



Hormonal modulation of reproduction in *Polistes fuscatus* social wasps: Dual functions in both ovary development and sexual receptivity

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ABSTRACT

Hormones are often major regulators of complex behaviors, such as mating and reproduction. In insects, juvenile hormone (JH) is integral to many components of reproductive physiology and behavior, but its role in female sexual receptivity is not well understood. To investigate the influence of JH on receptivity, we utilized the social wasp *Polistes fuscatus*. In *Polistes*, mating behavior is temporally separated from other components of reproduction, which allows for examination of the physiology and behavior of mating, disentangled from fertilization and egg-laying. We reared virgin gynes (reproductive females) in the lab and divided them into four groups, in which gynes received multiple topical treatments of either 20 µg, 10 µg, 5 µg, or 0 µg of the JH analog methoprene. Gynes were then placed in petri dishes with 2 unrelated males and we recorded attempted and successful mating. Additionally, we measured gyne ovarian development and survival in each group. We found that methoprene increased both sexual receptivity and ovarian development, but was associated with a decrease in long-term survival. Receptivity increased linearly as methoprene treatment increased, but the effect of methoprene on ovarian development was independent of dose. These results demonstrate the importance of JH in sexual receptivity and mating behavior. We argue that the relatively understudied *Polistes* gyne has potential as a model for mating and reproduction, and for the internal and external regulation of this complex behavior.

1. Introduction

Changes in behavior are a necessary component of maturation and life-stage shifts, allowing animals to generate developmentally appropriate responses to their environment. Hormones are frequently key to the regulation of behavior, as they can coordinate modifications of behavior and physiology across multiple different tissues within an individual (Adkins-Regan, 2005). Hormones can also regulate different components of a complex behavior. Changes in multicomponent behavioral “states” (e.g. juvenile vs adult) involve alterations of multiple individual behavioral responses, each of which may develop over different time scales. For example, it is well documented that hormones play a major role in animal reproductive behavior (Barth and Lester, 1973; Flatt et al., 2005), but sexual receptivity and mating is a specific component of reproductive state, which may be uncoupled from reproduction itself, and influenced by many environmental and social factors. How hormones control different temporally separated behavioral developmental events, as part of an overall shift in behavioral state, is generally less well understood.

In insects, hormones play a major role in the shift from non-

reproductive to reproductive behavior (Nijhout, 1994). Typically, juvenile hormones (JH) coordinate the onset of gonadotropic activity and reproductive behaviors including ovary activation, egg maturation, egg-laying (Nijhout, 1994; Hartfelder, 2000), sex pheromone production (Cusson and McNeil, 1989), and, in some insects, mating activity in males (Pener, 1967; Teal et al., 2000). While the above relationships are well established, the relationship between JH and female sexual receptivity is less clear. Female receptivity is a critical behavior for reproduction, and in many cases JH appears to be necessary to induce receptivity (Lea, 1968; Loher and Huber, 1966; Barth and Lester, 1973). However, in a review of sexual receptivity in insects, Ringo (1996) cautions against claiming that JH is required for receptivity because many early findings relied on negative evidence alone (the removal of the corpora allata led to an absence of sexual activity), and in some cases failed at replication (i.e. in the cockroach *Leucophaea maderae*: Englemann, 1960; Roth and Barth, 1964; Englemann and Barth, 1968; and in the grasshopper *Gomphocerus rufus*: Hartmann et al., 1972). Additionally, there are several groups of insects in which receptivity is not affected by allatectomy nor application of JH (Ringo, 1996). Recent findings continue to be incongruent, showing that JH promotes

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receptivity in some cases (in *Drosophila melanogaster*: Ringo et al., 2005), and decreases receptivity in others (in the western tarnished plant bug *Lygus hesperus*: Brent and Hull, 2014). One further reason for caution is that many female insects store sperm in a spermatheca, so mating and fertilization/egg-laying are sometimes temporally separated (Klowden, 2013). Because of this, strong evidence that JH regulates ovary activation and oviposition behavior is not synonymous with evidence that JH controls receptivity. Our understanding of the role of JH in moderating female receptivity thus remains incomplete.

The life cycle of temperate *Polistes* paper wasps offers an appealing context to study female sexual receptivity. Unlike many other insects, mating and egg-laying in temperate paper wasps occur months apart from each other, after major behavioral state changes. Whereas workers are typically smaller (Dani, 1994), reared early in the spring/summer by founding females and experience a low feeding rate as larvae, reproductive females (gynes) are reared in late summer/fall by a team of workers and experience a high feeding rate and generally have larger body size and ample fat stores (Haggard and Gamboa, 1980; Hunt, 2007). Importantly for the current study, gynes emerge in reproductive diapause and do not initiate egg-laying until the next favorable season (Hunt, 2007). Mating occurs off the nest in aggregations (Matthes-Sears and Alcock, 1986; Beani and Turillazzi, 1988; Reed and Landolt, 1991; Beani et al., 1992; Polak, 1992; Polak, 1993; De Souza et al., 2017). Then, the inseminated females spend the winter in quiescence, usually clustered with other gynes in a sheltered place (Hunt, 2007). In spring, these females emerge from quiescence and begin to construct nests, at which point they are referred to as “foundresses” (Reeve, 1991). It is at this life stage, several months after mating, that they first fertilize and lay eggs (Hunt, 2007). Overall, the lifestyle of *Polistes* allows for examination of the physiology and behavior of mating, disentangled from fertilization and egg-laying for a clearer investigation of the nature of insect sexual receptivity.

The gyne is a relatively understudied life stage in social wasps (Judd et al., 2010). In contrast, much work has been done on the behavior of social wasp workers and foundresses, including the important role of JH in division of labor. When polistine wasp nests contain more than one foundress, a dominance hierarchy emerges (Pardi, 1948; Turillazzi and Pardi, 1977; Jandt et al., 2014), and the dominant individuals have higher JH titers than subordinates (Röseler et al., 1984; Tibbetts and Izzo, 2009; Tibbetts et al., 2011a) and more developed ovaries (Pardi, 1948; Röseler et al., 1985; Barth et al., 1975). In addition, JH seems to have different roles in queen and worker behaviors. In *Polistes canadensis* queens, high JH corresponds with higher ovarian development, but in workers high JH correlates with an early onset of the worker-biased behavior of guarding (Giray et al., 2005). Additionally, JH increases worker ovarian development in *Polistes dominula* (Tibbetts et al., 2011b). These previous studies show that JH coordinates behavioral states in foundresses, queens, and workers. However, the role of JH in gyne behavior has not been studied. Gynes engage in several important behaviors including mating, feeding on floral nectar, and overwintering. Because of JH's proposed role in reproductive behaviors, we investigated whether this hormone can modulate female sexual receptivity in the temperate paper wasp, *Polistes fuscatus*. Specifically, we conducted mating trials with JH-treated virgin gynes to test the hypothesis that JH increases sexual receptivity in gynes. Our results support this hypothesis. Additionally, we confirm previous findings that JH helps regulate ovarian development (Barth et al., 1975; Giray et al., 2005; Tibbetts et al., 2011a; Tibbetts et al., 2011b), but show that JH also affects gyne survival during quiescence which suggests that increased receptivity may be paired with a trade-off between investment in somatic and gonadal activity.

2. Materials and methods

2.1. Wasp collection and housing

Between July and October 2018, *Polistes fuscatus* wasp nests ($n = 22$) and free-flying males ($n = 256$) were collected throughout the Finger Lakes region of central New York, USA. A few additional wasp nests ($n = 3$) and males ($n = 45$) were collected from New Hampshire, Maine, and Iowa. Wasp nests were affixed to cardboard squares with a hot glue gun and housed in individual plastic containers (36.8 cm $\times$ 22.2 cm $\times$ 24.8 cm extra-large “Kritter Keeper”, Lee’s Aquarium & Pet Products). Males caught free-flying were housed with others from the same collection site. All wasps were provided with sugar cubes (Domino Brand Sugar) and water *ad libitum* and nests were additionally provisioned with wax moth (*Galleria mellonella*) larvae in plastic dishes for workers to forage upon. Wasps were maintained in an indoor animal rearing room at Cornell University’s Liddell field laboratory in Ithaca, NY at 26 °C and 50% humidity with full-spectrum lights set to a day-night cycle of 14 h light and 10 h dark.

Nests were checked daily and any newly-eclosed male wasps were removed and housed with other male nestmates to prevent newly-eclosed females from exposure to males or mating. Only sexually mature males caught free-flying were used in mating trials. Because of limited male numbers, many males were used in two trials, but always with 24 h rest between trials. Using males in more than one mating trial is consistent with natural mating behavior, as males will mate multiply in the wild (Hughes et al., 2008). Because the majority of females that emerge later in the season are gynes (reproductive females) (Haggard and Gamboa, 1980), all virgin females that eclosed in the lab were most likely gynes.

2.2. Methoprene treatments

In October 2018, virgin gynes were removed from their nests, weighed, individually-marked with enamel paint and housed individually in 6 oz polypropylene deli containers (Fabri-Kal) with sugar cubes, water, and pieces of construction paper. Gynes ($n = 236$) were randomly assigned to one of four treatment groups: 20 μ g ($n = 58$), 10 μ g ($n = 59$), 5 μ g ($n = 62$), or 0 μ g ($n = 57$) methoprene (Sigma Aldrich) in 1 μ L acetone. Methoprene is a commonly used analog for JH in Hymenoptera and other insects (O'Donnell and Jeanne, 1993), and 20 μ g is below dosages known to increase short-term mortality in *Polistes* species (Giray et al., 2005; Shorter and Tibbetts, 2009). Previous research has shown that such low doses of acetone alone do not affect wasp behavior or survival (Shorter and Tibbetts, 2009; Giray et al., 2005; Agrahari and Gadagkar, 2003). Wasps received topical application of the treatment directly on their abdomens every other day for at least one week. Methoprene has a high photodegradation rate with a half-life of about 1.5 h (Csondes, 2004), so any residual methoprene not absorbed through the gyne's cuticle will have dissipated by the time mating trials occurred the following day. We then conducted mating trials in 3 batches occurring every other day the following week. This experimental schedule allowed us to continue an every-other-day treatment application of methoprene/acetone through a second week so that all females received treatment the day before their mating trial. The experimental design did result in different overall numbers of treatments applications prior to mating trials, with approximately 1/3 of all gynes received 3 treatments (of their respective treatment concentration) ($n = 80$), 1/3 received 4 treatments ($n = 80$), and 1/3 received 5 treatments ($n = 76$). This difference in total treatments did not significantly affect any of the variables measured in this study (*female attractiveness*: Wald chi-squared test, $X^2 = 0.78$, $df = 2$, p -value = 0.78; *female receptivity*: Wald chi-square test: $X^2 = 2.8$, $df = 2$, p -value = 0.24; *ovary development*: ANOVA: $F = 3.14$, $df = 1$, p -value = 0.08; *average oocyte area*: ANOVA: $F = 2.98$, $df = 1$, p -value = 0.09; *survival*: Mantel-Haenszel log rank test: $X^2 = 0.1$, $df = 2$,

$p = 0.96$). All gynes continued to receive methoprene/acetone treatments for the entire two weeks, so all individuals received a total of 5 treatments (although mating data was collected prior for 2/3 of the gynes).

2.3. Mating trials and behavioral data collection

Virgin females were matched with two males of similar weight. Having the opportunity to mate with more than one male has previously been shown to increase female receptivity (Miller et al., 2018). All three individuals were from different collection locations. Mating trials were performed in a laboratory room at 24°C. Each mating group of three wasps was placed in a clean plastic petri dish (9 cm diameter $\times$ 2 cm depth) and trials were recorded with Lorex 4 K Varifocal HD video cameras. Each petri dish was illuminated by two 850 lm LED lights with cloth taped over the lighting fixture to create bright, diffuse lighting. Previous work in the same room and filming setup found that mating generally occurs within the first 15 min of a trial (Miller et al., 2018), so we recorded mating trials for 1 h to provide ample time for possible mating.

Behavioral data were collected by recording male and female behavior from the mating trial videos. The observer was blind to treatments while recording behavioral data. We recorded whether a male attempted to mate with the female, which is characterized by mounting the female and positioning his gaster in an attempt to lock genitalia. If the female rejected a male's attempt, by twisting her abdomen to avoid insertion and moving out from under the male, this was recorded as a failed mating attempt. If a male and female locked genitalia and remained so for 10 s or longer, this was recorded as a successful mating attempt (as per Miller et al., 2018). There is some evidence that genital locking may occur for longer than 1 min without sperm transfer (Post and Jeanne, 1983), so we cannot be sure that the recorded instances of mating resulted in sperm transfer. However, when females rejected a male's attempt to mate (in 65 of 93 trials), they would vigorously avoid any genital contact via constant movement, including elaborate backflips. So, even without a confirmation of sperm transfer, a mating bout of > 10 s was considered strong evidence that a gyne was receptive to mating.

2.4. Ovary dissections

At the conclusion of mating trials, a subset of gynes were sampled from each methoprene treatment: 0 μ g ($n = 14$), 5 μ g ($n = 6$), 10 μ g ($n = 16$), and 20 μ g ($n = 10$). Gynes were randomly collected without knowledge of mating status and flash frozen. We did not collect all gynes, because the others were overwintered to examine nest establishment as part of ongoing studies. We dissected ovaries out of the abdomen and measured the average oocyte area, a common method to determine ovarian development in paper wasps (Bonavita-Cougourdan et al., 1991; Gunnels, 2007; Molina and O'Donnell, 2007; Sasaki et al., 2007; Mora-Kepfer, 2011). To measure average oocyte area, all ovaries were photographed, along with a 1 mm resolution scale bar, with a Canon 6D SLR camera and MP-E 65 mm lens (Canon, Melville, NY, USA). We then measured the area of the five largest oocytes using imageJ (Schneider et al., 2012) by first scaling each image according to the scale bar and then drawing polygons around the five largest oocytes and computing the area of these polygons. If we were unsure of which oocytes were largest, we measured 8 oocytes and then determined the five largest by area after measurement. Additionally, we scored the development of each ovary on a scale from 1 to 4 (methods and results of ovary scores are reported in [Supplementary Information: Ovary Scores, Supplemental Table 1, Supplemental Fig. 2, Supplemental Table 2, Supplemental Table 3](#)). These two methods of determining ovarian development were highly correlated with each other (Pearson's product-moment correlation: $R = 0.77$, $t = 8.07$, $df = 44$, p -value < 0.0001 ; [Supplemental Fig. 1](#)).

2.5. Survival

At the conclusion of mating trials, gynes entered a lab-rearing protocol pipeline. By monitoring wasps through this next stage, we were able to examine potential effects of methoprene on survival over a longer time period. After mating trials were completed, we provided all gynes with the opportunity to mate by putting them in provisioned large plastic tubs with a 2:1 male:female ratio for a week. Wasps in mating tubs were maintained in the same conditions as before: at 26 °C and 50% humidity with full-spectrum lights set to a day-night cycle of 14 h light and 10 h dark. This was done to facilitate the potential formation of nests by mated foundress in subsequent months as part of other ongoing experiments in the lab. Next, gynes were transitioned into quiescence. Groups of five females from the same collection location were put in 16 oz polypropylene deli containers (Fabri-Kal) with sugar cubes, water, and several layered pieces of construction paper and placed in an incubator with a 9:15 day:night light cycle at 10 °C. After a week, lights were turned off permanently and temperature was reduced to 4 °C. During the period when wasps were in the incubator, we checked for mortality and refilled water once a week for a total of 13 weeks.

2.6. Statistics

After viewing, each mating trial was categorized as either “no mating attempt”, “failed mating attempt”, or “successful mating”. To investigate whether JH affects female receptivity specifically, we excluded from the analysis the trials in which no mating attempt occurred. For all analyses we used mixed effects models with the gyne's natal nest included as a random factor. We modeled the success of mating attempts predicted by female methoprene treatment group and total treatments received by the time of mating trial with a mixed effects logistic regression using the function “glmer” with a binomial error distribution in the R package “lme4” (Bates et al., 2015). We performed post-hoc contrasts of each treatment with a Tukey-adjusted p -value using the function “emmeans” in the R package “emmeans” (Lenth, 2017). To investigate the effect of JH on ovarian development, we modeled ovariole area predicted by methoprene treatment group with a linear mixed effects model using the “lmer” function in the package “lme4” (Bates et al., 2015). Survival was modeled with a Cox proportional hazards regression model with natal nest as a cluster using the R package “coxme” (Therneau, 2019). Statistical analyses were performed using R version 3.4.3 (R Core Team, 2017).

Upon plotting the data for the effects of methoprene on receptivity, ovarian development, and survival, we observed that methoprene treatment affected each in a distinctly different pattern. Based on visual inspection, we hypothesized methoprene increased receptivity linearly, increased ovarian development in one step between the control and all methoprene + treatments, and increased mortality in one step between the highest methoprene treatment and all other treatments. To determine whether these measures were best explained by different statistical models, we performed a model selection test. We fit each response variable (receptivity, ovarian development, and survival) to four different models of the treatment variable: 1) continuous (grams methoprene per gram of wasp body mass), 2) ordinal (four discrete treatments), 3) dose-independent (untreated (0 μ g) vs. all methoprene + treatments), and 4) high-dose dependent (high treatment (20 μ g) vs. all other treatments). For each response variable we compared the AICs of each model of methoprene treatment to determine which best described the pattern of effect. The model with the highest support (with the lowest AIC value) for each response variable was assigned a Δ AIC of 0. Models with a Δ AIC < 2 were considered to be similarly supported and models with a Δ AIC > 2 were considered poorly supported (Anderson and Burnham, 2004).

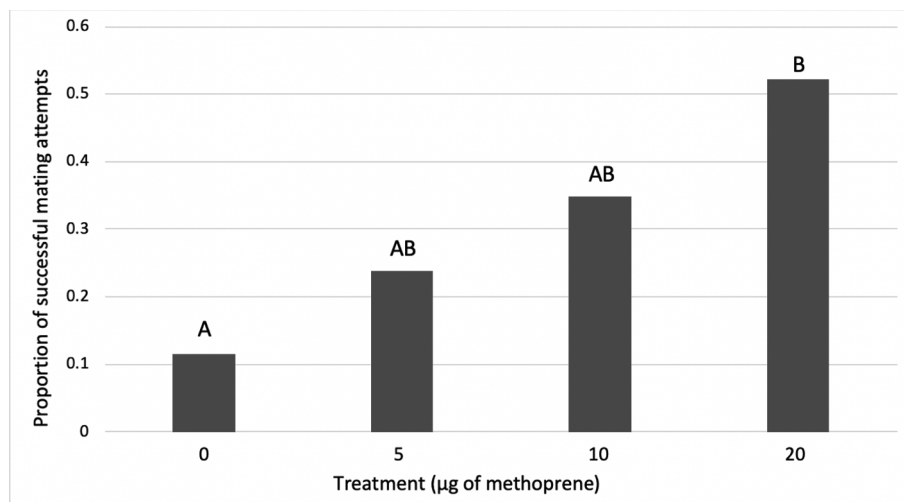


Fig. 1. Proportion of mating trials in which males that attempted to mate with females were successful. Although there is a trend that receptivity increases with the concentration of methoprene application, this effect was only significant in the 20 µg treatment (GLMM: z-ratio = -2.85, Tukey adjusted p-value = 0.022). The *n* of successful mating attempts are 3 of 26 (0 µg), 5 of 21 (5 µg), 8 of 23 (10 µg), and 12 of 23 (20 µg).

3. Results

3.1. Methoprene treatment increases female receptivity

The attractiveness of females to males was not affected by methoprene treatment. Males attempted to mate with the female in 93 of the 236 mating trials and treatment group did not affect whether males attempted to mate (Generalized linear mixed effects model: z-ratio = 0.51, p-value = 0.61).

Among trials in which mating attempts occurred, methoprene treatment significantly increased the likelihood of successful mating (Fig. 1; Generalized linear mixed effects model: z-ratio = 2.93, p-value = 0.003, *n* = 26 (0 µg), *n* = 21 (5 µg), *n* = 23 (10 µg), and *n* = 23 (20 µg) methoprene-treated gynes, indicating methoprene-treated females were more receptive. Pairwise comparisons indicated that only the difference between 20 µg and 0 µg was statistically significant (Generalized linear mixed effects model: z-ratio = -2.85, Tukey adjusted p-value = 0.022, Supplemental Table 3). We also measured receptivity when controlling µg of methoprene treatment per µg of gyne body mass (as measured prior to behavioral trials). This revealed a positive linear relationship between receptivity and methoprene per body mass (Generalized linear mixed effects model: z-ratio = -2.95, p-value = 0.003, *n* = 93, Supplemental Fig. 2).

3.2. Methoprene treatment increases ovarian development

All 3 methoprene treatments increased the average area of the 5 largest oocytes (Fig. 2.3; Linear mixed-effects model: *F* = 14.59; *df* = 3, 34.76; p-value < 0.0001). There were no differences between methoprene + treatments (results of posthoc pairwise comparisons reported in Supplemental Table 4).

3.3. Methoprene treatment decreases survival

Experimental treatment affected survival over the 13 weeks of monitoring (Fig. 3; Cox model: z-score = 2.205, *p* = 0.028 initial *n* = 42 (0 µg), *n* = 55 (5 µg), *n* = 45 (10 µg), and *n* = 48 (20 µg)).

3.4. Model selection

Because the effects of methoprene on receptivity, ovarian development, and survival, appeared to have different patterns, we used a model selection analysis for each of these response variables to provide a quantitative assessment of the observed patterns. We modeled methoprene treatment as either continuous, ordinal treatments, dose-independent, and high-dose-dependent patterns (models defined in

Methods). We found that continuous, dose-independent, and ordinal models similarly explained methoprene's effect on female receptivity, but not a high-dose dependent model (Table 1). Survival was best described by a continuous linear relationship with methoprene treatment per body mass (Table 1). The effect of methoprene on ovarian development was best described by the dose-independent model (Table 1).

4. Discussion

The regulation of mating behavior in insects has been difficult to disentangle from the regulation of reproductive behaviors. By utilizing a species in which the behavior and physiology of these states are naturally temporally decoupled, we have furthered an understanding of how hormones influence mating, separate from reproduction. In this study of *Polistes fuscatus* gynes, we found that treatment with the JH analog methoprene successfully increased sexual receptivity, increased ovary development, and slightly decreased survival when overwintering. Previous research has shown that JH is a gonadotropic coordinator in *Polistes* foundresses (Giray et al., 2005) as in most insects (Nijhout, 1994; Wasielewski et al., 2011; Smith et al., 2013; Shpigler et al., 2014). Thus, JH is often predicted to increase during a female's reproductive phase. However, because mating behavior and ovary activation/egg-laying occur temporally close together in most insect groups, it has previously been difficult to disentangle the role of JH in these two different behavioral states. Our results therefore provide an important addition to this body of research by showing that JH modulates mating in *Polistes*, even when this behavior is decoupled from (i.e. occurring a long period of time before) the egg-laying phase of reproduction.

Our results also suggest that juvenile hormone regulates mating behavior and ovarian development, but with a strikingly different pattern of dose responsiveness. Whereas female receptivity increased linearly with methoprene dosage, ovarian development increased in one step at the lowest methoprene + treatment and did not increase further with higher dosages. This suggests that JH does not coordinate female sexual behavior via the ovaries. Because of the mating biology of *Polistes* species, it is unlikely that JH could mediate receptivity via ovarian development. A defining characteristic of gynes is undeveloped ovaries (Toth et al., 2009; Hunt, 2007). So, gynes mate naturally without well-developed ovaries. Our results indicate that JH functions as both a regulator of sexual receptivity (perhaps via neuronal modification (Hartfelder and Engels, 1998; Adkins-Regan, 2005)) as well as a gonadotropin.

There has been much interest in the role of JH in the evolution of eusocial insect behavioral and physiological traits, and our findings add to our understanding of JH's role in eusocial evolution. Our findings

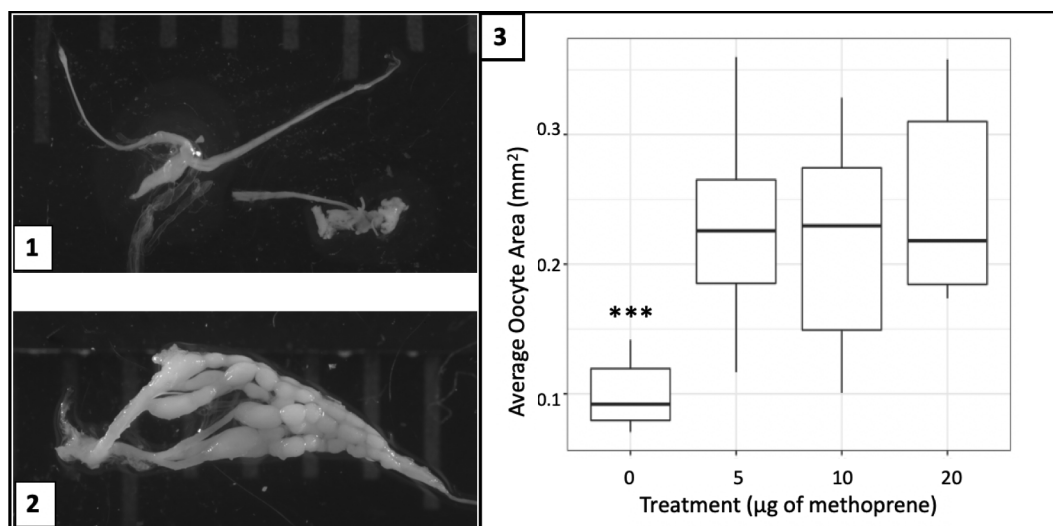


Fig. 2. Ovary development. 1) Undeveloped ovaries. 2) Well developed ovaries. 3) Average oocyte area for each treatment. All 3 methoprene treatments increased the average oocyte area (Linear mixed-effects model: $F = 14.59$; $df = 3, 34.76$; p -value < 0.0001), but there were no differences between methoprene treatments.

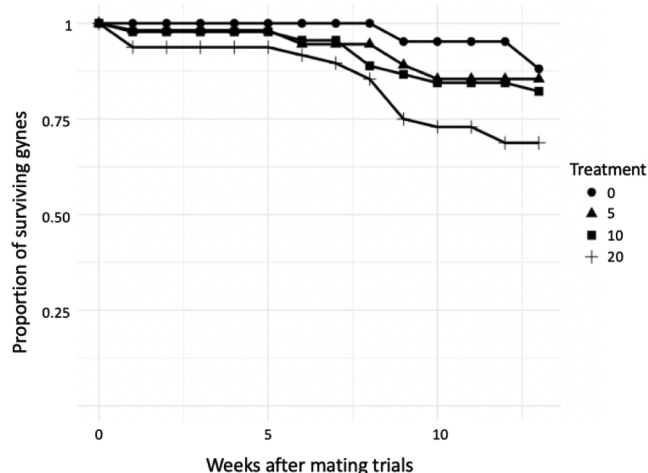


Fig. 3. Gyne survival post-mating. Methoprene treatment did not affect survival of quiescent gynes over the 12 weeks of monitoring (Cox model: z -score = 2.205, $p = 0.028$). Initial gyne counts for each treatment were 42 (0 μ g), 55 (5 μ g), 45 (10 μ g), and 48 (20 μ g).

may be interpreted as consistent with the “split-function hypothesis” of JH’s role in adult social Hymenoptera (West-Eberhard, 1996). This is in contrast to the “novel-function hypothesis” which posits that JH’s role in age-related shifts in behavior in social Hymenoptera workers evolved after its gonadotropic role was lost (Robinson, 1992). Rather, the “split-function hypothesis” proposes that JH acted as both a gonadotropin and a behavioral regulator in the adults of the solitary ancestor to modern social Hymenoptera (West-Eberhard, 1996; Robinson and Vargo, 1997; Giray et al., 2005; Tibbetts et al., 2013). The split-function hypothesis predicts that JH affects different members of the colony differently; regulating ovarian activation in queens and coordinating worker behavioral development, namely the shift from nest work to foraging (Robinson and Vargo, 1997; Giray et al., 2005). A split-function of JH in the queens and workers of social insects has had varying levels of support. In honey bees and some ants JH appears to regulate division of labor but not reproduction (Robinson, 1992; Corona et al., 2007; Dolezal et al., 2009; Bloch et al., 2002; Penick et al., 2011; Pamminer et al., 2016), which supports a novel function of JH in these groups. In workers of the primitively eusocial wasp *Ropalidia marginata* and the bumblebee *Bombus terrestris*, JH functions as a gonadotropin but not as

Table 1

Best of fit model selection for the effect of methoprene on 4 response variables. The models are continuous (grams methoprene per gram of wasp body mass), ordinal (four discrete treatments), dose independent (untreated (0 μ g) vs. all methoprene + treatments), and high-dose dependent (high treatment (20 μ g) vs. all other treatments). The most supported model (with the lowest AIC value) for each response variable was assigned a Δ AIC of 0 and is highlighted in bold. Models with a Δ AIC < 2 were considered to be similarly supported and are also highlighted in bold, while models with a Δ AIC > 2 were considered poorly supported (Anderson and Burnham, 2004). Models are in order from most to least supported for each response variable.

Response	Model	p-value	AIC	Δ AIC
Receptivity	Continuous	0.0032	106.53	0
	Dose independent	0.017	107.96	1.43
	Ordinal	0.005	108.14	1.61
	High-dose dependent	0.015	108.92	2.39
Average Oocyte Area	Dose independent	< 0.0001	-106.68	0
	Ordinal	< 0.0001	-91.21	15.47
	High-dose dependent	0.019	-81.59	25.09
	Continuous	0.00044	-78.48	28.20
Survival	Continuous	0.001	371.08	0
	High-dose dependent	0.009	383.44	12.36
	Ordinal	0.028	386.53	15.5
	Dose independent	0.093	386.78	15.7

a regulator of behavior (Agrahari and Gadagkar, 2003; Shpigler et al., 2016), which suggests that JH has retained its ancestral role in these species. In support of the split-function hypothesis, there is evidence that JH has dual roles as a gonadotropin and behavioral regulator in the facultatively eusocial sweat bee *Megalopta genalis* (Smith et al., 2013), and the paper wasps *Polistes canadensis* (Giray et al., 2005) and *Polistes dominula* (Shorter and Tibbetts, 2009; Tibbetts et al., 2018). Previous discussion and research of these hypotheses has focused on queens and workers (and in one study, males (Giray and Robinson, 1996)) and has not considered gyne behavior nor mating receptivity, leaving an important gap in our understanding of JH’s influence in behavioral traits relevant to eusociality. Like the transition from nest work to foraging behavior, the onset of sexual receptivity represents a process of behavioral maturation, and may be regulated by JH. The gyne life stage is akin to a solitary wasp in that gynes do not participate in most social behaviors on the nest and primarily engage in self-maintenance behaviors such as resting and feeding. Therefore, an extension of the split-function hypothesis to gynes would predict both roles of JH to co-occur in the same individual. Our results showing that JH affects both

receptivity to mating and ovarian development in *Polistes* gynes are consistent with this hypothesis. However, a more thorough interrogation of the split-function hypothesis would investigate how JH affects behaviors not directly associated with reproduction, including aggression and level of activity.

In addition to reproductive effects, we also observed an increase in mortality among gynes treated with methoprene. This heightened mortality may be the result of several potential influences of increased juvenile hormone. First, increased mortality may be a result of increased successful mating. The trade-off between reproduction and survival is well documented across taxa (Röseler et al., 1985), including in social insects (Toth et al., 2016; Pamminger et al., 2016). Mating in insects incurs life history costs, including decreased immunity (Rolff and Siva-Jothy, 2002). However, there is evidence that successful mating increases the survival of quiescent *Polistes dominula* gynes (Izzo, 2011), as well as other social insect species with long-lived queens (Schrempf, et al., 2005). This study did not test for a direct connection between mating and survival, so further research into how mating affects immunity in gynes could illuminate a possible fitness tradeoff between copulation and survival in *Polistes*. Second, the increased ovarian development of methoprene-treated individuals may have decreased survival during overwintering. Gynes tend to have low ovarian development and high lipid stores prior to overwintering, and the two are negatively correlated across *Polistes* castes (Toth et al., 2009). Thus, investment in ovarian development in lieu of lipid storage may decrease longevity. Third, it may be that increased JH titers affected survival more generally by retarding the transition into diapause (Bohm, 1972). If gynes failed to enter a quiescent state, they may have depleted lipid stores at a higher rate than the other gynes, ultimately leading to lower survival.

At a more practical level, our results have important implications to the development of *Polistes* as a model system for the study of social behavior. *Polistes* paper wasps have been an important scientific workhorse for answering proximate and ultimate questions about social behavior (Starks et al., 2006; Jandt et al., 2014). However, successful and productive rearing in the lab has proven challenging. Although *Polistes* have been studied effectively in the lab throughout their life cycle, establishing multiple generation lab lines has been a major hurdle for polistine biologists (Gibo, 1977; Post and Jeanne, 1982; Starks, 1998; Kudô, 2003; Jandt, et al., 2015). Along with replicating overwintering conditions, stimulating nest-founding, and promoting colony growth, successfully inducing mating in the lab is a major challenge for lab-rearing *Polistes* (personal communication, several social wasp biologists). Previous studies have demonstrated varying degrees of success at promoting wasp mating in the laboratory (Miller et al., 2018; de Souza et al., 2014; Izzo and Tibbetts, 2012; Sen et al., 2010). Our findings suggest that JH is a good target for inducing mating in the laboratory. Topical application of methoprene is a relatively easy procedure and increased female receptivity by 5 times. Although methoprene also increased mortality, survival of gynes in the highest methoprene treatments were still fairly high.

Overall, our results contribute to the long-understood role of JH as a master regulator of behavioral maturation. JH helps regulate normal development in larvae and acts as a gonadotropin in adults (Hartfelder, 2000). Here we demonstrate the importance of JH in sexual receptivity and mating behavior in a eusocial insect. By examining sexual receptivity in *Polistes* paper wasps, we can utilize the annual life cycle and temporal separation between gyne and foundress phases to study mating behavior decoupled from other reproductive behavior. The unique, but understudied *Polistes* gyne has potential as a model of female sexual receptivity and mating behavior, and for how internal and external environment regulate this behavioral state.

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Competing interests

The authors declare no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jinsphys.2019.103972>.

References

- Adkins-Regan, E., 2005. Hormones and animal social behavior. Princeton University Press.
- Agrahari, M., Gadagkar, R., 2003. Juvenile hormone accelerates ovarian development and does not affect age polyethism in the primitively eusocial wasp, *Ropalidia marginata*. *J. Insect Physiol.* 49 (3), 217–222.
- Matthes-Sears, W., Alcock, J., 1986. Hilltopping behavior of *Polistes commanichus navajoe* (Hymenoptera: Vespidae). *Ethology* 71 (1), 42–53.
- Anderson, D.R., Burnham, K., 2004. Model selection and multi-model inference. Springer-Verlag, Second. NY, pp. 63.
- Barth, R.H., Lester, L.J., 1973. Neuro-hormonal control of sexual behavior in insects. *Annu. Rev. Entomol.* 18 (1), 445–472.
- Barth, R.H., Lester, L.J., Sroka, P., Kessler, T., Hearn, R., 1975. Juvenile hormone promotes dominance behavior and ovarian development in social wasps (*Polistes annularis*). *Experientia* 31 (6), 691–692.
- Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.* 67 (1), 1–48. <https://doi.org/10.18637/jss.v067.i01>.
- Beani, L., Cervo, R., Lorenzi, C.M., Turillazzi, S., 1992. Landmark-based mating systems in four *Polistes* species (Hymenoptera: Vespidae). *J. Kansas Entomol. Soc.* 211–217.
- Beani, L., Turillazzi, S., 1988. Alternative mating tactics in males of *Polistes dominulus* (Hymenoptera: Vespidae). *Behav. Ecol. Sociobiol.* 22 (4), 257–264.
- Bloch, G., Wheeler, D.E., Robinson, G.E., 2002. Endocrine influences on the organization of insect societies. In: Hormones, brain and behavior. Academic Press, pp. 195–235.
- Bohm, M.K., 1972. Effects of environment and juvenile hormone on ovaries of the wasp, *Polistes metricus*. *J. Insect Physiol.* 18 (10), 1875–1883.
- Bonavita-Cougourdan, A., Theraulaz, G., Bagnères, A.G., Roux, M., Pratte, M., Provost, E., Clément, J.L., 1991. Cuticular hydrocarbons, social organization and ovarian development in a polistine wasp: *Polistes dominulus* Christ. *Comp. Biochem. Physiol. Part B: Comp. Biochem.* 100 (4), 667–680.
- Brent, C.S., Hull, J.J., 2014. Characterization of male-derived factors inhibiting female sexual receptivity in *Lygus hesperus*. *J. Insect Physiol.* 60, 104–110.
- Corona, M., Velarde, R.A., Remolina, S., Moran-Lauter, A., Wang, Y., Hughes, K.A., Robinson, G.E., 2007. Vitellogenin, juvenile hormone, insulin signaling, and queen honey bee longevity. *Proc. Natl. Acad. Sci.* 104 (17), 7128–7133.
- Csondes, A., 2004. Environmental fate of methoprene. Department of Pesticide Regulations, Sacramento, CA.
- Cusson, M., McNeil, J.N., 1989. Involvement of juvenile hormone in the regulation of pheromone release activities in a moth. *Science* 243 (4888), 210–212.
- Dani, F.R., 1994. Caste size differences in *Polistes gallicus* (L.) (Hymenoptera Vespidae). *Ethology Ecol. Evol.* 6 (sup1), 67–73.
- de Souza, A.R., Junior, C.A.M., do Nascimento, F. S., and Lino-Neto, J., 2014. Sexy faces in a male paper wasp. *PLoS ONE* 9 (5), e98172.
- de Souza, A.R., Lino-Neto, J., Tibbetts, E.A., Turillazzi, S., Beani, L., 2017. The leks of *Polistes dominulus* paper wasps: tiny abdominal spots play a critical role in male attacks toward potential rivals. *Ethology Ecol. Evolution* 29 (4), 410–419.
- Dolezal, A.G., Brent, C.S., Gadau, J., Hölldobler, B., Amdam, G.V., 2009. Endocrine physiology of the division of labour in *Pogonomyrmex californicus* founding queens. *Anim. Behav.* 77 (5), 1005–1010.
- Engelmann, F., 1960. Hormonal control of mating behavior in an insect. *Experientia* 16 (2), 69–70.
- Engelmann, F., Barth Jr, R.H., 1968. Endocrine control of female receptivity in *Leucophaea maderae*. *Ann. Entomol. Soc. Am.* 61, 503–505.
- Flatt, T., Tu, M.P., Tatar, M., 2005. Hormonal pleiotropy and the juvenile hormone regulation of *Drosophila* development and life history. *BioEssays* 27 (10), 999–1010.
- Gibo, D.L., 1977. A method for rearing various species of social wasps of the genus *Polistes* (Hymenoptera: Vespidae) under controlled conditions. *Can. Entomol.* 109 (7), 1013–1015.
- Giray, T., Robinson, G.E., 1996. Common endocrine and genetic mechanisms of behavioral development in male and worker honey bees and the evolution of division of labor. *Proc. Natl. Acad. Sci.* 93 (21), 11718–11722.
- Giray, T., Giovanetti, M., West-Eberhard, M.J., 2005. Juvenile hormone, reproduction, and worker behavior in the neotropical social wasp *Polistes canadensis*. *Proc. Natl. Acad. Sci.* 102 (9), 3330–3335.

- Gunnels IV, C.W., 2007. Seasonally variable eusocially selected traits in the paper wasp, *Mischocyttarus mexicanus*. *Ethology* 113 (7), 648–660.
- Haggard, C.M., Gamboa, G.J., 1980. Seasonal variation in body size and reproductive condition of a paper wasp, *Polistes metricus* (Hymenoptera: Vespidae). *Can. Entomol.* 112 (3), 239–248.
- Hartfelder, K., 2000. Insect juvenile hormone: from “status quo” to high society. *Braz. J. Med. Biol. Res.* 33 (2), 157–177.
- Hartfelder, K., Engels, W., 1998. 2 Social Insect Polymorphism: Hormonal Regulation of Plasticity in Development and Reproduction in the Honeybee. In: *Current topics in developmental biology* Vol. 40. Academic Press, pp. 45–77.
- Hartmann, R., Wolf, W., Loher, W., 1972. The influence of the endocrine system on reproductive behavior and development in grasshoppers. *Gen. Comp. Endocrinol.* 3, 518–528.
- Hughes, W.O.H., Ratnieks, F.L.W., Oldroyd, B.P., 2008. Multiple paternity or multiple queens: two routes to greater intracolony genetic diversity in the eusocial Hymenoptera. *J. Evol. Biol.* 21 (4), 1090–1095.
- Hunt, J.H., 2007. *The evolution of social wasps*. Oxford University Press.
- Izzo, A.S., 2011. Spotting the top male: sexual selection in a lek-mating paper wasp, *Polistes dominulus*. Ph.D. thesis. University of Michigan.
- Izzo, A.S., Tibbetts, E.A., 2012. Spotting the top male: sexually selected signals in male *Polistes dominulus* wasps. *Animal behavior* 83 (3), 839–845.
- Jandt, J.M., Tibbetts, E.A., Toth, A.L., 2014. *Polistes* paper wasps: a model genus for the study of social dominance hierarchies. *Insectes Soc.* 61 (1), 11–27.
- Jandt, J.M., Thomson, J.L., Geffre, A.C., Toth, A.L., 2015. Lab rearing environment perturbs social traits: a case study with *Polistes* wasps. *Behav. Ecol.* 26 (5), 1274–1284.
- Judd, T.M., Magnus, R.M., Fasnacht, M.P., 2010. A nutritional profile of the social wasp *Polistes metricus*: Differences in nutrient levels between castes and changes within castes during the annual life cycle. *J. Insect Physiol.* 56 (1), 42–56.
- Klowden, M.J., 2013. Physiological systems in insects. Academic Press.
- Kudô, K., 2003. Growth rate and body weight of foundress-reared offspring in a paper wasp, *Polistes chinensis* (Hymenoptera, Vespidae): no influence of food quantity on the first offspring. *Insectes Soc.* 50 (1), 77–81.
- Lea, A.O., 1968. Mating without insemination in virgin *Aedes aegypti*. *J. Insect Physiol.* 14 (3), 305–308.
- Lenth, R., 2017. <https://CRAN.R-project.org/package=emmeans>.
- Loher, W., Huber, F., 1966. Nervous and endocrine control of sexual behaviour in a grasshopper (*Gomphocerus rufus* L.). *Symp. Soc. Exp. Biol.* 20 38 1–400.
- Miller, S.E., Legan, A.W., Flores, Z.A., Ng, H.Y., Sheehan, M.J., 2018. Strong, but incomplete, mate choice discrimination between two closely related species of paper wasp. *Biol. J. Linn. Soc.*
- Molina, Y., O'Donnell, S., 2007. Mushroom body volume is related to social aggression and ovary development in the paper wasp *Polistes instabilis*. *Brain Behav. Evol.* 70 (2), 137–144.
- Mora-Kepler, F. (2011) Context-dependent behavior, reproduction and brain structure in newly-established colonies of the primitively eusocial wasp, *Mischocyttarus mexicanus*. Dissertation, University of Miami.
- Nijhout, H.F., 1994. *Insect hormones*. Princeton University Press.
- O'Donnell, S., Jeanne, R.L., 1993. Methoprene accelerates age polyethism in workers of a social wasp (*Polybia occidentalis*). *Physiol. Entomol.* 18 (2), 189–194.
- Pamminger, T., Treanor, D., Hughes, W.O., 2016. Pleiotropic effects of juvenile hormone in ant queens and the escape from the reproduction-immunocompetence trade-off. *Proc. R. Soc. B: Biol. Sci.* 283 (1822), 20152409.
- Pardi, L., 1948. Dominance order in *Polistes* wasps. *Physiol. Zool.* 21 (1), 1–13.
- Pener, M.P. (1967). Comparative studies on reciprocal interchange of the corpora allata between males and females of adult *Schistocerca gregaria* (Forsk.) (Orthoptera: Acrididae). In *Proceedings of the Royal Entomological Society of London. Series A, General Entomology* (Vol. 42, No. 10–12, pp. 139–148). Oxford, UK: Blackwell Publishing Ltd.
- Penick, C.A., Liebig, J., Brent, C.S., 2011. Reproduction, dominance, and caste: endocrine profiles of queens and workers of the ant *Harpegnathos saltator*. *J. Comp. Physiol. A.* 197 (11), 1063.
- Polak, M., 1992. Distribution of virgin females influences mate-searching behavior of male *Polistes canadensis* (L.) (Hymenoptera: Vespidae). *J. Insect Behav.* 5 (4), 531–535.
- Polak, M., 1993. Competition for landmark territories among male *Polistes canadensis* (L.) (Hymenoptera: Vespidae): large-size advantage and alternative mate-acquisition tactics. *Behav. Ecol.* 4 (4), 325–331.
- Post, D.C., Jeanne, R.L., 1982. Recognition of former nestmates during colony founding by the social wasp *Polistes fuscatus* (Hymenoptera: Vespidae). *Behav. Ecol. Sociobiol.* 11 (4), 283–285.
- Post, D.C., Jeanne, R.L., 1983. Relatedness and mate selection in *Polistes fuscatus* (Hymenoptera: Vespidae). *Anim. Behav.* 31 (4), 1260–1261.
- R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Reed, H.C., Landolt, P.J., 1991. Swarming of paper wasp (Hymenoptera: Vespidae) sexuals at towers in Florida. *Ann. Entomol. Soc. Am.* 84 (6), 628–635.
- Reeve, H.K., 1991. *Polistes*. In: Ross, K.G., Matthews, R.W. (Eds.), *The Social Biology of Wasps*. Cornell University Press, Ithaca, NY, pp. 99–148.
- Ringo, J., 1996. Sexual receptivity in insects. *Annu. Rev. Entomol.* 41 (1), 473–494.
- Ringo, J., Talyn, B., Brannan, M., 2005. Effects of precocene and low protein diet on reproductive behavior in *Drosophila melanogaster* (Diptera: Drosophilidae). *Ann. Entomol. Soc. Am.* 98 (4), 601–607.
- Robinson, G.E., 1992. Regulation of division of labor in insect societies. *Annu. Rev. Entomol.* 37 (1), 637–665.
- Robinson, G.E., Vargo, E.L., 1997. Juvenile hormone in adult eusocial Hymenoptera: gonadotropin and behavioral pacemaker. *Archives of Insect Biochemistry and Physiology: Published in Collaboration with the Entomological Society of America* 35 (4), 559–583.
- Rolff, J., Siva-Jothy, M.T., 2002. Copulation corrupts immunity: a mechanism for a cost of mating in insects. *Proc. Natl. Acad. Sci.* 99 (15), 9916–9918.
- Röseler, P.F., Röseler, I., Strambi, A., Augier, R., 1984. Influence of insect hormones on the establishment of dominance hierarchies among foundresses of the paper wasp, *Polistes gallicus*. *Behav. Ecol. Sociobiol.* 15 (2), 133–142.
- Röseler, P.F., Röseler, I., Strambi, A., 1985. Role of ovaries and ecdysteroids in dominance hierarchy establishment among foundresses of the primitively social wasp, *Polistes gallicus*. *Behav. Ecol. Sociobiol.* 18 (1), 9–13.
- Roth, L.M., Barth Jr, R.H., 1964. The control of sexual receptivity in female cockroaches. *J. Insect Physiol.* 10 (6), 965–975.
- Sasaki, K., Yamasaki, K., Nagao, T., 2007. Neuro-endocrine correlates of ovarian development and egg-laying behaviors in the primitively eusocial wasp (*Polistes chinensis*). *J. Insect Physiol.* 53 (9), 940–949.
- Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9 (7), 671.
- Schrempf, A., Heinze, J., Cremer, S., 2005. Sexual cooperation: mating increases longevity in ant queens. *Curr. Biol.* 15 (3), 267–270.
- Sen, R., Samudre, S., Shilpa, M.C., Tarak, R.C., Gadagkar, R., 2010. Middle aged wasps mate through most of the year, without regard to body size, ovarian development and nestmateship: a laboratory study of the primitively eusocial wasp *Ropalidia marginata*. *Insectes Soc.* 57 (1), 95–103.
- Shorter, J.R., Tibbetts, E.A., 2009. The effect of juvenile hormone on temporal polyethism in the paper wasp *Polistes dominulus*. *Insectes Soc.* 56 (1), 7–13.
- Shpigler, H., Amsalem, E., Huang, Z.Y., Cohen, M., Siegel, A.J., Hefetz, A., Bloch, G., 2014. Gonadotropic and physiological functions of juvenile hormone in bumblebee (*Bombus terrestris*) workers. *PLoS One* 9 (6), e100650.
- Shpigler, H.Y., Siegel, A.J., Huang, Z.Y., Bloch, G., 2016. No effect of juvenile hormone on task performance in a bumblebee (*Bombus terrestris*) supports an evolutionary link between endocrine signaling and social complexity. *Horm. Behav.* 85, 67–75.
- Smith, A.R., Kapheim, K.M., Pérez-Ortega, B., Brent, C.S., Weislo, W.T., 2013. Juvenile hormone levels reflect social opportunities in the facultatively eusocial sweat bee *Megalopta genalis* (Hymenoptera: Halictidae). *Horm. Behav.* 63 (1), 1–4.
- Starks, P.T., 1998. A novel ‘sit and wait’ reproductive strategy in social wasps. *Proc. R. Soc. Lond. B Biol. Sci.* 265 (1404), 1407–1410.
- Starks, P.T., Turillazzi, S., West-Eberhard, M.J., 2006. *Polistes* paper wasps: emergence of a model genus. *Ann. Zool. Fenn.* 43 (5–6), 385–386.
- Teal, P.E.A., Gomez-Simuta, Y., Proveaux, A.T., 2000. Mating experience and juvenile hormone enhance sexual signaling and mating in male Caribbean fruit flies. *Proc. Natl. Acad. Sci.* 97 (7), 3708–3712.
- Tibbetts, E.A., Izzo, A.S., 2009. Endocrine mediated phenotypic plasticity: condition-dependent effects of juvenile hormone on dominance and fertility of wasp queens. *Horm. Behav.* 56 (5), 527–531.
- Tibbetts, E.A., Izzo, A., Huang, Z.Y., 2011a. Behavioral and physiological factors associated with juvenile hormone in *Polistes* wasp foundresses. *Behav. Ecol. Sociobiol.* 65 (5), 1123–1131.
- Tibbetts, E.A., Levy, S., Donajkowski, K., 2011b. Reproductive plasticity in *Polistes* paper wasp workers and the evolutionary origins of sociality. *J. Insect Physiol.* 57 (7), 995–999.
- Tibbetts, E.A., Mettler, A., Donajkowski, K., 2013. Nutrition-dependent fertility response to juvenile hormone in non-social *Euodynerus foraminatus* wasps and the evolutionary origin of sociality. *J. Insect Physiol.* 59 (3), 339–344.
- Tibbetts, E.A., Fearon, M.L., Wong, E., Huang, Z.Y., Tinghitella, R.M., 2018. Rapid juvenile hormone downregulation in subordinate wasp queens facilitates stable co-operation. *Proc. R. Soc. B: Biol. Sci.* 285 (1872), 20172645.
- Therneau, T.M. (2019). coxme: Mixed Effects Cox Models. R package version 2.2-14. <https://CRAN.R-project.org/package=coxme>.
- Toth, A.L., Bilof, K.B.J., Henshaw, M.T., Hunt, J.H., Robinson, G.E., 2009. Lipid stores, ovary development, and brain gene expression in *Polistes metricus* females. *Insectes Soc.* 56 (1), 77–84.
- Toth, A.L., Sumner, S., Jeanne, R.L., 2016. Patterns of longevity across a sociality gradient in vespid wasps. *Curr. Opin. Insect Sci.* 16, 28–35.
- Turillazzi, S., Pardi, L., 1977. Body size and hierarchy in polygynic nests of *Polistes gallicus* (L.) (Hymenoptera Vespidae). *Monitore Zoologico Italiano-Italian. J. Zool.* 11 (1–2), 101–112.
- Wasielowski, O., Wojciechowicz, T., Giejdasz, K., Krishnan, N., 2011. Influence of methoprene and temperature on diapause termination in adult females of the overwintering solitary bee, *Osmia rufa* L. *J. Insect Physiol.* 57 (12), 1682–1688.
- West-Eberhard, M.J., 1996. Wasp societies as microcosms for the study of development and evolution. In: Turillazzi, S., West-Eberhard, M.J. (Eds.), *Natural History and Evolution of Paper Wasps*. Oxford University Press, Oxford, pp. 290–317.