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Juvenile hormone levels reflect social opportunities in the facultatively eusocial sweat bee *Megalopta genalis* (Hymenoptera: Halictidae)

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ABSTRACT

The evolution of eusociality is hypothesized to have involved de-coupling parental care from reproduction mediated by changes in endocrine regulation. While data for obligately eusocial insects are consistent with this hypothesis, we lack information from species representative of the transition from solitary reproduction to eusociality. Here we report the first evidence for a link between endocrine processes and social behavior in a facultatively eusocial bee, *Megalopta genalis* (Halictidae). Using females that varied in social, reproductive, and ecological context, we measured juvenile hormone (JH), a major regulator of colony caste dynamics in other eusocial species. JH was low at adult emergence, but elevated after 10 days in all nesting females. Females reared in cages with ad lib nutrition, however, did not elevate JH levels after 10 days. All reproductive females had significantly more JH than all age-matched non-reproductive females, suggesting a gonadotropic function. Among females in established nests, JH was higher in queens than workers and solitary reproductives, suggesting a role for JH in social dominance. A lack of significant differences in JH between solitary reproductives and non-reproductive workers suggests that JH content reflects more than reproductive status. Our data support the hypothesis that endocrine modifications are involved in the evolutionary decoupling of reproductive and somatic effort in social insects. These are the first measurements of JH in a solitary-nesting hymenopteran, and the first to compare eusocial and solitary nesting individuals of the same species.

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Introduction

An important source of evolutionary novelty may be the rearrangement of existing regulatory and physiological processes (Gerhart and Kirschner, 1997; West-Eberhard, 2003). The evolution of eusocial insects (species with reproductive queens and sterile workers) from solitary ancestors provides an excellent example of this repurposing (West Eberhard, 1987). While solitary insects both reproduce and forage, eusociality requires de-coupling reproduction (the tasks of the queen phenotype) from parental care (the tasks of the worker phenotype). Juvenile hormone (JH) is one of the primary regulators of insect ovarian development and activity (Flatt et al., 2005; Hartfelder, 2000). In many social insects, JH also influences dominance status and division of labor (reviewed in Bloch et al., 2009; Hartfelder, 2000). Thus, West-Eberhard (1996, 2003) hypothesized that JH contributes to regulating the switch between queen-like behaviors (reproduction) and worker-like behaviors (foraging) in solitary bees and wasps. Increasing or suppressing JH could lead to more consistently queen- or worker-like behavior, respectively, functioning as a mechanism for de-coupling reproduction from parental care, and thus promoting division of labor.

Studies of primitively eusocial insects (species without morphologically distinct behavioral castes), including the bumble bee *Bombus terrestris*, the paper wasps *Polistes dominulus*, *P. canadensis*, and *P. metricus*, and the halictid bee *Lasioglossum zephyrum*, generally demonstrate a role for JH in regulating division of labor. In these species, JH correlates with ovarian development and reproduction: queens have large ovaries and high JH titers, while workers have small, non-reproductive ovaries and low JH titers (Bell, 1973; Bloch et al., 2000; Giray et al., 2005; Röseler and Röseler, 1978; Röseler et al., 1980; Tibbetts and Izzo, 2009; Tibbetts and Sheehan, 2012; Tibbetts et al., 2011a). JH titers are also influenced by social context, nesting opportunity, and nutrition. In both *B. terrestris* and *P. dominulus*, worker JH titers increased following experimental removal of the queen, suggesting that queen dominance suppresses JH levels in nestmates (Bloch et al., 2000; Tibbetts and Huang, 2010). *L. zephyrum* females do not enlarge their

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ovaries without access to nesting substrate (Bell, 1973), and the effects of increased JH are greater in better-fed *P. dominulus* individuals (Tibbetts and Izzo, 2009). Together these findings generally support the hypothesis that the increased dominance and reproduction of queens is enabled by higher JH titers, and that queens suppress worker JH titers through aggressive interactions and/or control of nutrition. The role of JH in the evolutionary origins of queen and worker phenotypes is unknown, however, because JH has never been measured in a solitary-nesting bee or wasp to determine its influence on behavioral phenotype. Thus, it is impossible to evaluate how solitary regulatory mechanisms may have been modified to regulate social phenotypes without comparison to a solitary phenotype.

To fill this gap, we measured JH in a facultatively eusocial sweat bee, *Megalopta genalis* (Halictidae). *M. genalis* is not phylogenetically basal to *Bombus*, *Polistes*, or *Lasioglossum* (which represent independent origins of primitive eusociality in three different hymenopteran families). Rather, its significance is that a facultatively eusocial species permits direct comparison of solitary and social phenotypes in the same species, providing insights into factors that facilitated the evolutionary transition from solitary to social (Michener, 1985; Schwarz et al., 2007; Wcislo, 1997). We examined the role of JH in reproduction and dominance among females from both solitary nests (reproductive female without workers) and eusocial nests [reproductive queen with sterile worker(s)]. Based on the hypothesized link between reproduction and ovary development in solitary bees, we expected queens and solitary reproductive females to have higher JH than newly emerged adults with undeveloped ovaries. Additionally, we expected non-reproductive workers to have lower JH levels than their queens. We further compared these groups with females kept in the lab with adequate nutrition but without access to a nest, and with new nest foundresses, to measure the effect of ontogenetic, social, and ecological context on JH. If access to nutrition limits *M. genalis* JH levels independent of nesting context, then well-fed females in isolation should have high JH levels. If queen dominance limits JH levels, then females given access to nesting substrate without the presence of a queen should have elevated JH.

Methods

Natural history précis

M. genalis nests are initiated by a single female (Wcislo et al., 2004). These foundresses either become social queens or solitary reproductives. Eusocial nests form when a daughter remains at her natal nest as a subordinate non-reproductive worker that begins foraging 6–10 days after emergence. Workers and queens are readily distinguished through observation; the former do nearly all the foraging and the latter rarely leave the nest (Wcislo and Gonzalez, 2006). In a solitary nest, each offspring disperses approximately 6–10 days after eclosion (Kapheim, 2010; Kapheim et al., 2012) (ESM 1).

Observation nests and rearing conditions

All fieldwork was conducted on Barro Colorado Island (BCI), Panama (ESM 1). We collected bees from natural nests, and reared them under ambient conditions. When females emerged as adults, they were put into either cages or observation nests made of balsa wood between two sheets of plexiglass (ESM 2). Observation nests with newly emerged females were placed in the forest and monitored. Cage-reared bees were placed in circular 8 cm high × 7 cm diameter plastic containers and fed ad lib a honey:water:soy-protein (45:45:10 by volume) powder mixture (following Ref. Kapheim et al., 2012).

Overview of behavioral groups

In total, we measured JH content in seven groups of *M. genalis* (Table 1). 1) *Workers* were collected from social observation nests when 10 days old. We confirmed workers' status by marking nestmates and filming foraging flights. Only workers filmed returning from foraging trips were included in the study. 2) *Queens* were collected at the same time as the workers in their nests. 3) *Solitary reproductives* were collected from observation nests 10 days after their first offspring emerged, in order to match the treatment of the queens. These nests were not social because all offspring had dispersed. Average adult age at collection was 65.8 ± 5.8 SD days for queens and 65.8 ± 5.4 days for solitary females. 4) *Newly emerged bees* were collected the day after adult emergence. 5) *Ten-day old cage bees* were kept in social isolation for 10 days post-emergence with ad lib food, but no opportunity for nesting. 6) *Ten-day old observation nest foundresses* are those females we placed as singletons into empty observation nests when they emerged. We allowed them to initiate nesting, and then collected them when they were 10 days old. These females would have become solitary reproductives or social queens if left uncollected. 7) *Naturally dispersing foundresses* were females that had left their natal nests and initiated their own nests in sticks that had been regularly monitored for new nesting. They were collected within 4 days of nest initiation. The exact age of these bees is unknown, but they are likely ~10 days old, based on typical ages of females that disperse (Kapheim, 2010; Kapheim et al., 2012).

Reproduction assessment

At the time of collection, we noted the number and stage of each brood cell present in each nest for comparisons between queens and solitary reproductives (summed across pairs used in hormonal analysis — see below) with a Mann–Whitney U-test. All 10 day old observation nest foundresses and natural dispersers began nest construction [defined as building an entrance collar (Kapheim et al., 2012)], but had not laid an egg. We calculated a "nesting index" to quantify nest reproductive stage based on the following characteristics, summed for each pair used in hormonal analysis: nest collar only = 0, cell construction = 1, complete empty cell = 2, some pollen provisions = 3. We used this nesting index to compare reproductive stage between pairs of queens and solitary reproductives used for hormonal analysis with a 2 × 2 Fisher's exact test, based on whether or not either individual of a pair had a partially provisioned cell in their nest at the time of collection. We also used this nesting index to assess the relationship of JH and reproductive development among pairs of new nest foundresses with a Spearman's rank correlation.

JH extraction and analysis

Adult bees were collected between 26 February and 8 May 2010, at similar times of day, into individual glass vials containing 1 ml 50% acetonitrile, and stored at −80 °C until analysis. Whole-body extracts were used for JH extraction, and gas chromatography/mass spectrometry was used to determine JH content (following Brent and Vargo, 2003; Brent and Dolezal, 2009; Dolezal et al., 2009, 2012; Penick et al., 2011) (ESM 3). 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 d3-methoxyhydrin derivative of JHIII. Total abundance was quantified against a standard curve of derivatized JH III. We paired two randomly selected bees within each treatment group to extract enough JH for measurement. Sample sizes and values reported below refer to these pairs. We tested for differences between all groups with a Kruskal–Wallis test, followed by Conover post-hoc comparisons.

Results

There was significant variation in JH content among treatment groups (Kruskal–Wallis $\chi^2_6 = 40.21$, $p < 0.001$; Fig. 1). Queens had more JH than solitary reproductives (Conover post-hoc test; $p < 0.05$)