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Juvenile hormone influences precontest assessment behaviour in *Polistes dominulus* paper wasps

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Theory on animal contests often assumes that individuals can rapidly and accurately assess their own resource-holding potential (RHP). In empirical work, individuals behave as if they 'know' their own RHP, but little is known about how such assessment occurs. Here, we test how juvenile hormone (JH) influences precontest behaviour in *P. dominulus* paper wasps. Previous work has shown that *P. dominulus* have variable black facial patterns that are a signal of agonistic ability and that JH influences fighting ability. In this experiment, we tested how JH influences rival challenge behaviour in the absence of direct social competition. Wasps were given the opportunity to challenge or avoid a model wasp guarding a patch of food. Individuals treated with the JH analogue methoprene were more likely to challenge rivals than were control individuals, and they also had a shorter latency to challenge than controls. However, hormone treatment was only one of multiple factors that influenced behaviour, suggesting that assessment prior to contests is based on the interplay between individual physiology and social context. Contest decisions were also dependent on a wasp's own facial pattern and mass, as well as the facial pattern of its rival. Interestingly, these complex assessment decisions occurred in the absence of direct social cues that could be acquired during a contest. These results are consistent with JH playing a role in self-assessment of RHP. Hormones such as JH are influenced by the physical and social environment, so they provide diverse information about relative RHP and may be commonly involved in assessment behaviour.

Aggressive competition is costly, so many animals use assessment mechanisms to minimize the costs of conflict. The most common form of assessment is self-assessment, wherein animals make contest decisions based on their resource-holding potential (RHP) alone (Arnott & Elwood 2009). Mutual assessment is a more sophisticated type of assessment where individuals assess their own RHP relative to that of their opponent. Mutual assessment allows individuals with lower agonistic abilities than their rivals to rapidly terminate contests before investing in fights they are likely to lose. Mutual assessment is less common than simpler forms of assessment (Taylor & Elwood 2003). Some studies suggest that mutual assessment occurs across a range of taxa, including solitary wasps, fiddler crabs, cichlids and social paper wasps (reviewed in Arnott & Elwood 2009), while others suggest that true mutual assessment may be quite uncommon (reviewed in Elwood & Arnott 2012).

Most work on assessment mechanisms has focused on how individuals assess the RHP of their rival. Many species use body size as a cue of agonistic ability (Arnott & Elwood 2009). Other species have specially evolved signals of agonistic ability. For example, shell rapping in hermit crabs conveys information about an attacker's RHP and influences the defender's contest behaviour (Briffa & Elwood 2002). *Polistes dominulus* paper wasps have black facial patterns that signal agonistic ability. Individuals are less likely to challenge rivals with highly broken or wavy black facial spots than they are individuals with less broken facial spots (Tibbetts & Lindsay 2008; Tibbetts et al. 2010).

Less attention has focused on how an individual knows its own RHP, although self-assessment is essential to most theoretical and empirical work on animal contests. Self-assessment that occurs during contests is relatively easy to understand. Individuals compete until they reach a cost threshold. Individuals with high RHP have a higher cost threshold, so they are more persistent and more likely to win contests than individuals with low RHP (Mesterton-Gibbons et al. 1996). However, some species with mutual assessment compare their own RHP to that of their rival prior to engaging in a contest (Briffa 2008; Elias et al. 2008; Arnott & Elwood 2009). Assessing relative RHP prior to a contest is not a

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trivial challenge (Elwood & Arnott 2012). For example, in *P. dominulus* paper wasps, the decision to challenge or not to challenge a rival is dependent on the interaction between a wasp's own facial patterns and the facial patterns of its rival (Tibbetts et al. 2010). Wasps can easily observe their rival's facial pattern to gain information about rival agonistic ability. Wasps behave as if they 'know' their own facial pattern, but it is not clear how they acquire information about their agonistic ability without social information acquired during a fight.

Many different mechanisms may underlie assessment behaviour. Some mechanisms are likely to be cognitively complex, while others may be quite simple (le Roux & Bergman 2012). For example, competitive investment that is limited via a cost threshold is relatively simple and fits the commonly accepted criteria for self-assessment (Mesterton-Gibbons et al. 1996). On the other hand, some think it is problematic to use terms like self-assessment and mutual assessment without fully understanding the cognitive mechanisms underlying the behaviours. They argue that self-assessment can be considered an internal state that is difficult for a researcher to identify via outward behaviour alone. Here, we define assessment using outward behaviour without addressing the potential cognitive implications of assessment, as is common in the literature (e.g. Rudin & Briffa 2011). One goal of the current study was to identify simple, physiological mechanisms that may mediate apparently complex assessment behaviour.

Physiological information is one mechanism that individuals could use to rapidly assess their own RHP. Hormones like testosterone (T) and juvenile hormone (JH) are often associated with agonistic behaviour (Nijhout 1994; Adkins-Regan 2005). During contests among unfamiliar rivals, individuals with high T or JH titres are more likely to win fights than individuals with lower hormone titres (Drews 1993; Kou et al. 2009; Tibbetts et al. 2011a). Furthermore, JH and T influence aggression independent of dominance rank (Wingfield et al. 1987; Pearce et al. 2001). For example, in burying beetles, treatment with a JH analogue increases the probability of injuries from aggression, but not the probability of winning a fight (Scott 2006a). JH and T titres also vary with the physical and social environment (Pérez-Rodríguez et al. 2006; Trumbo 2007; Oliveira 2009; Tibbetts & Huang 2010), so they have the potential to integrate diverse information about an individual's abilities. Although we know that endocrine titres influence agonistic behaviour, no previous work has tested whether endocrine titres alone influence self-assessment prior to contests.

Here, we examine how an experimental increase in juvenile hormone influences behaviour prior to aggressive contests in *P. dominulus* paper wasps. *Polistes dominulus* use mutual rival assessment (Tibbetts et al. 2010). Facial patterns are associated with an individual's probability of being challenged, as well as the probability that they will challenge a rival. Previous work in *P. dominulus* has shown that JH is associated with an individual's ability to win a fight with a rival (Röseler et al. 1980; Röseler 1991; Tibbetts & Izzo 2009; Tibbetts et al. 2011a). We tested whether JH influences the process of mutual assessment prior to contests in the absence of direct competition and, if so, whether this effect swamps other factors or is one of many factors influencing assessment behaviour.

METHODS

We collected single foundress *P. dominulus* foundresses from their nests around Ann Arbor, Michigan, U.S.A., soon after nest foundation. At collection, each wasp was weighed on a scale accurate to 0.001 g and photographed for facial pattern analysis. Focal wasps were chosen randomly, so they reflected the wide range of weights and facial patterns found in the wild. Focal wasps weighed between 0.050 g and 0.15 g. We assessed the facial pattern of each individual 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 (Tibbetts 2010; Tibbetts et al. 2010, 2011a). First, we converted the area of the clypeus containing the population-wide badge variability into a 30×60 pixel bitmap. Then, we counted the number of pixels within each vertical column along the horizontal length of the clypeus. 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 5 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 and acts as a signal of fighting ability. Facial pattern brokenness of wasps in the present study was between 0 and 10.2 standard deviations.

Prior to the choice trials, focal wasps were treated three times per week for 2 weeks. Half the individuals were treated with 5 ug of methoprene in 1 ul of acetone, while the other half was treated with 1 ul of acetone alone as a control. Trials were performed 1 day after the last methoprene treatment. Methoprene is a JH analogue with behavioural and physiological effects similar to those of JH (O'Donnell & Jeanne 1993; Robinson & Vargo 1997; Giray et al. 2005). Furthermore, methoprene acts in ways similar to JH at the cellular level (Shemshedini & Wilson 1990; Parthasarathy & Palli 2009). The timing and amount of hormone treatment was chosen because it parallels previous work testing the effect of methoprene on dominance and reproduction in *P. dominulus* foundresses (Tibbetts & Izzo 2009; Tibbetts et al. 2011b). During treatment, wasps were housed in isolation to reduce the potential effect of direct social cues on self-assessment.

Each focal wasp was given the opportunity to challenge one model wasp for access to a patch of sugar. Trials were performed in a triangle-shaped arena (7 cm wide $\times$ 6 cm long). At the narrow end of the arena, there was a covered antechamber. At the opposite end of the arena, there was one sugar cube with a with a model wasp positioned on top of the sugar 'guarding' the sugar. Wasps eat nectar in the wild and are fed rock sugar in the laboratory. In the wild, food sharing among wasps occurs on and off nests. Guards and focal wasps were collected from sites at least 4 km apart to ensure they had not previously interacted. Guards were all freshly freeze-killed wasps. Guards originally had similar facial patterns to each other, but we altered facial patterns so that some guards had two spots on their face (2-spot guards), indicating high quality, while other guards had no spots on their face (0-spot guards), indicating low quality. This manipulation ensured that differences in the way that focal wasps treated 0-spot and 2-spot guards were caused by guard facial patterns rather than any correlates of facial patterns. Guards were of similar size (0.098–0.105 mg).

Focal wasps were placed in the closed antechamber for 5 min before they were released into the trial arena. Each trial lasted 20 min. Trials were videotaped for subsequent analysis by an observer blind to treatment and experimental predictions. The observer recorded whether the focal wasp challenged or avoided the guard as well as the focal wasp's latency to challenge the guard (time from release until challenge). A wasp was considered to challenge the guard if it ate the sugar or walked on the guard. Wasps that avoided physical contact with the guard were scored as 'no challenge'.

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