Advertised quality, caste and food availability influence the survival cost of juvenile hormone in paper wasps

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Life-history trade-offs are often hormonally mediated. Here, we provide a comparative perspective on the endocrine basis of life-history trade-offs by examining the invertebrate hormone juvenile hormone (JH). JH is often associated with benefits, including increased dominance and reproductive success. We tested whether JH reduced survival of *Polistes dominulus* wasps and whether this survival cost was influenced by factors such as advertised quality, food availability, caste and body size. Overall, JH reduced individual survival. Among fed and unfed queens, JH reduced survival in a dose-dependent manner. Among workers, JH had a stronger effect on survival of fed workers than unfed workers. Unfed workers died quickly and body size was the best predictor of survival. Surprisingly, queens and workers treated with JH survived longer when they had signals advertising high quality than when they had signals advertising low quality. The relationship between advertised quality and ability to withstand high levels of JH suggests that there are differential physiological costs associated with ornament elaboration that could play a role in maintaining signal accuracy over evolutionary time. Overall, the convergence of endocrine-mediated costs across diverse systems suggests that endocrine-mediated trade-offs may be an adaptive way to optimize resource allocation rather than a non-adaptive constraint specific to a particular hormone.

Keywords: juvenile hormone; testosterone; quality signal; conventional signal; social costs; honest communication

1. INTRODUCTION

Life-history traits are often negatively correlated (Stearns 1992). For example, individuals with high dominance and reproductive success may have reduced immune function and survival (Wingfield *et al.* 2001; Roff 2002; but see Koskimaki *et al.* 2004). Given the occurrence of certain life-history trade-offs across taxa, there has been interest in the physiological factors that mediate these trade-offs as well as the role of natural selection in shaping trade-offs (Hau 2007).

There is growing evidence that many classic life-history trade-offs are hormonally mediated. Most research on the endocrine basis of life-history trade-offs has focused on vertebrates, finding that testosterone (T) mediates key trade-offs. For example, T often increases dominance and reproduction while simultaneously reducing survival and immunity (Ketterson & Nolan 1999; Wingfield *et al.* 2001; Reed *et al.* 2006). Although T's precise actions are evolutionarily labile (Hau 2007), T mediates reproduction versus survival trade-offs across a range of diverse taxa.

In invertebrates, the hormone juvenile hormone (JH) may mediate trade-offs similar to those mediated by T. JH and T have different structures and work in different physiological backgrounds, but show surprising similarities. For example, JH increases dominance and reproduction in some taxa (Röseler 1991; Robinson & Vargo 1997; Trumbo 2007; Kou *et al.* 2008; Tibbetts &

Izzo 2009). JH may also reduce immune response (Rantala *et al.* 2003; Flatt & Kawecki 2007). Research on insects with discrete morphs suggests that JH may play a role in life-history trade-offs. Morphs show different investment into aspects of life history and morph development is influenced by JH (Nijhout 2003; Zera *et al.* 2007). Much less is known about how JH influences continuous variation in behaviour and physiology within a species. Studying the role of JH in life-history trade-offs is important for understanding how insects evolve as integrated units. In addition, it provides a nice comparison with previous work on T in vertebrates. Similarities across these independently evolved endocrine systems indicates that there has been convergent evolution in endocrine actions and suggests that the regulation of life-history trade-offs by a single hormone may be a common, adaptive solution to problems faced by diverse taxa.

Thus far, much of the research on endocrine-mediated trade-offs has focused on the overall costs and benefits associated with high endocrine titres rather than identifying how individual characteristics influence these costs and benefits. For example, individuals in good condition may be better able to withstand the costs of high endocrine titres than individuals in poor condition (Roberts & Peters 2009). Understanding individual variation in hormonal response is essential for identifying how selection acts on endocrine systems in the wild.

Measuring individual variation in hormonal response is also important because some models predict that the costs associated with high endocrine titres will vary across individuals. For example, a key prediction of

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honest signalling models is that the marginal net benefit associated with signal elaboration will decrease with increasing individual quality (Zahavi 1975; Getty 2006). One way for this to occur is if high-quality individuals suffer lower costs for a given level of signal elaboration than low-quality individuals. This prediction can be tested by imposing a cost (e.g. experimentally increase hormone titres) and assessing whether signal elaboration is correlated with an individual's ability to withstand this cost. A relationship between signal elaboration and ability to withstand high hormone titres may be particularly important for signals with social costs. When signals have social costs, signallers must back up their signal with agonistic behaviour (Tibbetts & Dale 2004; Senar 2006; Tibbetts & Izzo in press). Agonistic behaviour is often hormonally mediated, so individuals who signal high quality must withstand higher titres of the hormones that control agonistic behaviour than individuals that signal low quality (Tibbetts *et al.* in press). Given the costs associated with high endocrine titres, it may be difficult for some individuals to maintain the levels needed to back up a socially controlled signal. Therefore, differential costs associated with high endocrine titres could provide an important complement to behavioural cost in maintaining the accuracy of ornaments with social costs.

The hypothesis that physiological costs play a role in the evolutionary stability of signals makes two predictions. First, signal elaboration must have a consistent relationship with titres of the hormone that mediates agonistic behaviour that is prohibitively costly to break. Second, signal elaboration must be associated with an individual's ability to withstand high endocrine titres. In both vertebrates and invertebrates with signals of agonistic ability, there is evidence that the hormone which controls agonistic behaviour correlates with signal elaboration (Gonzalez *et al.* 2001; Tibbetts *et al.* in press). The second prediction has not been previously tested.

In this study, we will test how JH influences survival among nest-founding queens and workers of the paper wasp, *Polistes dominulus*. In addition, we test how individual factors such as body size and advertised quality influence the survival costs of JH. No previous studies have examined whether the physiological costs associated with high endocrine titres are influenced by advertised quality. Paper wasps have variable facial patterns that function as conventional signals of agonistic ability (Tibbetts & Dale 2004) whose accuracy is maintained via social costs (Tibbetts & Dale 2004; Tibbetts 2008; Tibbetts & Izzo in press). Wasps with more wavy or 'broken' black spots on their face are more dominant than those with less broken facial patterns (Tibbetts & Dale 2004; Tibbetts & Shorter 2009; Zanette & Field 2009) and are avoided by rivals (Tibbetts & Lindsay 2008; Tibbetts *et al.* 2010). As a result, advertised quality can be quickly assessed by quantifying each wasp's signal phenotype.

Previous work on JH in paper wasps has identified a number of behavioural and physiological effects associated with JH. In particular, high JH titres are associated with increased dominance and reproductive success among nest-founding wasp queens (Röseler 1991; Tibbetts & Izzo 2009; Tibbetts *et al.* in press). In workers, JH accelerates the transition from nest work to foraging as workers age (Giray *et al.* 2005; Shorter &

Tibbetts 2009). Juvenile hormone is also associated with reproductive dominance among some *Polistes* workers (Tibbetts & Huang in press). Are there costs associated with high JH titres that trade-off with these substantial benefits? Here, we test whether JH imposes survival costs in *Polistes* workers and foundresses. In addition, we will measure how food availability, individual quality and weight influences the effect of JH on survival.

2. MATERIAL AND METHODS

Nest-founding queens (foundresses) were collected from the wild and housed in individual containers until experimental treatment commenced in early July. Workers were collected from wild nests in mid to late July and housed individually until treatment commenced in late July. All workers came from nests where queens were marked prior to worker emergence to ensure that queens were not included in the worker sample. Although *Polistes* lack discrete castes, individuals that emerge in July are considered workers, while future reproductives emerge in mid to late August (Reeve 1991). Prior to treatment, individuals were weighed on a scale accurate to 1 mg.

We tested the role of food availability and hormone treatment in individual survival. The same experiment was performed independently in foundresses and workers because of the differences between the castes (O'Donnell 1998; Tibbetts 2006; Toth *et al.* 2009). For the feeding treatment, half the wasps were given unlimited sugar and water, while the other half were given water alone. Previous work has shown that a diet of water and sugar is sufficient for foundresses to survive the entire season in the laboratory (E. A. Tibbetts 2008, unpublished data). Wasps were also given one of three hormone treatments: 2 µl acetone (control), 5 µg methoprene in 1 µl acetone, or 10 µg methoprene in 2 µl acetone. This yielded a total of six treatment groups with approximately 25 wasps per group. Around 205 foundresses and 196 workers were included in the final analysis. Within the hymenoptera, methoprene has behavioural and physiological effects similar to those of JH (Robinson & Vargo 1997; Giray *et al.* 2005). Further, methoprene acts in ways similar to JH at the cellular level (Shemshedini & Wilson 1990; Ashok *et al.* 1998; Parthasarathy & Palli 2009). Previous studies on similarly sized hymenoptera have used doses of 25 to 250 µg methoprene per individual (Robinson & Vargo 1997; Giray *et al.* 2005; Shorter & Tibbetts 2009). Some previous work has focused on the short-term effects of a single hormone dose (i.e. Röseler 1991). This study was designed to test the costs associated with persistently high endocrine titres, so hormone treatments were performed three times a week for the entire experiment. Wasps were checked for survival daily during the first 20 days of the experiment and three times a week thereafter. Treatment and survival monitoring continued until 10 of the original wasps remained alive. In the foundress experiment, this occurred at 85 days. In the worker experiment, it occurred at 74 days.

After death, each wasp's face was photographed for facial pattern analysis. The signal phenotype of each individual was assessed by analysing a digital picture of the wasp's face with ADOBE PHOTOSHOP. 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. First, the area of the clypeus containing the population-wide badge variability was converted into a 30 × 60 pixel bitmap.

Figure 1. Mean ($\pm$ s.e.) survival of foundresses and workers treated with acetone (control), 5, and 10 μ g of the JH analogue methoprene. Fed individuals were given ad libitum food, while unfed foundresses were given no food (black bar, control; light grey bar, 5 μ g JH; dark grey bar, 10 μ g JH).

Then, the number of black pixels within each vertical column along the horizontal length of the clypeus was counted. The important parameter is total disruption of the black facial pattern, so the standard deviation of the black pigment deposition from pixels 5 to 55 along the horizontal gradient of the clypeus was calculated. The first and last five pixels were excluded 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 colouration and is the signalling aspect of the wasps' facial patterns (Tibbetts & Dale 2004; Tibbetts 2010; Tibbetts *et al.* 2010).

Analyses were performed in SPSS v. 17. Survival data were log-transformed for normality before analyses. The characteristics of foundresses and workers were compared using *t*-tests. Then, separate analyses were performed for each caste. Foundress analyses were performed using general linear models. Worker analyses were performed using linear mixed models that included nest as a random effect to control for possible similarity within nests. Linear mixed models are designed to analyse data that contain non-independent observations, as is the case when multiple individuals from the same nest are sampled. The first analyses within each caste tested how survival was influenced by feeding treatment (sugar or no sugar), JH treatment (control, 5 or 10 μ g), and their interaction. As feeding had a dramatic effect on survival, the data were split by feeding treatment to test how survival was influenced by JH treatment (control, 5 or 10 μ g), weight, facial pattern brokenness, the interaction between JH treatment and weight, and the interaction between JH treatment and facial pattern brokenness. Non-significant interactions were removed from all final models, though the interaction data are reported below.

3. RESULTS

Results from the foundress and worker survival experiments were analysed separately because of the differences between castes. Overall, foundresses survived

longer ($t_{354} = 3.3$, $p < 0.001$), were larger ($t_{354} = 14.6$, $p < 0.001$), and had facial patterns advertising higher quality ($t_{354} = 4.1$, $p < 0.001$) than workers.

(a) Foundresses

Foundress survival was influenced by feeding treatment ($F_{1,154} = 90.4$, $p < 0.001$; figure 1), JH treatment ($F_{2,154} = 9.8$, $p < 0.001$), and their interaction ($F_{2,154} = 3.7$, $p = 0.026$). Least significant difference (LSD) post hoc analysis illustrates that there was a significant difference in the survival of control individuals and those treated with 5 μ g hormone ($p = 0.015$) and 10 μ g hormone ($p < 0.001$), as well as individuals treated with 5 and 10 μ g hormone ($p = 0.035$). The significant interaction between JH treatment and feeding treatment probably stems from a greater reduction in survival among fed foundresses (control versus 5 μ g JH 45%, 5 versus 10 μ g JH 17%) than among unfed foundresses (control versus 5 μ g JH 36%, 5 versus 10 μ g JH 21%).

As feeding had a strong effect on foundress survival, the data were split to separately analyse the individual factors that influenced survival within fed and unfed foundresses. Among unfed foundresses, survival was influenced by JH treatment ($F_{2,77} = 3.8$, $p = 0.026$) and the interaction between JH treatment and facial pattern brokenness ($F_{2,77} = 3.9$, $p = 0.025$). Weight ($F_{1,77} = 0.94$, $p = 0.34$), facial pattern brokenness ($F_{1,77} = 0.5$, $p = 0.48$), and the interaction between JH treatment and weight ($F_{2,75} = 1.7$, $p = 0.18$) did not predict survival. The significant interaction between facial pattern brokenness and JH treatment probably occurred because there is a significant positive relationship between signal elaboration and survival among individuals treated with JH (figure 2; facial pattern brokenness $F_{1,55} = 7.8$, $p = 0.007$; JH dose (5 or 10 μ g) $F_{1,55} = 0.78$, $p = 0.38$; facial pattern by JH dose interaction $F_{1,54} = 0.37$, $p = 0.54$). Within individuals treated with JH, those advertising high quality survived longer than those advertising low quality (figure 2). However, there is no significant relationship between facial pattern and survival within foundresses treated with acetone ($F = 2.9$, $p = 0.10$).

Figure 2. Relationship between advertised quality and survival among unfed foundresses treated with 5 µg (filled circles, solid line) and 10 µg (triangles, dotted line) of the JH analogue methoprene.

Among fed foundresses, JH treatment significantly predicted survival ($F_{2,71} = 11.2$, $p < 0.001$), while weight ($F_{1,71} = 1.6$, $p = 0.21$), facial pattern brokenness ($F_{1,71} = 0.09$, $p = 0.77$), the interaction between JH treatment and weight ($F_{2,67} = 0.22$, $p = 0.80$), and the interaction between JH treatment and facial pattern brokenness ($F_{2,67} = 0.05$, $p = 0.95$) did not predict survival.

(b) Workers

Worker survival was influenced by feeding treatment ($F_{1,136} = 192$, $p < 0.001$; figure 1), JH treatment ($F_{2,136} = 5.4$, $p = 0.006$) the interaction between JH treatment and feeding treatment ($F_{2,136} = 5.5$, $p = 0.005$). LSD post hoc analysis illustrates that there was a significant difference in the survival of control individuals and those treated with 5 µg hormone ($p = 0.003$) and 10 µg hormone ($p = 0.009$), but not individuals treated with 5 and 10 µg hormone ($p = 0.73$). The significant interaction between JH treatment and feeding treatment probably occurred because JH treatment did not influence survival among unfed workers (figure 1), while it significantly reduced survival of fed workers (control versus 5 µg JH 49%, 5 versus 10 µg JH 17%).

As feeding had a strong effect on worker survival, the data were split to separately analyse the individual factors that influenced survival of fed and unfed workers. Among unfed workers, survival was influenced by individual weight ($F_{1,61} = 11.2$, $p = 0.001$), but not JH treatment ($F_{2,57} = 0.16$, $p = 0.85$), facial pattern brokenness ($F_{1,50} = 1.0$, $p = 0.32$), the interaction between JH treatment and facial pattern brokenness ($F_{2,56} = 1.8$, $p = 0.17$), or the interaction between JH treatment and weight ($F_{2,62} = 0.74$, $p = 0.48$). Because advertised quality was correlated with survival among unfed foundresses treated with JH, the relationship between advertised quality and survival was also analysed within unfed workers. Among workers treated with JH, survival was positively associated with advertised quality ($F_{1,46} = 5.7$,

$p = 0.03$), but not JH dose (5 or 10 µg JH; $F_{1,46} = 0.1$, $p = 0.76$) or the interaction between advertised quality and the JH dose ($F_{1,45} = 0.02$, $p = 0.88$). Among unfed workers treated with acetone, survival was not associated with facial pattern elaboration ($F_{1,1} = 2.4$, $p = 0.47$). Therefore, the relationship between facial pattern elaboration and survival is similar among unfed workers and foundresses.

Among fed workers, survival was influenced by JH treatment ($F_{2,51} = 9.1$, $p < 0.001$), but not weight ($F_{1,33} = 1.2$, $p = 0.28$), facial pattern brokenness ($F_{1,63} = 0.1$, $p = 0.75$), the interaction between JH treatment and weight ($F_{2,55} = 0.56$, $p = 0.57$), or the interaction between JH treatment and facial pattern brokenness ($F_{2,55} = 0.82$, $p = 0.44$).

4. DISCUSSION

Overall, JH reduced survival of both queens and workers. The survival cost of JH was not the same across all individuals, but varied with food availability, caste and advertised quality. JH had a strong, dose-dependent effect on survival of unfed queens and a weaker effect on the survival of fed queens (figure 1). Among workers, JH reduced survival of fed, but not unfed workers (figure 1). Unfed workers died quickly regardless of treatment and body size was the best predictor of survival. The effect of JH on survival was also influenced by advertised quality. The survival of unfed foundresses treated with JH was associated with advertised quality; individuals advertising high quality survived longer than those advertising low quality (figure 2). Post hoc analysis showed that there was a similar relationship between advertised quality and survival among unfed workers treated with JH, though it was weaker than the relationship in foundresses.

The results of this study provide compelling evidence that JH imposes substantial, dose-dependent survival costs. One other experiment examined the effect of JH on individual survival, finding that JH reduces the lifespan of unfed burying beetles (Trumbo & Robinson 2004). Other work also suggests that JH is associated with survival in invertebrates, as suppression of JH synthesis extends longevity of monarch butterflies, honeybees and *Drosophila* (Herman & Tatar 2001; Tatar *et al.* 2001; Flatt & Kawecki 2007). Although previous studies have not examined whether the effect of JH on survival is dose-dependent, there is consistent evidence that JH plays a role in the longevity of invertebrates. The mechanism underlying JH's effect on survival is not clear, though there are a number of possibilities, including decreased disease resistance, increased metabolism or accelerated ageing (Flatt *et al.* 2008; Heinze & Schrempf 2008; Toivonen & Partridge 2009).

Much more extensive research has been performed on the effect of T on vertebrate survival. T imposes survival costs in a range of vertebrates (Marler & Moore 1988; Duffy 1989; Nolan *et al.* 1992; Reed *et al.* 2006; Mills *et al.* 2009), though some studies failed to find an effect of T on survival (Brown *et al.* 2005). Overall, the actions of JH and T show remarkably similarities. For example, both hormones are associated with benefits such as increased reproduction and success during agonistic competition, as well as costs such as reduced survival and immune response (Nijhout 1994; Wingfield *et al.* 2001;

Adkins-Regan 2005). These endocrine systems have evolved independently, have different structures, and work in different physiological backgrounds, yet they mediate similar life-history trade-offs. The similarities in hormonal actions across the widely divergent endocrine systems suggest that there has been convergent evolution of endocrine systems. Therefore, the role of hormones in life-history trade-offs may be an adaptive way to optimize resource allocation rather than a constraint that is specific to a particular hormone.

Interestingly, the effect on JH on survival was not uniform, rather it varied with individual characteristics such as caste. The difference in JH's actions across queens and workers is particularly interesting given the lack of discrete castes in paper wasps. Queens and workers are both capable of reproducing and caste cannot be assessed via morphological characteristics (O'Donnell 1998). Although the morphological characteristics of queens and workers overlap, there are differences between the average queen and the average worker. Workers are smaller, have facial patterns indicating lower quality, and have lower fat content than foundresses (this study; O'Donnell 1998; Tibbetts 2006; Toth *et al.* 2009). The differences between queens and workers result in different survival responses. In particular, workers are less able to withstand periods without food, probably because of their smaller size and reduced condition. As a result, the effect of starvation on worker survival swamps the effect of JH on survival. Queens must withstand long periods without food during diapause and nest foundation (Reeve 1991), so they are in sufficiently good condition to withstand starvation. As a result, JH has a significant effect on the survival of both fed and unfed foundresses.

Advertised quality is another factor that had a dramatic effect on individual response to JH treatment. Individuals that advertise high quality were better able to withstand the costs of high JH than individuals that advertise low quality (figure 2). In most signalling systems, signalled or advertised quality is imperfectly correlated with an individual's true quality (Bradbury & Vehrencamp 2000). However, relationships like the one uncovered in this study indicate that advertised quality provides generally reliable information about the signaler's true abilities. The relationship between signal elaboration and ability to withstand JH may occur, in part, because facial patterns are associated with larval feeding and adult condition (Tibbetts & Curtis 2007; Tibbetts 2010). As a result, individuals with elaborate signals are in sufficiently good condition to withstand the costs of JH without food. Interestingly, facial pattern elaboration is not correlated with survival among JH-treated individuals given ad libitum food, suggesting that condition is an important mediator of the survival benefit associated with facial pattern elaboration. Food restriction is common in the wild, so the survival benefit associated with advertised quality is likely to be an important factor associated with survival in the wild.

The relationship between signal elaboration and ability to withstand high JH titres may have important implications for the evolutionary stability of ornaments. Models of honest signalling predict that the marginal net benefit of signal elaboration will vary with advertised quality; high-quality individuals will experience a larger

benefit to cost ratio for a given level of signal elaboration (Zahavi 1975; Getty 2006). One way for this to occur is if high-quality individuals suffer lower costs for a given level of signal elaboration than low-quality individuals. Few previous studies have tested whether variation in physiological costs mediates the evolutionary stability of animal ornaments.

This study shows that the cost of high endocrine titres varies with individual quality, suggesting that differential physiological costs could play a role in the evolutionary stability of animal ornaments. However, a relationship between signal elaboration and ability to withstand high hormone titres will only provide a honesty-ensuring cost of ornament elaboration if there is a stable link between ornament elaboration and hormone titres that is prohibitively costly to break. A relationship between signal elaboration and endocrine titres could occur for many reasons (Duckworth *et al.* 2004). However, costs associated with high endocrine titres will only play a role in signal honesty in taxa where this relationship is stable over evolutionary time. After all, an individual that decoupled signal elaboration from endocrine titres would receive the benefits of signalling high quality without suffering the costs of high endocrine titres. Therefore, some mechanism must maintain the link between endocrine titre and ornament elaboration for differential physiological costs to play a role in the honesty of animal ornaments. Two potential mechanisms could maintain this relationship over evolutionary time. First, social costs may maintain the link between ornament elaboration and hormone titres. Second, there may be physiological constraints that link signal elaboration with endocrine titres such that the signal provides an index of endocrine titres. Thus far, there is more support for social costs than physiological constraints.

In ornaments with social costs, signal elaboration must be linked with titres of the hormone that mediates agonistic behaviour because individuals need to back up their signal with the appropriate level of agonistic behaviour (Gonzalez *et al.* 2001; Tibbetts *et al.* *in press*). Individuals who cannot back up their signal with agonistic behaviour experience costly social interactions (Senar 2006). For example, signal elaboration and endocrine titres are typically correlated in paper wasps (Tibbetts *et al.* *in press*). When signal elaboration and JH titre are decoupled, wasps have difficulty establishing stable dominance relationships and receive increased aggression from conspecifics (Tibbetts & Dale 2004; Tibbetts & Izzo *in press*). This increase in aggressive conflict is often considered sufficient to maintain ornament accuracy over evolutionary time (Senar 2006). However, the results of this study suggest that social costs may have another important role: maintaining the link between signal elaboration and endocrine titre. With this type of stable link, the physiological costs required to withstand high endocrine titres would provide an additional cost of ornament elaboration. Individuals that signal high quality will suffer lower costs for having a high JH titre than individuals signalling low quality. As a result, the cost of backing up a signal with agonistic behaviour will vary with signal elaboration. Therefore, physiological costs could provide an important complement to behavioural costs in maintaining the evolutionary stability of animal ornaments. Future research in additional taxa

will be important to assess whether elaboration of social cost ornaments is commonly correlated with an individual's ability to withstand high endocrine titres. If so, physiological costs may offer a widespread complement to behavioural costs in maintaining ornament accuracy over evolutionary time.

The second mechanism that could maintain the required relationship between ornament elaboration and endocrine titres is if there is some mechanistic link between signal elaboration and endocrine titre (i.e. a physiological constraint). At first glance, physiological constraints seem to provide a viable mechanism for maintaining the correlation between JH and signal elaboration in wasps. After all, signal elaboration in *P. dominulus* is mediated by JH. Individuals with high JH during the late pupal stage develop facial patterns that advertise higher quality than individuals with low JH during the late pupal stage (Tibbetts *in press*). However, signal development occurs over a few days and there is no clear mechanism that would maintain the link between signal elaboration and JH-titre after signal production. Unfortunately, it is difficult to test the critical prediction of the physiological constraint hypothesis: that decoupling ornament elaboration and endocrine titre is prohibitively difficult or costly owing to physiological constraints. Although it is difficult to rule out the possibility of a physiological constraint that ties signal elaboration to JH titre throughout a wasp's life, such a constraint may not be evolutionarily stable. Individuals who 'cheat' by decoupling signal and endocrine titre would receive the benefits of the signal without suffering the physiological costs of high endocrine titres. In general, there is more support for the social cost hypothesis than the physiological constraint hypothesis, though future research that assess both hypotheses in additional taxa will be important.

Overall, JH reduced the survival of adult paper wasps in a dose-dependent manner. Factors such as food availability, caste and advertised quality influence the extent of this survival cost. The relationship between advertised quality and ability to withstand high JH titres is particularly interesting, as it suggests that differential physiological costs could play a role in the evolutionary stability of ornaments with social costs. Additional empirical work on a range of taxa will be important to assess how the costs of high endocrine titres vary across individuals and environments.

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