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# Octopamine levels relate to male mating tactic expression in the wolf spider *Rabidosa punctulata*

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## Abstract

In the wolf spider *Rabidosa punctulata*, upon encountering a female, males use one of two distinct strategies: (1) they court the female in an attempt to elicit a mating, or (2) they engage in a direct-mount tactic that involves extensive grappling with the female until a mating is achieved. The latter tactic appears more sexually aggressive, and both tactics come with the risk of being cannibalized. We explored the physiological mechanisms underlying this behavioral variation by assessing the relationship between circulating levels of the biogenic amine octopamine (OA), a neuromodulator suggested to play a role in “fight or flight” responses of arthropods and male mating tactic expression. We predicted, and found support for, a relationship between OA levels and tactic expression, with males adopting the direct-mount tactic expressing higher OA levels than courting males. Male mating tactic and mass also showed a significant interaction, with a negative trend in direct-mounting males and no relationship in courting males. Males had considerably higher levels of OA circulating in their hemolymph than females and female OA level increased with female mass. Our experimental design cannot disentangle cause from effect, but our results are consistent with the hypothesis that OA plays a role in regulating mating tactic expression in *R. punctulata*.

**Keywords:** aggression, alternative reproductive tactic, behavioral plasticity, biogenic amine, hormone

Mating behavior has been shown to vary both across individuals as well as within individuals across mating encounters (Gross, 1996). In males, variation in mating success has influenced the evolution of a wide array of distinct strategies used to acquire mates, with strategies encompassing such terms as “courtship”, “fighter”, “territorial”, “sneaker” and “satellite”. These distinct male strategies are often termed alternative reproductive tactics (reviewed in: Oliveira, Canario, & Ros, 2008; Shuster & Wade, 2003). While variability in mating behaviors may not be as obvious in females, variation among females in mating decisions is equally influential on the outcome of mating encounters (Jennions & Petrie, 1997). In fact, in some species, female reproductive decisions can be quite dramatic, as cannibalism may accompany female rejection (e.g. in spiders) (Aisenberg & Barrantes, 2011; Arnqvist & Henriksson, 1997; Arnqvist & Rowe, 2005; Elgar, Bruce, & de Crespiigny, 2003; Gage, 2005; Johnson, 2001). The considerable inter- and intraspecific variation in both male and female mating behaviors across the animal kingdom prompts questions of why and how such variation evolved and is maintained (Andersson, 1994; Gross, 1996).

Recent years have seen a growing interest in identifying the underlying physiological mechanisms responsible for the production of distinct reproductive behavior (Cunningham, Douthit, & Moore, 2014; Miles, Sinervo, Hazard, Svensson, & Costa, 2007). The release of hormones by the neuroendocrine system is known for its universal and widespread effects on organisms. For example, changes in circulating levels of testosterone can activate and/or modulate a variety of behaviors (e.g. locomotor performance, territoriality, vocalization, courtship displays) and can also influence the organization and structure of an individual’s morphology (e.g. body mass, muscle structure, secondary sexual characters) through alterations of developmental pathways (Miles et al., 2007; Oliveira et al., 2008). Variation in hormone type, quantity and timing of release have all been implicated as integral in the production of a wide array of distinct morphological and behavioral traits (e.g. sexual dimorphism, polymorphism, polyphenism; Nijhout, 2003). The broad distribution of hormones across animal taxa along with their diversity and flexibility in action provides one potentially universal proximate mechanism for the production of

male alternative reproductive tactics (both genetic polymorphisms and conditional tactics; Miles et al., 2007; Oliveira et al., 2008).

For many reproductive behaviors, variation in behavioral expression can be categorized by variation in aggression levels (e.g. dominant versus subordinate, territorial versus satellite, copulate versus cannibalize), and in invertebrates, the biogenic amine octopamine (OA) is considered an aggression hormone, associated with “fight or flight” type responses in multiple taxa (Orchard, 1982). OA has been suggested to play an integral part in arthropod arousal systems (Orchard, 1982), readying individuals to perform in a variety of vigorous activities (Roeder, 2005), potentially via its influence on muscle activity and output (Evans & Siegler, 1982; Malamud, Miszin, & Josephson, 1988) or sensory responsiveness (e.g. acoustic, visual, olfactory; Roeder, 2005). Not surprisingly, OA levels have been found to increase during stressful experiences (Bailey, Martin, & Downer, 1983; Davenport & Evans, 1984) and have been associated with various distinct behavior including courtship (Certel, Savella, Schlegel, & Kravitz, 2007), foraging (Schulz & Robinson, 2001), locomotion (Fussnecker, Smith, & Mustard, 2006; Saraswati, Fox, Soll, & Wu, 2004), flight (Adamo, Linn, & Hoy, 1995; Bailey et al., 1983), and even the honeybee waggle-dance (Barron, Maleszka, Vander Meer, & Robinson, 2007). In male *Drosophila*, OA appears integral in context-appropriate behaviors when presented with conspecifics (e.g. aggression when confronted with a male versus courtship when presented with a female; Certel et al., 2007). Despite its implication in aggressive interactions, studies are mixed in terms of the nature of the relationship between OA and aggression. While higher OA levels tend to decrease aggression in crustaceans (Kravitz, 1990), they typically increase aggressive behavior in insects (Hoyer et al., 2008; Stevenson, Dyakonova, Rillich, & Schildberger, 2005; Stevenson, Hofmann, Schoch, & Schildberger, 2000).

In arachnids, little is known about the relationships between OA and aggression and/or reproductive tactics. Seyfarth, Hammer, Spörhase-Eichmann, Hörner, and Vullings (1993) identified the distribution of neurons reactive to OA in the wandering spider *Cupiennius salei*, and suggested the dual action of OA in spiders as both a neurotransmitter and a neurohormone (Seyfarth et al., 1993). In the tarantula *Aphonopelma hentzi*, decreases in concentrations of OA in the brain following aggressive interactions with conspecifics suggest an activation of the octopaminergic system during these encounters (Punzo & Punzo, 2001). An activation of the octopaminergic system could enhance performance, as locally increased levels of OA have been found to increase the amplitude of skeletal muscle contraction in tarantulas (Grega, 1978) and enhance mechanosensory sensitivity (Torkkeli, Panek, & Meisner, 2011; Widmer, Hoyer, Meisner, French, & Torkkeli, 2005). OA levels have also been found to influence behaviors involved in interspecific interactions, as increased levels of OA shorten the duration of death feigning, an antipredator behavior in the orb-weaving spider *Larinoideus cornutus* (Jones et al., 2011). Interestingly, the occurrence and frequency of this death-feigning behavior has been identified as a part of a larger behavioral syndrome related to aggression (Pruitt et al., 2010; Pruitt, Riechert, & Jones, 2008). Here, we explore the relationship between OA and sexual aggression, or reproductive tactic expression, in the wolf spider *Rabidosa punctulata*.

Mating encounters in *R. punctulata* include a variety of aggressive behaviors. When encountering a female, male *R. punctulata* initially adopt one of two distinct mating tactics: (1) courtship, consisting of a multimodal display incorporating both visual (leg waves) and vibratory (tremulation, stridulation) components, or (2) direct mount, consisting of males grabbing and grappling with females until successful copulation. They can also use a mixed strategy, where they switch from one tactic to the other in se-

quence (Wilgers, Nicholas, Reed, Stratton, & Hebets, 2009). Tactic expression is not completely repeatable within males (of 28 males examined over multiple female–male interactions, 43% engaged in the same tactic repeatedly; Wilgers, 2007). During direct mounts, males and females commonly roll across the substrate as the female seemingly attempts to eject the male. Prior work has demonstrated that the expression of these mating tactics is dependent on the male’s body condition: larger males in better condition are more likely to express the direct-mount tactic, while smaller males in poor condition are more likely to express the courtship tactic (Wilgers et al., 2009). During female–male encounters it is not uncommon for females to attack males (27% of encounters), and females are known to be cannibalistic (7% of encounters) (Wilgers et al., 2009). The occurrence of cannibalism in this species is a suggested explanation for the general avoidance of the direct-mount tactic by smaller males, which might be more susceptible to cannibalism (Wilgers et al., 2009).

The variation in mating behavior expressed by both male and female *R. punctulata* during mating encounters makes them an ideal system for exploring underlying physiological mechanisms. This study specifically asks whether levels of OA are associated with male mating tactic expression and/or with exposure to females in male *R. punctulata*. Given the often aggressive nature of the behavior experienced by both sexes during *R. punctulata* mating encounters, we might expect levels of OA to elevate in response to (1) detection of females as well as (2) expression of the more aggressive mating tactic (direct mount). To test these expectations, we compared OA levels in unexposed males as well as exposed males that expressed distinct mating tactics. We also sought to compare overall patterns of OA expression in exposed versus unexposed females as well as unexposed males versus females. To achieve our aims, we measured circulating levels of OA in the hemolymph of male and female *R. punctulata*.

## Methods

### *Spider Collection and Maintenance*

We collected *R. punctulata* spiders in short and tall grasses from 30 August to 9 September 2010 approximately 21 km west of Lincoln, Nebraska, U.S.A. At the time of collection, all males were mature and all females were penultimate (one molt prior to sexual maturation). Thus, while exact age and mating history for the collected males was unknown, we can be confident that all collected females were virgins. Individuals were brought back to the laboratory where they were housed individually in plastic containers (84 × 84 × 110 mm). They were kept on a 12:12 h light:dark cycle and provided with a constant source of water in a climate-controlled environment. All spiders were fed two approximately body-size-matched crickets (*Acheta domestica*) once per week. Individuals were checked for molts every 2 days to determine their date of maturation.

### *Male Exposure and Mating Tactic*

We were interested in two distinct questions for males: (1) does exposure to a female influence circulating OA levels; and (2) does mating tactic expression predict circulating OA levels? To answer the first question, we haphazardly assigned 45 individual males to either a baseline control or an exposure treatment. For both treatments, males were placed in a circular plastic arena (diameter = 20.2 cm, height = 7.3 cm) surrounded with white walls and lined with a piece of white filter paper (diameter = 18.5 cm). All males were fed one cricket on the day before their trial and were weighed

immediately before being placed in the arena. Control males were placed into the arena and observed for 5 min. We chose a 5 min time period for our baseline control since prior research indicated that on average, males take about 4 min to adopt a mating tactic (Wilgers, 2009). As such, the time that males spent in the treatment arena would be comparable between the control and exposed males. Immediately after the 5 min elapsed, control males were captured and placed in a prepared plastic bag for hemolymph collection (see Hemolymph Collection).

Exposed males were placed in an arena with a sexually mature, virgin female (female age range 14–21 days post maturation). Females were placed in the arena to acclimate and deposit pheromone-laden silk for 1–4 h prior to the male's introduction. At the start of the trial, males were gently introduced and female–male pairs were observed real-time until either the male expressed a mating tactic (courtship or direct mount) or 15 min elapsed. As soon as a mating tactic was expressed and recorded, the spiders were separated and males were captured and placed in a prepared plastic bag for hemolymph collection, while females were returned to their home cage. Male hemolymph collection took place within 5 min of tactic expression.

#### Male versus Female

To determine baseline OA levels in female *R. punctulata*, we collected hemolymph from 48 females in the same manner as described for males. Twenty-four of these females had been exposed to males approximately 3 weeks prior, but remained unmated, and 24 had never been used in previous trials and were thus comparable to the control males described previously. Given that males were already mature in the field and thus older on average, we bled all of the females at an older age in an attempt to control age across the sexes ( $N = 48$ ; mean number of days post sexual maturation  $\pm$  SE =  $47.5 \pm 0.61$ ). However, by attempting to achieve more equitable age ranges between males and females, males were bled approximately 3 weeks before females, meaning that females were housed in the laboratory significantly longer than males.

#### Hemolymph Collection

Prior to the commencement of all trials, we set up an ice bath for the temporary cold storage of the buffer and hemolymph samples. We placed 45  $\mu$ l of buffer solution (0.2 M perchloric acid with 1  $\mu$ g/ml synephrine as an internal standard for OA) in Eppendorf tubes prelabelled with each spider's unique identification number and placed the Eppendorf tubes in the ice bath. For hemolymph collection, males were individually placed in a clean gallon-size (3.785-liter) Ziplock plastic bag that had a bottom corner cut. Males were gently maneuvered to the bottom of the bag and coaxed into placing their fourth walking leg through the open hole. The fourth walking leg of every male was cut approximately in the middle of the tibia with dissecting scissors. We collected 5  $\mu$ l of hemolymph in a graduated microcapillary tube from the cut leg and immediately transferred the hemolymph to the appropriately labelled Eppendorf tube containing buffer. We then vortexed each sample for approximately 5 s to evenly mix the hemolymph and buffer and placed the sample in a  $-20$  °C freezer to preserve until analysis. Hemolymph collection did not kill *R. punctulata* males (or females), and all individuals were subsequently returned to their home cages. Leg loss is not uncommon in wolf spiders (Brueseke, Rypstra, Walker, & Persons, 2001), and individuals with their fourth legs cut showed no abnormal behavior following the procedure (e.g. fed normally, etc.).

#### Octopamine Assays

Prior to analysis, samples were filtered through 0.22  $\mu$ m cellulose acetate (Costar Spin-x tube filters). Hemolymph was analyzed using high-performance liquid chromatography with electrochemical detection (HPLC-EC; Alexys Monoamines Analyzer). The mobile phase consisted of 10% MeOH with 50 mM phosphoric acid, 50 mM citric acid, 500 mg/ml 1-octane sulphonic acid sodium salt, at a pH of 3.25 (Antec, Boston, MA, U.S.A., 180.7050A rev 02). Samples were injected using an AS 110 autosampler into a flow of 50  $\mu$ l/min at 191.7 kg/cm<sup>2</sup> through an ALF-115 microbore column (150  $\times$  1 mm) with porous silica C(18) 3  $\mu$ m particle size at 35 °C. Monoamine detection was acquired at a 5 nA range for 60 min with a VT-03 cell set at 850 mV. Peaks were confirmed against known standards and by spiking samples with standards. Chromatogram analysis was performed with Clarity software (Solihull, U.K.), and concentrations of OA in lymph were estimated from the OA/SYN (analyte/internal standard) areas under the curve using the relative response factor of 0.969 (calculated from standard curves). We then factored in the fact that the hemolymph was diluted to 10% in each sample to determine actual amounts of OA per sample.

#### Cricket OA

To interpret our values of OA in the hemolymph of male and female *R. punctulata*, we assayed OA levels from five store-bought *Acheta domesticus* juvenile crickets. We knocked them out with CO<sub>2</sub> and collected hemolymph from an abdominal puncture. We collected 5  $\mu$ l of hemolymph into 95  $\mu$ l of buffer, with 1  $\mu$ g/ml SYN. Our OA assays were identical to those described above.

#### Statistical Analyses

Hormone concentrations are measured per unit of blood per hemolymph, and as such, most studies do not take an individual's size, mass or age into consideration. Nevertheless, one could imagine that an individual's overall condition (or even age) might influence its ability to synthesize and distribute hormones. Since very little is known about hormones in arachnids, and since tactic expression is condition dependent in *R. punctulata*, we included both mass and age (when possible) throughout our analyses. We unfortunately did not take body size measurements and so mass will be our best proxy of size and condition. We used least square regression models throughout with various combinations of the following predictor variables: exposure to the opposite sex (Y/N), mass, age (days post maturation molt) and sex, and the response variable of OA level (pg/ $\mu$ l). We used a nonparametric Wilcoxon test on the combined unexposed male and unexposed female data to compare circulating OA levels between the sexes as the data did not fit the requirements of a normal distribution. All analyses were run using JMP 8 (SAS Institute, Cary, NC, U.S.A.).

## Results

#### Chromatography

The chromatograms of lymph samples were very clean, with baseline noise less than 1.5 pA. Thus, even very small amounts of OA appeared as clear and distinct symmetrical peaks (mean  $\pm$  SE retention time =  $7.9 \pm 0.056$  min; Figure 1).

### Male Exposure and Mating Tactic

Our first analysis examined OA levels between males that were and were not exposed to females. We used a least squares regression model to examine the influence of exposure (Y/N), male mass, and the interaction between exposure \* male mass on the response variable of OA amount. Our overall model was not significant ( $F_{3,41} = 0.11$ ,  $P = 0.96$ ). Exposed males took a mean  $\pm$  SD of  $128.55 \pm 210$  s to express a tactic while all unexposed/control males were bled after 300 s.

Our second analysis examined only exposed males and compared the amount of OA in the hemolymph of males that expressed distinct mating tactics (courtship versus direct mount). We used a least squares regression model to examine the influence of tactic expression, male mass, and the interaction of tactic expression \* male mass on the response variable of OA amount. Our overall model was significant ( $F_{3,26} = 4.67$ ,  $P = 0.0097$ ). Our effect tests revealed an influence of tactic expression ( $F_{1,29} = 8.67$ ,  $P = 0.0067$ ) but no effect of male mass ( $F_{1,29} = 1.96$ ,  $P = 0.17$ ). Males that expressed a direct-mount tactic had higher OA levels than those that expressed courtship (Figure 2). We also found a significant interaction between tactic expression and male body mass ( $F_{1,29} = 6.3$ ,  $P = 0.019$ ). In males that courted, OA amounts showed no relationship with body mass ( $R^2 = 0.04$ ,  $P = 0.37$ ), while heavier males that expressed a direct mount had lower OA amounts ( $R^2 = 0.54$ ,  $P = 0.06$ ; Figure 3). There was no difference in the time to tactic expression between courting males ( $N = 69$ ) and direct-mounting males ( $N = 7$ ) (Wilcoxon signed-ranks exact test:  $Z = 0.28$ ,  $P = 0.77$ ).

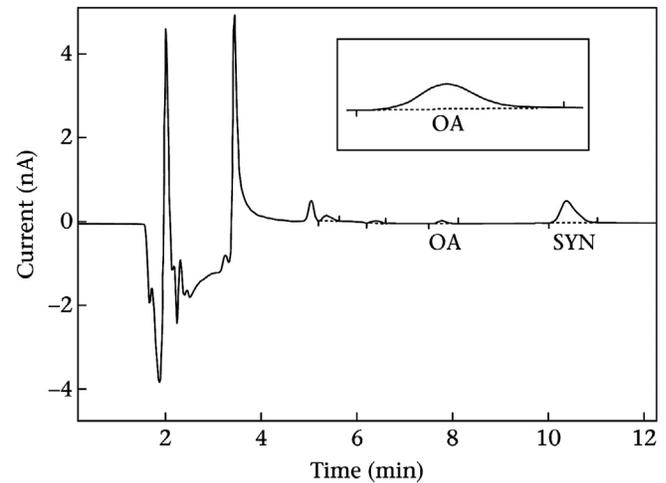
### Females and Males

We first compared OA amounts between females that had been exposed to males 3 weeks prior and unexposed/control females. We found an effect of exposure ( $F_{1,46} = 6.7$ ,  $P = 0.02$ ). Exposed females had slightly higher OA amounts than unexposed females (mean  $\pm$  SE: exposed:  $62 \pm 2.0$  pg/ $\mu$ l; unexposed:  $55 \pm 2.0$  pg/ $\mu$ l). Exposed females were slightly younger than unexposed females ( $F_{1,46} = 21.99$ ;  $P < 0.001$ ; mean  $\pm$  SE days post maturation molt: exposed:  $45.2 \pm 0.72$ ; unexposed  $50 \pm 0.72$ ) and they weighed slightly more than unexposed females ( $F_{1,46} = 17.2$ ,  $P = 0.001$ ; mean  $\pm$  SE: exposed:  $0.26 \pm 0.008$  g; unexposed:  $0.22 \pm 0.008$  g). Following from these observed differences between the groups, we used a least squares regression model to simultaneously examine the influence of exposure (Y/N), age post maturation and mass on our response variable of OA amount. Our initial model incorporated all interaction terms but as none were significant, we dropped these from the final model. Our model was significant ( $F_{3,44} = 4.89$ ,  $P = 0.005$ ) and our effect tests revealed that mass was the only significant predictor variable (exposure:  $F_{1,47} = 0.79$ ,  $P = 0.38$ ; age:  $F_{1,47} = 0.06$ ,  $P = 0.8$ ; mass:  $F_{1,47} = 6.23$ ,  $P = 0.02$ ). OA amounts were higher in heavier females (Figure 4).

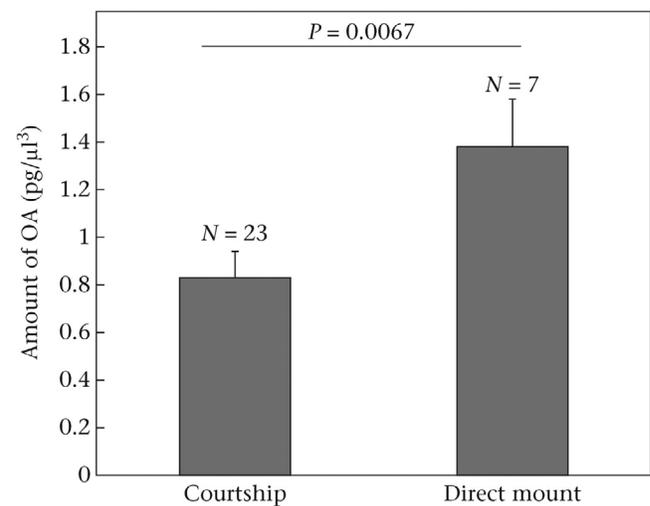
We compared OA amounts between males and females using only unexposed individuals in each group (females:  $N = 24$ , males:  $N = 15$ ). We were unable to transform our data to conform to assumptions of normality, so we used nonparametric statistics for this analysis. We found that unexposed males had significantly higher amounts of OA than unexposed females (Wilcoxon signed-ranks exact test:  $Z = 5.18$ ,  $N_{\text{males}} = 15$ ,  $N_{\text{females}} = 24$ ,  $P < 0.0001$ ; Figure 5).

### Cricket OA

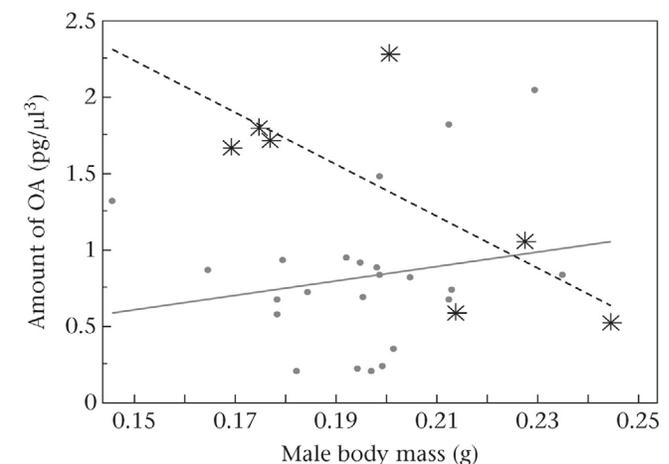
Mean  $\pm$  SD OA in the hemolymph of our assayed crickets was  $0.85 \pm 0.798$  pg/ $\mu$ l<sup>3</sup>. The crickets survived being anaesthetized and bled.



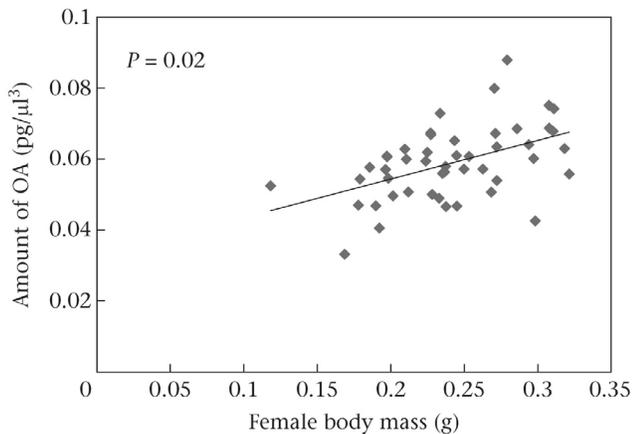
**Figure 1.** An example chromatogram of a male *Rabidosa* lymph sample using high-performance liquid chromatography (HPLC) with electrochemical detection. After an injection front, octopamine (OA) is detected in about 8 min, followed by synephrine (SYN) in about 11 min. OA peaks were distinct and symmetrical (inset).



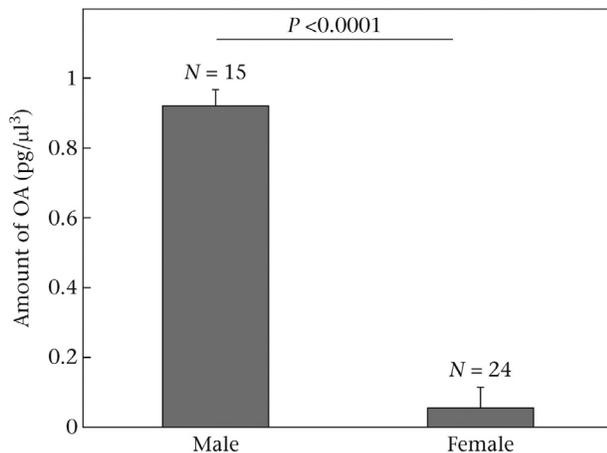
**Figure 2.** Levels of circulating octopamine (OA) in male *R. punctulata* that adopted different mating tactics.



**Figure 3.** Relationship between body mass and level of circulating octopamine (OA) in male *R. punctulata* that expressed courtship (circles, solid line) or a direct-mount (stars, dashed line) mating tactic.



**Figure 4.** Relationship between octopamine (OA) level and body mass in female *R. punctulata*.



**Figure 5.** Comparison of circulating octopamine (OA) levels in unexposed male and unexposed female *R. punctulata*.

## Discussion

We found considerable variation in the levels of circulating octopamine (OA) in the wolf spider *R. punctulata*. In males, OA levels were related to mating tactic expression and to the interaction between tactic expression and male mass. In females, OA levels were related only to female mass. This study provides evidence that the biogenic amine octopamine is involved in distinct mating tactic expression in this species and lays a foundation upon which future studies can explore the relationships between neurohormones and behavior in arachnids.

Male *R. punctulata* that adopted the direct-mount mating tactic during encounters with virgin females had higher levels of circulating OA as measured immediately following their tactic expression. The estimates of hemolymph OA concentration in males in this study were significantly higher than those observed in other arthropods, such as the crickets *Gryllus texensis* (range between ~40 pg/ $\mu$ l and 60 pg/ $\mu$ l; Adamo & Baker, 2011) and *A. domesticus* (38 pg/ $\mu$ l; Woodring, Meier, & Rose, 1988), the locust *Schiztocerca americana gregaria* (7.5 pg/ $\mu$ l with a 10-fold increase following stress) and the cockroach *Periplaneta americana* (4.4 pg/ $\mu$ l at rest with a three-fold increase with stress) (Davenport & Evans, 1984). Because of the magnitude of difference in our observed male OA amounts, we assayed a small number of crickets (*A. domesticus*) to compare with our results. We found substantially higher levels of OA in our cricket samples (average = 854 pg/ $\mu$ l) than previously reported for

crickets, however, the values of OA in our cricket samples are in line with the values that we calculated for male *R. punctulata*. Despite the discrepancy in our values compared to other studies, we are confident in the relative differences in circulating OA observed in this study as the methods were consistent throughout. The unusually high levels of OA we observed in both spiders and crickets compared to previous studies, however, is intriguing and we have been unable to come up with a satisfactory explanation (despite significant effort). We report our concentrations based on ratios to an internal standard in each sample, but should note that these values are consistent with estimates using a standard curve calibration for OA. Future studies will hopefully resolve these discrepancies. While this makes across-study comparisons difficult, within-study comparisons should be robust. Following from this, similar to our results, Adamo and colleagues documented an increase in OA levels in crickets following both agonistic interactions and courtship (Adamo et al., 1995). Unlike in the present study, however, hemolymph was sampled from the field crickets multiple times, enabling within-individual comparisons of OA level across behaviors. Their within-individual technique demonstrated that OA levels actually increased, as opposed to reflecting baseline differences across individuals, during agonistic and reproductive behavior, and that this increase happened on a relatively short timescale (i.e. within minutes; Adamo et al., 1995). We suspect a similar pattern in *R. punctulata* males. Reproductive interactions in *R. punctulata* frequently encompass agonistic interactions (e.g. direct-mount mating tactic, sexual cannibalism), and we hypothesize that this interaction results in the release of OA.

Contrary to our expectations, we found no relationship between males that were or were not exposed to females and their level of circulating OA. Again in the field cricket, antennal contact alone elicited increased OA levels (Adamo et al., 1995), and we expected that similar exposure to female chemical cues (through their silk), visual cues and vibratory cues might cause an increase in male OA levels. One potential explanation for our negative result is that OA levels were increased in all males, regardless of exposure treatment. Unexposed males were similarly placed in a novel environment for 5 min prior to hemolymph collection, and this transfer to a novel environment alone may have increased OA levels for all males. In hindsight, an additional control would have been to collect hemolymph from males upon capture from their home cages. This protocol was used for female hemolymph collection and may help explain why female levels were so much lower than males (see discussion below).

In insects, the release of OA has been suggested to relate to the need for mobilizing energy stores (Adamo et al., 1995; Davenport & Evans, 1984) and for priming individuals for strenuous activities through its effects on the skeletal-muscular system, where it is known to increase both the speed and power of muscular contractions (Grega, 1978; Roeder, 1999). In spiders, the effects of OA on sensory and motor systems are complex, but OA is suggested to function in increased sensitivity to vibrational stimuli. Even a brief activation of OA receptors is likely to improve a spider's ability to detect vibrations through a sensitization of mechanosensory neurons (Torkkeli et al., 2011). This increased sensitivity to vibrations could be crucial during potentially fatal female-male agonistic interactions. Together, the potential differential need for excess energy and power and/or increased sensitivity to vibrations in small versus large males may help explain our observed pattern of higher OA levels in small males that direct-mount versus lower OA levels in large males that direct-mount (see Figure 3). In the intense and aggressive interactions that take place during direct mounts, a male's speed, strength and sensitivity to female movements are at a premium as larger females are known

to end direct-mount attempts with cannibalism rather than copulation (Wilgers et al., 2009). Simply because of their size, larger males may require less priming or mechanosensory sensitivity (i.e. lower OA levels) than smaller males to achieve a successful mounting of a female while simultaneously avoiding cannibalism. Given that the sample size of this group was quite low, however, further direct inquiry into the relationship between mass (as well as other size measurements) and OA levels during the direct-mount tactic is needed.

In a pattern opposite to that observed in males that direct-mounted, we observed a pattern of increased OA levels associated with increased mass in females. While any interpretation of these data is pure speculation at this point, we postulate a few scenarios. One potential explanation is that females in better “condition” are better able to synthesize OA, and heavier females in our study were in better condition. Unfortunately, we did not acquire body size measurements and thus we were unable to calculate a proxy of body condition. A second scenario is that heavier females have more eggs yolked and that egg yolk requires mobilization of energy stores and thus OA synthesis. Again, these are pure speculation, but each is a testable hypothesis that could be addressed in future studies.

Our observed circulating OA levels in females were significantly lower than those observed in males. Unfortunately, several confounds make it impossible to provide a satisfactory interpretation of these observations. One distinct possibility is that lower OA levels in females simply reflect a longer time in captivity in the laboratory. In an attempt to make our ages (i.e. days post maturation molt) similar across the sexes, we chose to collect hemolymph from our females approximately 3 weeks after males (males were collected mature, making it impossible to know their precise age, but allowing us to infer that they were at least a few weeks older than females). Effects of captivity are known from several animals groups (Koene, 2013), and this potential explanation remains a distinct possibility. In addition, females, in contrast to males, were not transferred to a novel environment prior to bleeding. Thus, transfer of males to a novel environment prior to bleeding may be responsible for the high OA levels we observed. Another arguably more interesting possibility relates to the general demeanor of *R. punctulata* females. Across spiders, females are typically the larger and more aggressive sex during reproductive encounters, with spiders encompassing many prominent examples of sexual cannibalism (Andrade, 1996; Schwartz, Wagner, & Hebets, 2013, 2014). Despite this, we found female *R. punctulata* to have much lower levels of circulating OA than males. In *R. punctulata*, however, cannibalism rates are relatively low (7% of encounters) compared to some other species (range 0 – nearly 100%; Elgar, 1992; Wilder & Rypstra, 2008). In contrast, the rates of male direct mounts can be as high as 21%; considerably higher than the risk of cannibalism. The relative difference in the frequencies of these aggressive behaviors may help explain why males have dramatically elevated levels of OA (~17 $\times$ ) when compared to females. In a closely related species, *Rabidosa rabida*, larger females are much more likely to be cannibalistic than smaller females, and while males are not aggressive at all during mating encounters, females are highly cannibalistic (19% of encounters) (Wilgers & Hebets, 2012). An interesting comparison would be to investigate OA levels in males and females of *R. rabida*. Such a comparative study might provide insight into how variation in OA may mediate aggression during mating encounters and thus influence sexual selection through its potential role in determining mating systems and mate choice. Despite our in-

ability to provide a satisfactory interpretation of our female versus male OA results, so little is known about hormones in arachnids that we hope these initial observations will lay the groundwork for future studies addressing specific hypotheses.

Because of our hemolymph collection protocol, it is impossible to ascertain the timing of OA release. Unfortunately, multiple hemolymph collections from the same individual were not possible using our protocol as individuals could not survive this procedure. Based upon similar studies in other arthropod taxa, however, we suspect that our observed differences in OA across male mating tactics resulted from an increase in OA release as opposed to reflecting different baseline levels across males. In addition, OA levels are known to increase in stressful situations (Bailey et al., 1983; Davenport & Evans, 1984), making it likely that the witnessed increase in OA in direct-mounting males was a product of a stressful interaction. Future research is now needed to investigate the timing of OA release across behavioral contexts.

In summary, across various insect groups, OA has been identified as playing an important role in the physiological bases of behavioral flexibility. In crickets it has been associated with agonistic behavior, flight and courtship (Adamo et al., 1995). In burying beetles, the octopaminergic system is associated with resource defense, alternative mating tactics, social tolerance and indirect parental care (Cunningham et al., 2014). In spiders, most studies have thus far examined its proximate role in mechanoreception (Torkkeli & Panek, 2002; Torkkeli et al., 2011; Widmer et al., 2005), but a few have examined its role in behavioral variation as well (Jones et al., 2011; Punzo & Punzo, 2001). Our results on alternative mating tactics and OA levels in spiders add to the growing evidence accumulating across diverse arthropod taxa suggesting a fundamental role of neurohormonal regulation in behavioral flexibility. While we focused solely on OA in this study, there is clearly a role for other biogenic amines in regulating spider behavior as well (Jones et al., 2011), which should be the focus of future research. Finally, given the hypothesized relationship of chelicerates to the other arthropod groups (Rota-Stabelli et al., 2011), additional data on more arachnids regarding the function of biogenic amines in behavioral variation may shed light on the distinct patterns we currently observe across the insects and crustaceans.

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