

Behavioral and physiological factors associated with juvenile hormone in *Polistes* wasp foundresses

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Received: 14 June 2010 / Revised: 3 November 2010 / Accepted: 24 November 2010
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Abstract Although there is increasing interest in the evolution of endocrine systems, relatively little is known about the factors associated with natural endocrine variation in invertebrates. Here, we assess juvenile hormone (JH) titers among nest-founding queens of the wasp *Polistes dominulus* over 2 years. We allowed unfamiliar wasps to battle for dominance and examined the relationships between dominance rank, JH, ovarian development, and facial patterns. The relationship between JH-titer and dominance varied across years; there was a stronger relationship between JH-titer and dominance in 2006 than in 2008. Across years, wasps that won dominance contests had facial patterns with more broken black spots than wasps that lost dominance contests. There was no relationship between dominance rank and ovarian development. The individual characteristics associated with JH-titer were also tested; JH-titers were correlated with facial pattern brokenness and ovarian development. This study adds to previous work indicating that *P. dominulus* facial patterns function as a signal of fighting ability. Furthermore, the

correlation between JH-titers and facial patterns parallels previous work on testosterone and vertebrate signals and suggests that links between signals of fighting ability and hormones that mediate fighting ability may be common across taxa. Overall, individual JH-titers vary, though they are typically associated with factors related to individual reproductive success, including dominance, fertility, and facial pattern brokenness. Future studies in additional contexts and taxa will be important to test how and why JH-titers vary.

Keywords Badge of status · Testosterone · Aggression · Quality signal · Juvenile hormone · Dominance

Introduction

There has been increasing interest in research that integrates behavior, morphology, and physiology (Ketterson et al. 1992). As a result, researchers are focusing more attention on individual variation in hormone titers and how hormone titers are associated with behavioral and physiological variation within natural populations (Adkins-Regan 2005). Although there has been extensive research on vertebrate hormones, relatively less is known about the hormonal basis of insect behavior (Zera et al. 2007). Social insects provide a good model for exploring the endocrine underpinnings of behavior and physiology because they have complex behaviors with striking inter-individual variation. Furthermore, because vertebrates and invertebrates have similar behaviors but different endocrine systems (Nijhout 1994; Adkins-Regan 2005), comparing the mechanisms underlying behavior across taxa can provide insight into the evolution of endocrine regulation (Emlen and Nijhout 2000).

Communicated by O. Rueppell

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One key insect hormone is juvenile hormone (JH). JH is a versatile hormone that influences multiple aspects of insect behavior and physiology including metamorphosis, diapause, sexual behavior, migration, and parental care (Nijhout 1994). JH can function as a gonadotropin and is associated with fertility in a range of taxa including paper wasps (Röseler 1991; Robinson and Vargo 1997; Tibbetts and Izzo 2009). In addition, JH regulates foraging behavior in many social insect workers (Robinson and Vargo 1997; Giray et al. 2005). Researchers have found varying relationships between JH-titers and dominance: a positive relationship in bumblebees and cockroaches (Bloch et al. 2000; Chen et al. 2005), a negative relationship in a ponerine ant (Sommer et al. 1993; Brent et al. 2006), and no relationship in crickets and burying beetles (Adamo et al. 1994; Scott 2006b).

Here, we measure the relationship between JH-titers and individual behavior, physiology, and quality signal elaboration in wild, nest-founding queens of the paper wasp, *Polistes dominulus*. Paper wasp foundresses show complex social and agonistic behaviors. Aggressive competition is an important facet of foundress social life and competition over rank occurs both before and after nest foundation (Röseler 1991). Prior to nest foundation, foundresses spend a few weeks assessing nesting locations and competing with other foundresses before settling down to start a nest (Röseler 1991). Nest composition remains flexible for weeks after colony foundation, so foundresses continue to compete with potential nest usurpers and joiners (Nonacs and Reeve 1995). *P. dominulus* have variable, black facial patterns that function as a conventional signal of agonistic ability and may help minimize the costs of dominance competition. Facial pattern brokenness is associated with dominance (Tibbetts and Dale 2004; Tibbetts and Shorter 2009, but see Cervo et al. 2008) and wasps use each other's facial patterns to assess rivals prior to interacting (Tibbetts et al. 2010).

Previous work indicates that a foundress' ability to dominate rivals is associated with multiple factors. In addition to facial patterns, dominance is correlated with the size of the corpora allata (the organ that produces JH), body size, and ovarian development (Pardi 1948; Röseler et al. 1984; Röseler 1991). However, these factors are typically studied in isolation within nests that have been established for weeks, so we do not fully understand how these factors interact with each other to mediate the outcome of aggressive competition over dominance status. In this study, we assess multiple factors concurrently to test how foundress dominance is associated with JH-titers, egg development, and facial patterns. Second, we test the individual characteristics associated with JH-titer by measuring how a foundress' JH-titer is associated with her ovarian development, body size, and facial pattern brokenness.

The relationships between hormone-titer and behavior or physiology are often context-dependent and may vary with the environment, social circumstances, or individual traits (Nijhout 1994; Adkins-Regan 2005). As a result, we performed the experiment in two different years. In 2006, we assessed foundresses approximately 10 days after nest foundation. In 2008, we assessed foundresses at the time of nest foundation. Measuring these factors across 2 years provides useful information about the consistency of the relationships, though the experimental design provides limited information about the precise basis of any variation across years.

Materials and methods

Nest-founding *P. dominulus* wasps were collected around Ann Arbor, MI, after they emerged from diapause (May 7–17, 2006 and May 5–7, 2008). Foundresses were at different stages of nest foundation across the 2 years, as 2008 was a cooler spring than 2006 and the nesting cycle was delayed. In 2006, wasps had begun nest construction and were on approximately ten to 15 cell nests. In 2008, we caught wasps at the moment of nest foundation, so nests were very small, approximately one to two cells. All foundresses used in the experiment were collected in the early morning and were the only individual on their nest. Therefore, they were not part of a cofoundress association. Immediately after collection, each wasp was marked with an individual-specific paint mark and weighed on a scale accurate to 1 mg. Within a few hours of collection, wasps were paired with a similarly sized rival (within 8 mg) to battle for dominance in a 2-hour videotaped bout.

The dominance contests occurred in a neutral 8×8×2 cm clear plastic arena that contained no resource and wasps displayed a full range of aggressive behaviors as they battled for dominance, including darts, mounts, bites, and grapples. This experimental design mimics the aggressive contests that are common among foundresses during the early founding stage (Röseler et al. 1984, 1985). Dominance contests occur both on and off nests throughout the first weeks of the founding cycle (personal observation, Röseler 1991). Two-hour contests were chosen to standardize the amount of interaction across all individuals. Wasps typically have prolonged interactions before establishing dominance (average time to dominance=80 min). Rivals were collected from sites at least 3 km apart to ensure they had not previously interacted. At the end of the 2-h contest, wasps were sacrificed following cold anesthetization. Wasp abdomens were stored in 95% ethanol before dissection. Wasps were dissected, and the ovaries were photographed using an Infinity 1 camera. Then, all oocytes were measured using Infinity Analyze software. We used the number of oocytes

greater than 1 mm in length as the measure of ovarian development (mean=7 eggs, range=0–14 eggs).

Juvenile hormone titers

Immediately after each dominance bout, wasps were cooled and decapitated, and hemolymph was collected with microcapillary tubes (Drummond wiretrol, baked at 500 °C for 3.5 h to reduce JH absorption). The amount of hemolymph collected from each wasp was measured (range=0.93–16.6 µl). The final JH-titer was calculated as a concentration (nanograms per milliliter which was equivalent to pictograms per microliter), so the particular volume of blood does not influence the measured JH as long as it is within the range covered by the standard curve (3–10,000 pg; Huang and Robinson 1995). The hemolymph was mixed with 500 µl chilled acetonitrile. JH was determined according to well-established procedures used in honey bees (Huang et al. 1994; Jassim et al. 2000). Briefly, JH III in the hemolymph sample was extracted with 2×1 ml of hexane; then, the pooled hexane extracts were evaporated using a vacuum centrifuge (Speedvac) linked to a condenser (Savant SS21), which trapped the solvent at –98°C. The dried JH in the sample tube was dissolved in 100 µl of methanol, and a 20-µl aliquot (in duplicate) was taken out, dried, and mixed with 200 µl of buffer containing anti-JH anti-serum (1:14,000 dilution) and 10,000 disintegrations per minute (DPM) of [$^{10^{-3}}$ H(N)]-JH (Perkin Elmer, 647.5 Gbq/mmol). 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 ice-water mixture for 2.5 min, then centrifuged (2000×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 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 racemic JH III (Sigma) was run each day. The amount of JH in the samples was corrected by dividing by 2 because the racemic mixture of JH standard overestimates the 10R JH in biological samples by a factor of 2.

KaleidaGraph (Synergy Software, PA, USA) was used to generate a standard curve. The standard curve was obtained by using DPM bound as the dependent variable, JH amount (after log transformation) as the independent variable, to fit a five-parameter formula, using nonlinear regression. The five-parameter formula was described in Huang and Robinson (1996). Excel (Microsoft, USA) 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). The amount of JH in the

samples was corrected by dividing by 2 because the racemic mixture of JH standard overestimates the 10R JH in biological samples by a factor of 2.

To verify that we were measuring JH III and there was no significant impurity in the unprocessed hemolymph samples to interfere with the binding between JH and its antibody, we conducted a thin-layer chromatography (TLC). TLC was performed in a solvent system of hexane/ethyl acetate/glacial acetic acid (66:33:1). Radioactive JH III (10,000 dpm) was loaded on one lane, and a pooled sample of wasp hemolymph sample was loaded in another. After completion, each lane was divided into nine fractions, and the TLC silicon dust removed carefully with a razor blade. For the radiolabelled JH sample, dusts from each fraction were put into scintillation vials directly and DPM assessed by the liquid scintillation counter. For the hemolymph sample, each fraction was washed with hexane (1 ml×2) and dried, and the amount of presumptive JH measured as described above. The TLC experiment was replicated two times.

Facial pattern analysis

We assessed the signal phenotype of each individual by analyzing a digital picture of the wasp's face with Adobe Photoshop (v. CS3). A wasp's facial pattern “brokenness” is the best predictor of dominance and takes into account the number, size, and shape of black spots on the wasp's clypeus (Tibbetts and Lindsay 2008; Tibbetts et al. 2010). Facial patterns with more and wavier spots are higher on the brokenness index and signal higher dominance than facial patterns with fewer, smoother spots. First, the area of the clypeus containing the population-wide badge variability was converted into a 30×60-pixel bitmap. Then, the number of black pixels within each vertical column along the horizontal length of the clypeus was counted using a simple program created in Mathematica (Wolfram Research, IL, USA). We were interested in the total disruption of the black facial pattern, so we calculated the standard deviation of the black pigment deposition from pixels five to 55 along the horizontal gradient of the clypeus. We excluded the first and last five pixels from the brokenness analysis because the edges of the clypeus are black. As a result, wasps with black in the first and last five pixels have facial patterns that appear less broken than individuals with black spots that extend to the edge of the clypeus. The standard deviation of the black pigment deposition or “brokenness” of a wasp's face measures the amount of disruption in the black coloration.

Dominance

Videos of the dominance bouts were later scored to assess which of the two individuals successfully dominated her rival. Mounting behavior was used to establish which

individual was behaviorally dominant. During a mount, the dominant wasp climbs on top of the subordinate while the subordinate crouches and lowers her antennae (West-Eberhard 1969). After dominance is established with a mount, it remains fixed through subsequent interactions between individuals (personal observation, Röseler 1991; Tibbetts and Izzo 2010). Dominance in each bout was scored by two independent observers who were blind to the experimental predictions. In 2006, there were 53 trials with dominance established, and in 2008, there were 41 trials with dominance established. Individuals from trials where dominance was not established were excluded from further analyses. In 2006, one individual had contaminated blood and was excluded from JH analysis and one individual lacked measures of ovarian development. In 2008, one individual lacked information about ovarian development.

Statistical analysis

The data were analyzed in two different ways to test (1) the factors associated with dominance and (2) the factors that correlate with JH-titers. The dominance data were analyzed using paired logistic regressions. Regression models were chosen by comparing Mallow's C_p statistic for all potential models. Final models that include only the factors significantly associated with dominance rank are reported below. Information about non-significant factors is presented, but non-significant factors were not included in the final models. JH-titers and facial pattern brokenness were log-transformed for normality before analysis. Simple comparisons across years were performed using t tests that did not assume equal variance across years. The relationship between JH-titers and weight, ovarian development, year, and facial pattern brokenness were analyzed using a general linear model. Non-significant interactions were excluded from the final model. Analyses were performed using SPSS v. 17 and Minitab v. 15.

Results

Verification of JH measurement

TLC results indicated that hemolymph samples can be directly assayed for JH because there was no significant interference from impurity. This is because RIA immunoreactivities in the TLC fractions of the hemolymph sample matched well with the radioactivities of the radio-labeled JH standard (Fig. 1). The small amount of immunoreactivity in fraction 9 most likely stemmed from the spillover from fraction 8, due to a slight delay of retention times in the biological sample, as the was a slight delay for JH in a honey bee sample using an HPLC in a previous study (Huang et al. 1994).

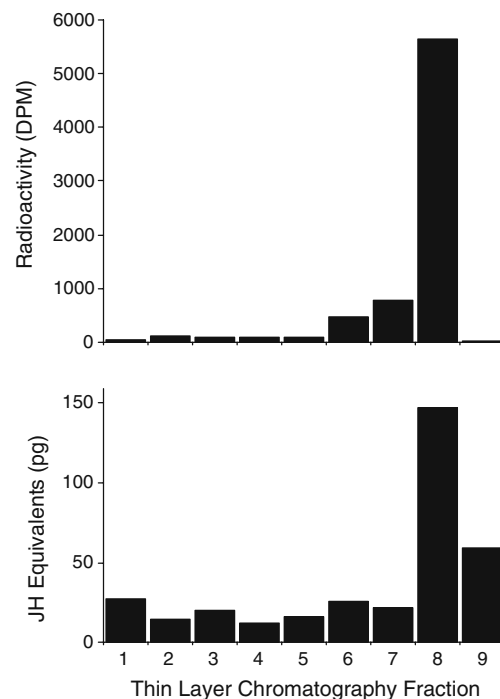


Fig. 1 Comigration of JH immunoreactivity of wasp hemolymph and radioactive JH standard. *Top*: distribution of radioactivity among thin-layer chromatography fractions of a radio-labeled JH standard (10,000 dpm). *Bottom*: distribution of immunoreactivity of a pooled wasp hemolymph sample. There is a clear match of the two samples, indicating the radioimmunoassay was measuring JH3 in the hemolymph and suggesting there are no other chemicals reacting with the antibody

Individuals collected in 2006 and 2008 showed significant differences in JH-titer ($t_{118}=7.5$, $p<0.001$), number of mature eggs ($t_{156}=8.8$, $p<0.001$), and weight ($t_{180}=-2.2$, $p=0.03$), but not facial pattern ($t_{177}=-1.2$, $p=0.24$). As a result, dominance data from the 2 years were analyzed separately.

Factors associated with dominance

Paired logistic regression were fitted to determine how differences in JH-titers, facial pattern, and ovarian development were associated with dominance rank, using Mallow's C_p statistic to assess which factors should be included in the final model (Table 1). In 2006, among wasps collected after nest establishment, dominance was associated with JH-titers and

Table 1 Factors associated with dominance rank: factors chosen by comparing Mallow's C_p statistic for all potential models

	2006	2008
Facial pattern brokenness	+	+
JH-titer	+	-
Ovarian development	-	-

(+) factors improve the overall model while (-) factors do not improve the overall model

facial patterns (overall $\chi^2=7.47$, $r^2=0.18$, $p=0.02$, $n=51$ pairs; JH-titer Wald $\chi^2=4.1$, $p=0.04$; facial pattern brokenness Wald $\chi^2=2.7$, $p=0.10$, Figs. 2 and 3). There was no association between dominance and the number of developed eggs (Wald $\chi^2=1.0$, $p=0.30$) or body weight (Wald $\chi^2=0.42$, $p=0.52$). The lack of relationship between size and dominance is not surprising, as foundresses were paired for similar weight.

In 2008, among wasps collected at nest establishment, dominance was positively associated with facial patterns alone (Table 1, overall $\chi^2=15.1$, $r^2=0.41$, $p<0.001$, $n=41$ pairs; facial pattern brokenness Wald $\chi^2=8.4$, $p=0.004$, Figs. 2 and 3). Although dominant wasps appear to have higher JH-titers than subordinate wasps (Fig. 2), this difference is only statistically significant in an independent analysis (paired t test, $t_{40}=2.1$, $p=0.037$). In the whole model that included facial patterns, JH-titers, eggs, and weight as independent variables, there was no significant association between dominance and JH-titers (Wald $\chi^2=1.2$, $p=0.28$), the number of developed eggs (Wald $\chi^2=0.25$, $p=0.62$), or body weight (Wald $\chi^2=0.18$, $p=0.89$).

Correlates of juvenile hormone

A general linear model was used to assess how JH-titers were associated with facial patterns, ovarian development,

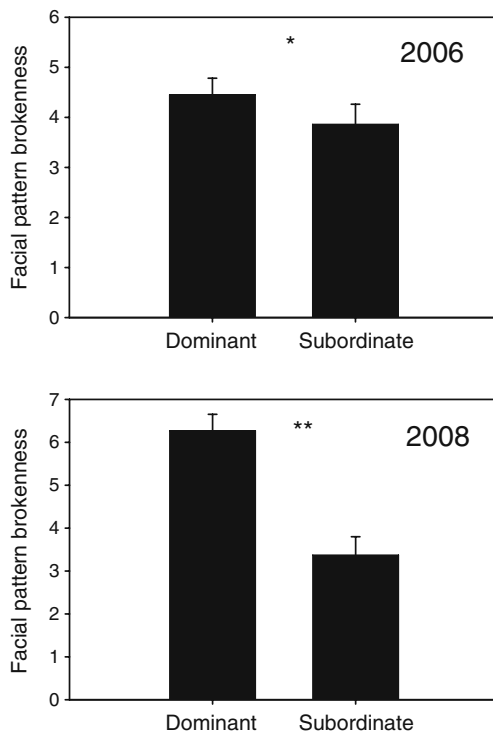


Fig. 2 Mean (+SE) facial pattern brokenness of individuals that won versus lost the dominance contests. In 2006, foundresses were collected from established nests ($n=51$ pairs, $p=0.10$). In 2008, individuals were collected at nest foundation ($n=41$ pairs). * $p=0.10$, ** $p<0.05$

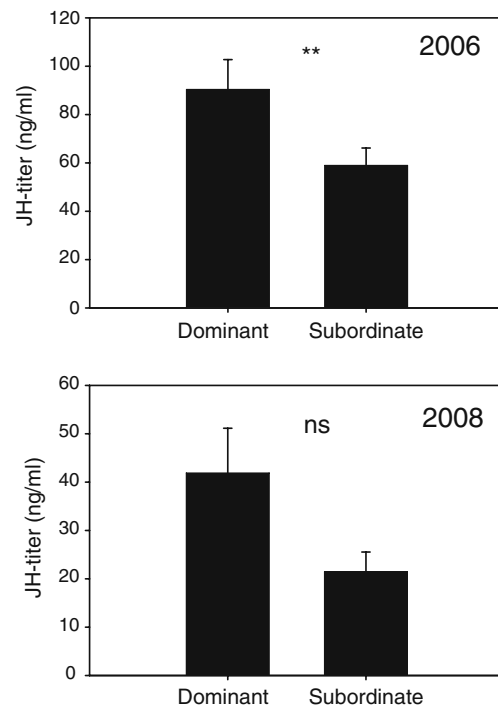


Fig. 3 Mean (+SE) JH-titer of individuals that won versus lost the dominance contests. In 2006, foundresses were collected from established nests ($n=51$ pairs). In 2008, individuals were collected at nest foundation ($n=41$ pairs). ** $p<0.05$, ns $p>0.10$

weight, year, and the interactions between year and the other variables. The final model shows that JH-titers are positively associated with ovarian development ($F_{1,179}=7.45$, $p=0.007$), facial pattern brokenness ($F_{1,179}=4.3$, $p=0.04$), the interaction between year and weight ($F_{1,179}=14.9$, $p<0.001$), and the interaction between year and facial pattern brokenness ($F_{1,179}=5.2$, $p=0.023$). Weight ($F_{1,179}=1.1$, $p=0.29$), year ($F_{1,179}=0$, $p=0.99$) and the interaction between year and ovarian development ($F_{1,178}=0.39$, $p=0.53$) were not significantly associated with JH-titer.

Discussion

Factors associated with dominance

Two factors were associated with an individual's ability to successfully dominate rivals: JH-titer and facial pattern brokenness (Figs. 2 and 3). However, the relative importance of these factors varied. In 2006, among wasps on established nests, dominance rank was more strongly associated with JH-titers than facial pattern. In 2008, among wasps that had just started nests, dominance was associated with facial pattern, but not JH-titers. In both years, ovarian development was not associated with dominance. These results, in combination with previous

studies, indicate that the conventional signal of agonistic ability in *P. dominulus* may be particularly important during aggressive competition among unfamiliar individuals, while factors such as differences in JH-titer, ovarian development, and cuticular hydrocarbons may be more important for maintaining dominance ranks on established nests (Sledge et al. 2004; Izzo et al. 2010).

The general relationship between facial pattern and dominance supports previous work indicating that facial patterns in *P. dominulus* wasps function as a conventional signal of fighting ability. Previous work demonstrated that *P. dominulus* with more facial spots are more likely to win fights, both at the time of nest foundation (Tibbetts and Dale 2004) and later, during usurpation battles over nest ownership (Tibbetts and Shorter 2009). Furthermore, wasps use facial patterns to assess the agonistic abilities of rivals (Tibbetts 2008; Tibbetts and Lindsay 2008; Tibbetts et al. 2010). However, previous work has largely examined facial patterns in isolation. This study is the first to examine how dominance rank is associated with physiological factors such as JH-titers and ovarian development, as well as facial pattern. The results show that the conventional signal of quality in *P. dominulus* can be more reliably associated with dominance than certain physiological factors.

The strength of the relationship between facial patterns and dominance varied across years, as they were more strongly associated with dominance in 2008 than in 2006. Multiple factors could account for this variation. First, social context varied across the 2 years. In 2008, wasps had started nests within a day or two of the experiment. The moment of nest foundation is a time of extreme instability and social conflict, as wasps typically battle with many different rivals before nest foundation (Röseler 1991). Signals of agonistic ability are predicted to be particularly important during battles with unfamiliar rivals (Senar 2006; Tibbetts and Safran 2009), so individuals may be particularly attuned to the signal of agonistic ability in early spring. In 2006, the wasps had been on established nests for approximately 10 days before being tested. At this stage, individuals fight with fewer unfamiliar rivals and signals of agonistic ability may be less important. Although it is initially surprising that receiver responses to a signal could change so rapidly, the importance of signals often changes with social context (Bradbury and Vehrencamp 1998). For example, signal responses in *P. dominulus* vary with context, including the value of the resource being contested and the relative quality of the rivals (Tibbetts 2008; Tibbetts et al. 2010). The variation in the relationship between facial patterns and dominance across years could also reflect stochastic variation or year-to-year variation in signal information content (Chaine and Lyon 2008). An ornament's signal value may often vary across years, populations, or social contexts (Cockburn et al. 2008; Kasumovic et al.

2008). Identifying the causes and behavioral consequences of this variation is an important direction for future communication research.

JH-titers were associated with foundress dominance in 2006. In 2006, wasps who won dominance contests had higher JH-titers than wasps who lost dominance contests. JH has long been thought to correlate with dominance in *Polistes*, as previous work demonstrated that the size of the corpora allata is associated with dominance rank (Röseler et al. 1984, 1985; Sledge et al. 2004). Furthermore, application of the JH analog methoprene increases foundress dominance (Röseler et al. 1984; Tibbetts and Izzo 2009). However, previous studies have not examined endogenous JH-titers. Some previous work suggests that endogenous JH-titers may be influenced by social interactions (Scott 2006a; Kou et al. 2008; Tibbetts and Huang 2010). Although it is not clear how rapidly JH-titers change during social interactions, behavior during dominance trials could contribute to the difference between dominant and subordinate JH-titers. Nevertheless, the results of this study, combined with previous work involving experimental JH alteration and CA volume measurement, indicate that JH is associated with foundress dominance.

This study was not designed to explicitly test the basis of the variation across years, so multiple factors may account for the varying relationship between JH and dominance. First, social context varied across the years; wasps were collected a few days later in the founding period in 2006 than in 2008. Nest foundation is an important milestone in a queen's life, so it may initiate neuroendocrine changes. For example, hormones such as JH and testosterone are often more strongly associated with dominance rank during periods of social instability (Wingfield et al. 1990; Scott 2006b; Tibbetts and Huang 2010). Alternatively, there may be year-to-year variation in the strength of the relationship between JH and dominance. Researchers have found varying relationships between JH-titer and dominance in other taxa: a positive relationship in bumblebees and cockroaches (Bloch et al. 2000; Chen et al. 2005), a negative relationship in a ponerine ant (Brent et al. 2006), and no relationship in crickets and burying beetles (Adamo et al. 1994; Scott 2006b). Considering the complexity of the hormone/dominance relationship within and between vertebrate species (Adkins-Regan 2005), it is not surprising that JH's effects on dominance behavior are complex and not fully understood. Additional research on a range of taxa, social circumstances, and environments will be important to provide a nuanced perspective on the factors that influence the relationship between JH and behavioral dominance.

Although some previous studies have suggested that ovarian development is associated with dominance in paper wasps, we found no evidence that dominant wasps have

more developed eggs than subordinate wasps. The studies that found associations between dominance and ovarian development examined stable associations of co-founding queens (Dropkin and Gamboa 1981; Pardi 1948; Turillazzi and Pardi 1977). The ovaries of subordinate foundresses within cooperative associations regress because subordinates rarely reproduce (Reeve 1991). As a result, it is not surprising that dominance rank is correlated with ovarian development on established wasp nests. However, ovarian development is not required for dominance, as an ovariectomized queen can still become the dominant foundress (Röseler et al. 1985). Therefore, the relationship between dominance rank and ovarian development found in previous studies is likely a consequence rather than a cause of dominance rank.

Factors associated with JH

Ovarian development and facial pattern brokenness were associated with individual JH-titers. Individuals with higher JH-titers had more developed eggs and facial patterns advertising higher quality than individuals with lower JH-titers. Body weight was not associated with JH-titers.

The relationship between JH-titers and ovarian development matches previous work indicating that JH functions as a gonadotropin. Across a range of social and non-social insects, JH is associated with ovarian development (Nijhout 1994; Robinson and Vargo 1997). However, the relationship between JH and ovarian development is not universal, as JH is decoupled from fertility in some taxa (honeybees Hartfelder 2000, queenless ants Brent et al. 2006). The effect of JH on ovarian development can even vary within a single taxon. For example, methoprene application increases ovarian development in large *P. dominulus* queens, but not in small *P. dominulus* queens (Tibbetts and Izzo 2009). JH also increases foraging, rather than fertility among paper wasp workers (Giray et al. 2005; Shorter and Tibbetts 2009). In this study, queens were examined when they were starting nests and laying eggs (Röseler 1991), so JH and ovarian development may be more strongly associated during this period than in other social contexts.

The quality signal in *P. dominulus* is also correlated with JH-titers. Individuals with more broken facial patterns have higher JH-titers than individuals with less broken facial patterns. *P. dominulus* facial patterns function as conventional signals of fighting ability (Tibbetts and Lindsay 2008), so it makes sense that ornamentation is correlated with JH, a hormone that mediates agonistic behavior. The correlation between JH-titers and facial pattern brokenness provides further evidence that facial patterns provide useful information about their bearer's agonistic abilities. The results also parallel some previous work in vertebrates which has found correlations between testosterone and

signals of agonistic ability (Zuk et al. 1995; Papeschi et al. 2000; Gonzalez et al. 2001; Mougeot et al. 2004). The parallels in the hormone/ornament relationships across vertebrates and invertebrates are striking and suggest that the links between hormone-titer and ornament brokenness may be an adaptation rather than a non-adaptive byproduct of a particular endocrine system.

Thus far, most research in social insects on the relationship between signals, JH, fertility, and dominance has focused on cuticular hydrocarbons (CHCs). CHCs are associated with dominance rank, JH, and fertility in many social insects (Peeters et al. 1999; Sledge et al. 2001; Heinze et al. 2002; Cuvillier-Hot et al. 2004; Brent et al. 2006). CHCs are flexible traits that change with fertility and colony rank (Peeters et al. 1999; Sledge et al. 2001; Liebig 2010), so they are quite different than the visual signals in *Polistes* wasps. Visual signals in paper wasps have more in common with agonistic signals in non-social species than with CHCs (e.g., Tibbetts and Safran 2009). Visual signals develop during pupation based on larval condition and remain fixed during adulthood (Tibbetts 2010). They are useful for identifying the agonistic abilities of unfamiliar foundresses during the nest-founding phase, but are unlikely to be used on established nests. In contrast, CHCs are used to identify the reproductive queen on stable nests (Monnin 2006; Liebig 2010).

Overall, there was variation in the endocrine relationships among nest-founding queens across year, though JH-titers are typically associated with factors related to an individual's reproductive success: dominance, fertility, and facial pattern brokenness. Vertebrate research has shown that endocrine relationships often vary substantially within and between taxa (Adkins-Regan 2005; Gill et al. 2007; Hau 2007), but there has been relatively little research on the context-dependence of insect endocrine relationships. Therefore, continued research on JH across contexts and taxa will be important to assess how biotic and abiotic factors influence individual endocrine variation in invertebrates.

Acknowledgments Funding was provided by the University of Michigan and Michigan State University Agricultural Experimental Station. The JH anti-serum was generous gift from David Borst. Thanks are due to A. Mettler, J. Shorter, and M. Wells for research assistance as well as two anonymous reviewers for helpful suggestions.

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