

**Spotting the top male: sexual selection in a lek-mating paper wasp,
*Polistes dominulus***

By

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For Butters: the best friend anyone could ever have.
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Abstract

Acquiring mates is a problem ubiquitous in the animal kingdom. Males typically face the brunt of the burden by bearing the elaborate weaponry and ornamentation required to compete for and attract females. Despite prolific interest in understanding the dynamics of male-male competition and female choice, many questions remain. In particular, there is little understanding of how sexual selection operates in systems lacking obviously costly ornamentation, or what drives female choice in systems in which males provide no tangible resources. *Polistes* paper wasps serve as a uniquely-suited model organism with which to address these questions: they epitomize intense competition as they have lek-based mating systems; females are genetically monogamous, suggesting strong female choosiness; and females hibernate between mating and producing offspring, making male ejaculate quality an important factor for female fitness. Finally, male paper wasps are unique among lekking organisms in that they do not appear to possess the obviously costly ornamentation typical of lekking males. Despite these interesting facets of paper wasp reproductive biology, it remains poorly studied. Here, I examine multiple aspects of the mating system of *Polistes dominulus* paper wasps, with the following objectives: (1) to identify sexually selected signals, (2) to determine whether identified signals

advertise quality information, and (3) to determine how females benefit from choice in a lekking system. I show that while chemical information is unrelated to reproductive success, male morphology functions as condition dependent sexually selected quality signals: males use dorsal abdominal spots in both inter- and intra-sexual competition, and larval diet influences the development of these spots. Further, female choice for attractive spots results in direct benefits to females, with females whose mates advertise high quality surviving hibernation longer than females whose mates advertise low quality. Overall, these results demonstrate that male ornaments are important mediators of mating dynamics in *Polistes dominulus*. Moreover, they show that females can gain direct benefits in non-economic mating systems, providing an alternative hypothesis to “good genes” models of female choice on leks. This work builds a foundation necessary for paper wasps to become model organisms for studying sexual selection and lekking dynamics.

Chapter I: Introduction

Competition is rampant in nature. Organisms must struggle not only to survive but to reproduce: without continued genetic propagation, a lineage becomes an evolutionary dead-end. Competition for reproductive opportunities is termed sexual selection, and may take two main forms: competition with rivals over access to mates (usually males competing over females; i.e. intra-sexual selection or male-male competition), and competition to be chosen by the opposite sex (usually females choosing males; i.e. inter-sexual selection or female choice) (Darwin 1859; Darwin 1871). This competition can become so intense that the competing sex may evolve structures that help gain mates yet are detrimental to survival. These paradoxical structures and traits, such as the massive antlers of Irish Elk (*Megaloceros giganteus*) are what initially sparked interest in studying sexual selection (Gould 1974).

Over the past twenty years, the field of sexual selection has blossomed into a large and successful research program with great theoretical and empirical advances (Andersson and Simmons 2006). The initial aims of research into sexual selection sought to understand why males have extravagant ornaments, weapons, or displays, what role females play in shaping selection on male traits, and how male-competition and female choice interact to shape male and female fitness. Although understanding has grown tremendously, many questions

remain. For example, the ubiquity of ornament use across taxa is unknown, making it difficult to understand the universality of current sexual selection theory. Further, there is contradiction between theory and observation on the functionality of ornaments that lack obvious production costs: theory argues that such ornaments would function poorly at best as quality indicators, while it appears that organisms are in fact utilizing such traits as quality signals (Torok et al. 2003; Griffith et al. 2006; Tibbetts and Curtis 2007). Finally, why females remain particularly choosy for seemingly arbitrary male ornaments in systems where males provide no tangible resources or parental care is quite perplexing; traditional models of “good genes” benefits do not appear to adequately address this issue (Møller and Alatalo 1999).

At the crux of sexual selection are male-based ornaments. These are traits such as weapons, songs, or other aspects of male morphology that serve to mediate the costs of competition for and attraction of mates. Ornaments serve as honest indicators of male quality, which may range from dominance or agonistic ability, size, condition (such as vigor or health), immunological resistance, and genetic constitution. The precise nature of the information conveyed by quality signals depends on the contexts in which they are used; for example, male rivals may be concerned with the condition or strength of a rival, while a choosy female may be interested in information regarding the genetic constitution, current infective status, or foraging ability of potential mates. The same ornament may be used in one or both contexts of sexual selection.

Much research on male ornamentation has focused on traits with obvious production costs (Zahavi 1975). For example, the elaborate tail plumage of peacocks (*Pavo*) and widowbirds (*Euplectes*) are obviously expensive to produce and maintain, and only males in good condition can survive with these “handicaps.” These costs are necessary to maintain the honesty of the signal, and signals and ornaments that lack these costs pose a problem: what maintains signal honesty or accuracy in these traits? Examples of sexually selected traits that lack production costs are few (but see (Torok et al. 2003; Hegyi et al. 2007; Kemp and Rutowski 2007; McGlothlin et al. 2007), but this may be due in part to publication or research bias. Theory has proposed that other costs may be used in lieu of production costs: namely social or maintenance costs (Szamado 2010), but relatively few examples exist to strengthen these arguments (but see (Tibbetts and Dale 2004). We are left with a self-reinforcing problem of believing these “cost-free” signals (i.e. those lacking production costs) would function poorly to advertise quality, while simultaneously overlooking them in research programs that would aim to test their actual signaling function.

Why individuals should pay attention to ornaments is also an interesting question. In some instances, the ornament may serve an obvious purpose: the large horns or antlers observed in many ungulate species serve as excellent indicators of strength and fighting ability. And in fact, males use these weapons to deter rival males and guard a group of females. However, in other cases, males may use seemingly arbitrary traits in male-male competition. For example, in many birds “badges of status” are melanized patches of plumage that typically

correlate with rank or dominance, and are used to mediate competition within a group (Strassmann 2004). Why a color patch (versus a weapon, for example) should deter rivals is not immediately obvious. Similarly, male ornaments that are used to attract females (i.e. are used in female choice) are relatively straightforward to understand when the ornament provides reliable information regarding traits directly useful to the female. For example, there is little confusion surrounding why female house finches prefer redder males: male redness correlates positively with both male foraging ability and paternal care (Hill 1991). Conversely, it is harder to understand why females of other species have preferences for traits that serve no obvious purpose. For example, why should female waterboatmen (*Micronecta scholtzi*) prefer males with such loud calls? Males of these aquatic insects attract females by producing the loudest calls possible, yet there are no demonstrable benefits for loud calling in males (Sueur et al. 2011). Understanding what information is gained through signal use, when the signals themselves appear somewhat arbitrary in nature, can provide valuable insight into the dynamics of fitness effects for individual species. In some cases, this can lead to new insights and new theory to explain why individuals make the choices they do during sexual selection.

This problem of female choice for arbitrary male traits has been particularly perplexing. Various hypotheses have been proposed to address why so many females across taxa are so choosy, especially in those situations where males do not provide tangible resources or paternal care to females. Some hypotheses rely on probability: runaway selection, which was proposed by Fisher

(Fisher 1915), does not require that females benefit from their choices in mates apart from gaining genes that will enable them to also produce such sexy sons (and daughters that prefer sexy males). In other words, female choice of male traits can initiate through an arbitrary choice that, over time, leads to genetic correlations between the trait and preference for the trait. Other explanations for the evolution of female choosiness focus on the benefits females gain. For example, the “good genes” model of choice states that females receive high quality genes from their males, such that their offspring will also be of high quality (Hamilton and Zuk 1982). In other words, in situations where males do not provide females with resources or paternal care, male ornaments indicate overall male quality, so that female choice selects for superior male genotypes. Unfortunately, it has been extremely difficult to quantify good genes effects (Møller and Alatalo 1999). Further, competing models, such as the genetic compatibility model, argue that females should select mates whose genomes are most compatible, so not all females should select the same males (Tregenza and Wedell 2000). That type of explanation fails to address the incredible consistency of female choice seen in mating systems such as leks. Unfortunately, we are still left without a consensus solution for the problem of explaining why females are so choosy when males do not provide them with resources.

Polistes paper wasps serve as a uniquely-suited model organism with which to address these outstanding questions. Paper wasps have lek-based mating systems, where both male-male competition and female choice play key

roles. Males must compete for rank in dominance hierarchies, and females choose as mates the most attractive males amongst the highest ranking. Therefore, competition is intense and quality signals are expected to be utilized to mediate the costs. Further, females have been shown to primarily use the sperm from single males (i.e. they are genetically monogamous) (Strassmann 2001), and mating occurs prior to over-wintering, so females must store sperm before fertilizing eggs; therefore it is likely that male ejaculate quality is important for female fitness. These lines of evidence further strengthen the role of female choice in the system. Finally, male paper wasps are unique among lekking organisms in that they do not appear to possess the obviously costly ornamentation typical of lekking males. Remarkably, despite these interesting facets of paper wasp reproductive biology and their potential for dramatic sexual selection (Baer 2003), there has been little research on sexual selection in paper wasps.

Here, I examine multiple aspects of the mating system of the lek-mating paper wasp, *Polistes dominulus*, with the following objectives: (1) to identify sexually selected signals, (2) to determine whether identified signals advertise quality information, and (3) to determine how females benefit from choice in a lekking system. The goal of my dissertation is to examine sexual selection in paper wasps by identifying male ornaments, determining a signaling role for and information content of these traits, and ultimately testing for benefits to female choice on male ornamentation. The work presented here consists of a comprehensive approach for examining sexual selection in paper wasps. First, in

chapter 2, because chemical communication is extremely important in social insects, I explored the potential for chemical signaling in males. Second, in chapter 3, I examined a potential visual signal in males, starting with identifying a sexually dimorphic trait that correlated with inter- and intra-sexual selection. Following correlative experiments, I performed manipulative experiments to demonstrate a signaling role of the trait. Third, in chapter 4, I investigated the development of the signal, using a nutritional manipulation experiment to test the condition-dependence of the signal. Condition-dependence functions both to maintain variation in a signal, and enables sexually selected signals to advertise individual quality. Finally, in chapter 5, I tested why females may pay attention to the visual signal by looking for an effect of advertised mate quality on female hibernation success. By linking female survival with preference for male ornamentation, I demonstrated why females should pay attention to male ornamentation.

Chapter II: Male cuticular hydrocarbons do not correlate with mate choice, but do correlate with immune response

Abstract

Cuticular hydrocarbons (CHCs) are utilized by insects for a variety of physiological and communicative purposes. Social insects have capitalized on the vast potential of CHCs as an information source, using them as signals to regulate social interactions. Currently, only female CHC profiles have been explored, and little is known regarding the CHC profiles of males. Here, using *Polistes dominulus* paper wasps, I examine the factors associated with individual variation in male cuticular hydrocarbon profiles to explore their potential function. Male CHC profiles correlate with immune function (i.e. encapsulation) and juvenile hormone levels, and to a lesser extent, agonistic ability. Conversely, CHC profiles do not have any association with mating success. Therefore, social insect males may use CHCs to mediate social interactions such as mediating competition on leks, but CHCs are unlikely to function in mate attraction. Further work is required to test for potential signaling roles of male CHCs, as male chemical profiles are as information-rich as female chemical profiles.

Introduction

Insect communication is dominated by chemical information, with cuticular hydrocarbons (CHCs) comprising a major source of social information (Howard and Blomquist 2005). CHCs are chains of fatty acids covering the exoskeletons of insects that serve a variety of communicative and physiological functions, such as to prevent desiccation, protect the organism from infection, and to help mediate social interactions (Singer 1998; Howard and Blomquist 2005). Across taxa, these chemical compounds are used to communicate kinship, social status, physiological state, species, sex, and in some cases, quality information (Lorenzi et al. 1997; Cuvillier-Hot et al. 2001; Sledge et al. 2001; Ruther et al. 2002; Dapporto et al. 2004; Sledge et al. 2004). For example, male attractiveness as mates is determined by their cuticular pheromone blend in crickets, *Drosophila*, and many beetles (Howard 1993; Howard et al. 2003; Ali and Tallamy 2010).

In social insects in particular, the potential communicative power of cuticular hydrocarbons is vast, as social interactions are frequent and not limited to mating bouts. Social insect communication has been intensely studied, and social insect CHCs have a variety of known signaling roles (Howard 1993; Turillazzi and West-Eberhard 1994). For example, CHCs regulate nest membership by providing information on kinship and nest of origin, and mediate on-nest interactions by communicating rank and reproductive status (Panek et al. 2001; Sledge et al. 2001; Ruther et al. 2002; Sledge et al. 2004). CHCs are also able to provide information regarding the physiological state of the bearer, such as communicating fertility level or infective status (Richard et al. 2008; Izzo et al. 2009). Interestingly, despite the rich literature on social insect CHCs, all of the

known communicative functions of CHCs have only been demonstrated in females (Bruschini et al. 2010). Males remain largely neglected in these studies.

Despite the lack of widespread interest in social insect males, chemical information may be as important in mediating male-male and male-female dynamics as it is for female-female interactions. As CHCs mediate female social interactions and regulate female dominance hierarchies by providing information regarding rank, fertility, and relatedness (Dapporto et al. 2004; Sledge et al. 2004; Izzo et al. 2009), it is likely they serve as important mediators of male social and reproductive interactions as well. For example, males may use CHCs both during the period when they are still on the nest as well as to aid in mating decisions. Chemical information may be important for kin recognition for both sexes to help avoid incestuous matings, to broadcast information regarding male physical state such as overall health or immune function, to attract females for mating, or even to form dominance hierarchies on leks. Clearly, the potential for chemical based communication by males is vast and further research is required.

In particular, it has been suggested that males use chemical information in sexual selection, especially to attract mates. Males have been shown to use CHCs to assess the relatedness and mated status of encountered females (Ryan and Gamboa 1986). Additionally, males have been observed dragging their abdomens across perches in their mating territories; this behavior has been hypothesized to function as territorial scent-marking or to attract mates (Beani and Turillazzi 1988).

Here I examined the cuticular hydrocarbon profiles of male *Polistes dominulus* paper wasps in order to test the hypothesis that males utilize chemical information in sexual selection. If the function of male scent-marking is to attract females as mates, then male CHC profiles should correlate with male mating success. I also tested the relationships between male CHCs and agonistic ability, to determine if male CHCs provide dominance information. Finally, in order to facilitate comparisons of male and female profiles, I measured the relationships between male CHCs and the physiological parameters of immunity (by encapsulation rate) and juvenile hormone level.

Methods

To explore the information conveyed by male cuticular hydrocarbons, we tested the relationships between male CHCs, behavior, and physiology. 200 virgin *Polistes dominulus* males of known age were collected from 41 mature nests (1-20 males/nest). Nests were collected from sites around Ann Arbor, Michigan during July 2009, and brought to the lab. Males that emerged in the lab were collected off the nest and housed individually in deli cups, where they were provided with *ad libitum* water and rock candy sugar. Prior to use in the experiment, each male was weighed and had a cuticular hydrocarbon sample collected.

Chemical Analysis

Prior to use in dominance bouts, each male had a cuticular hydrocarbon sample collected by swabbing for 10 seconds on the thorax with sterile cotton. Cotton swabs were placed in individual screw top glass vials and kept at -20C until further analysis. To prepare samples for GC/MS analysis, each sample was submerged in 500 μ L of pentane for 10 minutes. After 10 minutes, the swab was removed, and the pentane was dried under a nitrogen stream. The sample was re-eluted with 175 μ L of heptane for analysis. 1 μ L of the sample was injected into a Shimadzu GCMS-QP2010, with a 25 meter long DB-1 capillary column installed in a split injector (ratio is 1:100), with a 0.2 mm I.D. and a 0.33 micron film thickness. Each sample was run on the column for 40 minutes in a splitless mode. The temperature of the column was programmed from 80°C to 300°C, at 10° C min⁻¹. The column was held at 80°C for 3 minutes after injection, and the final temperature of 300°C was held for 30 minutes. Integration of peak areas was performed with Shimadzu's Post-Run Analysis software. The GC was coupled to a Trace mass spectrometer, operated at 70 eV. Components were characterized by analysis of their mass spectra. The method is the same as that used to assess female profiles in (Izzo et al. 2009).

Dominance Contests

Unrelated males were randomly paired and placed into 8 cm x 8 cm x 1.5 cm clear plastic arenas. Male pairs were allowed to freely interact for 2-hour video-taped bouts. To assess male behavior, all videos were scored by viewers blind to the purpose of the experiment. A male was determined to have won the bout when he successfully submitted his rival through an uncontested mount. A

successful, uncontested mount occurred when one male (the dominant) rapidly taps on his opponent's head with his antennae; the opponent (the subordinate) crouches, lowers his antennae, and accepts the antennal drumming. Previous work has shown that mounting indicates dominance, and aggression between males is significantly reduced after dominance establishment (Izzo & Tibbetts 2011).

Mating Bouts

Thirty minutes after each dominance contest, a female was added to the arena of each male pair. Male pairs were given access to sugar and water between dominance contests and mating bouts to minimize stress. All wasps were allowed to freely interact for 1-hour videotaped mating bouts. Videos were scored blind the purpose of the experiment and identity of the wasps used, and the number and duration of copulations were recorded for each male. Female preference was scored as the copulation duration of male 1 – the copulation duration of male 2. Copulation occurs when a male links his antennae around the female's antennae, climbs on her back, and they engage their genitals. Females can reject males prior to the mounting behavior (through avoidance, stinging, biting, or darting at him), and can also reject males once mounting has occurred by avoiding genital contact. Successful copulation requires full female participation.

Immunity Assay

Immediately following mating bouts, males were chilled then had a 2 mm monofilament implanted into their abdomen. Filaments were inserted on the

ventral side, between 3rd & 4th abdominal segments. To insert each filament, a hole was made with a needle, then the filament was pushed through with forceps. Males were returned to their individual deli cups for 24 hours and kept in the dark to minimize activity and stress. 24 hours after implantation, males were chilled then dissected to retrieve the filament. This is a common method that measures encapsulation, which is the primary immune response toward multicellular pathogens (Pathak 1993; Doums and Schmid-Hempel 2000; Baer and Schmid-Hempel 2006). During an encapsulation response, haemocytes gather around and attach to the target, melanize, and form a hard case. Higher immune response to a filament will result in a higher proportion of the filament covered in dark yellow – brown globules, whereas a lower immune response will contain overall less melanized area and will appear lighter in color.

Each dissected-out monofilament was photographed under standardized light conditions using a stereo microscope with an infinity digital camera. The encapsulation of the monofilament was analyzed using Adobe Photoshop's color histogram to measure reflectance across the RGB channels. The resulting values are counterintuitive, as lighter colors have higher RGB reflectance than darker colors though lighter colors indicate lower encapsulation. As a result, the values were transformed such that higher values correspond to greater encapsulation while lower values correspond to lower encapsulation.

Juvenile Hormone Analysis

Endogenous JH-titers of 138 males was measured. Hemolymph was collected 24 hours after the behavioral trials were concluded, immediately

preceding the removal of the monofilament. The amount of hemolymph collected from each wasp was measured (range = 12.2 – 3374.14 ng/μl), then the hemolymph was stored in 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 x 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 antiserum (1:14000 dilution, generous gift from David Borst) and 10,000 DPM of [10^{-3}H(N)]-JH (Perkin Elmer, 647.5 Gbq/mmol). The mixture was incubated at room temperature for 2 h, 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 ice-water mixture for 2.5 min, then centrifuged (2000x g for 3 min), and the supernatant, which contained bound JH, was decanted into scintillation vials. Liquid scintillation counting was performed using a Beckman LS 6500. A standard curve with various amounts (0, 3, 10, 30, 100, 300, 1000, 3000 and 10,000 pg) of standard JH (Sigma) was run each day. 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, and 5 unknown parameters, 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). TLC was used to verify that this method accurately assesses hemolymph JH-titer (Tibbetts et al. 2011).

Statistical Analysis

Cuticular profiles were obtained for 174 males, with a total of 76 pairs. 22 Males whose profiles were measured did not belong to a matched pair because the samples were not prepared properly, and could not be analyzed. 54 long-chain branched unbranched alkanes and alkenes were found in the wasps analyzed; compounds that appeared in less than 10% of the wasps were thrown out (Izzo et al. 2009), and the remaining 33 compounds were used in the analysis. Sample reduction in this way is required for proper statistical analysis. Peak areas for each compound were obtained using Shimadzu's post analysis software, and the resulting areas were standardized into percentages of the total peak area for a given profile. The 33 standardized compounds were reduced by principal components analysis. For dominance and female preference data, the difference between peak areas within a pair was obtained before principal components analysis using JMP 8. For this paired (i.e., difference) data, the first 6 components explained 71.754% of total variance (n=74 profile pairs), and for the non-paired CHC data, the first 6 components explained 76.798% of total variance (n=174 profiles). The resulting component scores were analyzed using multivariate statistics and backwards stepwise regression using JMP 8, and all components used in the analyses had eigenvalues >1. Data that was analyzed

for dominance and female preference was analyzed using paired logistic regression in JMP 8. Differences in the data were calculated by subtracting the values of wasp 1 from the values of wasp 2; when wasp 1 was behaviorally dominant (i.e., the winner of the fighting bout) or preferred by a female, the pair was assigned a 1; if wasp 1 was behaviorally subordinate (i.e., the loser of the bout) or not preferred by the female, the pair was assigned a 0. The resulting 1s and 0s were used as the response in the logistic regression model.

Dominance was established in 60 pairs, and females selected mates in 52 pairs. Not all males survived filament implantation, so immunity data was collected on 161 males. A total of 138 males had survived and yielded sufficient hemolymph for analysis of endogenous JH measures. Sample sizes lower than these values represent comparisons in which two or more types of data were not obtained for a particular pair of males or individual. All analyses were run using the maximum sample size possible.

Results

Because dominance and choice data are paired, separate analyses were required to test the relationships between behavior and chemical profile expression.

There is no relationship between mating success and male cuticular hydrocarbon profiles. A logistic regression model run on paired PCA components 1 – 6 and preferred/not preferred (as determined by the difference in male copulation durations, with “preferred” males copulating longer than non-preferred

males) shows that females do not use CHC profiles to assess male attractiveness $P=0.99$, $n=56$.

The relationship between dominance and cuticular hydrocarbon profiles is somewhat unclear. Using the paired PCA factors in a logistic regression model with dominance (i.e. win vs. loss), the full model shows no relationship between dominance and chemical profile $P=0.349$, $n=49$. However, stepwise backward regression shows that component 2 does have a significant relationship with dominance: $P=0.042$, $n=49$, adj. $R^2=0.06$ (Fig. 2.1). Discriminant analysis (DA) fails to classify subordinate and dominant males based on cuticular hydrocarbon profiles, classifying only 73.47% of males correctly (100% of variation explained by Function 1, Wilks' Lambda = 0.87, $P=0.41$). Interestingly, DA has a higher misclassification rate when only component 2 is used to distinguish between dominant and subordinate males, but the model is significant (61.22% of males classified correctly; 100% of variation explained by Function 1, Wilks' Lambda = 0.92, $P=0.05$). This suggests that males may be able to provide a small amount of information regarding agonistic ability through their chemical profiles.

Next, I used an MANOVA to test if immunity and juvenile hormone titers correlate with cuticular hydrocarbon profiles, and if so, the relative contributions of each on chemical profile expression. The whole model, which includes JH, immune response, and the non-paired PCA components 1-6 was significant (whole model: Wilks' Lambda = 0.786, $F(12,208)=2.215$, $P=0.012$). This is mainly attributed to the relationship between CHCs and immune function ($P=0.0149$, $F=2.786$), rather than the relationship between CHCs and JH

($P=0.08$, $F=1.89$). While both immune response and hormone titers are associated with chemical profiles, the relationship between CHCs and immune function is stronger than the relationship between CHCs and JH.

Juvenile hormone is marginally related to immune response. Males with higher JH levels tend to have lower immunity, although the trend is not statistically significant: $P=0.085$, $n=119$, adj. $R^2=0.016$ (standard least squares regression model; Fig. 2.2).

Discussion

Male CHC profiles do not have an association with male mating success, making it very unlikely that males use CHC profiles to attract females as mates. I found no support for the sexual-attractant hypothesis, as there were no aspects of male profiles that served to facilitate male mating success. Therefore, the territory-marking behaviors observed by (Beani and Turillazzi 1988) must either serve another function (such as to advertise ownership of territories to other males) or males are using chemicals not detectable by this method to attract females. Regardless, males are not utilizing CHC profiles in mate attraction and further experimentation is required to determine what chemicals are secreted during scent-marking.

Male CHC profiles do have an interesting relationship with agonistic ability. While profiles are not distinct between dominant and subordinate males, parts of the profile do associate with dominance. Males may use CHCs to aid in assessing rivals, in conjunction with other cues or signals of dominance (such as their dorsal abdominal spots, which are sexually selected quality signals) (Izzo &

Tibbetts, in review). Males may advertise this information with their scent-marking behavior in an effort to deter rivals from encroaching on territories. Further experimentation is required to determine whether males pay attention to chemical advertisement of agonistic ability, and whether this information is used to mediate the costs associated with aggression.

Interestingly, individual variation in male cuticular hydrocarbon profiles exhibits a strong association with variation in immune response, as measured by encapsulation. Additionally, I found that JH and immunity are marginally and negatively related to one another, with a secondary association between JH and male CHC profile expression. This suggests that JH may function to modulate the chemical expression of immunity in male paper wasps. Juvenile hormone has been shown to regulate the innate immune response in insects as well as to influence pheromone and chemical profile expression (Rantala et al. 2003; Tian et al. 2010), and JH addition to insects typically results in decreased immune function (Rantala et al. 2003). Further, application of JH has been shown to change both female behavior and CHC profiles (Sledge et al. 2004; Lengyel et al. 2007; Izzo et al. 2009). The results presented here demonstrate that males have the potential to advertise information regarding the state of their immune systems, yet whether or not this information is used in communication or is simply a byproduct of physiology remains to be tested.

Whether male cuticular hydrocarbons have signaling roles or function as cues, it is important to understand who is paying attention to the information. CHCs are typically important mediators of reproductive interactions (Howard

1993), and may be especially important in male-male competition in social insects. I found that male CHCs are marginally associated with dominance, and strongly associated with immunity. Paper wasp males form long-term associations on leks (Beani 1996), and the information broadcast by CHCs may help individuals make decisions about which lek to join. It has been hypothesized that mate choice in paper wasps may rely on male longevity on a lek (Beani 1996), so males may benefit by choosing to lek with weaker opponents. It is not likely that immunity information can be easily faked as it is tightly controlled by physiological processes, and so males could benefit by paying attention to the status of rivals. Alternatively, males may preferentially lek with brothers, and so both dominance and immunity information may be used to regulate the social interactions and dominance hierarchy on the lek. Weaker individuals could gain indirect fitness by helping stronger, healthier kin.

Across a variety of organisms, females gain fitness benefits by paying attention to the immunological status of males by selecting immunocompetent mates (Westneat and Birkhead 1998). Male ornaments often have a hormonal basis to their development, and may be used to advertise the strong immune system of the bearer. The immunocompetence hypothesis, which predicts that while hormones enhance male ornamentation, they also suppress immune function; therefore males with elaborate ornamentation must by necessity have especially strong immune systems (Folstad and Karter 1992). CHCs are often used during mate choice in insects and can function as ornaments (Peschke 1987; Howard 1993; Higginson et al. 2000; Howard et al. 2003; Ferveur 2005), so it

might be expected under the immunocompetence hypothesis that both immunity and CHCs should relate to mate choice in paper wasps (if CHCs are used as ornaments). However, we found no evidence that CHCs were involved in female choice in paper wasps, suggesting that there is little potential for males to utilize chemical ornamentation. However, paper wasp males do not participate in parental care, and so male immunity may be inconsequential to female fitness. Previous work in paper wasps suggested that males use CHCs to attract females, as males have been observed “scent marking” their territories (Beani and Turillazzi 1988). However, while CHCs may serve to advertise the presence of a male, our results do not support the potential for CHCs to indicate male attractiveness.

In short, males have been nearly systematically ignored in studies on chemical communication in social insects, resulting in little understanding of the dynamics of male social interactions or the role of chemical information during male-oriented communication. Much work is needed in order to fully understand the social biology of males, and how chemical information mediates those interactions. We have shown that males have the potential for complex interactions mediated by their cuticular hydrocarbon profiles, as their CHCs correlate with immunity, hormone titer, and agonistic ability.

Figures and figure legends

Figure 2.1: Dominance is significantly related to PCA component 2, suggesting that male CHC profiles may provide information regarding agonistic ability. 1 on the Y-axis refers to fight winners, while -1 refers to fight losers. The boxes show the upper and lower quartiles, while the whiskers represent data that falls within 1.5 inter-quartile range (IQR).

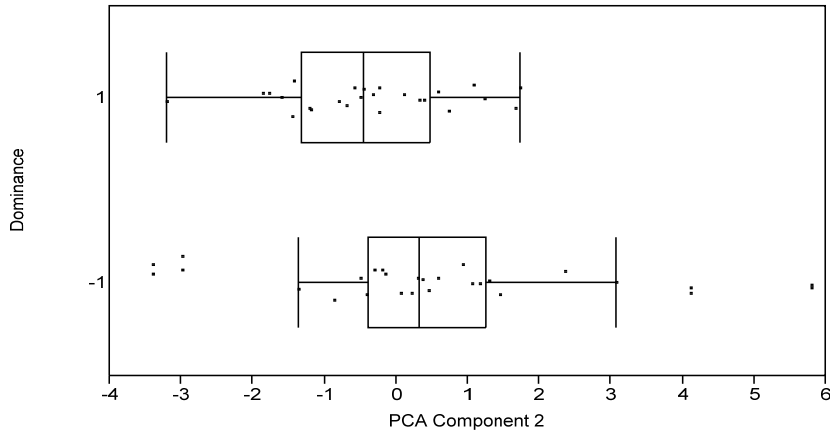
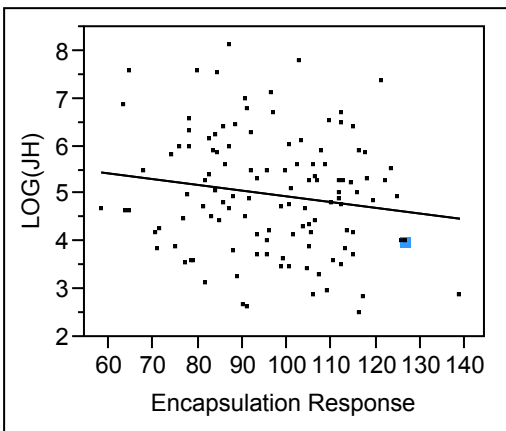


Figure 2.2: The relationship between circulating juvenile hormone (pg/ul) and encapsulation response. Encapsulation response is measured by the average RGB reflectance of the monofilament implant; values have been transformed for the graph such that high values of RGB correspond to higher encapsulation.



Chapter III: Sexually-selected signals in male paper wasps

Abstract

Although sexually selected ornaments are common across diverse taxa, little is known about mate choice and sexually selected ornamentation in social insects. *Polistes dominulus* paper wasps are a good system in which to study sexual selection, as males have a lek-based mating system as well as sexually dimorphic abdominal spots. Here, we demonstrate that the abdominal spots of male *Polistes dominulus* paper wasps function as signals in both inter- and intra-sexual selection. Males with smaller, elliptically-shaped spots are more dominant over male rivals and are more preferred by females than males with larger, irregularly shaped spots. Further, experimental manipulation of male spots demonstrates that spot morphology acts as a signal. Males with experimentally reduced abdominal spots win a greater proportion of fights and are preferred by females as mates over control males. These results demonstrate that male ornaments are an important mediator of mating dynamics in *Polistes dominulus* paper wasps. Further, they highlight the importance of additional research examining sexually selected ornamentation in social insects and can serve as a starting point to examine mating dynamics in haplodiploid systems.

Introduction

Many organisms use sexually selected signals to minimize the costs associated with competition for mates. Over the past several decades, there has been increasing research on sexually-selected quality signals, which convey information regarding their bearer's current condition (such as foraging skill or agonistic ability) and/or genetic constitution (i.e. good genes, parasite resistance) (Zahavi 1975; Hamilton and Zuk 1982; Gonzalez et al. 1999; Andersson and Simmons 2006). These signals may be used during aggressive competition over mates or resources required for mating (i.e. male-male competition), during non-aggressive competition for mating opportunities (i.e. female choice), or both. Many different types of traits function as sexually-selected quality signals (or "ornaments"), from structures built by animals, such as bowerbird bowers or fiddler crab sandcastles, to physical traits of the organism, such as exaggerated plumage, bright coloration, enlarged weaponry, or diverse song repertoires (Hill 1991; Evans and Hatchwell 1992; Searcy 1992; Borgia 1995; Backwell and Passmore 1996; Berglund et al. 1996; Fitzpatrick 1998).

Although sexually selected ornaments are common across diverse taxa, there has been little research on ornamentation or quality signaling in social insects. There is potential for dramatic sexual selection within the social insects (Baer 2003), as many species have mating systems with high variance in male reproductive success. For example, many social insect species have lek- or swarm-based mating systems that result in high reproductive skew among males (Reeve 1991; Beani et al. 1992; Polak 1993; Beani 1996; Cole and Wiernasz 1997). Males in many species have pheromones that attract females (Post and

Jeanne 1983; Reed and Landolt 1990), suggesting that sexual signaling may be important in social insects. However, sex attractant pheromones do not necessarily function as ornaments; pheromones may provide information about male presence rather than male quality. Currently, no examples of sexually selected quality signals have been described in social insects

Although the possibility of sexually selected ornamentation has received little attention in social insects, social insects have become important models for other aspects of sexual selection. For example, an interesting consequence of the haplo-diploid genetic system of hymenoptera is that males produce clonal sperm, making them ideal subjects for studying sexual conflict and sperm competition (Sundstrom and Boomsma 2000; Baer 2003; Baer and Boomsma 2006). Studies on bumblebees and several ant species have shown that this is an exciting field worth pursuing (Baer 2003; Baer and Boomsma 2006; den Boer et al. 2010). Despite the growing body of literature, there is still much to be learned about the role of sperm competition across the Hymenopterans (Baer 2003; Baer and Boomsma 2006).

Polistes paper wasps are a good system for studying sexually selected ornamentation. Paper wasps have a lek-based mating system with intense competition for mates (Wenzel 1987; Beani and Turillazzi 1990; Beani et al. 1992; Polak 1992; Polak 1993; Beani 1996). Both male-male competition and female choice play important roles on leks. Males establish dominance hierarchies during competition over position on the lek. However, high dominance rank does not guarantee copulation; females are quite choosy on leks

and select the most attractive males for mates (Hamilton and Zuk 1982; Pruett-Jones and Pruett-Jones 1990; Gratson et al. 1991; Gibson and Bachman 1992; Gibson 1996). Typically, one to a few males monopolize the majority of the copulations. This high skew in male reproductive success produces strong sexual selection on male traits, which often leads to dramatic male ornamentation (Balmford 1991; Kirkpatrick and Ryan 1991; Cuervo and Moeller 1999). Male ornamentation may be used to mediate male-male competition, female choice, or both. *Polistes* paper wasps exhibit typical lekking behavior: males compete vigorously for dominance positions on the lek, and females are choosy in their mate selections (personal observation, (Beani 1996). However, the possibility of male paper wasp ornamentation has not previously been examined.

Here, we examine the paper wasp *Polistes dominulus* to determine if males utilize sexually selected ornaments. Male *P. dominulus* paper wasps possess a pair of yellow dorsal abdominal spots that are a good candidate to function as sexually selected signals (Fig. 3.1, 3.2). First, the spots are highly variable in both size and shape. Second, the variation is sexually dimorphic: both sexes possess these spots, but male spots are larger and more variable than female spots. Third, the location and coloration of the spots make them highly conspicuous. We tested whether dorsal abdominal spots function as sexually selected signals by testing whether 1) variation in spot shape and size is associated with male dominance and/or female preference and 2) variation in

spots is used as a signal during male-male competition and/or female choice, such that spots function as ornaments.

Methods

Wasps:

Mature *Polistes dominulus* nests were collected from sites throughout Ann Arbor, Michigan during July of 2007 and 2008. Nests were brought to the laboratory and placed in nestboxes, and the wasps currently on the nest were marked with Testor's model paint. All males present on the nest as well as newly eclosing males and gynes were removed from the nest, weighed, and housed individually with *ad libitum* water and sugar. Only gynes that eclosed in the lab, with known age and virginity were used in trials; males that eclosed in the lab, as well as those previously on the nest, were used in trials. All males were sexually mature upon use in trials. Males on the nest at the time of nest collection spent at least 7 days in the laboratory before use in a trial. All males with known emergence dates were used between 10 – 21 days post emergence. No differences were found between the morphology or behavior of males that eclosed in the lab versus in the wild. All wasps that interacted in dominance and mating bouts were from nests that were at least 1 mile apart to ensure test subjects were not related.

Experiments:

To determine whether dorsal abdominal spots in male *Polistes dominulus* paper wasps are used as a sexually-selected signal, we first examined sexual

differences among males and females in spot size and shape, and then tested for an association between spot morphology and dominance/female preference. Finally, we tested the signaling function by assessing the effect of male spot manipulation on dominance and female preference.

Sexual Dimorphism

To examine sexual dimorphism of spots, we compared the spot shape index (SI; see methods section “morphological analysis,” below) of males and females (See Fig. 3.2, 3.3). A total of 162 males from 16 nests were measured and compared to a total of 50 females from 10 nests. All individuals used to test sexual dimorphism were wasps caught in 2007 that had pupated in the wild.

Correlations Between Male Morphology and Dominance, Mating Success

In 2007, the relationship between spot morphology and male dominance/female preference was tested using male-male dominance bouts and mating trials. Dominance bouts were conducted by randomly pairing two unfamiliar and unrelated males in an 8 cm x 8 cm x 2 cm clear plastic arena, and allowing them to battle for dominance in 2 hour video-taped bouts. In the trials, males showed a wide range of aggressive and dominance behaviors similar to those described by others (Beani and Turillazzi 1988; Polak 1993). After the dominance trial, males were returned to their individual deli cups for 24 hours so they could rest and eat prior to the mating trials. Then, the relationship between spot morphology and mating success/female preference was tested by placing

an unrelated female in the arena with a pair of males that fought previously. All mating trials occurred 24 hours after the males' initial dominance battles and had a 1-hour duration. A total of 113 trials, containing males from 37 nests (with 1-15 males per nest) were included in the dominance analysis. Not all male pairs were utilized for mating trials, so the sample size for mating trials is lower than that for dominance bouts (100 choice trials were conducted, with mating occurring in 81 trials), Males used had a size range of 0.051g – 0.135g. In order to test the effect of size on male dominance, unrelated males were randomly paired. The difference in size between males within a pair ranged from 0g – 0.056g. Immediately following the mating trial, all males were frozen for photographic analysis of their spot morphology.

Manipulations: Testing for a Signaling Role

In 2008, manipulative experiments were performed to test whether male abdominal spot morphology functions as a signal during male competition and/or female choice (Jacobs 1955; Moore 1990). To test whether spots act as a signal of dominance, unfamiliar males of similar size were paired and allowed to battle for dominance in 2-hour videotaped bouts. One male was randomly designated as the “receiver,” and remained un-manipulated. The other male was designated as the “sender” and received manipulations. In half the trials (experimental trials) the sender had a portion of his spots concealed with black paint. This makes the spots smaller and more elliptically shaped, a phenotype associated with high dominance. In the other half of the trials (control trials), the sender was sham

painted: he received a similar amount of paint in the same area of the abdomen as the experimental males, but the appearance of his spots was not changed. The control trials are important to ensure that paint alone did not influence behavior and enabled us to explicitly test whether spots alone are used during rival assessment and/or mate choice. Senders and receivers were chosen randomly, so sender controls are predicted to win half the dominance trials. However, if spots act as dominance signals, males whose spots were reduced in the experimental treatment are predicted to win a greater proportion of fights than expected by random chance alone. Therefore, the manipulated male should win in significantly more than 50% of the experimental trials. A total of 110 trials containing males from 47 nests (with 1-10 males per nest) were included in the dominance analysis. All of the included trials had dominance established; 43 trials were experimental trials, 67 were control trials. Comparisons in the results are of the proportion of trials won versus lost. Males used had a size range of 0.044g – 0.130g. Males were paired by size to minimize differences among them: the difference in size between males within a pair ranged from 0g – 0.005g.

Following dominance bouts, males were returned to their individual deli cups. 24 hours later, the males were re-paired (into the same pairs) and placed in the trial area with a randomly selected unrelated virgin gyne for a one hour videotaped mating trial. As senders and receivers were chosen randomly, control senders are predicted to be preferred by females in half the trials. However, if spots act as signals during female choice, the female is predicted prefer the

experimentally altered sender males in significantly >50% of the trials. Not all male pairs were utilized for mating trials, so the sample size for mating trials is lower than that for dominance bouts (70 choice trials were conducted, with mating occurring in 60 trials, 30 each of experimental and control). These trials used 140 males from 45 nests.

Behavioral analysis:

All behavioral tapes were scored independently by two viewers. Each viewer was blind to experimental treatment and predictions. Only trials in which dominance was established and consistently scored by both viewers were used in analyses. During male-male dominance trials, typical aggressive behaviors such as biting, darting, and grappling were exhibited by males (Beani and Turillazzi 1988; Polak 1993). The total number of darts performed by a single male in the trials ranged from 0-350, the total number of bites performed by a single male in a trial ranged from 0-24, and the total number of mounts performed by a single male ranged from 0-4. Mounts were unidirectional, such that only one male successfully mounted his rival in a given trial (although that male may have mounted multiple times). A male was said to have won the dominance contest when he subordinated his rival through an uncontested mount (the “subordinate” becomes submissive, crouches, and lowers his antenna (West-Eberhard 1969)). Dominant males (those that mount and subordinate rivals) initiate significantly more aggression than their rival male (mounts correlate with total number of darts, bites, and grapples, $p=0.004$, standard least

squares regression). After dominance has been established, there is a significant reduction of aggression between the two males, with males engaging in non-aggressive contact such as huddling or grooming.

During mating trials, females visually assessed and antennated both males and explored the arena before mating. The number and duration (in seconds) of copulations and attempted copulations were recorded. Copulation occurs when a male mounts a female, links his antennae around the female's antennae, and makes genital contact. Females are typically larger and more dominant than males, so females exert strong choice by rejecting males (Reeve 1991; Polak 1992; Baer 2003; Kovacs et al. 2008). Attempted copulations occur when a male attempts to grab and mount a female and she rejects him. Females reject males in several ways: females can prevent males from mounting by avoiding the male, forcefully rejecting his mounting attempts (through biting, darting at or stinging him), or by allowing the male to mount but moving their abdomens to avoid male genitalia (making copulation mechanically impossible). The frequency of successful and rejected copulations varied across females (matings per female: mean =3, range =0-17; rejections per female: mean =5, range =0-26). In the trials, females copulated with neither (19 out of 100 trials), one male (37 out of 100 trials), or both of the males (44 out of 100 trials) and "female preference" was scored as the difference in total time spent in copula between the two males (i.e. "male 1" copulation duration – "male 2" copulation duration). Females spent more time huddling and grooming with preferred males (those that were permitted to copulate for longer durations), and males with long

copulation durations never received aggressive rejections. As females in many *Polistes* species are genetically monogamous (Strassmann 2001), it is likely that postcopulatory sexual selection is important in this species.

Morphological analysis:

Each male was photographed for detailed morphological analysis of his spots (see Fig. 3.1). The length (L) and height (H) of the left and right spots (in pixels) and the area (“A,” in pixels) was measured using Adobe Photoshop CS. As no single measurement adequately captures the variation in size and shape of the spots, an index (the “spot-shape index,” hereafter SI) was created using the following formula:

$$SI = A/[Pi*(1/2L)*(1/2H)].$$

The length (L) and height (H) used for each wasp was the average between the left and right spots’ lengths and heights, respectively; area (A) was the average of the measured area for the left and right spots. The SI was used in all analyses, with high SI values corresponding to large, irregular spots, and small SI values corresponding to small, elliptical spots (see Fig. 3.2). This index was created to facilitate comparison of actual spots to an idealized ellipse: ellipses were chosen as the most regular male spots (and the typical female spots) are elliptical in nature. Comparing spots to the idealized ellipse provides a measure

of the irregularity, or 'blobbiness' of the spot. SI values are size-independent, thereby facilitating comparison among individuals.

All morphological measurements were made using Adobe Photoshop CS, and all statistical analyses were run using JMP 8 and PASW 18. Variation between males and females was tested using an F-test to compare variance between the sexes, and spot shape index means were compared across sexes using a Standard least squares analysis (including wasp weight and with nest as a random factor to control for any similarity within nests). The relationship between SI and dominance rank was analyzed using a paired logistic regression that tested how spot morphology and weight relates to dominance (win/loss). Standard least squares regression models were fitted to examine mate preference data. As there were two males in each mate choice trial (i.e. choice trials were paired), the analysis was run by testing the relationships between the difference in the males' SI values versus the difference in their total copulation durations. Multiple males from the same nest were included in the dominance and choice trials, but nest cannot be included as a random effect in paired analyses. As a result, we performed additional, unpaired analyses to test whether nest of origin influenced dominance or female choice. In both analyses, nest of origin was not an important factor (dominance: $p=0.88$, variance parameter = 0.001; choice: $p=0.9$, variance parameter = 0). The data for the spot manipulation experiments were analyzed using paired proportion tests, to determine whether the percentage of trials in which manipulated males won

dominance contests or were preferred by females deviated from the null expectation of 50%.

Results

Correlations:

Dorsal abdominal spots are sexually dimorphic, with males possessing significantly greater variation in size and shape than females: $p < 0.001$, $F_{161, 49} = 9.77$ (two-sided F-test; standard deviation for males = 0.276, standard deviation for females = 0.088). Females have smaller, more elliptical spots than males which translates into a smaller SI than males: mean female SI = 1.076, mean male SI = 1.376; difference in means: $p < 0.0001$ adj. $R^2 = 0.37$, $n = 212$; sex: $p < 0.001$, weight: $p = 0.76$; standard least squares regression including sex and weight as factors and “nest” as a random factor.

Male dominance correlates with spot morphology, such that males with smaller, more regular (i.e. elliptical) spots are more likely to win fights than males with larger, irregularly shaped spots, and dominance is un-related to male size (paired logistic regression model $p = 0.0065$, adj. $R^2 = 0.065$ $n = 113$; weight: $p = 0.4$, spot SI: $p = 0.004$; Fig. 3.4).

Male mating success is also associated with male spot morphology. Males with small SI spend greater amounts of time in copula with females than males with large SI, yet male size and dominance status do not influence female mate choice. Fitting a standard least squares regression model with the difference in time in copula predicted by the difference in male spot SI, difference

in male weight, and male dominance status shows that females prefer males with smaller spot SI (model $p=0.07$, adj. $R^2=0.057$, $n=81$; male spot SI: $p=0.01$; male weight: $p=0.59$, male dominance status: $p=0.5$, Fig. 3.5).

Manipulations:

The signal value of male spots was tested by assessing how experimental manipulation of abdominal spots influenced male success during competition and choice.

Males with experimentally reduced spots were more likely to win fights than expected due to random chance: manipulated males won 31/43 (72%) of trials ($p=0.0015$, $n=43$, $Z=3.213$; paired proportion test, see Figure 3.6 A). Painting alone had no effect on dominance. Control males that were painted without changing their spot size were just as likely to win fights as their unpainted partner: control males won 35/67 (52%) of trials ($p=0.372$, $n=67$, $Z=0.3276$; paired proportion test, see Figure 3.6 B). Therefore, males pay attention to spot morphology, and they use spot size and shape to assess the agonistic ability of their rivals.

Similarly, females pay attention to male spot morphology, as females spent more time copulating with experimentally altered males. Males with experimentally reduced spot size were preferred by females in a greater proportion of trials than their non-altered partner. The experimentally altered male was preferred in 22/30 (73%) of the trials ($p=0.004$, $n=30$, $Z=2.837$; paired proportion test, see Figure 3.7 A). Conversely, males who were painted without altering their spot size or shape in control trials were no more preferred than their

partner. Control males were preferred in 16/30 (53%) of trials ($p=0.35$, $n=30$, $Z=0.386$; paired proportion test, see Figure 3.7 B). These manipulations demonstrate that spot morphology alone strongly influences female choice

Discussion

Overall, the dorsal abdominal spots of male *Polistes dominulus* paper wasps are sexually-selected signals. Spots are sexually dimorphic, with males exhibiting larger spots with greater variance in spot morphology than females. Additionally, spot morphology is associated with male dominance and reproductive success, such that males with smaller, more elliptically-shaped spots are both more dominant over male rivals and more preferred by females than males with larger, more irregularly shaped spots. Further, dorsal spots are used as a signal during male-male competition and for female choice. Males with experimentally reduced spot size were more likely to win fights with rivals and were preferred by females. Sham painting had no effect on male dominance or reproductive success. Therefore, male dorsal spots function as sexually selected signals, providing valuable information about males that is used during aggressive competition and non-aggressive mate choice.

Although few previous studies have examined ornaments used during sexual selection in social insects, *Polistes* paper wasps have the type of mating system that is predicted to select for male ornamentation. Males form leks on landmarks such as fence posts, walls, roof peaks, in trees, etc., where they compete over small mating territories (personal observation, (Beani and

Turillazzi 1988; Beani et al. 1992; Beani 1996). Males frequently engage in mid-air battles or fight on the substrate. Losers retreat from the contest and typically fly away from the lek (personal observation, (Polak 1993). Females visit leks to choose mates, and appear to assess males during repeated visits before selecting a mate (Beani 1996). The intense mate competition within leks produces high reproductive skew and ornamentation in many lekking species, including paper wasps (this study, (Gibson and Bradbury 1985; Wilkinson and Riello 1994; Sherman 1999).

Although the same ornament is used during both competition and choice, females do not choose mates based on male dominance. This result suggests that male ornamentation reflects overall male quality such that both males and females benefit by paying attention to the signal. However, the male characteristics involved in competition and choice are distinct enough that dominance rank alone does not predict choice. The lack of relationship between dominance and choice is particularly interesting because both males interacted freely with the female during the choice trial, so aggressive interactions between the males could theoretically influence choice behavior. Nevertheless, male dominance rank alone was unrelated to female preference. Additional research on the precise information conveyed by male abdominal spots as well as the temporal overlap between mating contexts will be useful for understanding more about the dynamics of choice and competition during mating in paper wasps.

The disconnect between dominance and female choice in paper wasps is initially surprising because lekking models sometimes assume that dominance

rank is the basis of female choice. For example, the “hotshot” model of lek formation posits that leks form around the most dominant males, with active female choice having a diminished role (Beehler and Foster 1988). Relatively few studies have sought to explicitly disentangle the relationship between dominance and choice on leks as it is sometimes assumed that the most preferred male is also the most dominant. However, a disconnect between male dominance and reproductive success may be quite common in both lekking and non-lekking species (Thery 1990; Gratson et al. 1991; Qvarnström and Forsgren 1998; Young et al. 2009), and several models of lek formation posit that male mating success may actually be unrelated to male dominance (Bradbury and Gibson 1983; Gibson and Bradbury 1986). Our data add to these studies, as they indicate that dominance and female choice are not inherently linked and need to be tested separately.

Much research on animal ornamentation has focused on the mechanisms that maintain signal accuracy. A popular hypothesis is the handicap hypothesis which posits that ornaments must be differentially costly to provide reliable information about their bearer’s quality (Zahavi 1975). Examples of handicaps include carotenoid signals, exaggerated plumage, or weaponry (Zahavi 1975; Hamilton and Zuk 1982; Johnstone 1995; Andersson and Iwasa 1996; Olson and Owens 1998). Many ornaments are thought to have costs associated with signal production, but paper wasp dorsal abdominal spots lack obvious production costs. Dorsal spots are composed of the same yellow and black pigments that cover the paper wasps’ body, so it is unlikely to be differentially costly to produce

spots of varying size or shape. Further, the pigments that compose the signal, pterins and melanins, are thought to have minor costs associated with their production (Jawor and Breitwisch 2003; Rutowski et al. 2005).

What, then, maintains the accuracy of *P. dominulus* abdominal spots? Relatively little is known about the costs of melanin and pterin deposition in insects, so there may be hidden costs associated with producing some aspect of the abdominal spot. Alternatively, spots may have other types of production or maintenance costs. Although production costs are often proposed as the key mechanism that maintains signal accuracy, there has been less empirical work testing the true costs of ornament production and maintenance in many systems (Szamado 2010). Therefore, other types of costs, such as social costs or efficacy-based costs, may be surprisingly widespread (Szamado 2010). A growing body of literature has begun to document seemingly “cost free” signals, such as the depigmented white wing patches used in displays of collared flycatchers (Torok et al. 2003). Further studies will be important to explore the mechanisms maintaining accuracy of paper wasp abdominal spots. Initial results suggest that signal development may play an important role in signal accuracy, as male spots are condition-dependent quality signals (Izzo & Tibbetts, unpublished data). Condition-dependence provides a mechanism that may maintain variation in the signal. Overall, the results of this study highlight the value of studying sexually selected traits that lack obvious production costs as well as the importance of exploring diverse mechanisms that maintain ornament honesty over evolutionary time (Szamado 2010).

Although social insects have been important models for research on a range of topics, their reproductive biology has received relatively little attention (Baer 2003; Boomsma et al. 2005). Here we provide striking evidence that sexual selection is an important selective pressure in *Polistes* paper wasps. Males have variable dorsal abdominal spots that are important signals used during inter- and intra-sexual selection. This work highlights the value of research exploring mating dynamics in social insects, and serves as a starting point to develop social insects as model organisms for studying lekking dynamics.

Figures and Figure Captions

Figure 3.1: Photographs of male wasp abdomens demonstrating the location of the dorsal abdominal spots and how they were measured. A) Dorsal view of wasp with spots highlighted with white box; B) Top view of male spots (highlighted by white box), anterior is towards the left; C) Lateral view, box highlights the wasp's left spot, anterior is towards the left; D) Single spot with idealized ellipse drawn around it, length and height marked; E) Single spot with area in pixels measured.

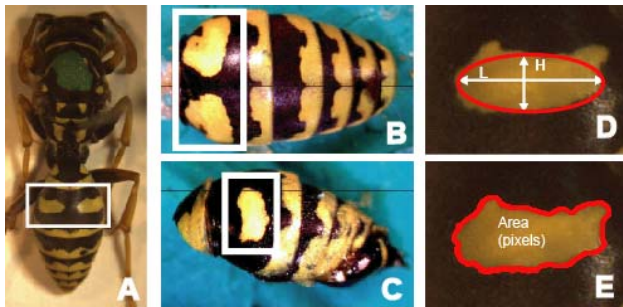


Figure 3.2: Male abdominal spot photographs arrayed from low to high Spot-shape Index (SI). Spots with low SI are found on males that are more dominant and more preferred by females; spots with high SI are found on males that are less dominant and less preferred by females.



Figure 3.3: Photographs of female dorsal abdominal spots; note the lack of variation among spots in shape and size as compared to the variation in male spots (Fig. 1, 2). Left-most panel is the dorsal abdominal view; the smaller photographs on the right are close-ups of the left spot on four different female wasps.

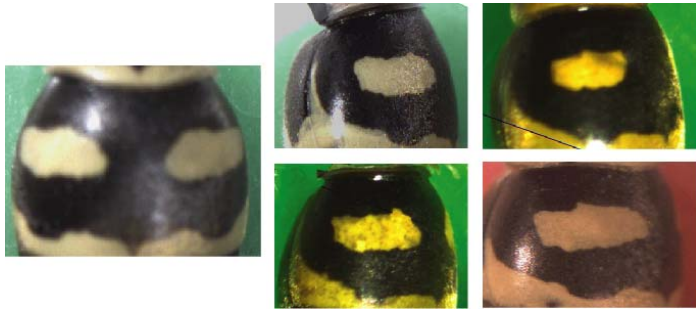


Figure 3.4: Relationship between male dominance (scored as win/loss) and male spot-shape index (referenced as SI). Males with low SI are more likely to win fights than males with high SI. Arrows indicate mean SI of dominant and subordinate wasps.

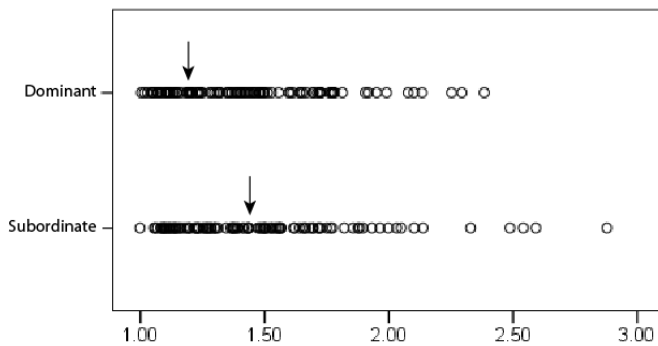


Figure 3.5: Relationship between female preference and male SI. Females prefer to mate with males with low SI. The difference in copulation duration (in seconds) plotted against the difference in male spot-shape index (SI). Males with comparatively smaller SI were permitted to copulate for longer durations than males with larger SI.

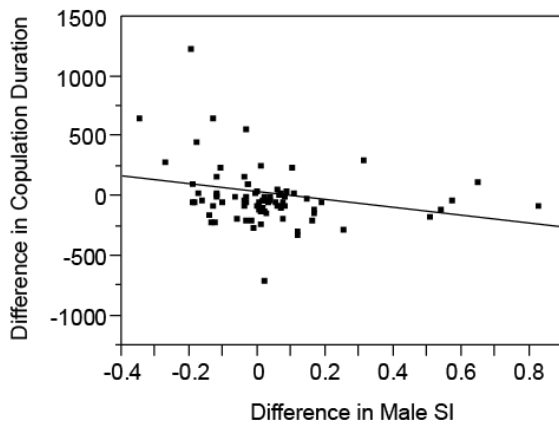


Figure 3.6: Proportions of fights won by painted (black bars) versus non-painted (gray bars) males. In Experimental trials the experimentally altered (i.e. painted) male's spots were concealed with black, to reduce the SI. Experimentally altered males won a significantly greater proportion of fights than non-altered males. In Control trials, the control male received a sham treatment: a similar amount of black paint was applied in the same area as the experimentally altered male, but spot shape and size was left unchanged. Control males and non-altered males won a similar proportion of fights. Stars represent significant differences in proportions; the dotted line represents null expectation of 50%.

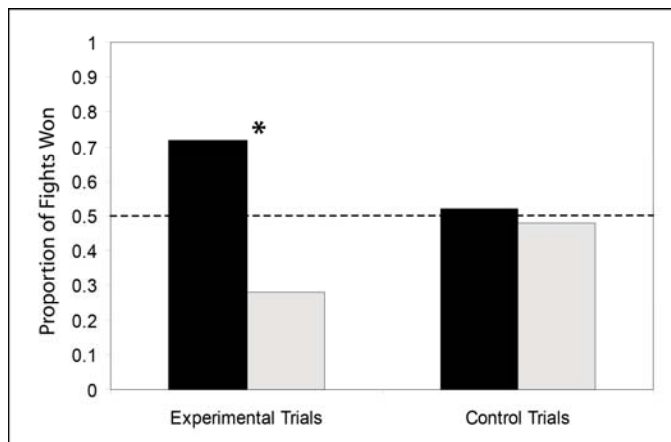
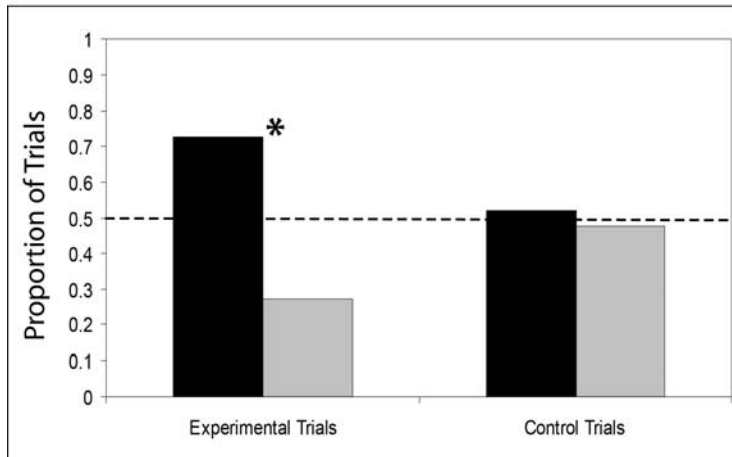


Figure 3.7: Proportions of trials in which females preferred painted (black bars) versus non-painted (gray bars) males. In Experimental trials the experimentally altered (i.e. painted) male's spots were concealed with black to reduce their SI. Experimentally altered males were preferred in a significantly greater proportion of trials than non-altered males. In Control trials the control male received a sham treatment: a similar amount of black paint was applied in the same area as the experimentally altered males, but spot shape and size was left unchanged. Control and non-altered males were equally preferred in control trials. Stars represent significant differences in proportions; the dotted line represents null expectation of 50%.



Chapter IV: Heightened condition dependence of a sexually selected signal

Abstract

Sexually selected signals are expected to exhibit heightened condition dependence as compared to non-signaling traits. This link to condition enables them to provide information regarding individual quality, as well as to provide a mechanism for maintaining signal accuracy over developmental time. Currently, little is known regarding the condition dependence of traits lacking obvious production costs and the extent to which such traits can accurately reflect individual quality. Male *Polistes dominulus* paper wasps are an excellent system in which to study condition dependence of sexually selected signals lacking obvious production costs. Males have yellow-on black spots which function as sexually selected signals, and as males are entirely covered in yellow and black, the signals do not appear especially costly to produce. Further, males have non-signaling thoracic stripes comprised of the same pigments, which are excellent candidates as control traits. Here, I performed an experimental manipulation of diet by restricting access to protein during larval development to test the condition dependence of the sexually selected signal and the non-signaling control trait. Males reared on diets with abundant protein developed small, elliptical spots similar to those seen on dominant, attractive males while males reared on diets with restricted protein developed large, irregularly shaped spots.

The control trait (thoracic stripes) did not differ between diet treatments. This demonstrates that sexually selected signals are developmentally independent from non-signaling traits comprised of the same pigments, and that sexually selected traits do not require obvious production costs to advertise individual quality.

Introduction

Sexually selected signals function almost universally to advertise the individual quality of their bearer. This quality information may vary based on context, but typically involves information regarding the individual's genetic constitution, condition, rearing environment, or a combination of these factors (Andersson 1994). By advertising quality information, these signals help to mediate the costs associated with assessment in agonistic contexts, mate choice, or both. Signal costs, such as production, maintenance, social, etc., are required in order to maintain signal honesty over evolutionary time.

In order to accurately advertise individual quality, sexually selected signals are expected to express heightened condition-dependence as compared to non-signaling traits (Cotton et al. 2004). Condition dependence of these signals maintains signal accuracy by linking trait development to the organism's condition (Rowe and Houle 1996). This environmental influence on trait expression enables signals to provide accurate information regarding individual quality, and provides information regarding the individual's developmental environment, foraging skills, etc. In fact, this expectation of condition-

dependence of sexually selected signals is so well accepted, that traits whose signaling functions are unknown are often assumed to be involved in sexual selection if they have been shown to be condition-dependent (Peters et al. 2008).

Much research on condition-dependence has focused on signals with obvious production costs. Because such signals are difficult to produce and maintain, only individuals in good condition can possess elaborate ornaments (Zahavi 1975). Linking condition dependence, individual quality, and production costs is relatively straight-forward. For example, carotenoid-based plumage and color patterns have been intensely studied, as carotenoids cannot be synthesized *de novo*, and are considered to be difficult to obtain (Jawor and Breitwisch 2003). As such, only high quality individuals (i.e. those with good foraging skills, in this example) are able to obtain enough carotenoids to develop bright red plumage. The costliness of signals maintains their ability to honestly advertise aspects of quality, and condition dependence helps maintain the accuracy of signals by adding an environmental component to signal production.

The longstanding assumption that condition dependence is inherently linked to signals with high production costs has led to the counter-assumption that traits lacking obvious production costs could not exhibit condition dependence, and as such would function as poor indicators of individual quality (Griffith et al. 2006). This arise from the argument that if signals are not costly to produce, how could their production be linked to and individual's condition? However, despite the focus on signals with obvious production costs, such costs are not an inherent requirement for the condition dependence of signals

(Szamado 2010). Social and maintenance costs can also serve to maintain signal honesty, but may be more difficult to quantify than production costs. Because of this, signals comprised of melanins, pterins, structural colors, or depigmented white patches have received less attention than carotenoid based signals. Since animals are capable of synthesizing these pigments, it has been assumed that such non-carotenoid colors should lack the condition dependence necessary to advertise quality information (Griffith et al. 2006).

Nevertheless, recent work has begun documenting the plethora of signals comprised of non-carotenoid (i.e. apparently “non-costly”) pigments (Torok et al. 2003; McGlothlin et al. 2007; Roulin et al. 2008), and a recent meta-analysis demonstrated the fallacy in thinking that such seemingly non-costly pigments could function at best as poor quality traits (Griffith et al. 2006). So, despite the widespread belief that lacking production costs should prevent such traits from functioning as condition-dependent quality indicators, the evidence to support those assumptions is lacking. This opens an exciting new avenue of research to determine whether there truly are limitations on signaling properties of non-carotenoid pigment patterns.

Here I test the hypothesis that sexually selected signals show heightened condition dependence as compared to non-signaling traits. I focus on the yellow-on-black dorsal abdominal spots of male *Polistes dominulus* paper wasps, which have previously been shown to be sexually selected signals (Izzo & Tibbetts, in review). These spots are used by males to solve agonistic encounters and by females to aid in mate choice. Males with smaller, more elliptically shaped spots

win more fights and are selected as mates more frequently than males with larger, irregularly shaped spots. Although the spots have been experimentally demonstrated to function as a sexually selected signal, we lack information regarding their development. This signal is a particularly interesting trait to test condition dependence because the signal is comprised of melanins and pterins, which lack obvious production costs (Stoehr 2006). Further, wasps are covered in the same pigments, so they provide an excellent opportunity to compare the condition dependence of signaling and non signaling traits comprised of the same pigment. Few experimental tests of the condition dependence of sexual signals includes control traits; fewer still have control traits that could be expected to share the same developmental pathway (Cotton et al. 2004).

Specifically, I tested whether manipulation of natal nutrition would significantly alter the male abdominal spots that function as a sexually selected signal more than non-signaling traits composed of similar pigments. Natal nutrition influences adult condition (Tibbetts and Curtis 2007), so manipulation of larval nutrition is a good way to test condition dependence. Females must mate at least 6 months prior to nest initiation (and store sperm for the duration) (Turillazzi and West-Eberhard 1994), so if male nutritional condition impacts male spermatophore condition, then male sexual signals may function to indicate spermatophore viability. I subjected wasp nests to different diet treatments, where I manipulated the amount of caterpillars available as a food source for developing wasp larvae. Half of the nests were subjected to a protein-limited diet, and males from these nests were predicted to develop signals that advertise

low quality; the other half of the nests were not on a restricted diet, and were predicted to develop signals that advertise high quality. The non-signaling (control) patterns were not predicted to be significantly different between treatment groups. These results would demonstrate heightened condition dependence of sexually selected signals as compared to a non-signaling control trait. Condition dependence of these signals would enable male paper wasps to provide information regarding individual quality to both rival males and mates.

Methods

In spring 2006, *Polistes dominulus* foundresses and their nests were collected from sites around Ann Arbor, MI and brought to the laboratory. Each foundress and her nest was placed into nestboxes, and randomly divided between two diet treatments, where the amount of waxworm caterpillars was manipulated. In the high-caterpillar treatment, nests received unlimited caterpillars, while in the low-caterpillar treatment nests were given approximately 40% fewer caterpillars per nest cell. Caterpillars are consumed by both adults and larvae, and provide the main protein source for each. Adult phenotype is fixed upon adult eclosion (emergence from pupation), so larval nutrition is important for adult fitness. The number of caterpillars eaten by each colony was recorded three times per week to ensure that high and low treatments were eating significantly different amounts. High-caterpillar colonies ate more caterpillars per nest cell per day than low-caterpillar colonies: High: mean = 0.034, standard deviation = 0.0018 caterpillars per nest cell per day; Low: mean

= 0.0156, standard deviation = 0.00021 caterpillars per nest cell per day; $p < 0.0001$, $t = 6.7$, $n = 23$. All nests were provided with unlimited water, sugar and paper for nest construction. Nests were monitored throughout the spring and summer, and foundresses not continuing nest construction in the lab were removed from the study. At the end of the summer, all males were removed from nests and were weighed on a scale accurate to 0.001 g and photographed for analysis of their color patterns.

In total, 76 males from 14 nests (6 high-caterpillar, 8 low-caterpillar) were collected and measured for the experiment. Each male was weighed while alive, and then frozen for photographic analysis. Photographs were taken of male dorsal abdominal spots (i.e. the sexually selected signal in this species, (Izzo & Tibbetts, in review; Figure 4.1, Figure 4.2), as well as the control trait (Figure 4.2). The control trait used was the thoracic stripes, as these stripes do not exhibit obvious variation in size or shape, and do not appear to be sexually dimorphic. Control traits (sensu (Cotton et al. 2004) were measured to show that the sexually selected signal shows heightened condition dependence relative to other traits, and that males reared on better diets were not just overall different from males reared on poor diets

Polistes dominulus paper wasps possess a pair of dorsal abdominal spots, which are yellow on a black background and function as a sexually selected signal (Izzo & Tibbetts, in review). The variation in size and shape exhibits sexual dimorphism, and males use these spots both in agonistic encounters and during mating bouts. Males with smaller, more elliptically shaped spots win more fights

and are more likely to be chosen as mates over males with larger, irregularly shaped spots. To capture and describe spot shape variation, a “spot-shape index” (SI) was created. This index compares the average measured size of the spot to an idealized ellipse calculated by the length and height of the spots. Males with smaller, elliptical spots have low SI values while males with large, irregularly shaped spots have high SI values. For each male in the experiment, the length (L), height (H), and area (A) of the dorsal abdominal spots was measured in pixels. The spot shape index (SI) was calculated using the formula $SI = A / (\pi * 1/2L * 1/2H)$ (Izzo & Tibbetts, in review). This index is size-independent. For the control trait, dorsal thoracic stripes, the area (in pixels) was measured, and this measurement was size-corrected using the head-width (i.e. male’s structural size) of the wasp. Size-correction was done by regressing stripe area on head-width and saving the residuals; the residuals then become the new data points. All photographs were taken using Infinity Analyze software with a camera mounted on a microscope. Photoshop CS was used for all photographic measurements.

To determine whether male dorsal abdominal spots show condition-dependence, data was analyzed using a standard least squares regression model with diet and nest (as a random factor) included as the predictors. Nest was included as a random effect in order to account for within-nest variation. All analyses were run using JMP 8.

Results

Diet treatments had a significant impact on male phenotype and body condition. Males reared on high-caterpillar diets had significantly larger body size than males reared on low caterpillar diets: weight: $p < 0.01$, adj. $R^2 = 0.65$, $n = 76$ (standard least squares, with weight predicted by diet and nest as a random factor); head-width: $p = 0.002$, adj. $R^2 = 0.51$, $n = 76$ (standard least squares, with head-width predicted by diet and nest as a random factor).

The male dorsal abdominal spots that function as a sexually selected signal also exhibit condition-dependence. Males reared on high caterpillar diets signal higher advertised quality (smaller SI) than males reared on low caterpillar diets: $p < 0.01$; diet $p = 0.0227$, adj. $R^2 = 0.507$, $n = 76$ (standard least squares, with SI predicted by diet and nest as a random factor; Figure 4.3). SI is a size-independent measure, yet to demonstrate that the increase in male size on high caterpillar diets was not responsible for the condition-dependence of SI, I ran a model to test whether SI was predicted by both diet and size (with nest as a random factor). The overall model retains significance yet does not perform very well, and size does not explain the variation in male signals: overall model $p < 0.001$, adj. $R^2 = 0.05$; SI is diet-dependent ($p = 0.04$), yet unrelated to size (head-width: $p = 0.76$). Therefore, the male spots exhibit condition dependence and this is not due to the increase in male size.

However, the control traits, male thoracic stripes, did not exhibit condition-dependence. There was no difference in the size-corrected area of stripes for males reared on high-caterpillar versus males reared on low-caterpillar diets:

$p=0.98$, $n=76$ (standard least squares, with the stripe area head-width residuals predicted by diet and nest as a random factor; Figure 4.4).

Discussion

The strong influence of diet treatment on signal expression shows that the sexually selected dorsal abdominal spots of male paper wasps exhibit condition dependence. Males reared on high-caterpillar diets, where protein was not a limited resource, developed spots with smaller SI than males reared on low-caterpillar diets, where protein and fat were limited resources. Males with small SI have previously been shown to win more agonistic bouts and are more preferred as mates than males with larger SI. Now I demonstrate that this difference in spot shape and size is condition dependent, and fits predictions for the development of sexually selected signals. Further, non-signaling control traits comprised of similar pigments do not exhibit condition dependence. This comparison demonstrates that male phenotype does not simply show correlated changes due to natal protein availability. Instead, condition dependence of the sexual signal is highly trait specific.

Because the sexually selected signal exhibits heightened condition dependence as compared to non-signaling traits, the sexual signal conveys information regarding adult condition that is dependent on the natal developmental environment. Information about male condition may be particularly useful for females assessing potential mates. Natal nutrition impacts a variety of physiological functions, including a male's ability to sequester

compounds for spermatophore provisioning (Boggs 1990; Gwynne and Brown 1994). As females mate in the fall and hibernate prior to initiating nests in the spring, it is especially important to have high-quality sperm (and well-provisioned spermatophores) from mates. Females must store and utilize sperm for up to 11 months in the future, and there are no opportunities for re-mating in the spring (males do not overwinter) (Turillazzi and West-Eberhard 1994). Therefore, female fitness is nearly entirely dependent on male spermatophore quality, which in turn is dependent on male natal nutrition.

A noteworthy strength of these data stems from the use of our particular control trait. Many experiments on condition dependence fail to utilize proper control traits, which is problematic because in order to determine if a sexually selected signal provides information regarding individual quality, it needs to be compared to a non-signaling trait. It is not sufficient to simply measure the condition dependence of the sexually selected signal alone, as any trait may exhibit some degree of condition dependence. Therefore, sexually selected signals must show heightened condition dependence in relation to non-signaling traits (Cotton et al. 2004). I have measured whether a non-signaling color pattern comprised of the same pigments as the sexually selected signal exhibits condition dependence, and found that diet treatment does not impact the size of the non-signaling thoracic stripes, indicating that the control trait is not condition dependent. Therefore, the development of the non-signaling thoracic striping is independent of the sexually selected dorsal abdominal spots. This further

strengthens the findings of condition dependence of the sexually selected spots, as it demonstrates that there is not simply an overall change in male phenotype.

An interesting aspect of this signaling system in particular is that the condition dependent signal is comprised of melanins and pterins. These pigments lack obvious production costs, especially in this system: wasps are entirely covered in the same yellow and black pigments. Further, since our control trait is comprised of similar pigments to the signal, the basic assumption would be that both color patterns could be under the same developmental control. There is no difference in relative cost between either pattern, yet only the signal's developmental pathway is linked to natal nutrition. By careful selection of similar traits, one with a signaling function and one without, I was able to explicitly demonstrate that sexual signals are sensitive to environmental influence while non-signaling traits are not, regardless of the presence (or absence) of obvious production costs. This lack of obvious costs has lead many to the assumption that these pigment types would function at best as poor indicators of quality. However, quality information is not dependent on production costs—condition dependence itself is the mechanism linking signals to quality information.

Although previous work has suggested that ornament pigmentation is linked with condition dependence (Griffith et al. 1999; Siefferman and Hill 2003; Siitari et al. 2007), my results add to a growing body of literature indicating that the information conveyed by a signal has a more important influence on condition dependence than the pigment that composes the ornament. Despite the belief

that production costs are required for condition-dependent quality signals, similar results of heightened condition dependence of non-costly ornaments have been seen in other taxa, such as the white plumes of juncos and flycatchers (Torok et al. 2003; McGlothlin et al. 2007), the structural (i.e. UV) coloration in birds and butterflies (Kemp and Rutowski 2007; Siefferman and Hill 2007; Peters et al. 2008), and the melanin-based status badges of female paper wasps (Tibbetts and Curtis 2007) and some birds (Hegyi et al. 2007; Roulin et al. 2008). This growing body of work demonstrates that multiple pigment types, regardless of their level of production costs, can be used to signal condition and quality (Griffith et al. 2006).

But the question remains as to what costs maintain signal honesty? Other costs, such as social costs or maintenance costs, may suffice (Szamado 2010). For example, the status badges of female paper wasps are kept honest through social costs; inaccurate signalers are punished with high levels of aggression from conspecifics (Tibbetts and Dale 2004). These costs are not well understood and quantifying both types can be difficult. Nevertheless, they may be important in maintaining signal honesty over evolutionary time (Szamado 2010). Clearly, the particular costs of “non-costly” signals require further investigation, but the question of how honesty is maintained in the system should not be confused with the question of whether or not a particular trait (or pigment type) can adequately convey quality information.

In summary, I have demonstrated that a sexually selected ornament comprised of pigments thought to lack production costs exhibits condition

dependence, and can function as a signal of quality. While it is widely accepted that sexually selected signals are highly condition dependent, there are many flaws in the empirical support of this belief. Many studies lack the control traits necessary to demonstrate a heightened level of condition dependence of the sexual signal, and other studies did not explicitly manipulate condition. Therefore it is difficult to be certain of the actual ubiquity of condition dependence of sexual signals (Cotton et al. 2004). The data presented here overcome these problems, and show that male paper wasps can provide quality information through sexually selected signals while simultaneously adding to the growing body of evidence that pigment signals lacking obvious production costs can function as good indicators of individual quality.

Figures and Figure Captions

Figure 4.1: Array of male dorsal abdominal spots, arranged from low to high SI. Males with low SI are more dominant SI to, and are more preferred as mates, than males with high SI.



Figure 4.2: Dorsal view of male wasp, showing the location of control traits (thoracic stripes, top circle) and male ornaments (dorsal abdominal spots, bottom circle).



Figure 4.3: Male SI is condition dependent, with males reared on high protein diets having significantly smaller SI than males reared on low protein diets ($p=0.0227$, standard least squares regression). Star denotes $p < 0.05$. Low SI advertises high quality, and males with spots with low SI are more dominant and more preferred as mates than males with high SI. Points have been jiggered for easier viewing. The boxes show the upper and lower quartiles, while the whiskers represent data that falls within 1.5 inter-quartile range (IQR).

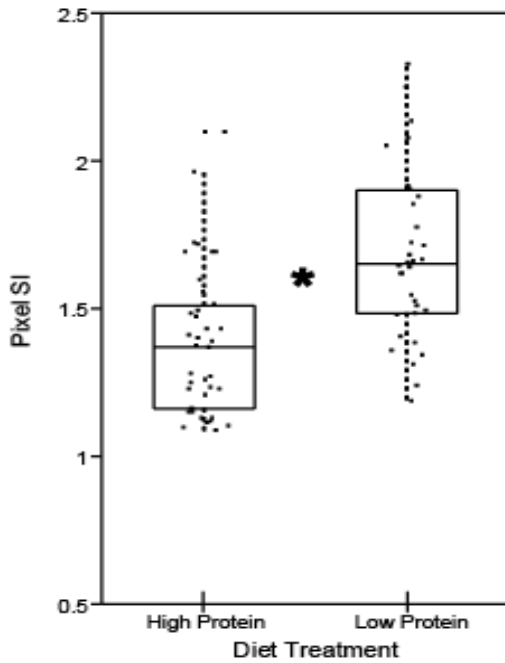
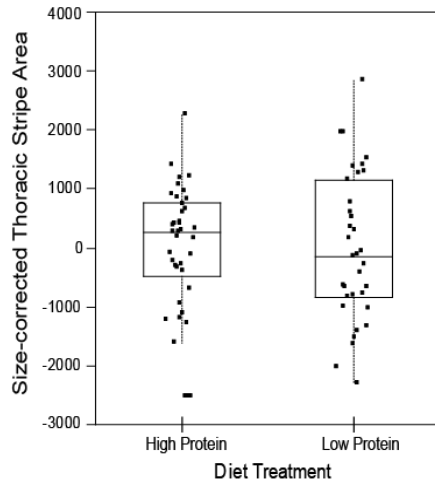


Figure 4.4: The control trait, the thoracic stripe, is not condition dependent. There is no difference in the size-corrected area of the stripe between high protein and low protein diets ($p=0.98$, standard least squares regression). Points are jittered. The boxes show the upper and lower quartiles, while the whiskers represent data that falls within 1.5 inter-quartile range (IQR).



Chapter V: Direct benefits to female choice

Abstract

The adaptive nature of female choice is no longer the contentious issue it once was, as a prolific body of literature exists documenting the selective power of this phenomenon (Andersson 1982; Møller 1988; Zuk et al. 1990; Wilkinson and Riello 1994; Jones et al. 2000). Nevertheless, questions remain regarding why females have strong preference for ornamented males in lek-based mating systems. As females receive nothing other than seminal fluid, “good genes” are often cited as the main benefit to choice, yet they have been difficult to demonstrate empirically (Moeller and Alatalo 1999). Here we provide an alternative explanation for the benefits of choosing ornamented males by showing that females receive direct benefits through male seminal products on leks. Females of lekking *Polistes* paper wasps have increased hibernation success when mated to preferred males as compared to those mated to non-preferred males. While it is known that ejaculates can manipulate female behavior and physiology (Cordero 1995; Chapman 2001), previous work has not tested whether females choose males whose ejaculates can provide increased benefits, or whether male ornaments correlate with ejaculate quality. We propose that ejaculate-based benefits may be a common mechanism underlying female choice across non-economic mating systems.

Introduction & Results

Female choice for highly ornamented males is widespread, though the benefits of female mate preference remain controversial in many species. It may be relatively obvious why particular males are preferred when males provide resources or parental care; for example, female house finches benefit by choosing males with redder plumage because red plumage coloration is associated with increased male parental care (Hill 1991). However, in many systems with strong female preference, males provide nothing other than sperm and the accompanying ejaculates. What do females get by mating with preferred males? Indirect benefits of choice, such as good genes, Fisherian benefits, or mate compatibility are frequently cited as the basis for female choice in these systems (Fisher 1915; Hamilton and Zuk 1982; Tregenza and Wedell 2000), but evidence of indirect effects is weak (Moeller and Alatalo 1999). Until now, male ejaculates have not been examined as sources for choice-based benefits.

Male ejaculates contain chemicals that have the potential to dramatically influence female behavior and physiology. For example, male accessory compounds in houseflies and *Drosophila* reduce female receptivity and attractiveness to future mates, and can even induce oviposition (Leopold et al. 1971; Leopold et al. 1971; Xue and Noll 2000). Conversely, ejaculates may also benefit females: in many lepidopterans, males transfer nutrients and other compounds used for egg provisioning (Boggs and Gilbert 1979; Bezzerides et al. 2004), and mating positively impacts female longevity in the ant, *Cardiocondyla obscurior* (Schrempf et al. 2005).

Even though male seminal products can manipulate females, it is unclear whether male ornaments provide information about male seminal characteristics. Such a link could provide an important benefit for females who choose ornamented males. Here, we test whether females receive direct benefits through male ejaculate compounds in a lek-breeding paper wasp *Polistes dominulus*. Females use variable abdominal spots to choose males; males with smaller, more elliptically shaped spots are preferred by females (Izzo & Tibbetts, in review; Fig. 5.1). Females receive nothing other than sperm from males. After mating, females hibernate before emerging the following year to build nests and produce offspring.

We test whether females derive direct benefits in the form of hibernation success from mating. We also test whether survival benefits are associated with male advertised quality such that females that mate with preferred males survive better than females that mate with less preferred males. Females were randomly assigned to mate with males of varying advertised quality (as assessed by male spot morphology), and then placed into an environmental chamber for overwintering. Survival was significantly correlated with mating status, such that mated females survived longer than unmated control females, $p=0.004$ (survival analysis, $n=299$, $X^2=8.3023$, $df=1$; Fig. 5.2).

To further examine all factors that may be involved with female survival, a standard least squares regression model was created which included female weight, female facial pattern (previously identified female signals of quality (Tibbetts and Dale 2004)), mated status, and all possible two way

interactions, as well as the random factors of nest and hibernation cup; all non-significant interactions were dropped from the model. The final model shows that female weight and the interaction between female weight and mated status were significant predictors of survival (overall model $P=0.001$, $n=299$, adj. $R^2=0.66$). Large size is an important parameter as females require sufficient fat stores to survive hibernation (female weight: $P<0.001$). The significant interaction term of female weight and mated status ($P=0.014$) shows that mating itself confers a survival advantage, but is size dependent. In other words, there is a larger effect of mating on female survival among large females than small females. Small females will not survive the winter regardless of mating, yet as females gain sufficient fat stores, ejaculate compounds will increase the chances of survival further. Mated status alone was not sufficient for survival, as small females simply do not have the nutritional stores to overcome the stress of hibernation (mated status $P=0.64$). Female quality alone (facial pattern $P=0.49$) did not significantly influence survival, nor did the interactions of weight with female quality ($P=0.09$) or mated status with female quality ($P=0.88$) and were dropped from the model. Clearly, female size is the most important parameter for hibernation survival as survival is dependent on adequate fat storage. However, mating itself confers an advantage for females of sufficient size, while female advertised quality is not an important indicator for female hibernation survival.

Remarkably, within mated females, there was a significant relationship between male advertised quality and female survival. Females mated to males with smaller, more elliptically shaped abdominal spots (i.e. males that are more

highly preferred) survive longer than females mated to males with larger abdominal spots (i.e. males that are not preferred). When male advertised quality is categorized as “high” or “low” (high advertised quality males categorized as those with Spot-shape Index (SI) values that fall below the population mean SI, and low quality males categorized as those with SI values that fall above the population mean SI), a survival analysis shows that quality is positively and significantly related to survival: $p=0.019$ ($n=60$, $X^2=5.483$, $df=1$; Fig. 5.3).

To examine how the continuous variation in male quality impacts female survival, a standard least squares model was created using male SI, female weight, female facial pattern, and all two-way interaction terms, with nests of origin and hibernation cup as random factors. All non-significant interaction terms were dropped from the model. The final model includes male advertised quality (SI), female weight, and female advertised quality (facial pattern), and shows that male advertised quality is important for female survival (overall model $P<0.001$, $n=60$, adj. $R^2=0.32$). Male advertised quality, the spot shape index (SI) is strongly associated with female survival ($P=0.03$), indicating that male advertised quality correlates with ejaculate quality. Within mated females, female size became less important to female survival ($P=0.1$), and female quality did not predict female survival ($P=0.9$). There were no significant interaction terms, and all were dropped from the final model. The effect of male advertised quality did not depend on female weight (SI x female weight $P=0.2$) or female advertised quality (SI x female facial pattern $P=0.43$). Overall female quality did not affect female survival (female weight x facial pattern $P=0.09$). Females with mates

advertising high quality survive longer than females with mates advertising lower quality. All females were randomly assigned to their mates, so these results indicate that advertised quality correlates positively with ejaculate quality.

Clearly, mating has an important impact on female survival in *Polistes* paper wasps. Mated females showed higher survival than unmated females when body size is accounted for. Within mated females, the quality of a female's mate also has a significant effect on female survival: females mated to males with higher advertised quality have higher survival than females mated to males with lower advertised quality. These findings are important when examining the evolution of female preferences, especially in non-economic systems like those of lekking species. What drives female preference, and what do males advertise with quality signals? These data suggest that females choose males to maximize direct benefits, which here is increased over-wintering survival. Male *Polistes dominulus* advertise quality through the size and shape of their dorsal abdominal spots (Izzo & Tibbetts, in review), and females use these spots when selecting mates. As female hibernation success correlates with male advertised quality, it is likely that male ornamentation is associated with ejaculate quality.

Seminal products have gained increased scrutiny for their role in sexual selection (Eberhard and Cordero 1995). Males transfer not only sperm to females, but also a multitude of other chemicals from their accessory glands. In many instances, the substances found in male ejaculates cause harm to females (Wolfner 2002). This is likely due to the polyandrous nature of the species in which these effects were identified and contrasts sharply with mating behavior of

social insects. The majority of social insects, *Polistes* included, are genetically monogamous (Strassmann 2001) and intra-ejaculate sperm competition does not occur due to the clonal nature of their sperm (Baer and Boomsma 2004). These factors create an expectation of sexual cooperation in *Polistes*, which would cause a predisposition towards beneficial (and not harmful) effects of ejaculates. Across diverse insect taxa, males have been shown to provide females with water, nutrients, and defensive compounds for eggs (Boggs and Gilbert 1979; Sakaluk 1985; Simmons 1995; Lewis et al. 2004). And, not all males are created equal: ejaculate provisioning can be costly to males (Eberhard and Cordero 1995), and individual males differ in their ability to sequester and transfer nutrients and other compounds to the female at mating (Boggs 1990).

Overall, these data are important for understanding the evolution of female mate choice in systems where male ejaculates are important for females. Here we show that female choice for highly ornamented males directly benefits females, as the ejaculates of ornamented males increases female hibernation success. Further, we propose that preferences due to ejaculate-based benefits have the potential to be common, and can act as the mechanism maintaining choosiness in a variety of mating systems. Besides in non-economic mating systems, ejaculate based benefits are likely common in organisms with expected sexual cooperation, such as in many monogamous species (Korner and Schmid-Hempel 2003; Baer and Schmid-Hempel 2005). With the difficulty in identifying and quantifying indirect benefits (Moeller and Alatalo 1999), we suggest that

researchers change their focus from looking for “good genes” effects and instead endeavor to find “good sperm” effects to explain female choice.

Methods Summary

Mature *Polistes dominulus* paper wasp nests were collected during July and August, 2008 from sites around Ann Arbor, Michigan. Newly emerging males and gynes were collected from these nests, housed individually in deli cup containers, and provided with *ad libitum* water and sugar. In total, 300 females were collected from 34 nests. Half of the females were kept as unmated controls, while the rest were randomly paired with an unrelated male and allowed to mate; mating trials were videotaped to confirm copulation. Females were then housed in deli cups in groups of 5 with water, sugar, and construction paper. All deli cups were placed into an environmental chamber on October 16, 2008 for hibernation. The chamber was kept at 80% humidity, and the temperature was decreased from 20°C to a final temperature of 3°C over 4 days. All hibernating females were censused on a weekly basis for survival. Survival was easy to distinguish due to female posture. Living females retain a huddled posture, are able to cling to walls, and have flexible antennae, while dead females fold their legs up and turn belly-up, and have rigid antennae. In March 2009, the temperature of the chamber was increased by 1.5°C degrees daily until all wasps were active (final temp =19°C). All wasps were censused for a final time after the final temperature of 19°C was reached. The total duration of hibernation was

220 days; the end lines of the survival graphs indicate wasps that survived the entire duration.

The advertised quality of males was calculated by measuring their dorsal abdominal spot characteristics, as in (Izzo & Tibbetts, in review). In brief, digital photographs of the left and right spots had their area (A), length (L), and height (H) measured in pixels using Adobe Photoshop CS. The spot shape index (SI) was calculated by $SI = A / [\pi * (1/2L) * (1/2H)]$. The index compares the area of an idealized ellipse found using the average length and height of the spots to the average measured area of the spot. The resulting size-independent value provides a measurement of the shape of the spots: values close to 1 correspond to small, elliptical spots; increasing values correspond to large, irregularly shaped spots. All statistical analyses were performed using Jmp 8.

Figures and Figure Captions

Fig 5.1: Photographs depicting location of dorsal abdominal spots (top panel) and variation in male spot morphology (i.e. the sexually selected ornaments of *Polistes dominulus* paper wasps), arranged from low SI (HIGH quality individuals) to high SI (LOW quality individuals) (bottom panels).

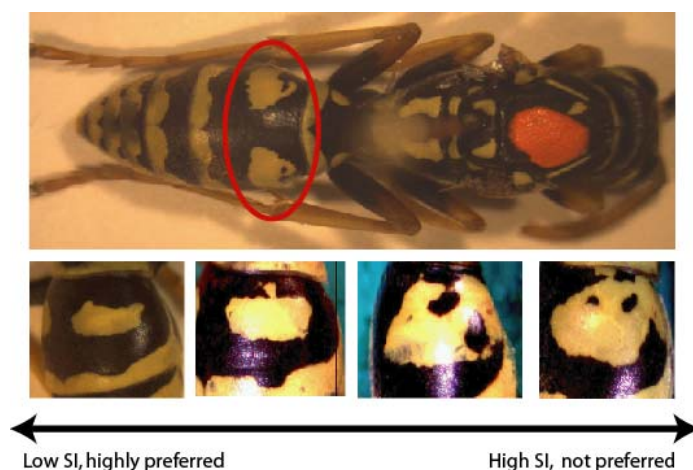


Fig. 5.2: Survival graph of mated (blue line) and unmated (red line) wasp gynes. Mating alone confers a significant survival benefit.

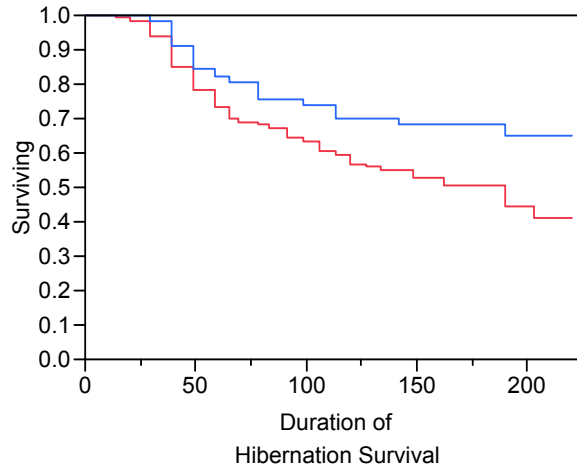
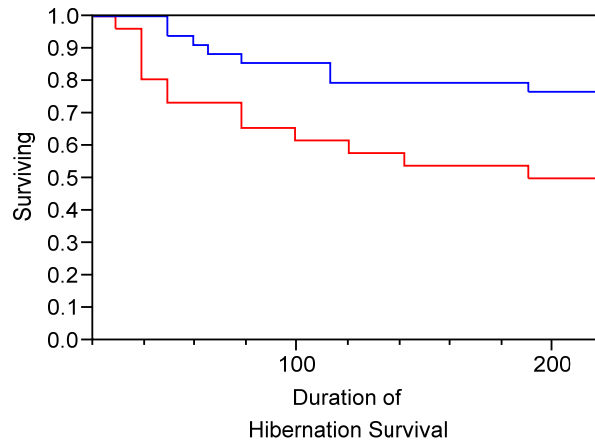


Fig 5.3: Survival graph within mated gynes, comparing females with mates that advertise high quality (blue line) to those with mates advertising low quality (red line). Mate quality is based on analysis of male dorsal abdominal spots.



Chapter VI: Conclusions

Sexual selection has long held the interest of naturalists and biologists, due in large part to the exotic and bizarre adaptations it creates. How organisms compete for access to mates or to entice members of the opposite sex into mating opportunities are not only fascinating, but also some of the most dynamic—and important—processes in nature. Despite a rich history examining the processes of male-male competition, female choice, and their interactions (Alexander and Borgia 1979; Andersson 1982; Arnold 1983; Andersson 1994), it remains difficult to understand how organisms lacking enlarged weaponry or obviously expensive ornaments are able to mediate the costs associated with these competitive interactions, and why females are choosy about mates in situations where males provide them with no tangible resources.

In this dissertation I utilized a series of manipulative experiments to develop paper wasps as a system to address questions surrounding sexual selection of lekking organisms; specifically I address what traits males use as ornaments, the contexts in which these ornaments are used, and how females benefit from their choices of male ornamentation. As one of the few lekking organisms that are tractable for laboratory experimentation, paper wasps are poised to become model organisms of sexual selection. Previous work on social

insects has demonstrated the lekking behavior of paper wasps and hinted at a role of chemical communication for lekking males (Beani and Turillazzi 1988; Beani 1996). Work on *Bombus* bumblebees and *Atta* leafcutter ants has highlighted the potential for interesting dynamics of sperm competition and argued that social insect males should serve as model organisms to study sexual selection (Baer 2003; den Boer et al. 2010). However, until now, no examples of social insect sexually selected signals have been identified, which are necessary for further studies of sexual selection in these organisms. The work presented here is the first in-depth investigation aimed at understanding ornamentation used in male-male competition and female choice in a social insect. Below I summarize the results from my four primary research chapters.

Chapter II: Male cuticular hydrocarbons do not correlate with mate choice, but do correlate with immune response

Social insects rely heavily on chemical communication to mediate multiple aspects of social behavior on the nest. Prior observations noted interesting male behaviors on leks: males appear to “scent-mark” their lekking territories (i.e. perches in mating arenas) (Beani 1996). This behavior has been hypothesized to attract mates through chemical signals (Beani 1996). In this chapter I examined individual male variation in cuticular hydrocarbon profiles to determine whether males use chemical information in sexual selection. While paper wasp chemical communication has been intensely studied (Panek et al. 2001; Sledge et al. 2001; Dapporto et al. 2004; Sledge et al. 2004), all the available information

pertains to females. Previously, the only information known regarding male chemical information is that male profiles are distinct from female profiles (Ryan and Gamboa 1986). I conducted the first analysis aimed at examining the information content of male CHCs, and demonstrated that male CHCs are not likely to be used in sexual selection for mate choice because there is no association between male CHCs and female choice. However, male CHCs do correlate weakly with agonistic ability, suggesting that males may use chemical profiles during agonistic encounters. Further, CHCs correlate with immunity (as measured by encapsulation rate) and to a lesser extent, with endogenous levels of juvenile hormone (JH). This immunity information may be used to regulate social behavior on the nest or during lek-based interactions. Further work is needed to show if and how this information is utilized, but this serves as the necessary starting point for such studies.

Chapter III: Sexually selected signals in male paper wasps

Previous work in *Polistes* paper wasps demonstrated that females are able to utilize socially-mediated visual signals of dominance (Tibbetts and Dale 2004). As paper wasps are visually-orienting predators, and have excellent eyesight, I explored the potential sexual signaling roles of morphological patterns on males. Males possess a pair of highly variable dorsal abdominal spots, with the variation exhibiting sexual dimorphism (males are variable, yet female spots are not). These yellow-on-black spots are highly conspicuous, and their location makes them ideally suited for assessment by females or rival males on leks.

Through correlative experimentation, I demonstrated that variation in these spots is related to both male competitive success and female mating preference: males with small, elliptically-shaped spots are more likely to win fights and are more preferred as mates over males with large, irregularly-shaped spots. Further, manipulative experimentation, where spots were decreased in size with paint, demonstrated that both male and female signal receivers pay attention to spot morphology: males whose spots were experimentally decreased in size won more fights and were preferred as mates more frequently than expected due to random chance. Males whose spots were sham-painted (i.e. a similar amount of paint was applied in the same area, yet spot morphology was unchanged) showed no difference in dominance or mating success than would be expected due to random chance. These results provide definitive evidence that male spots act as sexually selected signals in both male-male competition and for female choice. Yellow dorsal abdominal spots act as male ornaments.

These results are the first to describe a sexually selected ornament in a social insect, and serves as the necessary ground work for developing social insects into a model system for the study of lekking dynamics.

Chapter IV: Condition dependence of male signals

Sexual signals are predicted to exhibit heightened condition dependence as compared to non-signaling traits (Cotton et al. 2004). Through this phenomenon, signal expression is linked to environmental influence and thereby able to provide reliable and accurate information regarding the quality of the

ornament's bearer (Siitari et al. 2007). Interestingly, despite widespread belief of this prediction, rigorously-tested empirical support is weak (Cotton et al. 2004).

In this chapter I explicitly test the prediction that sexual signals, here the male-based dorsal abdominal spots, should exhibit heightened condition dependence as compared to a non-signaling control trait. Males were reared on nests that underwent experimental manipulation of diet: some received restricted amounts of protein while others did not. Male spot size and shape was significantly related to diet treatment, with males on high-protein diets exhibiting the small, elliptical spots indicative of dominant, preferred males. Males on low-protein diets produced significantly larger, irregularly shaped spots. The control trait, non-signaling thoracic stripes (that were comprised of the same pigments as the abdominal spots), did not vary between diet treatments. By utilizing a control trait comprised of the same pigments as the signaling trait, I am able to definitively show that spots are condition dependent while non-signaling traits are not. This serves to enable spots to reliably signal male condition, which in this system is natal nutrition. Further, these results demonstrate independent development of closely related morphological patterns, and shows that male phenotype does not simply show overall correlated changes. An interesting aspect of this system (and indeed this experiment), is that since males are covered in these same yellow and black pigments, male ornaments do not appear to be especially costly. Here I show that contrary to widespread assumption, these non-costly pigments are able to function as informative quality signals (Griffith et al. 2006). This highlights the need to examine traits lacking

obvious production costs and to re-examine our definitions of what costs truly are required for trait honesty.

Chapter V: Direct benefits to female choice

In systems where males provide no tangible resources to females, it can be especially difficult to understand why females are so choosy. The most accepted explanation for the choosiness of females in these resource-less (or “non-economic”) mating systems such as leks is the “good genes” hypothesis (Hamilton and Zuk 1982). This hypothesis proposes that when females choose males based on male ornamentation, they are using those ornaments to select high-quality males, or ones with superior genomes. Despite the widespread acceptance of the good genes hypothesis, empirical support has been rather difficult to produce, and little data exists that rigorously supports the “good genes” model (Møller and Alatalo 1999).

In this chapter I test a previously overlooked idea: that females may in fact be choosy on leks for *direct* benefits. The direct benefits hypothesis proposes that the sperm or seminal fluid from highly ornamented males (or preferred males) must benefit females more than the sperm or seminal fluid from less ornamented/less preferred males. As female paper wasps mate and then must hibernate prior to nest initiation, I tested whether females gain survival advantages due to mating with males that advertise high-quality through their dorsal abdominal spots. I found that indeed, females who were mated to males advertising high quality had a higher probability of hibernation success than

females mated to males advertising low quality, and that overall mated females survived better than non-mated females. These results demonstrate that females gain direct benefits through their choice in ornaments, such that male ejaculates help female survival. Further, they suggest that male quality is correlated with ejaculate quality. This “good sperm” hypothesis may serve as a viable alternative to the “good genes” model: we are just beginning to explore the potential for ejaculate-based effects on females, and this phenomenon may be quite widespread through lekking organisms.

Synthesis

This dissertation utilized an integrative approach to study sexual selection in a social insect, and builds a foundation which can be used to enable paper wasps to become model organisms of sexual selection. Prior to this work, little was known regarding the dynamics of male-male competition and female choice in social insects. The highly competitive nature of social insect mating systems, combined with the small size and local abundance of many species makes social insects ideal candidates for use in sexual selection studies; however, assumptions regarding the difficulties in observing matings, and the widespread (and erroneous) belief that males were nothing more than “flying sperm bags” (Tsuji 1996) resulted in little effort in studying social insect reproduction.

Several important theoretical findings stem from this dissertation. First, my results demonstrate that social insects do experience sexual selection despite lacking obvious ornamentation. Theory predicts that intense competition leads to

intense sexual selection, and leks are epitomized by extreme competition. Due to the seeming lack of extreme weaponry or exaggerated ornaments on social insect males, many researchers assumed that sexual selection was weak or absent in social insects. Here, I demonstrate that we don't in fact have a paradox of high mating competition and a lack of quality signals: ornamentation is present in paper wasps, and is used to mediate both male-male competition and female choice.

Next, my work lends support to a relatively recent argument: that seemingly "non-costly" pigments (i.e. those lacking production costs) can in fact function as informative and accurate quality signals (Griffith et al. 2006). Much work on ornamentation focused on those traits that were expensive to produce or acquire, and theory argued that pigments (or traits) that lacked those costs could not provide much information regarding individual quality, and were less likely to exhibit condition dependence than more obviously costly traits. However, recent observations of organisms using melanins, pterins, structural colors, or even depigmented white patches as condition-dependent quality signals (Torok et al. 2003; Kemp and Rutowski 2007; McGlothlin et al. 2007; Peters et al. 2007; Siefferman and Hill 2007; Hegyi et al. 2010) has begun to (slowly) change popular opinion. My work adds to the growing evidence that these signals can in fact accurately reflect quality. While we have a poor understanding of what, if not production costs, then maintains signal honesty, we now have the impetus to start searching for the answers.

Finally, my dissertation work has provided a novel hypothesis for the maintenance of female choosiness in non-economic systems. In many systems, we lack adequate explanations of why females are choosy. By demonstrating that females do in fact directly benefit from the seminal products of males, I was able to put forth a new theory: that perhaps females are selecting males based on “good sperm” that will be more immediately beneficial (to the females themselves) instead of the “good genes” effects for their offspring. Perhaps ornament quality is indicative of ejaculate quality, and females select males with superior ornaments because those males will have the most beneficial ejaculate compounds. These effects have the potential to be widespread across lekking organisms, and may in fact be found in many types of mating systems; we are just beginning to understand the complex nature of male ejaculate substances and their myriad effects on female behavior and physiology (Chapman et al. 1995; Cordero 1995; Civetta and Clark 2000; den Boer et al. 2010).

Future directions

The results presented here serve as the necessary ground work for paper wasps to serve as model organisms for sexual selection and the study of lekking dynamics. Multiple aspects of paper wasp biology not studied here make them interesting candidates with which to study sexual selection. The haplo-diploid nature of social insects has several ramifications: first, males produce clonal sperm, which eradicates intra-ejaculate sperm competition. This sets up the ability for interesting dynamics for sperm competition if females mate multiply,

and can make these insects quite useful for looking at patterns of cryptic female choice. Therefore, social insects can be developed as models for testing hypotheses on sperm competition and cryptic female choice. Second, the interesting effects on relatedness and genetic structure within familial lines will change the effects of male-female interlocus conflict. Males fitness benefits by the production of daughters, as his sperm will not be utilized to make sons. Therefore, it is not expected that male genomes would carry alleles that would harm females or provide greater benefits to males, as is seen in many diploid systems (Andres and Morrow 2003; Stewart et al. 2005). These traits combine to make social insects excellent models to compare with drosophila (which are used to study intralocus conflict), to determine how trait evolution should occur in the absence of conflict. Finally, males produce no sons, but do produce grandsons: this makes it difficult to visualize how male ornaments would get passed down, as clearly the “sexy sons” hypothesis doesn’t work (Fisher 1915). However, perhaps there is selection for “sexy grandsons.” After all, while selection may not be very strong, it does need to reach into future generations in order to be effective! While there are no details for a model of this type yet worked out, it would be able to operate in much the same manner as x-linked inheritance (T. M. Connallon, personal communication). Clearly, apart from the interesting facets of paper wasp reproductive biology highlighted in the dissertation, there are many more reasons that would make these species interesting models for sexual selection.

In terms of the work started in this dissertation, there are multiple avenues on which to follow up. In particular, chemical communication in males requires great deal of attention. My data suggests that males may utilize chemical information regarding agonistic ability; much work on the precise nature of the information conveyed, as well as to determine whether or not individuals actively pay attention to chemical information is needed. Further, the strong relationship between chemical information and immunity is rather curious: is this simply a physiological side-effect, or do males actively signal immune function? Should colony members perceive and respond to immunological information, it could be interesting to test the dynamics of disease transmission in a group, or to use paper wasp colonies as “real-life” models to determine how groups minimize the spread of infection within a group. Finally, there are many more aspects of male chemical information to examine, both in terms of potential signaling roles of the CHC profile and in terms of non CHC-based compounds; the correlations studied in this dissertation are in no way an exhaustive list.

Finally, as mentioned previously in my work, paper wasps can serve as valuable models with which to study lekking dynamics. I have shown their tractability for laboratory experimentation, and have an appendix of observations of wild behavior. Many lekking organisms are not tractable for observation or experimentation (as they are large bodied, rare in nature or endangered, found in exotic locations, etc), so a small and abundant insect with easily observed behavior is a valuable resource. Additionally, technology is growing at a rapid rate, and will greatly facilitate natural observations: specifically, RFID tagging

would be an incredible tool to use to understand the individual interactions on a given lek. While the technology is growing, the cost is decreasing, and it will soon be feasible to tag and follow every individual on multiple leks. This will allow unparalleled information regarding specific interactions and encounters, greatly illuminating the dynamics of lekking individuals, such as: how signal use relates to success on a lek; how groups form/who individuals lek with; how social selection shapes male/male alliances or interactions; how many males females will sample before mating; how many leks females visit or how far females will travel to find leks; how long particular males participate on a lek and if males travel amongst leks; and many others.

Appendix: Notes on natural behaviors (lekking)

Background on Lek-based mating systems:

When it comes to mating, many animals employ exaggerated courtship methods to entice mates. However, one of the most bizarre and perplexing strategies seen in the animal kingdom is lekking. A 'lek' is a group of males that defend small, resource-less territories in close proximity to one another. The purpose of the territory is to function as a display arena, where males perform to attract mates. Females wander through leks, observing and assessing potential mates; typically one male is the most impressive, and obtains the majority of the available matings on a lek (Krebs and Davies 1993).

Leks have baffled researchers for many reasons. First, if most males do not obtain even a single mate, why join the lek at all? One proposed reason for lek formation has been that multiple males are necessary to attract and excite females (Beehler and Foster 1988); but why should males cooperate to attract females for another male? Is it the only opportunity available, or do they simply make the best of a bad situation? In many species leks are often parasitized by satellite males, and in some species multiple strategies exist for lekking versus non-lekking males (Krebs and Davies 1993). But regardless, it is not clear why inferior males would choose to lek.

From the female standpoint, it is simultaneously difficult and obvious why lekking is beneficial. Females are able to assess multiple males in one location, and should therefore expend less energy and time locating mates (Krebs and Davies 1993). The perplexing aspect of females on lekking arises from female choosiness: males provide no parental care, no resources—nothing other than sperm. So why are females so choosy on leks? It is thought that females gain genetic benefits (“good genes”) and more fit offspring by selecting the “best” male among the lekking males. However, it has been extremely difficult to identify tangible fitness benefits (Moeller and Alatalo 1999), and direct benefits may be overlooked in many systems (see chapter IV).

Finally, the most debated issue surrounding leks centers on the maintenance of genetic variation. As female preference on leks is characterized by consistent, strong, and directional choice, what maintains genetic variation in male traits? Currently the most widely accepted answer is that sexually selected traits have greater amounts of variation than previously thought, and most ornaments have multiple genes (Pomiankowski and Møller 1995). Combined with the condition-dependence of most sexual traits (Rowe and Houle 1996; Kotiaho et al. 2001), it does not seem likely that female choice can purge the variation in male traits.

Lek-based mating systems are taxonomically diverse, yet relatively rare. In other words, leks are found throughout numerous taxonomic groups, but lekking is not the predominant mating system in any. Interestingly, within social insects, a large number of species utilize lek-based mating (Beani 1996). *Polistes*

paper wasps lek, are widespread, abundant, and tractable for experimentation, making them ideal subjects to study the dynamics of lekking. Unfortunately, sexual selection and reproductive biology are woefully understudied in paper wasps (and in fact most Hymenopterans).

Here, I spent time in the field collecting observations of paper wasp mating behavior. As little information exists regarding lekking dynamics in paper wasps, it was of interest to determine whether matings and male-male interactions could easily be observed. The purpose of these observations was to verify that male behavior seen in the lab corresponds to natural behaviors; in other words, do dominance contests and female choice bouts in laboratory settings match the types of interactions in which wild wasps engage?

Paper wasp lekking

Paper wasps have been observed to have lek-based mating systems, and reproduction in the wild has been predominantly studied in Italy. It is notoriously difficult to observe matings in the wild (Beani), due in part to the long period of assessment females spend before selecting mates. In Italy, males in the wild have been shown to utilize two strategies: the predominant strategy appears to be lek-based territory holders, with an alternative strategy of transient males (Beani). However, without detailed information with which to compare fitness and genetic basis of these two strategies, it is not possible to categorize them, or to determine whether they are condition-dependent or evolutionary stable strategies.

Observations of paper wasp mating behavior in the wild were collected during the late summer/early falls of 2008, 2009, and 2010. Observations were collected at the Matthai Botanical Gardens in Ann Arbor, MI, various Metroparks in Washtenaw County, and Oakland University in Rochester, MI.

During 2008, observations occurred at Oakland University, twice weekly during September. Each observation lasted for 2 – 3 hours, with one observation occurring at 1 pm each week and the second observation occurring at 4 pm each week. 8 Nests had males painted with colony-specific marks; two nests had 45-50 males marked, one nest had 37 males, one nest had 25 males, three nests had 10 males, and the remaining nest had 12 males marked. During the observation periods, males from three of the marked nests were no longer present in the nestboxes, and could not be located. Until after September 20, females were still present on most nests in the area, and males did not engage in lekking behavior. After September 20, males began lekking in abandoned nestboxes during favorable weather (see below) as well as on a western-facing top corner of an abandoned laboratory building.

During August 2009, 10 nests from Oakland University were marked (all males present on the nest were marked with colony-specific marks). Observations began late September, and continued twice-weekly for the following three weeks; observation periods occurred from 2 – 5 pm. Marked nests contained 34, 25, 25, 23, 16, 15, 14, 13, 7 and 4 males at the time of marking. While most males were still present on nests during observation periods (including many unmarked males, which may have emerged from pupation after

marking occurred), few male groups actively lekged during the observation periods (due to unfavorable weather conditions).

During 2010, observations occurred at the Matthai Botanical Gardens and Hudson Mills Metropark during September and October. Observations began at Matthai on September 22 and ended October 15th, with a total of 5 observation periods at Matthai, and 2 observation periods at Hudson Mills; each period occurred between 1:30 and 5:30 pm. At Matthai Botanical Gardens, 1 lek with up to 5 individuals was found, as well as numerous single males on goldenrod. At Hudson Mills Metropark, 3 leks of *Polistes fuscatus* were located; one consisted of roughly two-dozen males, the other two were under 10 males.

Timing of Lekking

Male paper wasps form leks in mid-August, and continue lekking until the first hard frost of the year, typically in mid-October (final observations occurred September 30, 2008, October 16, 2009, and October 15, 2010). Males are sexually mature at about 7-10 days post emergence (Giannotti 2004), and once they leave the nest to go to lek sites, do not return to the nest. Males visit the same lek repeatedly, and it appears that males are permanent residents on a lek; most lek sites contain crevices or areas males may use to spend the night.

Lekking males become active on the lek during the afternoon, typically after 1-2 pm, and remain active until dusk. The late afternoon sun is preferred, with most lek sites occurring on western-facing buildings, roof peaks, etc. Males are only active during sunny conditions (temperatures above 75°F) with mild wind; males do not actively lek during overcast, windy, or cooler days.

Lekking sites

Males lek on landmarks, typically roof peaks, pavilions or outhouse-type buildings in parks, corners of buildings, and tall flowering trees. Male leks form near nesting locations, but do not appear to occur on or near resources needed by females. All leks located were found on buildings that were isolated in grassy fields, or flowering trees that were noticeably taller than the surrounding shrubbery. At Oakland University, males aggregated in abandoned nestboxes, which were tall landmarks in the middle of grassy fields. Male aggregations occurred in the box which previously housed their own nest, or the neighboring nestbox (all females were no longer on the nest). Single males can be found on goldenrod, and goldenrod appears to grow in the vicinity of many leks.

Lekking partners/cooperation

Observations at Oakland University suggest that males primarily lek with brothers. Males were marked with nest-specific markings during July or August, prior to leaving the nest to begin lekking. Over 2 years, a total of 375 males from 18 nests were marked. Leks/male aggregations were comprised of nestmates, with an occasional male from a neighboring nestbox (which due to female site philopatry, is likely a cousin or other distant kin). Males within an aggregation typically contained the same paint mark, with 6 – 35 males in the aggregation; in 3 of the observed nestbox aggregations (out of 12 nestbox aggregations), 2-3 males bore paint marks from a neighboring nestbox. Unmarked males were found in all nestbox aggregations (up to another 12 unmarked males), however, these males could easily have been nestmates that pupated after colonies were

marked. Males were not observed to fight in these nestmate associations, but fights could have occurred prior to observation periods. Additionally, not all marked males were observed in the nestbox aggregations, so males may have left to join distant leks. Leks comprised of closely related males such as brothers would benefit the males, as lower-quality males would gain indirect fitness through the success of their higher-quality brothers. Whether leks at other locations are comprised of brothers is unknown, as only males at Oakland University were able to be marked on the nest.

Recognition

There is some observational evidence to suggest that males utilize chemicals to recognize kin. An aggregation of brothers at Oakland University was observed to cooperatively attack an intruding male, who bore a paint mark from a distant nest. Upon landing in the aggregation, the 4 related males attacked the intruder, by biting and grappling with him. The intruder flew off, and aggression ceased among the remaining (related) males. A female approached this same aggregation of males, and the males made an attempt to grab and copulate with the female. The female was from the same nest, and once a male had grabbed and antennated her, she was released and unmolested. Therefore, it seems likely that males use cuticular hydrocarbon profiles to identify kin and non-kin, both to attack intruding males and to avoid incestuous matings.

Leks Observed

Despite the abundance of *Polistes dominulus*, the species on which my dissertation focuses, it was relatively difficult to locate active male leks. During

September of 2010, a lek was located on the roof of a pavilion in the Matthai Botanical Garden. Up to 5 males were observed on the roof, with one male consistently patrolling the top, western-facing roof peak. 3-4 males would patrol the lower roof peak during the late afternoon. Males attempting to patrol the top peak were consistently chased off. These males were captured for use in an experiment (a “create-a-lek” experiment), and no matings were observed.

Conversely, a native species of paper wasp, *Polistes fuscatus*, had more abundant and easily found leks. At the Hudson Mills Metropark in Dexter, MI, several large leks were observed in October 2010. The largest lek consisted of roughly 2 dozen individuals patrolling the roof and western wall of a men’s outhouse building. The building was in a clearing/large field, neighboring deciduous forest and patches of goldenrod. The building provided ample areas to overnigh (wall panels were loose, and males were observed to crawl out of the spaces in the morning), and neighbored several park buildings with nests on them. By 2 in the afternoon, the lek was very active, with at least 12 individuals actively patrolling at any given time. Males were observed to engage in aerial grapples and fights, where they would tumble to the ground. After each fight, one wasp would resume patrolling around the roof, and the other (presumably the “loser”) would fly off. Males also engaged in grapples and aggressive behavior on the walls of the building. Within one hour of observation, two attempted matings were seen: in both cases, females sat perched on the wall and males approached. The wasps antennated one another, the male attempted to mount

the female, and the female aggressively rejected the male then flew away. These *P. fuscatus* leks were active, dynamic, and 3-dimensional in nature.

Problems with lek observations/failed experiments

Lekking is inherently difficult to study, as active leks are typically 3-dimensional in nature and large in scale (so video-taping is problematic). Tagging individuals with RFID tags would greatly facilitate observation, as individuals are small and may be high in the air. When weather conditions are not ideal, males are quite inactive, so it can be difficult to locate and observe active leks.

Experimentation repeatedly failed with the focal species (*Polistes dominulus*) due to the difficulties in finding tractable leks to work with. In several years, wasp populations were low due to difficult winters with repeated freeze/thaw episodes in the spring.

Summary

While it was not possible to test how male signals, i.e. the dorsal abdominal spots of male *Polistes dominulus* function in the wild, it was possible to observe male aggression and attempted copulation in the wild (but mostly in a different species). Males engage in fights and aggressive contests in the same manner as observed in the lab, and attempted copulations mirror those observed in lab experiments. Female rejection is more pronounced in the field than in the lab, but nonetheless lends credence to our laboratory protocols. Overall, the hours spent in the field demonstrated that males and females have ample

opportunity to assess lekking males by visual signals, and that chemical cues should be investigated further.

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