**The effects of experience with different courtship modalities on unimodal and multimodal
preferences in a wolf spider**

B. Stoffer* and George W. Uetz

ABSTRACT

Females often benefit from the ability to assess courting males using multiple signal modalities. Differences in the transmission properties of each signal modality, however, may hinder a female's ability to detect signals, and result in variation in individual sensory experience. Such variation in sensory experience would include, for example, variation in courtship modality experience. It is unknown, however, whether differences in courtship modality experience affect preferences for unimodal and multimodal signals. We tested the effects of juvenile courtship modality experience on adult mate preferences in the wolf spider, *Schizocosa ocreata* (Hentz). Male *S. ocreata* use a combination of visual and vibratory signals as a multimodal signal, and female *S. ocreata* (without previous experience) have been shown to weigh unimodal vibratory and unimodal visual signals equally. Whether individual variation in courtship modality experience affects the relative weight of unimodal signals is unknown not only in *S. ocreata*, but is poorly understood across animal taxa. We used playback techniques to manipulate the courtship modality experience (unimodal visual signals, unimodal vibratory signals, multimodal signals, or no signals) of penultimate females. Upon maturation, we counted the number of receptivity displays towards each unimodal signal and a multimodal signal on three consecutive days of single presentation studies. In no-choice adult trials, courtship modality experience affected unimodal, but not multimodal, preferences. Female *S. ocreata* preferred the familiar unimodal signal in no-choice presentations (e.g., females exposed to vibratory signals were more receptive toward those signals). However, females preferred multimodal signals over unimodal signals, regardless of their juvenile courtship modality experience. In two-choice trials, female *S. ocreata* significantly preferred visual signals, regardless of their experience. Taken together, an individual's courtship modality experience has the ability to affect unimodal

44 courtship preferences, but may be limited based on whether adult females are presented
45 unimodal signals in no-choice or two-choice trials.
46 KEY WORDS: behavioral plasticity, sensory environment, social environment, experience
47 effects, *Schizocosa*, wolf spiders

INTRODUCTION

Multimodal courtship signals might have evolved, at least partly, because they elicit enhanced responses in comparison to unimodal signals (Partan & Marler 1999, 2005), owing to (1) their ability to transmit in multiple sensory environments (Candolin 2003, Hebets & Papaj 2005) or (2) perceptual variability among signal receivers (Hebets & Papaj 2005, Ronald et al. 2012). Taken further, increased perception of one modality over the other due to multiple sensory environments (Candolin 2003, Hebets & Papaj 2005) might result in perceptual variability (Hebets & Papaj 2005, Ronald et al. 2012) among females. In other words, if females have repeated sensory experience with a particular courtship modality (i.e., their courtship modality experience varies relative to other individuals), they might exhibit plasticity in their preferences for unimodal signals by weighing each signal modality differently compared to individuals with other courtship modality experience.

Examining the influences of courtship modality experience on signal modality preferences is important given the increasing amount of evidence that female preferences are impacted by previous social experience (Kasumovic & Brooks 2011). For example, females may adjust mating preferences based on experience with varying sex ratios (Madden & Whiteside 2013), the cumulative number of potential mates (Stoffer & Uetz 2015), variation in the phenotypic expression of secondary sexual characters (Stoffer & Uetz 2016, Walling et al. 2008, Fowler-Finn & Rodríguez 2012), and males with familiar vs. novel traits (Johnson et al. 1993, Hebets 2003, Witte et al. 2000, Rutledge et al. 2010). Many studies, however, have focused on female responses after social experience with a single modality (e.g., visual experience: Stoffer & Uetz 2015, 2016; Walling et al. 2008; acoustic experience: Swanger & Zuk 2015, Bailey & Zuk 2008, Dawson & Ryan 2012, Anderson et al. 2014; vibratory experience: Fowler-Finn & Rodriguez

2012). In contrast, few studies have investigated how different sensory experiences (in this case, experience with male cues of different signal modalities) may affect subsequent female mating preferences (Bailey 2011, Rutledge et al. 2010). Identifying whether different signal modalities can both result in plasticity in mate preferences or whether plasticity is modality-dependent can ultimately suggest which sensory systems might be capable of neuroplasticity within a particular species. Prior to such investigation, however, it is important to examine whether variation in courtship modality experience can result in a shift in modality preferences for familiar or novel signal modalities. To the best of our knowledge, however, no studies have previously examined whether varying courtship modality experience might shift relative female preferences for unimodal courtship signals or preferences for unimodal vs multimodal signals.

The brush-legged wolf spider, *Schizocosa ocreata*, is a useful model organism to study the effects of courtship modality experience on female unimodal and multimodal preferences for several reasons. First, male *S. ocreata* use multimodal communication in their courtship, including a combination of visual and vibratory signals, to elicit female receptivity displays that signal their willingness to mate. They possess dark tufts of tibial bristles (a condition-dependent trait: Uetz et al. 2002) that females visually assess during courtship. Studies have shown that females are more receptive to males with larger tufts (Scheffer et al. 1996, McClintock & Uetz 1996). However, females also demonstrate increased receptivity towards males that have a higher amplitude and peak frequency of percussive strikes in their vibratory signals (Gibson & Uetz 2008), demonstrating the importance of vibratory signals as a condition-dependent trait (Gibson & Uetz 2012). Further, using live courting males, behavioral studies have demonstrated that isolated courtship signals in each modality (unimodal visual and unimodal vibratory signals) elicit similar responses when presented to females (Scheffer et al. 1996; Uetz et al. 2009). Specifically,

female *S. ocreata* demonstrated no significant difference in the likelihood to be receptive (Scheffer et al. 1996) or the number of receptivity displays (Uetz et al. 2009) when each courtship modality was isolated. Though female *S. ocreata* weigh unimodal signals equally, they demonstrated more receptivity towards multimodal signals than either unimodal signal (Scheffer et al. 1996, Uetz et al. 2009). These studies, however, were conducted using female *S. ocreata* without any previous experience with mature courting males, so whether experience with a particular sensory modality affects unimodal or multimodal preferences.

Second, *S. ocreata* live in a complex, leaf litter habitat in deciduous forests, with considerable microhabitat variation (e.g., substrate composition, amount of leaf litter, levels of sunlight), which could affect the efficacy of male courtship signal transmission. For example, *S. ocreata* can be found on rocks, bare soil, or wood, even though their vibratory courtship signals transmit more effectively on leaf litter (Gordon & Uetz 2011). Thus, males might court on substrates in which their vibratory signals are inefficient, but their visual signals transmit efficiently. Further, female *S. ocreata* might be able to assess vibratory signals better than visual signals if males are courting on leaves in the shade or at night. In fact, visual and vibratory courtship signals have different active spaces, when measured as the proportion of females that respond to each unimodal signal across a gradient of distances from the source of the courtship signal (Uetz et al. 2013). Thus, females might only be able to detect and assess one signal modality, depending on the distance from the male signaler (Uetz et al. 2013). Specifically, vibratory signals might be more easily detected at shorter distances (10-20cm), while visual signals might be more easily detected at greater distances (20-40cm) based on results from field studies (Uetz et al. 2013). Additionally, males may modulate their use of one signal modality over the other depending on their ability to transmit signals effectively in variable environments (Gordon & Uetz 2011), suggesting further

possible variation in courtship modality experience (e.g., increased use of visual signals in some microhabitats). Taken together, variation in the microhabitat has the potential to lead to variation in the sensory modality through which female *S. ocreata* are likely to experience male courtship.

Finally, *S. ocreata* demonstrate asynchrony of maturation, and in some cases males mature 2-3 weeks on average before females (Roberts & Uetz, unpublished data). During this time, non-reproductive juvenile females might be exposed to mature males. Further, the mature males could be courting nearby mature females or mature female silk, resulting in exposure to male courtship as well. Female *S. ocreata* can, therefore, use this period of time to assess the social environment, and in lab simulations of the social environment, have been shown to adjust their mate preferences based on the perceived availability of potential mates (Stoffer & Uetz 2015) and varying tuft size of potential mates (Stoffer & Uetz 2016). Thus, these studies, and others in *Schizocosa* (Hebets 2003, Hebets & Vink 2007, Rutledge et al. 2010) have demonstrated the importance of juvenile experience in subsequent adult preferences. Taken together, female *S. ocreata* may be exposed to male courtship as penultimate instar females, but the signal modality that they experience might vary from individual to individual, raising intriguing questions on the effects of courtship modality experience on subsequent modality preferences.

Overall, we hypothesized that courtship modality experience of penultimate instar females would affect relative preferences for unimodal signals as adults, but would not affect their overall preference for multimodal signals relative to unimodal signals. Other studies in *Schizocosa* have demonstrated that females prefer familiar phenotypes (Stoffer & Uetz 2016, Hebets 2003). Therefore, we predicted that females that had visual experience would be more receptive toward unimodal visual signals than unimodal vibratory signals, while those with vibratory experience would exhibit opposite effects. This is also based on an assumption that females with repetitive

use of a sensory system (1) might allocate more resources toward the development of that sensory system upon molting to adulthood or (2) experience neuroplasticity in particular neural regions associated with the sensory system (as seen in exposure to song in birds, reviewed in Bischof 2003). Alternatively, females might consider the “familiar” modality to be more reliable after repeated experience with that modality. Thus, adult female *S. ocreata* might be more responsive towards the modality that they experience as penultimate instar females. Because *S. ocreata* exhibit strong preferences for multimodal signals relative to unimodal signals (Uetz et al. 2009, Scheffer et al. 1996) and multimodal signals would still contain a familiar modality within the overall signal, we predicted that females would have significantly more receptivity displays in response to multimodal courtship signals than unimodal signals, irrespective of their experience.

METHODS

Animal Care

Female *S. ocreata* were collected from the Cincinnati Nature Center (CNC; located near the city of Milford, Claremont County, USA) in March 2013 (no-choice trials) and March 2014 (two-choice trials). All individuals used in this study were collected as juveniles, 2-3 molts before maturity. In nature, male and female *S. ocreata* typically mature and enter their breeding season in late April to early June (Stratton & Uetz 1981, Cady 1984, Stratton 2005). Mature males typically die prior to the dispersal of spiderlings from the female’s abdomen. Therefore, the penultimate instar stage is perhaps the only life stage in which female *S. ocreata* would be exposed to courtship prior to maturity. As a result, all individuals were collected prior to the presence of any mature males in the field (indicated by the presence of tufts on their forelegs), ensuring that females had no previous experience with male courtship other than the experience provided in this study during their penultimate instar stage. The research lab collects at least 100

individual spiders per collection, and not a single mature male was collected at the time that juvenile females were collected for this study. Each spider was isolated in an opaque plastic container with available water in a room with controlled relative humidity (65-75%), temperature (23-25°C), and light cycle (L:D 13:11h). All females were fed 2-3 juvenile crickets (approximately 3mm in length) twice per week. When females were exposed to male courtship, spiders were fed crickets only after trials were concluded for that day to ensure feeding did not distract from assessment of courtship signals.

Overall Design

Female *S. ocreata* were given different courtship modality experience treatments during their penultimate instar stages and were measured for their preferences for unimodal and multimodal male courtship signals as adults. Adult preferences were measured in single presentation (no-choice) and two-choice designs, using separate females. In these separate experiments, however, experience was provided using identical protocols. During courtship modality experience and adult no-choice and two-choice trials, each trial lasted five minutes. All adult trials began at 10-15 days post-maturity (dpm) All trials were recorded using a digital camera (Sony Handycam HDR-XR260).

Courtship Playback: Vibratory and Visual signals

All exposures took place in an arena (diameter: 15.5cm, height: 6.5cm) with a piezoelectric element placed in front of an iPod, allowing for the same arena to be used for vibratory playback, video playback, or both. In short (see below for further discussion), visual and vibratory courtship signals were all based on population averages. Therefore, the playback of courtship signals was identical when providing experience to penultimate instar females and when measuring their subsequent adult preferences. Additionally, based on Uetz et al. (2013), the

chosen arena dimensions ensure that playback of both vibratory and visual signals were within the typical active space (i.e., both signal modalities should be able to be detected within the arena, regardless of where the spider is located).

Vibratory playback was provided through the use of piezoelectric elements (APC International). The original vibratory recording was from the same individuals that were used to provide visual courtship signals in this study and previous studies (Roberts et al. 2007, Stoffer & Uetz 2015, 2016). The vibratory recordings were played from an Apple iPod (4th edition) that was connected to an amplifier (Pyle Mini 2x40W Stereo Power Amplifier). The output of the amplifier was connected to leads that were connected to the piezoelectric element via speaker wire. The piezoelectric element was attached to the substrate (Strathmore 300 series Bristol weight fine drawing paper) of the arena with minimal tape, and a piece of parchment paper was placed on top of the element, acting as the substrate through which the spiders would come in direct contact. Prior to any exposures, the vibratory recording was calibrated to match that of the average percussive amplitude in our population of *S. ocreata*. In this process, 25 randomly selected male *S. ocreata* (age: 10-15dpm, collected from CNC) were recorded using a PDV-100 laser Doppler vibrometer (125 mm/s/V sensitivity, 100 mm/s max, 96 mm standoff distance). The laser Doppler vibrometer (LDV) was connected to an external sound card (Roland QuadCapture) and calibrated with a 1 kHz tone (50% FS). The processing of male vibratory signals was conducted in SpectraPLUS-SC (24 kHz sampling rate, 2048 FFT, Hanning window) in order to calculate the amplitude (root mean square) of each percussive element. This allowed us to calculate the mean percussive amplitude for each male and the mean percussive amplitude for the population. Once this value was obtained (mean RMS: 4.565 mm/s), the previously described arena was used to play the vibratory courtship signal from the aforementioned

vibratory signal, and the LDV was used to calibrate the percussive amplitude. The amplifier was then adjusted until it matched the population mean as measured by the LDV. In summary, 25 males were recorded in order to match the amplitude of the single vibratory courtship stimulus to the population average.

Video playback was provided through the use of Apple iPod Touch units (4th edition), and used a courting male stimulus that has been previously used in other studies (McClintock and Uetz 1996, Roberts et al. 2007, Moskalik and Uetz 2011, Stoffer and Uetz 2015, 2016). The iPod units provide a high-resolution display of courting males that are the same body size and tuft size, based on population means from Roberts et al. (2007). The courting male video had a background of a digital photo of leaf litter to simulate natural visual contrast (Clark et al. 2011). In treatment groups in which females were not exposed to visual courtship signals (i.e., females that were exposed to vibratory signals alone or females from the sham control group), the leaf litter background was displayed on the iPod Touch unit without a courting male.

Taken together, a single male's courtship was used as the stimulus for vibratory playback, video playback, and multimodal playback. All characteristics within the vibratory courtship modality (e.g., the percussion and stridulation rates) as well as within the visual courtship modality (e.g., body size, tuft size, leg-tapping vigor) used in this study matched the population averages. Therefore, we expect any results from this study to accurately depict the results that would be found the majority of the time for *S. ocreata* in our population. Further, despite criticisms of video playback (Oliveira et al. 2000), including concerns regarding pseudoreplication, previous research in our lab has been discussed as examples that satisfy rigorous criteria (D'Eath 1998, Fleishman et al. 1998, Rosenthal 1999). Because population metrics used in our courtship stimuli are based on a single population, our results do possess

limitations on the generalization based on choices made within our experimental design (see McGregor 2000 for discussion within the context of playback experiments). Further, while multiple exemplars could be used to examine how the presence vs absence of particular modalities interacts with other male traits, our goal was to initially measure changes in preferences in the context of mean phenotypic values (see Rosenthal 2000 for further discussion).

Courtship Modality Experience

Exposures began the day after females molted to their penultimate instar stage and ended as they molted to maturity. Treatment groups included females that were exposed to (1) unimodal visual courtship signals, (2) unimodal vibratory courtship signals, or (3) multimodal courtship signals. Two additional treatment groups served as control groups: a group of females that were not provided any experience (control) and a group of females that were placed in the playback arena during their penultimate instar stage without any courtship signals (sham control). All females (except the control group) were placed into the playback arena twice per day.

No-choice Adult Trials

A total of 80 female *S. ocreata* were used to measure the effects of courtship modality experience on unimodal and multimodal preferences in no-choice trials (control: N = 12, sham control: N = 14, vibratory: N = 20, visual: N = 16, multimodal: N = 18). No-choice adult trials occurred during a three-day series of preference trials. During the first two days, females were placed in the same arena to test female preferences for unimodal courtship signals. Females were presented, in a random order, either a unimodal vibratory signal through the element or a unimodal visual signal through the iPod. On the third day, females were presented a multimodal signal (visual and vibratory).

Two-choice Unimodal Adult Trials

In a separate experiment (i.e., using different females), courtship modality experience was provided to penultimate females in the same exact manner as the experience for females in no-choice trials. A total of 110 female *S. ocreata* were used to measure the effects of courtship modality experience on unimodal and multimodal preferences in two-choice trials (control: N = 15, sham control: N = 22, vibratory: N = 25, visual: N = 21, multimodal: N = 27). However, females were given a choice between a unimodal vibratory signal and a unimodal visual signal within the same arena. Consequently, a different arena was required and was calibrated as previously described for the arena used to provide courtship modality experience and measure adult no-choice preferences. Individuals in this study were not tested for multimodal preferences in a two-choice design, as experience did not affect the relative weight of multimodal vs unimodal preferences in no-choice trials (see Results). In the circular arena, the iPod unit (video playback) and the piezoelectric element (vibratory playback) were originally separated by 90°. Females were placed in the center of the arena, equidistant from the iPod and piezoelectric element.

Once adult preference trials were commenced, initial observations clearly demonstrated that females strongly preferred visual signals to vibratory signals in this two-choice design. Specifically, the observer running the trials (BS) did not observe any receptivity displays towards the unimodal vibratory signal after conducting the first 62 trials. Results from other studies have demonstrated that the spatial separation of unimodal courtship signals affects female unimodal preferences in *S. ocreata* (Kozak & Uetz unpublished data). To ensure that our initial results were not due to the arena design, we measured a group of females (N = 62) in two-choice trials using a

277 90° separation of unimodal signals and a group (N = 48) of the females using a 180° separation.
278 The arena was re-calibrated when the degree of signal separation was changed.

279 *Ethical Note*

280 No legal requirements or protocols are required for the handling or use of spiders in research
281 conducted in Ohio, U.S.A. or at the University of Cincinnati. We did, however, make every
282 attempt to ensure proper maintenance and care for the spiders used in this study. To the best of
283 our knowledge, we followed the suggestions and adhered to the ASAB/ABS Guidelines for the
284 Use of Animals in Research. For example, using vibratory and video playback reduces the
285 number of individuals collected from the field. After the study, spiders were individually and
286 humanely euthanized with CO₂ anaesthetization, then placed in 70% ethanol.

287 *Data Analysis*

288 For each adult trial, the scorer (BS) was blind to which sensory treatment group females
289 experienced. While trials were muted during scoring of behavioral trials, it was impossible to
290 score female responses without seeing the digitally courting male. Taken together, the scorer was
291 blind to the treatment group that females were in, but not which sensory modality that females
292 were presented as adults. We measured the latency to approach the source of each signal to
293 determine if courtship modality experience affected how quickly females approached different
294 courtship signals. Each trial was also analyzed for female receptivity behaviors typical of female
295 *S. ocreata* (e.g., settle, tandem leg extensions, and pivots), and subsequent analyses included the
296 sum of these receptivity displays. These receptivity behaviors have been previously shown to lead
297 to successful copulation (Montgomery 1903, Uetz & Denterlein 1979, Scheffer et al. 1996,
298 Norton and Uetz 2005) and may signal their willingness to mate with (and not cannibalize)
299 courting males. Receptivity displays were only counted if they were within a zone that was the

width of the iPod (60mm), near the source of the courtship signal. This was the same zone that was used to analyze a female's latency to approach (i.e., the latency to enter the zone).

Data Analysis: Latency to Approach in No-choice Trials

We used the 'coxme' statistical package (Therneau 2012) in the R statistical program (R Core Team 2013, version 2.15.3) to use a Cox proportional hazards model. This R package allows the use of proportional hazards models with repeated measures designs, treating individuals as a random effect for each model. This is particularly useful for the current experimental design, as each female was either tested on three consecutive days (no-choice trials) or was given a choice between both unimodal signals (two-choice trials). Originally, statistical models with combinations of courtship modality experience, adult modality (i.e., the sensory modality they were presented as adults), and their interaction as factors were constructed with individual ID as a random effect and latency to approach as the response variable. Log-likelihood ratio tests were used to compare models to each other or to compare models to an intercept-only model with individual ID still used as a random effect. Post hoc tests were conducted using the 'multcomp' package in R (Hothorn et al. 2008), while using Tukey's all-pair comparisons with corrected p-values using a single-step method.

Data Analysis: Receptivity Displays in No-Choice Trials

In comparisons of receptivity displays, statistical analyses were conducted using the R statistical package (R Core Team 2013, version 2.15.3). Separate analyses were conducted to compare the effects of courtship modality experience on unimodal visual vs unimodal vibratory signals, unimodal visual vs multimodal signals, and unimodal vibratory vs multimodal signals. The number of receptivity displays in these binary comparisons were bound together using the 'c-bind' function in R. Consequently, when a generalized linear model (family = binomial) was

conducted, the response variable included a matrix with two columns: the number of receptivity displays in one adult preference trial and the number of receptivity displays in another adult preference trial. Log-likelihood ratio tests were used to compare models with courtship modality experience as a factor to an intercept-only model (also a binomial family generalized linear model). Post hoc tests were conducted using the ‘multcomp’ package in R (Hothorn et al. 2008), while using Tukey’s all-pair comparisons with corrected p-values using a single-step method.

Because courtship modality experience clearly had a significant effect on the relative weight of unimodal and multimodal signals, one-way ANOVAs were initially conducted for each independent response variable. These additional analyses are useful, as they might identify whether courtship modality experience results in variation of female preferences in response to visual signals, vibratory signals, or both. Each response variable (receptivity displays towards unimodal vibratory signals, receptivity displays towards unimodal visual signals, receptivity displays towards multimodal signals) was not normally distributed, as demonstrated by Shapiro-Wilk tests. Consequently, each response variable was analyzed using a generalized linear model with a Poisson distribution with courtship modality experience as a factor. Each model was then compared to a null model using likelihood ratio tests to determine if experience had a significant effect.

Data Analysis: Receptivity Rate in No-choice Trials

Because there was a significant effect of the signal modality tested as adults on female latency to approach (see Results), we performed additional analyses that compared female receptivity as a rate (‘receptivity rate’). This rate was defined as the number of receptivity displays (i.e., the sum of pivots, tandem leg extensions, and settles) divided by the amount of time remaining in the trial once the female approached the signal source. In calculating this rate, responses were no longer

whole integers, preventing the use of generalized linear models with a binomial family. Consequently, we used generalized linear models with the difference in the receptivity rates as the response variable to test the effects of courtship modality experience on relative preferences. The difference in receptivity rates was normally distributed for each statistical comparison as demonstrated by Shapiro-Wilk tests. Post hoc tests were conducted using the ‘multcomp’ package in R (Hothorn et al. 2008), while using Tukey’s all-pair comparisons with corrected p-values using a single-step method.

Data Analysis: Latency to Approach in Two-choice Trials

We used the ‘coxme’ statistical package (Therneau 2012) in R (R Core Team 2013, version 2.15.3) to use a Cox proportional hazards model, similar to analyses of latency to approach in no-choice trials. Originally, statistical models with combinations of courtship modality experience, adult modality, and their interaction as factors were constructed with individual ID as a random effect and latency to approach as the response variable. Log-likelihood ratio tests were used to compare models to each other or to compare models to an intercept-only model with individual ID still used as a random effect. For each statistical model that included an interaction, we compared each model to the same model without the interaction. Because it became evident that there were no significant interactions (all $p > 0.05$) for any combinations of courtship modality experience (control, sham control, visual, vibratory, multimodal), adult modality (visual or vibratory), or experimental design (90° or 180° separation), we report the results of the individual variables only. Post hoc tests were conducted using the ‘multcomp’ package in R (Hothorn et al. 2008), while using Tukey’s all-pair comparisons with corrected p-values using a single-step method.

Data Analysis: Receptivity Displays in Two-choice Trials

To analyze the number of receptivity displays in two-choice trials, we used a generalized linear model (family=binomial) with ‘Experimental Design’ (90° or 180° separation) and ‘Courtship Modality Experience’ as factors. Identical to no-choice trials, the number of receptivity displays were bound together using the ‘c-bind’ function in R (R Core Team 2013, version 2.15.3). Likelihood-ratio tests were used to compare models to an intercept-only model to test for the effects of experimental design and courtship modality experience on female preferences for unimodal courtship signals. Post hoc tests were conducted using the ‘multcomp’ package in R (Hothorn et al. 2008), while using Tukey’s all-pair comparisons with corrected p-values using a single-step method.

Data Analysis: Receptivity Rate in Two-choice Trials

Identical to our analysis of receptivity rate in no-choice trials, we used the difference in the receptivity rates as the response variable to test the effects of courtship modality experience and experimental design (90° vs 180° signal separation) on relative preferences using generalized linear models in R (R Core Team 2013, version 2.15.3). The full statistical model and subsequent reduced models were compared to intercept-only models using likelihood-ratio tests. Post hoc tests were conducted using the ‘multcomp’ package in R (Hothorn et al. 2008), while using Tukey’s all-pair comparisons with corrected p-values using a single-step method.

RESULTS

No-choice Trials: Latency to Approach

There was significant overall variation in the latency for female *S. ocreata* to approach male courtship signals in no-choice presentations (likelihood ratio test: $\chi^2_{14} = 61.935$, $P < 0.0001$). The adult courtship modality significantly affected their latency to approach male courtship signals (Fig. 1; likelihood ratio test: $\chi^2_4 = 50.661$, $P < 0.0001$), such that females approached visual ($Z =$

5.448, $P < 0.0001$) and multimodal ($Z = 6.713$, $P < 0.0001$) signals faster than vibratory signals in post hoc tests. There was no significant difference in the latency to approach visual signals in comparison to the latency to approach multimodal signals in post hoc tests ($Z = 1.513$, $P = 0.284$). There was no significant effect of courtship modality experience (likelihood ratio test: $\chi^2_4 = 3.024$, $P = 0.5538$) or the interaction of courtship modality experience and adult modality (likelihood ratio test: $\chi^2_4 = 7.521$, $P = 0.4816$). Taken together, adult female *S. ocreata* approach courtship signals faster if playback includes visual signals (video playback or multimodal playback), but this relationship is not affected by previous courtship modality experience during their penultimate instar stage.

No-choice Trials: Receptivity Displays

Females demonstrated a significant preference overall for visual signals over vibratory signals in no-choice unimodal trials, when excluding any effects of courtship modality experience (paired t-test: $t_{79} = 2.725$, $P = 0.0079$). However, female preferences for these unimodal signals varied based on their courtship modality experience during their penultimate instar stage, demonstrating more receptivity displays towards the unimodal signal modality that was provided to them during their penultimate instar stage (Fig. 2). Specifically, courtship modality experience explained a significant amount of variation in a female's relative weight of receptivity displays toward unimodal visual signals and unimodal vibratory signals (likelihood ratio test: $\chi^2_4 = 25.975$, $P < 0.0001$). Subsequent post hoc tests demonstrated that females with vibratory experience weighed unimodal signals significantly differently than females without any experience ($Z = 3.130$, $P = 0.149$), females with multimodal experience ($Z = 3.542$, $P = 0.0036$) or females with visual experience ($Z = 4.297$, $P < 0.001$). Of interest, female *S. ocreata* that had only vibratory

experience were more receptive toward vibratory signals than visual signals, while females with visual experience were more receptive toward visual signals than vibratory signals (Fig. 2).

Females demonstrated a significant preference overall for multimodal signals over unimodal vibratory signals, when excluding any effects of courtship modality experience (paired t-test: $t_{79} = 7.988$, $P < 0.0001$). However, courtship modality experience explained a significant amount of variation in a female's relative weight of receptivity displays toward unimodal vibratory signals and multimodal signals (likelihood ratio test: $\chi^2_4 = 12.964$, $P = 0.0115$). Subsequent post hoc tests, however, demonstrated that the relative weight of multimodal and unimodal vibratory signals was not significantly different across treatment groups, likely due to adjustment of p-values for multiple comparisons ($P > 0.05$ for all comparisons).

Females also demonstrated a significant preference overall for multimodal signals over unimodal visual signals, when excluding any effects of courtship modality experience (paired t-test: $t_{79} = 5.525$, $P < 0.0001$). However, courtship modality experience explained a significant amount of variation in a female's relative weight of receptivity displays toward unimodal visual signals and multimodal signals (likelihood ratio test: $\chi^2_4 = 13.618$, $P = 0.0086$). Subsequent post hoc tests demonstrated that females with vibratory experience weighed multimodal vs unimodal visual experience significantly differently than females with only arena experience ($Z = 3.262$, $P = 0.0096$) or visual experience ($Z = 2.817$, $P = 0.0388$).

Courtship modality experience had a significant effect on female receptivity displays towards unimodal vibratory signals (likelihood ratio test: $\chi^2_4 = 11.262$, $P = 0.0238$). Subsequent Tukey's post hoc tests revealed that female *S. ocreata* with vibratory experience as penultimate instar females had more receptivity displays to vibratory courtship signals as adults than females with visual or multimodal experience. Courtship modality experience had a significant effect on

female receptivity displays towards unimodal visual signals as well (likelihood ratio test: $\chi^2_4 = 15.496$, $P = 0.0038$). Subsequent Tukey's post hoc tests revealed that females with visual experience as penultimate instar females had more receptivity displays to visual courtship signals as adults than females with vibratory experience. Finally, courtship modality experience also had a significant effect on female receptivity displays towards multimodal signals (likelihood ratio test: $\chi^2_4 = 15.496$, $P = 0.0038$). Subsequent Tukey's post hoc tests revealed that females with vibratory experience had significantly more receptivity displays towards multimodal signals as adults than females in the sham control group, although this may be due to a general lower amount of receptivity from females in the sham control treatment group (see Figure 2).

No-choice Trials: Receptivity Rate

There was no significant difference overall in the receptivity rates in response to visual courtship signals and vibratory courtship signals (Fig. 3; paired t test: $t_{76} = 1.585$, $P = 0.1172$). However, there was a significant effect of courtship modality experience on the difference in receptivity rates between unimodal visual and unimodal vibratory signals (Fig. 3; likelihood ratio test: $\chi^2_4 = 17.299$, $P = 0.0015$). Subsequent post hoc tests demonstrated that females with vibratory experience weighed the unimodal signals differently than females with visual ($Z = 3.585$, $P = 0.0033$) or multimodal ($Z = 3.418$, $P = 0.0059$) experience.

The receptivity rate was significantly greater towards multimodal courtship signals in comparison to visual courtship signals, regardless of courtship modality experience (paired t test: $t_{76} = 5.351$; $P < 0.0001$). Courtship modality experience had no significant effect on the difference in receptivity rates in response to multimodal courtship signals and visual courtship signals (likelihood ratio test: $\chi^2_4 = 1.992$, $P = 0.1602$).

The receptivity rate was significantly greater towards multimodal courtship signals in comparison to vibratory courtship signals, regardless of courtship modality experience (paired t test: $t_{76} = 6.862$; $P < 0.0001$). Courtship modality experience had no significant effect on the difference in receptivity rates in response to multimodal courtship signals and visual courtship signals (likelihood ratio test: $\chi^2_4 = 10.902$, $P = 0.0573$).

Two-choice Unimodal Trials: Latency to Approach

The spatial separation of unimodal signals (likelihood ratio test: $\chi^2_1 = 0.034$, $P = 0.8547$) and courtship modality experience (likelihood ratio test: $\chi^2_4 = 2.668$, $P = 0.6149$) had no significant effect on the latency to approach the source of the courtship playback. The courtship signal modality tested as adults had a significant effect on the latency to approach courtship playback (Fig. 4; likelihood ratio test: $\chi^2_1 = 116.57$, $P < 0.0001$). Specifically, post hoc tests demonstrated that females approached visual signals sooner than vibratory signals ($Z = 10.86$, $P < 0.0001$).

Two-choice Unimodal Trials: Receptivity Displays

The full model (courtship modality experience, experimental design, and their interaction) was a significant improvement upon an intercept-only model (likelihood ratio test; $\chi^2_9 = 18.447$, $P = 0.0303$). Subsequent tests revealed that there was no significant effect of courtship modality experience on receptivity displays towards vibratory vs visual signals in a two-choice design (likelihood ratio test; $\chi^2_4 = 7.560$, $P = 0.1091$), but there was a significant effect of experimental design on female preferences for vibratory vs visual signals (Fig. 5; likelihood ratio test: $\chi^2_1 = 12.193$, $P = 0.0005$). Specifically, there was a greater difference in receptivity displays (i.e., stronger preference for visual signals to vibratory signals) when the unimodal signals were separated by 180° than 90° (paired t test: $t_{108} = 2.643$, $P = 0.0094$). Even though this difference exists, there were still significantly more receptivity displays directed toward visual courtship

signals than vibratory courtship signals, regardless of treatment group (paired t test: $t_{109} = 8.159$, $P < 0.0001$).

Two-choice Unimodal Trials: Receptivity Rate

The full model (courtship modality experience, experimental design, and their interaction) was not a significant improvement upon an intercept-only model (likelihood ratio test; $\chi^2_9 = 0.0014$, $P = 0.0579$). Models with each explanatory variable alone, however, suggest that the experimental design (90° vs 180° separation) had a significant effect on the difference in receptivity rates (likelihood ratio test: $\chi^2_1 = 0.001$, $P = 0.0200$), while courtship modality experience did not (likelihood ratio test: $\chi^2_4 < 0.001$, $p=0.5650$). Post hoc tests demonstrated that differences in receptivity rates were significantly greater when signals were separated by 180° than 90° ($Z = 2.327$, $P = 0.02$). Overall, however, there was a significantly greater receptivity rate towards visual signals than those towards vibratory signals (paired t test: $t_{109} = 7.8319$, $P < 0.0001$).

DISCUSSION

The courtship modality experience of female *S. ocreata* during the penultimate instar stage affected the relative weight of responses to unimodal courtship signals in no-choice, but not two-choice, trials. While females without previous social experience have been demonstrated to display similar preferences for unimodal vibratory signals and unimodal visual signals (Scheffer et al. 1996, Uetz et al. 2009), courtship modality experience provided in this experiment resulted in increased receptivity towards the familiar courtship modality in no-choice trials. Specifically, if female *S. ocreata* had experience with courtship signals in the vibratory modality only during their penultimate instar stage, they were more receptive towards unimodal vibratory signals as adults relative to unimodal visual signals. If they had experience with visual courtship signals,

however, they were more receptive towards unimodal visual signals relative to unimodal vibratory signals.

Whether female *S. ocreata* would ever experience only unimodal courtship signals in their natural environment (deciduous leaf litter forest) as juveniles remains unknown. Variation in their microhabitats, however, could differentially affect transmission properties of each courtship signal modality. An abundance of curled leaves, for example, could obstruct proper reception of visual courtship signals, but still allow vibratory signals to be transmitted. In contrast, a rocky microhabitat could obstruct proper signal transmission of vibratory courtship signals (Gordon & Uetz 2011), while still allowing proper transmission of visual courtship signals. While the current study intentionally chose extreme courtship modality experiences (i.e., some spiders experience only visual signals or only vibratory signals), future studies could examine modality preferences after females experience a gradient of signal modalities (e.g., experience unimodal vibratory signals in 25% of their simulated encounters with courting males and unimodal visual signals in 75% of their encounters, and vice versa). Regardless, this study demonstrates the potential for plasticity in courtship modality preferences, alluding to intriguing differences in sensory systems that might arise due to differences in courtship modality experience.

While there was initially an overall preference for visual signals in no-choice trials, this appears to be due to differences in the latency to approach unimodal visual vs unimodal vibratory signals. Female *S. ocreata* took longer to approach vibratory courtship signals than visual courtship signals, regardless of their courtship modality experience as penultimate females. Once receptivity was converted to a rate (correcting for differences in latency), there was no longer an overall preference for visual signals (i.e., females weighed unimodal signals equally overall). However, courtship modality experience still had a significant effect on

receptivity rate in no-choice trials. Because as a whole, females (i.e., regardless of courtship modality experience) took significantly longer to approach vibratory courtship signals, repeated experience with a particular sensory modality does not appear to affect an individual's ability to subsequently detect the sensory modality. Instead, courtship modality experience appears to have affected the ability of females to recognize and respond appropriately to a familiar sensory modality with receptivity displays (i.e., an actual mating decision and not just signal detection). Further underlying behavioral or neural mechanisms of this remain unclear at this time. We suggest, perhaps, that female *S. ocreata* may be better able to assess an individual's quality if they have previous experience with that particular modality. While there are connections between individual variation in sensory systems and mating decisions (see Ronald et al. 2012 and citations within), it remains unclear whether this could be in part due to individual variation in courtship modality experience. In humans, however, sensory deprivation during development in one modality can result in reallocation of brain regions to be used by a different sensory modality (Neville et al. 1983, Kujala et al. 1997, Sadato et al. 1996). Whether experience with a familiar unimodal courtship signal results in a reallocation of spider ganglia regions from the unfamiliar modality to the familiar modality remains to be examined.

While courtship modality experience affected female preferences for unimodal signals, female *S. ocreata* strongly maintained their preferences for multimodal signals. Previous studies in *S. ocreata*, using females without previous experience with courting males, demonstrated that females prefer multimodal courtship signals to unimodal signals of either signal modality (Uetz et al. 2009, Scheffer et al. 1996). Increased mating behaviors in response to multimodal signals in comparison to an isolated unimodal signal is not uncommon across animal taxa (flies: Rybak et al. 2002; frogs: Reichert & Höbel 2015, Preininger et al. 2013, Taylor et al. 2007, Rosenthal et

al. 2004; spiders excluding *Schizocosa* spp.: Wilgers & Hebets 2011, Elias et al. 2006) or in closely-related *Schizocosa* spp. (Hebets & Uetz 1999, Stafstrom & Hebets 2013). Therefore, that such a preference for multimodal signals exists, and is not variable with courtship modality experience, is not surprising. When females with unimodal (vibratory or visual) experience were tested for their preference for multimodal signals, such a multimodal signal inherently contains a combination of a familiar signal modality and a novel signal modality. Consequently, females could have an enhanced response to multimodal signals partly because of the combination of a familiar and novel modality. We argue, however, that because females without experience also preferred multimodal signals in this study and others (Uetz et al. 2009, Scheffer et al. 1996) that female preferences for multimodal signals in *S. ocreata* do not require learning or experience.

There appear to be some constraints on the effects of courtship modality experience as penultimate instar females on adult unimodal preferences in *S. ocreata*, given the differences in results from the no-choice (females preferred the ‘familiar’ unimodal signal) and two-choice (females preferred visual signals) experiments. In two-choice experiments, female *S. ocreata* not only preferred visual signals to vibratory signals regardless of courtship modality experience, but also approached them more quickly. It remains unclear, however, whether this preference in a two-choice design is a product of innate sensory biases (as suggested by Persons & Uetz 1998 in a predator-prey context) or whether it is a product of the experimental design. On one hand, visual signals may provide a target for females that can more easily be identified as a conspecific courting male in comparison to vibratory signals. This may also explain why the latency to approach visual signals is shorter than the latency to approach vibratory signals in both no-choice and two-choice experiments. Further, vibratory courtship signals may alert females of the presence of a nearby courting male, while visual signals may be used to better identify the male’s

source. While female *S. ocreata* are capable of finding the source of vibratory courtship signals and responding with receptivity displays when in isolation, visual signals may override such a response when each unimodal signal is simultaneously present in a two-choice design. It should be additionally noted that the inherent constraints of our experimental design allow female *S. ocreata* to simultaneously detect both unimodal signals even though the source of these signals is spatially separated. While we intentionally constructed this arena to allow female *S. ocreata* to detect each signal regardless of their location within the arena, it remains plausible that the overall preference for visual signals in two-choice trials is due to the ability of females to still be able to detect vibratory signals while at the source of the visual signals. Because spatial separation of unimodal signals can affect female preferences for unimodal courtship signals in *S. ocreata* (Kozak & Uetz unpublished data), we conducted the two-choice experiment in two manners. In both instances, however, females clearly preferred visual signals to vibratory signals – though this preference was stronger when unimodal signals were separated by 180°.

The effects of social experience on subsequent mating behaviors have been demonstrated in a variety of taxa – both invertebrate (Stoffer & Uetz 2015, Swanger & Zuk 2015, Atwell & Wagner 2014, Tinghitella 2014, Fowler-Finn & Rodriguez 2012, Rutledge et al. 2010, Hebets 2003) and vertebrate (Anderson et al. 2014, Asaba et al. 2014, Guevara-Fiore 2012, Walling et al. 2008). In some cases, a multimodal experience is provided, allowing the focal individuals to experience conspecifics using a combination of sensory modalities (Tinghitella 2014, Rutledge et al. 2010, Hebets 2003). In many cases, however, social experience provided by a single sensory modality is sufficient to result in variation in mating behaviors (Stoffer & Uetz 2015, Swanger & Zuk 2015, Atwell & Wagner 2014, Walling et al. 2008). To our knowledge, this is the first study to demonstrate that female preferences for unimodal signals can vary based on an individual's

courtship modality experience. We encourage other studies to investigate whether social experience has different effects on plasticity in mating preferences in a modality-dependent manner across a variety of animal taxa. Such studies would certainly increase our understanding of the relationship between sensory biology and behavioral plasticity, and comparative studies would improve our understanding of the evolution of such plasticity and the underlying mechanisms required for such plasticity to occur. Further, examining the effects of courtship modality experience in closely-related species could elucidate our understanding of how multimodal signals may have evolved. While some species have demonstrated preferences for novel traits due to a pre-existing sensory bias (see review in Ryan & Cummings 2013), it remains uncertain whether courtship modality experience could be a mechanism in which such a sensory bias may develop and promote speciation over evolutionary time.

608 ACKNOWLEDGEMENTS

609 Funding was provided by NSF Award IOS-1026995 (to GWU) and a University of Cincinnati
610 Biological Sciences Department Wieman-Wendell-Benedict grant (to BS). Research was
611 conducted as part of the dissertation requirements for the Ph.D. in Biological Sciences from the
612 University of Cincinnati. We would like to thank the undergraduate (Madeline Lallo, Corey
613 Vaughn, Rebecca Wilson, Maggie Williams) and graduate (Rachel Gilbert, Elizabeth Kozak, and
614 Alex Sweger) students in the Uetz lab for assistance with animal care, lab maintenance and
615 feedback.
616

REFERENCES

- Anderson, R. C., Peters, S., & Nowicki, S. (2014). Effects of early auditory experience on the development of local song preference in female swamp sparrows. *Behavioral Ecology and Sociobiology*, 68, 437–447.
- Asaba, A., Okabe, S., Nagasawa, M., Kato, M., Koshida, N., Osakada, T., Mogi, K., & Kikusui, T. (2014). Developmental social environment imprints female preference for male song in mice. *PLoS ONE*, 9:e87186. doi:10.1371/journal.pone.0087186.
- Atwell, A., & Wagner, W. E. (2014). Female mate choice plasticity is affected by the interaction between male density and female age in a field cricket. *Animal Behaviour*, 98, 177–183.
- Bailey, N. W., & Zuk, M. (2008). Acoustic experience shapes female mate choice in field crickets. *Proceedings to the Royal Society B*, 275, 2645–2650.
- Bischof, H-J. (2003). Neural mechanisms of sexual imprinting. *Animal Biology*, 53, 89–112.
- Candolin, U. (2003). Use of multiple cues in mate choice. *Biological Reviews*, 78, 575–595.
- Clark, D. L., Roberts, J. A., Rector, M., & Uetz, G. W. (2011). Spectral reflectance and communication in the wolf spider, *Schizocosa ocreata* (Hentz): simultaneous crypsis and background contrast in visual signals. *Behavioral Ecology and Sociobiology*, 65, 1237–1247.
- D'Eath, R. B. (1998). Can video images imitate real stimuli in animal behaviour experiments? *Biology Reviews*, 73, 267–292.
- Dawson, B., & Ryan, M.J. (2012). Female preferences are not altered by early acoustic experience in the neotropical frog *Physalaemus pustulosus*. *Journal of Herpetology*, 46, 535–538.
- Elias, D.O., Hebets, E. A., & Hoy, R. R. (2006). Female preference for complex/novel signals in a spider. *Behavioral Ecology*, 17, 765–771.

640 Fleishman, L. J., McClintock, W. J., D'Eath, R. B., Brainard, D. H., & Endler, J. A. (1998).
641 Colour perception and the use of video playback experiments in animal behaviour. *Animal*
642 *Behaviour*, *56*, 1035–1040.

643 Fowler-Finn, K. D., & Rodríguez, R. L. (2012). The evolution of experience-mediated plasticity
644 in mate preferences. *Journal of Evolutionary Biology*, *25*, 1855–1863.

645 Gibson, J. S., & Uetz, G. W. (2008). Seismic communication and mate choice in wolf spiders:
646 components of male seismic signals and mating success. *Animal Behaviour*, *75*, 1253–1262.

647 Gibson, J. S., & Uetz, G. W. (2012). Effect of rearing environment and food availability on
648 seismic signaling in male wolf spiders (Araneae: Lycosidae). *Animal Behaviour*, *84*, 85–92.

649 Gordon, S. D., & Uetz, G.W. (2011). Multimodal communication of wolf spiders on different
650 substrates: evidence for behavioral plasticity. *Animal Behaviour*, *81*, 367–375.

651 Guevara-Fiore, P. (2012). Early social experience significantly affects sexual behavior in male
652 guppies. *Animal Behaviour*, *84*, 191–195.

653 Hebets, E. A. (2003). Subadult experience influences adult mate choice in an arthropod: exposed
654 female wolf spiders prefer males of a familiar phenotype. *Proceedings of the National*
655 *Academy of Sciences, U. S. A.*, *100*, 13390–13395.

656 Hebets, E. A., & Papaj, D. R. (2005). Complex signal function: developing a framework of
657 testable hypotheses. *Behavioral Ecology and Sociobiology*, *57*, 197–214.

658 Hebets, E. A., & Uetz, G. W. (1999). Female response to isolated signals from multimodal male
659 courtship displays in the wolf spider genus *Schizocosa* (Araneae: Lycosidae). *Animal*
660 *Behaviour*, *57*, 865–872.

661 Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric
662 models. *Biometrical Journal*, *50*, 346–363.

663 Johnson, K., Rosetta, D., Burley, N. D. (1993). Preferences of female American goldfinches
664 (*Carduelis tristis*) for natural and artificial male traits. *Behavioral Ecology*, 4, 138–143.

665 Kasumovic, M. M., & Brooks, R. C. (2011). It's all who you know: the evolution of socially
666 cued anticipatory plasticity as a mating strategy. *Quarterly Review of Biology*, 86, 181–197.

667 Kujala, T., Alho, K., Huottilainen, M., Ilmoniemi, R. J., Lehtokoski, A., Leinonen, A., Rinne, T.,
668 Salonen, O., Sinkkonen, J., Standertskjold-Nordenstam, C. G., Naatanen, R. (1997).
669 Electrophysiological evidence for cross-modal plasticity in humans with early- and late-onset
670 blindness. *Psychophysiology*, 34, 213–216.

671 Madden, J. R., & Whiteside, M. A. (2013). Variation in female mate choice and mating success
672 is affected by sex ratio experience during early life. *Animal Behaviour*, 86, 139–142.

673 McClintock, W. J., & Uetz, G. W. (1996). Female choice and pre-existing bias: visual cues
674 during courtship in two *Schizocosa* wolf spiders (Araenae: Lycosidae). *Animal Behaviour*,
675 52, 167–181.

676 McGregor, P. K. (2000). Playback experiments: design and analysis. *Acta Ethologica*, 3, 3–8.

677 Montgomery, T. H. (1903). Studies on the habits of spiders, particularly those of the mating
678 period. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 55, 59–149.

679 Moskalik, B., & Uetz, G. W. (2011). Female hunger state affects mate choice of a sexually
680 selected trait in a wolf spider. *Animal Behaviour*, 81, 715–722

681 Neville, H. J., Schmidt, A., & Kutas, M. (1983). Altered visual-evoked potentials in congenitally
682 deaf adults. *Brain Research*, 266, 127–132.

683 Norton, S., & Uetz, G. W. (2005). Mating frequency in *Schizocosa ocreata* (Hentz) wolf spiders:
684 evidence for a mating system with female monogamy and male polygamy. *Journal of*
685 *Arachnology*, 33, 16–24.

686 Partan, S. R., & Marler, P. (1999). Communication goes multimodal. *Science*, 283, 1272–1273.

687 Partan, S. R., & Marler, P. (2005). Issues in the classification of multimodal communication
688 signals. *American Naturalist*, 166, 231–245.

689 Persons, M. H., & Uetz, G. W. (1998). Presampling sensory information and prey density
690 assessment by wolf spiders (Araneae, Lycosidae). *Behavioral Ecology*, 9, 360–366.

691 Preininger, D., Boeckle, M., Freudmann, A., Starnberger, I., Sztatecsny, M., & Hodl, W. (2013).
692 Multimodal signaling in the small torrent frog (*Micrixalus saxicola*) in a complex acoustic
693 environment. *Behavioral Ecology and Sociobiology*, 67, 1449–1456.

694 R Core Team. (2013). R: A language and environment for statistical computing. R Foundation
695 for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL [http://www.R-](http://www.R-project.org/)
696 [project.org/](http://www.R-project.org/).

697 Reichert, M. S., & Höbel, G. (2015). Modality interactions alter the shape of acoustic mate
698 preference functions in gray treefrogs. *Evolution*, 69, 2383–2398.

699 Roberts, J. A., Taylor, P. W., & Uetz, G. W. (2007). Consequences of complex signaling:
700 predator detection of multimodal cues. *Behavioral Ecology*, 18, 236–240.

701 Ronald, K. L., Fernández-Juricic, E., & Lucas, J. R. (2012). Taking the sensory approach: how
702 individual differences in sensory perception can influence mate choice. *Animal Behaviour*,
703 84, 1283–1294.

704 Rosenthal, G. G. (2000). Design considerations and techniques for constructing video stimuli.
705 *Acta Ethologica*, 3, 49–54.

706 Rosenthal, G. G., Rand, A. S., & Ryan, M. J. (2004). The vocal sac as a visual cue in anuran
707 communication: an experimental analysis using video playback. *Animal Behaviour*, 68, 55–
708 58.

709 Rutledge, J. M., Miller, A., & Uetz, G. W. (2010). Exposure to multiple sensory cues as a
 710 juvenile affects adult female mate preferences in wolf spiders. *Animal Behaviour*, 80, 419–
 711 426.

712 Ryan, M. J., Cummings, M. E. (2013). Perceptual biases and mate choice. *Annual Review of*
 713 *Ecology Evolution and Systematics*, 44, 437–459.

714 Rybak, F., Sureau, G., & Aubin, T. (2002). Functional coupling of acoustic and chemical signals
 715 in the courtship behaviour of the male *Drosophila melanogaster*. *Proceedings of the*
 716 *Zoological Society of London B*, 269, 695–701.

717 Sadato, N., Pascual-Leone, A., Grafman, J., Ibanez, V., Deiber, M. P., Dold, G., & Hallett, M.
 718 (1996). Activation of the primary visual cortex by Braille reading in blind subjects. *Nature*,
 719 380, 526–628.

720 Scheffer, S. J., Uetz, G. W., & Stratton, G. E. (1996). Sexual selection, male morphology, and
 721 the efficacy of courtship signalling in two wolf spiders (Araneae: Lycosidae). *Behavioral*
 722 *Ecology Sociobiology*, 38, 17–23.

723 Stafstrom, J. A., & Hebets, E. A. (2013). Female mate choice for multimodal courtship and the
 724 importance of the signaling background for selection on male ornamentation. *Current*
 725 *Zoology*, 59, 200–209.

726 Stoffer, B., & Uetz, G. W. (2015). The effects of social experience with varying male availability
 727 on female mate preferences in a wolf spider. *Behavioral Ecology Sociobiology*, 69, 927–937.

728 Stoffer, B., & Uetz, G. W. (2016). Social experience affects female mate preferences for a visual
 729 trait in a wolf spider. *Behavioral Ecology*, 27, 252–261.

730 Swanger, E., & Zuk, M. (2015). Cricket responses to sexual signals are influenced more by adult
 731 than juvenile experiences. *Journal of Insect Behavior*, 28, 328–337.

732 Taylor, R. C., Buchanan, B. W., & Doherty, J. L. (2007). Sexual selection in the squirrel treefrog
 733 *Hyla squirella*: the role of multimodal cue assessment in female choice. *Animal Behaviour*,
 734 74, 1753–1763.

735 Therneau, T. (2012). coxme: Mixed Effects Cox Models. R package version 2.2-3.
 736 <http://CRAN.R-project.org/package=coxme>.

737 Tinghitella, R. M. (2014). Male and female crickets modulate their courtship behaviour
 738 depending on female experience with mate availability. *Animal Behaviour*, 91, 9–15.

739 Uetz, G. W., & Denterlein, G. (1979). Courtship behavior, habitat and reproductive isolation in
 740 *Schizocosa rovnieri* Uetz & Dondale (Araneae: Lycosidae). *Journal of Arachnology*, 7, 121–
 741 128.

742 Uetz, G. W., Papke, R., & Kilinc, B. (2002). Influence of feeding regime on body size, body
 743 condition and a male secondary sexual character in *Schizocosa ocreata* wolf spiders
 744 (Araneae, Lycosidae): Condition-dependence in a visual signaling trait. *Journal of*
 745 *Arachnology*, 30, 461–469.

746 Uetz, G. W., Roberts, J. A., Clark, D. L., Gibson, J. S., & Gordon, S. D. (2013). Multimodal
 747 signals increase active space of communication by wolf spiders in a complex litter
 748 environment. *Behavioral Ecology and Sociobiology*, 67, 1471–1482.

749 Uetz, G. W., Roberts, J. A., & Taylor, P. W. (2009). Multimodal communication and mate
 750 choice in wolf spiders: female response to multimodal versus unimodal signals. *Animal*
 751 *Behaviour*, 78, 299–305.

752 Walling, C. A., Royle, N. J., Lindström, J., & Metcalfe, N. B. (2008). Experience-induced
 753 preference for short-sworded males in the green swordtail, *Xiphophorus helleri*. *Animal*
 754 *Behaviour*, 76, 271–276.

755 Wilgers, D. J., & Hebets, E. A. (2011). Complex courtship displays facilitate male reproductive
756 success and plasticity in signaling across variable environments. *Current Zoology* 57, 175–
757 186.

758 Witte, K., Hirschler, U., & Curio, E. (2000). Sexual imprinting on a novel adornment influences
759 mate preferences in the Javanese manikin *Lonchura leucogastroides*. *Ethology*, 106, 349–
760 363.

761

FIGURE LEGENDS

Figure 1:

The proportion of females that approached the source of male courtship signals over time in no-choice presentations. There was no significant effect of courtship modality experience on latency to approach courtship signals as adults (likelihood ratio test: $\chi^2_4 = 3.0239$, $P = 0.5538$). Thus, all experience treatment groups were pooled together to demonstrate that the adult courtship modality had a significant effect on latency to approach (likelihood ratio test: $\chi^2_4 = 50.661$, $P < 0.0001$). Post hoc tests revealed that females took significantly longer time to approach unimodal vibratory signals in comparison to visual ($Z = 5.448$, $P < 0.0001$) or multimodal ($Z = 6.713$, $P < 0.0001$) signals.

Figure 2:

The mean ($\pm$ SE) number of receptivity displays by adult female *S. ocreata* in response to unimodal and multimodal signals after varying courtship modality experience. Courtship modality experience had a significant effect on a female's relative preference for unimodal and multimodal signals (likelihood ratio tests – vibratory vs visual: $\chi^2_4 = 25.975$, $P < 0.0001$; vibratory vs multimodal: $\chi^2_4 = 12.964$, $P = 0.0115$; visual vs multimodal: $\chi^2_4 = 13.618$, $P = 0.0086$). Lower-case letters indicated the post hoc results for relative unimodal preferences (vibratory vs visual), while upper-case letters indicate post hoc tests for visual vs multimodal preferences. If columns have the same letter, there was no significant difference in females' relative preferences for these signals.

Figure 3:

The mean ($\pm$ SE) receptivity rate by adult female *S. ocreata* in response to unimodal and multimodal signals after varying courtship modality experiences. Courtship modality experience

had a significant effect on the difference in receptivity rates between unimodal visual and unimodal vibratory signals (likelihood ratio test: $\chi^2_4 = 17.299$, $P = 0.0015$). Subsequent post hoc tests demonstrated that females with vibratory experience weighed the unimodal signals differently than females with visual ($Z = 3.585$, $P = 0.0033$) or multimodal ($Z = 3.418$, $P = 0.0059$) experience.

Figure 4:

The proportion of females that approached the source of male courtship signals in two-choice presentations. Only the adult sensory modality had a significant effect on the latency to approach (likelihood ratio test: $\chi^2_1 = 116.57$, $P < 0.0001$). Specifically, post hoc tests demonstrated that females took significantly longer to approach vibratory signals in comparison to visual signals ($Z = 10.86$, $P < 0.0001$).

Figure 5:

The mean ($\pm$ SE) number of receptivity displays towards unimodal signals, based on experimental design in two-choice trials. There was no significant effect of courtship modality experience on receptivity displays towards unimodal signals in a two-choice design (likelihood ratio test: $\chi^2_4 = 7.56$, $P = 0.1091$), but there was a significant effect of experimental design (likelihood ratio test: $\chi^2_1 = 12.193$, $P = 0.0005$). There was a stronger preference for visual signals to vibratory signals when the unimodal signals were separated by 180° than 90° (paired t test: $t_{108} = 2.643$, $P = 0.0094$).

805 **FIGURES:**

806 **Figure 1:**

807

808

809

810 **Figure 2:**

811

812

813 **Figure 3:**

814

815

816

817 **Figure 4:**

818

819

820 **Figure 5:**

821

822

ACKNOWLEDGEMENTS

Funding was provided by NSF Award IOS-1026995 (to GWU) and a University of Cincinnati Biological Sciences Department Wieman-Wendell-Benedict grant (to BS). Research was conducted as part of the dissertation requirements for the Ph.D. in Biological Sciences from the University of Cincinnati. We would like to thank the undergraduate (Madeline Lallo, Corey Vaughn, Rebecca Wilson, Maggie Williams) and graduate (Rachel Gilbert, Elizabeth Kozak, and Alex Sweger) students in the Uetz lab for assistance with animal care, lab maintenance and feedback.