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Endocrine mediated phenotypic plasticity: Condition-dependent effects of juvenile hormone on dominance and fertility of wasp queens

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A B S T R A C T

There has been increasing interest in the mechanisms that mediate behavioral and physiological plasticity across individuals with similar genotypes. Some of the most dramatic plasticity is found within and between social insect castes. For example, *Polistes* wasp queens can nest alone, dominate a group of cooperative queens, or act as worker-like subordinates who rarely reproduce. Previous work suggests that condition-dependent endocrine responses may play a role in plasticity between castes in the hymenoptera. Here, we test whether condition-dependent endocrine responses influence plasticity within castes in the wasp *Polistes dominulus*. We experimentally manipulate juvenile hormone (JH) titers in nest-founding queens and assess whether JH mediates variation in behavior and physiology. JH generally increased dominance and fertility of queens, but JH's effects were not uniform across individuals. JH had a stronger effect on the dominance and fertility of large individuals and individuals with facial patterns advertising high quality than on the dominance and fertility of small individuals and those advertising low quality. These results demonstrate that JH has condition-dependent effects. As such, they clarify how JH can mediate different behaviors in well nourished queens and poorly nourished workers. Many *Polistes* queens nest cooperatively with other queens, so condition-dependent hormonal responses provide a mechanism for queens to adaptively allocate energy based on their probability of successfully becoming the dominant queen. Research on the endocrine basis of plasticity often focuses on variation in endocrine titers alone. However, differential endocrine responses are likely to be a widespread mechanism mediating behavioral and physiological plasticity.

Introduction

A single genotype can produce organisms with widely divergent phenotypes, depending on their developmental environment. The ability of a genotype to produce a range of morphologies, physiologies, and/or behaviors across different environments has been termed “developmental plasticity.” Plasticity has been attracting increasing attention as a key source of variation within populations (West-Eberhard, 2003; Pigliucci, 2005). Understanding the regulation of plasticity is challenging because environmental effects must trigger divergent developmental pathways that are present within the same genotype (Flatt et al., 2005; Zera et al., 2007). As a result, researchers are just starting to understand the developmental mechanisms that underlie plasticity and how selection shapes these mechanisms (Dufty et al., 2002).

Hormones are one of the key mechanisms mediating plasticity, as they coordinate suites of traits and synchronize an organism's internal environment with the external environment. A hormone can coordinate complex, multi-faceted phenotypes through its pleiotropic effects on multiple traits (Adkins-Regan, 2005). For example, juvenile hormones plays an important role in mediating morphological and physiological polymorphisms in horned beetles (Emlen and Nijhout, 2000; Moczek, 2009) and locusts (Zera and Zhao, 2004). Endocrine regulation is also a key mediator of behavioral and physiological responses to environmental variation. For example, testosterone (T) titers vary seasonally in many temperate bird species. As T-titers change, they influence a cascade of behavioral and physiological traits including territoriality, brain development, moult, and singing behavior, all of which prepare a bird for the breeding season (Adkins-Regan, 2005).

Although much phenotypic variation can be attributed to endocrine variation, relatively little is known about the role of inter-individual endocrine variation in more subtle behavioral and physiological plasticity. Understanding the basis of subtle individual variation is more difficult than broadly context-dependent effects (Kempenaers et al., 2008; Williams, 2008). One strategy for investigating individual variation is to assess: (1) whether there are factors that consistently predict variation in endocrine systems and (2) how the variation influences individual phenotypes. For example, do individuals in good physical condition have different hormone titers than individuals in poor condition? How do these hormonal differences influence behavior and physiology? Although researchers are just beginning to tackle individual variation in endocrine systems,

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there is growing appreciation for the importance of individual variation for understanding plasticity and the evolution of complex traits (Kempenaers et al., 2008; Williams, 2008).

One of the challenges associated with studying the endocrine basis of plasticity is that phenotypic variation may be caused by differences in hormone titers or in responses to hormone titers. Measuring variation in hormone titers is an important first step toward understanding plasticity (Zera, 2007). However the same amount of hormone can have very different effects on different individuals based on inter-individual variation in hormonal receptors or binding proteins. Relatively little is known about variation in receptors and binding proteins due, in part, to the methodological challenges associated with studying these parameters in natural circumstances (Adkins-Regan, 2005; Ball and Balthazart, 2008). A method that has potential for understanding individual variation in hormone responsiveness is to give a similar amount of a hormone to numerous individuals and test what individual characteristics are associated with differences in hormonal response.

Social insects provide good models for studying the mechanisms underlying developmental plasticity because they have some of the most striking examples of plasticity. Within a single colony, there are dramatic morphological, physiological, and behavioral differences among individuals. The caste difference between queens and workers is well known and there has been extensive research on the endocrine regulation of queen/worker plasticity (Wheeler, 1986; Emlen and Nijhout, 2000; West-Eberhard, 2003). However, there can also be dramatic variation within a single caste. For example, nest founding queens (foundresses) in the *Polistes* paper wasps can nest alone, dominate a group of cooperative queens, or act as worker-like subordinate queens who rarely reproduce (West-Eberhard, 1969; Nonacs and Reeve, 1995). Much less progress has been made in understanding the basis of within-caste plasticity. Are there consistent differences in endocrine function among foundresses that can be used to predict individual physiology or behavior?

Here, we explore how juvenile hormone influences nest founding queens (foundresses) of the paper wasp *Polistes dominulus*. Juvenile hormone (JH) is a versatile hormone that influences multiple aspects of insect behavior and physiology including metamorphosis, diapause, dominance, and caste-development (Nijhout, 1994). JH can function as a gonadotropin and is associated with fertility (Barth et al., 1975; Robinson and Vargo, 1997). JH is also associated with aggression and dominance in some taxa (Röseler et al., 1985; Trumbo, 2007; Kou et al., 2008). In many social insects, including paper wasps, JH mediates age-related division of labor among workers (Giray et al., 2005; Robinson and Vargo, 1997; Shorter and Tibbetts, 2009).

Here, we test how JH influences dominance and fertility of nest-founding paper wasps. We also assess whether JH has a uniform effect across foundresses or whether the effect of JH varies with foundress quality. *Polistes dominulus* foundresses often start nests with other foundresses and dominance rank within foundress groups is associated with two factors: body size (Turillazzi and Pardi, 1977) and individual facial patterns (Tibbetts and Dale, 2004; Tibbetts and Shorter, 2009; Zanette and Field, 2009). Body size is thought to be a good predictor of fighting ability (Turillazzi and Pardi, 1977; but see Seppa et al., 2002) and *P. dominulus* facial patterns function as condition-dependent signals of agonistic ability (Tibbetts and Curtis, 2007; Tibbetts and Lindsay, 2008). Do size and signal elaboration also predict endocrine response? Variation in endocrine response across individuals of different condition could play an important role in mediating the behavioral and physiological plasticity within foundresses.

Materials and methods

Nest founding *Polistes dominulus* wasps were collected after they emerged from diapause in early May 2008. After collection, each wasp was weighed and housed in an individual container with *ad lib* sugar and water. Wasps were randomly assigned to hormone treatment. Half of the individuals were treated with 5 μg of the JH analog methoprene in 1 μl acetone, while the other half was treated with 1 μl acetone. Within the hymenoptera, methoprene has behavioral and physiological effects similar to those of JH (O'Donnell and Jeanne, 1993; Bloch et al., 1996; Robinson and Vargo, 1997; Giray et al., 2005). Further, methoprene acts in ways similar to JH at the cellular level (Ashok et al., 1998; Shemshedini and Wilson, 1990; Parthasarathy and Palli, 2009). Previous studies on similarly sized hymenoptera have used doses of 25 to 250 μg methoprene per individual; these doses do not have detrimental effects on adults (Robinson and Vargo, 1997; Agrahari and Gadagkar, 2003; Giray et al., 2005; Shorter and Tibbetts, 2009). We chose a conservative, 5 μg dose of methoprene, because individuals were treated multiple times over 2 weeks. In total, wasps were treated with methoprene or acetone three times a week for 2 weeks. 110 foundresses were used in the experiment, 55 methoprene- and 55 acetone-treated individuals.

After 2 weeks, each wasp was paired with a similarly sized rival (within 0.008 g) to battle for dominance in 2 h videotaped bouts. Each pair consisted of one JH-treated wasp and one acetone-treated wasp. Individuals within each pair were collected from sites at least 3 km apart to ensure they had no previous interactions. After the 2 h contest, wasps were sacrificed. Wasp abdomens were stored in ethanol before dissection. The number of mature oocytes (i.e. those greater than 1 mm in length) in each individual were measured. 6 individuals were excluded from egg analyses because of errors in storage or dissection of ovaries.

We assessed the signal phenotype of each individual by analyzing 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 and Dale, 2004; Tibbetts et al., in press). First, the area of the clypeus containing the population-wide badge variability was converted into a 30×60 pixel bitmap. Then, the number of pixels within each vertical column along the horizontal length of the clypeus was counted. 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 5 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 wasps' face measures the amount of disruption in the black coloration and is associated with dominance (Tibbetts and Dale, 2004).

Videos of dominance trials were 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. Dominance in each bout was scored by 2 independent observers who were blind to treatment. 11 dominance trials were excluded from the final analysis because neither individual successfully mounted her rival and 1 trial was excluded because of videotaping errors.

Results

Over all individuals, experimental addition of the juvenile hormone analog methoprene significantly increased the number of mature eggs in an individual's ovaries ($t_{102} = 6.54$, $p < 0.001$).