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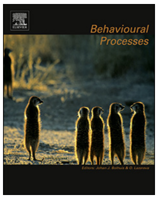

Male attraction to female airborne cues by the net-casting spider, *Deinopis spinosa*

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A R T I C L E I N F O

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A B S T R A C T

For many animals, finding a mate can be a difficult task. For males, it often involves actively searching for conspecific females, sometimes over great distances. This mate-searching can be aided through chemical or visual signals or cues produced by sexually receptive females. Here, we investigate the roles of olfaction and vision in mate-searching in a strictly nocturnal net-casting spider, *Deinopis spinosa.* First, we used an olfactometer assay to determine if mature male *D. spinosa* respond to conspecific airborne cues. We found that mature males, but not mature females, were attracted to airborne cues of mature female conspecifics. We next investigated the relative importance of olfaction and vision in male mate-searching. While manipulating airflow and light levels in screened enclosures in the laboratory, we tested freely moving mature males for mate-searching success. We found no effect of our airflow treatment on mate-searching success. Light levels, however, affected mate-searching in an unexpected way - males were more likely to locate females in complete darkness when compared to dim-light conditions. Our results suggest that visual cues are not necessary for successful male mate-searching in *D. spinosa,* but that the visual environment can nonetheless influence male behavior. In summary, we provide evidence suggesting that airborne cues, but not visual cues, are important in *D. spinosa* male mate-searching efforts, though the source of these chemical airborne cues remains unknown.

1. Introduction

Finding a mate can be a challenging task, especially when the adults of a species tend to be solitary and dispersed. Additionally, mate-searching can be quite costly, as it can increase the likelihood of predation, and the act of searching itself can be metabolically expensive (Fromhage et al., 2016). In many species, mature males actively search for conspecific females, not the other way around. Females may aid males, however, by producing attractive signals or cues that indicate their presence or location (Andersson, 1994; Birkhead and Møller, 1998; Simmons, 2001; Fromhage et al., 2016). Such advertisements can be produced and received across different sensory modalities, as they can encompass visual, acoustic, or chemical forms.

Among invertebrates, chemical communication is ubiquitous and the range over which chemical signaling operates can vary greatly. For example, surface chemicals, such as the hydrocarbons contained within insect cuticle, often require direct contact for detection - *i.e.* contact chemoreception (Lacaille et al., 2007; Park et al., 2006; but see Ozaki et al., 2005). These low volatility compounds are often utilized in species recognition or to elicit courtship at close range (Ozaki et al., 2012). In contrast, high volatility compounds - *e.g.* airborne chemical cues - travel much farther distances. For example, in the emperor moth, *Pavonia pavonia*, males can detect and respond to airborne chemical cues as far as 11 km away from the chemical's source – *i.e.* a conspecific female (Regnier and Law, 1968). In animals with solitary and dispersed adults, such airborne chemicals may be especially important for mate attraction. Volatile airborne chemicals can also afford multiple benefits to receptive females looking to attract a mate, as they are long-lasting, can be effective over long distances, and tend to be inexpensive to produce (Regnier and Law, 1968; Greenfield, 1981; Cardé and Baker, 1984). Unsurprisingly, many arthropods use such airborne chemicals in sexual communication (Ants: [Holldobler and Bartz, 1985], Paper wasps: [Jeanne, 1996], Moths: [Baker and Carde, 1979; Linn et al., 1986; Phelan, 1997]).

Among the arthropods, chemical signal production and reception has been well-studied in the insects (Roelofs, 1995; Ayasse et al., 2001; Yew and Chung, 2015), but much less is known about chemical signaling in arachnids such as spiders (Class: Arachnida, Order: Araneae). Male spiders have, however, been shown to alter their behavior when exposed to female chemical cues (Gaskett, 2007; Uhl and Elias, 2011; Uhl, 2013). While many of these behavioral responses appear to be mediated by close-range, contact chemoreception (Gaskett, 2007),

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there is evidence that spiders from a broad range of families use airborne chemical cues in a mating context (Agelenidae: [Papke et al., 2001], Araneidae: [Herberstein et al., 2002; Gaskett et al., 2004], Linyphiidae: [Watson, 1986], Lycosidae: [Roberts and Uetz, 2004], Salticidae: [Nelson et al., 2012; Cross and Jackson, 2013], Theridiidae: [Kasumovic and Andrade, 2004], Thomisidae: [Stellwag and Dodson, 2010]). Airborne chemical cues may be most important for spiders living in three-dimensionally complex environments, such as tropical forest understories, where dense plant growth might impede visual signaling. Furthermore, nocturnally active spiders in these habitats may have additional challenges of navigating through these complex environments under low-light conditions. Some spiders, however, have special adaptations for nocturnal vision - *e.g.* net-casting spiders – that might aid in nocturnal navigation and mate-searching.

Net-casting spiders (Family: Deinopidae) in the genus *Deinopis* are known for their bulbous and massively enlarged Posterior Median Eyes [PMEs] which are generally regarded as wide-angle motion sensors (Blest and Land, 1977; Land, 1985). These unusual eyes - located in the middle of the spider's second eye row - are the largest eyes of any spider (Blest and Land, 1977; Mammola et al., 2017). Their size, in addition to their short focal distance and large photoreceptors (20 μm wide, 110 μm long), makes these eyes well-adapted for detecting motion at night (Blest and Land, 1977; Laughlin et al., 1980; Land and Nilsson, 2012). Indeed, evidence suggests that *Deinopis spinosa* relies heavily on PME vision for nocturnal foraging. In a recent sensory ablation study, Stafstrom and Hebets (2016) occluded the PMEs of *D. spinosa* in both field and laboratory environments and assessed foraging efficiency. They showed that when PMEs were occluded, spiders were less likely to capture ground-dwelling prey. Thus, the enlarged eyes of *D. spinosa* seem to specifically aid foraging spiders in capturing prey off of the ground. The method of capturing these ground-dwelling prey is quite unique and, here, warrants further mention.

Deinopids use a form of nocturnal foraging called “net-casting.” First, the deinopid builds a non-sticky frame, similar to the frame built in the initial stages of most orb-weaving spiders (Coddington, 1986; Coddington and Sobrevila, 1987). Once frame construction is completed, the spider creates a specialized structure called a “capture snare.” The spider then positions itself upside down, hanging by a silken line from the middle of the frame, and grasps the capture snare using outstretched front legs (Robinson and Robinson, 1971). Upon detection of a potential prey item, the spider lunges at, and actively entangles, the prey using the capture snare (Robinson and Robinson, 1971). Throughout each night of foraging, spiders hang motionless in their frame and typically remain in the same general location for more than a week (J. Stafstrom, pers. obs.). Upon sexual maturation, however, only females persist in using this foraging behavior.

Mature male deinopids no longer hunt for prey, but instead focus on finding mates. Concomitant with this loss of net-casting behavior in mature male *D. spinosa* is a reduction in PME diameter – mature male PME diameters are ∼25% smaller when compared to those of penultimate males (Blest and Land, 1977; Stafstrom et al., 2017). The decrease in diameter of these specialized eyes in mature males, and not mature females, suggests that these nocturnal motion sensors are not particularly useful for mate-searching. As such, we expect sensory modalities other than visual motion detection to play larger roles in mature male mate-searching behavior in *Deinopis.* To date, however, the sensory information mature male deinopid spiders use for their mate-searching has not been investigated experimentally.

Here, we investigate the potential use of both airborne chemical cues and visual cues in the mate-searching behavior of *Deinopis spinosa.* This species is strictly nocturnal and inhabits three-dimensionally complex subtropical environments. Like other deinopids, mature females are dispersed throughout the habitat, yet they can remain in a specific location for long periods. We propose that chemical cues play an important role in male *D. spinosa* mate-searching, while visual cues play no important role. To test these hypotheses, we used live conspecifics as stimuli in olfactometer assays in which we investigated the potential detection of conspecific airborne cues. We also used enclosures with live female/male pairs and manipulated both airflow and light levels to further explore the roles of chemical and visual environments in the context of mate-searching.

2. General methods

2.1. Spider collection and maintenance

We collected both mature and immature *D. spinosa* from Gainesville, Florida, USA in September 2016 and August 2017, and transported spiders to the University of Nebraska – Lincoln, USA. We housed spiders individually under 12:12 light:dark conditions in cylindrical enclosures with the dimensions of 14 cm (height) x 10 cm (width) x 10 cm (depth), with filter paper lining the bottoms. We allowed spiders water *ad libitum* and fed each spider (except mature males, because they do not forage once mature) two crickets (*Acheta domesticus*) once per week by placing crickets in the bottom of their enclosures.

3. Methods - conspecific detection through airborne cues

3.1. Olfactometer design

To test whether male or female *D. spinosa* were attracted to conspecific airborne cues, we used a Y-shaped olfactometer previously constructed for similar experiments with amblypygids (Class Arachnida, Order Amblypygi; Walsh and Rayor, 2008). The olfactometer was comprised of three, 75 mm diameter, clear acrylic tubes. The “introduction arm” was 61 cm long, while the two shorter “stimulus arms” were both 48 cm in length (Fig. 1A).

The stimulus arms were connected to the introduction arm by a “choice chamber” - a black 3-way plumbing joint covered by a clear plexiglass viewing window (Fig. 1A). This window was created by excising the top portion of the plumbing joint and adhering a “trapezoid-shaped” layer of plexiglass cut to fit this incision. This window had a short edge of 65 mm, a parallel long edge of 130 mm, and two sides of equal length at 100 mm (Fig. 1). The window allowed us to easily view and record the movement of spiders from above.

The end of each stimulus arm was covered with metal insect screen with a square mesh pattern (Phifer, Brite Aluminum Screening; mesh dimensions = 2 mm x 2 mm) so as to close off the end of the tube. Since the plastic is difficult for these spiders to grip, we also added strips of the same insect screening to three sides of the inner tube of all three arms (one introduction arm and two stimulus arms) and along the bottom of the choice chamber to aid the spider's locomotion. Finally, the introduction arm had a removable window (*i.e.* a rectangle cut out of the tube that could be removed and placed back in) through which we introduced a focal spider at the beginning of a trial. All three arms, as well as the choice chamber, were removable such that each stimulus arm could be randomly assigned a side for each trial and the inside of all tubes could be cleaned in between trials with ethanol. While glass is generally a preferable substrate for olfactometer trials due to the ease with which surface chemicals can be cleaned off, this particular olfactometer was already made and available and prior results from use of this olfactometer demonstrated that build-up of chemicals was not a concern (Walsh and Rayor, 2008).

To provide airborne chemical cues, we attached "stimulus containers" that could house live spiders to the distal end of each stimulus arm. The stimulus containers were made of black PVC plastic cylinders covered on one end with the same insect screen used above. When the stimulus containers were attached to the end of the stimulus arm, it created a screened-in space in which we could place live conspecific spiders as stimuli. The insect screen was dense enough (2 mm x 2 mm) to prevent focal and stimulus spiders from touching, while still allowing airflow and potential airborne cues to pass from the stimulus container

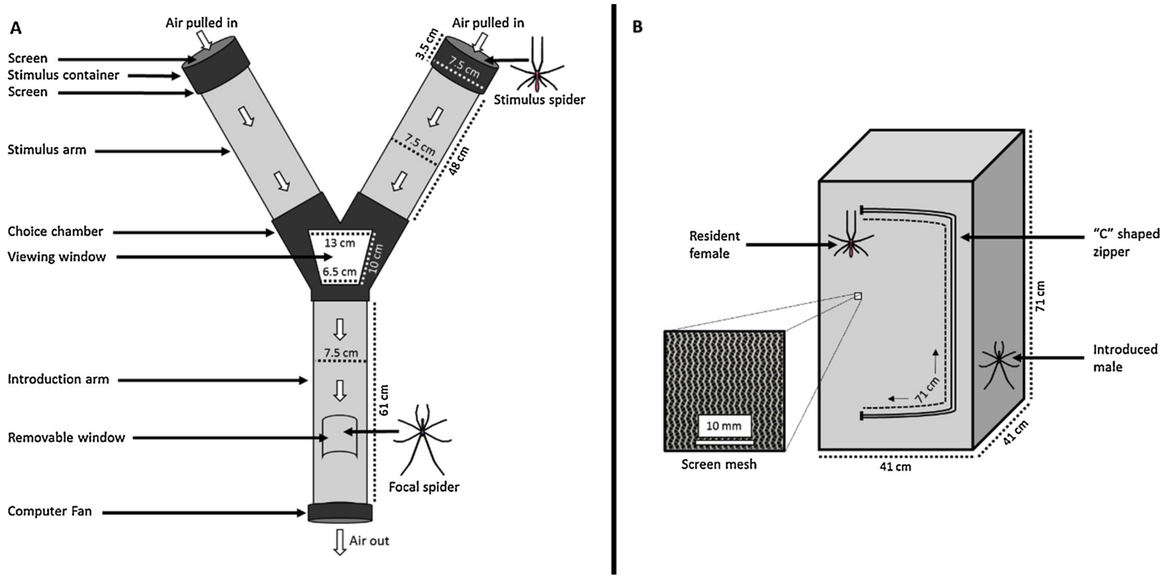

Fig. 1. Depiction of the devices used in (**A**) olfactory discrimination trials and (**B**) mate-search trials. Details of the olfactometer trials (**A**): A computer fan pulled air through stimulus containers, through each stimulus arm, into the choice chamber, and out through the bottom of the introduction arm. Stimulus spiders were placed inside stimulus containers that were double-screened, allowing for airborne cues to pass through into the stimulus arm while also not allowing spiders to interact. To begin trials, focal spiders were placed inside the introduction arm through a removable window. Trials were recorded using an infrared video camera focused on the choice chamber, which had a transparent viewing window. Details of mate-search trials (**B**): resident females were placed in screened enclosures 24 h prior to trial commencement. Airflow and lighting conditions were altered using desktop fans and a dimmed headlamp. The zipper flap was partially opened to place introduced males into screened enclosures. A photograph of the mesh pattern (inset) lining the enclosure. Mate-search success (yes/no) was scored in real-time.

to the stimulus arm.

A computer fan (Sunon, model #KDE1208PTS1-6), adhered to a circular adapter made of PVC plastic, was affixed to the end of the introductory tube, such that it faced away from the olfactometer. This fan, when plugged into the wall, was powered by AC electricity. When switched on, this fan pulled air through the stimulus arms, through the choice chamber, and into the introduction arm. Thus, when we placed a focal spider into the introduction arm, they were exposed to airborne chemical cues from both stimulus arms. Though we did not have the equipment to accurately measure fan speed, the speed of the fan was consistent across all trials as it was non-adjustable (either “on/off”), as used previously (Walsh and Rayor, 2008).

To preclude visual detection and restrict our assay to airborne chemical cues, we ran all trials in a darkened room, devoid of visible light sources (< 0.02 lx). Infrared light, from our recording device (see below), was the only light quantifiably present. While we did not actively prevent potential vibratory communication between stimulus and focal spiders, such communication has not been described for deinopid spiders, and no obvious stridulatory or tremulatory movements were observed. Additionally, the vibratory transmission properties of the thick acrylic and PVC plastic used to construct the olfactometer and stimulus containers should preclude transmission of vibrations in animals of this size (Elias and Mason, 2014).

3.2. Experimental design

We conducted three sets of olfactometer assays – (i) one-stimulus trials with males as focal animals, (ii) one-stimulus trials with females as focal animals, and (iii) two-stimulus trials with males as focal animals. During one-stimulus trials, one of the stimulus containers held a mature female *D. spinosa,* while the other stimulus container remained empty. During two-stimulus trials, one stimulus container held a mature female, while the other stimulus container held a penultimate male. The two-stimulus trials followed the one-stimulus trials and were conducted to determine whether males were generally attracted to conspecifics or explicitly to mature females. All males ($N = 18$) were used in both one-stimulus and two-stimulus trials, but two were removed from one-stimulus analyses due to inactivity. All females ($N = 10$) were used only once in the one-stimulus trial type. We never used stimulus spiders or focal spiders more than once per trial type.

All olfactometer trials took place at night between 20:00 and 03:00 Central Standard Time. Trials began about one hour after the beginning of the dark photoperiod. During each experiment type (one-stimulus and two-stimulus), we randomly chose the side(s) in which a stimulus spider was first placed. Stimulus sides were alternated for successive trials in each pair. For example, if we began a one-stimulus trial with the stimulus spider on the right, the next trial that evening would have the stimulus spider on the left, and so on. We cleaned the apparatus between trials using paper towels wetted with 70% EtOH, followed by paper towels wetted with water purified *via* reverse osmosis (RO water). The inside of all olfactometer arms, the choice chamber, and the stimulus chambers were all cleaned.

Once placed into the introduction arm, focal spiders moved freely within the olfactometer. We videotaped trials using a Sony Handycam HDR – HC9 video camera in NightShot mode, placed on a tripod above the choice chamber. The only light exposed to the focal spider was infrared light emitted from the video camera, localized to the choice chamber. We recorded the first stimulus arm visited by each focal spider (“first visit”), as well as how long it took to make the first visit in seconds (“latency to first visit”). We also recorded which stimulus arm was “chosen”, and how long it took to make this choice in seconds (“latency to choice”). A “choice” was defined as staying in one of the stimulus arms for over 5 min without leaving. We used this 5 min cutoff as it had been used successfully in previous experiments with amblypygids (Hebets, unpublished data). Trials lasted 30 min or until a focal animal made a choice and, if no choice was made within 30 min, individuals were removed from further analyses.

3.3. Statistical analyses

For all one and two-stimulus trials, we compared: (a) which stimulus arm was visited first, (b) the latency to first visit, (c) the stimulus arm chosen, and (d) the latency to choice. To determine if focal individuals were more likely to choose a particular stimulus arm first, we

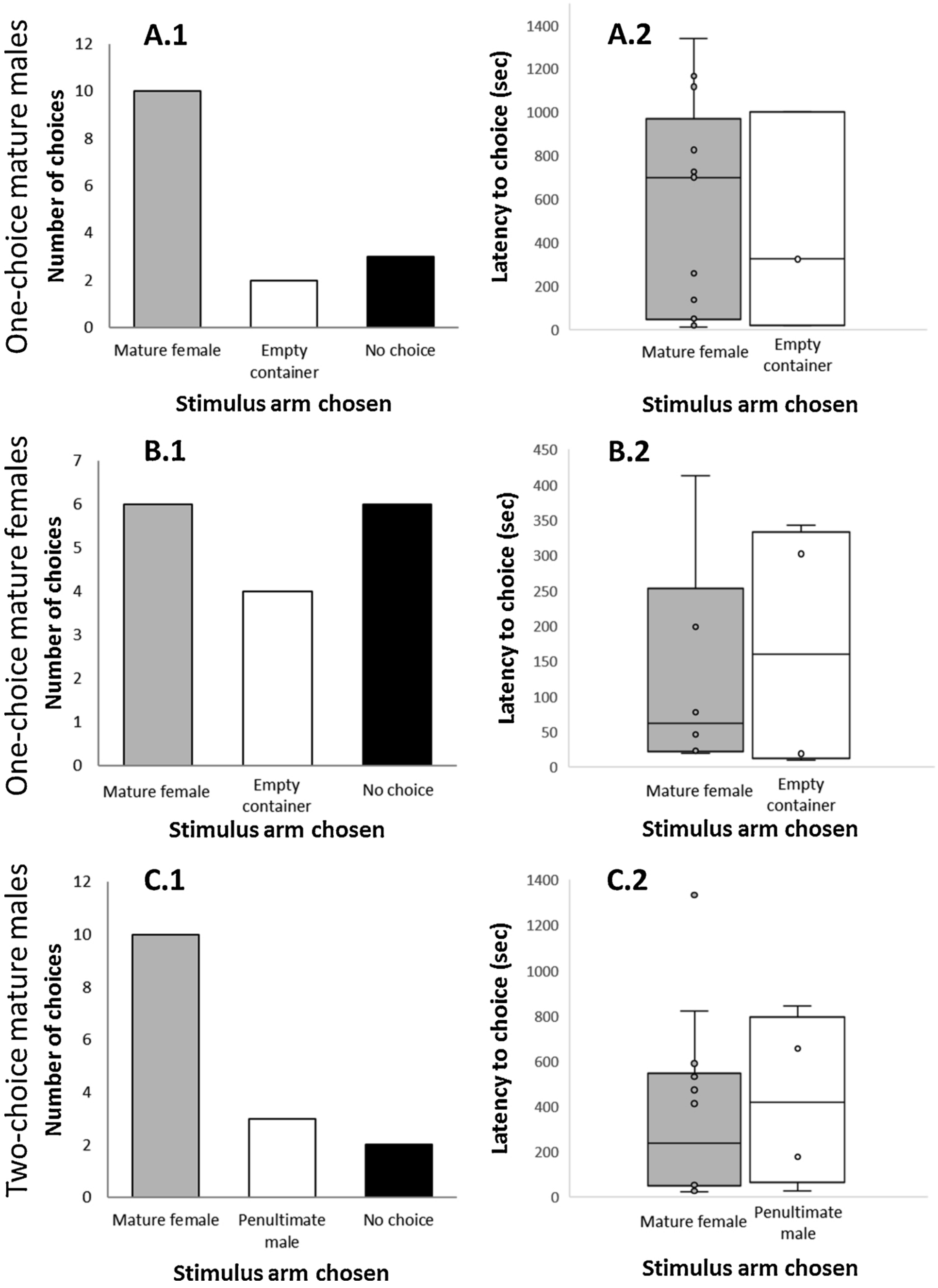

Fig. 2. Olfaction-based conspecific detection. Graphical representation of mature male one-stimulus trials (**A.1, A.2**), mature female one-stimulus trials (**B.1, B.2**), and mature male two-stimulus trials (**C.1, C.2**). Number of choices made over all trials summed in (**A.1, B.1, C.1**), while latencies for each choice is depicted in (**A.2, B.2, C.2**). Mature males, in both one-stimulus (N = 16) and two-stimulus (N = 18) trials, were more likely to "choose" the stimulus arm containing mature female airborne cues. In contrast, mature females (N = 10) had no obvious tendencies regarding which stimulus arm was chosen. Across all trials, latency to choice did not significantly differ dependent on which stimulus arm was chosen.

used chi-square tests. To determine whether the latency to visit a first stimulus arm depended on the specific stimulus arm visited, we used Mann-Whitney U tests. To evaluate whether focal spiders tended to choose either stimulus arm more often than by random chance, we used chi-square tests. To determine whether the latency to make a choice depended on which stimulus was chosen, we used Mann-Whitney U tests. All statistical analyses were run using SPSS (IBM) version 22.0.

4. Results - conspecific detection of airborne cues

4.1. One-stimulus trials - mature males as focal animals

There was no significant difference between which stimulus arm was first visited by mature males (N = 16, $\chi^2 = 0.25$, P = 0.617). The latency to visit either stimulus arm was not significantly different (U = 26.00, Z = 0.582, P = 0.56). Mature males more often chose (*i.e.* remain in a given stimulus arm for 5 min) the stimulus arm containing a mature female when compared to an empty stimulus arm (N = 16, $\chi^2 = 6.25$, P = 0.012; Fig. 2A.1). The latency to choice was not significantly different between stimuli (U = 17.00, Z = 0.336, P = 0.8; Fig. 2A.2). Overall, 56.25% of males (9 out of 16) chose to stay in the stimulus arm they first visited.

4.2. One-stimulus trials - mature females as focal animals

There was no significant difference between which stimulus arm was first visited by mature females (N = 10, $\chi^2 = 0.00$, P = 0.999). The latency to visit either stimulus arm was not significantly different (U = 17.50, Z = 0.080, P = 0.936). Unlike mature males, mature females did not significantly more often choose the stimulus arm containing a mature female when compared to an empty stimulus arm (N = 10, $\chi^2 = .500$, p = 0.779, Fig. 2B.1). The latency to choice was not significantly different between choice types (U = 10.50, Z = .321, P = 0.748, Fig. 2B.2). Overall, 100% of females (10 out of 10) chose to stay in the stimulus arm they first visited.

4.3. Two-stimulus trials - mature males as focal animals

Mature males significantly more often first visited the mature female stimulus arm when compared to the penultimate male stimulus arm (N = 18, χ^2 = 5.556, P = 0.018). The latency to visit either stimulus arm was not significantly different (U = 18.00, Z = 1.062, P = 0.327). Mature males more often chose the mature female stimulus arm when compared to the penultimate male stimulus arm (N = 18, χ^2 = 5.556, P = 0.018; Fig. 2C.1). The latency to choice was not significantly different between choice types (U = 23.00, Z = 0.531, P = 0.645; Fig. 2C.2). Overall, 72.22% of males (13 out of 18) chose to stay in the stimulus arm they first visited.

5. Methods - mate-search trials

5.1. Experimental design

To explore the relative importance of olfaction and vision in male *D. spinosa* mate-searching, we conducted mate-search trials where freely moving mature males could locate and interact with freely moving mature females under altered airflow and lighting conditions. These trials took place inside screened “butterfly enclosures” (Trasfit, ASIN# B01LN8ETBS; Fig. 1B) with the dimensions of 71 cm (height) x 41 cm (width) x 41 cm (depth). The screen mesh was nylon with a complex mesh pattern (see Fig. 1B, inset). Enclosures could be opened and closed using a zipper along a C-shaped zipper chain (72 cm in length, Fig. 1B) that was located along one of the long lengths of the enclosure.

We manipulated airflow and lighting conditions to alter the chemical and visual sensory environment of mate-searching spiders. We had two airflow treatments: (i) increased airflow (hereafter: “fans present”) and (ii) unmanipulated airflow (hereafter: “fans absent”). In the fans present treatment, we placed AA battery powered desktop fans (Travelon, ASIN# B003DGPWLK) outside of testing enclosures at a distance of 5 cm. Fans were placed on the side of the enclosure closest to settled females within the enclosure, between 15–20 cm away from females in total distance. The variability in distance reflected our compensation for where the female settled inside the enclosure (details regarding female placement in enclosure is forthcoming). Fans were pointed away from the enclosure to pull air through the enclosure. Increased airflow in fans present enclosures was visually confirmed by using dense water vapor blown inside an enclosure. We witnessed increased air turbulence in the fan present conditions when compared to the fan absent conditions, but did not have a means of quantifying this difference. Fan batteries were replaced prior to each night of trials. In the fans absent treatment, identical methods were followed except that fan blades were removed from operating fans. This allowed spiders in the fans absent treatment to experience vibrations associated with operating fans, but without airflow being altered.

Similar to our airflow treatments, we had two visual treatments that differed in the amount of light experienced by spiders: (i) dimly-lit treatment (hereafter: “light present”) and (ii) complete darkness treatment (hereafter: “light absent”). Light present trials were run with a headlamp (Black Diamond Spot headlamp, Item# BD620634OCTNALL1) turned to the lowest light intensity and pointed away from testing enclosures. These lighting conditions (~0.1 lx) mimicked the illuminance of a full moon (maximum illuminance ~0.3 lx, estimated average illuminance ~0.05-0.1 lx; Kyba et al., 2017). Light absent trials were run in complete darkness (< 0.02 lx). Light levels were quantified using a handheld, digital luxmeter (HDE, ASIN# B00992B29I).

Twenty-four hours prior to mate-searching trials, single mature females were placed inside testing enclosures and allowed to acclimate. All females were found to be stationary and hanging from newly deposited silk draglines prior to the start of each trial. Trials began when a mature male was placed in the testing enclosure. To introduce a male spider, we partially unzipped the side of the enclosure, placed the male on the bottom of the enclosure, and zipped the enclosure closed. All males started at a similar location due to the physical constraints of their introduction location (*i.e.* where the zipper opening was), but their location varied with respect to their distance from the stimulus female. Most females, however, hung in the top corners of their enclosures and thus the typical distance between a male's starting location and his stimulus female was between 50 cm and 60 cm.

Trials took place at night between 22:00 and 03:00 Central Standard Time, about three hours after the beginning of the dark photoperiod. Trials lasted 30 min, or until a male interacted with a female *via* touching or courtship. We used a repeated-measures, 2 × 2 factorial design of airflow treatment (fans present *vs.* fans absent) and lighting treatment (light present *vs.* light absent) such that each male was used in four different trials. Males were never allowed to mate with the female and they were removed from the enclosure as soon as they encountered the female. As such, we reduced the impact of using males multiply. Additionally, given our repeated measures design and the potential for varying cues across females, individual males were always used with the same female as a stimulus. Mate-search success (yes/no) was scored in real time.

5.2. Statistical analyses

To investigate how each individual treatment affected mate-searching success, a general linear mixed model (GLMM) was fit in which fixed factors were airflow treatment (fans present/fans absent) and vision treatment (light present/light absent). The response was binary (mate-searching successful/not successful), and spider ID was a random effect. A likelihood ratio test was used to determine model significance against an intercept-only model. To investigate the significance of fixed effects within the model, likelihood ratio tests were used to compare the full model (vision treatment and airflow treatment) against models omitting either fixed effect. GLMM likelihood ratio tests were run using the lme4 package (Bates et al., 2015) in R Statistics package version 3.3.1 (Bolker, 2015).

6. Results - mate-search trials

Our GLMM fit our data significantly better than an intercept-only model with identical random effects structure (χ^2 = 9.011, P = 0.011). Within this full model, the vision treatment significantly affected mate-searching success (χ^2 = 9.011, P = 0.003), while a model excluding airflow treatment did not differ from the full model (χ^2 = 0.00, P = 0.999). These results indicate that the light environment experienced during testing significantly affected mate-searching (Fig. 3). Specifically, males experienced decreased mate-searching success in the light present treatment. In contrast, airflow exhibited no detectable effect.

7. Discussion

Our results strongly suggest that mature male *Deinopis spinosa* are attracted to airborne chemical cues of mature female conspecifics. By using olfactometer assays, we determined that mature males were more likely to associate with airborne cues of a mature female when compared to cues originating from either a container devoid of conspecific cues or a container holding a penultimate male. Through mate-searching assays conducted under manipulated airflow and lighting conditions, we failed to find an effect of air-flow on mate-searching success, while lighting conditions did significantly affect success rates. Contrary to our predictions, males located females more often in complete darkness as compared to under dimly lit conditions. Together, our data support a role of chemical cue detection, but not visual cue detection, in close range (< 1 m) mate-searching in *D. spinosa*.

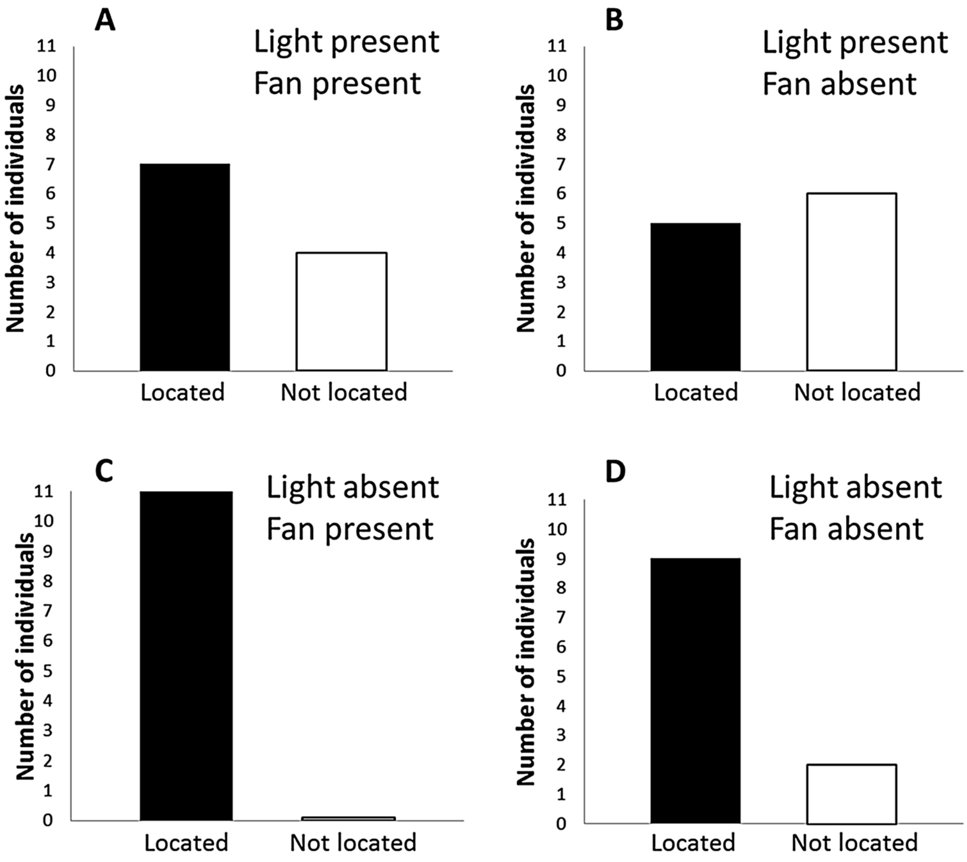

Fig. 3. Mate-search trials. Total mate-searching successes per treatment are summed in (**A-D**). Treatments are as follows: (**A**) light present, fan present; (**B**) light present, fan absent; (**C**) light absent, fan present; (**D**) light absent, fan absent. Males were significantly more likely to locate and interact with females in the dark (**C,D**) than in the light (**A,B**), while airflow treatment was not a significant factor in determining mate-search success (**A,C** *vs.* **B,D**).

Results of our olfactometer assays indicate that male *D. spinosa* can use chemical cues to locate mature females. The use of chemical cues in mate-searching has been similarly demonstrated in other web-building spiders (reviewed in Gaskett, 2007). In our one-stimulus olfactometer experiment, mature males preferred to associate with mature female airborne cues when compared to cues from an empty stimulus container. Our two-stimulus experiment further demonstrated that this affinity to mature female cues was not simply due to conspecific attraction in general. In these two-stimulus trials, males tended to remain in stimulus arms containing mature female cues for longer periods of time (5 min) when compared to stimulus arms containing penultimate male cues. Since all of our olfactometer trials were conducted in the dark, our results indicate that chemical cues from conspecific females are sufficient for mate detection. As *D. spinosa* are strictly nocturnal and inhabit complex subtropical environments, successful mate-searching in the absence of visual cues (*i.e.* through olfaction) may be particularly beneficial for mature males.

In contrast to mature males, *D. spinosa* females showed no evidence of discriminating between airborne cues of conspecific females and an empty container. Females also tended to move between stimulus arms less than males - all females chose to stay in the stimulus arm they first visited, while about 50% of males sampled both stimulus arms prior to making a choice. Our results provide no indication that females either seek out or avoid conspecific females. There are three potential explanations for these findings: (1) females cannot detect airborne chemical cues, (2) females can detect airborne chemical cues, but not those relevant to conspecific females, or (3) females can detect airborne chemical cues relevant to conspecific females, but this does not affect their behavior in our olfactometer design. Given our results, we cannot directly assess which explanation is most plausible, yet we suspect that females simply do not respond to conspecific airborne chemical cues even if detected. Future studies assessing the behavior of freely moving females in a natural environment would be useful in disentangling what role, if any, airborne chemical cues might play in the behavior of mature female *D. spinosa.*

Similar to previous arachnid olfactometer studies (Dodson et al., 2013; Walsh and Rayor, 2008), we found differences in male spiders' tendency to "first visit" a specific stimulus arm. This effect, however, was only observed under two-stimulus conditions in which mature males experienced potential cues from both mature females and penultimate males. It is possible that this "first visit" effect resulted from the simultaneous attraction and aversion to the two stimuli provided – *e.g.* attraction to mature female cues and aversion to penultimate male cues. While attraction to a potential mate can be beneficial to mate-searching males, repulsion to either a non-receptive conspecific or potential competition may be similarly beneficial.

While mature males appear to be attracted to female airborne cues, the source of the presumed chemical attractant remains unclear. Across multiple web-building and non-web-building spiders, a common method of cue deposition in mature females is to lay silk containing chemical cues (Gaskett, 2007). Though often associated with contact-chemoreception (the act of sensing chemicals *via* touch), airborne detection and attraction to female silk cues has been described in multiple spider families (Gaskett, 2007). For instance, in the redback spider *Latrodectus hesperus,* webs of virgin females, absent of female spiders, have been shown to attract conspecific males (Kasumovic and Andrade, 2004).

When *Deinopis* spiders are removed from their web and placed into a new environment, they quickly attach a dragline to any available surface and then settle into a cryptic posture (J. Stafstrom, pers. obs.). We observed this behavior when placing stimulus females into the stimulus containers of our olfactometer experiment. As such, stimulus containers held both female silk and live females, and any potential chemical cues originating from either source. Our experimental design, then, cannot discriminate between chemical cues originating from female silk or from the female herself. Mature females, for example, might possess a cuticular chemical profile that is attractive to males at short distances. Similar to flies, beetles, wasps, and ants (Singer, 1998; Cross and Jackson, 2013), males of multiple spider species are known to be attracted to extracts of female cuticle perceived *via* airborne cues (Gaskett, 2007; Cerveira and Jackson, 2013). Future research is now necessary to locate the source of these putative chemical cues.

In our second experiment, by manipulating airflow and lighting conditions, we explored the relative roles of olfaction and vision in mate-searching. Given that our olfactometer assays demonstrated a role of olfaction in mate searching, we expected our airflow treatment to influence a male's ability to locate a female in live mate-searching trials. Contrary to this expectation, however, airflow did not influence the mate-searching success of *D. spinosa* males. We suspect that this is due, in large part, to the specifics of our experimental design and again, to the potential for female silk to contain chemical cues.

Our fans present treatment was intended to decrease the active signal space of airborne chemical cues. However, this manipulation of airflow would not have affected potential contact chemical cues associated with female silk. During their 24 h acclimation period, mature females laid silk as they moved around the enclosure prior to settling in one location. As previously mentioned, it is possible, and even probable, that mature female *D. spinosa* silk is a source of contact chemical cues. Contact chemoreception is common among spiders, as they use chemoreceptive hairs, often located on the pedipalps, to detect substrate-borne chemical cues (Foelix, 2011). This type of chemical detection occurs when chemoreceptors physically contact substrate-borne chemicals, and thus, should not be significantly altered by airflow. We suspect that the presence of silk lines, and presumed substrate-borne chemical cues, negated the effects of increased airflow on any chemically mediated mate-searching in our enclosure trials. Indeed, our design may have inadvertently addressed questions of very close range – *i.e.* within the range of silk deposition - *versus* longer-range female attraction, as airborne chemical cues may be used over longer distances and contact chemoreception may be used at close range.

In our mate-searching experiment, the manipulated light levels influenced mate-searching success in an unexpected way. Given the decreased PME size of mature male *D. spinosa,* our expectation was that light environment would not significantly affect mate-searching behavior. However, we found that males were more likely to locate females in complete darkness as compared to under dimly lit conditions. One possible explanation for our results is that the light intensity experienced in the dimly lit treatment negatively affected mate-searching behavior. Even though the light intensity of our dimly lit treatment (~0.1 lx) falls within the range of naturally occurring nocturnal light intensities (maximum illuminance ~0.3 lx, estimated average illuminance ~0.05-0.1 lx; Kyba et al., 2017), this intensity mimics full moon conditions, previously shown to negatively affect nocturnal behavior. For example, in some nocturnal animals including snakes (Clarke et al., 1996; Campbell et al., 2008; Weaver, 2011), birds (Brigham et al., 2001), and scorpions (Kaltsas and Mylonas, 2010; Kaltsas et al., 2008), activity levels are known to decrease during full moon periods. This reduction in activity is presumably to avoid visually guided predators under these more lighted conditions. Thus, it is possible that mature male *D. spinosa* decrease activity under light intensities similar to that of a full moon. Though all mature male *D. spinosa* moved once placed inside our testing enclosure, we unfortunately did not quantify the extent of movement during our trials – *e.g.* the proportion of time active or the total distance traveled. Thus, it is possible that males simply decreased activity in our light present treatment, which would lead to poorer performance in these trials. Regardless, as *D. spinosa* males successfully located females in complete darkness, our results clearly indicate that visual cues are not necessary for successful close-range (< 1 m) mate-searching in this species.

In summary, while conducting the first investigation of mate-searching behavior in a net-casting spider, we provide evidence for chemical cue-mediated mate attraction in *Deinopis spinosa*. Considering the complex natural habitat and nocturnal activity patterns of *D. spinosa,* the ability to locate conspecific females in the absence of visual cues may prove integral to successful reproduction. In addition, our work illustrates decreased mate-searching success under light intensities similar to that of a full moon, potentially due to decreased activity driven by avoidance of visually guided predators. Though we provide novel information regarding the sensory ecology of net-casting spiders, we also uncover new questions to answer. While a paucity of behavioral research has been conducted on this enigmatic family of spiders, we are excited to contribute to a foundation for future investigations of deinopid sensory ecology.

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