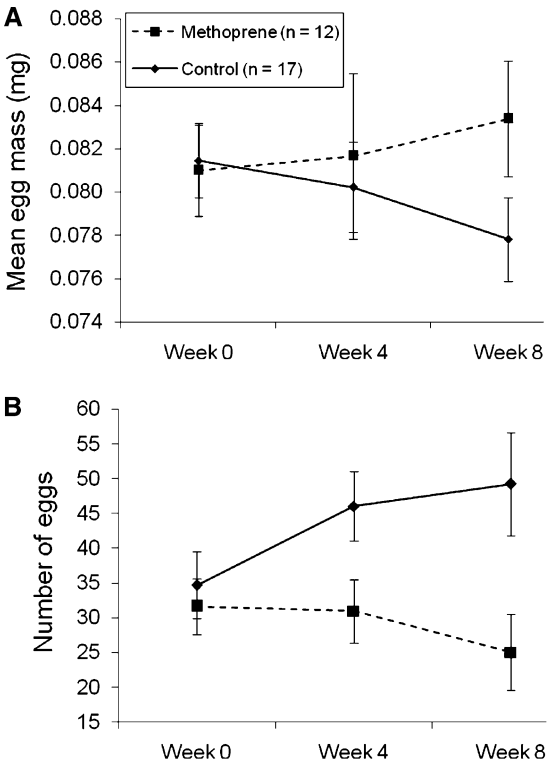

Fig. 4 **a** Mean egg mass (±SE) and **b** egg number (±SE) across treatments over time

with unusually low ecdysteroid levels was excluded ($F_{1,25} = 4.64$, $P < 0.05$; Fig. 5).

Queen dissection

Queens under different hormonal regimes did not differ in the number of active ovarioles (Student's t test, $t_{14} = 1.00$, $P = 0.33$), vitellogenic oocytes ($t_{14} = 0.14$, $P = 0.89$) or the number of follicular remnants ($t_{14} = 0.02$, $P = 0.99$).

Trophic egg production

Queens treated with methoprene produced significantly more trophic eggs than those given the control treatment (Kruskal–Wallis test, $P < 0.02$; Fig. 6).

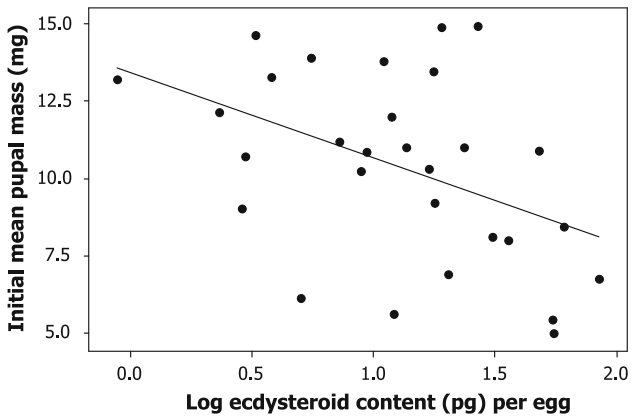

Fig. 5 Relationship between the ecdysteroid content of eggs (log-transformed) and the initial mean pupal mass of the colony prior to hormone treatments. $n = 26$, $R^2 = 0.18$

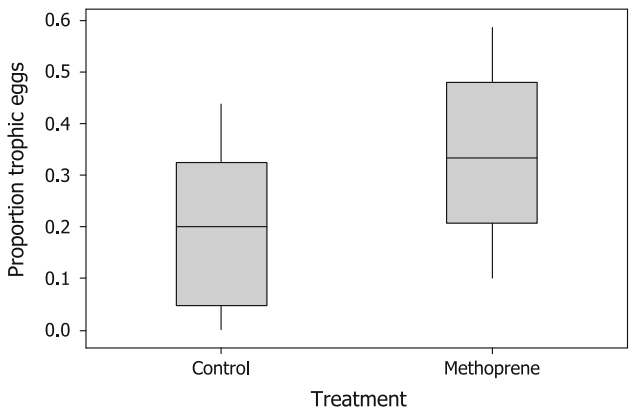

Fig. 6 *Boxplot* of proportions of trophic eggs present in egg batches laid by queens within 3 days of the last experimental treatment (week 8) of either methoprene ($n = 13$) or control solvent ($n = 17$). The distribution differed significantly between treatments ($p < 0.02$)

Discussion

The results of this study show an intergenerational effect of maternal hormone status on offspring body size. Increasing maternal JH levels increased the adult body mass of worker offspring by 50%. Interestingly, this effect did not appear to be mediated by shifts in maternal ecdysteroid deposition into the eggs, a factor identified as a correlate of progeny caste determination in *Pogonomyrmex* (Schwander et al. 2008). JH-mediated size differences may have been evoked by hormonally induced changes in the maternal RNA or proteins that become sequestered in eggs, or changes in egg size, trophic egg availability or brood/worker ratio may have altered the nutritional environment of developing larvae. In contrast, natural variation in worker body size across colonies prior to experimental manipulation was significantly negatively associated with egg ecdysteroid concentration, suggesting that there are multiple alternative routes by which queens may influence offspring body size.

Juvenile hormone has two recognized functions in adult eusocial insects: as a gonadotropin, although this has been secondarily lost in some groups, and as a behavioral regulator (Robinson and Vargo 1997; Hartfelder and Emlen 2005; Giray et al. 2005). In the ants studied to date, JH appears to drive the process of vitellogenesis and oocyte maturation (Vargo and Laurel 1994; Brent and Vargo 2003; Dolezal et al. 2009), although exceptions are found in the highly derived queenless ants *Streblognathus peetersi* (Brent et al. 2006) and *Harpegnathus saltator* (C.A. Penick, J. Liebig and C. S. Brent, in prep). We found little evidence that JH is directly involved in driving oogenesis. Instead, queens receiving methoprene produced fewer eggs than controls (Fig. 4b). This could be indicative of some degree of cumulative toxicity from the weekly treatments, or the queens' resources may have been redirected to produce fewer but higher quality eggs which could explain their enhanced size (Fig. 4a).

In addition to its recognized functions, the results of this study suggest a possible third role for JH as a regulator of offspring phenotypes. Increasing maternal JH levels led to a significant increase in offspring worker body size over time, shifting pupae from the apparent body size ceiling typical of laboratory colonies to that of mature field colonies despite being reared by a worker population two orders of magnitude smaller in number (Figs. 1, 2). Although JH has been shown to increase adult body size when applied directly to developing brood (Robeau and Vinson 1976; Passera and Suzzoni 1979; Wheeler and Nijhout 1981), this is the first demonstration that altering adult JH titers can translate into a shift in body size in the subsequent generation. JH levels in all eggs were near the limit of detection regardless of treatment, indicating that

the effect was not caused by direct transfer or leakage of the JH treatments to the offspring.

Given the generally antagonistic relationship between JH and ecdysteroids found in a number of physiological contexts, and the previous finding that egg ecdysteroid content can influence caste fate in *Pheidole pallidula* (Suzzoni et al. 1980) and *Pogonomyrmex* (Schwander et al. 2008), we predicted that a maternal effect of JH on body size would most likely be mediated by a reduction in ecdysteroid deposition into eggs. We did not find evidence that such a pathway was responsible in our manipulations. Although offspring body size did increase when maternal JH was elevated, the eggs laid by these queens did not differ significantly in free ecdysteroid content from the control treatment. The relationship with the conjugated ecdysteroids packaged into the eggs may be different, given that the antibody used is not very sensitive to those forms of the hormone (Warren and Gilbert 1986). However, the detectable unbound ecdysteroids are likely to be in proportion with the conjugated forms, suggesting that the maternal ecdysteroids played a limited role in altering offspring size.

Although we cannot directly identify the mechanism responsible for offspring response to maternal JH, our results suggest three possibilities. First, maternal hormonal state may determine the quantity of nutritional resources sequestered in the egg. Circulating JH appears to increase the rate of vitellogenin uptake by the ovaries of fire ant queens (Vargo and Laurel 1994). We found that egg size diverged significantly across treatment groups in the direction expected if JH-enhanced vitellogenesis (Fig. 4a), as has been documented in a number of insect species (Glinka and Wyatt 1996; Cruz et al. 2003; Parthasarathy et al. 2010). Whether larger eggs actually translate into larger adults, however, is unclear. Larger eggs produce initially larger larvae in the termite *Reticulitermes speratus*, but this apparently accelerates offspring development rather than leading to larger individuals (Matsuura and Kobayashi 2010). In ants, the little evidence available is mixed. Queens of *Myrmica rubra* and *Formica rufa* lay larger eggs at certain times of year that are more likely to develop into the larger reproductive caste than the smaller worker-biased eggs (Gösswald and Bier 1953; Brian 1980). In *F. selysi*, however, the larger-bodied monogyne queen morph develops from significantly smaller eggs than the polygyne morph (Meunier and Chapuisat 2009).

Another route by which the hormonal state of the queen can regulate offspring development is by altering maternal RNA transcript levels. An increase in JH has been demonstrated to affect the expression of transport proteins in the ovaries and developing oocytes which are necessary for uptake processes such as vitellogenesis and the sequestration of maternally inherited mRNAs and regulatory proteins (Dubrovsky et al. 2002). These maternal factors are essential to orchestrating early embryonic development in insects (Farley and Ryder 2008; Tadros and Lipshitz 2009), and may influence the growth of maturing offspring.

Queens may also be affecting offspring size indirectly, by altering the nutritional environment available during larval development. The significant increase in worker body size was accompanied by a reduction in the number of individuals being reared to adulthood, with the majority of JH-addition colonies ceasing to produce pupae altogether by the eighth week (Fig. 3). This appeared to be partially due to a shift toward higher trophic egg production, although the sharp reduction in productivity suggests that the hatching rate of non-trophic eggs also probably decreased (Fig. 6). Low egg viability may have directly impacted resource availability for those larvae that did hatch successfully. Newly hatched larvae are not spatially segregated from the egg pile until they are considerably larger than the eggs. This provides larvae the opportunity to consume neighboring eggs, and egg piles typically contain multiple damaged eggs and/or empty egg shells suggestive of larval oophagy. Although methoprene treatment reduced egg laying rates, which would reduce resource availability to microlarvae, egg viability was reduced even more strongly, reducing competition among microlarvae and increasing the availability of egg resources during early development.

Similarly, the reduction in total number of larvae available for rearing may have allowed workers to allocate more resources per individual larva, increasing eventual adult body size. This would most properly be categorized as a queen–worker interaction effect, whereby queens influence the rearing decisions of workers by limiting (or increasing) the brood-to-worker ratio. Numerical egg limitation is hypothesized to underlie consistent differences in worker size distributions between polygynous and monogynous colonies in a number of ant species (Greenberg et al. 1985; Goodisman and Ross 1996; Schwander et al. 2005). Polygyne colonies, whose queens collectively produce a surfeit of eggs, have significantly smaller workers than monogyne colonies where only one queen is present and may not be able to produce as many eggs as the available resources could support. It is possible that worker rearing decisions may be further regulated, even within single-queen colonies, by JH-mediated shifts in egg-laying rate and viability.

In contrast to experimental manipulations, natural variation in worker body size in colonies prior to experimental manipulation was negatively correlated with egg ecdysteroid content (Fig. 5), and not with egg size. This suggests that a distinct ecdysteroid-mediated pathway may be an important maternal mechanism mediating worker body size during colony growth. Recent work on the proximate

factors influencing reproductive caste in this species also implicated ecdysteroid content in eggs, and our results matched levels observed in worker-producing colonies in that study (Schwander et al. 2008). The finding that ecdysteroids are associated with both body size and caste raises the possibility that the two outcomes are developmentally regulated along a single endocrine continuum, such that decreasing quantities of maternally deposited ecdysteroids progressively increase worker body size and reproductive capacity until progeny reach a size threshold for switching development toward the queen trajectory. A functional link between body size and reproductive caste is consistent with the observed positive relationship between body size and ovarian function in workers in both queenright and queenless conditions (Smith et al. 2007; Helms Cahan et al. in prep), as well as between worker body size and representation in the queen caste across matrilines in polygynous *Acromyrmex echinatior* colonies (Hughes and Boomsma 2008).

At the same time, the discovery of an alternative JH-mediated maternal effect suggests that size and caste can potentially be dissociated from one another evolutionarily. Notably, JH manipulation did not enhance the occurrence of intra-lineage, queen-destined progeny, which were completely absent from the subset of progeny genotyped at week 8, and even the single reproductive female pupa produced was of the worker-typical inter-lineage genotype. If the effect of JH addition is indeed mediated nutritionally, this would suggest that size-biasing mechanisms available to queens and workers have very different properties: queens can bias both size and reproductive potential of female offspring by varying ecdysteroid deposition, while workers can make changes to worker size via feeding regimes but are less able to use this power to control reproductive caste allocation decisions. Thus, the diversity of physiological mechanisms influencing development may prove to have important implications for the resolution of queen–worker conflicts over timing of reproduction or optimal sex ratio (Ratnieks et al. 2006).

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