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EXPLORING SOURCES OF SELECTION ON THE MULTIMODAL COURTSHIP DISPLAYS OF TWO SISTER SPECIES OF WOLF SPIDERS: SCHIZOCOSA CRASSIPALPATA AND SCHIZOCOSA BILINEATA

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EXPLORING SOURCES OF SELECTION ON THE MULTIMODAL COURTSHIP
DISPLAYS OF TWO SISTER SPECIES OF WOLF SPIDERS: *SCHIZOCOSA*
CRASSIPALPATA AND *SCHIZOCOSA BILINEATA*

By

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University of Nebraska, 2011

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We examined multiple sources of selection on the multimodal courtship display of two sister species of wolf spiders, *Schizocosa crassipalpata* and *Schizocosa bilineata*. We first experimentally altered body condition in these two species by employing divergent diet treatments. We then tested for differences in adult male seismic courtship displays, visual courtship displays, and foreleg morphology; as well as adult female foreleg morphology. We then simultaneously examined both content and efficacy-based sources of selection on the visual and seismic multimodal courtship display of both species by conducting mate choice trials with high and low diet-quantity individuals across manipulated signaling environments and by conducting mate choice trials where the level of male ornamentation was experimentally manipulated.

Our diet treatments diverged body condition indices between HD and LD males and females of both species. We found condition dependent expression of male *S. crassipalpata* seismic courtship display and foreleg length as well as male *S. bilineata* foreleg length and brush size, and female *S. bilineata* foreleg length. We found no difference in the pattern of copulatory success for either species between males that had their level of foreleg ornamentation experimentally altered. However, we did find a difference in the signal efficacy function of male multimodal courtship displays between species. Male *S. crassipalpata* courtship displays were dominated by the seismic signal, across our treatments copulation success was highest when it was present. Conversely,

male *S. bilineata* courtship displays were dominated by the visual signal, and across our environmental treatments, copulation success was highest when it was present. Male *S. bilineata* also courted significantly more in the visual positive environment. An ecological shift in activity patterns may have caused the initial split between *S. crassipalpata* and *S. bilineata* and a shift in female preference for a more visual display by *S. bilineata* females may have subsequently reinforced reproductive isolation between these two species.

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CHAPTER 1

CONDITION-DEPENDENT EXPRESSION OF SEXUAL TRAITS DIFFERS
BETWEEN TWO SISTER SPECIES OF WOLF SPIDER

Mitchel D. Bern

INTRODUCTION

Sexual selection theory predicts that exaggerated sexual traits will be favored if individuals with larger trait values achieve higher mating success (Darwin 1871). Most research to date on sexually selected traits has focused on male traits involved in courtship and mate acquisition because such traits are often found to be targets of strong directional selection (Delcourt and Rundle 2011). Under this type of selection, expression of the male trait will become relatively greater than the homologous trait in females. Often the directionality of sexual selection does not match that of natural selection. In this case, the benefit to sexual fitness associated with expression of the male trait is offset by the cost of survival, making sexually selected traits costly (Cotton et al. 2004b, Bonduriansky 2007).

Theory predicts that due to the cost associated with exaggerated traits they should develop a higher degree of condition-dependence than non-sexually selected traits, so that higher condition individuals will be able to allocate more metabolic resources toward trait expression than low condition individuals (Rowe & Houle 1996, Cotton et al. 2004a, Bonduriansky 2007). This should lead to a positive covariance between the level of trait expression and level of condition. In this way, the level of trait expression can serve as a reliable indicator of a male's phenotypic quality and thus, underlying genetic quality (Zahavi 1975, Andersson 1986, Grafen 1990, Row and Houle 1996, Iwasa and Pomiankowski 1994, 1999, Cotton 2004b). A popular method for experimentally altering condition is by manipulating diets and examining the effects on one or more sexual traits. Using this method, numerous studies have found support for heightened condition dependence in sexually dimorphic traits that function as courtship signals (Blount et al.

2003, Cotton et al. 2004a, Bonduriansky and Rowe 2005, Punzalan et al. 2008, McGuigan 2009).

One group where condition-dependent expression of sexual traits has been tested are wolf spiders (Araneae: Lycosidae), particularly the genus *Schizocosa*. Male *Schizocosa* wolf spiders court females by producing a series of substrate borne seismic vibrations. Males produce these vibrations by one or more mechanisms including foreleg, palpal or abdomen drumming, body tremulation, or stridulation, using a stridulatory organ located at the tibio-tarsal joint of the males' pedipalps (Rovner 1975, Stratton 2005, Elias et al. 2006). Females lack these stridulatory organs, and do not appear to drum or produce vibrations in any way (Rovner 1975, M. Bern personal observation). Males of some species also possess forelegs with dark pigmentation and or black tibial brushes (Stratton 2005, Framenau and Hebets 2007). These presumed ornaments are presented to females during a leg waving display that varies by species. Numerous studies have found condition-dependent expression of both sexual ornaments (Uetz et al. 2002, Hebets 2008, Shamble et al. 2009, Hebets et al. 2011) and courtship behaviors (Gibson & Uetz 2007, Hebets et al. 2011) in *Schizocosa* wolf spiders. The relative importance of visual and seismic signaling varies greatly among closely related species (Hebets 2005, 2008, Rundus et al. 2010a, b, Hebets et al. 2011), making it an especially attractive system for studying the expression of condition-dependent sexual traits and displays. In this study we used two sister species of *Schizocosa* wolf spider, *S. crassipalpata* and *S. bilineata* (Stratton 2005, Vaccaro et al. 2010) to explore questions about the expression of condition-dependent sexual traits.

Schizocosa crassipalata and *S. bilineata* are found throughout Eastern North America. Their distributions largely overlap, and both sympatric and allopatric populations are known (M. Bern, unpublished data). Their distribution ranges from Southern Canada South to Georgia and Texas, and from the East coast to as far west as South Dakota and Nebraska (Figure 1; Comstock 1940, Kaston 1948, Dondale and Redner 1990, Sierwald et al. 2005, Stratton 2005, Vaccaro et al. 2010). These two species vary greatly in both adult male morphology and courtship display (Emerton & Dondale 1978, Stratton 2005). *Schizocosa crassipalata* males lack foreleg ornamentation and females are slightly larger than males (Emerton & Dondale 1978, Stratton 2005). Males court females in the seismic modality by producing a series of substrate borne seismic vibrations. Courtship in the visual modality is characterized by an asymmetrical tapping of the un-ornamented forelegs. The visual display typically follows the seismic display. *Schizocosa bilineata* males possess dark pigmentation and dark brushes on the tibia of the forelegs at maturation (Emerton & Dondale 1978, Stratton 2005, Vaccaro et al. 2010), and females are also slightly larger than males (Stratton 2005). Males court females in the seismic modality by producing a series of substrate borne seismic vibrations. Courtship in the visual modality is characterized by an incremental lowering of one or both ornamented forelegs culminating in a quick tap of the substrate (Vaccaro et al. 2010). The seismic and visual displays of *S. bilineata* occur in synchrony with one another.

In order to examine the effects of past foraging history and nutrient uptake on the expression of the adult male seismic courtship displays, visual courtship displays, and foreleg morphology; as well as adult female foreleg morphology, we induced divergence in condition by artificially manipulated the diet quantity of *S. crassipalata* and *S.*

bilineata immature individuals. Our aim was to identify if the aforementioned characters are currently under intersexual selection by comparing secondary sexual traits between sexes and/ or diet treatments. If traits are currently under intersexual selection we expect these traits to vary between sexes and/or within sexes of differing diet treatments (divergent condition).

METHODS

Collection and Maintenance - Immature *S. crassipalata* (N = 271) were collected from an open grassy habitat (canopy height < 10") in the Bath Nature Preserve in Summit County, OH (41° 10' 35.7414" N, 81° 38' 52.7928" W) from March 17-21 2009. All *S. crassipalata* individuals were 1-2 molts from maturity at collection. Immature *S.*

bilineata (N = 436) were collected in an open grassy habitat (canopy height < 8") along a riparian zone near the north end of Ohio State University campus at Newark, Licking County, OH (40° 4' 30.9144" N, 82° 26' 32.8272" W) from March 29-31, 2010. All *S. bilineata* individuals were 2-3 molts from maturity at collection. We collected immature spiders both to ensure that they were virgins and to allow for enough developmental time for diet manipulations to take effect (see below). After collection, all spiders were immediately transported back to the University of Nebraska-Lincoln and housed individually in 5.9cm x 5.9cm x 7.7cm clear plastic containers (Amac Plastic Products, Petaluma, CA) with visual barriers between containers. Spiders were maintained on a 12:12, light:dark cycle and provided a constant source of water.

Diet Manipulations – We manipulated the diet of individuals to examine the effects of past foraging history and nutrient uptake on the expression of adult male (i) seismic courtship displays, (ii) visual courtship displays, and (iii) foreleg morphology; as well as

adult female (iv) foreleg morphology. Immature spiders of both species were randomly assigned to one of two diet treatments: 1) Low quantity diet (LD) - one cricket (*Acheta domestica*, Bassetts cricket ranch, CA, USA) of a size visually approximate to the body of the spider (prosoma + opithosoma) once a week and 2) High quantity diet (HD) - one size match cricket as above twice a week. All spiders were examined at least every two days for molts in order to accurately estimate the date of their final maturation molt. Assigned feeding regimes were started immediately upon set-up in the laboratory. Because immature individuals of both sexes are indistinguishable, the sex of individuals was not known. Once mature, each spiders' weight was recorded.

Following experimentation, spiders were sacrificed via cold storage. Cephalothorax widths were then measured for each individual spider by first photographing each spider using a Leica light microscope (Leica Microsystems, Bannockburn, IL, USA) fitted with a SPOT Flex 15.2 64 Mp camera (SPOT Imaging Solutions, Sterling Heights, MI, USA). Using the photographs each individual's cephalothorax width was measured three times using Image-Pro Discovery software (Media Cybernetics, Bethesda, MD, USA) and the mean of the three measurements was calculated. Prior to analysis of the data we created a metric of body condition for both males and females by comparing the ratio of body mass at maturation to cephalothorax width. This body condition index was chosen over other potential indices (e.g. residuals) because it is better suited for examining differences between heterogeneous groups (e.g. populations, Jakob et al. 1996), such as the distinct size groups created by our diet manipulations.

Seismic Signal Quantification – We recorded the seismic courtship signals of a total of 30 (15 HD and 15 LD) *S. crassipalpata* males and 28 (14 HD and 14 LD) *S. bilineata* males. Due to a lack of information on age-specific courtship performance in either species, we attempted to control for male age across treatment groups. Recordings were made on a vibration isolation table (Minus K 50BM-8C, Minus K Technology, Inglewood, CA, USA) in a sound isolation chamber measuring 50cm × 37cm × 43 cm. The chamber was insulated with sound absorbing foam (Super Soundproofing Co., San Marcos, CA, USA) and fitted with a Vita-Lite full spectrum florescent bulb (Duro-Test Lighting Inc., Philadelphia, PA, USA). The temperature in the testing chamber was kept at 22° C ($\pm 1^\circ$). Recordings were made with a Laser Doppler Vibrometer (Polytec PDV100, Polytec, Irvine, CA, USA), set for a peak velocity measurement range of ± 20 mm/sec, with a low pass filter at 22kHz, and at a 24 bit 48 kSa/a sample rate. Output from the vibrometer was recorded on an Apple PowerBook using a power 1401 A-to-D converter and Spike 2 version 5 (Cambridge Electronic Design Ltd, Cambridge, UK). All recordings were exported as uncompressed WAV files for signal analysis.

A testing arena was placed inside the chamber on the vibration isolation table and lined with filter paper upon which female chemical silk cues had been deposited. The silk cues used were derived from three high diet conspecific females. These females were allowed to deposit silk cues on filter paper for two hours, after which the filter paper was cut into sections and used in the trials. In this way, males were exposed to cues from all three females during the trials. Male *Schizocosa* have been shown to assess female reproductive status and receptivity based on silk cues (Roberts & Uetz 2005), and this design increased experimental power by controlling for variability in female silk cues.

Additionally, using three separate females allowed us to overcome potential issues associated with using only a single stimulus source. A 0.5cm × 0.5cm piece of retroreflective tape (3M Diamond Grade, 3M, Saint Paul, MN, USA) was placed in the center of the filter paper to increase the signal strength of the vibrometer. Males were placed in the testing arena and allowed to acclimate for up to ten minutes. If the male did not begin to chemo-explore the arena by the end of the acclimation period, it was removed from the arena and the trial was ended. If a male did not begin to court in the first five minutes after beginning chemo-exploration, or did not produce more than one courtship bout, then again, the male was removed from the arena and the trial was ended. For males that did court, five minutes of courtship were recorded. If a male did not produce more than two bouts of courtship during the recording period the recording was discarded and not used in our analysis.

Because males were allowed to move freely in the arenas during trials, we do not have information about a male's exact location relative to the laser while they were courting. It was thus impossible to analyze characteristics of signal amplitude from our recordings. Additionally, both species' signals were broadband in nature and we did not attempt to analyze the frequency component of signals. The signal characteristics scored for both species were all temporal in nature and included: the number of courtship bouts, number of pulses, number of trills, time spent courting, number of inter-bout intervals, and number of inter-pulse intervals (Figure 2). All signal character measurements were made using Raven Pro version 1.3 (Cornell Laboratory of Ornithology, Ithaca, NY, USA).

Many of the signal characters measured relate to the amount of time males spent courting. There was thus, a high degree of inter-correlation between many of the signal characters. In order to analyze the variation in the amount of courtship for each species we carried out principle component analyses on the covariance matrices of both species. The signal characters analyzed for *S. crassipalpata* (N=30) included: the number of courtship bouts, number of pulses, number of trills, and time spent courting. The first principal component (PC1) of this analysis, which had an eigenvalue of 1039.62, accounted for 98.22% of the variation in the amount of courtship. In all subsequent analysis we used PC1 as our index for the amount of seismic courtship signaling for *S. crassipalpata*. The signal characters analyzed for *S. bilineata* (N=28) included: the number of courtship bouts, number of pulses, number of trills, time spent courting. The first principal component (PC1) of this analysis, which had an eigenvalue of 5091.3, accounted for 78% of the variation in the amount of courtship. In all subsequent analysis we used PC1 as our index for the amount of seismic courtship signaling for *S. bilineata*.

Visual Movement Quantification – Using a different set of males than those used for the seismic signal recordings, we made high speed video recordings of 23 (11 HD and 12 LD) *S. crassipalpata* and 29 (14 HD and 15 LD) *S. bilineata* courting males. Because we lack information on age-specific courtship performance in either species, we again controlled for male age across treatment groups.

High speed video recordings were made using a Fastcam 1024PCI high-speed digital camera (Photron USA, San Diego, CA, USA) at 500 fps. Individuals were illuminated from above with two Lumina fiber-optic lights (Chiu Technical Corp., Kings Park, NY, USA) and filmed from the side in clear plastic arenas measuring 13 cm wide,

13.5 cm high and 3.5 cm deep. A grid of squares (13mm diameter) was fastened to the back of arenas so that movements could be tracked and analyzed in a 2-dimensional plane (x and y axes). The shallow depth of the arenas helped to facilitate this 2-dimensional analysis, by limiting the spiders' movement in the z axis. The bottom of the arena was lined with filter paper upon which silk cues, from 3 conspecific high diet females had been placed (see rationale for this design in "*Seismic Signal Quantification*" methods). Males were placed in the arena and allowed to acclimate for up to ten minutes. As with the seismic signal recordings, if a male did not begin to chemo-explore within the ten minute acclimation period, it was removed and the trial was ended. If a male did not begin to court in the first five minutes after beginning chemo-exploration, then again, the male was removed from the arena and the trial was ended. For males that courted, the first courtship bout was recorded and analyzed.

The videos of both species courtship were analyzed using Pro-Analyst motion tracking software (Itronx Imaging Technologies Inc. Westlake Village, CA, USA). The leg wave characteristics measured were the same for both species. The height (mm) of the highest leg wave and the speed (mm/s) of that leg wave were measured. These leg wave characters were chosen because they likely reflect information about male body condition (i.e. males with higher body condition will be able to raise their legs higher and wave them at a faster speed). Motor movements which are near the anatomical and physiological production limits, like higher and faster leg waves, likely reflect information about male condition to females (Byers et al. 2010). The height of the leg wave was determined by measuring the distance from the tip of the last leg segment (tarsus) to the substrate directly below it. To control for the effect of body size on the

height of males' leg waves (i.e. larger males will inherently be able to raise their legs higher off the substrate), we analyzed the height of each male's leg wave relative to its cephalothorax width. The speed of the wave was determined by measuring the time it took the tip of the tarsus to reach the substrate from the highest point of the leg wave.

Male and Female Foreleg Morphology Quantification – Following experimentation, spiders were sacrificed via cold storage. The forelegs from 102 (52 HD and 50 LD) male *S. crassipalata* and 59 (30 HD and 29 LD) female *S. crassipalata* were then removed (Figure 3A). Similarly forelegs from 159 (87 HD and 72 LD) male *S. bilineata* and 60 (30 HD and 30 LD) female *S. bilineata* were removed (Figure 3B). One of the two forelegs from each spider was haphazardly chosen and the exterior lateral side was photographed using a Leica light microscope (Leica Microsystems, Bannockburn, IL, USA) fitted with a SPOT Flex 15.2 64 Mp camera (SPOT Imaging Solutions, Sterling Heights, MI, USA). Using these photographs, measurements of the total foreleg length (tarsus – femur) were taken. Additionally, brush measurements were taken for all photographs of *S. bilineata* male forelegs. Brush measurements included total brush height (dorsal brush + ventral brush) and total brush area.

Measurements were taken using Image-Pro Discovery software (Media Cybernetics, Bethesda, MD, USA). Prior to analysis, the measurement of total foreleg length was regressed against cephalothorax width for each individual and the residuals were used as a measure of relative foreleg length. Measurements of brush height and brush area for *S. bilineata* males were also regressed against cephalothorax width and the residuals of these regressions were used as measures of relative brush size.

Statistical Analysis – Body condition indices were compared between diet treatments for males and females using Wilcoxon Signed-Rank Tests. Differences in adult age (i.e. the number of days post maturation) between diet treatments were also compared using Wilcoxon Signed-Rank Tests. A repeated measures ANOVA was used to examine how courtship effort, as defined here by the # of courtship bouts per minute, changed over time and whether this change was dependent upon male diet treatment. Male diet treatment was used as the dependent variable and the # bouts of courtship in minute 1, in minute 2, in minute 3, in minute 4, and in minute 5 were the independent variables. Nominal logistic regressions were used to analyze the seismic signal data. Male diet treatment was used as the dependent variable and the index of the amount of courtship (PC1) and inter-bout interval and inter-pulse interval as independent variables reflecting courtship rate. Nominal logistic regressions were used to analyze the male leg wave data as well. Male diet treatment was used as the dependent variable and the height and speed of the males' leg wave were used as independent variables. Least Squares Regressions were used to compare foreleg measurements between males and females of both species as well as to make comparisons between diet treatments within each sex. The independent variables in these models were the individuals' sex, diet, and the interaction between the two. Significant effects were further examined using Likelihood Ratio tests. For *S. bilineata* males, a Nominal Logistic regression was used to test for differences between HD and LD individuals in brush area and brush height. Diet treatment was used as the dependent variable while brush height, brush area, and the interaction between the two were used as the independent variables. Significant effects were further examined using Likelihood Ratio tests. A Bonferroni correction was applied to all regression

models to control for the effect of multiple comparisons. All statistics were carried out using JMP v 6.0 (SAS Institute Inc., Cary, NC).

RESULTS

Diet Manipulations – Schizocosa crassipalata HD females had significantly higher body condition indices than LD females (Likelihood Ratio, $\chi_1^2 = 65.503$, $P < 0.001$), and HD males had significantly higher body condition indices than LD males (Likelihood Ratio, $\chi_1^2 = 36.213$, $P < 0.001$). *Schizocosa bilineata* HD females had significantly higher body condition indices than LD females (Likelihood Ratio, $\chi_1^2 = 98.511$, $P < 0.001$), and HD males had significantly higher body condition indices than LD males (Likelihood Ratio, $\chi_1^2 = 43.955$, $P < 0.001$).

S. crassipalata Seismic Signal Quantification – Schizocosa crassipalata males ranged between 26 and 30 days post maturation, with an average age of 28.47 days post maturation at the time of testing. HD males averaged 28.4 and LD males averaged 28.53 days post maturation. Between treatment groups (HD vs. LD males), there was a significant difference in the number of bouts per minute ($F_{(1,28)} = .193$, $p = .0275$) with HD males producing more bouts per minute than LD males (Figure 4). Within individuals, regardless of their diet treatment, all males decreased the number of courtship bouts/minute over time ($F_{(4,25)} = 2.15$, $p < 0.0001$). There was no interaction between time and diet treatment ($F_{(4,25)} = .083$, $p = 0.72$), indicating that HD and LD males did not differ in how they decreased their bouts/minute over time (Figure 4).

To determine whether courtship amount or courtship rate were good predictors of male diet treatment, we ran a nominal logistic regression with PC1-courtship amount,

inter-bout interval, and inter-pulse interval as our three independent variables, and male diet treatment as our dependent variable. The over-all model was significant ($\chi^2 = 8.42$, $df = 3$, $p = 0.038$). Mean inter-bout interval and inter-pulse interval were not good predictors of male diet (inter-bout interval $\chi^2 = 4.91$, $p = 0.68$; inter-pulse interval $\chi^2 = .42$, $p = 0.52$), but PC1-courtship amount was a good predictor of male diet treatment ($\chi^2 = 4.91$, $p = 0.027$).

S. crassipalata Visual Movement Quantification – *Schizocosa crassipalata* males ranged between 42 and 51 days post maturation, with an average age of 47.09 days post maturation at the time of testing. HD males averaged 47.45 days post maturation and LD males averaged 46.75 days post maturation. To determine if our diet treatments influenced a males' leg wave characteristics, we carried out a nominal logistic regression with the height of the leg wave/cephalothorax width, speed of the wave (mm/s), and the interaction between these two variables as our independent variables and male diet treatment as our dependent variable. We found no significant differences between diet treatments for either the speed of males' leg waves or the interaction between the speed and the height of leg waves (speed of the wave: $\chi^2 = 1.264$, $df = 1$, $P = 0.261$; speed \times height of the wave: $\chi^2 = 0.375$, $df = 1$, $P = 0.540$), while the height of males' leg wave was a significant predictor of male diet treatment ($\chi^2 = 6.205$, $df = 1$, $P = 0.0127$). *Schizocosa crassipalata* HD males waved their legs higher than LD males relative to cephalothorax width (Likelihood Ratio, $\chi^2_1 = 4.083$, $P = 0.043$).

S. crassipalata foreleg measurements – We found a significant effect of both sex and diet on foreleg length for *S. crassipalata*, but no effect of the interaction between these two variables (Whole model test: $R^2 = 0.37$, $df = 3$, $P < 0.001$). *Schizocosa crassipalata* HD males had longer forelegs than HD females (Likelihood Ratio, $\chi_1^2 = 32.176$, $P < 0.001$) and LD males had longer forelegs than LD females (Likelihood Ratio, $\chi_1^2 = 27.967$, $P < 0.001$). Relative to cephalothorax width, HD males had significantly longer legs than LD males (Likelihood Ratio, $\chi_1^2 = 13.481$, $P < 0.001$), but there was no significant difference in leg length between female diet treatments (Likelihood Ratio, $\chi_1^2 = 1.293$, $P = 0.256$).

S. bilineata Seismic Signal Quantification – *Schizocosa bilineata* males ranged between 37-39 days post maturation. Both HD and LD males average age at the time of testing was 37.14 days post maturation. Between treatment groups (HD vs. LD males), there was no difference in the over-all # of bouts per minute ($F_{(1,26)} = .0008$, $p = 0.89$). Within individuals, regardless of their diet treatment, all males decreased the number of courtship bouts/minute over time ($F_{(4,23)} = 1.7$, $p = 0.0001$), with the decrease occurring between minutes 1 and 2 (see Figure 5). There was no interaction between time and diet treatment ($F_{(4,23)} = 0.12$, $p = 0.61$), indicating that HD and LD males did not differ in how they decreased their bouts/minute over time (Figure 5).

To determine whether courtship amount or courtship rate were good predictors of male diet treatment, we ran a nominal logistic regression with PC1-courtship amount, inter-bout interval, and inter-pulse interval as our three independent variables, and male diet treatment as our dependent variable. The over-all model was not significant (Whole

model test: $\chi^2 = 2.64$, $df = 3$, $p = 0.45$), indicating that none of our measured variables associated with seismic courtship signaling were good predictors of male diet treatment.

S. bilineata Visual Movement Quantification – *Schizocosa bilineata* males ranged between 44 and 46 days post maturation at the time of testing, and both HD and LD males averaged 45.07 days post maturation. To determine if our diet treatments influenced a males' leg wave characteristics, we carried out a nominal logistic regression with the height of the leg wave, speed of the wave (mm/s), and the interaction between these two variables as our independent variables and male diet treatment as our dependent variable. We found no significant differences between diet treatments for any of the leg wave characters we tested (Whole model test: $\chi^2 = 2.388$, $df = 3$, $P = 0.496$).

S. bilineata foreleg/brush measurements – We found a significant effect of both sex and diet on foreleg length for *S. bilineata*, but no effect of the interaction between these two variables (Whole model test: $R^2 = 0.27$, $df = 3$, $P < 0.001$). Relative to cephalothorax width, *S. bilineata* HD males had longer forelegs than HD females (Likelihood Ratio, $\chi_1^2 = 14.599$, $P < 0.001$) and LD males had longer forelegs than LD females (Likelihood Ratio, $\chi_1^2 = 28.041$, $P < 0.001$). Relative to cephalothorax width, HD males had longer forelegs than LD males (Likelihood Ratio, $\chi_1^2 = 10.653$, $P < 0.002$), and HD females had longer forelegs than LD females (Likelihood Ratio, $\chi_1^2 = 10.198$, $P < 0.002$).

When examining male brush measurements, relative to cephalothorax width between diet treatments we found a significant effect of brush area, but not brush height or the interaction between brush area and brush height (Whole model test: $\chi^2 = 22.748$,

df= 3, $P < 0.001$). Relative to cephalothorax width, HD males' brush area was significantly greater than that of LD males (Likelihood Ratio, $\chi^2_1 = 19.712$, $P < 0.001$).

DISCUSSION

Condition dependence may have important implications for many evolutionary processes, including the evolution of mating preferences and sexual dimorphism (Cotton et al. 2004b). There has been extensive research conducted on condition dependent expression of sexual traits; however few studies have taken a multivariate approach to its study (Delcourt and Rundle 2011). Mate preferences often target multiple traits simultaneously (Hebets and Papaj 2005, Bro-Jørgensen 2010). Here we used divergent diet treatments to investigate the condition dependence of multiple sexual traits in two sister species of wolf spiders, unornamented *S. crassipalpatata* and ornamented *S. bilineata*. Our diet treatments significantly diverged body condition indices between HD and LD males and females of both species. Below we will discuss the implications of divergent body condition on the expression of the multiple secondary sexual characters we examined: seismic courtship display, visual courtship display and foreleg morphology.

Seismic Courtship – Schizocosa crassipalpatata males reduced the number of courtship bouts they produced over the five minute trial period, but HD males produced more seismic courtship bouts per minute than did LD males. The number of courtship bouts per minute a male can produce then, is condition dependent, suggesting that this attribute of the seismic signal of *S. crassipalpatata* has experienced/is experiencing directional intersexual selection. In another species of wolf spider, *Hygrolycosa rubrofasciata*, males produce seismic courtship signals by drumming their abdomens against their substrate.

This drumming display is condition-dependent (Kotiaho 2000) and has been shown to greatly increase a male's metabolic rate (Kotiaho 1998). Similarly, the metabolic cost of courtship in *S. crassipalpata* (and *S. bilineata*) is likely high and probably explains the reduction in the number of courtship bouts over time (Figures 4&5). Due to the inherent cost of producing an extended seismic courtship song, these types of courtship displays could be used by females to gauge male body condition and or genetic quality (Byers et al. 2010). Future work should address the probable link between male courtship vigor and female mate choice in *S. crassipalpata*.

The ornamented *S. bilineata* males also reduced the number of courtship bouts they produced throughout their trial periods, with the majority of the decline in signally occurring between the first and second minute of the trial. However there was no difference in the number of bouts produced by HD males or LD males. Based on our results, the seismic courtship signal of *S. bilineata* males may not have experienced strong directional intersexual selection. One possible explanation for this result is that, although the signal characters we analyzed appear to be under selection by *S. crassipalpata* females, these same signal characters are not under selection by *S. bilineata* females. Because they are so closely related, but have distributions which largely overlap, it is not unexpected that different signal characters would be selected by females of each species. Future studies examining the types of selection on *S. bilineata* seismic signals may uncover signal characters that are under intersexual selection.

Visual Courtship – We found a significant difference in the height of the leg wave displays between diet treatments for *S. crassipalpata* males. However, there were no differences between diet treatments for either *S. bilineata* in the height or speed of their

leg wave displays. These courtship characters seemed likely to correlate with male condition because they should reflect a male's ability to perform fine motor skill at near the limits of its physiological and/or anatomical limit. We thus, have evidence for condition dependence of the height of leg waves in *S. crassipalpata* but not *S. bilineata*. It seems odd that the unornamented species, *S. crassipalpata*, would show a difference between diet treatments in a leg wave character but not the ornamented species, *S. bilineata*. A possible explanation for this result is that as *S. bilineata* originally evolved its brushed ornaments to enhance the efficacy of its display of motor skills (height of their leg waves). Over time female *S. bilineata* mate choice preferences may have stopped selecting for the motor display itself and began selecting for the brushes alone, while *S. crassipalpata* female mating preferences have continued to select for male displays which incorporate higher leg waves.

It is also possible that we did not detect differences in the height of *S. bilineata* leg waves and the other leg wave characters we measured in both species because we did not measure these characters for long enough during a male's courtship display – differences may manifest over time, as HD and LD males might be differentially able to keep up vigorous courtship displays. We only analyzed the first courtship bout and future comparisons of the first courtship bout with one later in the display may yield more interesting and informative results. Extended measurements were difficult, however, given our experimental design. For example, while we purposely used experimental arenas with small dimensions to help facilitate our video analysis (see methods for justification), small dimensions inhibit courtship performance over time. When males contacted the sides of the arenas, they tended to stop courting and spent more time

walking along the edges of the arena. In our analysis of the seismic courtship display, both species decreased the amount of courtship the longer they courted. If future studies are able to overcome the methodological constraints we faced in this study, they may find condition-dependent differences in males' leg wave speed and height over an extended courtship period.

Foreleg Morphology – For *S. crassipalpata*, foreleg length was both sexually dimorphic and condition-dependent for males. Theory predicts that due to the selection on exaggerated sexual traits they should develop a high degree of condition-dependence. This evolutionary scenario seems likely in this case. Despite lacking foreleg ornamentation, male *S. crassipalpata* vigorously wave their forelegs during courtship displays. Though there is no foreleg “ornament” per se, females may use the entire foreleg as an indicator of male condition. Alternatively, selection on foreleg length may be independent of courtship per se, but may facilitate male mounting and reduce the likelihood of sexual cannibalism during this risky behavior.

Like the unornamented forelegs of *S. crassipalpata* males, the ornamented forelegs of *S. bilineata* males showed condition-dependent expression of length. The same hypothesis regarding why *S. crassipalpata* males displayed condition dependent leg length also likely applies to *S. bilineata* male foreleg length. Perhaps more interesting is the finding that *S. bilineata* females also display some level of condition dependent expression of foreleg length. There are two likely evolutionary scenarios that may explain these results. First, females may display condition dependent forelegs as a result of a shared genetic architecture with males. If females exert directional selection on male foreleg length so that male forelegs become condition-dependent, female forelegs may

also display a level of condition-dependence due to their shared genetic architecture with males. Second, males may be exerting directional selection on females for longer foreleg length. During courtship, female *Schizocosa* wolf spiders display their receptivity toward males by displaying a behavior known as a settle (Hebets and Uetz 1999). When females “settle” they lower their bodies toward the substrate and extend their first two pairs of legs in front of them. If males are more likely to mate with a female if she has longer forelegs then this could explain why females too display condition dependent foreleg length. Future work should investigate these hypotheses. Relative to cephalothorax width, *S. bilineata* HD males had larger tibial brushes than LD males. These results fit well the presumption that foreleg brushes are sexual ornaments. Like other sexual traits, sexual ornaments are predicted to be costly and heightened expression of an ornament should correlate with body condition (Cotton et al 2004b). Males of a related species of wolf spider, *Schizocosa ocreata*, also possess foreleg tibial brushes which have been shown to exhibit condition-dependence (Uetz et al. 2002). When females of this species are unable to detect males’ seismic signals, they have decreased receptivity toward males with shaved versus intact brushes (Scheffer et al. 1996) as well as to males with smaller brushes (McClintock and Uetz 1996). However, female receptivity did not vary with male ornamentation in experiments using a video playback experiment where male brushes were either artificially removed (no ornamentation), enlarged (enhanced brushes) or not altered (control) (McClintock and Uetz 1996). Additionally trials involving males with shaved brushes versus intact brushes also did not result in mating frequency differences (Scheffer et al. 1996). In this study it was suggested that tibial brushes in *S. ocreata* may function to increase the signal efficacy of courtship displays, so that when

signaling in environments where the seismic signal is not able to be effectively transmitted males may still be able to effectively court and mate with females (Scheffer et al. 1996). Future study of the courtship displays of both *S. bilineata* and *S. crassipalpata* should carefully test the signal function of each signal component independently in order to fully explore their functions.

Conclusions – We used divergent diet treatments to investigate the condition dependence of multiple sexual traits in two sister species of wolf spiders, unornamented *S. crassipalpata* and ornamented *S. bilineata*. Our diet treatments significantly diverged body condition indices between HD and LD males and females of both species. Additionally, we found differences between diet treatments in some but not all male display characteristics we measured. A possible explanation for why we did not detect differences in some of the seismic and visual characters we measured is the advanced age of the males we used. The males used in these courtship trials were older than those normally used in mate choice trials. Although none of the males used in these courtship trials seemed to court any less vigorously, the possibility remains that their age could have influenced the vigor with which they were able to court and also could have affected our ability to detect differences between diet treatments. Future studies should be mindful of how a male's age could influence their courtship performance.

We did find condition dependent expression of male *S. crassipalpata* seismic courtship display, visual courtship display and foreleg length as well as male *S. bilineata* foreleg length and brush size, and female *S. bilineata* foreleg length. That these male sexual traits demonstrate condition dependence suggests that they have experienced/are currently experiencing directional intersexual selection. Future studies should address this

hypothesis by investigating whether female mating preferences in these two species match the patterns in this study.

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FIGURES

Figure 1: Known distribution of *Schizocosa crassipalata*, *Schizocosa bilineata* and their areas of overlap.

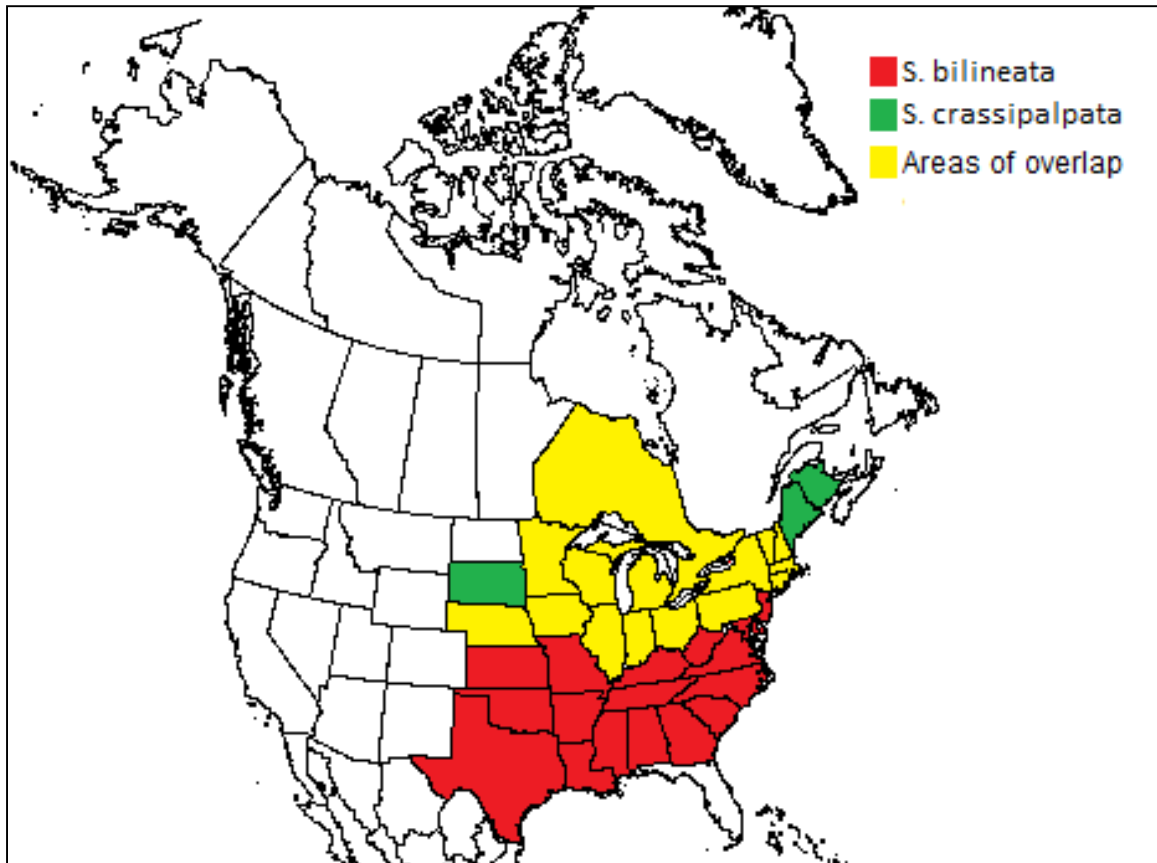


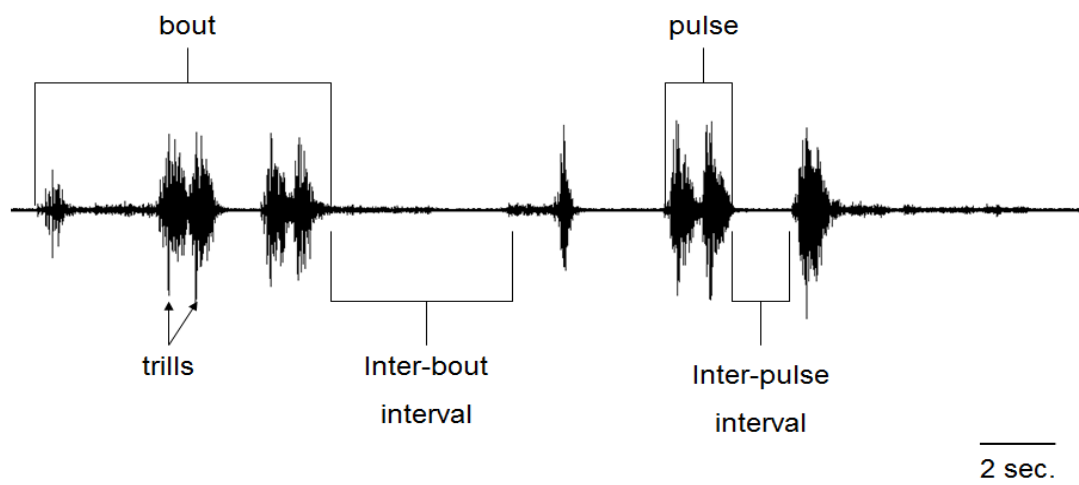
Figure 2A: *Schizocosa crassipalpa* seismic signal

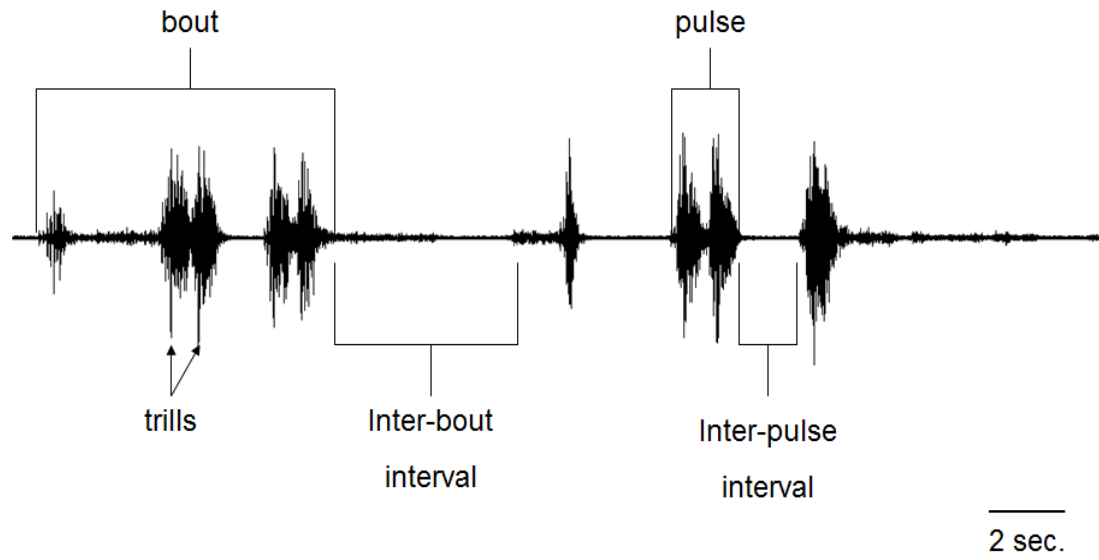
Figure 2B: *Schizocosa bilineata* seismic signal

Figure 3A: Unornamented
Schizocosa crassipalata
male and female forelegs



Figure 3B: Ornamented
Schizocosa bilineata male
and unornamented female
forelegs

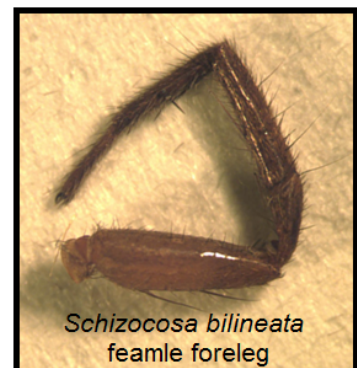


Figure 4: Number of courtship bouts per minute produced by high diet and low diet treatment *Schizocosa crassipalata* males.

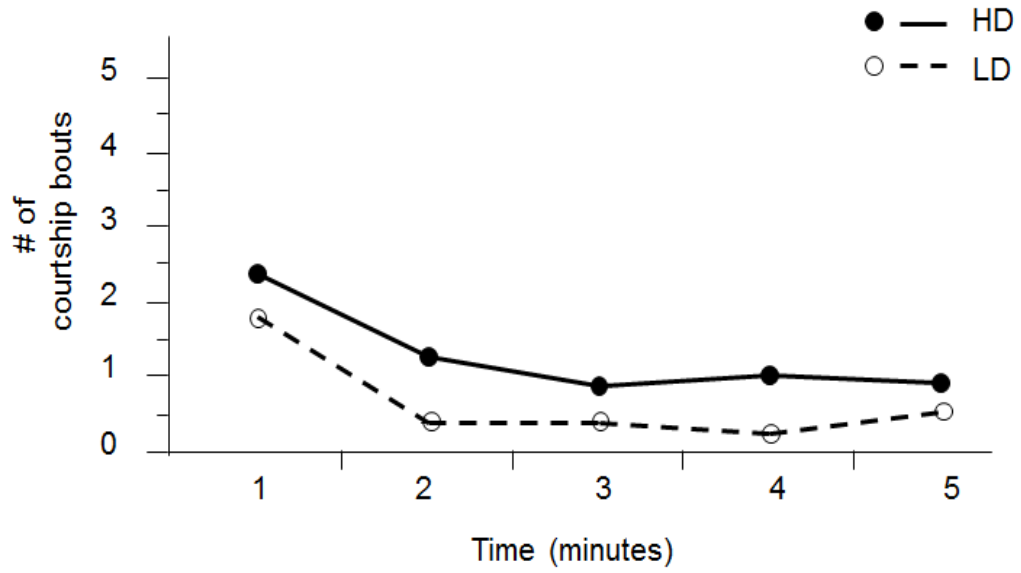
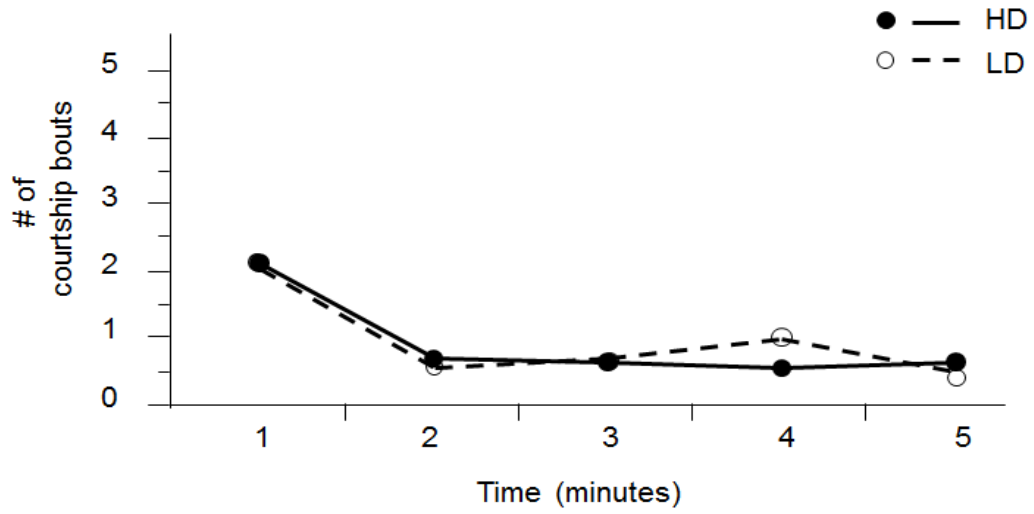


Figure 5: Number of courtship bouts per minute produced by high diet and low diet treatment *Schizocosa bilineata* males.



CHAPTER 2

SIGNAL MODALITIES DIFFERENTIATE MATE CHOICE IN TWO SISTER
SPECIES OF WOLF SPIDER

Mitchel D. Bern

INTRODUCTION

Perhaps one of the most compelling questions in contemporary sexual selection theory concerns its role in the speciation process. One way that sexual selection is thought to influence the speciation process is through the co-evolution of diverging courtship signals and receiver preferences between isolated populations. This disparate evolutionary coupling of signals and preferences among populations is purported to result in the divergence of signals, and ultimately, behavioral isolation between populations (Boughman 2001, Guerra and Santiago 2008, Sullivan-Beckers and Coccoft 2010). In this way, courtship signals and the female preferences for them have been proposed to be of special importance, because they have the potential to cause rapid divergences between populations and species (Lande 1981, West-Eberhard, 1983, Andersson 1994).

Recently, though, the way we think about courtship signaling has shifted. In the past, most studies of courtship signals considered only signals in one modality, in isolation; that is signals which stimulate a single type of sensory receptor through a single sensory channel (modality) (Partan and Marler 1999). We now realize the potential importance and prevalence of complex signals in all animal communication, including courtship (Candolin 2003, Hebets & Papaj 2005, Partan and Marler 2005). Complex signals are categorized in numerous ways (Partan and Marler 1999, Hebets and Papaj 2005), one of which concerns the number of incorporated sensory modalities. For example, signals which are transmitted in a single sensory modality but which might have multiple components are termed unimodal, and differ from multimodal signals, a term which describes displays in which two or more signals are transmitted in two or more

sensory modalities (Scheffer *et al.* 1996, Hebets and Uetz 1999, Partan & Marler 1999, 2005, Hebets & Papaj 2005).

In the past decade the number of papers on complex signaling has increased dramatically (Rybak *et al.* 2002, Scheuber *et al.* 2004, Elias *et al.* 2006, Rundus *et al.* 2007, Ratcliffe and Nydam 2008, Wilgers and Hebets 2011). These studies, and others, have helped generate testable hypotheses for identifying the types of selection that have shaped the evolution and function of complex signaling. A more thorough understanding of complex signal evolution may in turn shed light on the divergence of courtship signals across closely related species and ultimately, on our understanding of species divergence.

Selection on signals can be divided into two categories: content based selection (how well a signal relays information) and efficacy based selection (how well a signal propagates through the environment and is perceived by the receiver) (Hebets & Papaj 2005, Partan and Marler 2005). It is important to note that these two types of selection are not mutually exclusive and may interact (Hebets and Papaj 2005, Bro-Jørgensen 2010). In order to develop a more complete view of how complex courtship signals may have evolved and potentially contributed to species divergences, it is important that we utilize experimental techniques which examine multiple sources of selection simultaneously. Understanding the role of both content based and efficacy based selection and their potential interactions in complex signal evolution is best accomplished by using experiments that simultaneously manipulate both signal efficacy and signal content, and then assess receiver response to both isolated and combined signal components (Hebets & Papaj 2005).

Wolf spiders in the genus *Schizocosa* are an excellent model system for the study of complex courtship displays (Rundus et al. 2010). Some members of this genus have courtship displays which are classified as unimodal, consisting of only a seismic (vibratory) signal, while others employ a more complex multimodal display consisting of a seismic signal plus a visual signal in the form of leg waving or arching patterns. Additionally, males of some species have conspicuous dark pigmentation and/or black brushes on their forelegs. Recent studies have shown that the function of male *Schizocosa* multimodal courtship signals varies greatly across closely related species (Hebets 2005, 2008, 2011, Rundus et al. 2010a, b) and courtship divergence has previously been shown to serve as a behavioral isolating mechanism in one sister species complex (Stratton & Uetz 1981, 1986). Here, we explore another sister species complex (Stratton 2005, Vaccaro et al. 2010), *S. crassipalpata* (Roewer 1951) and *S. bilineata* (Emerton 1885). Courtship displays and associated morphologies in these two species are divergent (Dondale and Redner 1978, Stratton 2005). *Schizocosa crassipalpata* males lack foreleg ornamentation and females are slightly larger than males (Dondale and Redner 1978, Stratton 2005). Males court females in the seismic modality by producing a series of substrate borne seismic vibrations. Courtship in the visual modality is characterized by an asymmetrical tapping of the un-ornamented forelegs. The visual display typically follows the seismic display. *Schizocosa bilineata* males possess dark pigmentation and dark brushes on the tibia of the forelegs at maturation (Dondale and Redner 1978, Stratton 2005, Vaccaro et al. 2010), and females are also slightly larger than males (Stratton 2005). Males court females in the seismic modality by producing a series of substrate borne seismic vibrations. Courtship in the visual modality is characterized by an

incremental lowering of one or both ornamented forelegs culminating in a quick tap of the substrate. The seismic and visual displays of *S. bilineata* occur in synchrony with one another.

Schizocosa crassipalpa and *S. bilineata* are thought to occur throughout Eastern North America. Their known distribution ranges from Southern Canada South to Georgia and Texas, and from the East coast to as far west as South Dakota and Nebraska (Comstock 1940, Kaston 1948, Dondale and Redner 1990, Sierwald et al. 2005, Stratton 2005, Vaccaro et al. 2010). Sympatric populations have been found in these areas of overlay, but where their ranges do not overlap, stable allopatric populations have also been found (M. Bern, unpublished data). Both species are typically found in grassy habitats with a canopy height of 8-12 inches (M. Bern, personal observation).

In an effort to inform us on the extent to which both content and efficacy based selection may have influenced the current form of the courtship displays, and potentially contributed to behavioral isolation of these two sister species, we examine the function of complex courtship signaling in both species. Our examination of complex signal function simultaneously examines (1) current content-based selection by exploring the relationship between female mate choice and condition-dependent signal expression, and (2) current efficacy-based selection by examining the role of isolated signal components in mating success.

To diverge signal content, we conducted mate choice trials where we manipulated male body condition using diet treatments. If information about foraging history relayed in male courtship displays has been selected for by female preferences, we would expect

females to mate more frequently with males whose current condition reflects past foraging success (i.e. males with higher body condition and/or more elaborate foreleg ornaments). To alter signal efficacy, we created artificial signaling environments in which we independently controlled the presence and absence of both the seismic and visual displays. Mate choice trials were conducted across all possible combinations of these experimental manipulations and the frequency of copulation occurrence in each was measured. Additionally, to further examine the role of content based selection in shaping the development of male foreleg ornamentation in these two species, we conducted a second set of mate choice trials where we did not vary male or female diet or signaling environment, but did vary the level of male foreleg ornamentation. We were, thus able to assess the relative importance of both content and efficacy based selection on the function of male courtship displays for each species by examining how female mate choice decisions varied across these different mate choice trials and treatments.

METHODS

Collection and Maintenance - Immature *S. crassipalpa* (N = 271) were collected from an open grassy habitat (canopy height < 10") in the Bath Nature Preserve in Summit County, OH (41° 10' 35.7414" N, 81° 38' 52.7928" W) from March 17-21 2009. All *S. crassipalpa* individuals were 1-2 molts from maturity at collection. Immature *S. bilineata* (N = 436) were collected in an open grassy habitat (canopy height < 8") along a riparian zone near the north end of Ohio State University campus at Newark, Licking County, OH (40° 4' 30.9144" N, 82° 26' 32.8272" W) from March 29-31, 2010. All *S. bilineata* individuals were 2-3 molts from maturity at collection. We collected immature spiders of each species both to ensure that they were virgins and to allow for enough

developmental time for diet manipulations to take effect (see below). After collection, all spiders were immediately transported back to the University of Nebraska-Lincoln and housed individually in 5.9cm x 5.9cm x 7.7cm clear plastic containers (Amac Plastic Products, Petaluma, CA) with visual barriers between containers. Spiders were maintained on a 12:12, light:dark cycle and provided a constant source of water.

Diet Manipulations – We manipulated diet to examine effects of past foraging history on the expression of adult reproductive traits/behaviors. Immature spiders of both species were randomly assigned to one of two diet treatments: 1) Low quantity diet (LD) - one cricket (*Acheta domesticus*, Bassetts cricket ranch, CA, USA) of a size visually approximate to the body of the spider (prosoma + opithosoma) once a week and 2) High quantity diet (HD) - one size match cricket as above twice a week. All spiders were examined at least every two days for molts in order to accurately estimate the date of their final maturation molt. Assigned feeding regimes were started immediately upon set-up in the laboratory. Because immature individuals of both sexes are identical, the sex of individuals was not known. In total we created four diet treatments for each species (HD females, HD males, LD females, and LD males).

Signaling Environment Manipulations – Mating arenas consisted of circular plastic arenas 12.5 cm in diameter (Pioneer Plastics Inc. Dixon, KY). All arenas were surrounded with white paper to control for any visual cues outside the arenas, and were placed on foam blocks to minimize ambient vibrational noise. To determine the importance of each signaling modality for copulation success, we manipulated the signaling environment such that particular signaling channels (visual versus seismic) were isolated. We used a 2 x 2 design in which we controlled the presence/absence of

visual signaling and the presence/absence of seismic signaling independently (similar to Hebets 2005, 2008 and Rundus et al. 2010a, b).

The manipulation of the visual signal consisted of light versus dark conditions (light = visual signal present, dark = visual signal negative). All light treatments were illuminated using two Virta-Lite full spectrum 30-watt florescent bulbs (Duro-Test Lighting Inc., Philadelphia, PA, USA) while dark treatments were illuminated by only infrared illuminators (Supercircuits, Austin, TX, USA) and observed using Rigel 3200 night vision goggles (Rigel Optics Inc. Washougal, WA). The infrared illuminator emitted light at a wavelength of ~850 nm. Electroretinogram recordings taken from a variety of wandering spiders, including wolf spiders, have provided no evidence that they are able to detect light at this wavelength (DeVoe 1969, DeVoe et al. 1972, Barth 1993).

The manipulation of the seismic signal consisted of filter paper versus granite substratum (filter paper substratum = seismic signal present, granite substratum = seismic signal negative). The vibration present treatment trials were run in an arena lined with white filter paper substratum (Schleicher and Schuell, Keene, NH, USA). The seismic negative treatment was run in a bottomless arena placed on a granite substratum. Previous work using a laser Doppler vibrometer to record male *Schizocosa* wolf spiders producing vibrational signals have shown that granite is not effective at transmitting a male spider's signals (Rundus et al. 2010b).

Diet and Environment Manipulation Mating Trials – Given that we had two diet treatments for both males and females, we had four possible female-male combinations (HD female/HD male; HD female/LD male; LD female/HD male; and LD female/LD

male). Additionally, we had four possible signaling environments (visual/seismic = V+/S+; V+/S-; V-/S+; and V-/S-). Ultimately, this resulted in sixteen possible mating treatments. Individuals were randomly assigned across these sixteen treatments. At the time of testing, groups of four visual present trials were run back-to-back with groups of four visual negative trials. Visual present trials were comprised of two replicates of the seismic present and two replicates of seismic negative treatments. Similarly visual negative trials were comprised of two replicates of seismic present and two replicates of seismic negative treatments.

Approximately 24 hours before the start of trials, females were fed one size matched cricket to minimize the potential for pre-copulatory cannibalism. On the day of their mating trial male and female weights were recorded prior testing. Females were placed in their respective arenas at least 1 hour prior to the start of the trial to acclimate and deposit silk cues. These cues alone are enough to elicit courtship behaviors from both male *Schizocosa* wolf spiders (Roberts and Uetz 2005, Vaccaro et al. 2010, M. Bern, personal observation). Trials began when males were introduced into the arena, and each pair was allowed to interact for up to 30 minutes. Trials were scored for the following behaviors: all occurrences of male courtship (leg waves), female receptivity displays (turns & settles), male attempted mounts, copulation, female attacks on males and cannibalism. Following each trial, all individuals were sacrificed via cold storage and preserved in 70% ethyl alcohol. Additionally, cephalothorax widths were measured for each individual spider by first photographing each spider using a Leica light microscope (Leica Microsystems, Bannockburn, IL, USA) fitted with a SPOT Flex 15.2 64 Mp camera (SPOT Imaging Solutions, Sterling Heights, MI, USA). Using the photographs

each individual's cephalothorax width was measured three times using Image-Pro Discovery software (Media Cybernetics, Bethesda, MD, USA) and the mean of the three measurements was calculated. Prior to analysis of the data we created a metric of body condition for both males and females by comparing the ratio of body mass at maturation to cephalothorax width. This body condition index was chosen over other potential indices (e.g. residuals) because it is better suited for examining differences between heterogeneous groups (e.g. populations, Jakob et al. 1996), such as the distinct size groups created by our diet manipulations.

Schizocosa crassipalata Foreleg Ornamentation Mating Trials – *Schizocosa*

crassipalata males have unornamented forelegs, yet they incorporate a foreleg wave in their courtship display. Past comparative studies of *Schizocosa* have suggested that ornamentation evolved to amplify pre-existing foreleg movements (Hebets and Uetz 2000) Here, we test whether adding ornamentation to the foreleg of *S. crassipalata* might increase the attractiveness of males. To test if females would mate more frequently with males if they had ornaments, we applied artificial ornaments to some males and left some males unornamented and then compared copulation frequency between these two treatments. All spiders used in these trials were raised on the high diet treatment (described above) Once mature, males were placed into one of two experimental treatment groups – (1) Painted: The tibia and patella of males' forelegs were painted with black acrylic paint (Anita's All Purpose Acrylic Craft Paint – 11002 Black/Noir Negro, Synta Inc., Clarkston, GA, USA) and (2) Unpainted: The tibia and patella of males' forelegs were "painted" with water. In order to paint the males, the corner of a Ziploc bag (SC Johnson, Racine, WI, USA) was cut off, males were placed in the bag and restrained.

Their forelegs were then pulled through the hole in the corner of the bag using soft tip forceps and painted. Males were painted the morning of the trials, approximately two hours before the beginning of trials. A small spot of the same black paint used to paint males legs was applied to the mating arenas at the same time males were painted, to control for odor cues between treatment groups.

Mating arenas consisted of circular plastic arenas 12.5 cm in diameter (Pioneer Plastics Inc. Dixon, KY). All arenas were lined with filter paper and surrounded with a picture of grass, to control for visual cues outside the arena and to simulate the natural habitat of *S. crassipalpa*. Freshly cut grass blades were placed into the arena on top of the filter paper. The use of a complex background and the addition of grass blades to the mating arena was implemented to increase the likelihood of detecting differences between painted and unpainted mating trials – if ornamentation increases the efficacy of visual movements, then we expect it would have a greater effect in a signaling environment with a lower signal:noise ratio (*e.g.* a more heterogeneous environment).

Approximately 24 hours before the start of trials females were fed one size matched cricket to minimize the potential for pre-copulatory cannibalism. On the day of their mating trial male and female weights were recorded prior to testing. Females were placed in their respective arenas at least 1 hour prior to the start of the trial to acclimate and deposit silk cues. Trials began when males were introduced into the arena, and each pair was allowed to interact for up to 30 minutes. Trials were scored for the following behaviors: all occurrences of male courtship (leg waves), female receptivity displays (turns & settles), male attempted mounts, copulation, female attacks on males and

cannibalism. Following each trial, all individuals were sacrificed via cold storage and preserved in 70% ethyl alcohol.

Schizocosa bilineata Foreleg Ornamentation Mating Trials – *Schizocosa bilineata* males have foreleg ornaments consisting of darkly pigmented tibial leg segments and tibial brushes. A previous study with another brushed *Schizocosa* species, *S. ocreata*, found that females were more receptive toward males which possessed brushes over males whose brushes had been removed (Scheffer et al. 1996), suggesting that brushes have evolved in response to female mate choice. To test if female *S. bilineata* prefer males with brushes, we paired females with males for which we artificially removed ornaments and with males possessing natural ornaments and compared copulation frequency between these two treatments. Once mature, males were placed into one of two experimental treatment groups – (1) Shaved: Tibial brushes were shaved off males using a syringe edge; (2) Unshaved: Tibial brushes were stroked with the unsharpened shaft of a syringe. In order to shave and sham shave males, the corner of a Ziploc bag (SC Johnson, Racine, WI, USA) was cut off, males were placed in the bag and restrained. Their forelegs were then pulled through the hole in the corner of the bag using soft tip forceps and shaved. Males were shaved or sham shaved approximately 24 hours prior to the start of their mate choice trials.

All spiders used in these mate choice trials were raised on the high diet treatment (described above). Mating arenas consisted of circular plastic arenas 12.5 cm in diameter (Pioneer Plastics Inc. Dixon, KY). All arenas were lined with filter paper and surrounded with a white piece of paper, to control for visual cues outside the arena. Approximately 24 hours before the start of trials females were fed one size matched cricket to minimize

the potential for pre-copulatory cannibalism. On the day of their mating trial male and female weights were recorded prior to the beginning of the trial. Females were placed in their respective arenas at least 1 hour prior to the start of the trial to acclimate and deposit silk cues. Trials began when males were introduced into the arena, and each pair was allowed to interact for up to 30 minutes. Trials were scored for the following behaviors: all occurrences of male courtship (leg waves), female receptivity displays (turns & settles), male attempted mounts, copulation, female attacks on males and cannibalism. Following each trial, all individuals were sacrificed via cold storage and preserved in 70% ethyl alcohol.

Statistical Analysis - Body condition indices were compared between diet treatments for males and females using Wilcoxon Signed-Rank Tests. Differences in the number of days post maturation between diet treatments were also compared using Wilcoxon Signed-Rank Tests. Nominal logistic regressions were used to investigate the effect of multiple variables and their interactions on copulation occurrence for the diet and environmental manipulation mating trials. During model selection several preliminary models which included all interactions between variables were generated. None of these interactions were significant, so in an attempt to minimize unnecessary inflation of the models' R^2 value only single variables were included in the final models reported here. Significant effects were further examined using Likelihood Ratio tests and Wilcoxon 2-sample tests. Nominal logistic regressions were used to investigate the effect of multiple variables and their interactions on copulation occurrence for the foreleg manipulation mating trials. Least squares regressions were used to examine the effect of multiple variables on copulation latency and males' courtship rate for all mate choice trials. Significant effects

were further examined using a Wilcoxon 2-Sample test. A Bonferroni correction was applied to all regression models to control for the effect of multiple comparisons. All statistics were carried out using JMP v 6.0 (SAS Institute Inc., Cary, NC).

RESULTS

Schizocosa crassipalpa

Diet and Environment Manipulation Mating Trials – A total of 130 males (62 HD, 68 LD) and 130 females (70 HD, 60 LD) were run in mating trials. At the time of the mating trials HD females had significantly higher body condition indices than LD females ($t_{126} = 7.016, P < 0.001$). At the time of the mating trials HD males had significantly higher body condition indices than LD males ($t_{128} = 5.235, P < 0.001$). At the time of the mating trials females ranged between 11-19 days post maturation with a mean age of 14.89 days post maturation. There was a significant difference in the average female age between diet treatments at the time of mating trials (Wilcoxon Signed-Rank test, $Z = 5.815, df = 1, P < 0.001$). HD females averaged 15.56 days post maturation while LD females averaged 14.11 days post maturation. This difference is due to differences in maturation time between the two diet treatments and given that it is less than 2 days difference, is likely not biologically meaningful. Males ranged between 12-37 days post maturation and averaged 17.92 days post maturation. There was no significant difference in the average age between male diet treatments at the time of mating trials (Wilcoxon Signed-Rank test, $Z = 1.239, df = 1, P = 0.123$).

Since we had a limited number of individuals to use across our numerous experimental treatments (16 treatments total) we stopped conducting trials in the seismic

negative environments after the first 48 mate choice trials (12 trials run in each of the four environmental treatments). At this point, only a single pair had copulated in the seismic negative environments (1 V+/S-; 0 V-/S-), while 17 pairs had copulated in the seismic present treatments (10 V-/S+; 7 V+/S+).

To determine if diet treatment or signaling environment had an effect on the likelihood of copulation, we carried out a nominal logistic regression with female age, female diet, male diet, visual environment, seismic environment, and courtship rate (# of courtship bouts/time spent courting) as our independent variables and copulation presence/absence as our response variable. Female age was included because it has previously been shown to be important in female mate choice decisions in a related species, *Schizocosa ocreata* (Uetz and Norton 2007), and because it differed significantly between the diet treatments (see above). We found no effect of female age or diet (male or female) on the likelihood of copulation; while the visual environment, seismic environment and courtship rate all had a significant effect on the likelihood of copulation (Table 1; whole model test: $\chi^2 = 65.469$, $df = 6$, $P < 0.001$). Copulations occurred significantly more in the visual negative environment (Likelihood Ratio, $\chi_1^2 = 30.408$, $P < 0.001$; Figure 1), as well as the seismic present environment (Likelihood Ratio, $\chi_1^2 = 4.457$, $P < 0.035$; Figure 1). Males which copulated had significantly higher courtship rates ($N = 66$, $\bar{x} = 0.05$ bouts/time spent courting) than males which did not copulate ($N = 64$, $\bar{x} = 0.02$ bouts/time spent courting) (Wilcoxon 2-sample test, $Z = 5.801$, $P < 0.001$). Additionally, males that courted below 0.03 bouts/minute were not able to gain copulations (Figure 2).

To examine if diet treatment or signaling environment had an effect on copulation latency we carried out a least squares regression with female diet, male diet, visual environment, seismic environment, and courtship rate (# of courtship bouts/time spent courting) as our independent variables and the latency of copulation as our response variable. We found no effect of female diet, male diet, or courtship rate but did find an effect of both the visual environment and seismic environment on copulation latency (Table 2; whole model test: $R^2= 0.24$, $df= 5$, $P< 0.001$). Pairs copulated much more quickly in the visual negative environments (Likelihood Ratio, $\chi_1^2 = 9.724$, $P< 0.002$) and the seismic positive environments (Likelihood Ratio, $\chi_1^2 = 19.777$, $P< 0.001$).

To examine whether male courtship effort was influenced by male diet treatment, female diet treatment and signaling environment, we carried out a least squares regression with male diet, female diet, visual environment, and seismic environment as our independent variables and courtship rate as our response variable. We found no effect of male diet, female diet, visual environment or seismic environment on a male's courtship rate (Table 3; whole model test: $R^2= 0.03$, $df= 4$, $P= 0.451$).

Foreleg Ornamentation Mating Trials – A total of 31 males (16 Painted, 15 Unpainted) and 31 females were run in mate choice trials. At the time of testing females ranged between 13-38 days post maturation with an average age of 31 days post maturation. Males ranged between 29-38 days post maturation with an average age of 33 days post maturation. Of the trials we conducted, 20 out of 31 (64.5%) resulted in copulations. Of the trials which resulted in copulations 11 (55%) occurred in trials with painted males and 9 (45%) occurred in trials with unpainted males.

To examine if copulation frequencies differed significantly between the two male leg treatment groups we carried out a nominal logistic regression for copulation occurrence with male leg treatment, courtship rate (bouts/time spent courting) and the interaction between the two as our independent variables. The overall model was insignificant (whole model test: $\chi^2 = 2.957$, $df = 3$, $P = 0.398$), indicating that none of these variables influences copulation occurrence. To examine if copulation latency was affected by male leg treatment, we carried out a least squares regression with copulation latency as our dependent variable and male leg color, copulation latency and the interaction between the two as our independent variables. The overall model was insignificant (whole model test: $R^2 = 0.08$, $df = 3$, $P = 0.507$), again indicating that none of these variables influences copulation latency.

Schizocosa bilineata

Diet and Environment Manipulation Mating Trials – A total of 192 females (104 HD and 88 LD) and 192 males (105 HD and 87 LD) were run in mating trials. Due to both pre and post preservation body decomposition, body condition indices could not be generated for 16 females (13 HD and 3 LD) and 14 males (6 HD and 8 LD). At the time of the mating trials HD females had significantly higher body condition indices than LD females ($t_{174} = 5.682$, $P < 0.001$). At the time of the mating trials HD males had significantly higher body condition indices than LD males ($t_{171} = 3.595$, $P < 0.001$). Females ranged in age from 11-16 days post maturation with a mean of 14.12 days post maturation. There was a significant difference in the mean age between female diet treatments (Wilcoxon Signed-Rank test, $Z = 2.811$, $df = 1$, $P < 0.005$). HD females averaged 14.29 days post maturation while LD females averaged 13.92 days post

maturation. Again, this difference is less than one day and is likely not biologically meaningful. Males ranged in age from 10-30 days post maturation with an average of 19.66 days. There was a significant difference in the average age between male diet treatments (Wilcoxon Signed-Rank test, $Z= 6.068$, $df= 1$, $P< 0.001$). HD males averaged 21.75 days post maturation while LD males averaged 17.13 days post maturation. As with *S. crassipalpata*, these differences in age across diet treatments are a result of differences in maturation time between diet treatments.

To examine if diet treatment or signaling environment had an effect on the likelihood of copulation we carried out a nominal logistic regression with female age, female diet, male diet, visual environment, seismic environment, and courtship rate (# of courtship bouts/time spent courting) as our independent variables and copulation occurrence as our response variable. We found no effect of female age, male or female diet (HD or LD) or the seismic environment on the likelihood of copulation; while the visual environment and courtship rate both had a significant effect on the likelihood of copulation (Table 4; whole model test: $\chi^2= 176.992$, $df= 6$, $P< 0.001$). Copulations occurred significantly more often in the visual present environment than in the visual negative environment (Likelihood Ratio, $\chi^2_1 = 40.707$, $P< 0.001$; Figure 3). Males that copulated had significantly higher courtship rates ($N= 69$, $\bar{x} = 0.04$ bouts/time spent courting) than males that did not copulate ($N= 123$, $\bar{x} = 0.01$ bouts/time spent courting) (Wilcoxon 2-sample test, $Z= 10.793$, $P< 0.001$). Males that courted at less than .08 bouts/minute were unable to gain copulations (Figure 4).

To examine if diet treatment or signaling environment had an effect on copulation latency we carried out a least squares regression with female diet, male diet, visual

environment, seismic environment, and courtship rate (# of courtship bouts/time spent courting) as our independent variables and the latency of copulation as our response variable. We found no effect of female diet, male diet, the visual environment, or the seismic environment. There was however, a significant effect of courtship rate on copulation latency (Table 5; whole model test: $R^2= 0.24$, $df= 5$, $P< 0.001$). To further explore this result, we carried out a linear regression of copulation latency by courtship rate for mate choice trials in which copulation occurred ($N=69$), and examined the line of best fit. The results of the regression show an inverse relationship, so that as courtship rate increased the more quickly a pair copulated (Figure 5; $df= 1$, $t\text{-ratio} = -3.74$, $P< 0.001$).

To examine if male courtship effort was influenced by male diet treatment, female diet treatment and signaling environment, we carried out a least squares regression with male diet, female diet, visual environment, and seismic environment as our independent variables and courtship rate as our response variable. We found no effect of male diet, female diet, or seismic environment on a male's courtship rate. We did, however find a significant effect of the visual environment on courtship rate (Table 6; whole model test: $R^2= 0.18$, $df= 4$, $P< 0.001$). Males courted at a significantly higher rate in the visual present environment ($N= 95$, $\bar{x} = 0.03$ bouts/time spent courting) than in the visual negative environment ($N= 97$, $\bar{x} = 0.01$ bouts/time spent courting) (Wilcoxon 2-sample test, $Z= 5.502$, $P< 0.001$).

Foreleg Ornamentation Mating Trials – A total of 20 males (10 Shaved, 10 Unshaved) and 20 females were run in mate choice trials. At the time of testing females ranged between 14-23 days post maturation with an average age of 19.2 days post maturation.

Males ranged between 22-31 days post maturation with an average age of 26.85 days post maturation. Of the trials we conducted, 9 out of 20 (45%) resulted in copulations. Of the trials which resulted in copulations 5 (55.6%) occurred in trials with shaved males and 4 (44.4%) occurred in trials with unshaved males.

To examine if copulation frequencies differed significantly between the two male leg treatment groups, we carried out a nominal logistic regression for copulation occurrence with male leg treatment, courtship rate (bouts/time spent courting) and the interaction between the two as our independent variables. We found a significant effect of courtship rate on the likelihood to copulation but no effect of male leg treatment or the interaction between leg treatment and courtship rate (whole model test: $\chi^2 = 20.939$, $df = 3$, $P < 0.001$). Males which copulated had a higher courtship rate than those which did not copulate (Likelihood Ratio, $\chi^2_1 = 12.481$, $P < 0.001$). To examine if copulation latency was affected by male leg treatment, we carried out a least squares regression with copulation latency as our dependent variable and male leg color, copulation latency and the interaction between the two as our independent variables. The overall model was insignificant (whole model test: $R^2 = 0.29$, $df = 3$, $P = 0.125$), indicating that none of these variables had a significant effect on copulation latency.

DISCUSSION

We found no influence of diet treatment on female mate choice for either *S. crassipalpata* or *S. bilineata*, despite having significantly diverged diet treatments with respect to their body condition indices. Copulation success was not dependent on either male or female diet treatment for either species we examined. Additionally, our ornamentation

manipulations showed no effect on copulation frequency for either species. Thus, information about male foraging history, and potentially about past as well current energy reserves (*i.e.* condition), does not seem to influence female mate choice decisions for either *S. crassipalpata* or *S. bilineata*. It is worth noting however, that in our ornamentation manipulation trials the females used were older than the females used in our signaling environment trials. Another study with a related species, *S. ocreata*, has shown that female choosiness decreases with age (Uetz and Norton 2007). In this study the authors found that *S. ocreata* females' receptivity peaked between 15 and 21 days post maturation (Uetz and Norton 2007). It seems plausible (especially for *S. crassipalpata*, where females averaged 31 days post maturation in the ornamentation manipulation trials vs. 15 days post maturation in the environmental manipulation trials) that our results were affected by females' age, so that females mated indiscriminately with regard to a male's level of ornamentation.

For both species, the signaling environment as well as male courtship rate significantly influenced mating success. For the un-ornamented *S. crassipalpata* males, mating success was dependent upon both visual and seismic signal efficacy. *S. crassipalpata* pairs were more likely to mate in the visual negative than in the visual present environment, and in the presence versus absence of a seismic signal – indicating a strong reliance on the seismic signal modality. In direct contrast to *S. crassipalpata*, for *S. bilineata*, the seismic signal appears to play little to no role in mating success, while visual signaling appears to be important. For the visually ornamented *S. bilineata* males, the likelihood of copulating was significantly higher in the visual present versus the visual negative, with no influence of the seismic signaling environment. Thus, these two

sister species appear to rely on signals sent through different sensory channels for reproductive success. Below, we discuss these results by each species first and then discuss their implications regarding the divergence of these two sister species.

Schizocosa crassipalpa – The seismic courtship signal appears crucial for mating success in *S. crassipalpa*, as copulation success was significantly higher in the presence versus absence of seismic signal transmission and pairs were more likely to mate in the visual negative versus the visual positive environment (when presumably only seismic or near-field sound signals could be perceived). Although *S. crassipalpa* males incorporate a visual leg wave into their courtship displays, only one copulation was observed in the presence of visual signal transmission but absence of seismic signal transmission (V+/S-; see Figure 1). While there is a clear reliance on seismic signaling in this species, the incorporation of a leg wave raises the possibility of an additional signaling modality – air particle displacement, or near-field sound transmission. This signaling modality has been suggested to be used in the male courtship display of another species of *Schizocosa* wolf spider, *S. retrosa* (Rundus et al. 2010a). Both male and female wolf spiders have sensory organs called slit sensilla (Foelix 1996), which are capable of receiving the pressure wave component of sounds if they are sufficiently loud (Barth 1982). Additionally spiders, as well as other arthropods, possess fine filiform hair sensilla. In the case of spiders these hairs are called trichobothria. To date most research on the function of trichobothria has been centered around their use in prey capture (Barth 2002), however, their use in intraspecific communication among arachnids has been demonstrated (Santer and Hebets 2008). Additionally, Rundus et al. (2010a) have shown that the leg wave displays of *S. retrosa* are of sufficient magnitude to displace air

particles at a high enough velocity to be detected by the trichobathria of females. It seems plausible that the leg wave display of *S. crassipalpata* males could also cause sufficient air particle displacement as to be perceived by females.

While reliance on seismic and potentially near field sound transmission fits with our finding that pairs mated more in the presence vs absence of seismic signaling, it does not necessarily aid in explaining why pairs mated significantly less in the visual present versus visual negative environment. While our data cannot address this finding explicitly, we put forth a few potential explanations. The first possibility is that our results simply reflect the activity patterns of *S. crassipalpata*. It is possible that this species has a predominantly nocturnal activity pattern, making it less likely to court in light environments. In opposition to this hypothesis, however, we found no effect of the signaling environment on courtship performance. Alternatively, the visual leg wave of males may function to inadvertently startle females under visual present conditions. In another species of related wolf spiders, *S. ocreata*, male use their fangs during sexual encounters with females, sometimes resulting in female haemolymph loss (Johns et al. 2009). This could help explain the copulatory pattern observed here as females stand to potentially incur significant costs when mating with overly aggressive males.

Higher levels of male *S. crassipalpata* courtship rate were also positively correlated with copulation success across all diet and environmental treatments. Males which courted at higher rates had a greater likelihood to copulate than those which courted at a relatively lower rate. Additionally there seems to be a minimum threshold level of courtship, below which no female accepted males for copulation. Above this minimum level of courtship a male's probability of copulating increased. Female

preference for high male courtship rate has been widely documented across a variety of taxa including fiddler crabs (Marai & Blackwell 2006), crickets (Tregenza et al. 2006), skinks (Stapley 2008) damsel fish (Knapp & Kovach 1991), and wolf spiders (Shamble et al. 2009, Hebets et al. 2011). Courtship is an energetically costly activity, and females may use it as an honest indicator of male condition. Future studies should directly address this hypothesis of female preference in *S. crassipalpata*.

Schizocosa bilineata – In contrast to *S. crassipalpata*, male *S. bilineata* possess dark brushes of hair on the tibia of the forelegs. For this visually ornamented species, the visual signaling environment significantly influenced reproductive success, with males courting at higher rates in the visual present versus visual negative environments and subsequently achieving more copulations in the visual positive versus visual negative environments. This is the first time that a male's visual signal has been demonstrated to be more important than the seismic signal for copulatory success in the genus *Schizocosa*. None-the-less, although copulatory success was much lower in the visual negative environment, copulations did still occur when only the seismic signal was present. Thus, the seismic signal is still sufficient for mating success in this species. It may be that the seismic signal operates as an efficacy back up for the visual signal, allowing females to assess males in environments where they are not able to detect the visual signal. An efficacy backup for the visual signal would be beneficial for males courting in low light conditions. Although *S. bilineata* seem to be more active during the day light hours, they have been observed and collected at night as well (M. Bern, personal observation). Additionally, *S. bilineata* live in a grassy habitat where visual detection is not always

optimal, and the seismic signal may be better suited to alerting conspecifics when vegetation is particularly dense and the range of visual detection is low.

Support for the signal efficacy back up function of multimodal courtship signaling has been demonstrated in other species of wolf spider (Hebets and Papaj 2005, Gordon and Uetz 2011, Wilgers and Hebets 2011). An alternative explanation for this pattern of copulatory success is provided by the efficacy trade-off hypothesis. Under this hypothesis, the seismic signal might be more effective at raising female detection rates over relatively long distances while the visual signal might allow females to more easily locate males in close proximity. Males and females were in relatively close proximity to each other during our mating trials, but females in the in the V-/S+ environment were able to orient toward courting males. Male *S. bilineata* may use the seismic signal over longer distances to alert and guide females to their general area but rely more heavily on the highly conspicuous visual signal to court females at closer ranges. These two possible hypotheses are not mutually exclusive and could both prove fruitful areas of future research.

The finding that males adjust their courtship rate depending upon their signaling environment is intriguing. Two recent studies have demonstrated that male wolf spiders demonstrate plasticity in their courtship and mating behaviors across multiple environmental contexts (Gordon and Uetz 2011, Wilgers and Hebets 2011). In their natural habitat *S. bilineata* seem to be more active during the day than at night (M. Bern, personal observation). This diurnal activity pattern is the exact opposite of the activity patterns observed for most other *Schizocosa* species, including *S. crassipalpata* and may have contributed to a more visually based courtship display in *S. bilineata*.

While *S. bilineata* pairs were more likely to mate in environments which facilitated visual signal transmission, males also expressed higher courtship rates in these environments, making it difficult to determine the cause and effect of our environment-dependent copulation success. Due to the presence of conspicuous foreleg ornamentation, it is tempting to interpret our results through the lens of female choice for male ornamentation – females were able to detect the ornamentation in the visual positive vs. negative environment, resulting in higher mating success. However, in the absence of condition-dependent mate choice, this explanation seems unlikely. If female preferences for male ornaments were driving our observed pattern of copulation frequency across signaling environments, we might have expected females to prefer males with larger brushes (i.e. HD males) in the light – a pattern that we did not detect. Additionally, in the subsequent mate choice experiments we conducted in which *S. bilineata* males had their foreleg brushes experimentally removed while others had their brushes left intact, copulation frequency was independent of the presence/absence of male brushes. Nonetheless, while we suggest that females do not appear to make mate choices based on the presence or size of male brushes, the brushes may still increase the efficacy of males' leg wave by increasing its detectability or discriminability against complex backgrounds. This hypothesis has been suggested before for other species of wolf spider where foreleg ornamentation was not found to be under direct sexual selection (Shamble et al. 2009). Visual displays which increase the signal efficacy in complex or “noisy” backgrounds are known to exist (Ord et al. 2007) and could provide an important mechanism for the retention of male brushes in *S. bilineata*. Another possible explanation for the retention of male brushes in *S. bilineata* is that they may aid in displacing air particles to help

generate a near-field sound signal. As stated before, it has been shown that the leg waving display of *Schizocosa* wolf spiders are theoretically capable of producing this kind of signal (Rundus et al. 2010a). However, we find this explanation for the retention of brushes unlikely for *S. bilineata* because no copulations occurred in the V-/S- environment. If a near-field signal were important for male copulatory success we would have expected to have seen at least some copulations in this environment.

A similar hypothesis that might explain the presence of male brushes in *S. bilineata* despite the seeming lack of female preference for them is the motor performance enhancement hypothesis (Byers et al. 2010). This hypothesis predicts that male ornaments evolved not because of female preferences for the ornament itself, but because it enhances male's apparent motor skill during courtship displays. Motor performance displays during courtship may be selected for if they provide females with accurate information about males' motor skills in other context, such as foraging and predator avoidance. This could also contribute to why males courted at higher rates in the visual present environment. Courtship behavior is costly for a wide range of animal taxa including spiders (Kotiaho et al. 1998, Hoefler 2008). In a related species of wolf spider *S. stridulans*, it has been shown that male foreleg ornamentation reduces the level of courtship necessary to gain copulations (Hebets et al. 2011). A similar interaction may exist between the level of ornamentation and courtship rate in *S. bilineata* and future work should explore the possibility that male brushes evolved secondarily to increase a male's apparent motor performance.

Conclusions – Copulatory success was not significantly correlated with either male or female diet treatment for either species. Spiders were immature when they were collected

in the field, and it was impossible to know a priori how close they were to sexual maturity. Because of this, the possibility remains that spiders may not have been on their diet treatments long enough to meaningfully diverge aspects of male foraging history. Regardless, our results provide no reason to support that this is the case. We have no evidence that females of either species are using information about male body condition to make their mate choice decisions.

We found a stark difference in the signal efficacy function of male multimodal courtship displays between *S. crassipalpata* and *S. bilineata*. Despite the use of a leg tap in male courtship displays, female *S. crassipalpata* do not appear to use this visual signal directly in their mate choice decisions. Conversely male *S. bilineata* displays were dominated by the visual signal, and across our environmental treatments copulation success was highest when it was present, even when the seismic signal was absent. Male *S. bilineata* also courted significantly more in the visual positive environment. A courtship display consisting of only a seismic display is thought to be the ancestral state for *Schizocosa* wolf spiders (Stratton 2005). In their natural habitat *S. bilineata* seem to be more active during the day than at night (M. Bern, personal observation). This diurnal activity pattern is the exact opposite of activity patterns observed for most other known *Schizocosa* species, including *S. crassipalpata*. If an ecological shift in activity patterns caused the initial split between *S. crassipalpata* and *S. bilineata* then a shift in female preference for a more visual display by *S. bilineata* females may have subsequently reinforced reproductive isolation between these two species. Future work should address whether or not this is the case.

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TABLESTable 1: *Schizocosa crassipalpata* nominal logistic regression for copulation occurrence.

Source	df	Chi Square	P value
Female Age (random)	1	3.343	0.037
Female Diet	1	0.345	0.557
Male Diet	1	0.176	0.675
Visual Environment	1	11.369	<0.001*
Seismic Environment	1	33.335	<0.001*
Courtship Rate	1	20.692	<0.001*

* Denotes significant values after bonferroni correction

Table 2: *Schizocosa crassipalpata* least squares regression for copulation latency.

Source	df	t ratio	<i>P</i> value
Female Diet	1	-1.24	0.217
Male Diet	1	0.29	0.769
Visual Environment	1	2.90	0.004*
Seismic Environment	1	-2.63	0.009*
Courtship Rate	1	-0.74	0.462

* Denotes significant values after bonferroni correction

Table 3: *Schizocosa crassipalpata* standard least squares model for courtship rate.

Source	df	t ratio	<i>P</i> value
Female Diet	1	-1.370	0.298
Male Diet	1	-0.800	0.163
Visual Environment	1	-1.000	0.102
Seismic Environment	1	-0.570	0.716

* Denotes significant values after bonfferoni correction

Table 4: *Schizocosa bilineata* nominal logistic regression for copulation occurrence.

Source	df	Chi Square	P value
Female Age (random)	1	3.946	0.047
Female Diet	1	0.292	0.589
Male Diet	1	0.245	0.621
Visual Environment	1	11.133	<0.001*
Seismic Environment	1	2.676	0.102
Courtship Rate	1	122.595	<0.001*

* Denotes significant values after bonfferoni correction

Table 5: *Schizocosa bilineata* least squares regression for copulation latency.

Source	df	t ratio	P value
Female Diet	1	0.14	0.891
Male Diet	1	1.18	0.240
Visual Environment	1	-1.78	0.077
Seismic Environment	1	-1.86	0.643
Courtship Rate	1	5.65	<0.001*

* Denotes significant values after bonferroni correction

Table 6: *Schizocosa bilineata* standard least squares model for courtship rate.

Source	df	t ratio	<i>P</i> value
Female Diet	1	0.590	0.555
Male Diet	1	0.620	0.535
Visual Environment	1	-0.636	<0.001*
Seismic Environment	1	-1.140	0.257

* Denotes significant values after bonfferoni correction

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FIGURES

Figure 1: *Schizocosa crassipalata* copulation percentages in each environmental treatment.

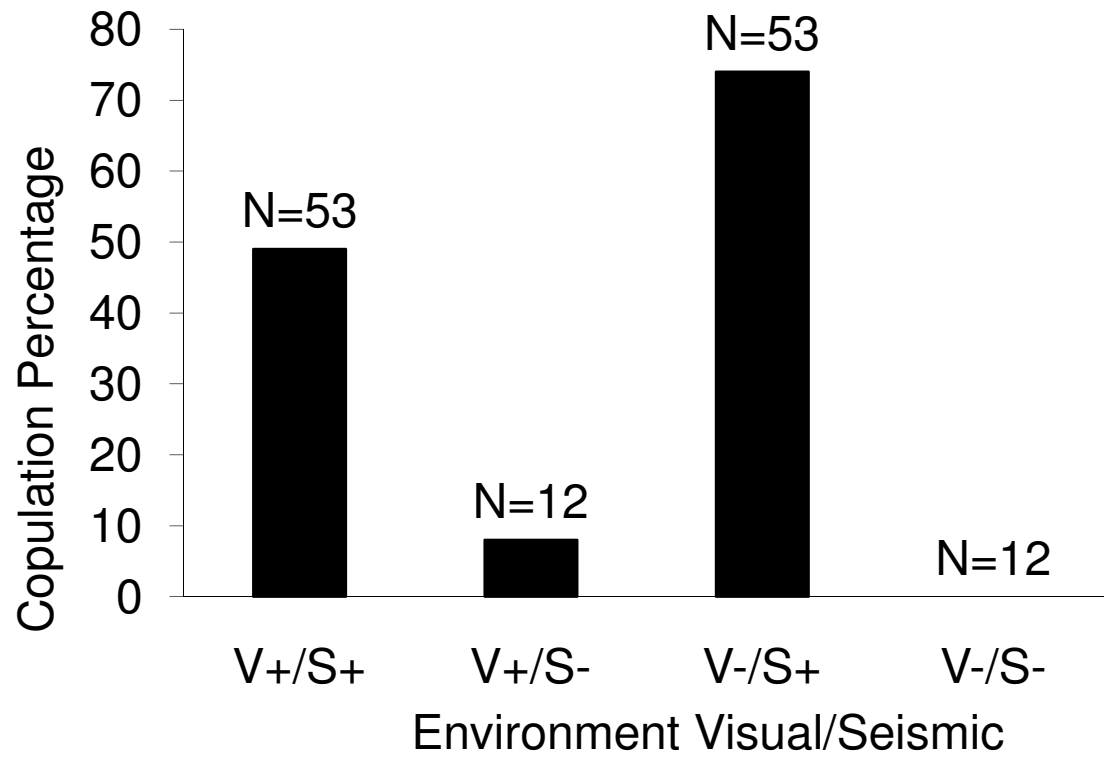


Figure 2: *Schizocosa crassipalata* probability of copulating by courtship rate. The dashed line represents the threshold for female acceptance.

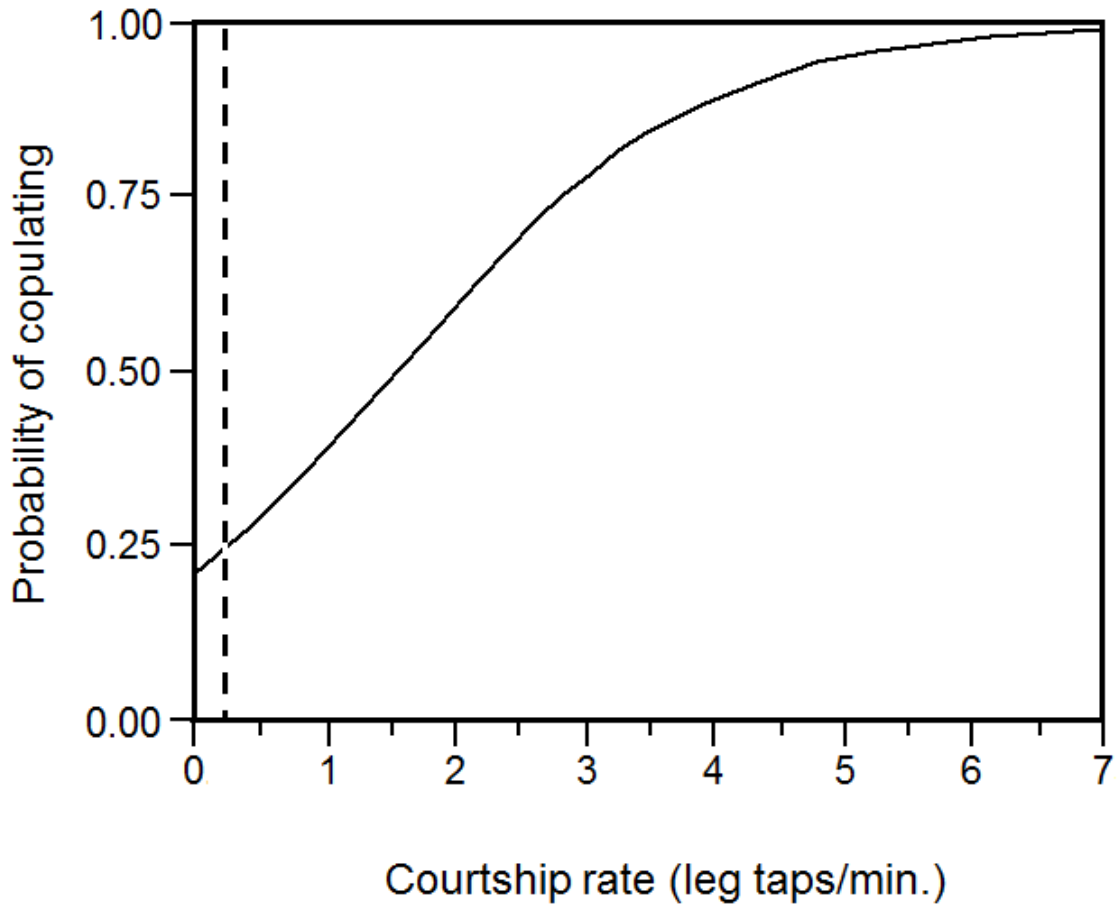


Figure 3: *Schizocosa bilineata* copulation percentages in each environmental treatment.

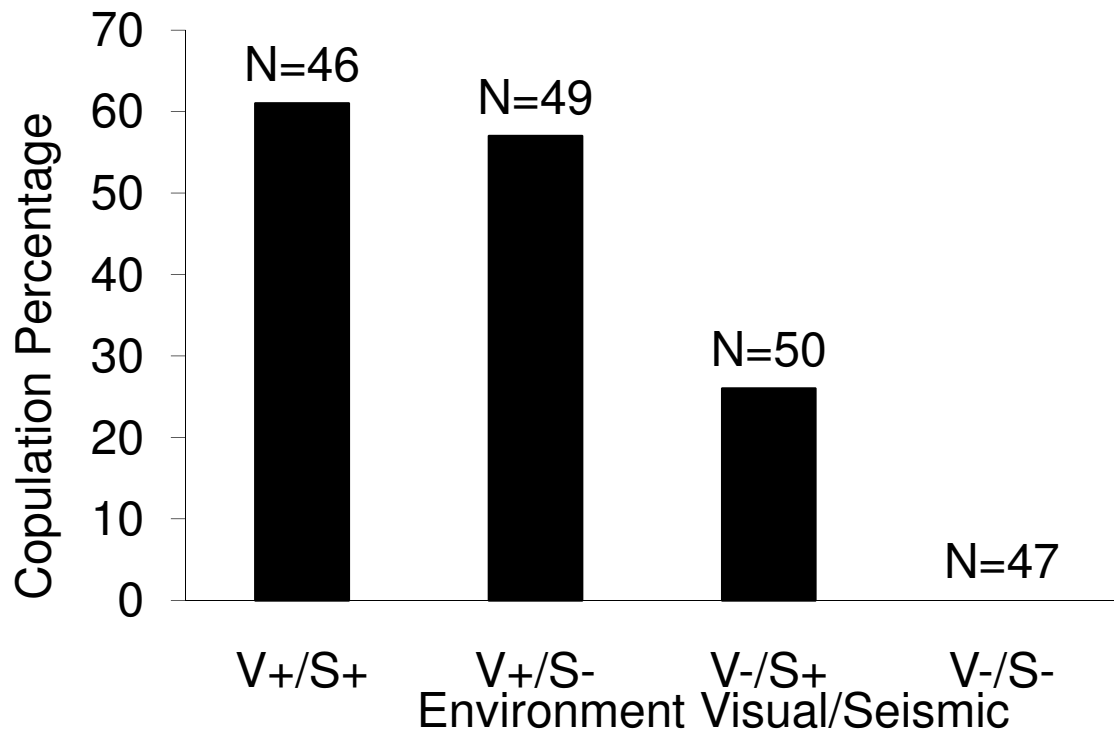


Figure 4: *Schizocosa bilineata* probability of copulating by courtship rate. The dashed line represents the threshold for female acceptance.

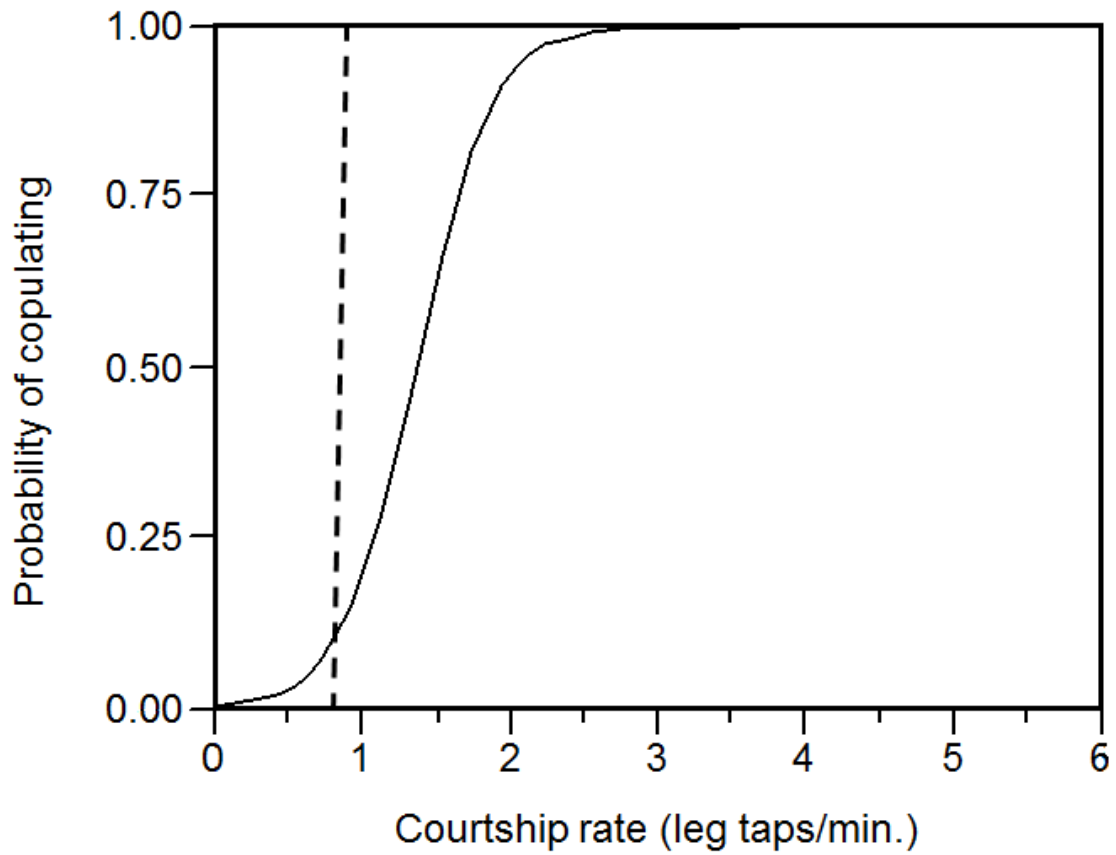


Figure 5: *Schizocosa bilineata* copulation latency by courtship rate for mate choice pairs which copulated (df= 1, t-ratio = -3.74, P< 0.001).

