ANDRÉ RODRIGUES DE SOUZA

After all, what do wasps want?

The choice of sexual partner in the paper wasps *Polistes*

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Doctor Scientiae*.

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Summary

Abstract

SOUZA, André Rodrigues de, D.Sc., Universidade Federal de Viçosa, May. 2015. **After all, what do wasps want? The choice of sexual partner in the paper wasps *Polistes.*** Advisor: José Lino-Neto. Co-advisors: Carlos Alberto Mourão-Júnior and Fábio Santos do Nascimento.

The choice of sexual partner is of fundamental importance, as there are deep consequences for the animal fitness. This is particularly important when females are genetically monogamic and must store male sperm for a long time and when incestuous copulations may increase probability for the expression of recessive deleterious alleles. Social paper wasps like *Polistes* fit this situation, but surprisingly little is known about how sexual selection operates in this taxon. Here, we examine proximal and ultimate causes involved in the choice of sexual partner in *Polistes* paper wasps. We conducted laboratory assays with *Polistes simillimus* to examine if male visual ornamentation mediates female choice. Also, we examined in the field wether visual ornamentation in *Polistes dominula* males is used to mediate male-male competition. Finally, we examined, under laboratory conditions, the occurrence of intersexual nestmate discimination by chemical cues in *Polistes versicolor*. The results suggest that mate choice is a widespread phenomena in *Polistes*; It is mediated by visual and chemical information; and fitness benefits associated with male individual quality and genetic compatibility are likely candidates to explain the ultimade causes for the evolution of mate choice in these wasps.

Resumo

SOUZA, André Rodrigues de, D.Sc., Universidade Federal de Viçosa, maio de 2015. **Afinal, o que querem as vespas: A escolha do parceiro sexual em vespas sociais *Polistes.*** Orientador: José Lino-Neto. Coorientadores: Carlos Alberto Mourão-Júnior e Fábio Santos do Nascimento.

A escolha do parceiro sexual é de fundamental importância, pois existem profundas consequências de aptidão para os indivíduos. Isto é particularmente importante quando fêmeas são geneticamente monogâmicas e devem armazenar os espermatozoides do macho por muito tempo e quando cópulas incestuosas podem aumentar a chances de alelos deletérios recessivos se expressarem. Vespas sociais como *Polistes* se enquadram nesta situação, mas surpreendentemente pouco se sabe a respeito de como a seleção sexual opera nesse táxon. Assim, Nós realizamos ensaios laboratoriais com *Polistes simillimus* para examinar se a ornamentação visual no macho media a escolha do parceiro sexual pela fêmea. Além disso, nós examinamos em campo se a ornamentação visual em machos de *Polistes dominula* é utilizada para mediar a competição entre machos. Finalmente, nós examinamos, em laboratório, se há ocorrência de reconhecimento intersexual de companheiro de ninho em *Polistes versicolor*. Os resultados sugerem que a escolha do parceiro sexual é um fenômeno comum em vespas sociais; tal escolha é mediada por informações visuais e químicas; e benefícios em aptidão associados à qualidade individual do macho e à compatibilidade genética são prováveis explicações para a evolução da escolha do parceiro sexual em vespas sociais.

Introduction

Intersexual selection is a process in which traits evolve because they improve the probability of an individual to be chosen by the opposite sex as a sexual partner, maximizing its reproductive success. This is one of the two components of sexual selection theory (the other is the intrasexual selection) proposed by Darwin (1871). The sexual preference for specific kind of partners can be easily demonstrated by experimentally manipulating the sexually selected trait (Andersson, 1982; Moller, 1989). However, the biggest challenge is to explain how sexual preferences evolve genetically. For this, a number of mechanisms have been proposed (Andersson & Simmons, 2006). One possibility is that the preference for a partner with a specific ornament evolves as a result of direct benefits of being choosy, given that the ornament may mirrors sexual partner`s individual quality. For example, the ability of males to provide resources such as good quality territory, nourishment, parental care or protection can be associated with its ornamentation, so that females can have direct benefits for choosing males according to their ornaments. Alternatively, the sexual preference for specific traits may evolve as a result of the benefits associated to copulating with a partner whose alleles complement the genome of who is choosing (genetic compatibility). For example, avoid copulation with related partners is adaptive, as incestuous copulations may impose fitness costs by allowing for the expression of recessive deleterious alleles and decreasing the allelic diversity in a gene pool (Fonseca et al., 2000).

To study sexual selection, model species should be suitable for field studies, since the main objective is to understand how sexual selection mechanisms work in the natural environment, where species evolve and acquire their traits. Genetic investigations and well controlled experiments that work only in the laboratory could then be interpreted under a supporting background about species biology, selective pressures and adaptations in their natural evolutive context (Andersson & Simmons, 2006). Sexual behavior in *Polistes* paper wasps (Hymenoptera:Vespidae:Polistinae) can be observed in the field and, at least in temperate places, it is relatively well know (Beani, 1996). Typically, males are produced during the summer, remains for only a few days at the nest and, then, they join to large male aggregations at specific sunny landmarks, where they form the ‘leks’. These male aggregations are visited by females and under these circunstances, copulations may occur. In this sense, leks are truly arenas in which one can observe males competing for reproductive opportunities. Model systems should also have sexual dimorphism, as this is frequently associated with strong sexual selection (Andersson & Simmons, 2006). *Polistes* wasps have many sexually dimorphic traits (Izzo & Tibbetts, 2012). Further, *Polistes* sexual behavior can be observed in big boxes (Beani & Turillazzi 1988), as well as in small glass arenas (Liebit *et al.* 2010; Izzo & Tibbetts 2012). Finally, lek mating systems, observed in many *Polistes* species, is also observed in many vertebrates and, therefore, it is of interest of a many researchers.

Here, we examine proximal and ultimate causes involved in the choice of sexual partner in *Polistes* paper wasps. In the chapter 1, by conducting laboratory assays with *Polistes simillimus*, we examined a potential visual signal in males, starting with identifying sexually dimorphic traits correlated with intra and intersexual selection. Following correlative experiments, we performed manipulative experiments to demonstrate a signalling role of a visual trait. In the chapter 2, we examined the potential for visual signal in *Polistes dominula* males under field conditions, by testing the response of lekking males toward lures (dead males), whose visual traits were experimentally manipulated. Finally, in the chapter 3, we examined, under laboratory conditions, the occurrence of intersexual nestmate discimination in *Polistes versicolor* paper wasps. To this, we promoted mating bouts among related and unrelated partners and compared male and female sexual interest towards each kind of partner.

Chapter 1: Sexually selected visual signals in male *Polistes simillimus* paper wasps*

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*de Souza AR, Júnior CAM, Nascimento FS, Lino-Neto J (2014) Sexy Faces in a Male Paper Wasp. PLoS ONE 9(5): e98172. doi:10.1371/journal.pone.0098172

Sexually selected visual signals in male *Polistes simillimus* paper wasps

Abstract

Sexually selected signals are common in many animals, though little reported in social insects. We investigated the occurrence of male visual signals mediating the dominance relationships among males and female choice of sexual partner in the paper wasp *Polistes simillimus*. Males have three conspicuous, variable and sexually dimorphic traits: black pigmentation on the head, a pair of yellow abdominal spots and body size differences. By conducting behavioral assays, we found that none of the three visual traits are associated with male-male dominance relationship. However, males with higher proportion of black facial pigmentation and bigger yellow abdominal spots are more likely chosen as sexual partners. Also, after experimentally manipulating the proportion of black pigment on males' face, we found that females may evaluate male facial coloration during the choice of a sexual partner. Thus, the black pigmentation on *P. simillimus* male's head appears to play a role as a sexually selected visual signal. We suggest that sexual selection is a common force in *Polistes* and we highlight the importance of this group as a model for the study of visual communication in insects.

Introduction

Males often present conspicuous traits that act as signals of quality mediating competition for reproductive opportunities (Andersson, 1994; Birkhead & Moller, 1998). Thus, nests, exaggerated plumage, bright colors, horns, acoustic and behavioral repertoires are commonly used to convey information about an individual’s ability, such as foraging, fighting, resistance to parasites and good genes (Zahavi,1975; Hamilton & Zuk, 1982; Hill, 1991; Evans & Hatchwell, 1992; Searcy, 1992; Borgia, 1995; Blackwell & Passmore, 1996; Berglund et al., 1996; Fitzpatrick, 1998; Gonzalez et al., 1999; Andersson & Simmons, 2006). These sexually selected signals drive the disputes between males for access to females (intrasexual competition), the female choice of sexual partners (intersexual competition), or both.

Males of many eusocial Hymenoptera are potentially subjected to a high sexual selection pressure (Baer, 2003). For example, in many species, the mating system is based on leks, where there is a high bias in reproductive success among males (Reeve, 1991; Beani et al., 1992; Polak, 1993; Beani, 1996; Coler & Wiernasz, 1997). In addition, females are typically monogamous (Strassmann, 2001), and consequently, the choice of sexual partner may be important. Thus, sexually selected signals are expected to mediate the competition for reproductive opportunities. However, there is a lack of data concerning sexually selected traits in social insects and some researchers have claimed that sexual selection in these insects is weak (Boomsma, 2005). To date, there is only one example of sexually selected signal in eusocial insects. Males of *Polistes dominula* have a pair of yellow spots on their abdomen that act as visual signals used to mediate both the dominance relationships among males and female choice of sexual partners (Izzo & Tibbetts, 2012). Males of this species form leks (Beani et al., 1992; Polak, 1993; Beani, 1996), in which they aggressively compete for dominance positions. Females visit several leks and evaluate males before mating (Beani, 1996). Additional research on the occurrence of sexually selected signals in *Polistes* is motivated because its body coloration is striking, highly variable and sexually dimorphic (Enteman, 1904). Further, in females, visual cues have been used to signal individual identity (Tibbetts, 2002) and individual quality (Tibbetts & Dale, 2004; Tibbetts & Sheehan, 2011; Tannure-Nascimento et al., 2008) and thus, the study of paper wasp coloration is a promising avenue for understanding visual communication in insects. We investigated the occurrence of sexually selected signals in males of the social wasp *Polistes simillimus*. First, we identified three conspicuous, variable and sexually dimorphic male visual traits: black pigmentation on the head, a pair of yellow abdominal spots and body size. Then, we performed behavioral essays in order to test whether each of these traits are associated with dominance relationships among males and/or female choice of sexual partner. Further, we also experimentally manipulated the black pigmentation on the head to test whether females evaluate this male coloration.

Materials and Methods

Wasp Collection and Maintenance

A total of 15 male/female-producing colonies of *P. simillimus* were collected in Viçosa, Minas Gerais state, Southeastern of Brazil (20^{0}48’ S, 42^{0}51’ W, elevation 800 m), between January and April 2013. In the laboratory, the nests were kept in plastic containers of 1 L. Adults emerged in the field (i. e. adults already emerged at the time of colony collection) were frozen. Adults emerged from nests after collection were individualized in plastic containers of 0.5 L and fed with honey *ad libitum*. Virgin males between 7–22 days and virgin females between 10–25 days after emergence were used in behavioral assays and subsequently frozen. Wasps used in a given experimental trial came from locations at least 1.5 km from each other. Each wasp participated in a single behavioral test.

Sexual Dimorphism

We analyzed sexual dimorphism by using adult males and females collected in the field, as well as new adults emerged in the laboratory. Before analyzes, wasps remained frozen at -20 ^{0}C for about 2–10 weeks. Freezing for this period does not change the shape or color of wasps (De Souza, personal observation). We searched for sexual dimorphism in the proportion of black pigment on the head, the morphology of abdominal yellow spots and the head size. These parameters were measured as follow: The head and abdomen of each individual were previously separated from the rest of the body. Images were captured with a digital camera (Canon A-620) attached to a stereomicroscope (Stemi 2000-C). We positioned heads in frontal view and in frontal view slightly inclined downward, and the abdomen in the right and left lateral views (Izzo & Tibbetts, 2012). We then analyzed the images by using Image Pro Plus 5.0 application. The black pigmentation on male's face is concentrated in the upper portion of the head, around the ocelli (Fig. 1.1). It may extend vertically from the line immediately below the antennal sockets to the line above the upper limit of the eyes. Laterally, the black pigmentation may extend to the inner edge of the eye. By using images of the head slightly inclined, we calculated the proportion of black pigmentation in this region, excluding the area of the ocelli and antennal sockets (Fig. 1.2). The pair of abdominal spots was evaluated by using the spot shape index, or SI (Izzo & Tibbetts, 2012). The index considers the width and height of the largest ellipse that can be inserted into the spots as well as the total area of the spots (Fig. 1.3). Thus, SI=A/(Pi*(1/2W)*(1/2H)), where width (W), height (H) and the area (A) corresponds to the mean values obtained for the right and left spot of a given wasp. For each wasp, we measured the maximum head width in front view (Fig. 1.4), as this measure is a strong predictor of body size (Eickwort, 1969).

Association between Male Visual Traits with Dominance and Mating Success

To investigate which traits are associated with male-male dominance and/or female's choice of sexual partner, we conducted (i) dominance trials, in which two unfamiliar males were introduced to each other and (ii) mating trials in which two unrelated males were simultaneously presented to an unrelated female. Both kinds of trials took place in a glass arena (8x8x2 cm) where wasps were allowed to interact during one hour. As there were always two males in each trial, one of them received a small amount of white paint on the dorsal part of the thorax, allowing individual identification. This experimental design is similar to that used to study *P. dominula* males' sexual signals (Izzo & Tibbetts, 2012).

Experimental Manipulation of Black Pigmentation on the Male Face

We tested whether females evaluate the black pigmentation on the male face during the choice of a sexual partner. To do so, the same kind of mating trial was performed (one female and two males), but this time we manipulated the proportion of black pigmentation on the male's face by using brown and black paints (Acrilex H). One male in each of these trials had a portion of his natural black pigmentation on the face covered with brown ink, and to control, he received a similar amount of black ink on a naturally black area. In the other male of the pair, black facial pigment was increased by the addition of black ink on a naturally brown portion of the head, and to control, a similar amount of brown ink was added on the original brown area. As a result, each male of a given pair had a greater or lesser proportion of black pigment in the face (Fig. 1.5), though both received a similar amount of brown and black inks. Wasps were painted one day before being used in the behavioral experiments. This period is sufficient for individuals to become familiar to the presence of ink on their body (De Souza et al., 2012). The experimental manipulation of ornaments to observe the response of the receptor has been frequently used to test whether a trait function as a sexual signal (Andersson, 1982; Moller, 1989; Hebets, 2005).

Behavioral Analysis

A single observer, blind to the males black pigmentation on the head, the morphology of abdominal spots and the body size, directly recorded all interactions between wasps. Aggressive behaviors such as biting, darting and mounting are common among *Polistes* males (Polak, 1993; Polak, 1992; Beani & Turillazzi, 1988) and are used to infer their dominance relationships (Izzo & Tibbetts, 2012). In each dominance trial, the observer recorded the direction and frequency of aggression between males. We categorized the most aggressive male as dominant and the less aggressive as subordinate. *Polistes* females are generally larger and more aggressive than males. Thus, it is expected that they are able to accept or reject mating (Reeve, 1991). In each sexual trial, the observer recorded the frequency of mating between female and each of the two males. We confirmed insemination by the presence of sperm in the spermatheca of the female a day after the behavioral test. Thus, we classified males that inseminated females as chosen and males who did not inseminated females as not chosen.

Statistical Analyzes

We studied sexual dimorphism on visual traits by using General linear models (GLM). First, we run a GLM to see how the proportion of vertex black pigmented is influenced by sex and body size; we also tested whether the spot shape index is influenced by sex and body size; finally, we run a GLM to verify how body size is influenced by sex. To test whether male visual traits were associated with male-male dominance or female mate choice, we performed two separated generalized mixed effects models (GLZ) for a binomial response variable. In one of the models, whether or not a male was dominant was input as the dependent variable, while body size, proportion of vertex black pigmented and spot shape index were entered into the analysis as the independent variables and finally, males' age, painting on the thorax and nest origin were included as random effects. The other model was similar, but the dependent variable was whether or not a male copulated instead of whether or not a male was dominant. We used the non parametric bilateral Fisher's exact test to verify if males experimentally manipulated to have a high or a low proportion of black pigment on the head differed in the probability of being chosen by the female as sexual partners, The same test was used to verify the effect of marking with white paint on the probability of a male being dominant and/or being chosen as the female sexual mate. Descriptive and inferential analyzes were performed by applications SPSS 15.0 and Statistica 12.0. The level of significance was set at 5%.

Results

Sexual Dimorphism

The proportion of black pigmentation on the head was influenced by sex ($F_{1,109}$ = 14.7991, P = 0.0002, N = 113) so that males' black pigmentation is higher and more variable than in females (males = 31 ± 10% (range: 12–64%), females =13 ± 3% (range: 7–19%), Fig. 1.6). Also, the black pigmentation was influenced by body size ($F_{1,109}$ = 22.3330, P = 0.0001, N = 113) and the interaction among body size and sex ($F_{1,109}$ = 12.0893,P = 0.0007, N = 113). It means that body size alone affects the black pigmentation on the head but this effect is different in males and females. Specifically, the proportion of black facial pigment is strongly negatively related with body size of males, but this relation seems to be weak in females (Fig. 1.7). The SI was influenced by sex ($F_{1,109}$ = 17.3797, P = 0.0001,N = 113), so that males had larger and more irregular abdominal spots than females (SI males abdominal spots: 0.013860.0032 (range: 0.0098–0.0244), females: 0.0121 ± 0.0014 (range: 0.0092–0.0156), N = 113; Figs. 2.8 and 2.9). Also, the SI was influenced by body size ($F_{1,109}$ = 17.3277, P = 0.0001, N = 113) and the interaction among body size and sex ($F_{1,109}$ = 17.1664, P = 0.0001,N = 113). It means that body size alone affects SI but this effect is different in males and females. Specifically, the SI is strongly negatively related with body size of males, but this relation seems to be weak in females (Fig. 1.10). Finally, body size is affected by sex ($F_{1,109}$ = 45.32, P = 0.0001,N = 113), so that males are smaller than females (males head size = 3.46 ± 0.13 (range: 3.11 – 3.71), females head size = 3.58 ± 0.13 (range: 3.24 – 3.74), Fig. 1.11).

Association Between Sexually Dimorphic Traits With Male Dominance And Female Choice

Aggressive behaviors were observed in 16 of 39 trials with unmanipulated males. In each trial, aggression between males was low and always unidirectional. Dominant males exhibited 0 – 5 darts and 0 – 3 bites on subordinate males. Dominance relationship among males was not associated with the proportion of black pigment on the head ($X^2 = 0.2627$, $P = 0.6082$, $N = 16$), the irregularity of the abdominal spots ($X^2 = 0.1390$, $P = 0.7091$, $N =16$) or the head size ($X^2 = 0.2786$, $P = 0.5975$, $N =16$). During the unmanipulated mating trials, aggressive interactions between males were not observed. Copulations occurred in 10 out of 44 trials. Females rejected males in different ways: when males tried to mount, females flew to another part of the arena or bite and/or stung males, yet some males were able to mount females. In this case, females moved the abdomen, avoiding genital contact. Alternatively, females accepted copulation by allowing genital interlocking. In each sexual trial, we observed 0 – 4 copulations, each lasting 6 – 20 s. Females copulated with only one of two males available in each trial. Copulations were always associated with sperm transfer as verified by checking the spermatheca. The female choice of sexual partner was associated with the male proportion of black pigment on the head ($X^2 = 9.4800$, $P = 0.0020$, $N = 10$), so that chosen males have bigger black spots than non chosen ones (Fig. 1.12). Female choice of sexual partner was also associated with male SI ($X^2 = 8.1154$, $P = 0.0043$, $N = 10$), so that chosen males have bigger yellow abdominal spots than non chosen ones (Fig 2.13.), and with the interaction between SI and black spots on the head ($X^2 = 9.6442$, $P = 0.0018$, $N = 10$). Head size was not associated with female choice ($X^2 = 0.3278$, $P = 0.5669$, $N = 10$).

Experimental manipulation of male black pigment on the Head

Copulations occurred in 11 out of 62 mating trials made with males whose proportion of black pigmentation on the head were previously manipulated. Males experimentally manipulated to have a higher proportion of black facial pigment were more chosen by the female as a sexual mate than males with apparent lower proportion (9 out of 11 males; Fisher's exact test: P = 0.01, N = 11; Fig. 1.14). White painting on the thorax did not affect female choice of sexual partners (Fisher's exact test: P = 1, N = 11).

Discussion

In *P. simillimus*, the black facial pigmentation of males proved to be a sexually selected signal. This pigmentation is dimorphic, as it is larger and more variable in males than in females. Furthermore, female choice of sexual partner is linked to the proportion of black pigment in the male's head so that males with high rather than low proportion of black pigment on the head are chosen as sexual partners. Finally, males experimentally manipulated to have higher black pigmentation on the head were preferred by females as sexual partner compared to those with low pigmentation, suggesting that females evaluate male coloration. Thus, the proportion of black pigment on the head of males is a sexually selected signal used by females during the choice of the sexual partner. One important point was the low rate of successful copulations in our experiments. A recent work showed that males of *P. dominula* avoid copulate with workers (Cappa et al., 2013). Since in our experiments we did not discriminate among future foundresses and late workers it is possible that *P. simillimus* males present the same preference. Anyway, our analyses focused only in reproductively receptive females.

We found no evidence that the black pigmentation on the head is associated with the establishment of dominance relationships among males. During each dominance trial between males of *P.simillimus*, the frequency of darts and bites were low, and mounting was not observed. In contrast, males of other *Polistes* species often exhibit such aggressive behaviors (Polak, 1993; Beani, 1996; Izzo & Tibbetts, 2012). The rarity in the frequency of aggressive behavior suggests that males of *P. simillimus* differ from other male *Polistes* studied so far. However, interpretation of these results should be considered with caution because of the lack of more complete information about the male behavior in this species.

The stronger negative correlation between the male proportion of black pigmented on the head and body size is quite surprising. In *Polistes*, it is known that quality signals are generally positively associated with body size (Tibbetts & Dale 2004; Tibbetts & Sheehan 2011; Tanure-Nascimento et al., 2008) and individual identity signals are not (Tibbettts, 2002). Our findings suggest that the signal we found for *P. simillimus* have different properties compared to previous *Polistes* visual signals studied so far. Most importantly, despite the association with the proportion of vertex black pigmented, body size doesn't predict female choice or male-male dominance, so that these two visual traits may not convey the same information for females.

The yellow spots on the second abdominal segment in *P. simillimus* are sexually dimorphic and despite not associated with male-male dominance relations, they are associated with female choice of sexual partner. Since female choice was affected by the interaction between the proportion of vertex black and the SI, it is likely that females evaluate both yellow and black spots before choose a male. The yellow spots on the second abdominal segment of *P. dominula* males, probably homologous to that of *P. simillimus*, are sexually selected signals (Izzo & Tibbetts, 2012). They are sexually dimorphic, and they are used to mediate both male-male dominance relations and female choice of sexual partners. Further experimental investigation will be necessary to clarify the function of *P. simillimus* abdominal spots. As long as we can tell, both abdominal spots in *P. dominula* and the black spot on *P. simillimus* males share similar properties, as they are sexually dimorphic, visual, conspicuous signals, and have continuous variation. However, they differ in location and in the kind of pigment they are composed. In wasps, yellow spots are formed by pterines while dark spots are formed by melanin (Ishay & Pertsis, 2002).

The independent evolved sexual signals in *Polistes* suggest that sexual selection may be common in these insects. In many systems, sexual signals provide useful information to the receiver. For example, females of *P. dominula* whose sexual partner had more regular spots ("preferred phenotype") survive longer than females whose sexual partner had less regular spots ("not preferred phenotype") (Izzo, 2011). Thus, females' choice based on male's abdominal spots maximizes their direct fitness (Izzo, 2011). Future studies with *P. simillimus* will be interesting to verify if independently evolved sexual signals convey the same type of information or if there are other factors mediating the evolution of female choice in social wasps.

The natural history of *Polistes* suggests a favorable scenario for the evolution of female choice. In most species, females are genetically monogamous (Strassmann, 2001). They copulate before winter, but only begin to fertilize the eggs in early spring. In addition, several generations of sterile workers are produced before future queens (Reeve, 1991). As a result, the sperm must be stored for several months, and thus, choosing good quality sexual partner may be important. The major challenge for the study of sexual selection in *Polistes* is observing sexual behavior in natural environments, essential for the interpretation of recent advances obtained from the laboratory experiments.

Visual signals in *Polistes* seem to be involved in communication at closer distances, as they are considered mediators of aggressive and/or sexual interactions (Izzo & Tibbetts, 2012; Tibbetts & Dale, 2004; Tannure-Nascimento et al., 2008). In both cases, the positioning of individuals face to face is common and thus communication is only effective if the visual acuity is appropriate. The compound eyes of insects have excellent spatial resolution at short distances (Land, 1972). In support, evidence suggests that wasps are able to perceive different characteristics of visual signals, such as patterns of disruption (Tibbetts & Dale, 2004), irregularity of contour (Izzo & Tibbetts, 2012) and the area of the body spots (Tannure-Nascimento et al., 2008). Thus, it seems that the social wasp's vision is sufficiently accurate to detect the variability in body coloration typical of *Polistes*.

Most research on insect communication focuses on chemical signals (Howard & Blomquist, 2005). For example, the role of cuticular hydrocarbons in communication among social insects is well known. These substances are used as signal of fertility, dominance and also for recognition of nest mates (Howard & Blomquist, 2005; Sledge et al., 2001; Gamboa, 2004; Monin, 2006; Dapporto et al., 2007). However, the visual communication can also be important. For example, in females of *Polistes fuscatus*, facial and abdominal marks are signals for individual recognition (Tibbetts, 2002). In females of *P. dominula*, *P. exclamans* and *P. satan*, facial markings are signals of quality that inform about the fighting ability of females (tibbetts & Dale, Tibbetts & Sheehan, 2011; Tannure-nascimento et al., 2008). Finally, in males of *P. dominula* and *P. simillimus*, visual signals are involved in intra and/or intersexual competition (Izzo & tibbetts, 2012). Together, this body of information suggests that the visual channel has been explored in different ways, highlighting the potential of social wasps as models for the study of visual communication in insects.

Figures and Legends

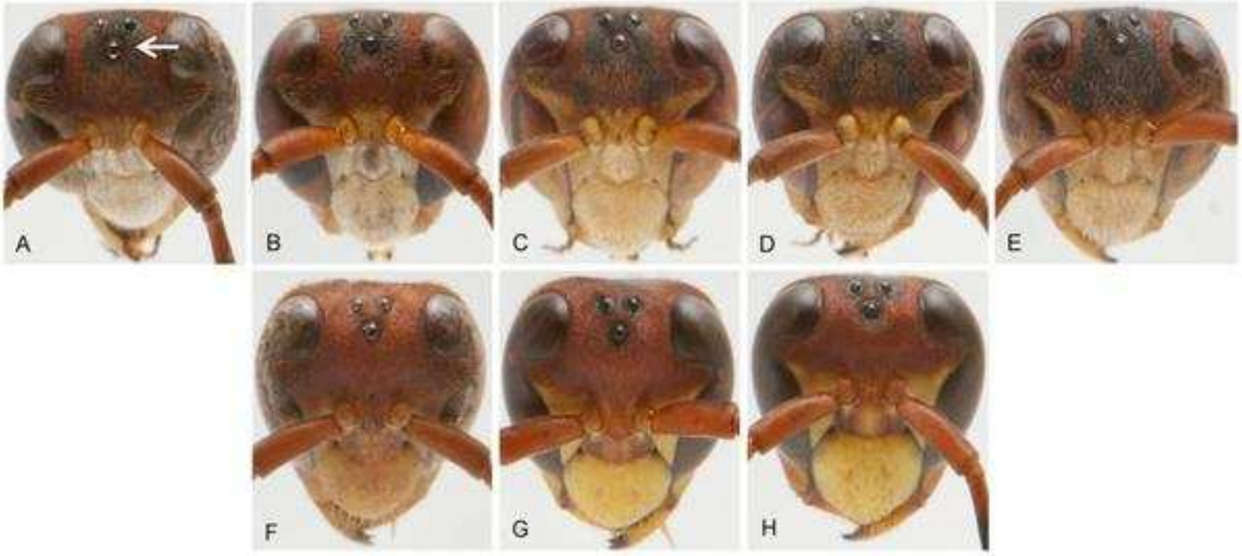

Figure 1.1. Variation in the proportion of black pigment in heads (arrow) of males (A–E) and females (F–H).

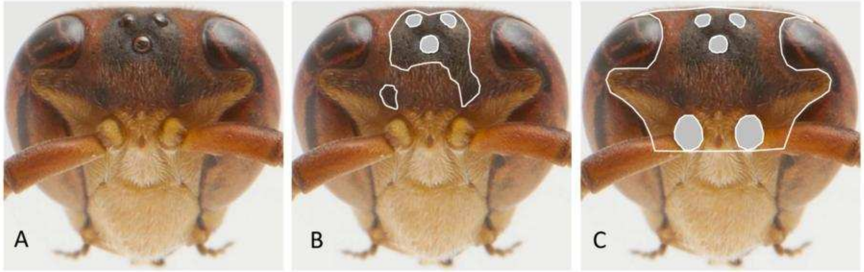

Figure 1.2. Proportion of black pigment on a male head (A), determined as the black pigment area enclosed by the white line in B, with respect to the area enclosed by the white line in C. Note the exclusion of the ocelli and antennal sockets area (grey circles).

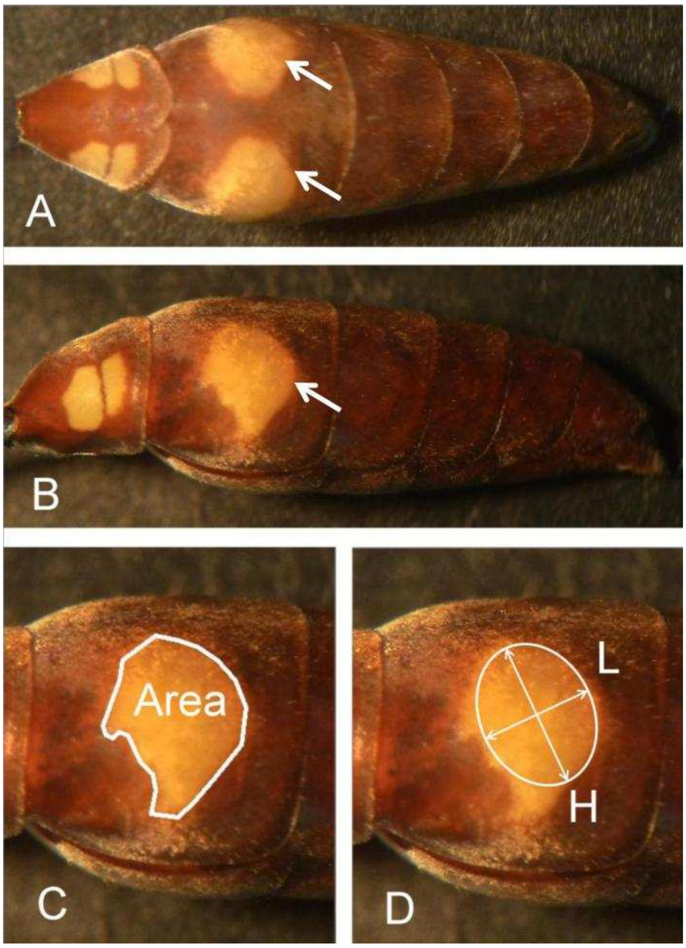

Figure 1.3. Spots of a male second gastral tergite. Dorsal (A) and lateral (B) views of the abdomen showing the spots (arrows). The spot shape index was calculated from the area of each spot (C) and the length (L) and height (H) of the largest sphere that can be inserted into each spot. In all images, the male anterior side is to the left.

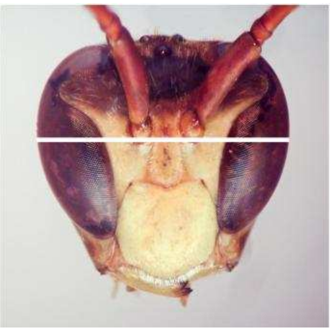

Figure 1.4. Measurement of maximum head width (white line), used to infer the body size.

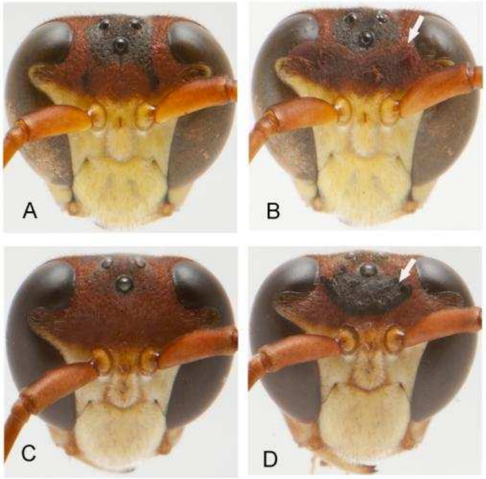

Figure 1.5. Experimental paint manipulation of males' black pigmentation on the head. Males were manipulated to have low (A, before; B, after manipulation) or high black pigmentation (C, before; D, after manipulation). The arrows indicate the manipulated area.

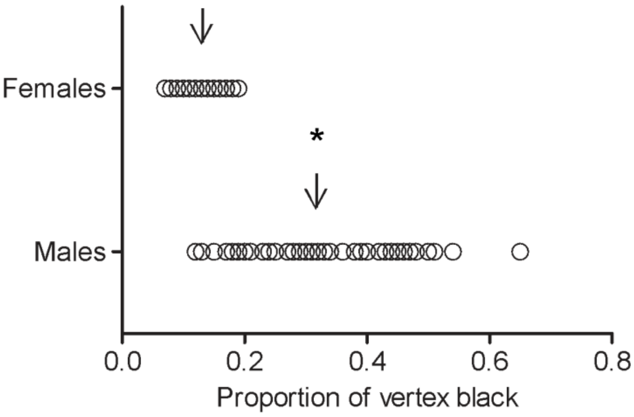

Figure 1.6. Variation in the proportion of black pigment in heads of males and females. The proportion of black pigmentation on the head is higher and more variable in males than in females. Each circle represents one individual and the arrows indicate the mean. * Indicates statistical difference between the classes (see text).

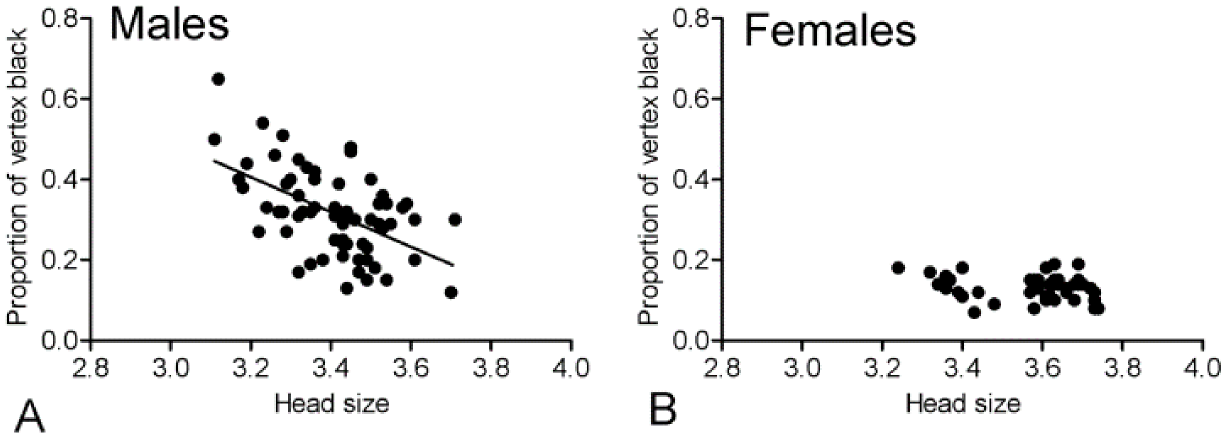

Figure 1.7. Relation between head size and proportion of vertex black pigmented. Males have a stronger negative relation compared to females.

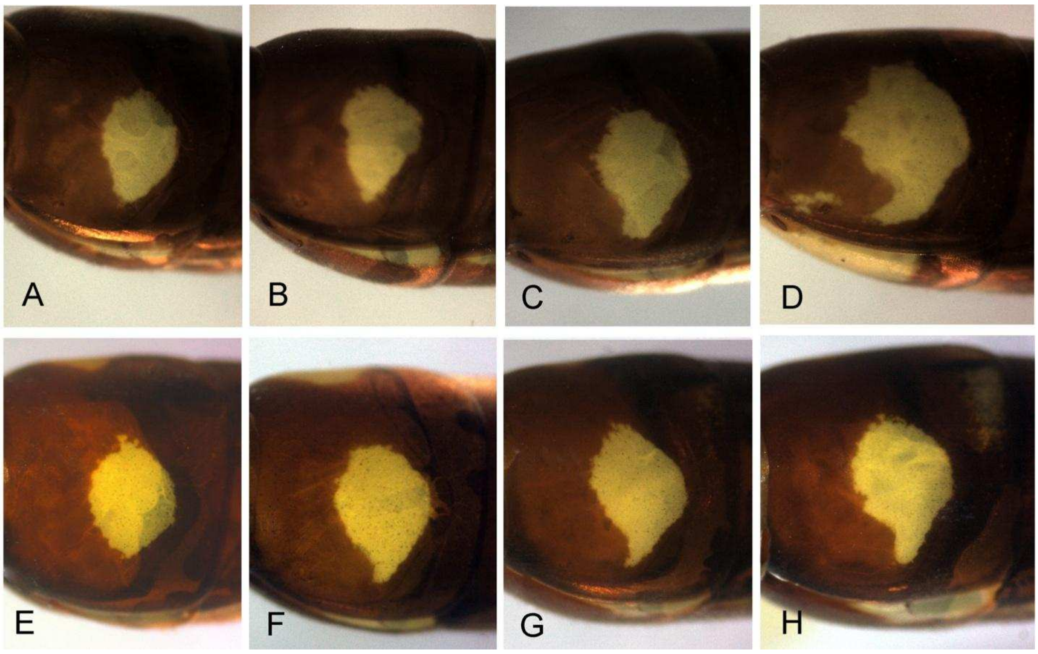

Figure 1.8. Abdominal spots in males (A–D) and females (E–H). Each image shows one of the two spots in each individual and tin all images the wasp's anterior side is to the left.

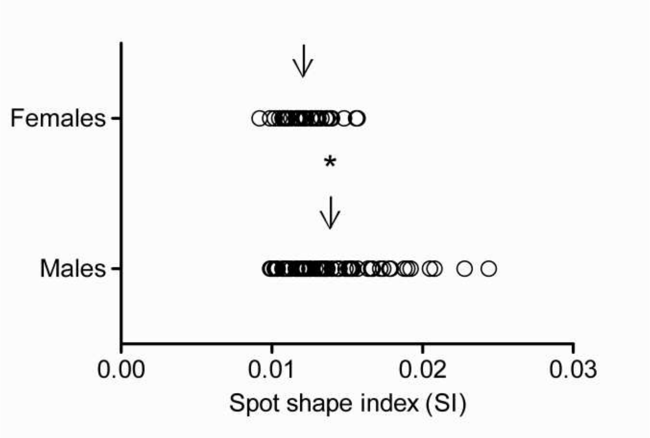

Figure 1.9. Spots shape index in males and females. Each circle represents one individual and the arrows indicate the mean. * Indicates statistical difference between the classes (see text).

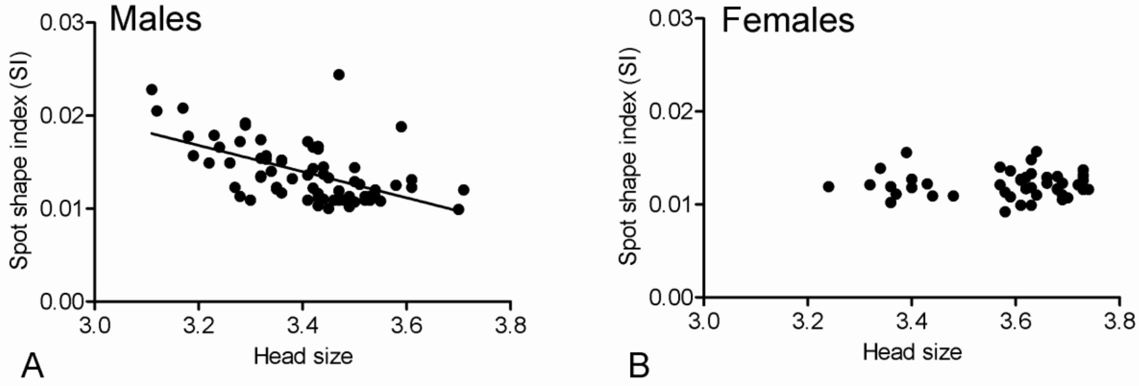

Figure 1.10. Relation between head size and SI. Males have a stronger negative relation compared to females.

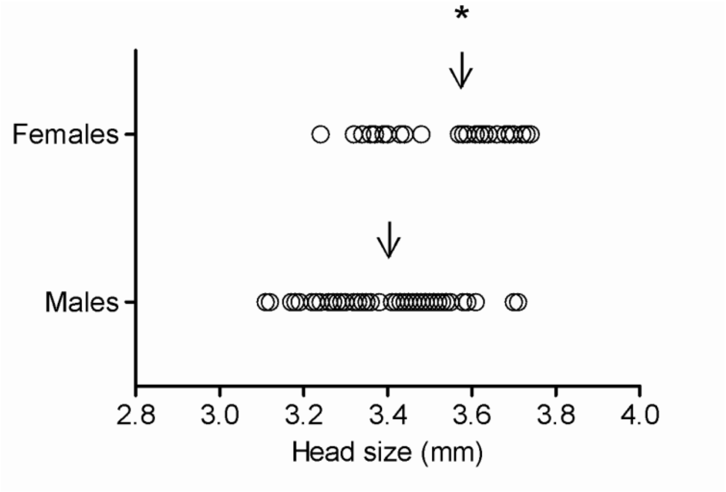

Figure 1.11. Variation in head size of males and females. Males are smaller than females. Each circle represents one individual and the arrows indicate the mean. * Indicates statistical difference between the classes (see text).

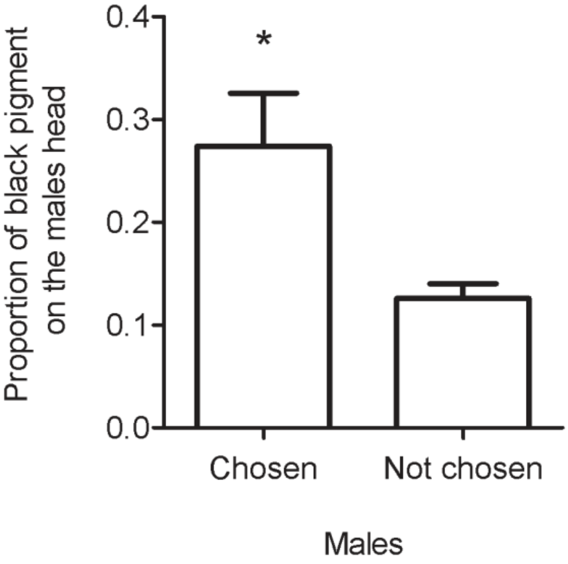

Figure 1.12. Proportion of black pigment of males chosen and not chosen by females as sexual partners. Mean and standard deviation are presented. * Indicates statistical difference between the classes (see text).

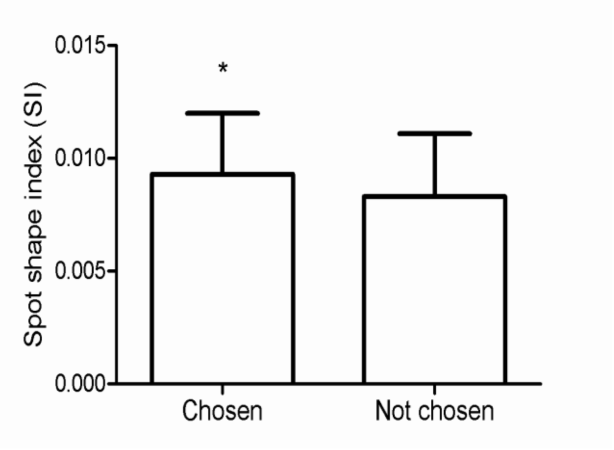

Figure 1.13. SI of males chosen and not chosen by females as sexual partners. Mean and standard deviation are presented. * Indicates statistical difference between the classes (see text).

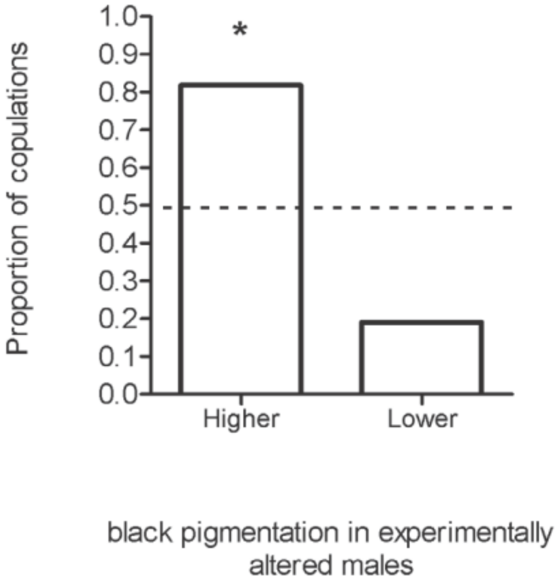

Figure 1.14. Proportion of copulations of males experimentally manipulated to have high and low black pigmentation on the head. * Represents statistical difference in proportions (see text). The dotted line represents the null expectation of 50%.

CHAPTER 2 – A role for male sexually selected visual signals in leks of *Polistes dominula* paper wasps

A role for male sexually selected visual signals in leks of *Polistes dominula* paper wasps

Abstract

Leks are characterized by strong male-male competition and female mate choice. While there is convincing evidence that female choice on leks may be based on male ornaments, little is known about the possibility that the same traits can also mediate conflicts among rivals. Males of *Polistes dominula* paper wasps have variable yellow abdominal spots that may function as sexually selected signals: regularly round spots increase fighting and mating success under laboratory conditions. By conducting a decoy challenge in a natural lek, we observed that lures manipulated in their spots' shape received more aggressions by territorial males when they had round than irregular spots. Since round spots are sexually selected quality signals, lekking males could use these ornaments to quickly assess the fighting ability of rivals and to mediate aggressive interactions. Visual signals may be involved in the dynamics of male competition as well as in the evolution of female choice based on ornaments that lack high production costs but represent a reliable cue of fighting ability. In this sense, dual function ornaments could play an important role in lek-based mating systems.

Introduction

Male-male competition and female mate choice are selective forces driving reproductive dynamics, as it is observed in lekking mating systems. Here, males congregate on a communal display area during the breeding season, the leks, where they spend most of the time establishing and defending resource-less territories from conspecific intruders and using these sites for bizarre exhibition and courtship. Females visit a lek to select a partner (Bradbury, 1981). “Lek,” a Scandinavian word meaning “play”, was used by Darwin to define the nuptial arenas of some galliforms (*apud* Beani, 1996); the rules of this play are very complex, due to the “dual function” of male secondary sexual characters: mediators of male-male competition and female mate choice (Berglund et al., 1996). Therefore, lekking systems provide an opportunity to study how intra and intersexual selection shape male traits.

Intuitively, in a lek, male-male interactions are far more common than male-female interactions. Surprisingly, research on sexual ornaments in leks has focused mainly on the female perspective, as we frequently interpret male ornamental traits as primarily subjected to female mate choice (Berglund et al., 1996). For example, it is well known that female criteria to select a partner may rely on ornamental signals, assumed to inform females about male quality (Clutton-Brock et al., 1988; Höglund et al., 1990; Robertson, 1990; Petrie et al., 1991). However, little is known about whether ornaments are used to mediate male-male competition.To date, there is only associative (peacocks: Loyau et al., 2005; great bustards: Alonso et al., 2010) or not robust evidence (cichlid fishes: Martin & Genner, 2009) that lekking males use ornaments to mediate their aggressive interactions. Given that the components of sexual selection may act in the same as well as in opposing directions (Hunt et al., 2009), quantifying the different contexts in which ornaments are used is essential to understand how selection shaped these traits.

In social insects like Hymenoptera, there is the potential for a dramatic sexual selection among males (Baer, 2003). The role of sexual selection in the evolution of eusociality is a topic of growing interest (Baer, 2014; Beani et al., 2014). At the level of pre-copulatory behavior, many species have lek- or swarm- or scent-based mating systems characterized by a high frequency of aggressive interactions and a high reproductive skew among males (social wasps: Reeve, 1991; Beani et al.,1992; Polak, 1993; Beani, 1996; bumblebees: Baer, 2003; ants: Cole, & Wiernasz, 1997).

If competition for mating opportunities is intense, male-male assessment of rival`s competitive ability in both lekking and swarming social hymenopterans is likely to occur. In social wasps, there is only limited laboratory evidence that sex dimorphic traits (abdominal stripes and spots) are used as ornamental signals mediating the aggressive interactions between males (Stenogastrinae wasps: Beani & Turillazzi, 1999; Polistinae wasps: Izzo & Tibbetts, 2012). There is no experimental field evidence supporting the role of sexual ornaments in social insects.

Males of the social wasp *Polistes dominula* are good models for investigating the role of visual signals in sexual selection. Males are produced mostly in the summer, in a relatively short time window. They spend the first few days of adult life on the nest and then join large male aggregations at sunny landmarks (Beani et al., 1992), where they may intercept receptive females. These “hotspots” (Bradbury & Gibson, 1983), located near nesting, foraging and hibernation sites (Beani, 1996), are occasionally visited by females (Beani & Turillazzi, 1988), so that intrasexual male competition for a mating opportunity is intense. In this compact lek system, a male can defend and mark a number of small perching sites, its exclusive territory, such as a branch, a few leaves and so on (Beani, & Turillazzi, 1988). Since territory holding is associated with a higher reproductive success, territory ownership is contested and aggressive interactions between rivals at perching sites are common (Beani, & Turillazzi, 1988; Beani, & Zaccaroni, 2014). Therefore, ornaments could minimize social costs by allowing males to assess each other`s fighting ability before engaging in escalated aggressive interactions.

Indeed, males of *P. dominula* have spots that may be sexual signals, but their function has not been tested in the field. The pair of yellow spots on the black surface of the second gastral tergite is sexually dimorphic (Izzo & Tibbetts, 2012). Sexual dimorphism is typical in traits under strong sexual selection (Anderson & Simmons, 2006). A laboratory study on an invasive population from United States suggests that the shape of these spots is used as a signal to mediate both male aggressive interactions and female mate choice (Izzo & Tibbetts, 2012). When wasps interact in a small glass arena, round spotted males are more likely to win intrasexual dominance bouts and to be chosen as sexual partners than irregular spotted males. These results remained consistent even after paint manipulation of abdominal spots, suggesting that wasps do pay attention to the male abdominal spots before interacting.

In this field study, we reassess the hypothesis of male abdominal spots as signals of fighting ability in *P. dominula*. At least in females of this species, the status signal of black spots on the yellow clypeus vary across populations, probably due to environmental influence on signal expression (Cervo et al., 2008; Green & Field, 2011; Tibbetts et al., 2011). Thus, it is important to evaluate spot dimorphism in female and male wasps from different populations. Moreover, the small arena used in the laboratorial study (Izzo & Tibbetts, 2012) represents a simplified but constrained environment to study both male competition and female mate choice (Beani et al., 2014; Cappa et al., 2013). Whether or not wasps pay attention to the abdominal spots in a more natural situation still remains an open question. Thus, we examined the effects of simulated territorial intrusions by using washed lures (to control for odor and behavior), manipulated for their spot shape (round versus irregular spots). We tested whether 1) wasp abdominal spots are sexually dimorphic in a native *P. dominula* population and 2) males pay attention to the abdominal spots of rivals on natural leks.

Materials and Methods

Study Area

During the summer of 2014 (July-September), we carried out studies in a native population of *P. dominula* in the surroundings of Florence, Italy. This area consists of a flat landscape with a set of visually conspicuous landmarks (trees, poles, vertical silhouettes, etc), in which male leks can be easily found (Beani & Turillazzi, 1988). We made the collections and behavioral experiments between 10 a.m. to 3 p. m., as this is the time when males are more active at leks (Beani & Turillazzi, 1988).

Sexual Dimorphism

First, we examined the sexual dimorphism of the wasp`s yellow spots over the black part of the second gastral tergite. To do this, we compared male and female spot shape indexes (SI, see Tibbetts, & Izzo, 2012). Previous work suggests that SI is the parameter of male spots that receives pay attention. Round spotted wasps have lower SI values while irregular spotted wasps have higher SI values. In males, the lower the SI value, the higher the male agonistic ability and its sexual attractiveness to females (Tibbetts, & Izzo, 2012). A total of 80 males captured on fences, flowers and bushes were measured and compared with a total of 76 females captured at the same sites.

Lure Preparation

Pairs of *P. dominula* male lures (Fig. 2.1) were used in behavioral experiments at natural male lekking sites. Each of the 30 pairs of lures consisted of pinned dead males matched by head width (difference in head width was 0.0067± 0.0217 mm), washed in pentane for 24 h, mounted a with a similar abdominal elongation, but with abdominal spots experimentally altered with yellow paint: one of the males was painted to have round spots and the other was painted to have irregular spots. Paints dried for 24 hours before using lures in behavioral assays. Even though the paint might not correspond exactly to the natural spot coloration, both males received the same kind of paint, so painting alone could not account for differences across pairs of lures. Males used as lures were collected from lekking sites at least 1 Km away from the leks they were tested in, to minimize the possibility of previous interactions.

Lure Presentation

The decoy challenge consisted of introducing a pair of experimentally manipulated lures (with different abdominal spots) in a male perch at a lekking site. First, we identified a perch defended by a male (a leaf in a tree). As soon as the male left the perch, we placed a pair of lures over the leaf and waiting for the male`s return. After 2-5 minutes, the territorial male started to interact with lures showing different kinds of possible interactions. The male could simply “inspect the rival”: a male approaches one of the lures flying in a zig-zag pattern, without touching the rival and immediately flies off; the territorial male could also “strike the rival”: after approaching one of the lures, the male strikes it by dropping onto its back, and immediately flies off (Beani & Turillazzi, 1988). An observer, positioned 1.5-2 m away from the pair of lures, recorded the frequency at which the territorial male displayed each of these two types of behavior towards each of the lures for three consecutive minutes after the first interaction. In each trial, the pair of lures was placed in a parallel orientation, three centimeters away from each other, always under direct sunlight (Fig. 2.1). The position of the lures in relation to the perch (left and right side) was randomly assigned. Each pair (N= 30) of lures and territorial perch was used only once.

Statistical Analysis

The sexual dimorphism was tested by using a Generalized Estimating Equations (GLZ) in which SI was introduced as the dependent variable, while sex was the factor and head width was the covariate. The behavioral responses of focal males towards the rivals were compared with Wilcoxon signed rank test, at 5 % level of significance. We used the software SPSS 15.0.

Results

Sexual Dimorphism

SI is associated with sex (GLZ: Wald`s $X^2 = 25.410$, $P < 0.0001$, N = 76 females and 80 males), so that males have larger and more irregular abdominal spots than females (male SI: 1.5802 ± 0.7771, range 0.9728-3.6192; female SI: 1.1283 ± 0.2855, range 0.6458-1.4665). SI is not associated with head size (Wald`s $X^2 = 0.389$, $P < 0.533$, N = 76 females and 80 males).

Lures Presentation

In our decoy challenge, the interaction between territorial males and lures was variable: males could inspect or strike only one of the lures, both lures, or neither.

Lekking males inspected both lures at a similar rate (number of inspections received in a 3-min interval: round spotted lures: 1.1666 ± 1.4793, irregular spotted lures: 0.9666 ± 0.8899; Wilcoxon: W = 51, P = 0.3979, N = 30, Fig. 2.2a), but they were more likely to strike round rather than irregular spotted lures (number of strikes received over a 3-min interval by round spotted lures: 1.3666 ± 1.2452, irregular spotted lures: 0.5333 ± 0.8193, Wilcoxon: W = 186, $P < 0.001$, N = 30,Fig. 2.2b).

Discussion

The sexually dimorphic abdominal spots seem to function as signals of fighting ability in *P. dominula* lekking males. When faced with two conspecific rivals at their perches, males prefer to challenge the opponent with more rounded spots. Rivals were experimentally manipulated (Fig. 2.1) to have a similar position at the perch, similar body size and no odor cues, and they received the same kind of paint; thus, the only consistent difference was the spot shape in our short-term trials. Therefore, under field conditions, males inspect the lures (Fig. 2.2a), paying attention to rival`s abdominal spots before physically interact and focusing the aggression on round spotted ones (Fig. 2.2b). Since a spot shape is associated with male behavioral dominance in both un-manipulated and manipulated individuals (Izzo & Tibbetts, 2012), lekking males might assess rival`s fighting ability through this signal. Therefore, signal discrimination – the first requirement of a reliable signal assessment – is demonstrated by variation in male response towards the two decoys.

One aspect of the results that is initially surprising is that males preferentially challenged the rival with abdominal spots associated with higher fighting ability. Intuitively, we expected rivals to challenge individuals signaling lower fighting ability. However, when territorial males compete without weapons, for example in butterflies (Kemp et al., 2001), as well as in *P. dominula* (Beani & Turillazzi, 1988; Beani et al., 2014), attacks may be directed preferentially towards superior rivals, as they are a greater threat as potential usurpers of the territory. Female *P. dominula* wasps are more likely to copulate with a male on a perch if the male is the owner of the territory (Beani & Zaccaroni, 2014). Males should invest energy to maintain their territory ownership, so that competition over limited resources, i.e. the favorite encounter sites inside a compact lek, is costly. In recent decoy challenge trials, territorial white-crowned sparrows responded more aggressively towards decoys with enhanced status badge (Laubach et al., 2013). Similarly, our results demonstrate that *P. dominula* lekking males are more likely to challenge potentially more dangerous rivals in case of extremely valuable resources, such as a territory inside a lek, especially if the conflict consists of quick strikes with no serious damage, as in the case of paper wasps. Obviously, the trend of the aggressive response toward a decoy with a conspicuous ornament may be the opposite (Pryke et al., 2001), in relation to the context and the value of the contested resource (Tibbetts, 2008). An alternative hypothesis is that males interact more frequently with round spotted lures because they look like females, due to a considerable overlap between spot shape in females and high quality males (see Izzo & Tibbetts, 2012); however, we never observed any sexual interaction (grasp, attempted copulation) towards our lures, whereas “strike” is a typical aggressive intra-sexual behavior (Beani & Turillazzi, 1988).

The abdominal spots in male *P. dominula* can be considered conventional signals, because they lack a required (inherent) link with bearer`s agonistic ability (Guilford & Dawkins, 1995), as in the case of other body coloration traits (Part & Qvarnstrom, 1997; Pryke & Andersson, 2003; Rohwer, 1975; Tibbetts & Dale, 2004). As a result, it is not clear how these spots could honestly convey information about fighting ability (Senar, 2006; Whitfield, 1987). The handicap hypothesis states that ornaments must be costly to provide reliable information about their bearer’s quality (Zahavi, 1975). It is unlikely that pigments involved in spot production provide the cost that keeps the signal honest. Dorsal spots are composed by pterins (yellow) surrounded by melanin (black). These pigments seem to have some production cost, particularly in herbivores that are nitrogen limited (Jawor & Breitwisch, 2003; Rutowski et al., 2005). Wasps are not nitrogen limited and these pigments are present across their whole body, so spot production is unlikely to be highly costly (Izzo & Tibbetts, 2012).

Alternatively, the receiver’s behavioral response toward lures is consistent with the social control hypothesis, which states that individuals displaying the most dominant signals will experience increased levels of aggression (Rohwer, 1977). We experimentally demonstrated that decoys signaling higher agonistic ability, i.e. round abdominal spots, received more aggressive acts than decoys signaling lower agonistic ability, i.e. irregular spots. If individuals that signal high status receive more frequent or intense aggression than those that signal low status, only individuals with high fighting ability could afford to have a signal advertising high fighting ability. The possibility of social costs maintaining signal accuracy must be considered with caution because under laboratory conditions (small glass arena) there is no evidence that signal inaccuracy is costly. In fact, individuals that signal inaccurately high agonistic ability are more likely to win the dominance bouts and more likely to be chosen as sexual partners than controls (Izzo & Tibbetts, 2012). Of course, the costs of signal inaccuracy likely vary across contexts and inaccuracy may be more strongly disfavored on natural leks. Further investigation is needed to identify the mechanisms maintaining signal honesty over evolutionary time (Számadó, 2011) and the physiological and genetic background of abdominal spots.

Signals of fighting ability are expected to evolve when aggressive interactions among unfamiliar individuals are common (Rohwer, 1982; Shreeve, 1987; Zulandt-Schneider et al.,1999). This scenario may be achieved in a lek system, as residents vigorously defend their territory against intruders. Sexually selected signals - evolved as “armaments and ornaments” in a Darwinian dual perspective (Beani & Zuk, 2014) - are poorly documented in social wasps (Beani & Turillazzi, 1999; de Souza, et al., 2014; Izzo & Tibbetts, 2012). Perhaps, this is a consequence of the long term negligence regarding studies on male behavior in social insects (Baer, 2003, 2014; Beani, et al. 2014; Boomsma, 2005). This lack of studies is somewhat surprising given that male hymenopterans represent useful model organisms to study sexual selection theory in unconventional ways (Beani et al., 2014). For example, the subtle cognitive bases of male behavior is unclear; how are they able to evaluate tiny quality signals of rivals? Finally, visual ornaments may represent only a single component of the multimodal signaling process involved in male courtship (Herberstein et al., 2014). In *Polistes* leks, male size, fighting ability, scent-marking and male endurance could play a critical role in determining mating success (Beani et al., 2014). Thus, the role of single components inside the overall signaling system should still be interpreted with caution.

There is no evidence of geographic variation in male *P. dominula* signals, as in both the native population and the Michigan invasive one (Izzo & Tibbetts, 2012) abdominal spots are sexually dimorphic, with males having larger and more variable shapes than females. It is likely that this sexually selected signal is widespread among *P. dominula* males, in contrast to the socially selected facial pattern among the females of the same species. In North America populations, foundressess use a black facial pattern on the clypeus to signal fighting ability to conspecific females (Tibbetts & Dale, 2004; Tibbetts & Lindsay, 2008). However, the same black facial pattern does not appear to work as a signal in native populations from Italy and Spain (Cervo et al., 2008; Green & Field, 2011; Tibbetts et al., 2011). Since there is a negative association between the frequency of black facial pattern and warmer temperature (Green et al., 2012; Tibbetts et al., 2011), authors have argued that environmental constraints on black facial pattern expression may explain the lack of a signaling function in some populations. Interestingly, male abdominal spots work as a sexually selected visual signal, regardless of populations and environmental constraints. The occurrence of *P. dominula* male quality signals provides an additional avenue to investigate the evolution of visual communication in paper wasps.

The brief nature of male-female interactions, compared to the prolonged frequent male-male interactions suggests that intrasexual male competition may play a critical role in leks. Females of lekking species may have reduced opportunity to test whether a male signal reflects its quality, especially in the case of ornamental colors that lack high production costs. By selecting mates through signals of fighting ability (Izzo, & Tibbetts, 2012), females can improve their chance of choosing mates based on an honest quality signal. According to Berglund et al. (1996), this is because males rather than females may be better able to recognize cheaters, since males can immediately test the rival’s quality in combat. In addition, it may be more costly to fake the trait in male-male contests than in courtship. Finally, ornaments with dual function could keep the habit of females exerting their choice in aggregations of males, where the trait is likely to be put in trial during male-male contests. If they cannot be faked, females may use them as a reliable criterion to identify valuable mates: the armament-ornament model is “parsimonious” in an evolutionary perspective (Berglund et al., 1996). Therefore, we propose that dual function ornaments could play an important role in the evolutionary stability of lekking behavior.

Figures and Legends

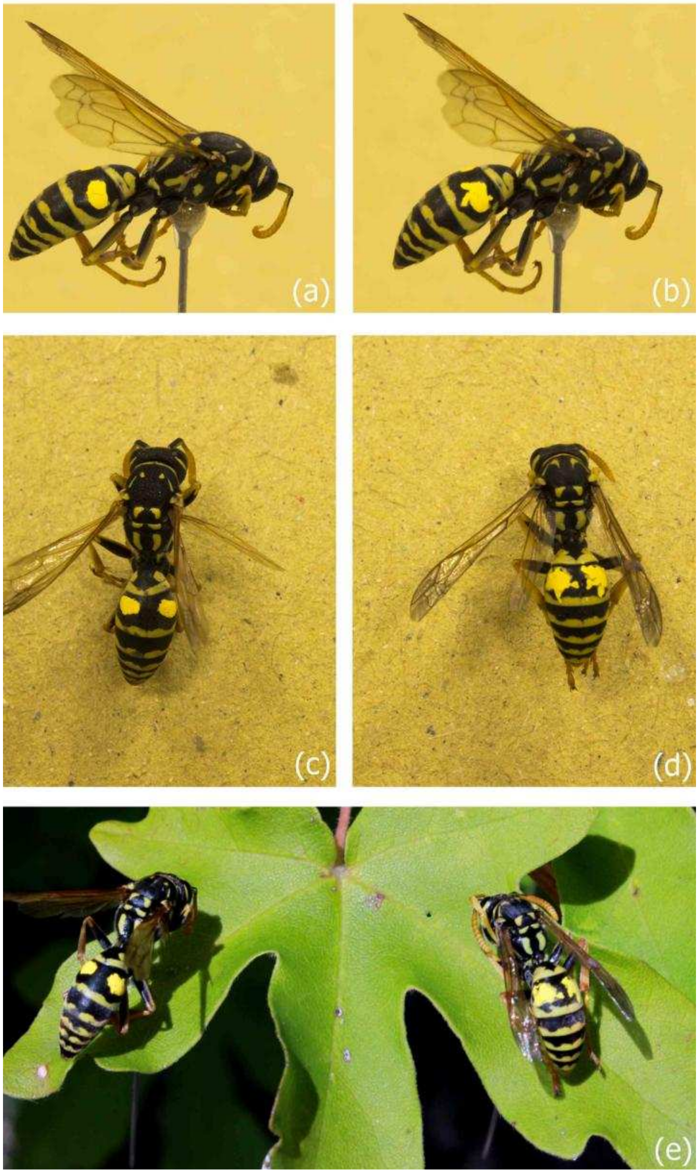

Figure 2.1. Experimental paint manipulation of lures' abdominal spot shape. One of the lures was manipulated to have more rounded spots (a,c), associated with high quality, while the other was manipulated to have spot irregular spots (b,d), associated with low quality. Then, they were simultaneously presented to territorial males of *P. dominula* (e). Note that lures were pinned at the perch in a similar position and under similar light conditions.

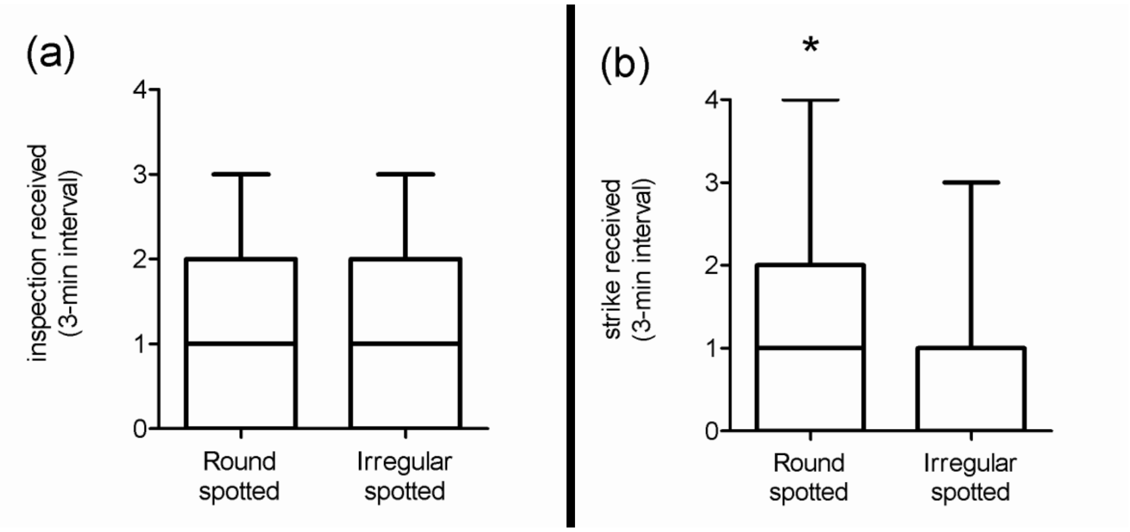

Figure 2.2. Frequency of inspection (a) and strike (b) received by experimentally manipulated lures, with round or irregular yellow spots on the abdominal tergite. The line represents the median, the bottom and top of each box represents the 25th and 75th percentile, the whiskers show the maximum values. * = P<0.001.

CHAPTER 3 – Intersexual nestmate recognition *Polistes versicolor* paper wasps

Intersexual nestmate recognition *Polistes versicolor* paper wasps

Abstract

Polistes paper wasps mating occur mainly out of nests so that wasps can potentially find both related and unrelated partners. Mating with a related partner imposes fitness costs. Thus, mechanisms to prevent inbreeding are expected. We tested whether *P. versicolor* wasps have intersexual nestmate discrimination ability. By conducting laboratory behavioral assays in which a female was simultaneously presented to both a nestmate male and a non-nestmate male, we showed that sexual behavior is associated with relatedness between sexual partners. Regardless male`s age and structural size, females were more aggressive when facing a nestmate male than a non-nestmate male. Males attempted to copulate more frequently with non-nestmate females than with nestmate ones. Thus, *P. versicolor* wasps have intersexual nestmate discrimination ability. This can potentially explain the absence of diploid males, a result of inbreeding, in Brazilian populations of this species. The potential mechanism underlying this ability is discussed.

Introduction

Nestmate recognition is the differential treatment of conspecifics on the basis of colony origin (Gamboa, 1996). Although female-female nestmate discrimination has been largely demonstrated among social insects, the extension in which recognition is observed between gender and the underlining mechanisms of such ability remain poorly understood. Paper wasps have been fit this situation, as laboratory mating contexts have been showed males and females avoiding to mate with genetically related sexual partners (Shelmann-Reeve & Gamboa, 1985; Ryan & Gamboa, 1986; Starks et al., 1998; Liebert et al., 2010). However, this preference seems not to be consistent across paper wasp species (Sen et al., 2010) or even between populations of the same species (Beani & Turillazzi, 1988; Liebert et al., 2010). Intersexual nestmate recognition is thought to be adaptive because it can be a mechanism of inbreeding avoidance. Inbreeding results in fitness costs by allowing for the expression of deleterious recessive alleles and decreasing allelic diversity within the gene pool (Fonseca et al., 2000; Tsutsui et al., 2000; Zeisset & Beebee, 2003; Rasner et al., 2004). Additionally, in hymenopteran societies, females are diploid while males haploids, but inbreeding can produce diploid males, which do not contribute to colony productivity and are functionally sterile (Liebert et al., 2004). As a result, in populations subjected to inbreeding, it is expected selection for mechanisms of avoiding incestuous copulations.

Polistes versicolor is a Neotropical paper wasp species with a widely distribution, from Costa Rica to Argentina (Richards, 1978). Their colonies are started by one or a few inseminated females, which produces some generations of workers, followed by males and reproductive females (Gobbi, 1977). Copulations occur mainly out of the nest, where wasps can potentially find both related and unrelated sexual partners so that inbreeding may exist. Indirect evidence suggests that at least in some Brazilian populations of *P. versicolor*, inbreeding can be rare or even absent because no diploid males, a consequence of inbreeding, have been reported (Nagamati-Júnior et al., 2010). This finding leads to the suspect that some mechanism to avoid inbreeding could be operating. We tested whether wasps are able to recognize their nestmates in a laboratory mating bioassay.

Materials and Methods

Between February and March 2012 and 2013, male/female producing colonies of *P. versicolor* were located in the municipality of Juiz de Fora, Minas Gerais State, southwestern of Brazil (21^{o} $46^{'}$S, 43 ^{o} 21’W, 800 m elevation). Daily visits between 7-8 pm were conducted to collect newly emerged wasps. To discriminate newly emerged individuals from older ones, we first paint marked all adult wasps on the nest, so that on the next days, non-painted wasps could be newly emerged ones or foragers that were out of the nest by the time of paint marking. Newly emerged wasps were easily discriminated from non-marked foragers because the former presented black eyes and they could not fly, while the later had brown eyes and well developed flight ability. The newly emerged wasps were left less than 24 h on their natal nests and then they were collected and individually placed in circular plastic containers of 500 ml and provided with water and honey *ad libitum* until the beginning of behavioral assays. As a result, at the day of the behavioral assay, males were 7-36 days old and females were 16-48 days old. These wasps were paint marked on the thorax with Acrilex® paint (De Souza et al., 2012), 24 h before the mating trials, to allow their identification. We used a total of 20 females and 40 males. We assumed that males and females were virgins at the time of collection, because right after the emergence, *P. versicolor* males are not sexually mature. It takes a time to sperm cells migrate from the testes to the spermatic vesicle. The seminal vesicle of 2-day-old *P. versicolor* males has no sperm (De Souza, personal observation) and thus, males at this age are not able to successfully inseminate females.

The behavioral assay consisted in observing a triad comprising a female, a nestmate male and a non-nestmate male during one hour, in a glass arena of 20x20x20 cm. An observer, blinded to the wasps’ nestmembership, directly registered the frequency of behavioral interactions: (i) the number of bites a female directed towards each male, (ii) the number of attempt copulations that each male directed towards the female, defined as the number of times a male mount on the female but fail to establish genital interlocking and (ii) the number of successful copulations, defined as the number of times male-female genital interlocking was observed. Assays were conducted between 10 am and 6 pm in a laboratory, under room conditions. After each trial, the arena was washed and cleaned with ethanol 70% to minimize the effects of polar chemical residues on social wasps’ behavior

(Liebert et al., 2010). A different set of wasps was used in each trial. The experimental design avoided familiarity between nestmate males and females, because in each trial, nestmate males were at least two days older or younger than nestmate females. As a result, they have never met each other in the maternal colony, as they were always removed from their colonies less than 24 h after emergence. In each trial, we used unrelated males and female collected from colonies distant at least 1 km apart. After experiments, males were freeze-killed and then, the maximum head width was measured with a caliper rule.

Males in a given assay differed in nestmembership, age and structural body size (inferred from the head size). As there were two males in each mate choice trial (i.e. choice trials were paired), we decided to compute the relative differences among males of a given choice trial rather than the absolute values (Izzo & Tibbetts, 2012). Thus, in each essay, we estimated the male’s difference in: the aggression received from the female; the attempted copulations; structural size and age. Then, a multiple regression analysis was used to test whether male-female nestmembership, male age and male body size predict female`s aggression towards a male. Another multiple regression analysis was used to test whether male-female nestmembership, male age and male body size predict males’ sexual interest toward the female (attempt copulations). Descriptive and inferential analyses were executed by the software Graph Pad InStat 3.0 and SPSS 15.0.

Results

The sequence of sexual behaviors displayed by successful males of *P. versicolor* started with fast movements of the antennae close to the female. Then, the male climbed the female body (mounting) and finally a genital interlocking was observed. However, successful copulations were rarely observed (2/20 trials) and most of times females rejected males in different ways: When a male tried to mount, the female could fly away to another part of the arena or even bite the male; nevertheless, a male sometimes could successfully mount on the female. In these cases, the female could move her abdomen, avoiding genital contact, making copulation mechanically impossible. Aggression between males was not observed.

Female behavior during sexual interactions (aggression towards a male) is associated with male-female nestmembership ($F_{1,20} = 32.42$, $P < 0.0001$, $N = 20$; Fig.

4.1a) and the number of attempted copulations a male directs towards her ($F_{1,20}$ = 14.03, P < 0.001, N = 20), but it is not associated with male structural size ($F_{1,20}$ = 1.04, P = 0.3148, N = 20) or male age ($F_{1,20}$ = 3.82, P = 0.0581, N = 20).

Male behavior during sexual interactions (attempted copulations towards a female) is associated with male-female nestmembership ($F_{1,20}$ = 27.3500, P < 0.0001, N = 20, Fig.4.1b) as well as the amount of aggression the male receive from the female ($F_{1,20}$ = 14.0359, P < 0.001, N = 20) but it is not associated with male size ($F_{1,20}$ = 2.4668, P = 0.1255, N = 20) or male age ($F_{1,20}$ = 3.8240, P = 0.0581, N = 20). One of the two copulations (genital interlocking) observed was between nestmate partners and another copulation was between unrelated partners.

Discussion

Polistes versicolor wasps were able to recognize whether or not a partner is a nestmate during a laboratory mating context. Once inbreeding can poses fitness costs associated with diploid male and triploid female production (Liebert et al. 2004, 2006), it is expected selection for both males and females recognizing and avoiding nestmate matings. Indeed, females were more aggressive toward nestmate males than toward non-nestmate ones, and non-nestmate males attempted to copulate with females more than nestmate ones. Although the results demonstrated that wasps were able to recognize intersexual nestmates, it is not known if wasps use this ability to decide whether to mate with a potential partner. This is because (i) although males differed in the frequency of attempted copulation, both nestmates and non-nestmate males attempted to copulate and (ii) although females were more aggressive toward nestmate males, it was observed that copulation with both related and unrelated partners are possible. Since no diploid males or triploid females, a consequence of inbreeding, have been registered in *P. versicolor* populations in Brazil (Nagamati-Júnior et al., 2010), a mechanism of inbreeding avoidance is likely to occur and thus, intersexual nestmate recognition seems to be a good candidate.

If inbreeding is costly and wasps are able to discriminate between related and unrelated sexual partners, why do they invest in nestmate copulations? The conspecific acceptance threshold model (Reeve, 1989) provides a theoretical framework to understand the context dependence of nestmate recognition and inbreeding avoidance. In this model, an individual evaluator (Liebert & Starks, 2004) compares the cues of a potential mate with an internal template, and either accepts or rejects such mate based on an ”acceptance threshold”. This threshold is determinate by balancing the relative costs of mate with a relative (“acceptance error”) versus not mating with a non-relative (“rejection error”), given the probability of encounter related and unrelated mates. As a result, this model predicts some flexibility in mating decisions rather than a simple fixative rule, which can explain the low attempted copulations by males toward nestmate females and the low rate of females that copulate with nestmates in paper wasps (Shlemann-Reeve & Gamboa, 1985; Ryan & Gamboa, 1986; Starks et al., 1998; Liebert et al., 2010; Beani & Turillazzi, 1988; Sen et al., 2010).

By preventing nestmates to share visual cues for recognition before the behavioral experiment, it is possible to suppose that cues for intersexual nestmate recognition in *P. versicolor* are odors, already learned by the end of the first day of emergence. In field studies, *Polistes* males have been observed dragging their abdomens across perches in their mating territories, probably applying some odor (Beani & Turillazzi, 1988). While this behavior has been previously hypothesized to function as territorial scent-marking or to attract mates (Beani & Turillazzi, 1988), it is also possible that a nestmate recognition function is involved. These results further add to the body of evidence showing that the choice of sexual partner, both from the female and male point of view, is an important aspect of *Polistes* reproductive biology (Izzo & Tibbetts, 2012; Cappa et al, 2013; De Souza et al., 2014) and that mate choice may be a compromise with fitness relevant traits, like genetic compatibility.

Figures and Legends

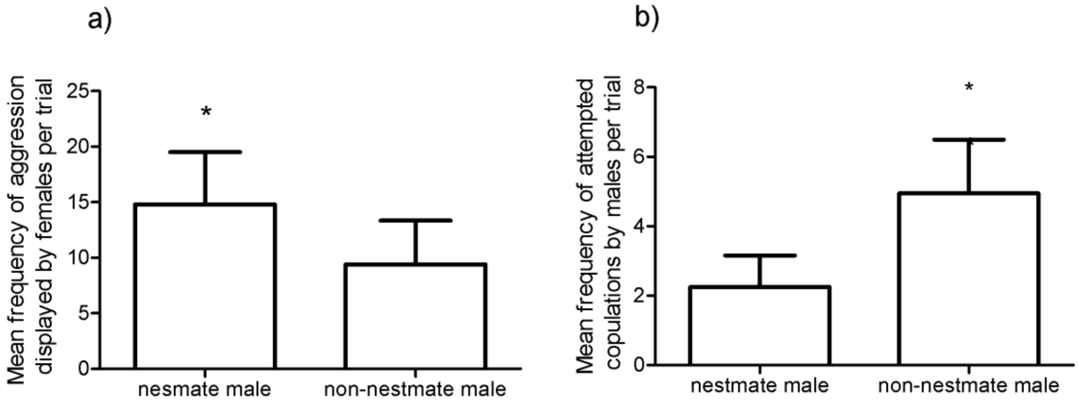

Figure 3.1. Mean (±SE) of (a) aggression directed toward males by females and (b) attempted copulations directed toward females by males. * indicates statistical differences between classes.

Conclusions

The results of experiments described in this dissertation indicate that:

(i) mate choice is a common component of reproductive dynamic in *Polistes* paper wasps;

(ii) such choice is based on visual and probably also chemical information;

(iii) fitness benefits associated to male individual quality and genetic compatibility are likely to explain the evolution of mate choice in paper wasps.

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