

# The Challenge Hypothesis in an Insect: Juvenile Hormone Increases during Reproductive Conflict following Queen Loss in *Polistes* Wasps

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**ABSTRACT:** The challenge hypothesis was proposed as a mechanism for vertebrates to optimize their testosterone titers by upregulating testosterone during periods of aggressive competition. Here we test key predictions of the challenge hypothesis in an independently evolved endocrine system: juvenile hormone (JH) in a social insect. We assess how social conflict influences JH titers in *Polistes dominulus* wasps. Aggressive conflict was produced by removing the queen from some colonies (experimental) and a low-ranked worker from other colonies (control). Queen removal produced competition among workers to fill the reproductive vacancy. Workers upregulated their JH titers in response to this social conflict; worker JH titers were higher in queenless than in queenright colonies. Furthermore, JH titers were associated with an individual's ability to dominate rivals; the worker that took over as the replacement queen had a substantially higher JH titer than did other workers. Finally, JH titers were positively associated with aggression in queenless colonies, but there was no relationship between JH and aggression in stable, queenright colonies. Overall, these results match key predictions of the challenge hypothesis and parallel much of the work on testosterone in vertebrates. Social modulation of hormone titers is not confined to a particular endocrine system but is likely to be an adaptive feature of endocrine systems across diverse taxa.

**Keywords:** challenge hypothesis, juvenile hormone, testosterone, aggression, dominance, competition.

## Introduction

Studying endocrine systems in multiple contexts is important for understanding how hormones coordinate behavior and physiology as well as how selection acts on endocrine systems in the wild (Zera 2007). As a result, researchers are focusing more attention on the factors that influence interindividual variation in hormone titers as well as the role of social context in endocrine variation

within wild populations (Ketterson et al. 1996; Ball and Balthazart 2008).

In vertebrates, much research on the context dependence of hormonal actions has focused on social modulation of the steroid hormone testosterone (T). T is often associated with benefits such as increased competitiveness, fertility, and mating success, but high T titers are also associated with costs such as reduced immune function and survival (Wingfield et al. 2001; Adkins-Regan 2005). Individuals are hypothesized to mitigate these trade-offs by upregulating T titers during times of aggressive competition (Wingfield et al. 1990). This hypothesis, termed the challenge hypothesis, has received empirical support across a range of taxa and competitive contexts. Many vertebrates upregulate T titers during periods of social competition (Hirschenhauser and Oliveira 2006; Goymann et al. 2007). The variation in T titers across contexts means that it is difficult to understand T's actions without studying its effects in multiple contexts. For example, the strength of the relationship between T and dominance or aggression varies with the extent of social competition. There is typically a strong correlation between T and dominance or aggression during times of aggressive competition, but there may be no relationship during periods of social stability (Wingfield et al. 1990; Adkins-Regan 2005).

Although there has been extensive research on vertebrate endocrine systems, much less is known about endocrine regulation in invertebrates. Both vertebrates and invertebrates have complex social behaviors, but their endocrine systems have evolved independently. As a result, vertebrate and invertebrate hormones have different structures and work in different physiological backgrounds (Nijhout 1994; Adkins-Regan 2005). Comparing the factors that influence endocrine variation across independently evolved taxa can provide insight into the evolution of endocrine systems. Similarities in endocrine responses suggest that there has been convergent evolution of endocrine systems and indicate that the hypotheses devel-

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oped for vertebrates reflect adaptive solutions to problems faced by diverse taxa.

The insect hormone juvenile hormone (JH) is a key hormone that provides a good model for studying endocrine variation in wild invertebrates. It is a versatile hormone that influences multiple aspects of insect behavior and physiology including metamorphosis, diapause, sexual behavior, migration, parental care, and caste development (Nijhout 1994). JH can also function as a gonadotropin and is associated with fertility (Barth et al. 1975; Robinson and Vargo 1997). In many social insects, JH mediates age-related division of labor among workers (Robinson and Vargo 1997; Giray et al. 2005; Shorter and Tibbetts 2009).

JH provides a particularly good comparison with work on T, as JH has some effects that parallel those of T. For example, both JH and T are associated with life-history trade-offs. In the endocrine systems of both vertebrates and invertebrates, high hormone titers are often associated with benefits, such as increased fertility and dominance, as well as costs, such as reduced immune function (Nijhout 1994; Wingfield et al. 2001; Rantala et al. 2003; Adkins-Regan 2005; Amdam et al. 2007). Given the costs and benefits associated with high JH titers, insects may benefit by using social information to modulate their JH titers.

In this study, we test three key predictions of the challenge hypothesis in an insect model. The challenge hypothesis critically predicts that (1) individuals upregulate their endocrine titers during periods of aggressive competition and (2) high endocrine titers are associated with increased reproductive success in competitive contexts. The challenge hypothesis also predicts that (3) aggression will be more strongly associated with endocrine titers during periods of heightened aggressive competition than during periods of low conflict (Wingfield et al. 1990; Goymann et al. 2007). Promising work has provided some support for the challenge hypothesis in insects, as JH may increase during social competition (Scott 2006a; Kou et al. 2008). However, less is known about the other predictions of the challenge hypothesis in taxa lacking T (Scott 2006b; Trumbo 2007).

We test predictions of the challenge hypothesis by assessing hormonal response during an important competitive context within social insects: queen replacement. In social-insect colonies there is intense competition to become the primary reproductive following queen loss. The competition is particularly intense in colonies of primitively eusocial insects that lack discrete castes, because all workers are capable of reproducing (O'Donnell 1998). Worker competition to fill the reproductive vacancy has dramatic effects on the colony. Workers become more aggressive, colony productivity declines, and workers even show reduced immune function (Gobin et al. 2003; Strass-

mann et al. 2004; Bocher et al. 2008). Despite extensive work on worker takeover in social insects, the physiological factors associated with queen replacement have not been addressed.

JH titers were examined in wild nests of *Polistes dominulus* paper wasps. JH plays an important role in mediating dominance and aggression among *Polistes* queens (Röseler et al. 1984; Röseler 1991; Tibbetts and Izzo 2009; E. A. Tibbetts, M. Izzo, and Z. Y. Huang, unpublished data). Among workers, nothing is known about the role of JH in dominance and aggression; previous work focused on the role of JH in modulating age-based division of labor (Giray et al. 2005; Shorter and Tibbetts 2009). *Polistes* are primitively eusocial insects that lack discrete castes (O'Donnell 1998), so workers compete to fill the reproductive vacancy left after queen loss (Strassmann et al. 2004). Here we compare the JH titers and behavior of workers in queenright colonies with the JH titers and behavior of workers in colonies following queen loss. We test whether workers upregulate JH during the period of competition following queen loss. Furthermore, we test whether JH titer is associated with the individual who takes over as the replacement queen following queen loss. Finally, we test whether there is a stronger relationship between JH and aggression within queenless colonies than within queenright colonies.

## Methods

Colonies used in this study were located at the University of Michigan botanical garden in Ann Arbor, Michigan. Soon after nest foundation in mid-May, all nest-founding queens were marked with individual-specific paint marks. Only single-foundress nests that contained their original queen were used in this experiment. Workers emerged between the end of June and early July. The experiment began in mid-July 2008, when nests contained at least six workers. Before the experiment, all workers on each nest were marked with unique paint marks to allow for individual identification. On day 1, the queen was removed from half of the colonies (experimental) and a low-ranked worker was removed from the other half of the colonies (control). Removals were performed early in the morning, when all colony members were cool and inactive, to avoid disturbing the nest. The collected queens and workers were immediately weighed and bled for JH analysis. On day 4 (approximately 75 h after queen or worker removal), colonies were videotaped for 2 h. The next day, colonies were collected early in the morning while all colony members were present. Queen- and worker-removal nests were collected simultaneously to ensure that there was no difference in collection time or collection date of the control and the experimental nests. Therefore, any diel variation

in JH would not influence the results (Zhao and Zera 2004). Twelve nests were used in the experiment: six queen removal and six worker removal. A total of 122 individuals were on the nests. One worker died over the multiday experiment. Colony size ranged from seven to 15 individuals (mean = 10.2, SE = 0.89). Colony size was similar between experimental and control colonies ( $t = 0.73$ ,  $P = .48$ ).

Later, nest videotapes were scored by an observer who was blind to treatment and experimental predictions. For each individual wasp, we recorded the time off the nest as a measure of foraging activity. Two hours of behavioral observations are sufficient to accurately assess worker foraging behavior (Shorter and Tibbetts 2009). The average forager spends 35% of her time off the nest and makes multiple trips within a 2-h observation period (J. R. Shorter and E. A. Tibbetts, unpublished data). In addition, we recorded the number of mounts each individual initiated and received. Mounts are the most aggressive type of interaction on *Polistes* nests. Dominant individuals commonly mount individuals who are subordinate to them, but subordinates never mount individuals who are dominant to them (West-Eberhard 1969; Tibbetts and Dale 2004). The identity of the most dominant colony member was also recorded. The most dominant colony member is straightforward to identify using information about aggression, foraging, and position on the colony. Dominants initiate the most mounts, but they never receive mounts. In addition, dominants do not forage for food, and they spend most of their time on the center of the nest comb. Sixteen individuals eclosed during the experiment. All were unmarked, so they could not be individually identified and are not included in the behavioral analyses. Newly eclosed individuals do not participate extensively in behavioral interactions, as they initiate and receive little aggression. JH was determined according to well-established procedures used with honeybees (Huang et al. 1994; Jassim et al. 2000). First, wasps were cooled, and then hemolymph was collected with microcapillary tubes (Drummond Wirtrol, baked at 500°C for 3.5 h to reduce JH absorption). Hemolymph was collected by cutting the antennae of each individual, gluing its mouth shut, and centrifuging. This method is useful because it prevents contamination from gut contents. A few samples were contaminated with frass (eight of 109 workers and three of 12 queens). Such contamination is easy to identify, as the sample is gray instead of pale yellow. The amount of hemolymph collected from each wasp was measured, and the hemolymph was mixed with 500  $\mu$ L of chilled acetonitrile. JH titers of 11 individuals were not measured because the hemolymph sample was contaminated with other body fluids. JH-III was extracted from the hemolymph sample with  $2 \times 1$  mL of hexane, and the pooled hexane extracts were evaporated

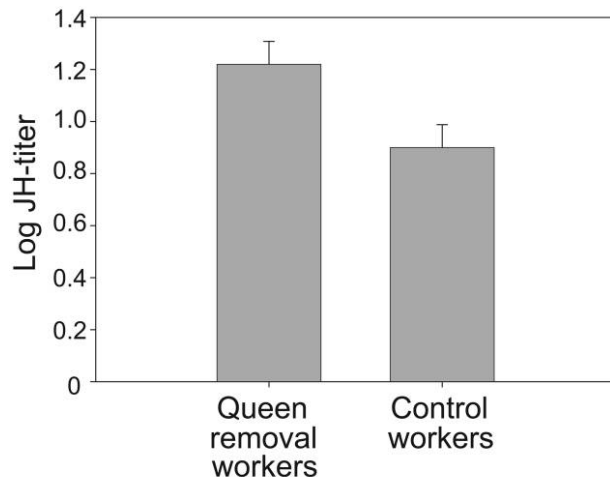
using a vacuum centrifuge (Speedvac) linked to a condenser (Savant SS21), which trapped the solvent at  $-98^{\circ}\text{C}$ . The dried JH in the sample tube was dissolved in 90  $\mu$ L of methanol, and a 30- $\mu$ L aliquot (in duplicate) was removed, dried, and mixed with 200  $\mu$ L of buffer containing anti-JH antiserum (1 : 14,000 dilution; a generous gift from D. Borst) and 10,000 DPM of [ $10\text{-}^3\text{H(N)}$ ]-JH (Perkin Elmer; 647.5 Gbq  $\text{mmol}^{-1}$ ). The mixture was incubated at room temperature for 2 h, and then 0.5 mL of dextran-coated charcoal suspension (Sigma) was added to each sample tube to absorb the unbound JH. This mixture was incubated in an icewater mixture for 2.5 min and then centrifuged (2,000 g for 3 min), and the supernatant, which contained bound JH, was decanted into scintillation vials. Liquid scintillation counting was performed using a Tricarb 2100TR (Packard), which gave the radioactivity in disintegrations per minute (DPM) for each sample. A standard curve with various amounts (0, 3, 10, 30, 100, 300, 1,000, 3,000, and 10,000 pg) of standard JH-III (Sigma) was run each day. KaleidaGraph (Synergy Software) was used to generate a standard curve. The standard curve was obtained by using DPM bound as the dependent variable and JH amount (after log transformation) as the independent variable to fit a five-parameter formula using nonlinear regression. The five-parameter formula was described by Huang and Robinson (1996). Excel (Microsoft) was used to calculate the amount of JH in each sample by reversing the five-parameter formula (solving for JH with known DPM and the five fitted parameters). JH titer in each wasp was then calculated after factoring the volume of hemolymph and the dilution factor (usually 1 : 3). We corrected the JH titers in the samples by dividing by 2 because the “standard” JH sample was a racemic mixture with 50% having biological activity. Extraction efficiency of JH from the hexane/water/acetonitrile partitioning was not corrected because in general it was over 95% and did not vary across different samples.

JH titers and aggression measures were transformed for normality as  $\log(x + 1)$ . The  $\log(x + 1)$  transformation was used to ensure that zero values were defined after transformation. Analyses were performed in SPSS, version 17. Analyses were performed using linear mixed models, including nest as a random effect to control for possible similarity within nests. Linear mixed models are designed to analyze data that contain nonindependent observations, as is the case when multiple individuals from the same nest are sampled. Unless otherwise noted, comparisons are within individuals collected on the same date. For example, queen versus worker comparisons in worker-removal colonies include only those workers collected on the same day as the queen, to ensure that any day-to-day variation in JH titer will not influence the results.

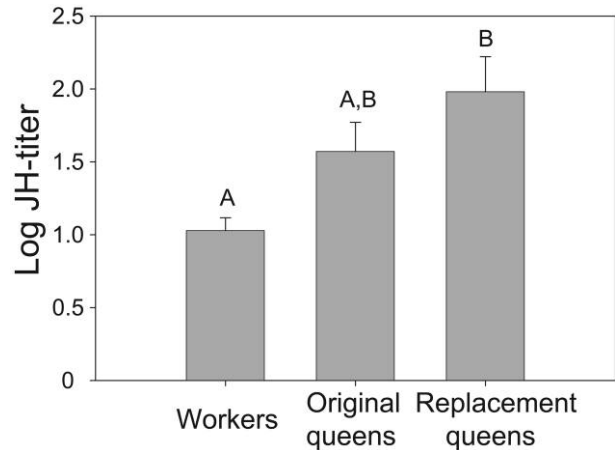
## Results

Experimental removal of queens significantly influenced worker JH titers. The JH titers of workers in queenless colonies were significantly higher than the JH titers of workers in queenright colonies (fig. 1;  $F_{1,9} = 5.69$ ,  $P = .041$ ; queen-removal mean = 1.2; worker-removal mean = 0.87; 95% confidence interval [CI] for the difference between queen and worker removal: 0.019–0.72). JH titers were also associated with dominance among workers. Individuals that became the replacement queens after queen removal had substantially higher JH titers than individuals that remained low ranked (fig. 2;  $F_{1,42} = 14.1$ ,  $P = .001$ ; replacement-alpha mean = 2.0; worker mean = 1.1; 95% CI for the difference between replacement alpha and worker: 0.40–1.33).

The relationship between queen dominance and JH titer is more complicated. In worker-removal colonies, there was no significant difference between the JH titers of queens and workers ( $F_{1,43} = 2.7$ ,  $P = .11$ ; queen mean = 1.47; worker mean = 0.91; 95% CI for the difference between queen and worker:  $-0.12$  to  $1.25$ ). The lack of statistical significance may be due, in part, to the small sample size for this analysis, as there was contamination in three of the six queen hemolymph samples from the worker-removal colonies and so their JH titer could not be measured. After both queen- and worker-removal colonies were included in the analysis, queens had significantly higher JH titers than did workers ( $F_{1,96} = 4.5$ ,  $P = .034$ ; worker mean = 1.08; queen mean = 1.52, 95% CI for the difference between queen and worker: 0.034–



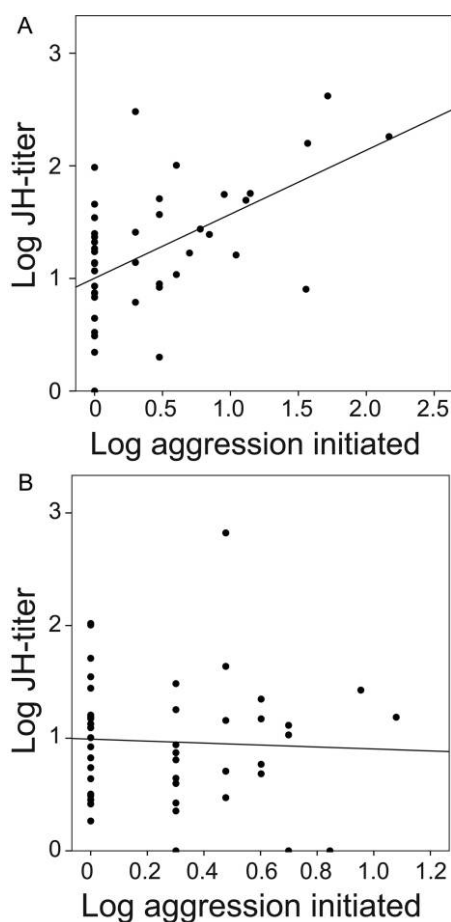
**Figure 1:** Mean (+SE) juvenile hormone (JH) titers of workers from experimental colonies, where the queen was removed ( $n = 46$ ), and from control colonies, where a low-ranked worker was removed ( $n = 49$ ).



**Figure 2:** Mean (+SE) juvenile hormone (JH) titers of workers ( $n = 43$ ), original queens ( $n = 6$ ), and replacement queens ( $n = 6$ ) within colonies where the queen was removed. Original queen JH titer was measured at queen removal, 3 days before the JH titers of replacement queens and workers were measured. Different letters indicate significant differences (least significant difference post hoc analysis).

0.83). However, in queen-removal colonies, the queen was removed from the nest 4 days before the workers and the replacement queen, so day-to-day variation in JH titers could potentially influence the results. The JH titers of the original queen, replacement queen, and workers were also compared within queen-removal colonies alone. The overall model shows that a wasp's role is strongly associated with her JH titer (fig. 2;  $F_{2,46} = 7.98$ ,  $P = .001$ ; worker mean = 1.1, 95% CI = 0.9–1.3; queen mean = 1.6, 95% CI = 1.1–2.0; replacement-queen mean = 2.0, 95% CI = 1.5–2.4). Least significant difference post hoc analysis shows that worker JH titers are lower than replacement-queen JH titers ( $P = .001$ ). However, there was no significant difference in the JH titers of workers and the original queen ( $P = .06$ ) or of the replacement and the original queens ( $P = .13$ ). Therefore, there is a trend toward queens having higher JH titers than workers, while replacement queens have substantially higher JH titers than workers. Although queen-versus-worker JH comparisons are hampered by a relatively small sample of queens, queen JH titer is not relevant to the key predictions of the challenge hypothesis tested in this experiment.

JH titers are most strongly correlated with aggression during the period of aggressive competition following queen removal. In queen-removal colonies, a worker's JH titer was strongly correlated with the number of aggressive acts it initiated (fig. 3A;  $F_{1,40} = 19.0$ ,  $P < .001$ ). However, among workers on queenright colonies, there was no relationship between initiated aggression and JH titer (fig. 3B;  $F_{1,38} = 0.22$ ,  $P = .88$ ). Similarly, there was no rela-



**Figure 3:** Relationship between juvenile hormone (JH) titers and the number of aggressive acts initiated by workers on (A) colonies following queen removal ( $n = 46$ ) and (B) queenright colonies ( $n = 49$ ).

relationship between initiated aggression and JH titer in queenright colonies after including both the queen and the workers in the analysis ( $F_{1,26} = 0.0$ ,  $P = .98$ ). There was also no relationship between JH titer and the amount of aggression an individual received on both queen removal nests ( $F_{1,34} = 1.6$ ,  $P = .21$ ) and on worker removal nests ( $F_{1,17} = 1.4$ ,  $P = .25$ ).

The relationship between JH titer and foraging behavior was statistically significant only when queens were included in the analysis. Over workers and queens, there was a negative relationship between JH titer and time off the nest ( $F_{1,86} = 5.45$ ,  $P = .022$ ), probably because queens and replacement queens have high JH and never forage. This relationship disappears if the queens and the replacement queens are excluded from the analysis ( $F_{1,32} = 0.98$ ,  $P = .33$ ). Similarly, across queens and workers, individuals that spent more than 5 min off the nest had lower JH titers than did individuals that remained on the

nest for the entire 2 h of observation ( $F_{1,86} = 64.6$ ,  $P = .034$ ). However, there was no relationship between JH titer and whether individuals spent more than 5 min off the nest when the queens and the replacement queens were excluded from the analysis ( $F_{1,31} = 1.12$ ,  $P = .30$ ). Therefore, foraging behavior is not correlated with JH titer among workers.

## Discussion

The experimental results fit key predictions of the challenge hypothesis and suggest that social context plays an important role in mediating JH titers. JH titers increased during the period of aggressive competition following queen loss (fig. 1). Furthermore, JH titers were associated with dominance rank and reproductive success. Individuals who succeeded the queen as the dominant reproductive had substantially higher JH titers than did subordinate, nonreproductive workers (fig. 2). Finally, JH titers were positively associated with aggression during the period of social competition following queen removal, but there was no relationship between JH titers and aggression in stable, queenright colonies (fig. 3). Overall, these results provide the first comprehensive support for the challenge hypothesis in a system without testosterone.

The relationships between aggression, competition, dominance, and JH match key predictions of the challenge hypothesis and parallel much of the work on T in vertebrates. First, across a range of taxa and contexts, social conflict increases vertebrate T titers (Wingfield et al. 1990; Hirschenhauser et al. 2003; Hirschenhauser and Oliveira 2004; Oliveira 2004) in the same way that JH titers increased during the period of competition following queen loss (this study). Furthermore, a high T titer is often correlated with dominance rank and reproductive success (Ramenofsky 1984; Adkins-Regan 2005; Beehner et al. 2006), just as JH titer was associated with dominance and reproduction in *Polistes dominulus* workers (this study). Finally, T typically has a stronger relationship with aggression and dominance rank during periods of social instability than during periods without conflict (Wingfield et al. 1990; Oliveira 2004). This study found similar results with JH. The parallels in the endocrine responses of JH and T are striking and suggest that social modulation of hormones is not confined to a particular endocrine system but instead may be a common adaptive solution to problems experienced by diverse taxa.

The challenge hypothesis was proposed as a mechanism for individuals to maximize the benefits of high T titers while minimizing the costs associated with prolonged high T titers. The original challenge hypothesis considered the reduced parental care associated with high T titers as one of the primary costs favoring social modulation of hor-

more titers (Wingfield et al. 1990). Subsequent studies have identified numerous costs associated with high T titers that may favor social modulation of T, including reduced immune function, survival, and fat reserves (Wikelski et al. 1999; Wingfield et al. 2001; Roberts et al. 2004). In paper wasps, reduced parental care is unlikely to be an important factor favoring social modulation of endocrine titers: the dominant reproductive wasp engages in little parental care after worker emergence (Reeve 1991). Instead, other costs associated with high JH have likely selected for social modulation of JH. Although there has been relatively little work performed on the costs associated with high JH titers, there is evidence that JH may reduce immune function (Rantala et al. 2003). In honeybees, high JH is associated with low vitellogenin levels, and vitellogenin protects against oxidative damage (Amdam et al. 2007). Future research that explicitly examines the trade-offs mediated by JH will provide important insight into the selective pressures favoring social modulation of endocrine titers in invertebrates.

The challenge hypothesis was originally developed to understand the relationship between T titers and male-male competition (Wingfield et al. 1990; Gill et al. 2007). This study takes a different perspective by examining the hormone titers of females during social competition. Although female-female competition in eusocial taxa is a new context for the challenge hypothesis, it is one in which social modulation of endocrine titers could be particularly important. Colony reproductive success depends on cooperation, so high titers of the hormones that mediate aggression may impose social costs by disrupting colony social dynamics and reducing reproductive success. As a result, both the social and the individual costs of high endocrine titers may favor social modulation of endocrine titers within eusocial or cooperatively breeding taxa.

A few studies have suggested that social competition can influence endocrine titers in insects. For example, competition increases JH titers in burying beetles (Scott 2006a). Additional work will be required to assess whether the challenge hypothesis applies to burying beetles, as the increase in JH during competition does not appear to increase dominance or reproductive success in this species (Scott 2006b; Trumbo 2007). In cockroaches, individual JH titers increase after winning a fight (Kou et al. 2008). Overall, insect endocrine systems are likely to be more responsive to social stimuli than previously realized, though additional research will be important to identify the relevance of the challenge hypothesis across diverse nonvertebrate taxa.

This study focused on the role of JH during aggressive competition, though previous work suggests that JH is also associated with fertility. JH is correlated with the fertility of *Polistes* foundresses (Röseler 1991; E. A. Tibbetts, M.

Izzo, and Z. Y. Huang, unpublished data), and JH application increased ovarian development of some foundresses (Röseler 1991; Tibbetts and Izzo 2009). In social insect colonies, the dominant individual is also the primary reproductive, so the pleiotropic effect of JH on dominance and ovarian development makes sense. Whenever hormones have multifaceted effects, it can be difficult to attribute changes in hormone titer to one specific aspect of behavior or physiology. The results of this study suggest that JH may be more strongly associated with aggressive competition than fertility. For example, although the original queen is the most fertile individual in the colony, her JH titer was only marginally higher than those of the workers (fig. 2). In comparison, the replacement queen had dramatically higher JH than did other workers. Furthermore, JH titer was strongly correlated with aggression in removal colonies, suggesting that JH plays an important role in mediating conflict. However, this study was not designed to identify the precise relative effects of JH on dominance and fertility. Regardless of JH's precise effects, the results demonstrate that individuals pay attention to their social environment and modulate hormone titers to optimize life-history investment on the basis of the variation within the social environment.

Work on JH in social insect workers has primarily focused on the effect of JH on foraging behavior. For example, in honeybees, foraging workers have higher JH titers than do nurses, and application of JH accelerates the transition from nursing to foraging (Huang et al. 1991; Elekonich et al. 2001). Similar results were found in *Polybia* wasps (O'Donnell and Jeanne 1993). In *Polistes* wasps, application of JH accelerates worker transition from nest work to foraging (Giray et al. 2005; Shorter and Tibbetts 2009). Therefore, it is interesting that this study found no relationship between worker foraging behavior and JH titer.

If JH accelerates the onset of worker foraging behavior in *Polistes*, then why do foraging workers not have higher JH titers than nonforaging workers? One possibility is that a short-term spike in JH initiates worker foraging but workers do not maintain high levels of JH after they begin foraging. Another possibility is that JH has context-dependent effects on worker behavior (West-Eberhard 1996). After all, JH influences dominance, aggression, and foraging in workers. Dominance and foraging behaviors are often thought to be at opposite ends of the behavioral spectrum, as dominant reproductive individuals in worker-phase colonies do not forage and foragers do not reproduce (Hunt 1991; Reeve 1991). One way for JH to mediate such diverse behaviors within a single taxa is through context-dependent effects. For example, JH may increase dominance, aggression, and reproduction among well-fed individuals while increasing foraging behavior in

poorly fed individuals (West-Eberhard 1996; Giray et al. 2005). JH has this type of nutrition-dependent effect in *P. dominulus* queens: it increases the dominance and fertility of only large foundresses in good physical condition (Tibbetts and Izzo 2009). The effect of nutrition on JH's effects within paper wasp workers has not been explicitly tested. If the effect of JH on workers varies with nutritional context, the relationship between JH titer and foraging would be confined to workers in poor condition. Therefore, without nutritional information, it will be difficult to assess the role of natural JH titers on worker foraging.

Overall, JH titers of female paper wasps increase during periods of social competition and are associated with dominance rank and aggression. The responsiveness of JH to social stimuli parallels previous work on testosterone titers in vertebrates and matches key predictions of the challenge hypothesis. The similarities in endocrine response across vertebrate and invertebrate endocrine systems suggest that social modulation of hormones may be a common, adaptive mechanism for balancing the costs and benefits associated with high endocrine titers.

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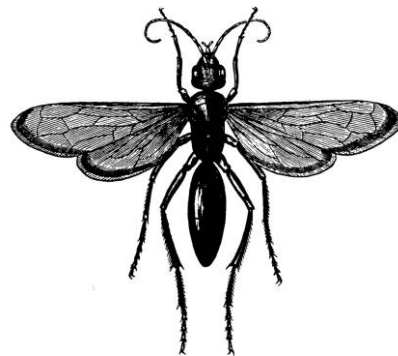
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Mud dauber, *Pompilus formosus*. “The large, red-winged ‘Tarantula Killer’ ... takes its prey by stinging, thus instantly paralyzing every limb of its victim. The effects of the introduction of its venom is as sudden as the snap of the electric spark. The wasp then drags it, going backwards to some suitable place, excavates a hole five inches deep in the earth, places its great spider in it, deposits an egg under one of its legs, near the body, and then covers the hole very securely.” From “The Tarantula Killers of Texas” by G. Lincecum (*American Naturalist*, 1867, 1:137–141).