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Potential role of foreleg trichobothria in the reception of air particle movement during wolf spider courtship

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Abstract. Spiders use information received from trichobothria for capturing prey or escaping predators. However, we have no extensive information on if or how trichobothria might be used in conspecific communication. This study tests the hypothesis that female wolf spiders *Schizocosa retrorsa*, (Banks, 1911) use their foreleg trichobothria to detect and assess courting males. Prior studies have shown that male *S. retrorsa* can mate successfully in signaling environments where females cannot detect visual or vibratory signals. Despite this, higher rates of male leg waving still predicted successful mating. In addition to their visual conspicuousness, these rapid leg waves can generate air particle movement that may be detectable by a female's trichobothria. If females use trichobothria to detect and assess male leg waving, then we might predict the following. When female trichobothria are compromised, we expect (i) lower overall mating success and (ii) no relationship between male mating success and leg waving rate. To test these predictions, we ran mating trials in environments unconducive to visual or vibratory signals and compared the mating success and mating predictors of female/male *S. retrorsa* pairs across two female treatments groups: (a) foreleg trichobothria unablated and (b) ablated. We found no significant difference between the mating success of the two groups. However, mating success was higher for males that waved their legs at a faster rate in the unablated, but not the ablated treatment groups. Our results indicate that the ablation of female trichobothria interferes with the detection and assessment of male leg waving, supporting a role of trichobothria in receiving air particle movement signals.

Keywords: Mate choice, multimodal, *Schizocosa retrorsa*, near-field sound
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Arthropods possess an array of specialized sensory structures that often function in ways unique to the animal's natural history. Within arachnids alone, multiple orders have evolved their own novel sensory organs and/or have evolved specializations in sense organs shared by other groups. Order-specific sensory structures include the malleoli of solifuges (Brownell & Farley 1974) or the pectines of scorpions (Wolf 2017). Specialization of sensory organs can be seen in the specialized eyes of jumping spiders (Land 1969; Harland & Jackson 2002) and the elongate antenniform legs of amblypygids (Santer & Hebets 2011b). Significant research has been devoted to exploring the functions of these taxon-specific structures and specializations. For example, research suggests that solifugid malleoli detect chemical signals (Brownell & Farley 1974; Sombke et al. 2019). Similarly, we now know that scorpion pectines can function as chemo-sensors as well as mechano-sensors (Gaffin & Brownell 1997; Wolf 2017). The eyes of jumping spiders and their associated visual acuity have been explored in the context of foraging (Li & Lim 2005; Taylor et al. 2014), navigation or orientation (Tarsitano & Jackson 1992; Cross & Jackson 2019), and courtship and mating (Taylor & McGraw 2013; Echeverri et al. 2017; Girard et al. 2018). Finally, the antenniform legs of amblypygids, shown to detect airborne odors (Hebets & Chapman 2000), are used in agonistic interactions (Fowler–Finn & Hebets2006; Santer & Hebets 2008, 2011a) and are critical for the animal's successful nocturnal navigation (Hebets et al. 2014; Bingman et al. 2016; Wiegmann et al. 2019).

Most arachnids, as well as insects and crustaceans, possess particularly fine filiform sensilla, known as trichobothria in arachnids (Görner & Andrews 1969; Barth 2002; Breithaupt 2002; Shimozawa et al. 2003). These thin elongate sensilla are extremely sensitive to very small particle displacement (Görner & Andrews 1969; Tautz & Markl 1978; Barth 2000; Breithaupt 2002; Shimozawa et al. 2003) – e.g., frequencies as low as 50–150 Hz as far as 70 cm away (Tautz & Markl 1978; Barth 2000). Among spiders, trichobothria function has been studied extensively in *Cupiennius salei* (Keyserling, 1877) (Trechaleidae) (Barth et al. 1993; Friedel & Barth 1997; Barth & Holler 1999). The trichobothria are known to deflect at specific angles (Barth et al. 1993, 1995) and provide directional information (Friedel & Barth 1997). Air particle movement detection with trichobothria is important for spiders to catch prey (Friedel & Barth 1997) and escape predators (Suter 2003). Whether they are used in receiving intraspecific signals, however, is yet to be confirmed. Nonetheless, recent studies strongly suggest that air particle movement is important in the complex courtship displays of *Schizocosa retrorsa* (Banks, 1911) wolf spiders (Rundus et al. 2010; Choi et al. 2019; Kundu et al. 2022).

Schizocosa retrorsa has emerged as an important species for elucidating the evolution and function of multimodal communication in wolf spiders (Hebets et al. 1996, 2008; Rundus et al. 2010; Choi et al. 2019). Despite having a courtship display that appears to include visual and substrate-borne vibratory (hereafter - 'vibratory') signaling, *S. retrorsa* males can successfully copulate in the absence, or reduction, of signals in either/both signaling modality (Rundus et al. 2010; Choi et al. 2019; Kundu et al. 2022). Curiously, the rate of leg waving by the males (previously thought to be a visual courtship display) has consistently been found to predict mating success, even in signaling environments where visual and vibratory signals could not be transmitted (Rundus et al. 2010; Choi et al. 2019). Such results have suggested the potential role of an additional signaling modality – air particle movement or near field sound.

To explore the potential for leg waving to generate air particle movement signals used during courtship communication, a recent study tested whether *S. retrorsa* mating success was affected by the presence of noise in the near field. The study found that mating success was higher in the absence, versus presence, of artificially introduced air particle movement (i.e., near field 'noise'; hereafter 'noise'). Furthermore, the study found a relationship between leg waving rate and mating success in the absence, but not presence, of noise. Although these findings are consistent with a role of air particle movement in *S. retrorsa* courtship displays, males also

exhibited courtship plasticity across signaling environments—males engaged in lower rates of leg waving in the presence of noise (Kundu et al. 2022). Given the observed plasticity in male courtship intensity across noise environments, it remains unclear the extent to which female detection of air particle movement, or male courtship plasticity, was the primary driver of the most recent results.

The present study directly tests the role of female trichobothria in receiving and assessing *S. retrorsa* male courtship displays. We tested this by ablating the trichobothria of females and recording the outcome of female-male interactions in environments that prevented visual and vibratory signal transmission. More specifically, we conducted mating trials with females who had the trichobothria on their first two pairs of legs intact (unablated) versus cut (ablated). If females use foreleg trichobothria to assess male courtship based on air particle movement generated during leg waving, then we expect (i) ablated trials should have lower mating rates and (ii) males that acquire matings in the unablated groups should display higher leg waving rates than males that do not mate, but this pattern should not be present in males from the ablated trials.

To ensure that the ablation manipulation did not dramatically influence female behavior, we also assessed the foraging behavior of unablated and ablated females. We were interested primarily in whether foraging behavior, a presumably "normal" behavior, was or was not noticeably altered. To explore this, we collected data on foraging success. Given that spiders may use trichobothria during foraging (Friedel & Barth 1997), we would not have been surprised if we observed lower attack success of ablated females. Nonetheless, if our manipulation was minimally intrusive as we had hoped; our expectation was to observe similar foraging success, as spiders are likely to use information beyond air particle movement (e.g., tactile, chemo-sensation) for foraging (Hoefler et al. 2002; Hostettler & Nentwig 2006; Meza et al. 2021).

METHODS

Study animals.—We collected juvenile *S. retrorsa* wolf spiders from Panola Co., Mississippi, U.S.A. near Sardis Dam T8S R6W Sect. 13 (34°23′N, 89°47′30″W) on May 5th, 2021. We housed the spiders individually in plastic cages (5.8 × 5.8 × 7.9 cm, AMAC Plastic Products, USA) after we transported them to the laboratory at the University of Nebraska-Lincoln. To visually isolate the individuals, the cages were covered with masking tape. The inside of the cages had metal mesh on 2 sides for the spiders to climb. We fed the spiders through a hole in the top of the cage fitted with a removable cork. Twice a week, we fed the spiders with two 1/16th inch (1.59 mm) crickets (*Gryllodes sigillatus* from Ghann's Cricket Farm). A constant source of water and humidity were provided by a dental wick that fit into a hole in the bottom of the cage. All cages sat on top of mesh wire in plastic tubs (65x37x14.5 cm) filled with 2–3 cm of water in which the dental wicks rested. We placed the cages in a controlled light (12 hours light, 12 hours dark) and constant temperature (25°C) environment. We checked the spiders for molts (shed exoskeleton indicates growth and often sexual maturation) every 1–2 days.

Preparation of study animals.—We randomly separated all adult females of our study into two treatment groups: (a) unablated and (b) ablated. Methods for ablating or disabling sensilla have previously been tested on different arthropods, e.g., sensilla on the back of caterpillars (*Barathra brassicae L.*) (Markl & Tautz 1975; Tautz & Markl 1978), trichobothria on fishing spiders (*Dolomedes triton* (Walckenaer, 1837)) (Dolomedidae) (Suter 2003), Labyrinth spiders

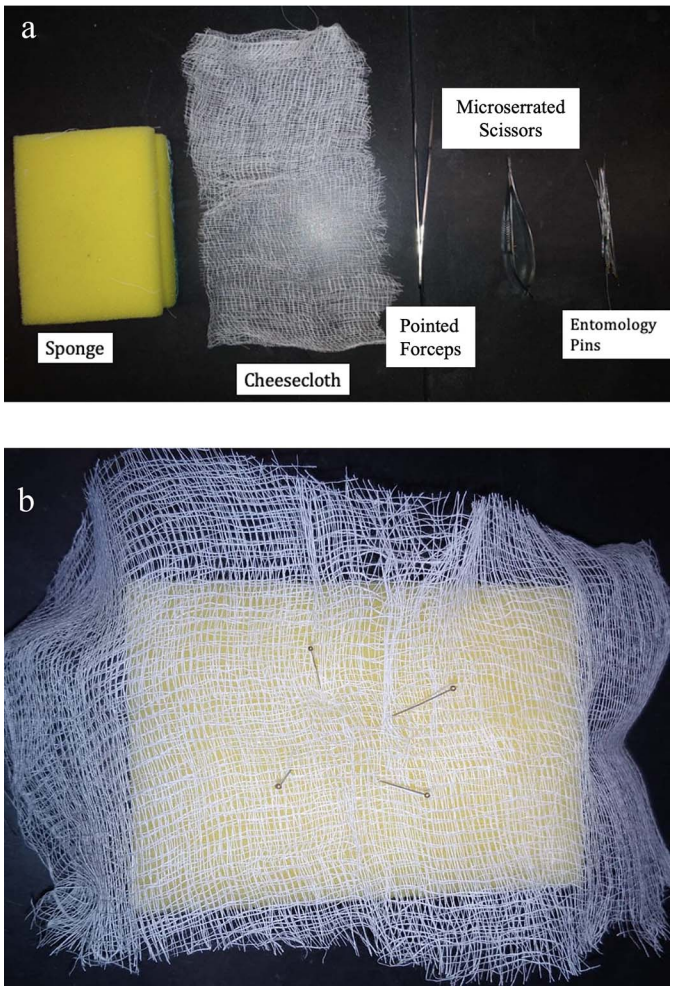

Figure 1.—The sponge, cheesecloth, pointed forceps, microserrated scissors and entomology pins (a) were used to immobilize the females to ablate their trichobothria of the first 2 pairs of legs or just hold them down for 3–4 minutes. (b) Three layers of cheesecloth along with entomology pins were used over the sponge.

(*Agelena labyrinthica* (Clerck, 1757)) (Agelenidae) (Görner & Andrews 1969), long-jawed orb weavers (*Leucauge mariana* (Taczanowski, 1881)) (Tetragnathidae) (Segura-Hernández et al. 2020), and amblypygids (*Phrynus marginemaculatus* C. L. Koch, 1840) (Phrynidae) (Santer & Hebets 2011a). Using a method like Segura-Hernandez and colleagues (Segura-Hernández et al. 2020), we ablated the trichobothria on the front 2 pairs of legs in the ablated group of females. These legs can have 22–40 trichobothria per leg spread out over their femur, patella, tibia, metatarsus, and tarsus (See Supplemental Table S1, online at https://doi.org/10.1636/JoA-S-22-065.s1). Depending on the placement of the trichobothria, it was difficult to cut trichobothria without injuring the spider. Nonetheless, we used all care to make sure that the spiders were unharmed and did not autotomize their legs during the trichobothrial ablation process.

Again, like Segura-Hernandez and colleagues, (Segura-Hernández et al. 2020) we used a sponge, a piece of cheesecloth, pointed forceps, microserrated scissors (Brand: Fine Science Tools, Item No.: 15071-08 Curved/Serrated/8cm) and entomology pins (Segura-Hernández et al. 2020) to restrain spiders during the ablation/sham ablation procedures (Fig. 1a). We placed each female on the sponge and quickly trapped

them with 3 layers of cheesecloth (Fig. 1b). We used entomology pins to hold down the cheesecloth and keep the spider in position. We placed 1–2 pins in front of the head of the spider, one at the tip of the abdomen, one on each side of the abdomen and one on each side of the cephalothorax between the second and third pairs of legs to keep the spider in position. We did not use CO_2 as an anesthetic, as prior studies in our laboratory have demonstrated negative effects of CO_2 on spiders (unpublished data).

Using the index finger and thumb of our non-dominant hand to hold down the cheesecloth with the female in position, we (author PK) used pointed forceps to gently guide the two pairs of forelegs out through the holes in the cheesecloth. During this process, we tried to make sure that we pulled out each of the legs through individual holes in the cheesecloth to reduce the chances of the spider escaping. We were also careful not to pull too hard on the legs, which might have caused the spiders to autotomize them. Then, we placed the whole setup under a dissecting scope. With the micro-serrated scissors in our dominant hand, we looked through the scope, identified the trichobothria and cut them as close to the exoskeleton as possible. We worked with one leg at a time. Once we ablated the trichobothria on all four legs (first two legs on both sides of the body), we moved the sponge to a container. We took out the pins and slowly lifted the cheesecloth while letting the spider pull back her legs through the holes. We allowed each spider to rest for a few minutes before putting her back in her cage. Following the ablation, we determined that we ablated approximately half of the trichobothria present on all focal legs, as there were 13–21 trichobothria left per leg after the ablation process (Supplemental Table S1). Our manipulation created a significant difference in the number of trichobothria on the front two pairs of legs between the unablated and ablated females (Supplemental Table S1). The ablation of trichobothria was also visually notable for the front two pairs of legs as compared to the remaining walking legs.

The unablated females experienced the same handling and procedure but without actual ablation of trichobothria. Instead of cutting the trichobothria, the unablated female's trichobothria were touched with the pointed forceps while held under the cheesecloth to elicit a similar sensation to the ablated group females. These females were held under the same conditions for 3–4 minutes before being released and put back into their cages.

Foraging trials.—We ran foraging trials 2–5 hours after the unablation/ablation procedure and 15–20 hours prior to the mating trials. The spiders were fed 5–7 days prior to the foraging trials. We released the individual females into the "mating" arena (20 cm in diameter, in the dark, on a granite surface) where we allowed them to roam freely for 3–4 minutes. We then introduced a single ~1.59 mm (1/16th inch) cricket (*Gryllodes sigillatus*) into the arena opposite to the position of the spider. We left the spider and cricket to interact for 3 minutes, after this time we noted whether the spider ate the cricket or not (foraging success Y/N). After the foraging trial, the females were given one additional cricket if they ate during the trial or two crickets if they did not eat during the trial. The corresponding males were also fed at the same time in preparation for the mating trials.

Mating trials.—Our mating trial arena was a transparent plastic circular enclosure (diameter 20 cm) resting on a granite slab. We conducted all mating trials in a dark room with only an infrared light source (Brand: IR Illuminator, Model: CM-IR110) and on a surface of granite. The dark room and granite ensured that the pairs did not detect each other visually or through substrate-borne vibrations, since granite is an ineffective transmitter of the vibrations that are a part of spider courtship signals (Elias et al. 2004; Hebets 2005). We coated the top inner circumference of the arena wall with petroleum jelly to prevent spiders from climbing out. We cleaned the arena and granite slab with deionized water before each mating trial and cleaned everything with 70% ethanol at the end of each day. We used each individual only once.

Table 1.—Description of scored behavior from the infrared videos using BORIS.

Terms	Defined by	Description
Latency to Mate	Measured in seconds	From the time males first start courting until they successfully mate or until the end of the video for unmated males.
Rate of Leg Waving Bouts	# of leg waving bouts/latency to mate (#/s)	Leg waving bouts are defined as sets of leg waves interrupted by inactivity, walking, grooming, or push-up displays.
Rate of Push-ups	# of push-ups/ latency to mate (#/s)	Push-ups are a behavioral display shown by the males as a part of their mating ritual often before, after or in-between leg waving bouts.

On the day of the experiment, we weighed each female and then placed them in the mating arena, in the dark on granite for 30 mins for acclimatization. Then, we weighed the paired male. After the 30 mins, we introduced the male into the arena but confined him within a small removable barrier (a plastic cylinder, open on the ends; diameter 3 cm). We recorded all trials for later analysis with an infrared camera (Brand: Sony, Model: FDR-AX53) placed in front of the experimental setup at an angle of 10 degrees downwards from the horizontal. We set the infrared camera to record, released the male and allowed the pair to interact for 30 minutes. Our recording file names had descriptive information, but ablated and unablated trials were scored in a haphazard order.

Schizocosa retrorsa pairs mate for approximately 150–160 minutes (Hebets et al. 1996), making it likely that we would observe any successful mating. Prior studies have shown pairs to commence mating anywhere from 5 minutes to 78 minutes after first encounter (Hebets et al. 1996). We ran the trials for 30 mins, which is an accepted period of time used for this species previously (Choi et al. 2019; Kundu et al. 2022). We documented whether the pairs were mating or not at the end of 30 minutes. Additionally, we used our infrared video recordings to quantify the trials in detail.

As the spiders were nearing the end of their natural life (life span: ~1 year), we euthanized all spiders after they were used in a trial by freezing and preserved them in 70% ethanol. The specimens are preserved in our collection (Hebets laboratory) at the University of Nebraska-Lincoln.

Behavioral scoring.—We used BORIS - 2021-09-20: v. 7.12.2 to quantify the mating trial videos. A single individual (KP) analyzed all the videos, which again had descriptive file names, but unablated/ablated trials were mixed randomly during analysis. Details of scored behavior are in Table 1.

Statistical analyses.—To ensure that there were no differences between our groups (unablated/ablated) with respect to female/male age or weight, we compared the ages and weights of females and males using independent two-sample Welch t-tests. We used this same approach to compare age and weight differences of the females and males within a pair.

For the foraging trials, we used a Chi-square test to compare how many unablated versus ablated females successfully captured prey. We used independent two-sample Welch t-tests to see if mass influenced female foraging success for both groups.

To test our first prediction of higher mating success in unablated vs. ablated trials, we compared the two groups using a Chi-square test. We also used Kaplan Meier Survival analysis to explore potential differences in the 'latency to mate' between the female treatment groups (unablated/ablated) as well as an independent two-sample Welch t-test to investigate any differences between the average 'latency to mate' in the two groups. To ensure that males were courting with equal intensity across our treatments, we compared the rate of leg waving bouts from males in the unablated versus ablated groups using an independent two-sample Welch t-test.

Next, we wanted to explore factors that might influence mating success. We used a binomial logistic regression model (R package: stats, function: glm (specified with 'family=binomial') with initial predictor variables of: female treatment (unablated/ablated), rate of leg waving bouts, interaction between the female treatment and rate of leg waving bouts, rate of push-ups, interaction between treatment and rate of push-ups, and female and male weights and ages. We used backward selection to reduce the model to the following: female treatment, rate of leg waving bouts and interaction between the female treatment and rate of leg waving bouts (R package: AICcmodavg, function: aictab) (see Supplemental Table S2, online at https://doi.org/10.1636/JoA-S-22-065.s1). We performed a Wald test to understand the model's best fit criteria and summarized it with Anova Type II. Finally, to test our second prediction of courtship rate predicting mating success in unablated but not ablated mating trials, we performed a Mann-Whitney-Wilcoxon signed rank test (R package: rstatix, function: wilcox_test) to compare the rate of leg waving bouts of the mated and unmated males within each experimental group (unablated/ablated). This last analysis also allowed us to explore the suggested interaction between female treatment and leg waving bouts we observed in our logistic regression.

The data were analyzed using the packages – tidyverse, ggplot2, survival (function Surv), car (function Anova), ggpubr (function t_test), rstatix(function wilcox_test), stats (function glm) and AICcmodavg (function aictab), in the R 4.1.3 binary (for macOS 10.13 (High Sierra) and higher) through Rstudio Desktop.

RESULTS

Foraging trials.—There was no significant difference between the foraging success of the females of the unablated group and that of the ablated group. In the unablated group, 9/15 (60%) females ate and in the ablated group 10/15 (66.67%) ate ($\chi^2 = 0.144$, df = 1, $P = 0.705$). Overall, mass influenced foraging, i.e., the females of lower body mass tended to forage more than females with higher body mass (see Supplemental Table S3, online at https://doi.org/10.1636/JoA-S-22-065.s1).

Mating trials.—The average age of females was 14.33 ± 0.48 days post maturation and that of males was 25.63 ± 0.85 days post maturation. On the day of the mating trials, the average weight of females was 56.43 ± 6.34 mg while that of the males was 42.03 ± 3.76 mg. There was no significant difference between the ages and weights of females and males of the experimental groups or in the age or weight difference of the female-male pairs between the groups (see Supplemental Table S4, online at online at https://doi.org/10.1636/JoA-S-22-065.s1).

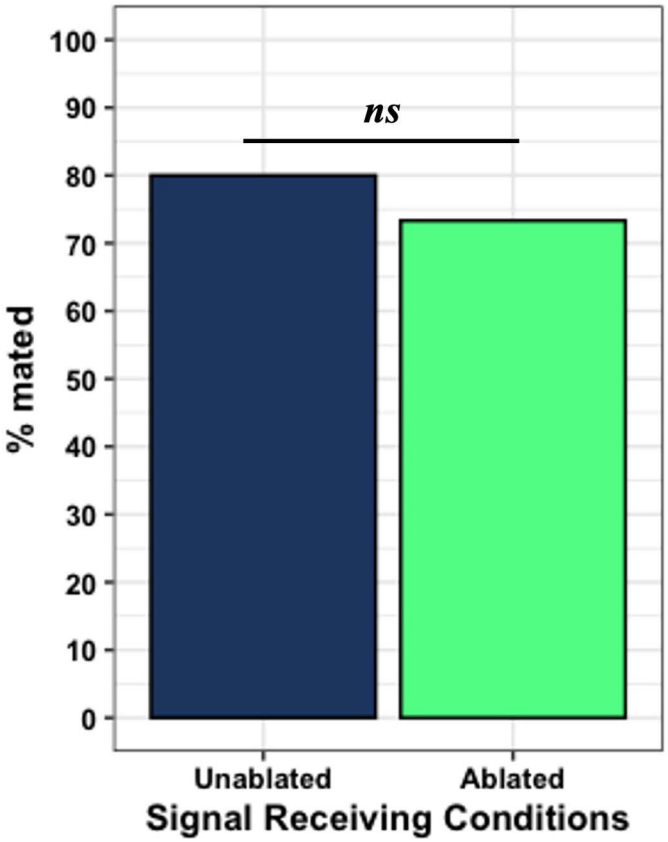

Figure 2.—No significant difference in the % of successful matings in the group where the females had unablated trichobothria compared to the group where the females had ablated trichobothria. 80% mated when the females were unablated while 73.333% mated when the females were ablated ($n = 15$, $P = 0.666$, ns: not significant).

In the unablated group, 80% ($n = 15$) mated successfully while in the ablated group 73.333% mated successfully (Fig. 2). In contrast to our first prediction, there was no significant difference in the successful mating of the 2 groups ($\chi^2 = 0.186$, df = 1, $P = 0.666$). The latency to mate for the unablated group was 996.846 ± 580.834 seconds while that for ablated group was 986.014 ± 606.372 seconds. The curves for the two groups are overlapping in the Kaplan Meier Survival analysis (Fig. 3). There was no significant difference in the latency to mate of the two groups (Test statistic (Welch t-test) = 0.133, df = 27.948, $P = 0.895$). Overall, there was no significant difference in the rate of leg waving bouts by males of the unablated and ablated groups (Test statistic (Welch t-test) = 0.061, df = 23.4, $P = 0.952$).

Our binomial logistic regression model predicting mating success using female treatment, rate of leg waving bouts and an interaction between female treatment and rate of leg waving bouts was not significant (Wald $\chi^2 = 5$, df = 3, $P = 0.17$). However, while the female treatment ($P = 0.779$) and the rate of leg waving ($P = 0.064$) were not significant, the model indicated a significant interaction between the two ($P = 0.022$*).

We found support for our second prediction that leg waving rate would predict mating success in the unblated but not ablated treatment group. Within the unablated group, males that mated had significantly higher rates of leg waving bouts than those that did not mate (Unablated: mated, 0.024 ± 0.008/s vs. unmated, 0.012 ± 0.004/s; Test-statistic (Wilcoxon test) = 34, $P = 0.018$*) (Fig. 4).

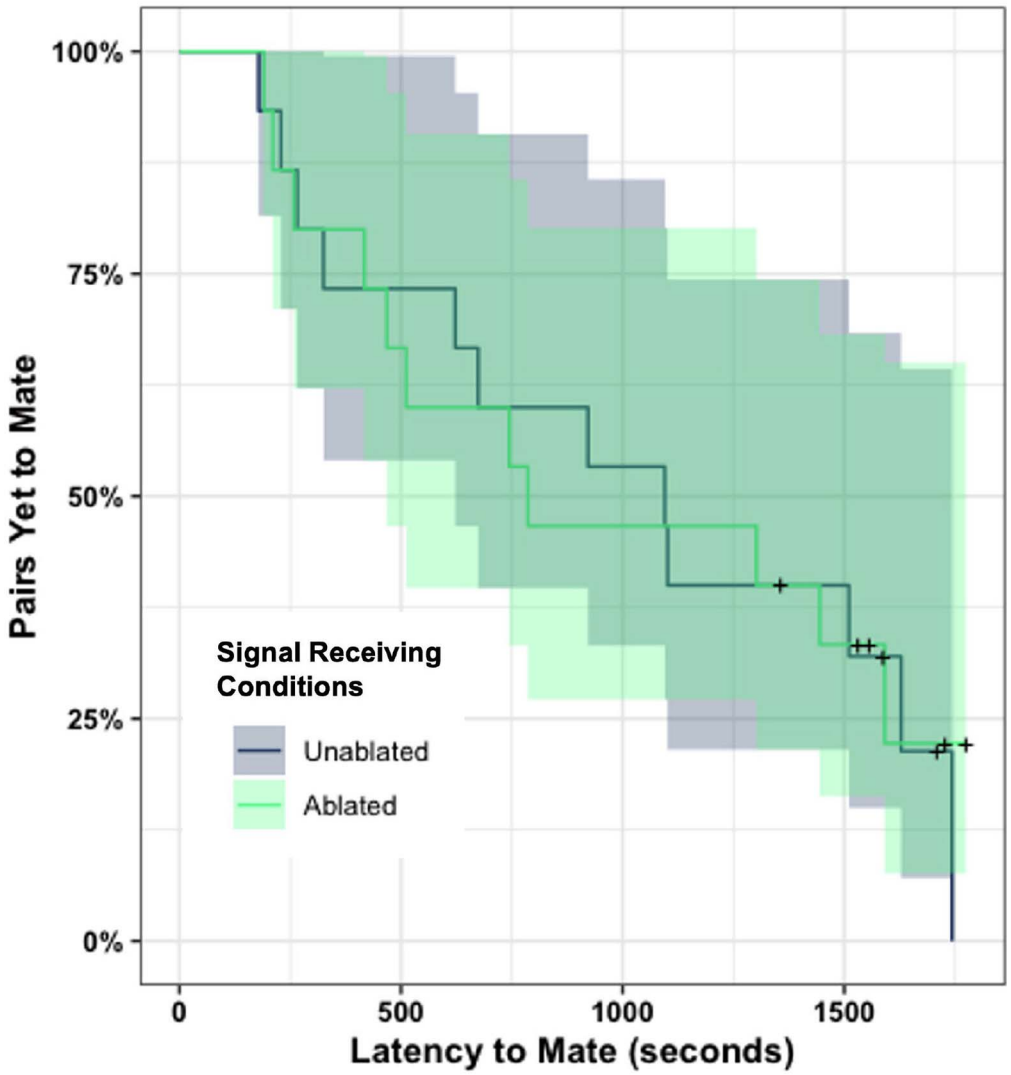

Figure 3.—No significant difference in the latency to mate across the two female treatment groups (unablated: 996.846 ± 580.834s, ablated: 986.014 ± 606.372s). The '+' signs on the Kaplan Meier survival analysis indicate pairs that did not mate (censored pairs, often superimposed) and the shaded regions are the 95% confidence interval at each time point.

However, there was no significant difference between the rate of the leg waving bouts of the males that mated versus not within the ablated group (Ablated: mated, 0.023 ± 0.015/s, unmated: 0.017 ± 0.014/s; Test-statistic (Wilcoxon test) = 27, $P = 0.571$) (Fig. 4).

DISCUSSION

Results of previous experiments that conducted mating trials in signaling environments that prevented or reduced the transmission of visual and vibratory courtship signaling suggested that a 'hidden' sensory modality must be important during male *S. retrorsa* courtship – i.e., air particle movement or near field sound (Rundus et al. 2010; Choi et al. 2019). Most recently, a study directly explored this modality by introducing artificial air particle movement (i.e., 'noise') and assessing its influence on mating success. The study found higher mating success in 'no noise' versus 'noise' environments, supporting the hypothesized role of air particle movement in *S. retrorsa* multimodal courtship displays (Kundu et al. 2022). However, this prior study also found differences in male courtship rate across treatments, indicating that the signaling environment also altered male investment in courtship (Kundu et al. 2022). Nonetheless, the rate of leg waving was still predictive of mating success across both noise/no noise treatments, suggesting that females were indeed assessing leg waving rate during mate choice decisions. Given the absence of visual and vibratory signal transmission in these prior experiments, results continue to support a role of air particle movement generated by leg waving during male *S. retrorsa* courtship.

Our study built upon these past results by using an experimental design that attempted to directly interfere with a female's ability to detect air particle movement. We attempted to remove the trichobothria on the first two pair of legs of females and then assess mating success. First, we importantly found that our experimental manipulations of females did not appear to influence overall behavior, which we assessed with a foraging trial. We found no difference in the number of females who successfully foraged between unablated and ablated females. This result provides us some reassurance that our manipulation did not dramatically alter female behavior in a way that would prevent interpretation of our mating results. Although

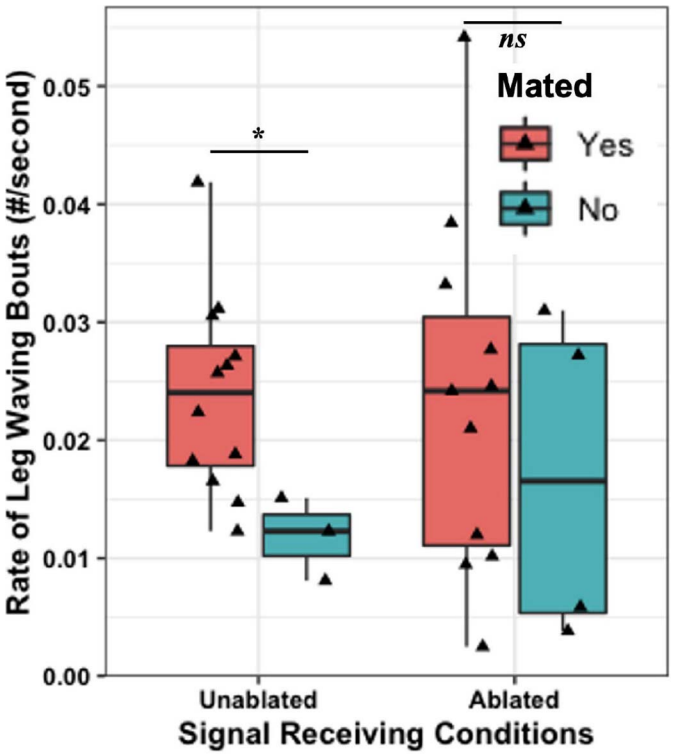

Figure 4.—In the treatment group with unablated females, the mating males had significantly higher rates of leg waving than the males that did not mate (Mated: 0.024 ± 0.008/s, Not mated: 0.012 ± 0.004/s, $*P < 0.05$). In the group with ablated females, there was no significant difference in the rates of leg waving between the males that mated compared to those that did not (Mated: 0.023 ± 0.015/s, Not mated: 0.017 ± 0.014/s)

spiders do use trichobothria to detect prey, they also use other mechanosensory and chemosensory information (Friedel & Barth 1997; Hoefler et al. 2002; Hostettler & Nentwig 2006). Additionally, in our trials, females still had the trichobothria of the two pairs of hind legs intact to function in prey detection.

Next, we tested our first prediction – that unablated females would show higher mating success than ablated females if foreleg trichobothria were used to assess male courtship. In contrast to our expectations, we found that overall mating rates did not differ between unablated and ablated female-male pairs. We also observed no difference in latency to mate between the groups. Importantly, the female treatment did not appear to influence male courtship behavior either, as we found no significant difference in the leg waving rates between males in the two groups.

When exploring factors that might have influenced mating success, we found a suggestion that there was an interaction between treatment (unablated/ablated) and male leg waving rate. Interestingly, this potential interaction lined up well with our second prediction – that in unablated pairings, males that court at a higher rate will acquire matings while the same pattern would not be present in ablated pairings. We tested this by comparing mated versus non-mated males in each group and indeed found support for this prediction. We found that in unablated treatments, pairs that mated had significantly higher male leg waving rates while this same pattern was not seen in ablated pairs.

If females are using trichobothria to detect air particle movement generated by leg waving displays in *S. retrorsa* male courtship, then we would have expected to see a difference in overall mating success between unablated versus ablated mating pairs. We found no such difference. In hindsight, however, this may not be surprising simply due to the large number of trichobothria found on all the walking legs of female *S. retrorsa* and the difficulty in removing/ablating all sensilla even on the first two pair of legs. We know, for example, that we were not successful in ablating all the trichobothria from the front two pairs of legs completely (Supplemental Table S1). There were still several trichobothria left that were either difficult to get to or were missed. Unfortunately, ablating such fine and small sensilla under a dissecting stereoscope came with the possibility of accidentally cutting a part of the leg or inducing the spider to autotomize its own leg. To avoid those possibilities, the ablation process was done carefully and conservatively, which meant trichobothria were sometimes missed. Additionally, the trichobothria on their third and fourth pair of legs were left intact and could have detected air particle movement from courting males, just as they likely detected crickets during the foraging trials. Thus, our 'ablation' protocol did not fully ablate a female's ability to use trichobothria to detect environmental stimuli and other sensory modalities remained intact. Nonetheless, trichobothria removal did likely reduce the efficacy of detecting air particle movement to some extent.

Other studies have used different methods of inactivating or ablating trichobothria and other sensilla. The trichobothria in fishing spiders were disabled with vacuum grease (Suter 2003) and the sensilla in caterpillars were thermocauterized (Tautz & Markl 1978) to understand predator avoidance. Fishing spiders are larger in size than the wolf spiders studied here and while the caterpillars have only eight sensilla, the wolf spiders here can have up to 40 trichobothria on a single slender limb. Thus, our approach of cutting trichobothria, at the time, seemed the most time efficient. In revisiting this study, we would spend longer with each ablated female spider to ablate as many more trichobothria as possible from all four pairs of legs. Using small amounts of vacuum grease is also a viable option that could be explored. Alternatively, a combination of both methods (cutting and vacuum grease) might also prove to be effective. If alternate ablation techniques were to be used, however, it might be important to increase the latency between inactivation/ablation of trichobothria and the mating trials to let the spiders recover better and get rid of trace chemical signals.

Given that prior studies have consistently demonstrated an influence of male leg waving rate on mating success in *S. retrorsa*, we predicted that if this leg waving was detected by female trichobothria, then we would only see an influence of leg waving rate in unablated mating pairs. We see a suggestion of this significant interaction in our overall model and thus explore the pattern of leg waving and mating success separately across unablated and ablated treatment groups. This analysis again provides support for our second prediction. For the unablated group, the males that mated had higher rates of leg waving compared to the ones that did not, i.e., successful mating depended on the rate of leg waving. However, the rate of leg waving did not influence mating success in the ablated group. These results suggest that the females' ability to detect air particle movement signals generated by the males' leg waving was compromised by the ablation of trichobothria. Unlike in previous experiments (Kundu et al. 2022), we did not observe plasticity in male courtship behavior across treatments and thus, our results suggest that the differences in mating success were driven by female mate choice as opposed to male behavior. A larger sample size would help bolster this finding.

This study adds to our growing knowledge of the role of air particle movement in intraspecific communication. We know that African cave crickets (*Phaeophilacris spectrum*) who lack stridulatory organs use filiform sensilla for intraspecific communication. Males conduct a series of wing flicks at 8–12 Hz (low frequency) towards the females, who respond by calming down and being receptive to copulation (Heidelbach et al. 1991; Heidelbach & Dambach 1997). Amblypygids, during agonistic contests, move their modified antenniform legs in quick succession close to their opponents' trichobothria at a frequency of 29 Hz (Santer & Hebets 2008). The leg movements are predictive of contest winners (Fowler–Finn & Hebets 2006) and their trichobothria are responsive to those frequencies (Santer & Hebets 2008). Although our study does not provide the definitive support for female trichobothria being used to detect air particle movement generated by male *S. retrorsa* leg waving displays, our results are consistent with this hypothesis. A larger sample size and a more effective way of ablating trichobothria would provide us with more clarity and understanding of the importance of air particle movement signals in mating.

Though our study focused on the detection of air particle movement, there are other sensory modalities that may be important in *S. retrorsa* reproductive interactions. We did not, for example, assess the potential for chemical communication to play a role in mating success. Indeed, our observation of similar overall mating rates between unablated and ablated females may reflect a reliance on chemical communication as well. Future studies should explore this possibility, especially given that visual and vibratory signaling do not appear necessary for successful mating in this species. It also remains possible that movement patterns and opportunities for physical contact also influence mating success in *S. retrorsa*. To explore this, we quantified the number of times females and males made contact during mating trials. We found no differences between the rates of contact in unablated and ablated groups (data not shown) and number of contacts did not predict mating success in prior studies with *S. retrorsa* (Kundu et al. 2022). While chemical communication remains a possibility, our data nonetheless support the role of air particle movement and add to the growing evidence suggesting this modality is important in the multimodal courtship display of *S. retrorsa*.

Air particle movement generated from male *S. retrorsa* leg waving is predicted to be detectable by females from as far away as 65 mm (Rundus et al. 2010). As in other *Schizocosa* species, males initiate courtship upon encountering silk from mature females (Foelix 1996; Roberts & Uetz 2005), and thus may begin courting even without visual contact with a female. Though sexual aggression is common in some spider species (e.g., *Dolomedes* Latreille, 1804: Johnson 2001; Johnson & Sih 2005), pre-copulatory cannibalism is uncommon in *S. retrorsa* (Hebets et al. 2013). Male *Schizocosa* are typically able to acquire a copulation when females engage in a "settle" behavior, indicating receptivity and allowing males to mount (Uetz & Denterlein 1979; Stratton & Uetz 1983; Uetz & Roberts 2002). Given that *S. retrorsa* tends to be found in microhabitats with less three-dimensional physical heterogeneity (i.e., open sand or pine litter versus dense, deep leaf litter, (Hebets et al. 1996)), air particle movement may be a more effective signaling modality than substrate-borne vibrations, which are dominant courtship signals in many other *Schizocosa* species (Hebets et al. 2013).

Ultimately, our results are consistent with a role of female trichobothria in detecting air movement generated by male *S. retrorsa* multimodal courtship displays. Results provide additional evidence that air particle movement is an important sensory modality in *S. retrorsa* courtship, as the rate of leg waving (which generates air particle movement) was once again a good predictor of male mating success. Studies such as this are important in helping to characterize the complete architecture of a communication display, from both the signaler and the receiver's perspective. Only when we know what all the elements are of a communication system can we use a more integrated framework for understanding their evolution and function (Hebets et al. 2016).

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SUPPLEMENTAL MATERIALS

Supplemental Tables S1-S4, available online at https://doi.org/10.1636/JoA-S-22-065.s1

Table S1.—Count of trichobothria on the front two pairs of legs of five unablated females and five ablated females.

Table S2.—Model selection.

Table S3.—Average mass of females that ate versus females that did not eat during the foraging trial.

Table S4.—Comparison of the age and weight of spiders at the time of mating trials, by sex (females vs. males) and signal-receiving condition (ablated vs. unablated females).

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