

Strong, but incomplete, mate choice discrimination between two closely related species of paper wasp

Sara E. Miller^{1*}, Andrew W. Legan¹, Zoe A. Flores¹, Hong Yu Ng¹, Michael J. Sheehan¹

¹Cornell University Department of Neurobiology and Behavior. Ithaca, NY, USA

*corresponding author

Abstract

Paper wasps (genus: *Polistes*) are one of the most species rich genera of social insect. Prior studies have found that male coloration, male color pattern, territory choice, and female caste are potential drivers of intraspecific mate choice in paper wasps. However, there has been no formal assessment of interspecific mate choice in this group, therefore the mechanisms driving diversification in paper wasps remains an open question. This study measured interspecific and intraspecific mating behavior between two closely related species of paper wasps, *Polistes fuscatus* and *Polistes metricus*. These two species have ample opportunity to interbreed as *P. fuscatus* and *P. metricus* forage, nest, and mate in the same habitats. We tested the strength of reproductive isolation between these species using no choice and choice mating trials. Our results show strong, symmetric, prezygotic isolation between *P. fuscatus* and *P. metricus*. Males discriminated between conspecifics and heterospecifics but attempted to mate with females of the other species in approximately 10% of heterospecific mating trials. Female wasps were more discriminating than males and likely evaluated species identity and male quality through visual or olfactory cues. We additionally report sexual dimorphism in *P. metricus* body size.

28 Introduction

29 Social hymenopterans are a highly successful group of organisms. Surprisingly, the
30 mechanisms that cause diversification in this group have received little attention (Boomsma,
31 Baer, & Heinze, 2005; Baer, 2014). One approach to studying the process of speciation is to
32 identify reproductive boundaries between species and to test the strength of these boundaries
33 (Coyne & Orr 2004). Understanding reproductive boundaries in social hymenoptera is of interest
34 because features of social insect mating systems predict that social insects should have high
35 specificity in mating signals, which can promote speciation and diversification (Coyne & Orr
36 2004). For example, populations of red-tailed bumblebees (*Bombus lapidarius*) show recent
37 divergence in male marking secretions, an important cue for mate attraction (Lecocq *et al.*, 2013).
38 However male social insects lack energetically costly mating displays and rarely display male
39 specific ornamentation characteristic of other systems with strong sexual selection (Boomsma *et*
40 *al.*, 2005; Baer, 2014; Beani *et al.*, 2014), leaving the cause of diversification in this group an open
41 question.

42 Both male and female social hymenoptera have highly skewed distribution in mating
43 success. The majority of female social hymenoptera are non-reproductive workers. Only a small
44 proportion of female social hymenoptera mate, and for primitively social species such as paper
45 wasps, many mated females join cooperative foundress associations and never reproduce
46 (Reeve, 1991). Male *Polistes dominula* paper wasps can distinguish between non-reproductive
47 workers and reproductive females (Cappa *et al.*, 2013). Mating in social hymenoptera typically
48 occurs in leks or groups, characterized by strong male competition and female choice (Boomsma
49 *et al.*, 2005; Beani *et al.*, 2014). Females are monogamous for most species of social hymenoptera

(Strassmann, 2001). Male social hymenoptera do not provide parental care or nuptial gifts, with rare exceptions (see Beani *et al.*, 2014), but contribute only gametes to offspring. As a result, a female likely chooses to mate with a male solely based upon her assessment of male quality. Together these features predict that social hymenopteran mating signals should show high specificity.

Polistes paper wasps are one of the most species rich genera of social insect making this an interesting candidate group in which to investigate social insect diversification. There are more than 200 recognized species of paper wasp (Carpenter, 1996). Species display wide variation in body and facial coloration both within and between species (de Souza *et al.*, 2017b; Carpenter, 1996; Tibbetts, 2004). Paper wasp species often have overlapping ranges, but there is little ecological differentiation among paper wasps (Buck, Marshall & Cheung 2008; Richter, 2000). Together, these observations suggest that diversification in these species may be driven or reinforced by specificity in mating signals. In support of this prediction, coloration has been shown to be an important signal for mate choice within species. Male stenogastrine wasps have sexually dimorphic stripes on their tergites, which are displayed during territorial competition and mating (Beani & Turillazzi, 1999). In *P. dominula*, the size of a yellow abdominal marking influences female mating choice and male-male competition (de Souza *et al.*, 2017a; Izzo & Tibbetts, 2012). Similarly, black facial pigmentation and yellow abdominal spots can impact the choice of sexual partner for *P. simillimus* (de Souza *et al.*, 2014), and these markings may be honest signals of male quality (de Souza *et al.*, 2018).

In temperate latitudes, paper wasp colonies are started by a single wasp or by a small group of female wasps (foundress association) in the spring (Sheehan *et al.*, 2015). Colonies

produce female worker offspring until mid-summer when nests transition to rearing males and reproductive females (gynes). Males leave their natal nests to gather at leks or defend small contiguous territories near the tops of vertical structures such as trees, buildings or telephone poles (Beani et al., 1992; Beani & Turillazzi, 1988; Beani & Turillazzi, 1990a,b; Beani & Zaccaroni, 2015; Matthes-Sears & Alcock 1986; Polak, 1993). Mating occurs when females approach lekking sites. However, smaller males may attempt to mate opportunistically with females near foraging areas or flight paths (Beani & Turillazzi 1988; Post & Jeanne 1983; Polak 1993). Mating territories commonly contain multiple species of paper wasp (Beani & Turillazzi 1990b; Reed & Landolt 1991). As a result of this overlap, unsuccessful heterospecific mating attempts have been observed in the wild (Post & Jeanne, 1983; Reed & Landolt 1991). These heterospecific mating attempts may be stimulated in part by a long-distance volatile pheromone produced by gynes, which has been shown to attract both conspecific and heterospecific males (Post & Jeanne 1984; Reed & Landolt 1990; Ono & Sasaki 1987). After mating, gynes diapause over winter, then the following spring, they build new nests and rear the first group of worker offspring. Due to the disproportionate female investment in offspring, paper wasps gynes may be choosier and more discriminating in mate choice than males.

This study assessed inter- and intraspecific mating behavior between two species of paper wasp, *Polistes fuscatus* and *Polistes metricus*. These species are sympatric throughout most of their range where they forage, nest, and mate in similar habitats, offering ample opportunity for these species to interbreed (Miller *et al.*, 2018b). Quantifying reproductive isolation between *P. fuscatus* and *P. metricus* may be particularly interesting because these species exhibit only weak

genetic divergence (Miller *et al.*, 2018a), suggesting that reproductive boundaries between these species may have formed recently or may be still in the process of forming.

Methods

Wasp Collection

In September 2017, *P. fuscatus* nests (N=24) were collected in New York (Schuyler and Thompson Counties) and *P. metricus* (N=11) nests were collected in Maryland (Frederick and Montgomery Counties). *P. fuscatus* and *P. metricus* are sympatric in Maryland, but only *P. fuscatus* is found in New York. Adult wasps were housed in plastic containers, segregated by nest and gender. Pupae hatching in mid-September were either male or assumed to be reproductive females (gynes). Nests were monitored daily for eclosed wasps. Newly emerged wasps were immediately removed and housed with other nest members of the same gender to ensure that individuals had no prior exposure to members of the opposite sex. Wasps were kept in a 12L:12D light cycle and fed water and sugar *ad libitum*.

Mating Experiment 1

Individuals were randomly assigned to mating trials (N=152) in one of four groups: *P. fuscatus* female x *P. fuscatus* male; *P. metricus* female x *P. metricus* male; *P. fuscatus* female x *P. metricus* male; and *P. metricus* female x *P. fuscatus* male. Male and female wasps from the same nest were never paired. Wasps were individually housed during the course of the experiment to prevent the transmission of social or chemical information.

More females eclosed in the lab (*P. fuscatus* N=77; *P. metricus* N=72) than males (*P. fuscatus* N=37; *P. metricus* N=23). Due to the discrepancy between the sexes, virgin females with no prior mating experience were used in all trials, but lab eclosed males were used multiple times, always with the same species of female. Using males in multiple mate choice trials is consistent with the experience of wasps in the wild as male wasps will mate multiple times if given the opportunity, whereas female wasps typically mate only once (Hughes *et al.*, 2008).

Mating trials followed a no-choice design. A single virgin female and a single lab eclosed male were introduced into a petri dish containing a vial of water. Wasps were allowed to interact for two hours and were weighed at the end of the trial. Trials were recorded with Sony HDR-CX405 video cameras. Males were allowed to recuperate for at least 24 hours between trials. All trials were conducted from 10:00-16:00 h.

Measurement of Behavior

Behavioral interactions between males and females were quantified for the first 30 minutes of each trial. Male interest was determined by measuring the latency and frequency of three male behaviors required for mating (based on Beani & Turillazzi 1988). (1) *Approach/inspection*: the male oriented his head and body in the direction of the female, walked towards the female, and attempted to contact the female with his legs or antenna. (2) *Mating Attempt*: the male mounted the female in preparation for mating. (3) *Mating*: genital linkage occurred for ten or more seconds. Matings that occurred in the remaining 90 minutes of each trial were also recorded.

To assess female aggression, we counted the number of snaps (opening and closing of mandibles in the direction of the other wasp), bites, and stings. Aggressive behaviors had a highly skewed distribution, driven by several extremely aggressive females. Therefore, we report aggression as the log of the sum of the number of aggressive behaviors observed in each trial. All behaviors were scored using BORIS (V6.2.2) by two observers blind to species identity.

Mating Experiment 2

There was a low proportion of successful matings in experiment 1. We therefore performed a second set of trials to test if female receptivity increased when females were given a choice of mating partners. Mating trials used the same pairings as Experiment 1 (N=99). Experiment 2 began two days following the final Experiment 1 trial. Most mating in Experiment 1 occurred within the first fifteen minutes of the trial, therefore we reduced the length of mating trials to thirty minutes. Trials followed the same procedure as Experiment 1, with the exception that each trial used two males.

Females in Experiment 2 had been previously used in a single trial in Experiment 1 but had not mated during that trial. Several males died prior to the start of Experiment 2, either as a result of female aggression or due to senescence. To increase the number of males in the experiment, additional wild-caught males collected on nests (*P. fuscatus* N=44; *P. metricus* N=22) or caught on the wing (*P. fuscatus* N=2) were included. Wasps of both genders were assigned to the same pairing as in Experiment 1. For each trial, we report the latency for the first male to attempt to mate, and the latency of the first copulation.

Statistical Analysis

Differences in male and female body size were assessed separately for each species using a linear mixed effects model with the nlme package (Pinheiro *et al.* 2018), including nest of origin as a random effect. Chi square tests were used to measure the difference in the frequency of male behaviors between heterospecific and conspecific mating trials. Due to the small number of counts in some groups, p-values were estimated using Monte-Carlo simulations (N=2000). Quantitative measurements of behavior were not normally distributed, as a result, the non-parametric Kruskal-Wallis test was used to assess differences in these behaviors between conspecific and heterospecific trials. Following Coyne and Orr (1989), the strength of prezygotic reproductive isolation was estimated as: $1 - (\text{frequency of heterospecific mating} / \text{frequency of conspecific mating})$. All statistics analyses were calculated with R (v3.4.3) (R Core Team, 2018).

Results

P. fuscatus and *P. metricus* were differentially sexual dimorphic in body weight. *P. metricus* females were smaller than *P. metricus* males (Fig 1A: $F_{1,100}=34.6$, $P<0.0001$). *P. fuscatus* females were larger than *P. fuscatus* males, but this result was not statistically significant after correcting for nest ($F_{1,124}=2.3$, $P=0.13$). This resulted in females that were smaller than males in the *P. metricus* male x *P. fuscatus* female pairings, in comparison to other pairings (Figure 1B).

Experiment 1

Male mating behavior in no-choice trials was simple and identical for *P. fuscatus* and *P. metricus*. The male approached the female early in the trial. If she was receptive, the male mounted the female and mating occurred. Males did not perform any visible courtship behavior, indicating that female choice likely relied solely upon visual or chemical cues of male quality.

Males inspected females in 145 trials (96%). Males were equally likely to inspect conspecific and heterospecific females ($\chi^2 = 2.0$, $P=0.75$), but were slower to inspect and spent less time inspecting females of the other species (Table 1; Figure 2A,D; Latency: $H_2 = 7.2$, $P=0.007$; Time: $H_2 = 33.9$, $P=5.7e^{-9}$). Males mounted females (hereafter “Attempt”): Mating attempts occurred in 40 of 152 trials (27%). Mating attempts were more likely to occur in conspecific trials ($\chi^2 = 41.7$, $P=5e^{-4}$). Males attempted to mate with conspecifics more rapidly (Figure 2B: Latency: $H_2 = 7.2$, $P=0.007$) and spent more time attempting to mate with conspecifics than heterospecifics (Figure 2E: Time mounting: $H_2 = 33.9$, $P=5.7e^{-9}$). Mating occurred in 19 trials (13%). All matings were between conspecifics ($\chi^2 = 25.3$, $P=5e^{-4}$). When mating occurred, the pair typically mated rapidly (median time: 308s).

This experiment had a low rate of mating success, largely driven by female rejection of males. Females rejected males by performing aggressive behaviors towards the male, preventing males from mating, or by moving her abdomen to prevent genital linkage after mounting occurred. Females were more aggressive towards conspecific males ($H_2 = 5.5$, $P=0.02$), but this difference was driven by the higher number of mating attempts by conspecific males than heterospecific males. The highest rates of aggression were observed during trials between *P. fuscatus* females and *P. metricus* males (Figure 2F).

There was uneven mating success among males. Only 6 of 19 males mated (32%) in *P. fuscatus* x *P. fuscatus* mating trials, with two males mating twice. Similarly, 8 of 20 males mated (40%) in *P. metricus* x *P. metricus* trials, with three males each mating twice. Mated *P. fuscatus* males were smaller than males that did not mate (paired t-test: $t = 2.5$, $df=11.6$, $P=0.03$). Conversely, *P. metricus* males were larger in trials with successful mating, but this difference was not significant (paired t-test: $t = -0.86$, $df=24.1$, $P=0.40$). The relative difference in male and female body size was not a predictor of mating success for *P. fuscatus* (paired t-test: $t = -1.1$, $df=9.1$, $P=0.31$) or *P. metricus* (paired t-test: $t = -0.24$, $df=32.4$, $P=0.81$).

Experiment 2

Males attempted to mount females in 34 trials (34%). As above, mating attempts were more likely to occur in conspecific trials (Table 2; $X^2 = 33.3$, $P=5e^{-4}$) but there was no difference in the latency to attempt to mate between conspecific and heterospecific trials (Figure 2G; $H_2 = 0.33$, $P=0.56$). Mating occurred in 24 trials (25%). Mating was more likely to occur between conspecifics ($X^2 = 20.8$, $P=5e^{-4}$), however we observed two interspecific matings. One mating occurred between a *P. fuscatus* female and a *P. metricus* male, and one mating between a *P. metricus* female and a *P. fuscatus* male. In both trials, the female had a smaller body weight than the male. The latency to mate was similar for conspecific and heterospecific trials (Figure 2H: $H_2 = 0.05$, $P=0.82$). The difference in the latency in mating attempts and mating observed between Experiment 1 and Experiment 2 was largely driven by a single heterospecific trial during which mating occurred almost immediately. The two female wasps that mated with heterospecifics were overwintered in the lab but neither female produced offspring. One female died in the early

spring before founding a nest. The second female joined a cooperative foundress association as a subordinate but did not lay eggs.

Including female choice led to a moderate increase in mating success (Figure 2I). Experiment 2 had more mating attempts (odds ratio = 0.68 (95% CI: 0.39-1.19), $P=0.18$) and successful matings (odds ratio = 0.45 (95% CI: 0.23-0.87), $P=0.019$), than experiment 1. Combining the results of experiments 1 and 2, we estimate prezygotic reproductive isolation between *P. fuscatus* and *P. metricus* as 0.91.

Males from different sources (wild caught or lab eclosed) attempted to mate (odds ratio = 1.54 (95% CI: 0.68-3.5), $P=0.30$) and mated at similar frequencies (odds ratio = 1.3 (95% CI: 0.51-3.53), $P=0.58$). Male body weight did not influence female mate choice in Experiment 2. *P. fuscatus* females did not preferentially mate with the smaller male (paired t-test: $t= 1.3$, $df=15.7$, $P=0.21$), nor did *P. metricus* females prefer the larger male (paired t-test: $t= -0.5$, $df=13.9$, $P=0.62$). In three of the conspecific trials (3%), the female mated with both males.

In five trials (5.1%), one male attempted to mate with the other male. Male-male mating occurred in only a single trial with the same species pairing. During this trial, a *P. fuscatus* male first mated with the *P. fuscatus* female. The same male then attempted to mate with the other male. The remaining four trials with male-male mating attempts were pairings between a *P. metricus* female and *P. fuscatus* males. In all four trials, the male initially inspected - but did not mate with - the female, prior to mounting the other male. Body weight did not predict which male mounted the other male (paired t-test: $t=-1.19$, $df=5.5$, $P=0.28$).

244 Discussion

245 Mate choice experiments showed strong, symmetric, prezygotic isolation between *P.*
246 *fuscatus* and *P. metricus*. Male wasps were less likely to attempt to mate with heterospecifics
247 than conspecifics, although males attempted to mate with females of the other species in
248 approximately 10% of heterospecific mating trials. Matching prior observations, males did not
249 perform any obvious courtship behavior towards females. As a result, female assessment of
250 species identity and male quality likely relied on visual or olfactory cues. Male wasps had skewed
251 mating success with some males mating multiple times, suggesting that certain males were
252 perceived to be of higher quality by females. Female wasps were choosy and frequently rejected
253 undesirable males through aggressive interactions. This led to a low level of mating success even
254 among correct species pairings, matching previous observations of mating in *P. fuscatus* (Post
255 and Jeanne, 1983). In these trials, successful mating was primarily determined through female
256 choice.

257 *P. fuscatus* females were larger than *P. fuscatus* males and conversely, *P. metricus* females
258 were smaller than *P. metricus* males. Boomsma *et al.* (2005) predicted that social insects will have
259 little sexual dimorphism, but when sexual dimorphism exists, males should be smaller than
260 females. The higher body weight in *P. metricus* males appears to contradict these findings.
261 Experiment 1 suggested that *P. fuscatus* females preferred smaller males while *P. metricus*
262 females preferred larger males, but this result was not significant in Experiment 2. However, one
263 possible confound was our use of body weight rather than body length to assess male size. Body
264 weight may be affected by the nutritional condition, therefore the role of male body size in mate
265 choice is still uncertain for these species.

Prezygotic discrimination between *P. fuscatus* and *P. metricus* was not complete. In two trials, females mated with heterospecific males. Neither of these trials produced offspring, but further studies are necessary to determine if additional post-mating isolating mechanisms exist between these species. Nevertheless, our results suggest the potential for hybridization and gene flow between *P. fuscatus* and *P. metricus* in the wild.

If mating success in these species is driven by female choice, interspecific mating is more likely to occur when females have incomplete or inaccurate information about male quality. This could occur when one species is rare, and the other species is common, such as at range limits. Alternatively, if females are using visual or olfactory cues to choose a mate, interspecific mating may occur in situations in which these signals are obscured. For example, crowded lekking sites containing many males of multiple species. Lastly, interspecific mating could occur when males are able to circumvent female choice, such as coercive mating between large males and small females, as may have been the case in the heterospecific matings in our study.

Poor mate choice decisions can also occur when high quality heterospecifics are confused with conspecifics, leading to a conflict between species choice and mate choice (Pfennig, 1998). In social hymenopterans, gender is determined through single-locus complementary sex determination. When a female mates with a male with the same allele at the sex-determination locus, half of diploid offspring produced will be sterile males, leading to a severe fitness cost to the nest (Crozier, 1977). A byproduct of inbreeding avoidance could be an increase in heterospecific matings. In addition, a poor mate choice decision may be better than not mating. Mated *Bombus* gynes have higher rates of winter survival than un-mated gynes (Baer & Schmid-Hempel, 2001; Gerloff & Schmid-Hempel, 2005), and there is some indication of similar benefits

of mating in *P. dominula* (Izzo, 2011), suggesting that males provide unknown supplemental resources for females.

Our experiments found that male wasps had a larger margin of error for incorrect species choice. This was evidenced by male attempts to mate with other males in Experiment 2. Male-male mating behavior in insects is thought to represent a case of mistaken identity and inaccurate mate choice (Scharf & Martin, 2013; Sales *et al.*, 2018). Male-male mating attempts occurred at a similar frequency to male attempts to mate with heterospecific females.

Overall, we found strong but incomplete mate choice discrimination between *P. fuscatus* and *P. metricus*. Future studies are needed to determine which cues are important for distinguishing species identity during mate choice and to determine the frequency of hybridization between these species in nature.

Acknowledgements

We thank Sarah Bluher for help collecting wasps. This work was supported by NSF postdoctoral fellowship (S.M) DBI-1711703, NSF Graduate Research Fellowship (A.L.), and NIH grant DP2-GM128202 (M.S.).

References

- Baer B, Schmid-Hempel P. 2001.** Unexpected consequences of polyandry for parasitism and fitness in the bumblebee, *Bombus terrestris*. *Evolution* **55**: 1639-1643.
- Baer B. 2014.** Sexual selection in social insects. In: Shuker DM, Simmons LW, eds. *The evolution of insect mating systems*. USA: Oxford University Press, 261-274.
- Beani L, Cervo R, Lorenzi CM, Turillazzi S. 1992.** Landmark-based mating systems in four *Polistes* species (Hymenoptera: Vespidae). *Journal of the Kansas Entomological Society* **1992**: 211-217.
- Beani L, Turillazzi S. 1988.** Alternative mating tactics in males of *Polistes dominulus* (Hymenoptera: Vespidae). *Behavioral Ecology and Sociobiology* **22**: 257–264.

- 316 **Beani L, Turillazzi S. 1990a.** Male swarms at landmarks and scramble competition polygyny in
317 *Polistes gallicus* (Hymenoptera: Vespidae). *Journal of insect behavior* **3**: 545–556.
- 318 **Beani L, Turillazzi S. 1990b.** Overlap at landmarks by lek-territorial and swarming males of two
319 sympatric polistine wasps (Hymenoptera: Vespidae). *Ethology Ecology and Evolution* **2**: 419–31.
- 320 **Beani L, Turillazzi S. 1999.** Stripes display in hover-wasps (Vespidae: Stenogastrinae): a socially
321 costly status badge. *Animal behaviour*, **57**: 1233–1239.
- 322 **Beani L, Dessi-Fulgheri F, Cappa F, Toth A. 2014.** The trap of sex in social insects: From the
323 female to the male perspective. *Neuroscience and Biobehavioral Reviews* **46**: 519–533.
- 324 **Beani L, Zaccaroni M. 2015.** Experimental male size manipulation in *Polistes dominula* paper
325 wasps: being the right size. *Ethology Ecology & Evolution*, **27**: 185–199.
- 326 **Buck M, Marshall SA, Cheung DK. 2008.** Identification Atlas of the Vespidae (Hymenoptera,
327 Aculeata) of the northeastern Nearctic region. *Canadian Journal of Arthropod Identification*, **5**:
328 1–492.
- 329 **Boomsma JJ, Baer B, Heinze J. 2005.** The evolution of male traits in social insects. *Annual*
330 *Review of Entomology* **50**: 395–420.
- 331 **Cappa F, Bruschini C, Cervo R, Turillazzi S, Beani L. 2013.** Males do not like the working class:
332 male sexual preference and recognition of functional castes in a primitively eusocial wasp.
333 *Animal Behaviour* **86**: 801–810.
- 334 **Carpenter JM. 1996.** Distributional checklist of species of the genus *Polistes* (Hymenoptera,
335 Vespidae, Polistinae, Polistini). American Museum novitates; no. 3188.
- 336 **Coyne JA, Orr HA. 1989.** Patterns of speciation in Drosophila. *Evolution*, **43**: 362–381.
- 337 **Coyne JA, Orr HA. 2004.** Speciation. Massachusetts: Sinauer.
- 338 **Crozier RH. 1977.** Evolutionary genetics of the Hymenoptera. *Annual Review of Entomology*, **22**:
339 263–288.
- 340 **de Souza AR, Júnior C, do Nascimento FS, Lino-Neto J. 2014.** Sexy faces in a male paper wasp.
341 PLoS One **9**: e98172.
- 342 **de Souza AR, Simões TG, Rantala MJ, Santos EF, Lino-Neto J, do Nascimento FS. 2018.** Sexual
343 ornaments reveal the strength of melanization immune response and longevity of male paper
344 wasps. *Journal of insect physiology* **109**: 163–168.
- 345 **de Souza AR, Lino-Neto J, Tibbetts EA, Turillazzi S, Beani L. 2017.** The leks of *Polistes dominula*
346 paper wasps: tiny abdominal spots play a critical role in male attacks toward potential
347 rivals. *Ethology Ecology & Evolution*, **29**: 410–419.

348 **de Souza AR, Turillazzi S, Lino-Neto J, Santini G. 2017.** Colder environments may select for
349 darker paper wasps. *Biological Journal of the Linnean Society* **120**: 700-704.

350 **DesJardins N, Tibbetts EA. 2018.** Sex differences in face but not colour learning in *Polistes*
351 *fuscatus* paper wasps. *Animal Behaviour* **140**: 1-6.

352 **Gerloff U, Schmid-Hempel P. 2005.** Inbreeding depression and family variation in a social
353 insect, *Bombus terrestris* (Hymenoptera: Apidae). *Oikos* **111**: 67-80.

354
355 **Hughes WOH, Ratnieks FLW, Oldroyd BP. 2008.** Multiple paternity or multiple queens: two
356 routes to greater intracolony genetic diversity in the eusocial Hymenoptera. *Journal of*
357 *Evolutionary Biology* **21**: 1090-1095.

358 **Izzo AS. 2011.** Spotting the Top Male: Sexual Selection in a Lek-Mating Paper Wasp, *Polistes*
359 *dominulus*. PhD. University of Michigan.

360 **Izzo AS, Tibbetts EA. 2012.** Spotting the top male: sexually selected signals in male *Polistes*
361 *dominulus* wasps. *Animal Behaviour* **83**: 839-845.

362 **Lecocq T, Vereecken NJ, Michez D, Dellicour S, Lhomme P, Valterova I, Rasplus JY, Rasmont P.**
363 **2013.** Patterns of genetic and reproductive traits differentiation in mainland vs. Corsican
364 populations of bumblebees. *PLoS One* **8**: e65642.

365 **Miller SE, Legan AW, Henshaw M, Ostevik KL, Samuk K, Uy FMK, Reeve HK, Sheehan MJ.**
366 **2018a.** Evolutionary dynamics of recent selection for enhanced social cognition. *bioRxiv*:
367 425215.

368 **Miller SE, Bluher SE, Bell E, Cini A, da Silva RC, de Souza AR, Gandia KM, Jandt J, Loope K,**
369 **Prato A, et al. 2018b.** WASPnest: a worldwide assessment of social *Polistine* nesting behavior.
370 *Ecology* **99**: 2405-2405.

371 **Ono M, Sasaki M. 1987.** Sex pheromones and their cross-activities in six Japanese sympatri
372 species of the genus *Vespa*. *Insectes sociaux* **34**: 252-260.

373
374 **Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. 2018.** nlme: Linear and nonlinear mixed
375 effects models. R package version 3.1-137. Available at: [https://CRAN.R-](https://CRAN.R-project.org/package=nlme)
376 [project.org/package=nlme](https://CRAN.R-project.org/package=nlme)

377 **Polak M. 1993.** Competition for landmark territories among male *Polistes canadensis* (L.)
378 (Hymenoptera: Vespidae): large-size advantage and alternative mate-acquisition tactics.
379 *Behavioral Ecology* **4**: 325–331.

380 **Post DC, Jeanne RL., 1983.** Male reproductive behavior of the social wasp *Polistes fuscatus*
381 (Hymenoptera: Vespidae). *Zeitschrift für Tierpsychologie* **62**: 157-171.

382 **Reed HC, Landolt PJ. 1990.** Sex attraction in paper wasp, *Polistes exclamans* Viereck
383 (Hymenoptera: Vespidae), in a wind tunnel. *Journal of Chemical Ecology* **16**: 1277-1287.
384

385 **Reed HC, Landolt PJ. 1991.** Swarming of paper wasp (Hymenoptera: *Vespidae*) sexuals at
386 towers in Florida. *Annals of the Entomological Society of America* **84**: 628-635.
387

388 **Reeve HK. 1991.** *Polistes*. In: Ross KG, Matthews RW, eds. *The social biology of wasps*. Ithaca:
389 Comstock Publishing Associates, 99-148.

390 **Sales K, Trent T, Gardner J, Lumley AJ, Behaviour RVA. 2018.** Experimental evolution with an
391 insect model reveals that male homosexual behaviour occurs due to inaccurate mate choice.
392 *Animal Behaviour* **139**: 51-59.

393 **Sheehan MJ, Botero CA, Hendry TA, Sedio BE, Jandt JM, Weiner S, Toth A, Tibbetts EA. 2015.**
394 Different axes of environmental variation explain the presence vs. extent of cooperative nest
395 founding associations in *Polistes* paper wasps. *Ecology letters* **18**: 1057-1067.

396 **Matthes-Sears W, Alcock J. 1986.** Hilltopping behavior of *Polistes commanchus navajoe*
397 (Hymenoptera: *Vespidae*). *Ethology* **71**: 42-53.

398 **R Core Team. 2018.** *R: A language and environment for statistical computing*. Vienna: R
399 Foundation for Statistical Computing. Available at: <http://www.R-project.org>.

400 **Richter MR. 2000.** Social wasp (Hymenoptera: *Vespidae*) foraging behavior. *Annual review of*
401 *Entomology* **45**: 121-150.
402

403 **Scharf I, Martin OY. 2013.** Same-sex sexual behavior in insects and arachnids: prevalence,
404 causes, and consequences. *Behavioral Ecology and Sociobiology* **67**: 1719–1730.

405 **Strassmann J. 2001.** The rarity of multiple mating by females in the social Hymenoptera.
406 *Insectes Sociaux* **48**: 1–13.

407 **Tibbetts EA. 2004.** Complex social behaviour can select for variability in visual features: a case
408 study in *Polistes* wasps. *Proceedings of the Royal Society B: Biological Sciences* **271**: 1955–1960.
409

Figure Legends

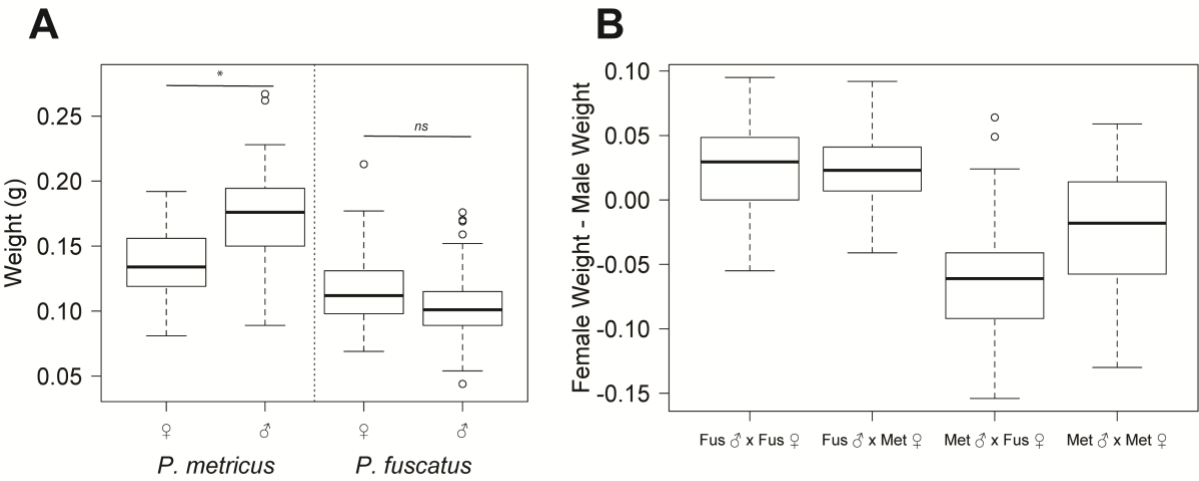
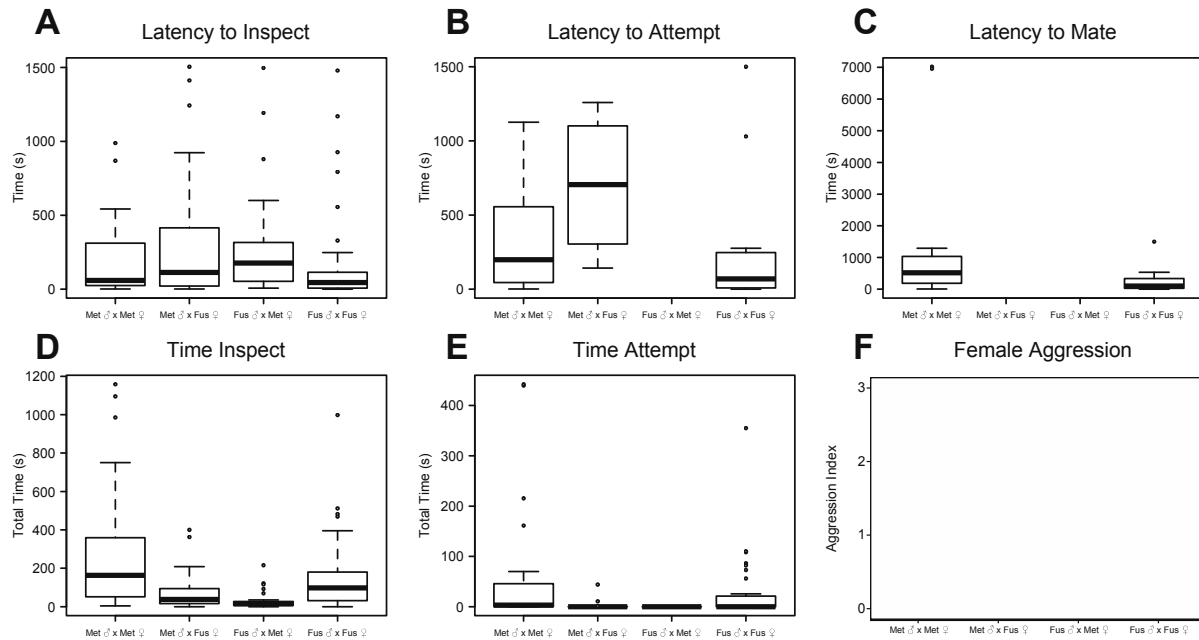
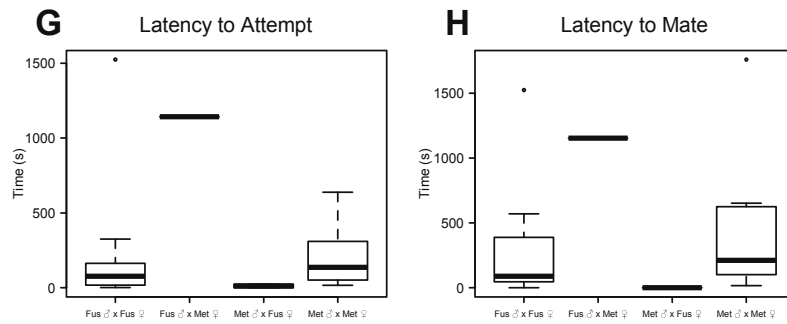


Figure 1: (A) Body weight for *P. metricus* and *P. fuscatus* females and males. (B) The difference in body weight in Experiment 1 between paired males and females from the four possible species pairings. Due to the larger body weight of *P. metricus* males, females were smaller than males in the *P. metricus* male x *P. fuscatus* female trials.

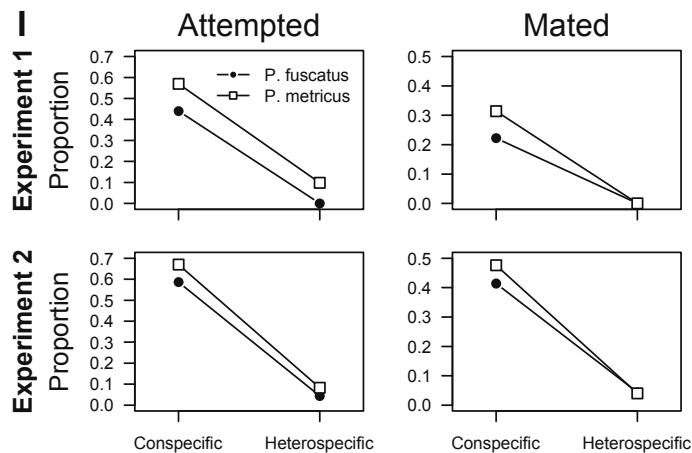
Experiment 1



Experiment 2



Summary



418

419 Figure 2: Quantitative measurements of male mating behavior in Experiment 1 shows that

420 males (A) approach, (B) mount/attempt to mate, and (C) mate with conspecific females more

421 quickly than heterospecific females. Males spend more time (D) approaching and (E)

422 attempting to mate with conspecific females during the first 30-minutes of each trial.

423 Heterospecific pairings did not mate during any trial in Experiment 1. Female *P. fuscatus* were

424 slightly more aggressive (F) than *P. metricus* females. Experiment 2 showed similar results with

425 males (G) attempting to mate, and (H) mating with conspecific females more quickly than

426 heterospecific females. There was a small increase in the proportion of trials during which

427 males attempted to mate and mated (I) in Experiment 2 compared to Experiment 1.

428

Type	Trials	Approaches	Latency to Approach	Attempts	Latency to Attempt	Matings	Latency to Mate	Female Aggression
fuscatus female x fuscatus male	36	35	187.6 ± 60.6	16	234 ± 105.5	8	303 ± 181.4	2.1 ± 0.31
fuscatus female x metricus male	41	40	297 ± 64.8	4	703.9 ± 248.0	0	NA	1.43 ± 0.29
metricus female x fuscatus male	39	35	262.7 ± 56.9	0	NA	0	NA	1.11 ± 0.19
metricus female x metricus male	35	35	188.6 ± 43.9	20	313.4 ± 71.2	11	1626.4 ± 807.3	1.62 ± 0.20

Table 1: Summary of results from Mate choice Experiment 1. Approaches, attempts, and matings refer to the number of trials during which a male performed that behavior. Latency to approach, latency to attempt, and latency to mate are the mean and standard error of the time it took a male to perform that behavior during the trial in seconds. Female aggression is the mean and standard error of the female aggression index (see methods).

Type	Trials	Attempts	Latency to Attempt	Matings	Latency to Mate
fuscatus female x fuscatus male	31	17	176.9 ± 87.2	12	281.2 ± 125.4
fuscatus female x metricus male	24	2	11.7 ±	1	0.01
metricus female x fuscatus male	23	1	1142.6 ±	1	1153.9
metricus female x metricus male	21	14	186.1 ± 47.7	10	456.3 ± 182.7

Table 2: Summary of results from Mate choice Experiment 2. Attempts, and matings refer to the number of trials during which a male performed that behavior. Latency to attempt, and latency to mate is the mean and standard error of the time it took a male to perform that behavior during the trial in seconds.