

# Female mate choice for multimodal courtship and the importance of the signaling background for selection on male ornamentation

Jay A. STAFSTROM\*, Eileen A. HEBETS

School of Biological Sciences, University of Nebraska, Lincoln, NE 68588-0118, USA

**Abstract** Conspicuous visual ornaments are frequently incorporated into complex courtship displays that integrate signal components from multiple sensory modalities. Mature male *Schizocosa crassipes* (Walckenaer, 1837) wolf spiders wave, arch, and tap their ornamented forelegs in a visual courtship display that simultaneously incorporates seismic components. To determine the importance of modality-specific signal components in female mate choice, we used a signal ablation design and compared the mating frequency of female-male pairs across signaling environments with manipulated modality-specific transmission properties. We found that the successful transmission of isolated visual or seismic signaling was sufficient for mating success; neither signaling modality was necessary. Additionally, the environment enabling the successful transmission of composite, multimodal displays yielded the highest mating frequencies. Our results indicate the presence of selection from *S. crassipes* females for multimodal courtship and suggest that multimodal signaling may facilitate mating across variable signaling environments. We next explored the influence of ornamentation *per se* on female choice by phenotypically manipulating males into two groups: (i) intact (brushes present) and (ii) shaved (brushes absent). We compared the mating frequencies of intact versus shaved males in the presence versus absence of seismic signaling. Males with brushes intact had higher mating frequencies than shaved males, but only under specific signaling conditions – in the presence of seismic signaling. Female choice for male brushes then appears dependent on the signaling background, making brushes themselves an unlikely target of direct selection. Our results emphasize the complex nature of female choice, highlighting the potential for both trait interactions and environment-dependent selection [*Current Zoology* 59 (2): 200–209, 2013].

**Keywords** Enhancement, Environmental variability, Inter-signal interaction, Sexual selection

Darwin first posited the theory of sexual selection, in part, to account for the abundance and variety of male secondary sexual characters, which he suggested evolve and are maintained by male-male competition or female mate choice (Darwin, 1871). Female mate choice has since been shown to exhibit strong selection pressure on male ornaments (e.g. Hamilton and Zuk, 1982; Hill, 1991; Andersson, 1994; Wilkinson and Reillo, 1994) and investigating how secondary sexual characters influence mating success continues to be a focus for evolutionary biologists (de Heij et al., 2011; Karino et al., 2011; Rick et al., 2011; Kekäläinen et al., 2010; Toomey and McGraw, 2012). However, not all traits deemed to be ornaments have been found to play a vital role in female mate choice (Saether et al., 2000; Westneat, 2006; Takahashi et al., 2008; Shamble et al., 2009), causing researchers to re-evaluate the putative function of such secondary sexual traits. In lieu of an explanation requiring direct selection on male ornaments, increased

support is surfacing for hypotheses suggesting that female mate choice is based upon combinations of traits or signal interactions (Kekäläinen et al., 2010; Grafe et al., 2012; Taylor et al., 2011) and/or upon motor performance, with ornamentation evolving secondarily in an enhancing role (see Byers et al., 2010; Barske et al., 2011; Hebets et al., 2011; Wilgers and Hebets, 2012). The former hypothesis proposes that separate signal components interact in various ways, dependent on signaling system, to alter female response. Such an interaction of signal components is demonstrated in squirrel treefrogs, where female response to auditory signal components is altered by the presence of a visual signal (Taylor et al., 2012). The latter hypothesis posits that motor performance accurately reflects whole-organism performance, with vigor indicating the repeatability of energetically expensive actions and skill indicating the aptitude to perform challenging motor tasks (see Byers et al., 2010). An assessment of motor performance then,

Received Nov. 19, 2012; accepted Feb. 26, 2013.

\* Corresponding author. E-mail: jstaf@huskers.unl.edu

© 2013 *Current Zoology*

potentially augmented by ornamentation, could provide females with more reliable and accurate information regarding a male's survival, genetic quality, and/or developmental history (Byers et al., 2010). The golden-collard manakin *Manacus vitellinus* provides an example in which selection appears to act secondarily on ornamentation. In this species, brightly colored males exhibit complex and physically demanding courtship displays. Females tend to choose males that perform specific parts of the courtship display faster, discriminating between male performances that differ by thousandths of a second (Barske et al., 2011).

Although ornaments are visual signals, they are frequently part of ritualized courtship displays, with components commonly transmitted in other modalities as well (Zuk et al., 1995; Backwell et al., 1999; Hebets and Uetz, 2000; Madsen et al., 2004; Loyau et al., 2005; Murai and Backwell, 2006). Much past research has focused primarily on modality-specific courtship signals (Andersson, 1982; Ryan and Rand, 1990; Basolo, 1990; Gerdhardt, 1991; Ryan and Rand, 1995), but more recent theoretical and empirical work highlights the importance of a more integrative approach, examining combinations of signal components and their interactions (for reviews see Partan and Marler, 1999; Partan and Marler, 2005; Candolin, 2003; Hebets and Papaj, 2005; Wiegmann and Morris, 2005). An integral first step in understanding the function and evolution of such multimodal courtship displays and the associated role of ornamentation is to investigate the relative importance of distinct signal components both in isolation and in concert with the entire display (Partan and Marler, 1999; Partan and Marler, 2005). A recent surge in multimodal signaling research follows this paradigm (e.g. Zeyl and Laberge, 2011; Grafe et al., 2012; Maruska and Fernald, 2012; Stoffer and Walker, 2012).

Numerous studies using a variety of approaches and techniques, [e.g. video playback (e.g. McClintock and Uetz, 1996; Hebets and Uetz, 2000; Uetz and Roberts, 2002; Hebets, 2005; Hebets, 2008), cue isolation (e.g. Scheffer et al., 1996; Hebets and Uetz, 1999; Uetz et al., 2009), and signal ablation (e.g., Hebets, 2005; Hebets, 2008; Rundus et al., 2010; Rundus et al., 2011)] have examined female mate choice for modality-specific, as well as combined, courtship displays in the wolf spider genus *Schizocosa*. Upon sexual maturation, males of multiple *Schizocosa* species develop a conspicuous pattern of pigmentation and/or brushes on appendages associated with courtship movements (reviewed in Stratton, 2005; Framenau and Hebets, 2007). The courtship

displays of various species in the genus are then composed, in part, of stereotyped movements (e.g. foreleg arches or taps) of ornamented legs. Together, the ornamentation and movements comprise the visual courtship display. In conjunction with visual courtship displays (when present), all *Schizocosa* species employ a species-specific seismic signal, and seismic signaling is the proposed ancestral condition for this genus (Stratton, 2005). The seismic signal is known to be crucial for mating success, even in species with foreleg pigmentation and stereotyped courtship movements (*S. uetzi*, Hebets, 2005; *S. stridulans*, Hebets, 2008; *S. floridana*, Rundus et al., 2011). Furthermore, visual ornamentation alone appears unimportant in the mate choice decisions of many previously studied species (*S. uetzi* – Shamble et al., 2008; *S. stridulans* – Hebets et al., 2011; *S. floridana* – Rundus et al., 2011). Male visual signaling associated with ornamentation does not predict male-male agonistic interactions either (at least not in *S. ocreata*), suggesting that it does not function in intra-sexual selection (Delaney et al., 2007). Such results then raise the questions: Why do so some *Schizocosa* species possess seemingly elaborate 'ornaments' and are females exerting selection on such male secondary sexual traits?

While males of many *Schizocosa* species develop dark pigmentation on portions of their forelegs upon maturation, others additionally develop large brushes of black hairs (reviewed in Stratton, 2005). Brushes have evolved independently multiple times within the genus (Stratton, 2005), yet much of our knowledge regarding the function of multimodal signaling and ornamentation in *Schizocosa* female mate choice comes from species that lack such large conspicuous foreleg brushes (e.g. *S. uetzi*, Shamble et al., 2009; *S. floridana*, Rundus et al., 2011; Rosenthal and Hebets, 2012; *S. retorsa*, Rundus et al., 2010; *S. stridulans*, Hebets, 2008, Hebets et al., 2011). In the present study, we examined *S. crassipes* (Walckenaer, 1837), one of only three conspicuously brush-legged *Schizocosa* species to be the focus of prior research (the others being *S. ocreata* and *S. bilineata*). A wealth of knowledge has been accrued over the years on *S. ocreata* (e.g. McClintock and Uetz, 1996; Scheffer et al., 1996; Uetz and Norton, 2007; Uetz et al., 2011; Clark et al., 2012), but only one descriptive study exists for *S. bilineata* (Vaccaro et al., 2010). Additionally, much of the data for *S. ocreata* comes from assessing patterns of female receptivity, which are used as a proxy of mating success; but recent methodological advances allow designs that enable direct female-male contact and thus direct assessment of mating success (e.g.

Hebets, 2005; Hebets, 2008).

Here, we focus on the conspicuously brush-legged species, *S. crassipes*, which incorporates active leg waving, tapping, and arching into its visual courtship display (Miller et al., 1998). Upon maturation, male *S. crassipes* develop brushes of hair that extend beyond the leg on the dorsal and ventral sides of their foreleg tibia. The femur and tibia of the forelegs additionally possess dark pigmentation (Stratton, 2005). These are the legs that are involved in the waving, tapping and arching that make up the active male courtship display (Miller et al., 1998). Prior cue isolation studies using *S. crassipes* found that females responded similarly across isolated and combined courtship signal modalities (visual only, seismic only, visual + seismic; Hebets and Uetz, 1999). Furthermore, a video playback study found no support for female choice based upon foreleg ornamentation (Hebets and Uetz, 2000). Here, we follow up on these studies using both more natural and biologically relevant experimental techniques that enable female-male interactions as well as larger sample sizes, and revisit the function of both multimodal signaling and male brushes in *S. crassipes*.

To determine the relative importance of visual, seismic, and combined multimodal courtship signaling, we first use a signal ablation approach and assess mating frequencies across four manipulated signaling environments which independently reduce modality-specific transmission efficacy. We next assess the importance of brushes *per se* by examining their influence on mating success in the presence versus absence of seismic signaling.

## 1 Materials and Methods

### 1.1 Spiders

Immature *S. crassipes* were collected at a single location near Newnan's Lake in Gainesville, Florida (N 29° 40.504', W 82° 13.582') on January 23rd, 2009. All spiders were brought back to the laboratory, housed individually in 5.9 cm × 5.9 cm × 7.7cm clear plastic containers (Amac Plastic Products, Petaluma, CA, USA), and provided with water *ad libitum*. They were kept on a 12:12 h light: dark cycle at room temperature (22–24 °C). Molt checks were conducted every other day to monitor development. All spiders were fed 1–4 crickets once per week.

### 1.2 Experiment 1: Importance of signaling modalities

To test the importance of distinct signaling modalities and modality combinations (visual and seismic) in fe-

male choice (as indicated by male mating success), we compared mating frequencies across four manipulated signaling environments encompassing every combination of the presence/absence of visual (V+/V-) and seismic (S+/S-) signaling. Spiders experienced a visual environment of light versus dark (V+/V-) and a seismic environment of filter paper substrate versus granite substrate (S+/S-). In all four signaling environments, mating trials took place in circular plastic arenas measuring 12.5 cm diameter × 7.5 cm height. To mimic natural conditions, we attached photographs of the collection site substrate to the sides of the arena and glued small construction paper leaves to the top to provide cover. Visual signal present trials (V+S+ and V+S-) were run under 2 Vita-Lite full spectrum 30-W fluorescent bulbs (Duro-Test Lighting Inc., Philadelphia, PA), while visual signal absent trials (V-S+ and V-S-) were run in complete darkness (less than .001 lux) and videotaped under infrared illumination. Seismic signal present trials (V+S+ and V-S+) were run in arenas with bottoms lined with a piece of Whatman #1 185-mm filter paper (Schleicher and Schuell, Keene, NH). Seismic signal absent trials (V+S- and V-S-) were run in arenas with the bottoms cut out and replaced by a granite slab, as granite does not allow for the effective propagation of spider seismic signals (Elias et al., 2004; Sullivan-Beckers and Hebets, 2011). All trials were run during the day under normal diurnal conditions for the spiders. All females and males were virgins and had never previously interacted with a mature conspecific. These spiders live a single year and we collected individuals more than a month prior to their maturation, long after adults from the previous generation had died.

Females and males were weighed immediately preceding mate choice trials. Females were placed in the arena and allowed to acclimate for 30 minutes while males acclimated within an inverted glass vial for one minute directly preceding trial commencement. Trials began when the glass vial was removed and males could freely move about the arena and ended after 30 minutes had passed or successful copulation. All trials were videotaped using a Sony Handycam HDR-HC9 video camera. Visual signal absent trials were videotaped using the NightShot recording option, which allows the camera to record using infrared illumination. Videotapes were scored for male courtship rate, copulation success, and latency to copulate. Male courtship rate was defined as the number of body bounces per minute within the first two minutes of courtship. Only males that initiated courtship were used in statistical analyses (8 out of 94

males failed to initiate courtship).

We used a Chi Square analysis to compare the mating success across our four signaling environments (V+/S+; V+/S-; V-/S+; V-/S-). We were interested specifically in whether pairs were more likely to mate in the presence of a complete composite display as opposed to an isolated visual or seismic display (*i.e.* V+S+ versus V+S-; V+S+ versus V-S+) and thus used a Chi Square analysis to conduct pairwise comparisons between isolated and combined signal components. Additionally, in order to directly assess the influence of each signaling modality, we used a similar analysis to compare the likelihood of copulation between each signal in isolation (V+S- versus V-S+). We used a non-parametric Kruskal-Wallis test to compare the time to mating across the four signaling environments.

Given that courtship rate is a variable known to be important in the mating success of other *Schizocosa* species (Delaney et al., 2007; Gibson and Uetz, 2008; Shamble et al., 2009; Hebets et al., 2011; Rundus et al., 2010, 2011), we used a non-parametric Kruskal-Wallis test to determine whether males courted at different rates across the four signaling environments. We conducted the same test on female age since this is another variable known to be important in mate choice in other *Schizocosa* species (Norton and Uetz, 2005).

### 1.3 Experiment 2: Importance of brushes in the presence/absence of seismic signaling

In Experiment 1, the highest mating frequency was in the signaling environment that enabled the transmission of both visual and seismic courtship components, demonstrating an additive effect of combining the two modalities (visual plus seismic) on female mate choice and male mating success. The visual components of *S. crassipes* male courtship consist of physical movements (*i.e.* the waving and tapping of forelegs) and ‘ornamentation’ (*i.e.* foreleg brushes and/or pigmentation). This second experiment focused on one component of visual signaling – foreleg brushes. We examined whether the presence of male brushes influences female mate choice when presented in isolation (S-) versus in combination with seismic signaling (S+). Similar to Experiment 1, we compared mating frequencies across four treatments - the treatments for this experiment, however, encompass every combination of the presence/absence of male brushes (intact/shaved) and the presence/absence of seismic signaling (S+/S-). All trials were run in the light and we haphazardly assigned males to their brush treatment: (i) intact – those with brushes left intact (control males) and (ii) shaved – those with foreleg

brushes removed. Copulation success was recorded for each trial. Courtship rate and latency to copulate were not recorded for these trials.

To shave off brushes, males were placed into a 16.51 cm x 15.24 cm Ziploc bag with a small hole cut into it allowing for the spiders forelegs to extend outside of the bag. A 25G BD hypodermic needle was then used to gently remove the male’s foreleg brushes without damaging the cuticle. Sham trials were conducted for males assigned to the intact ornament group (control males). The procedure for sham shaving was identical to actual shaving except that the dull side of the hypodermic needle was run back and forth over the brushes mimicking stimulation experienced by the shaved males. Males from both treatments were kept in the Ziploc bag for about 5 minutes and we observed no notable differences in behavior following manipulations.

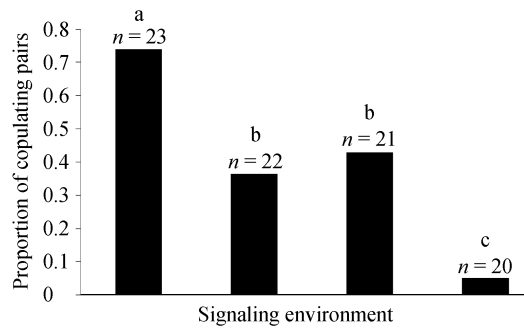
Similar to Experiment 1, we used a Chi Square analysis to compare mating frequencies across our four treatments. Since we were specifically interested in the influence of brushes on female choice in isolation versus in combination with seismic signaling, we conducted separate pairwise comparisons of mating frequency of intact vs. shaved males for each seismic signaling treatment (S+ intact vs. shaved; S- intact vs. shaved).

## 2 Results

### 2.1 Experiment 1: Importance of signaling modalities

A total of 86 females and 86 males were run through single choice mating trials across four variable signaling environments ( $n = 23$  V+/S+;  $n = 22$  V+/S-;  $n = 21$  V-S+;  $n = 20$  V-S-). Mating frequencies differed among the four treatments ( $\chi^2_3 = 24.361$ ;  $P < 0.0001$ , Fig. 1). Pairs were more likely to mate in the presence of both signaling modalities (V+S+) as compared to either signal in isolation (V+S+ versus V+S-,  $\chi^2_1 = 4.45$ ,  $P = .0349$ ; V+S+ versus V-S+,  $\chi^2_1 = 6.58$ ,  $P = 0.0103$ , Fig. 1). No difference was found between treatments where signals were isolated (V+S- versus V-S+,  $\chi^2_1 = 0.19$ ,  $P = 0.6633$ ). Mating frequency in the absence of both modalities (V-S-) was significantly lower than any other treatment ( $P < 0.002$  for all comparisons, Fig. 1). A non-parametric Kruskal-Wallis test indicated no significant difference among signaling environments in terms of time to copulation ( $\chi^2_3 = 4.98$ ,  $df = 3$ ,  $P = 0.17$ ).

Weights did not vary significantly across signaling environments for females (Kruskal-Wallis,  $\chi^2_3 = 3.532$ ,  $P = 0.32$ ) or males (Kruskal-Wallis,  $\chi^2_3 = 1.834$ ,  $P =$



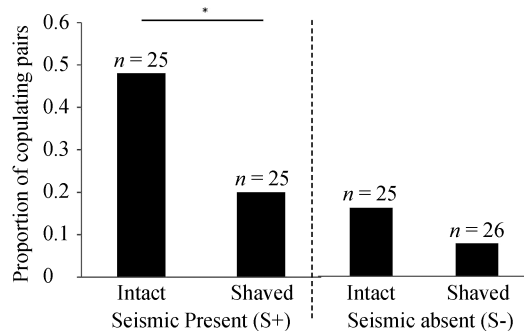
**Fig. 1** Proportion of copulating pairs across manipulated signaling environments

Mating frequency was dependent on signaling environment. Pairs were most likely to mate when both visual and seismic components were present. Isolating visual and seismic components resulted in equivalent mating frequencies. Different letters (a, b, or c) represent significant differences between treatments.

0.61). Male courtship rate (# of body bounces/min) did not vary across signaling environments (Kruskal-Wallis,  $\chi^2_3 = 2.295$ ,  $P = 0.513$ ), nor did female age (Kruskal-Wallis,  $\chi^2_3 = 1.728$ ,  $P = 0.63$ ).

## 2.2 Experiment 2: Importance of brushes in the presence/absence of seismic signaling

A total of 101 females and 101 males were run through single choice mating trials across four treatments ( $n = 25$  intact /S+;  $n = 25$  shaved /S+;  $n = 25$  intact /S-;  $n = 26$  shaved/S-). Mating frequencies differed among the four treatments ( $\chi^2_3 = 12.65$ ;  $P = 0.0055$ , Figure 2). Given that results from Experiment 1 indicated an additive effect of visual and seismic signaling, we were particularly interested in the influence of brushes *per se* in the presence/absence of seismic signaling. In the presence of seismic signaling, males with brushes left intact were more likely to achieve a copulation than those with brushes shaved ( $\chi^2_1 = 4.47$ ,  $P = 0.035$ ; Fig. 2). In the absence of seismic signaling, we



**Fig. 2** Proportion of copulating pairs for males across brush treatments

Seismic present trials (S+) are to the left of the dotted line, while seismic absent trials (S-) are to the right. Only in the seismic present treatment were intact males more likely to mate than shaved males.

found no difference in mating success between shaved males versus intact (control) males ( $\chi^2_1 = 0.86$ ,  $P = 0.35$ ; Fig. 2).

## 3 Discussion

### 3.1 Importance of signaling modalities

This study demonstrates that the visual and seismic components of the multimodal courtship display of *Schizocosa crassipes* are each sufficient for, and equivalent in, facilitating mating; yet mating frequency was highest when both signaling modalities were presented in combination. This additive effect of combined signaling modalities indicates female choice for multimodal versus unimodal courtship. None-the-less, the presence of a single modality (visual or seismic) is sufficient for mating in this species, demonstrating that the multimodal courtship display of *S. crassipes* can facilitate mating across variable environmental conditions (Candolin, 2003; Hebets and Papaj, 2005). Our results concerning the importance of unimodal and multimodal courtship signal function in *S. crassipes* are consistent with multiple sources of selection acting on complex signal evolution: selection from females for multimodality or complexity and selection for transmission efficacy across variable environments.

Despite the occurrence of species-specific courtship displays that incorporate tapping and waving of ornamented legs, there is little prior support for the importance of visual signaling in *Schizocosa* female mate choice (Hebets, 2005; Taylor et al., 2006; Hebets, 2008; Rundus et al., 2011). Seismic courtship is the proposed ancestral modality for *Schizocosa* wolf spiders (Stratton, 2005) and of the species studied to date the majority requires the successful propagation of seismic signals for female acceptance of males (Hebets, 2005; Hebets, 2008; Rundus et al., 2010; Rundus et al., 2011; Hebets et al., *in revision*). In this study of *S. crassipes*, although successful seismic signal transmission is not necessary for female mate choice, it is sufficient to initiate mating. Unlike the results of other previously studied species, however, the successful propagation of visual signaling is also sufficient for mating success. Female *S. crassipes* will accept males in the absence of seismic signaling as long as a visual display is present. Mating frequencies in the presence of isolated visual versus isolated seismic signaling do not differ, suggesting that the modality-specific display components can function as back-ups to each other and may potentially provide redundant information (Partan and Marler, 1999; Partan and Marler, 2005; Hebets and Papaj, 2005). While our study does

not address the potential information content of signal components, the importance of visual versus seismic signaling in female choice appears similar in *S. crassipes* and if they indeed convey redundant information, we would predict a tight co-variance between signals in these two modalities.

Our results are consistent with those of a prior cue isolation study conducted on *S. crassipes* that demonstrated that females were equally receptive to isolated visual versus seismic courtship displays (Hebets and Uetz, 1999). Similar results are also seen in *S. ocreata* and were argued to provide evidence of redundant signals (Uetz et al., 2009). We wished to more directly compare the importance of visual and seismic courtship signals between these two brush-legged species, and thus we calculated effect sizes for the influence of visual and seismic signals on mating success (see Hebets et al., in revision for details). Effect sizes for *S. ocreata* were calculated from data presented in Uetz et al. (2009) and are very similar to those we calculate for *S. crassipes* in this study (*S. ocreata* visual  $r = 0.42$ , seismic  $r = 0.33$ , calculated in Hebets et al., in revision; *S. crassipes*, seismic  $r = 0.38$ , visual  $r = 0.33$ ). The visual and seismic displays of these two brush-legged species appear to be of similar importance in female mate choice and our data for *S. crassipes* are consistent with an efficacy-based ‘multiple sensory environments’ backup hypothesis, where signals in each modality can act as a backup to the other in the presence of environmental variability that reduces modality-specific signal transmission (Candolin, 2003; Hebets and Papaj, 2005). We hesitate, however, to make a suggestion of signal redundancy for *S. crassipes* as we currently have no information regarding signal content – or the pattern of covariance between modality-specific signal components and signaler quality (see Hebets and Papaj, 2005).

While each signaling modality is sufficient for successful mating in *S. crassipes*, the combination of visual and seismic components resulted in higher mating frequencies than those observed under conditions of isolated signaling modalities. Such a synergistic effect has been categorized as enhancement – where the intensity of receiver response is greater to a combined display as compared to each isolated component (reviewed in Partan and Marler, 1999; Partan and Marler, 2005). Enhancement has not only been documented in wolf spiders (Uetz et al., 2009), but has also been observed in other taxa such as fruit flies (Rybak et al., 2002), squirrels (Partan et al., 2009; Partan et al. 2010), pigeons (Partan et al., 2005), butterflies (Costanzo and Monteiro,

2007), and fish (Künzler and Bakker, 2001). Künzler and Bakker (2001), for example, have shown that female sticklebacks exhibit strong preferences for males displaying three components of their courtship display over males displaying two or fewer components. Males with two courtship signal components were also seen as more attractive than males displaying any component in isolation. This enhancement of response to combined signaling modalities could have numerous underlying causes. For example, adding more components to a signal could enable males to convey additional information to females (e.g. species identity, genetic quality, etc.) (Multiple message hypothesis – Møller and Pomiankowski, 1993; Johnstone, 1996; Hebets and Papaj, 2005). Such additional information could aid females in mate choice decisions and increase the likelihood of them accepting a male. Alternatively, if females are basing mate choice decisions mostly on male motor performance, they may be more attracted to more complex displays such as those that incorporate multiple signals produced by distinct physical mechanisms (e.g. stridulation, leg-waiving). Similarly, a recent comparative study encompassing data from 10 *Schizocosa* species suggests that females select for courtship complexity *per se* (Hebets et al., in revision), an idea proposed for other diverse signaling spider groups as well (Elias et al., 2012). In contrast to more content-based hypotheses, increased mating frequencies for multimodal versus unimodal signaling males could also result from increased detectability or discriminability of courtship (Rowe, 1999); although we might also expect to see differences in the time to mating if this were the case, which we do not. An inter-signal interaction in which the response to one component is increased in the presence of another component (*sensu* Hebets and Papaj, 2005) could also lead to an observation of enhancement. These hypotheses all require additional tests, but the result remains - females are more likely to mate with males displaying multimodal versus unimodal courtship displays.

### 3.2 Importance of brushes

Results from our second experiment confirm that female choice for male brushes exists in *S. crassipes*. Importantly, however, this female choice for males possessing foreleg brushes is only realized in the presence of multimodal courtship displays. The conspicuous foreleg brushes (i.e. ornamentation) that male *S. crassipes* develop upon sexual maturation appear to influence female mate choice only when in combination with seismic courtship signaling. The influence of female

mate choice on male brushes in *S. crassipes* can thus vary across signaling backgrounds, indicating that brushes *per se* are not under direct selection from female choice.

Our second experiment compared mating frequencies between intact and shaved males across seismic signaling environments (S+ vs. S-). Intact males experienced higher mating success than shaved males, but only in the presence of seismic signaling. These results suggest that females may attend to male brushes more under particular environmental conditions, such as when they can also perceive seismic signals. Similar findings have been recognized in other wolf spiders, where ornamented males achieve higher mating success only in the presence (versus absence) of seismic signaling (e.g. *Rabidosa rabida*, Wilgers and Hebets, 2011) and females are more likely to display receptivity to more ornamented males only in the presence of seismic signaling (*S. uetzi*, Hebets, 2005). In each of these examples, it has been suggested that the seismic signal alters or focuses a female's visual attention. Future studies are required to test this specific hypothesis in *S. crassipes*, but it is clear that foreleg brushes are more beneficial to males in the presence of the composite display, providing additional evidence for an additive effect of combining signaling modalities for female mate choice. We point out that since the effect of ornamentation varied across signaling environments, an intact male mating advantage is unlikely to be explained by artifacts associated with the phenotypic manipulations themselves.

Prior research using video playback similarly failed to find differences in *S. crassipes* female receptivity responses to isolated visual stimuli that varied in the degree of foreleg ornamentation (Hebets and Uetz, 2000). Curiously, these results are in stark contrast to those of *S. ocreata*, where a cue isolation design involving intact and shaved males in the absence of seismic signaling revealed that a higher number of females displayed receptivity to intact versus shaved males and simultaneous choice trials resulted in equal mating frequencies between intact and shaved males (Scheffer et al., 1996). These incongruent results, found in two distinctly ornamented *Schizocosa* species, suggest that multimodal courtship signaling functions differently among species. Prior evidence of variable functions of multimodal signaling among closely related species has already been documented in *Schizocosa*. Multimodal signaling functions differently between *S. uetzi* and *S. stridulans*, where the seismic signal alters a female's visual attention in *S. uetzi* (Hebets, 2005) and the seis-

mic signal dominates the visual signal in *S. stridulans* (Hebets, 2008). Based upon phylogenetic evidence (morphological phylogeny, Stratton 2005; preliminary CO1 molecular phylogeny; Hebets et al., *in revision*), *S. crassipes* and *S. ocreata* represent independent origins of male foreleg brushes. Future comparative work incorporating additional brush-legged species will further inform our understanding of multimodal signal evolution and function in this genus.

Courtship displays are frequently repetitive and vigorous in nature (reviewed in Byers et al., 2010) and there has been increased interest in, and support for, the hypothesis that females choose mates based upon their motor performance during these vigorous, and presumably costly, displays (Husak et al., 2008; Byers et al., 2010 and references therein; Barske et al., 2011; Hebets et al., 2011; Neave et al., 2011; Spencer and MacDougall-Shackleton, 2011). Multiple studies focusing on different *Schizocosa* species have provided evidence of the importance of courtship rate in *Schizocosa* female mate choice (Shamble et al., 2009; Hebets et al., 2011; Rundus et al., 2011; Rosenthal and Hebets, 2012). Such advantages for males with higher courtship rates may indicate female mate choice for male motor performance. Courtship rate is also known to interact with male secondary sexual traits in one *Schizocosa* species. Prior work with *S. stridulans* has shown that male foreleg pigmentation is important only through its interaction with courtship rate and not through direct female mate choice – increased ornamentation eases a male's reliance on courtship rate to acquire a mating (Hebets et al., 2011). Unfortunately, we are unable to address this type of interaction in the present study since we do not have courtship rates for our intact/shaved trials and we were unable to obtain accurate, trustworthy brush measurements for our males from Experiment 1. Given the importance of both courtship rate and brush presence in *S. crassipes*, future work should examine a potential interaction between the two.

### 3.3 Conclusions

The patterns documented herein are unique thus far among *Schizocosa* wolf spiders. All prior species examined with a signal ablation approach demonstrate a reliance on seismic signaling for mating success (Hebets, 2005; Hebets, 2008; Rundus et al., 2011); while our results show that visual signaling is sufficient for female mate choice in *S. crassipes*. Furthermore, prior work on the brush-legged species *S. ocreata* indicates female mate choice for brushes in the absence, versus presence, of seismic signaling (Scheffer et al., 1996), a result op-

posite that documented here for *S. crassipes*.

Results of our first study demonstrate that the visual and seismic courtship signals of *S. crassipes* are each sufficient for successful mating, but females are more likely to mate with males when they can perceive the composite, multimodal display. The finding that females are equally likely to accept a male based upon only visual or only seismic courtship signaling suggests that males engaging in multimodal courtship can increase the likelihood of attracting a female across variable signaling environments (e.g. environments with reduced modality-specific signal transmission). Additionally, that females were more likely to mate with multimodal signaling males suggests that female mate choice, in addition to selection for transmission efficacy, is an important factor in the evolution of complex multimodal signaling in this species. Results from our second experiment, using phenotypic manipulations of males across signaling backgrounds, established that females base mate choice decisions on the presence/absence of male brushes only in the presence of seismic signaling. These results suggest that female mate choice in this species does not act directly on the male secondary sexual trait - foreleg brushes. Such results again highlight the potential for signal interactions and demonstrate the potential for selection from female choice to vary across signaling backgrounds. Approaches such as those taken here, which assess receiver responses to isolated and combined displays and manipulate display components across signaling environments, can ultimately lend insight into how different types of selection might act on communication systems and can reveal the complex, multivariate nature of selection.

**Acknowledgements** We would like to thank M. Rosenthal, A. Rundus, S. Cowles, R.A. Powers, N. Muchhala, S. Purandare, and K. Funk for their valuable feedback on earlier versions of this manuscript, and M. Hansen and A. Swoboda for their help with feeding spiders. M. Rosenthal, G. Stratton, and P. Miller were invaluable with their aid in locating and collecting *S. crassipes*. We would also like to thank GB Edwards for helping locate collection sites. This work was supported by the National Science Foundation (IOS – 0643179) to EAH.

## References

- Andersson M, 1982. Sexual selection, natural selection and quality advertisement. *Biology Journal of the Linnean Society* 17: 375–393.
- Andersson M, 1994. *Sexual Selection*. Princeton, NJ: Princeton University Press.
- Backwell PRY, Jennions MD, Christy JH, Passmore NI, 1999. Female choice in the synchronously waving fiddler crab *Uca annulipes*. *Ethology* 105: 415–421.
- Barske J, Schlinger BA, Wikelski M, Fusani L, 2011. Female choice for male motor skills. *Proceedings of the Royal Society B* 278: 3523–3528.
- Basolo AL, 1990. Female preference predates the evolution of the sword in swordtail fish. *Science* 250: 808–810.
- Byers J, Hebets E, Podos J, 2010. Female mate choice based upon male motor performance. *Animal Behaviour* 79: 771–778.
- Candolin U, 2003. The use of multiple cues in mate choice. *Biology Reviews* 78: 575–595.
- Clark DL, Roberts JA, Uetz GW, 2012. Eavesdropping and signal matching in visual courtship displays of spiders. *Biology Letters* 8: 375–378.
- Costanzo K, Monteiro A, 2007. The use of chemical and visual cues in female choice in the butterfly *Bicyclus anynana*. *Proceedings of the Biological Sciences* 274: 845–851.
- Darwin C, 1871. *The descent of Man, and Selection in Relation to Sex*. London: John Murray.
- de Heij ME, Gustafsson L, Brommer JE, 2011. Experimental manipulation shows that the white wing patch in collared flycatchers is a male sexual ornament. *Ecology and Evolution* 1: 546–555.
- Delaney KJ, Roberts JA, Uetz GW, 2007. Male signaling behavior and sexual selection in a wolf spider (Araneae: Lycosidae): A test for dual functions. *Behavior, Ecology, and Sociobiology* 62: 67–75.
- Elias DO, Maddison WP, Peckmezian C, Girard MB, Mason AC, 2012. Orchestrating the score: Complex multimodal courtship in the *Habronattus coecatus* group of *Habronattus* jumping spiders. *Biological Journal of the Linnean Society* 105: 522–547.
- Elias DO, Mason AC, Hoy RR, 2004. The effect of substrate on the efficacy of seismic courtship signal transmission in the jumping spider *Habronattus dossenus*. *Journal of Experimental Biology* 207: 4105–4110.
- Framenau VW, Hebets EA, 2007. A review of leg ornamentation in male wolf spiders, with the description of new species from Australia, *Artoria schizocoides* (Araneae, Lycosidae). *Journal of Arachnology* 35: 89–101.
- Gerhardt HC, 1991. Female mate choice in tree frogs: Static and dynamic acoustic criteria. *Animal Behaviour* 42: 615–635.
- Grafe TU, Preininger D, Sztatecsny M, Kasah R, Dehling JM et al., 2012. Multimodal communication in a noisy environment: A case study of the Bornean rock frog *Staurois parvus*. *PLoS One* 7(5): e37965.
- Gibson JS, Uetz GW, 2008. Seismic communication and mate choice in wolf spiders I: Components of male seismic signals and mating success. *Animal Behaviour* 75: 1253–1262.
- Hamilton WD, Zuk M, 1982. Heritable true fitness and bright birds: A role for parasites? *Science* 218: 384–387.
- Hebets EA, 2005. Attention-altering signal interactions in the multimodal courtship display of the wolf spider *Schizocosa uetzi*. *Behavioral Ecology* 16: 75–82.
- Hebets EA, 2008. Seismic signal dominance in the multimodal courtship display of the wolf spider *Schizocosa stridulans*. *Behavioral Ecology* 19: 1258–1266.
- Hebets EA, Papaj DR, 2005. Complex signal function: Developing a framework for testable hypotheses. *Behavioral Ecology and Sociobiology* 57: 197–214.



- Hebets EA, Stafstrom JA, Rodriguez RL, Wilgers DJ, 2011. Enigmatic ornamentation eases male reliance on courtship performance for mating success. *Animal Behavior* 81: 963–972.
- Hebets EA, Uetz GW, 1999. Female responses to isolated signals from multimodal male courtship displays in the wolf spider genus *Schizocosa* (Araneae: Lycosidae). *Animal Behaviour* 57: 865–872.
- Hebets EA, Uetz GW, 2000. Leg ornamentation and the efficacy of courtship display in four species of wolf spider (Araneae: Lycosidae). *Behavioral Ecology and Sociobiology* 47: 280–286.
- Hebets EA, Vink C, Sullivan-Beckers L, Rosenthal M, 2012. The dominance of seismic signaling and the evolution of signal complexity in the multimodal courtship displays of *Schizocosa* wolf spiders. *Behavioral Ecology and Sociobiology in revision*
- Hill GE, 1991. Plumage coloration is a sexually selected indicator of male quality. *Nature* 350: 337–339.
- Husak J, Fox S, Van Den Bussche R, 2008. Faster male lizards are better defenders not sneakers. *Animal Behaviour* 75: 1725–1730.
- Johnstone RA, 1996. Multiple displays in animal communication: ‘backup signals’ and ‘multiple messages.’ *Philosophical Transactions: Biological Sciences* 351: 329–338.
- Karino K, Ishiwatari T, Kudo H, Sato A, 2011. Female mate preference for a costly ornament in male guppies. *Behavioral Ecology and Sociology* 65: 1305–1315.
- Kekäläinen J, Valkama H, Huuskonen H, Taskinen J, 2010. Multiple sexual ornamentation signals male quality and predicts female preference in minnows. *Ethology* 116: 895–903.
- Künzler R, Bakker TCM, 2001. Female preference for single and combined traits in computer animated stickleback males. *Behavioral Ecology* 12: 681–685.
- Loyau A, Saint Jalme M, Cagniant C, Sorci G, 2005. Multiple sexual advertisements honestly reflect health status in peacocks *Pavo cristatus*. *Behavioral Ecology and Sociobiology* 58: 552–557.
- Madsen V, Balsby JS, Dabelsteen T, Osorno JL, 2004. Bimodal signaling of a sexually selected trait: Gular pouch drumming in the magnificent frigate bird. *Condor* 106: 156–160.
- Maruska KP, Fernald RD, 2012. Contextual chemosensory urine signaling in an African cichlid fish. *Journal of Experimental Biology* 215: 68–74.
- McClintock WJ, Uetz GW, 1996. Female choice and pre-existing bias: Visual cues during courtship in two *Schizocosa* wolf spiders (Araneae: Lycosidae). *Animal Behaviour* 52: 167–181.
- Miller CL, Stratton CE, Miller PE, Hebets EA, 1998. Geographic variation in male courtship behavior and sexual isolation in wolf spiders of the genus *Schizocosa*. *Animal Behaviour* 56: 937–951.
- Murai M, Backwell PRY, 2006. A conspicuous courtship signal in the fiddler crab *Uca perplexa*: Female choice based on display structure. *Behavioral Ecology and Sociobiology* 60: 736–741.
- Neave N, McCarty K, Freynik J, Caplan N, Hoenekopp J et al., 2011. Male dance moves that catch a woman’s eye. *Biology Letters* 7: 221–224.
- Norton S, Uetz GW, 2005. Mating frequency in *Schizocosa ocreata* (Hentz) wolf spiders: Evidence for a mating system with female monogamy and male polygamy. *Journal of Arachnology* 33: 16–24.
- Partan SR, Fulmer AG, Gounard MA, Redmond JE, 2010. Multimodal alarm behavior in urban and rural gray squirrels studied by means of observation and a mechanical robot. *Current Zoology* 56: 313–326.
- Partan SR, Larco CP, Owens MJ, 2009. Wild tree squirrels respond with multisensory enhancement to conspecific robot alarm behaviour. *Animal Behaviour* 77: 1127–1135.
- Partan S, Marler P, 1999. Behavior-communication goes multimodal. *Science* 283: 1272–1273.
- Partan S, Marler P, 2005. Issues in the classification of multimodal communication signals. *The American Naturalist* 166: 231–45.
- Partan S, Yelda S, Price V, Shimizu T, 2005. Female pigeons, *Columba livia*, respond to multisensory audio/video playbacks of male courtship behaviour. *Animal Behaviour* 70: 957–966.
- Preininger D, Boeckle M, Hödl W, 2009. Communication in noisy environments II: Visual signaling behavior of male foot-flagging frogs *Staurois latopalmaris*. *Herpetologica* 65: 166–173.
- Rick IP, Mehliis M, Bakker TCM, 2011. Male red ornamentation is associated with female red sensitivity in sticklebacks. *PLoS One* 6: e25554.
- Rosenthal MF, Hebets EA, 2012. Resource heterogeneity interacts with courtship rate to influence mating success in the wolf spider *Schizocosa floridana*. *Animal Behaviour* in press.
- Rowe C, 1999. Receiver psychology and the evolution of multi-component signals. *Animal Behaviour* 58: 921–931.
- Rundus AS, Santer RD, Hebets EA, 2010. Multimodal courtship efficacy of *Schizocosa retrorsa* wolf spiders: Implications of an additional signal modality. *Behavioral Ecology* 21: 701–707.
- Rundus AS, Sullivan-Beckers L, Wilgers D, Hebets EA, 2011. Females are choosy in the dark: Context-dependent reliance on courtship components and its impact on fitness. *Evolution* 65: 268–282.
- Ryan MJ, Rand AS, 1990. The sensory basis of sexual selection for complex calls in the túngara frog *Physalaemus pustulosus* (sexual selection for sensory exploitation). *Evolution* 44: 305–314.
- Ryan MJ, Rand AS, 1995. Female responses to ancestral advertisement calls in the túngara frog. *Science* 269: 390–392.
- Rybak F, Aubin T, Moulin B, Jallon JM, 2002. Acoustic communication in *Drosophila melanogaster* courtship: Are pulse- and sine-song frequencies important for courtship success? *Canadian Journal of Zoology* 80: 987–996.
- Saether SA, Fiske PJ, Kalas A, Gjul JM, 2000. Females of the lekking great snipe do not prefer males with whiter tails. *Animal Behaviour* 59: 273–280.
- Scheffer SJ, Uetz GW, Stratton GE, 1996. Sexual selection, male morphology, and the efficiency of courtship signaling in two wolf spiders (Araneae: Lycosidae). *Behavioral Ecology and Sociobiology* 38: 17–23.
- Shamble PS, Wilgers DJ, Swoboda KA, Hebets EA, 2009. Courtship effort is a better predictor of mating success than ornamentation for male wolf spiders. *Behavioral Ecology* 20: 1242–1251.
- Spencer KA, MacDougall-Shackleton SA, 2011. Indicators of development as sexually selected traits: The developmental stress hypothesis in context. *Behavioral Ecology* 22: 1–9.

- Stoffer B, Walker SE, 2012. The use of multimodal communication in mate choice decisions by female house crickets *Acheta domestica*. *Animal Behaviour* 83: 1131–1138.
- Stratton GE, 2005. Evolution of ornamentation and courtship behavior in *Schizocosa*: Insights from a phylogeny based on morphology (Araneae, Lycosidae). *Journal of Arachnology* 33: 347–376.
- Sullivan-Beckers L, Hebets EA, 2011. Modality-specific experience with female feedback increases the efficacy of courtship signaling in male wolf spiders. *Animal Behavior* 82: 1052–1057.
- Takahashi M, Arita H, Hiraiwa-Hasegawa M, Hasegawa T, 2008. Peahens do not prefer peacocks with more elaborate trains. *Animal Behaviour* 75: 1209–1219.
- Taylor PW, Roberts JA, Uetz GW, 2006. Mating in the absence of visual cues by *Schizocosa ocreata* (Hentz 1844) wolf spiders. *Journal of Arachnology* 34: 501–505.
- Taylor RC, Klein BA, Ryan MJ, 2011. Inter-signal interaction and uncertain information in anuran multimodal signals. *Current Zoology* 57:153–161.
- Toomey MB, McGraw KJ, 2012. Mate choice for a male carotenoid-based ornament is linked to female dietary carotenoid intake and accumulation. *BMC Evolutionary Biology* 12: 3–15.
- Uetz GW, Clark DL, Roberts JA, Rector M, 2011. Effect of visual background complexity and light level on detection of visual signals of male *Schizocosa ocreata* wolf spiders by female wolf spider receivers. *Behavioral Ecology and Sociobiology* 65: 753–761.
- Uetz GW, Norton S, 2007. Preference for male traits in female wolf spiders varies with the choice of available males, female age and reproductive state. *Behavioral Ecology and Sociobiology* 61: 631–641.
- Uetz GW, Roberts JA, 2002. Multisensory cues and multimodal communication in spiders: Insights from video/audio playback studies. *Brain Behavior and Evolution* 59: 222–230.
- Uetz, GW, Roberts JA, Taylor PW, 2009. Multimodal communication and mate choice in wolf spiders I: Female responses to multimodal vs. unimodal male signals in two sibling wolf spider species. *Animal Behaviour* 278: 299–305.
- Westneat DF, 2006. No evidence of current sexual selection on sexually dimorphic traits in a bird with high variance in mating success. *The American Naturalist* 167: 171–189.
- Wiegmann DD, Morris M, 2005. Search behavior and mate choice. *Recent Advances in Experimental and Theoretical Biology* 1: 201–216.
- Wilgers DJ, Hebets EA, 2011. Complex courtship displays facilitate male reproductive success and plasticity in signaling across variable environments. *Current Zoology* 57: 175–186.
- Wilgers DJ, Hebets EA, 2012. Age-related female mating decisions are condition dependent in a wolf spider. *Behavioral Ecology and Sociobiology* 66: 29–38.
- Wilkinson GS, Reillo PR, 1994. Female choice response to artificial selection on an exaggerated male trait in a stalk-eyed fly. *Proceedings of the Royal Society of London, Series B* 255: 1–6.
- Vaccaro R, Uetz GW, Roberts JA, 2010. Courtship and mating behavior of the wolf spider *Schizocosa bilineata*. *Journal of Arachnology* 38: 452–459.
- Zeyl JN, Laberge F, 2011. Multisensory signals trigger approach behavior in the fire-bellied toad *Bombina orientalis*: Sex differences and call specificity. *Zoology* 114: 369–377.
- Zuk M, Johnsen TS, MacLarty T, 1995. Endocrine-immune interactions, ornaments and mate choice in red jungle fowl. *Proceedings of the Royal Society of London, Series B* 260: 205–210.