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The effects of conspecific male density on the reproductive behavior of male *Schizocosa retrorsa* (Banks, 1911) wolf spiders (Araneae: Lycosidae)

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Abstract. In the context of competitive mate searching, males may use cues from conspecifics, such as movement cues and/or courtship signals, to locate mates. For ground-dwelling wolf spiders, substrate-borne vibratory cues may be particularly important sources of information, given the potential presence of many visual obstacles. This study explores the possible use of conspecific male cues in wolf spiders by asking: (i) Do male *Schizocosa retrorsa* (Banks, 1911) wolf spiders use vibratory cues from conspecific males to alter their searching or signaling behavior? (ii) Can males assess the density of conspecific males using conspecific male cues? (iii) Does the variation in conspecific male density affect the behavioral response of focal males to the conspecific cues? To answer these questions, we tested the effects of (i) the number of conspecific males and (ii) the activity of conspecific males (e.g., courtship yes/no) on a focal male's behavior. We recorded the following focal male behavior: (a) the presence/absence of courtship behavior, (b) temporal/structural signal characteristics of the multimodal courtship signaling, and (c) locomotory patterns. Our results suggest that, (i) *S. retrorsa* males assess their competitive environment through substrate-borne vibratory cues generated by courting or non-courting behavior of conspecific neighbors, (ii) *S. retrorsa* males may alter their reproductive behavior between mate searching and courtship signaling by the assessment of cues associated with conspecific male density, and (iii) the assessment and perception of density-dependent conspecific courtship signaling can be used as social information to adjust the reproductive behavior.

Keywords: Courtship plasticity, reproductive behavior, male-male competition, eavesdropping, substrate-borne vibration.

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In many animal species, males actively use direct or indirect cues produced by receptive females to aid in mate localization (Rypstra et al. 2009; Guevara-fiore et al. 2010). Using such cues, males can decide to change their direction of movement and/or their courtship activity to increase their chances of acquiring a mate. However, if males are searching for temporally or spatially scattered females, cues or signals produced by conspecific females may not always be sufficient for effective mate location. In such instances, eavesdropping males might locate receptive females by approaching conspecific males showing reproductive behavior (e.g., treefrogs - Bee 2007; katydids - Guerra & Mason 2005; newts - Park & Propper 2001). In particular, when mate searching is critical for male reproductive success (i.e., scramble competition; Nahrung & Allen 2004), eavesdropping behavior may promote conspecific male aggregation through a positive feedback loop of increasing numbers of conspecific males near receptive females and thus increased cue/signal production that could be used for eavesdropping (i.e., Beacon effect; Rohr et al. 2002; Guerra & Mason 2005; Bee 2007; Buxton et al. 2015).

Eavesdropping on conspecific male cues as a way to help locate potential mates may reduce male search costs, but the resulting spatial proximity among conspecific rivals may simultaneously increase the intensity of male-male competition through direct (i.e., male-male aggression; Jirotkul 1999) or indirect male-male competition (i.e., signal competition; Schwartz et al. 2002). Additionally, competitive interactions might be density-dependent. For example, theoretical studies on the effects of conspecific male density on male reproductive strategies suggest that males are more likely to get involved in competitive interactions with other males at intermediate male densities. At low or high male densities, males are predicted to switch their reproductive strategies from direct competition to searching for alternative mating opportunities (Knell 2009; Bretman et al. 2011). Thus, eavesdropping males may alter their response to conspecific male cues/signals depending on the perceived conspecific male density. Such plastic responses to male density have been observed in male eastern red-spotted newts, *Notophthalmus viridescens*, in which males are attracted to chemical cues from large groups of conspecific males (Rohr et al. 2002, 2005), but generally seek less competitive mating opportunities when the male density is too high (Rohr et al. 2005).

Eavesdropping is possible because males that have already located a receptive female often engage in some form of signaling (i.e., courtship) to entice the female to mate. Previous studies suggest that other searching males actively perceive cues from these conspecific males, i.e., they eavesdrop (Milner et al. 2010; Clark et al. 2012). But only a few studies have investigated whether the response to the eavesdropped cues/signals varies across conspecific male density. Guerra & Mason (2005), for example, showed that male katydids can locate a breeding aggregation and choose the intermediate male density by attending to the amplitude of a conspecific male chorus, without direct observation of the presence/number of conspecific males or females. Also, treefrogs locate the spatially scattered breeding site by following the conspecific chorus (Bee 2007), but reduce courtship effort and change the strategy to satellite males when the conspecific male density is too high (Perrill et al. 1982). The extent to which this density-dependent effect of eavesdropping is widespread, however, remains unknown.

To test the density-dependent effects of eavesdropping, we first need to verify that males can assess the conspecific male density through indirect cues from conspecific males such as courtship signals and/or locomotory cues. Then, we can determine if males alter their behavior across a range of perceived conspecific male density. Furthermore, if conspecific males produce multiple cues/signals, e.g., visual or vibratory cues from general locomotion and courtship, the behavioral response to eavesdropping of focal males may vary due to perceived information. We aimed to test the density-dependent effects of eavesdropping in the wolf spider *Schizocosa retrorsa* (Banks, 1911).

Male wolf spiders in the genus *Schizocosa* Chamberlin, 1904 (Lycosidae) competitively search for spatially scattered receptive females (Roberts et al. 2006), but the interactions among males rarely become aggressive (*Schizocosa crassipes* (Walckenaer, 1837); Aspey 1976, 1977). Previous studies showed that male wolf spiders exploit diverse cues to locate receptive females, including chemical cues from female silk (Bell & Roberts 2017) and vibratory cues from the locomotion of conspecifics (Rovner 1996). Considering the high encounter rate with conspecific males in their natural habitat (*Schizocosa ocreata* (Hentz, 1844); Cady 1984), males may easily detect the substrate-borne vibrations from the locomotion or courtship display of conspecific males. However, evidence that these male wolf spiders can assess male density through eavesdropping and alter their searching/courtship behavior accordingly is equivocal. For instance, Roberts et al. (2006) found no evidence that *Schizocosa ocreata* males alter their reproductive behavior according to visual or vibratory cues from conspecific males. However, other studies suggested that *S. ocreata* males adjust their courtship signaling based on the signaling rate of visual playbacks of a courting conspecific male (Clark et al. 2012) and showed that males increased the duration of courtship signaling with the number of conspecific males (1 vs. 2–3 conspecific males; Clark et al. 2015).

The present study builds upon this prior work by using the wolf spider *S. retrorsa* to ask three questions about the use of eavesdropping as a reproductive strategy. (i) Do *S. retrorsa* males use cues from conspecific rivals to alter their searching or signaling behavior? (ii) Can *S. retrorsa* males assess the density of conspecific rivals using conspecific male cues? (iii) How does the variation in conspecific male density influence a focal male's reproductive behavior (e.g., courting versus searching)? Unlike prior research with multimodal (i.e., visual + vibratory) playback stimuli (Roberts et al. 2006; Clark et al. 2012, 2015; De Young & Wilgers 2016), we focus explicitly on the effects of substrate-borne vibratory cues/signals to verify if males can alter their reproductive behavior without direct visual evidence of the presence/number of rivals. Given that the detection of visual signals may be often disturbed by visual obstacles in the natural habitats of ground-dwelling wolf spiders, substrate-borne vibratory signals may provide a more reliable source for eavesdropping (Rovner 1996; Lohrey et al. 2009; Uetz et al. 2013). Thus, we assume that by using substrate-borne vibratory cues/signals alone, we are employing a more natural scenario that reflects potential eavesdropping opportunities in the field. Additionally, vibratory signaling is assumed to be the ancestral signaling modality in *Schizocosa* courtship (Stratton 2005) and has been shown to be the most important signaling modality for many *Schizocosa* species (Hebets et al. 2013).

METHODS

Study animals.—We collected penultimate stage (one molt prior to the final maturation molt) *Schizocosa retrorsa* males and females from Marshall, Co., MS, USA (34°40′ N 89°28′ W) on April 28, 2017. After transportation to a laboratory space with a controlled light cycle (12L:12D cycle) and temperature (25 °C), we placed animals in individual plastic cages (60 × 60 × 80 mm) wrapped with masking tape to prevent visual interaction among individual spiders during maintenance. Although there is a possibility that our study animals in the same rearing room can perceive the presence of other individuals by volatile chemical cues like other wolf spiders (*Allocosa brasiliensis* (Petrunkevitch, 1910) and *Allocosa alticeps* (Mello-Leitão, 1944); Aisenberg et al. 2010), the effects of pre-experienced conspecific volatile chemical cues on our study may be minimal due to (i) the insignificant effects of volatile chemical cues on mate finding or courtship behavior of *Schizocosa* wolf spiders (*S. ocreata*; Bell & Roberts 2017) and (ii) the frequent encounter rate with conspecific males in their natural habitat (personal observation).

We provided water using a cotton wick partially resting into a reservoir below the cage, as well as within the cage. We fed spiders three small crickets (*Gryllodes sigillatus*), approximately half the body length of the spider, twice a week. We only used spiders after they reached 14 days post-maturation molt. We used this cut-off because of the known variation in the sexual receptivity of female *Schizocosa* wolf spiders throughout adulthood (Roberts & Uetz 2005). During the experimental period, we checked the body mass of individual spiders before each trial to ensure that the repeated trial did not affect the male body condition. Following experiments, we euthanized males by freezing before preserving them in 70% ethanol. We retained individuals as voucher specimens in our collection at the University of Nebraska-Lincoln, USA.

Experimental design.—In an acrylic experimental arena (Samsung construct, Seoul, South Korea; Fig. 1), we deployed one 'focal' male in a central hoop (diameter: 180 mm) with different numbers of 'neighbor' males (0, 1, 4, and 8). We deployed neighbor males individually in one of eight peripheral hoops (diameter: 90 mm; Fig. 1). We visually isolated all spiders from each other using opaque acetate film, but locomotion-induced vibrations and vibratory courtship signals transmitted freely through the filter paper floor that connected focal and neighbor males. To minimize the influence of ambient vibrations, we placed the experimental arena with 11 acrylic legs (height: 20 mm) on a granite slab.

To elicit courtship behavior of focal and neighbor males, a mature virgin female was allowed to deposit pheromone-laden silk (Tietjen 1979) on filter paper for one hour. Males were then exposed to the pheromone-laden filter papers during trials. The persistence of female pheromone on silk varies due to environmental condition, but the degree of male response to female silk remains stable for at least a day (*Schizocosa malitiosa* (Tullgren, 1905); Baruffaldi et al. 2010). To start a trial, we put neighbor males in arenas first, before the focal male, so that neighbor males could start courtship signaling before a focal male was placed in an arena. During a 15-min

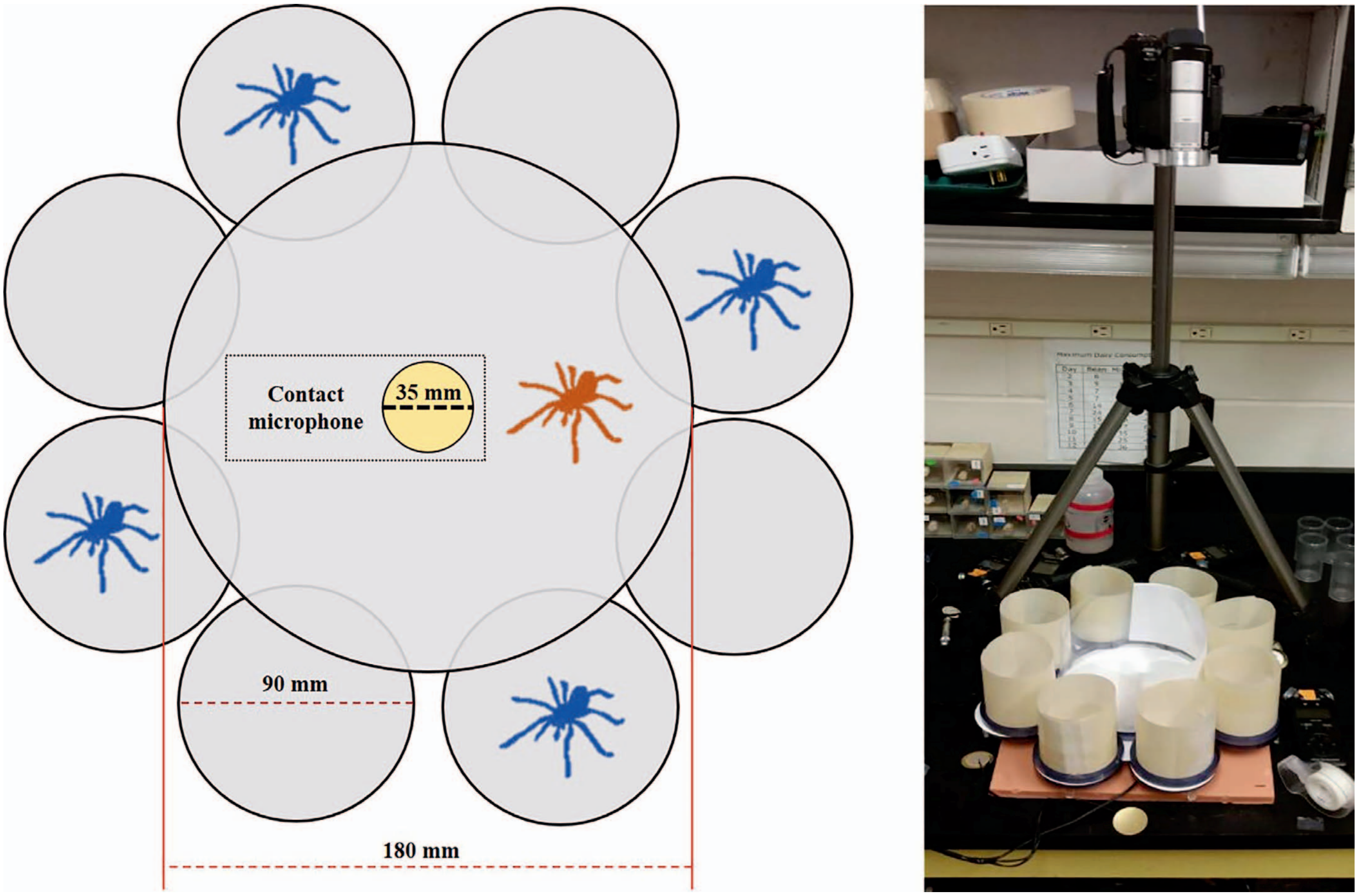

Figure 1.—The experimental setup. Bird's-eye view of a 4-neighbor trial (Left); side view of the experimental setup with video camera (Right)

trial, we recorded vibrations within an experimental arena using a contact microphone (35 mm diameter, Resonant impedance 300Ω max, Resonant frequency 2.9 ± 0.5 KHz, Goedrum Co., Chanhua, Taiwan) and TASCAM DR-05 audio recorder (44.1 kHz sampling rate, TEAC, Wiesbaden, Germany) attached to the underside of the central hoop. In 4-neighbor trials, we also recorded vibrations at peripheral hoops located in each cardinal direction. However, due to a limited number of contact microphones, we did not have contact microphones recording neighbor males in any other density treatments (1- and 8-neighbor trials). Using a Sony HDR-SR10 video camera (Sony Electronics Inc., Minato, Tokyo, Japan), we recorded the locomotion of focal and neighbor males during each trial (Fig. 1).

We used a repeated measures design in which focal males were first run in the absence of any neighboring males and then each male was run through three trials with different neighboring male densities (1, 4, and 8-neighbor males) in random order. A focal male's first trial was always in the absence other conspecific males, to control for the possibility that exposure to neighboring males' courtship elicits long-term changes in male signaling behavior. During four consecutive days, we conducted one trial per day and used most neighbor males only once, except for three males that we used twice. Also, we did not use focal males as neighbor males. We conducted experiments in a temperature-controlled room (25 °C) with light provided by a full-spectrum compact fluorescent light bulb (NaturesSunlite 30W, Naturallighting.com, Dickinson, TX, U.S.A.) from 1100 to 2200. The time of day did not affect the occurrence of courtship for either focal ($\chi^2_1 = 0.662$, $P = 0.416$) or neighbor males ($\chi^2_1 = 1.526$, $P = 0.217$).

Data analysis.—To investigate any variation in focal male reproductive behavior in relation to conspecific male density, we quantified the (ia) presence/absence and (ib) total duration of courtship signaling during each trial for each focal male. When courtship signaling was present, we also quantified (ii) temporal/structural characteristics of the courtship signal (e.g., latency to court; duration of courtship, etc.). Finally, we explored (iii) whether focal males moved towards the courting conspecific males in different male densities.

Acoustic analysis: A *bout* of the vibratory display of *Schizocosa retrorsa* consists of two *components* – pedipalpal drumming and foreleg taps (Hebets et al. 1996). Each component generates acoustically distinct substrate-borne vibrations in both the acoustic structure and pulse rates (Fig. 2). Pedipalpal drumming is characterized by a loud click sound associated with the "push-up" display. Pedipalpal drumming is often, but not always, followed by an extended foreleg tap, which produces a series of uniform pulses at a faster pulse rate than that of pedipalpal drumming (Hebets et al. 1996). There is no previous research on the acoustic characters of *S. retrorsa*, but we found significant differences

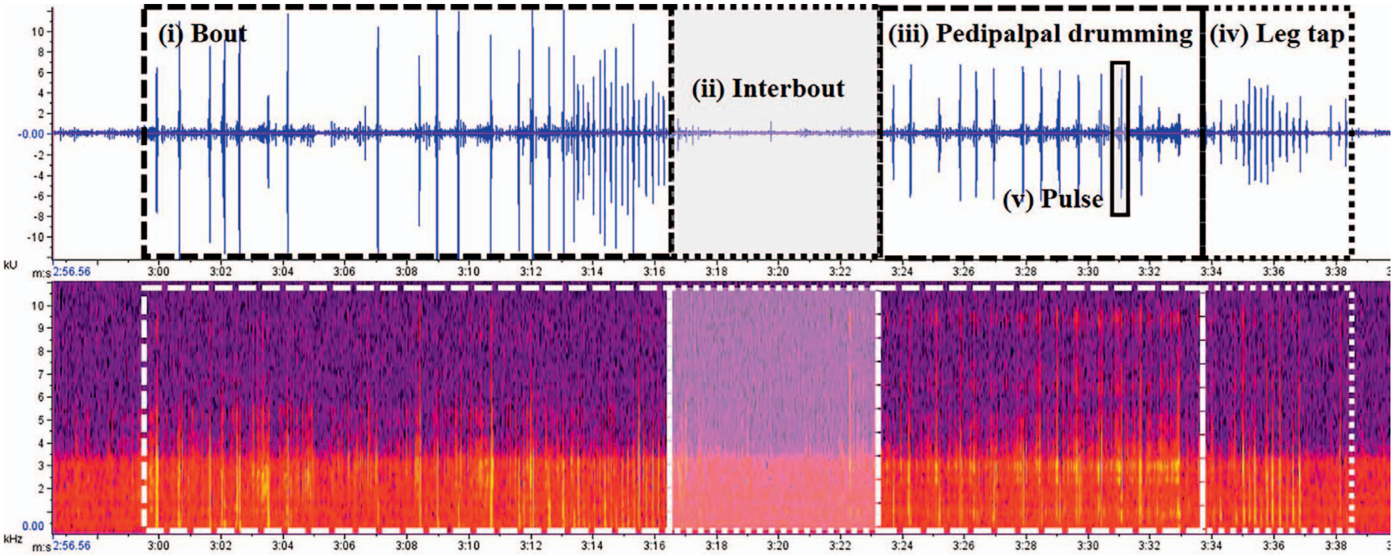

Figure 2.—Waveform and spectrogram of *S. retrorsa* courtship signal. A bout (i) consists of pedipalpal drumming (iii) and leg tapping (iv) which are a series of pulses (v).

in pulse rates between pedipalpal drumming (1.61 pulse/s) and leg tapping (3.74 pulse/s) in our study (Paired samples *t*-test; $t_{146} = -29.03$, $P < 0.001$), indicating that these vibratory display components are distinct and individually identifiable (Hebets et al. 1996).

Because the focal male was connected through a shared filter paper floor with each neighbor male, the acoustic recordings at the central hoop recorded courtship signals of all males – i.e., focal males as well as neighbor males. We could distinguish the courtship signals of focal males from those of neighbor males based on the spectrograms (Fast Fourier Transformation, 1024 Hz, Hanning window) (Fig. S1, online at https://doi.org/10.1636/JoA-S-20-079-.s1). For the focal male's recorded courtship display, we quantified the following: (a) latency to the first courtship bout from the time that focal male entered an arena (hereafter, the *latency to courtship*), (b) sum of durations of all courtship bouts (hereafter, the *total duration of courtship*), (c) *total number of courtship bouts*, (d) average duration of bouts from the beginning of pedipalpal drumming to the last pulse of leg tapping (hereafter, *bout duration*), (e) average interval between bouts (hereafter, *interbout interval*), (f) average of the pulse rate of pedipalpal drumming of each bout (number of pulses/pedipalpal drumming duration; hereafter, *pedipalpal drumming rate*), and (g) average of the proportion of pedipalpal drumming in a bout (pedipalpal drumming duration/bout duration; hereafter, *pedipalpal drumming proportion*) (Fig. 2). We filtered all recordings using the noise reduction function in version 2.2.2 of Audacity® using the noise profile of the ambient noise in each file. We used Raven Pro 1.5 for Windows (Cornell Lab of Ornithology, Ithaca, New York, USA) for sound analysis.

Video analysis: We tested whether focal males use the social information provided by vibratory signaling of conspecific males to locate potential mates by observing the locomotion of focal males during each trial using ezTrack software (Pennington et al. 2019) and pathfinder software (Cooke et al. 2019). We analyzed each focal male's spending time on each pixel of video recording and calculated the distance of movement during a trial in mm. To investigate the effects of the number of neighbors and their courtship activity on the spatial location of focal males, we constructed heatmaps based on the length of spending time on each of 70 grids consisting of a visual image of the central circle of the experimental arena. The size of the grid is automatically determined by the number of pixels consisting of a spider in video files (Cooke et al. 2019). After constructing heatmaps of all trials, we visually inspected the heatmaps and compared (a) the absence versus presence of neighbor male's courtship and (b) the density of neighbor males. In the 4-neighbor trials only (because this is the only treatment for which we had accurate neighbor male courtship behavior), we examined whether focal males moved towards neighbor males after their courtship signals. Due to the difference in the angle of camera, we did not analyse 0-neighbor trials.

Statistical analysis: We performed generalized mixed-effects models with individual as a random effect to investigate the effects of the number of neighbor males and the duration of neighbor male's courtship on various aspects of focal male's courtship behavior, including (a) the presence of focal male courtship signaling, (b) the latency to courtship, (c) the total duration of courtship, (d) the number of bouts, (e) the bout duration, (f) the interbout interval, (g) the pedipalpal drumming rate, (h) the pedipalpal drumming proportion, and (i) the moving distance of focal males during a trial. Among the predictor variables, we log-transformed the duration of neighbor male's courtship via a $\log(1+x)$ transformation to include data from trials in which neighbors did not court. Except for the logistic regression model for (a) the presence of focal male courtship signaling and (i) the moving distance of focal males, we did not include the interaction term between the fixed variables due to the limited sample size after excluding non-courting focal individuals, to construct regression models of other characters from (b) to (h). For the mixed effect model for (h) the pedipalpal drumming proportion, we used beta logistic regression

Table 1.—Descriptive statistics of acoustic features (mean ± s.d). (NON = number of neighbors, NCF = number of courting focal males, TNC = trials that neighbor males courted, NTD = neigbor males' total courtship duration, TD = total courtship duration, NB = number of bouts, BD = bout duration, IBI = interbout interval, PDR = pedipalpal drumming rate, PDP = proportion of pedipalpal drumming, MD = moving distance during a trial)

NON	NCF (n/total)	TNC (n/total)	NTD (s)	Latency (s)	TD (s)	NB	BD (s)	IBI (s)	PDR (n/s)	PDP	MD (cm)
0	6/24	-	-	179.83 ± 138.55	156.92 ± 76.80	23 ± 12.68	7.07 ± 1.49	27.53 ± 9.57	1.67 ± 0.19	0.18 ± 0.13	-
1	7/24	5/24	10.14 ± 21.91	247.10 ± 254.30	49.86 ± 38.36	8 ± 5.60	6.15 ± 0.92	90.68 ± 55.35	1.51 ± 0.14	0.14 ± 0.28	64.38 ± 32.51
4	7/24	14/24	11.50 ± 19.63	96.56 ± 153.32	123.35 ± 88.18	16.86 ± 10.33	6.89 ± 1.67	39.39 ± 22.45	1.62 ± 0.09	0.23 ± 0.20	52.49 ± 97.08
8	8/24	16/24	67.07 ± 89.65	199.03 ± 231.52	42.79 ± 54.71	5.63 ± 6.28	6.92 ± 1.50	69.72 ± 55.11	1.48 ± 0.26	0.20 ± 0.22	66.33 ± 28.36

analysis. *P*-values were obtained by the type III Wald chi-square test of the full model of each dependent variable using Anova() function from R package {car} (Fox & Weisberg 2018).

We conducted generalized mixed-effect regression analyses using glmer() functions from R package {lme4} (Bates et al. 2014). We used glmmTMB() functions from R package {glmmTMB} (Magnusson et al. 2019) to construct the mixed-effect beta logistic regression model for (h) the pedipalpal drumming proportion. When we found a significant fixed effect, subsequent post-hoc analyses were conducted using pairwise comparisons with Bonferroni correction of *P*-values using emmeans() functions from R package {emmeans} (Russell et al. 2019). Additionally, when we found a significant effect of the number of neighbor males in a model, we included values from 0-neighbor trials in the post-hoc analysis. We performed all the statistical analyses using R version 3.6.1 for Windows (R core team 2019).

RESULTS

Out of 25 focal male *Schizocosa retrorsa* used across our four repeated-measures design treatments (0, 1, 4, and 8 neighbors), most of the focal males produced courtship signals in one ($n = 5$) or two trials ($n = 6$), but some males were recorded in three ($n = 1$) or four trials ($n = 2$) across four repeated trials (0, 1, 4, and 8-neighbor males). There was no significant change in mass of focal males during the experimental period (Friedman's test; $\chi^2_3 = 4.729$, $P = 0.193$), and the average difference in mass between focal male and neighboring males was not significantly different among trials (one-way Analysis of Variance (one-way ANOVA); $F(1, 69) = 0.086$, $P = 0.77$) (Table 1).

Focal *Schizocosa retrorsa* males produced courtship signals independent of the number of neighbors ($\chi^2_2 = 1.142$, $P = 0.565$), the total duration of the neighbor male's courtship ($\chi^2_1 = 0.177$, $P = 0.674$), and the interaction term ($\chi^2_2 = 1.898$, $P = 0.387$). The latency to the courtship signals was, however, influenced by the number of neighbors ($\chi^2_2 = 7.383$, $P = 0.025$), but there was no significant difference in post-hoc pairwise comparisons (0-neighbor vs. 1-neighbor; $P = 1.000$, 0-neighbor vs. 4-neighbor; $P = 1.000$, 0-neighbor vs. 8-neighbor; $P = 1.000$, 1-neighbor vs. 4-neighbor; $P = 0.780$, 1-neighbor vs. 8-neighbor; $P = 1.000$, 4-neighbor vs. 8-neighbor; $P = 1.000$).

The total courtship duration of focal male courtship was influenced by the number of neighbors ($\chi^2_2 = 10.872$, $P = 0.004$), but not by the total duration of the neighbor male's courtship ($\chi^2_1 = 1.363$, $P = 0.243$) or the interaction term ($\chi^2_2 = 3.607$, $P = 0.165$) (Table 1). In the post-hoc analysis, focal males significantly decreased the total courtship duration when they had eight neighbors as compared to zero- and 4-neighbor males (vs. 0-neighbor; $P = 0.007$, vs. 4-neighbor; $P = 0.029$) while there was no significant difference in other pairwise comparisons (0-neighbor vs. 1-neighbor; $P = 0.128$, 0-neighbor vs. 4-neighbor; $P = 1.000$, 1-neighbor vs. 4-neighbor; $P = 0.324$, 1-neighbor vs. 8-neighbor; $P = 1.000$) (Fig. 3).

The number of courtship bouts was influenced by the number of neighbors ($\chi^2_2 = 10.735$, $P = 0.005$), but not by the

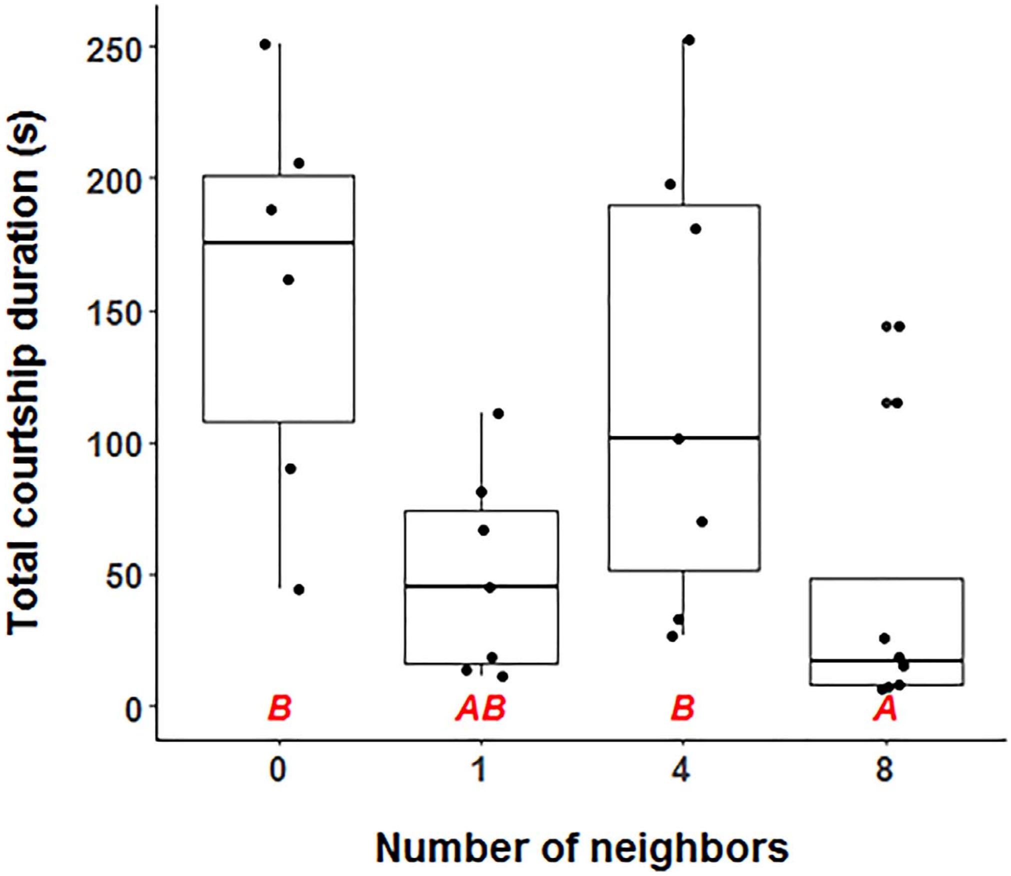

Figure 3.—Total courtship duration across the different number of neighbors. The top and bottom of the box represent the 25th and 75th percentile, and the line in the middle represents the 50th percentile. The whiskers represent 1.5 times the 25th and 75th percentiles. The dots represent data points from each individual. The dots are scattered centering around the corresponding number of neighbors to avoid overlap. The results of the post-hoc analysis are shown by letters under the box plots; plots sharing the same letter are not significantly different.

total duration of the neighbor male's courtship ($\chi^2_1 = 0.470$, $P = 0.493$) or the interaction term ($\chi^2_2 = 0.042$, $P = 0.979$) (Table 1). *Schizocosa retrorsa* males produced a greater number of courtship bouts in 0-neighbor trials than that in 1-neighbor ($P = 0.051$) and 8-neighbor trials ($P = 0.012$), but there was no significant difference between 0-neighbor and 4-neighbor trials ($P = 1.000$). There was no significant difference among trials in the presence of neighbors (1-neighbor vs. 4-neighbor; $P = 0.481$, 1-neighbor vs. 8-neighbor; $P = 1.000$, 4-neighbor vs. 8-neighbor; $P = 0.159$) (Fig. 4).

The duration of courtship bouts was not influenced by the number of neighbors ($\chi^2_2 = 5.182$, $P = 0.075$) or the total duration of the neighbor male's courtship ($\chi^2_1 = 0.451$, $P = 0.502$), but the interaction term was significant ($\chi^2_2 = 6.893$, $P = 0.031$). In 4-neighbor male trials, focal males reduced their bout duration when their neighbors signal longer as compared to the constant bout duration regardless of neighbor male's courtship in 1- and 8-neighbor male trials (Fig. 5).

The interbout interval of courtship signals was also influenced by the number of neighbors ($\chi^2_2 = 6.257$, $P = 0.044$), but not by the total duration of the neighbor male's courtship ($\chi^2_1 = 0.049$, $P = 0.824$) or the interaction term ($\chi^2_2 = 2.374$, $P = 0.305$) (Table 1). However, none of pairwise comparisons were significant (0-neighbor vs. 1-neighbor; $P = 0.252$, 0-neighbor vs. 4-neighbor; $P = 1.000$, 0-neighbor vs. 8-neighbor; $P = 0.928$, 1-neighbor vs. 4-neighbor; $P = 0.509$, 1-neighbor vs. 8-neighbor; $P = 1.000$, 4-neighbor vs. 8-neighbor; $P = 1.000$).

Schizocosa retrorsa males did not change their pedipalpal drumming rate based on the number of neighbors ($\chi^2_2 = 0.692$, $P = 0.708$), the total duration of the neighbor male's courtship ($\chi^2_1 = 0.348$, $P = 0.555$), or the interaction term ($\chi^2_2 = 1.074$, $P = 0.584$). However, focal males decreased the proportion of pedipalpal drumming in a bout when their neighbors courted longer ($\chi^2_1 = 4.127$, $P = 0.042$). The number of neighbors ($\chi^2_2 = 0.980$, $P = 0.613$) or the interaction term ($\chi^2_2 = 3.110$, $P = 0.211$) did not influence the proportion of pedipalpal drumming of focal males.

The locomotion of focal males during a trial was influenced by the total duration of the neighbor male's courtship ($\chi^2_1 = 5.868$, $P = 0.015$) and the interaction term ($\chi^2_2 = 6.809$, $P = 0.033$), but not by the number of neighbors ($\chi^2_2 = 3.486$, $P = 0.175$) (Table 1). In the 1-neighbor trial, focal males tended to move more with the total courtship duration of neighbor males (Fig. 6). However, there was no obvious difference in the spatial distribution of focal males in heatmaps (Figs. S2-S4, online at https://doi.org/10.1636/JoA-S-20-079-.s1).

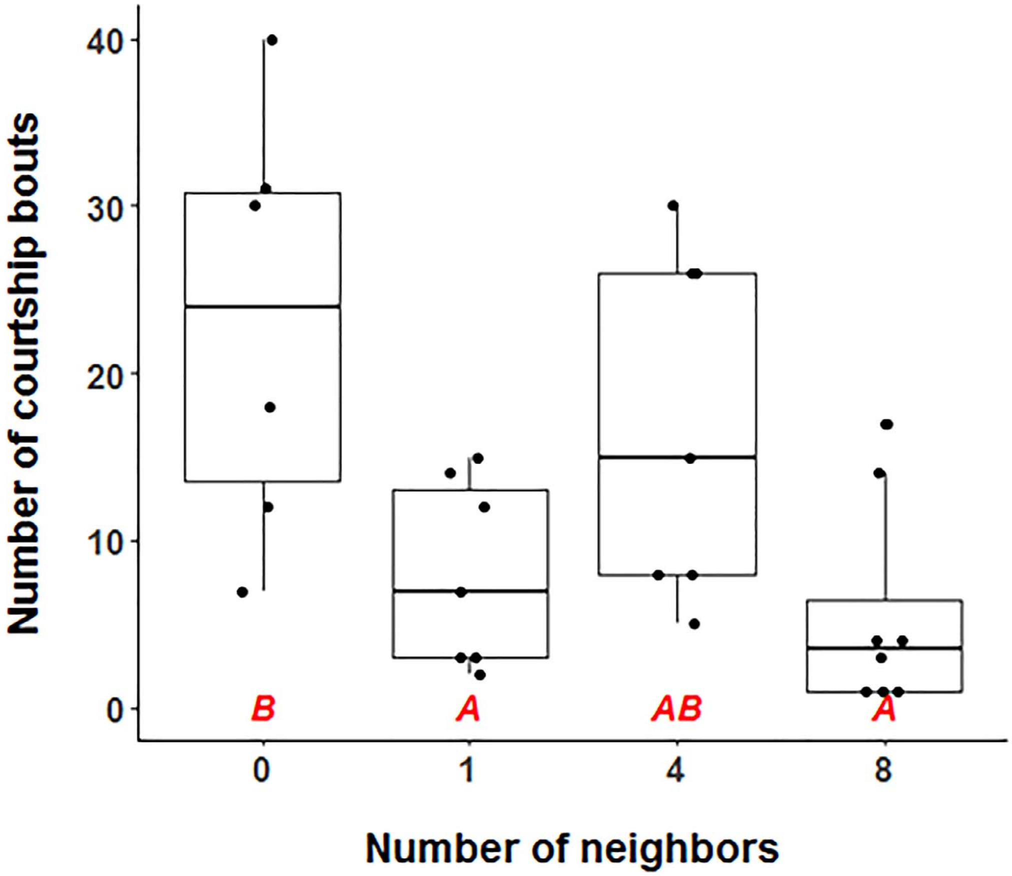

Figure 4.—The number of courtship bouts across the different number of neighbors. The top and bottom of the box represent the 25th and 75th percentile, and the line in the middle represents the 50th percentile. The whiskers represent 1.5 times of the 25th and 75th percentiles. The dots represent data points from each individual. The dots are scattered centering around the corresponding number of neighbors to avoid the overlap. The results of the post-hoc analysis are shown by letters under the box plots; plots sharing the same letter are not significantly different.

DISCUSSION

Our study provides evidence that substrate-borne vibratory cues of conspecific males are sufficient to elicit changes in the reproductive behavior of mature male *Schizocosa retrorsa*. Considering that prior work on this species demonstrated that substrate-borne vibratory courtship signals do not affect the likelihood of mating of *S. retrorsa* (Choi et al. 2019), our results may provide an interesting insight into how multiple intended and unintended receivers may affect the evolution of sexual communication. Not only did focal *S. retrorsa* males alter behavior in the presence of conspecific male vibratory cues, but their response was non-linear. This nonlinearity suggests that eavesdropping males may assess not only the potential risk of male-male competition, but also the likelihood of the presence of receptive females. Our results are similar to those found in other species, where males are competitively searching for spatially/temporally scattered females (Bee 2007; Milner et al. 2010; Webster & Laland 2013).

In our experiment, the number and courtship activity of neighbor males did not influence the presence/absence of the focal male's courtship signaling. We suspect that this is due to our experimental design. The constant occurrence of male courtship signaling across density treatments is likely due to the constant presence of female silk. Female silk and the associated pheromones are a direct cue for the presence of a sexually mature, virgin female in our experimental arena. Male *Schizocosa* spiders obtain various information about females from silk such as species (Roberts & Uetz 2004), sexual receptivity (Roberts & Uetz 2005), or age (Rundus et al. 2015); and silk alone is known to initiate male courtship (Tietjen 1979). Given that all focal males were exposed to mature female silk, it is not surprising that the presence of male courtship was independent of neighbor density and/or courtship activity.

Unlike the likelihood to court, the latency to initiate focal male courtship was influenced by the number of neighbors, but no differences were found between treatment groups. A previous study suggested that female preferences for initially courting males may affect the reproductive strategy of males, such as adjustments of courtship vigor by eavesdropping in *S. ocreata* (Stoffer et al. 2016), so the effects of the number of neighbor males on the latency may be a byproduct of active eavesdropping. However, we were unable to test the potential effects of the timing of conspecific courtship signals on the latency to male courtship signaling due to our limited sample size. The limited sample size also imposes a limit on our ability to verify any difference among treatment groups.

As opposed to influencing the presence/absence of courtship, focal males were more likely to use information from

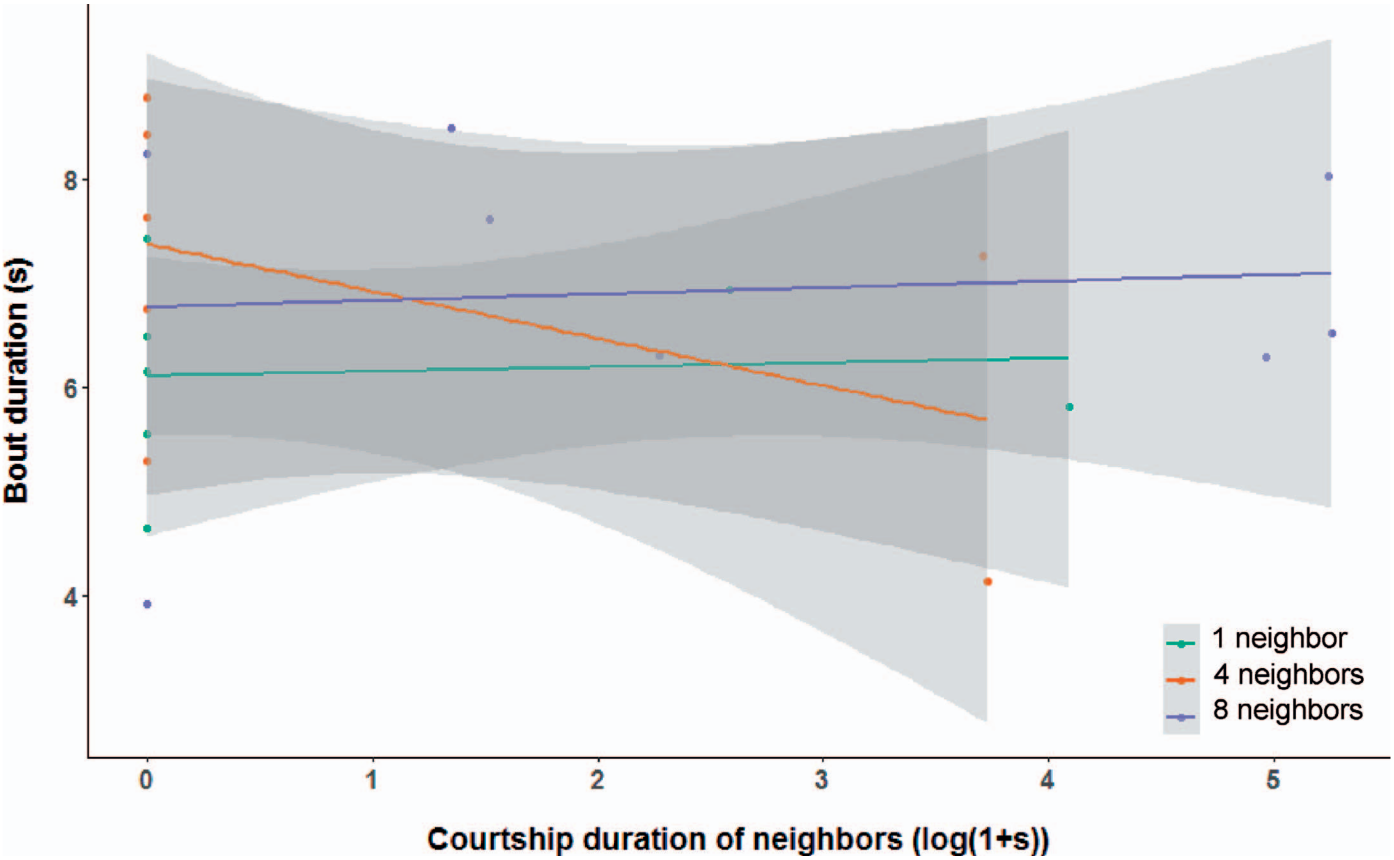

Figure 5.—Bout duration of focal males (in seconds, s) as a function of neighbors' courtship duration. The number of neighbors is color-coded (Green – 1-neighbor, Orange – 4-neighbor, Purple – 8-neighbor). The shaded area represents the 95 % confidence interval.

their neighbors to adjust or attune their signaling behavior. Once they started courtship signaling, *Schizocosa retrorsa* males adjusted the total courtship duration by changing the number of courtship bouts in response to variation in number of neighbor males across trials. Interestingly, focal males did not monotonically increase or decrease their courtship activity with the number of neighbors. As compared to the 0-neighbor trial, *S. retrorsa* males produced fewer courtship bouts at trials with small (single) and large (eight) numbers of conspecific males but restored the courtship activity with the intermediate (four) number of neighbors. Also, the total duration of the courtship signal of neighbor males affected the structure of the multimodal courtship signal (consisting of pedipalpal drumming and foreleg tapping). Another interesting result is the increased proportion of foreleg tapping with the duration of the neighbor male's courtship signal. In a previous study on the multimodal courtship signal of *S. retrorsa*, the rate of foreleg-tapping only provided relevant predictions about mating success (Rundus et al. 2010). Thus, the increased proportion of foreleg tapping with the courtship duration of neighbor males may be the result of acoustic competition among males. Regardless, together, our results suggest that *S. retrorsa* males can acquire information about neighboring males through vibratory cues, and this information, especially as it relates to neighbor male courtship behavior, influences focal male courtship in non-linear ways.

Our findings that the number of neighbor males, but not the courtship activity of neighbor males, influences focal male courtship duration and the number of courtship bouts suggests that *S. retrorsa* males may use non-courting substrate-borne vibratory cues to assess the male density around them. Although our experimental setup could not record substrate-borne vibrations quieter than courtship signals (e.g., vibrational cues from walking) from neighbor males due to the limited sensitivity of the contact microphone, recordings of courting males using laser vibrometer usually contain non-courting vibrations caused by walking or chemo-exploratory behavior (Sullivan-Beckers & Hebets 2011). We propose that *S. retrorsa* males in our experiment are indeed assessing spider density through perceived conspecific cues. We see this as more likely than an explanation posing that focal males are responding to potential vibratory cues associated with predation risk for the following reasons. Lohrey et al. (2009) showed that *S. ocreata* males can distinguish substrate-borne vibrations generated by avian predators from other non-threatening cues. Moreover, the most common response of courting males to predatory cues is the cessation of courtship signaling (Lohrey et al. 2009; Wilgers et al. 2014), yet males continued to court in our experiments. Finally, the non-monotonic variation in the total courtship duration suggests that the density-dependent behavioral response of *S. retrorsa* males is not an anti-predatory response.

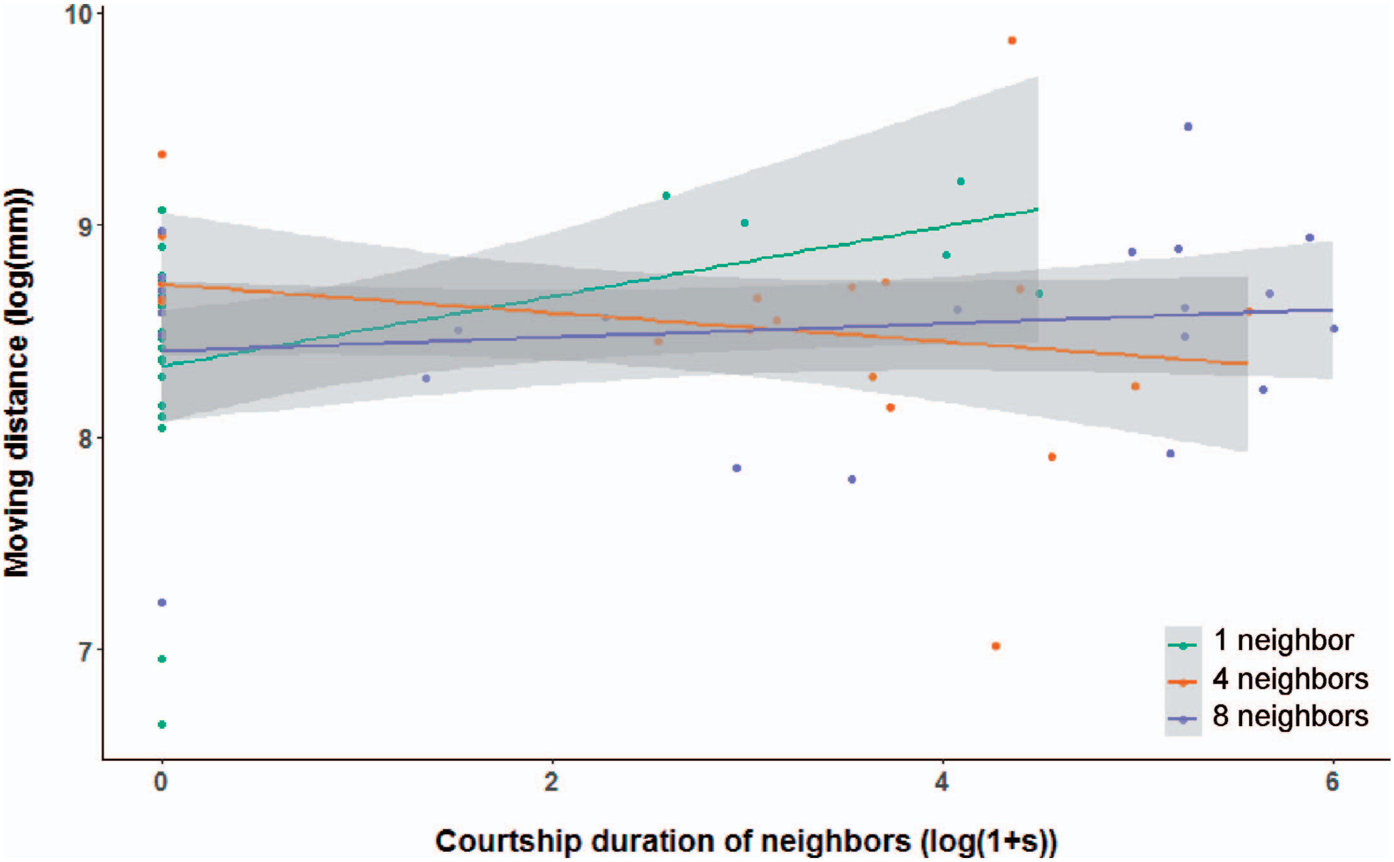

Figure 6.— Moving distance of the focal male as a function of the courtship duration of neighbors. The number of neighbors is color-coded (Green – 1-neighbor, Orange – 4-neighbor, Purple – 8-neighbor). The shaded area represents the 95 % confidence interval.

The non-linear response of focal males to variation in conspecific male density may suggest that males perceive two different types of information from vibratory cues of conspecific rivals – (i) the likelihood of the presence of receptive females and (ii) the potential risk of male-male competition. After *S. retrorsa* males initiate courtship signaling following contact with female pheromone-laden silk, they often pause their courtship and initiate additional chemoexploratory – or chemical searching – behavior. Males often alternate between chemoexploration and courtship until they come into visual contact with a female (personal observation). Thus, the density-dependent variation in total courtship duration observed in our study may reflect the differential switching of male reproductive behavior between mate searching and courtship signaling across neighbor densities. That is, after the detection of the female cue in low neighbor densities, males may spend more time courting and attempting to attract a female. In contrast, when males perceive a large number of neighbors, visually and chemically searching for the female may be a better strategy than adding courtship to an already crowded field of potential competitors. In intermediate neighbor numbers, the alternation between courtship and searching may be more even. To test this hypothesis, future studies will be needed to test the mate searching/courtship behavior of male *S. retrorsa* in choice experiments among multiple conspecific male densities from low to high (Rohr et al. 2005).

The density-dependent influence of neighbor males' courtship activity on focal male's locomotion during a trial may provide further support for the hypothesis that males alternate between courtship signaling and mate searching. With small and high numbers of neighbors, *S. retrorsa* males may use other males' courtship signals as a cue for locating females, so the increased courtship activity of neighbors may lead focal males to move more. However, with the intermediate number of neighbors, focal males were focused on courtship signaling, so they did not alter their behavior using the cue from neighbor courtship activity. This may suggest the alternative use of social information from other male's courtship signaling depending on the perceived intensity of male-male competition in *S. retrorsa*. However, due to our limited sample size of trials in which both focal and neighbor males courted (1-neighbor: *n* = 2, 4-neighbor: *n* = 2, 8-neighbor: *n* = 6), support for this explanation would require additional experiments.

While the moving distance of focal males was influenced by neighbor males, we did not find evidence of local enhancement, or social gathering facilitated by the presence/cues from an individual at the area (Webster & Laland 2013), in our heatmaps. Unfortunately, this finding is difficult to interpret, since a lack of observed local enhancement facilitated by conspecific courtship signals may simply be due to the size of the experimental arena. The maximum distance between focal and neighbor males in our experimental design was 270 mm, which is not very different from the estimated active space of the substrate-borne vibratory signal of *Schizocosa* wolf spiders (< 200mm – Uetz et al. 2013; Choi et al. 2019). Thus, focal males may increase searching effort within a central hoop of the experimental arena rather than moving towards courting conspecifics in response to conspecific courtship signals, because they are already well within the signals active space. Also, the distribution of female silk on the filter paper may affect the movement of focal males during a trial. While we gave females 1 hour to lay silk on filter paper, many females mostly stayed on the edge of filter paper amid efforts to escape. Considering that *Schizocosa* males follow female silk (*S. ocreata*; Bell & Roberts 2017), the uneven distribution of female silk may facilitate males to follow the trails of female silk instead of showing local enhancement towards vibratory cues from neighbor males. While the construction of heatmap using ezTrack provides a useful way to visualize the movement pattern, future research may need to test the male movement in a larger experimental arena and in the absence of direct cues such as female silk to investigate whether *Schizocosa* males show local enhancement by eavesdropping on neighbor males. Moreover, we cannot exclude the possibility that the contact microphones attached to peripheral hoops in 4-neighbor trial may affect the transmission of vibratory cues from neighbor males. Future study will also need to control the transmission characters of the entire experimental arena.

In summary, our experiment suggests that (i) *S. retrorsa* males assess their competitive environment through substrate-borne vibratory cues generated by courting or non-courting behavior of conspecific neighbors, (ii) *S. retrorsa* males may alter their reproductive behavior between mate searching and courtship signaling by the assessment of conspecific male density, and (iii) the assessment and perception of conspecific male cues as social information may provide information about the likelihood of the presence of females and the risk of male-male competition. To test whether the utilization of conspecific male cues and density-dependent reproductive strategy influence male reproductive success, future studies will be required to investigate the reproductive behavior of male wolf spiders in a more natural experimental setup with patchily distributed male aggregations across a heterogeneous vibratory environment that varies in conspecific male density.

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SUPPLEMENTARY FILES

Supplemental figures S1-S4, available online at https://doi.org/10.1636/JoA-S-20-079-.s1

Figure S1.—Waveform and spectrogram of courtship signals of neighbor and focal *Schizocosa retrorsa* males.

Figure S2.—Heatmaps of the focal males' movements during the 1-neighbor trial when neighbor males did not and did court.

Figure S3.—Heatmaps of the focal males' movements during the 4-neighbor trial when neighbor males did not and did court.

Figure S4.—Heatmaps of the focal males' movements during the 8-neighbor trial when neighbor males did not and did court.

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