University of Cincinnati

Date: 6/20/2017

I, Timothy Meyer, hereby submit this original work as part of the requirements for the degree of Master of Science in Biological Sciences.

It is entitled:
A Test of Two-axis Male Mate Choice in Schizocosa Ocreata (Hentz) Based on Experience and Cues Indicating Female State

Student’s name: Timothy Meyer

This work and its defense approved by:

Committee chair: George Uetz, Ph.D.
Committee member: Eric Maurer, Ph.D.
Committee member: Andrew Roberts, Ph.D.
A test of two-axis male mate choice in *Schizocosa ocreata* (Hentz) based on experience and cues indicating female state

A thesis submitted to the
Graduate School
of the University of Cincinnati
in partial fulfillment of the
requirements for the degree of
Master of Science
in the Department of Biological Sciences
of the College of Arts and Sciences
by

Timothy B. Meyer
B.S. Saint Louis University

June 2017
Committee Chair: George W. Uetz, Ph.D.
Cincinnati, OH
Abstract

Recent interest in male mate choice has prompted a re-examination of widely held beliefs regarding sex roles in animal mating systems. It is now known that males can be choosy based on female quality – namely with regards to sperm competition and fecundity. However, studies which directly compare aspects of female quality and their influence on male mate choice are relatively rare. The brush-legged wolf spider Schizocosa ocreata (Hentz) has a well-studied, conspicuous male courtship display, within which can be seen evidence of male mate choice based on female mating history and foraging history, as well as possible effects of experience and rearing environment. This makes it an excellent system to compare the importance of these two female states and examine how the choice patterns they give rise to may be influenced by the environment. By examining male courtship and female behavior under different circumstances and across environmental experience conditions, the relative effects of these factors can be inferred. Evidence collected in this study suggests that female mating status is the dominant predictor of both male and female mate choice, with males most often courting virgin females more vigorously regardless of context, as well as females showing higher levels of receptivity when unmated. An important exception to this trend is seen in field-reared males and their female partners, who show no preference or behavioral differences based on female state, suggesting that this preference is not static and may be influenced by experience. Altogether these results fit within the general pattern of two-axis preference studies by showing a trend to the importance of mating status with lesser importance of feeding status. Therefore, these results may help to better understand the influence of male mate choice on female sexual selection across taxa.
Acknowledgements

I would like to thank and acknowledge my advisor Dr. George Uetz for his encouragement, confidence, and support for these past three years and for remaining constantly available to his students despite the time constraints of his tenure as department head. I would also like to thank George and his wife Kitty for everything they do to make the lab a home and a family. I thank the members of my advisory committee, Dr. J. Andrew Roberts and Dr. Eric Maurer, for their patience and their support in extending my time here, as well as for their input on project design and theory. Thank you as well to the many graduate and undergraduate students that I have shared the lab with through times both stressful and enjoyable. Thank you to Brent, Rachel, and Alex for your advice and your examples in my first year and thank you to Emily, Brittany, Maddi, and Trinity for making me seem relaxed and carefree by comparison.
**Table of Contents**

Abstract ........................................................................................................................................... ii

Acknowledgments .......................................................................................................................... iv

List of Figures .................................................................................................................................. vi

Introduction ....................................................................................................................................... 7

Methods

  *Animal Maintenance and Care* ................................................................................................. 14

  *Treatment Groups* ....................................................................................................................... 14

  *Part I: Females exposed to video playback* ............................................................................... 15

  *Part II: Males exposed to female silk* ........................................................................................ 16

  *Part III: Live mating trials* ......................................................................................................... 17

  *Statistical Analyses* ..................................................................................................................... 17

Results

  *Part I: Females exposed to video playback* ............................................................................... 18

  *Part II: Males exposed to female silk* ........................................................................................ 18

  *Part III: Live mating trials* ......................................................................................................... 20

Discussion ......................................................................................................................................... 21

References ......................................................................................................................................... 29

Figures ............................................................................................................................................. 35
List of tables and figures

Figure 1: Percent of Female Response in Playback
Figure 2: Latency to Female Response in Playback
Figure 3: Strength of Female Response in Playback
Figure 4: Percent of Male Response on Silk
Figure 5: Latency to Male Response on Silk
Figure 6: Strength of Male Response on Silk ( Strikes per Bout)
Figure 7: Strength of Male Response on Silk (Courtship Bouts)
Figure 8: Strength of Male Response on Silk (Cheliceral Strikes)
Figure 9: Percent of Male Response in Live Mating
Figure 10: Percent of Female Response in Live Mating
Figure 11: Percent of Successful Copulations
Figure 12: Latency to Male Response in Live Mating
Figure 13: Latency to Female Response in Live Mating
Figure 14: Latency to Successful Copulation
Figure 15: Strength of Male Response in Live Mating ( Strikes per Bout)
Figure 16: Strength of Male Response in Live Mating (Courtship Bouts)
Figure 17: Strength of Male Response in Live Mating (Cheliceral Strikes)
Figure 18: Strength of Female Response in Live Mating
**Introduction**

Mating systems are an important factor in determining the composition of successive generations and are one of the most widely studied elements of animal behavior (Emlen and Oring 1977). Numerous mating strategies are utilized in the animal kingdom, as variable as the organisms which make use of them, with one commonly observed strategy being persuasive courtship. This strategy often follows a standard sequence of events where the first step involves locating and identifying a potential mate. The persuasive courtship step begins after the recognition phase is complete and can take on immense variety and complexity. This courtship is usually directed from the “promiscuous” sex towards the “choosy” sex and, at a point during or immediately following courtship, the choosier individual may signal their receptivity and the interaction will proceed to the final phase of copulation and fertilization. In this system, it is generally accepted that the promiscuous sex is the male and the choosy sex is the female, within which Bateman’s principles state that the fitness of the male will increase with increasing number of copulations while the fitness of the female will not (Bateman 1948; Arnold 1994; Collet et al 2014). Research in this area has therefore mainly focused on the principles underlying the concept of female choice – i.e. what makes a male attractive? Factors such as male size, coloration, health, ability to provide resources, and the genetic potential behind these qualities have been shown to influence a female’s willingness to accept a partner (Jennions and Petrie 1997; Ryan and Keddy-Hector 1992; Widemo and Sæther 1999).

However, this paradigm does not consider the possibility of male mate choice, consequently this area of research is significantly understudied (Tang-Martinez and Ryder 2005; Tang-Martinez 2016). More recent studies have shown that even within systems where Bateman’s principles hold true, and males may increase their fitness with multiple matings, they...
may still exhibit a degree of choosiness when there is a significant energetic cost to mating or when female quality is variable (Collet et al 2014; Edward and Chapman 2011; Bonduriansky 2001). From a male’s perspective, female quality can be reduced in two main ways, though few studies have examined their relative importance in influencing choice. First, due to first-male advantage in sperm competition, previously-mated females do not offer the same level of fitness benefits to a male as a non-mated female would (Parker 1970; Wedell et al 2002; Wigby and Chapman 2004). Since mated females possess fewer unfertilized eggs on average than non-mated females, males would benefit from the ability to discriminate between them and have been shown to preferentially associate with or engage in courtship toward the latter (Dewsbury 1982; Thomas 2011). Second, fecundity differences may lead to variation in female quality. Often, larger females tend to have higher fecundity, leading to strong male preference relating to body size (Bonduriansky 2001). Additionally, recent foraging history can also influence fecundity and behavior (Scharf 2016), which may provide advantages to males that can discern information relating to foraging history through cues other than body size (Avigliano et al, 2016; Barry, 2010; Maxwell et al 2010).

In spiders, male-female interactions take on another layer of complexity. Factors such as mating history and feeding history, which may already influence male mate choice, can have an increased effect due to their association with female behaviors that carry significant risk of physical harm. Due to the relatively high likelihood of sexual cannibalism in many spider species and their generally solitary lifestyle, intersexual encounters tend to be infrequent and potentially fatal for individual males (Elgar, 1992; Elgar and Schneider 2004; Prenter et al 2006). Since the outcome of these encounters may depend on whether a female considers a male a potential mate or potential prey (Blamires 2011; Berning et al 2012), it is important for males to be able to
accurately and reliably detect and identify female cues that indicate how she might respond to courtship. In sexually monandrous species, where the female will generally only mate once or otherwise be highly selective in choosing mates, successful copulation will reduce a female’s receptivity to successive male advances, making a previously-mated female less attractive to a male (Elgar 1998; Heberstein et al 2002; Suter 1990; Stoltz et al 2007; Baruffaldi and Costa, 2014). Additionally, research in some spider taxa has suggested the presence of female-associated cues containing information regarding hunger state, possibly representing an increased physical threat to the male, which may also influence male courtship behavior (Johnson et al 2011; Baruffaldi and Andrade, 2015).

In *Schizocosa ocreata* (Hentz), a common wolf spider which resides in deciduous forest leaf litter throughout eastern North America, the importance of intersexual communication, female physiological state, and individual experience have been well-established as factors influencing mate choice and determining the outcome of mating interactions. Although male *S. ocreata* are polygynous and will frequently mate multiple times if given the opportunity, females are generally monandrous and rarely consent to secondary matings (Norton and Uetz 2005). Intersexual encounters in this species normally begin with males locating and responding to a signal originating from the female that advertises her presence and a wide range of information regarding her identity and state (Roberts and Uetz 2005; Roberts and Uetz 2004). Although the composition of this cue remains unknown (Bell 2015), evidence suggests it is associated with the dragline silk produced and left behind by female *S. ocreata* as they move about their environment. Studies have shown that the information contained in this cue is contingent upon different female physiological states, namely mating and hunger status (Moskalik and Uetz 2011a; Roberts and Uetz 2005). Just as these states influence the cues these females produce,
they also influence the pre- and post-courtship behaviors they exhibit, making them more or less likely to display receptivity or aggression towards their male counterparts, and a likely source of selection on associated male behaviors (Moskalik and Uetz 2011a, b; Persons and Uetz 2005).

The males of this species possess secondary sexual characteristics in the form of brushes, or tufts of bristles, on the tibia of their forelegs. The tufts are utilized as visual signals in conjunction with leg-tapping and substrate-borne vibratory signals, forming multimodal courtship displays which males employ to identify themselves to females for species recognition and as persuasive courtship (Scheffer et al 1996; Uetz et al 2009). These courtship displays and ornaments are physiologically and energetically costly (Cady et al 2011), as well as condition dependent (Gibson and Uetz 2012; Gibson and Uetz 2008; Gilbert et al 2016; Uetz et al 2002), suggesting that male courtship is an honest indicator of current and past condition. Furthermore, males have been shown to vary courtship investment by altering aspects of this courtship display (i.e. the number or rate of certain components) in response to female characteristics such as those previously discussed. For instance, male *S. ocreata* will reduce the number of courtship bouts in response to cues from females that have already mated, females outside of a certain age range, and females that have been deprived of food (Moskalik and Uetz 2011a, Roberts and Uetz 2005), all of which correspond to female states which potentially reduce the likelihood of successful copulation by representing a low return on investment or greater risk of cannibalism.

Just as with the dynamics of female choice, patterns of male choice across the animal kingdom may not be static or universal. Across different contexts, age classes, or experience levels both males and females may modulate their preferences to take advantage of changing circumstances. Abiotic factors such as temperature, humidity, light, and environmental complexity, as well as biotic factors such as resource availability, predation risk, and the social
environment have been shown to affect different aspects of mating systems, as well as the
development of the traits involved (Callander et al 2012; Rypstra et al 2016). Additionally,
internal factors relating to an individual’s own social history and life history may play key roles
in determining mate preference (Cotton et al 2006; Schneider et al 2016). For example, terminal
investment theory suggests that an individual may increase its investment in reproductive effort
or alter its preferences as its future reproductive potential decreases due to age, illness, injury, or
other factors (Clutton-Brock 1984). This variation highlights the need for comparative studies
across different contexts to infer how mate choice might function in complex natural
environments.

Recent work has established the potential importance of individual experience and
environment history in S. ocreata as well. Several studies have shown that female S. ocreata may
alter their preferences for certain male traits and signal types depending on the social
environment they are exposed to before maturation (Rutledge et al 2010; Rutledge and Uetz
2014; Stoffer and Uetz 2015, 2016, 2017). Furthermore, experience may also influence the
courtship behavior and preferences of males of this species, as at least one study has shown that
previous experience with female chemotactile cues can result in changes in courtship vigor
toward food-deprived females (Moskalik and Uetz 2011a). Unfortunately, few studies have been
successfully conducted in which environmental conditions have been manipulated during S.
ocreata development (though there is evidence of behavioral differences between field caught
spiders and spiders reared in laboratory conditions; see Gibson and Uetz 2012; Clark et al 2015),
resulting in a poor understanding of exactly how past abiotic conditions may influence behavior.

Taken together, these factors illustrate the complexity and variability of this well-studied
mating system. To enhance our understanding of the relationship between these factors, it is
necessary to directly compare their effects within sample populations. Previous studies, like those referenced above, have examined the impact of variables such as mating history, feeding history, and experience as isolated variables, but there would be no separation of these among individuals in a natural population. This internal complexity would therefore lead to much greater variation in the combinations of physiological states, histories, and behaviors possessed and exhibited by the members of the community. Laboratory studies have demonstrated multiple preference axes that male *S. ocreata* respond to regarding female state and quality, but no studies have examined the relative importance of these states in influencing male behavior. It is unknown whether males display equivalent responses to changes in female quality due to different states, or if each state produces a unique level of response, or if these effects are interactive or independent of one another. Therefore, it is possible that combined factors may lead to complex interactions in behavior and mate choice that may have significant impact on overall fitness.

In mating systems with persuasive courtship, male mate choice is often demonstrated through modifications to courtship behavior (Edward and Chapman 2011). It is within this dynamic that intersexual communication becomes the critical means of both expressing and quantifying choice. At its simplest form, successful communication involves the transmission of some information-containing cue from one party – termed the signaler or sender – to one or more additional parties – termed the receiver. Often the production of these signals is an energetically costly process, making them honest indicators of both male quality and male investment (Endler 1992; Billen 2006; Bradbury and Vehrencamp 1998). In the most extreme form of mate choice, males may choose to forego engaging in courtship toward less attractive females, although less dramatic male choice may also be observed through changes in courtship vigor and energy
expenditure (Edward and Chapman 2011). This potential for modification not only allows males to optimize their reproductive investment depending on female quality, but also allows researchers to quantify and investigate the circumstances under which this occurs.

The research described herein will attempt to use this variation in male courtship communication and corresponding female behaviors to investigate the relative effects of female states on male mate choice along multiple preference axes. To do so, this study will test whether: 1) there is an additive effect of multiple female states (hunger and mating) on male behavior in the absence of any previous or immediate experience, 2) whether experience gained in the field affects this interaction, and 3) whether the behaviors observed when isolating sexes remain when repeated in a live male-female encounter. This research also includes an examination of how multiple states affect female behavior across the same contexts to determine whether changes in female behavior correspond to changes in male behavior. It is hypothesized that variation in multiple female states will have an additive effect on male courtship when males are presented with silk cues alone, resulting in a greater decrease in vigor and investment toward females that possess two negative states when compared to courtship towards females that possess only one or none. However, multiple female states may also result in a masking or overriding effect, where only one state may influence behavior. It is also hypothesized that variation in male experience will result in variation in male courtship behaviors across contexts and states, due to previous experience with female behavioral and cue variation in the field, where unpredictable conditions may favor the development of different behaviors. Furthermore, it is hypothesized that there may also be an additive or masking effect on female behavior when females are exposed to male courtship under video/vibratory playback conditions, resulting in differential receptivity and aggression. Finally, it is hypothesized that the behaviors exhibited in the isolated
male and female experiments described above will also be observed in live-mating trials, as these situations represent the greatest risk and reward to both parties.

**Methods**

*Animal Maintenance and Care*

All female *S. ocreata* and those males specified as “lab-reared” were collected from the Cincinnati Nature Center Rowe Woods in Clermont County, OH (N 39° 7’ 32.894” W 84° 14’ 57.692”) as juveniles and raised to maturity under a 13L:11D light cycle at 21 degrees Celsius. Collection occurred from March-May and August-October 2014-2016. Spiders specified as “field-reared” were collected from the same population as above, 1-2 weeks after mature males were first observed at the collection site (generally late May), and housed under the same environmental conditions until use (trial dates ranged from 1-21 days post-collection). All spiders were isolated from the collection date to the trial date in opaque plastic “deli dish” containers (diameter = 9 cm) with continuous access to water. Spiders were fed two or three 1/8”-juvenile crickets (*Acheta domesticus*) twice a week from the collection date until the start of the trial or when otherwise stated.

*Treatment Groups*

All experiments described herein utilized females from four treatment groups combining two female states (hunger and mating status) in a factorial design. These treatment groups are referred to as virgin-fed (VF), virgin-deprived (VD), mated-fed (MF), and mated-deprived (MD). Upon reaching 7-14 days post-maturity, females were randomly assigned to one of these four treatment groups. Females assigned to the mated groups (MF and MD) were then paired with similarly-aged males and allowed to mate. Females that failed to mate or cannibalized the male
were discarded from further use. All females assigned to food deprived groups (VD and MD) were then deprived of food for 7 days, while females assigned to fed groups (VF and MF) continued to be fed per the regular schedule. Across all studies, food-deprived females were confirmed to have significantly less mass than fed females (Wilcoxon Signed Ranks, \( z(325)=7.068, p<0.0001 \)). Spiders included in fed groups were fed a single cricket 24 hours prior to trials to eliminate hunger effects.

**Part I: Females exposed to video playback**

The aim of Part I was to establish a baseline of female behavior across reproductive and feeding states. In this experiment, females aged 14-21 days post maturity were exposed to a combined video and vibratory playback of male courtship (see Uetz and Roberts 2002 for discussion on the use and effectiveness of playback in *S. ocreata*). Playback was used to observe female interaction with males without the possibility of males adjusting their courtship behavior to compensate for female quality, thereby eliminating the effect of male mate choice on female behavior. Each female (total N=101, VF=25, VD=26, MF=25, MD=25) was placed in a circular arena (diameter = ~20cm) and exposed to 5 minutes of a playback stimulus. Playback consisted of a recording of a live male *S. ocreata* against a leaf litter background (Uetz et al 2011) shown to the focal female via a 4th Edition Apple iPod Touch. Video playback was synchronized with the vibratory recording of the same live male, which was transmitted through the parchment paper substrate via a piezoelectric disc bender at the population average amplitude (Roberts et al 2007). To reduce the effect of pseudo-replication, three different male exemplars were used to test female receptivity, representing the population average and upper/lower 95% interval of male condition and vigor (Roberts et al 2007). Each female was exposed to only one male exemplar and each female treatment group was assigned an approximately equal number of each
exemplar. All trials were recorded with a SONY Handycam HDR-XR260 and females were scored for latency to receptivity and number of receptive displays (pivot, tandem leg extend, settle, see McClintock and Uetz 1996 and Uetz et al 2009 for descriptions and significance).

**Part II: Males exposed to female silk**

The aim of Part II was to investigate how males with different levels of experience respond to female cues in the absence of feedback from a live female. First, female spiders aged 14-21 days post maturity (total N=45, VF=10, VD=12, MF=12, MD=11) were placed in individual circular arenas (diameter = 15cm) constructed from transparent plastic and allowed to deposit silk on a filter paper disk for 12 hours. The female was removed from the arena and the filter paper disk was then cut into two equal halves, creating 90 silk stimuli for eliciting male response. The scissors were cleaned with 70-percent ethanol before the first disk was cut and after each subsequent disk to remove any silk or chemical residue, preventing between-treatment contamination. Individual lab-reared naïve male spiders (N=85) aged 14-21 days post-maturity were then randomly paired with one of the previously prepared silk samples, placed on the filter paper, and recorded for 5 minutes. After completion of the observation period, males were removed from the filter paper and returned to their housing containers. Silk samples were then discarded and the test arenas cleaned with ethanol to remove chemical cues left behind. Silk samples were never used more than once, and were only used if there was an available male. This procedure was repeated using field-reared experienced males (N=80) and new females (total N=45, VF=11, VD=12, MF=11, MD=11). All trials were recorded with a SONY Handycam HDR-XR260 and scored for latency to begin courtship, number of courtship bouts, and number of cheliceral strikes (refer to Delaney et al 2007 for detailed descriptions of these behaviors).
**Part III: Live mating trials**

The aim of Part III was to investigate how male mate choice at different experience levels functions in a live mating encounter and the effects of that choice in terms of successful copulation. First experiment, female spiders aged 14-21 post-maturity (total N=87, VF=22, VD=23, MF=21, MD=21) were individually placed in a circular arena (diameter = 15 cm) on filter paper and allowed to deposit silk for 2 hours. A single lab-reared naïve male, aged 14-21 days post-maturity (total N=87), was then placed in each arena and the male and female were allowed to freely interact for 5 minutes. After the 5-minute interaction period, if copulation occurred, pairs were allowed to continue copulation until they separated or else were removed and returned to their housing containers. This procedure was then repeated using field-reared males (total N=67) and a new set of females (total N=67, VF=16, VD=18, MF=16, MD=17). All trials were recorded with a SONY Handycam HDR-XR260 and males were scored for latency to begin courtship, number of courtship bouts, and number of cheliceral strikes. Females were scored for latency to receptivity and number of receptive displays. The number of successful copulations, as well as the time they occurred, were also recorded.

**Statistical Analyses**

After trials were scored, the number of cheliceral strikes was divided by the number of bouts for each male to calculate strikes-per-bout as a measure of within-bout courtship vigor to supplement the number of bouts and cheliceral strikes. For live-mating trials, the number of female receptivity behaviors, as well as the number of male cheliceral strikes and courtship bouts, were divided by the total trial length (from start to copulation, cannibalism, or trial end) to calculate a time-relative measure of behavior. Measures of proportion or likelihood (e.g. to court, display receptivity, or copulate) were analyzed using a three-way contingency analysis using the
categorical female states and a categorical yes/no count of events. Latency (also to court, display receptivity, or copulate) was analyzed using a parametric survivorship analysis with a Weibull distribution. For Part I, the rate of female receptivity displays was analyzed via ANOVA with feeding and mating status as factors. For Part II, male courtship was analyzed via a MANOVA with the number of cheliceral strikes, courtship bouts, and cheliceral strikes per courtship bout as response variables and female mating status, female feeding status, and male rearing environment as factors. Additional univariate ANOVA tests were performed to investigate differences in each courtship measure, with a Bonferroni correction applied (α=0.017) and Tukey Kramer post-hoc tests performed when applicable. For Part III, male and female behaviors were analyzed in the same manner as in Parts I & II.

Results

Part I: Females exposed to video playback

No difference was found in the likelihood of females to display receptivity based on female state over a five-minute trial ($G^2=1.7$, df=4, p=0.7907; Figure 1). Likewise, a parametric survival analysis (Weibull) found no effect of female state on the latency to display receptivity toward the male stimulus ($X^2=2.6166$, df=3, p=0.4546; Figure 2). An ANOVA found no difference in the mean number of receptivity behaviors displayed by females across treatment groups (F=1.5517, df=3, p=0.2064; Figure 3).

Part II: Males exposed to female silk

No differences were found in the likelihood of lab-reared males to court females based on female state ($G^2=4.6$, df=4, p=0.372; Figure 4), while field-reared males were more likely to
court virgin females, regardless of their feeding status (full table: \(G^2=19.66, \text{df}=4, p=0.0006\); by mating status: \(G^2=15.7, \text{df}=1, p<0.0001\); Figure 4).

Lab-reared males engaged in courtship significantly sooner when exposed to silk from virgin females (whole model test: \(X^2=10.5236, \text{df}=3, p=0.0146\); by mating status: \(X^2=9.967, \text{df}=1, p=0.0016\); Figure 5b), while both female mating status and feeding status had an effect on the latency to courtship among field-reared males (whole model test: \(X^2=39.759, \text{df}=3, p<0.0001\); by mating status: \(X^2=33.655, \text{df}=1, p<0.0001\); by feeding status: \(X^2=4.1038, \text{df}=1, p=0.0428\); Figure 5a).

For male courtship behaviors on silk, the full model MANOVA was significant (Wilks’ Lambda \(F_{21,445}=7.2285, p<0.0001\)) and there was a significant effect of male rearing (Wilks’ Lambda \(F_{3,155}=30.3205, p<0.0001\)), female mating status (Wilks’ Lambda \(F_{3,155}=8.7106, p<0.0001\)), and female feeding status (Wilks’ Lambda \(F_{3,155}=3.6264, p=0.00144\)) – as well as significant interactions between male rearing and female mating status (Wilks’ Lambda \(F_{3,155}=8.9237, p<0.0001\)) and female mating and feeding status (Wilks’ Lambda \(F_{3,155}=3.4259, p=0.0187\)). Mean number of cheliceral strikes per courtship bout was significantly influenced (with Bonferroni corrected alpha of 0.017) by male rearing and female mating status (ANOVA whole model: \(F=7.0257, \text{df}=7, p<0.0001\); by male rearing: \(F=16.1533, \text{df}=1, p<0.0001\); by mating status: \(F=7.1822, \text{df}=1, p=0.0081\); Figure 6). Furthermore, there was a significant interaction between male rearing and female mating status (\(F=15.6505, \text{df}=1, p<0.001\)). The mean number of courtship bouts was also influenced by male rearing and female mating status (ANOVA whole model: \(F=8.3778, \text{df}=7, p<0.0001\); by male rearing: \(F=39.9616, \text{df}=1, p<0.0001\); by mating status: \(F=15.8824, \text{df}=1, p<0.001\); Figure 7). There was no significant difference in the mean number of cheliceral strikes (\(F=1.6209, \text{df}=7, p=0.1331\); Figure 8).
Part III: Live mating trials

Lab-reared males were significantly more likely to court virgin females (full table: $G^2=10.2$, df=4, $p=0.0372$; by mating status: $G^2=9.36$, df=1, $p=0.0022$; Figure 9), while field-reared males showed no significant difference ($G^2=1.34$, df=4, $p=0.8546$; Figure 9). Virgin females paired with lab-reared males were more likely to exhibit receptivity behaviors than their mated counterparts (full table: $G^2=21.06$, df=4, $p=0.0003$; by mating status: $G^2=19.5$, df=1, $p<0.0001$; Figure 10), while no significant differences were found based on any female state for females paired with field-reared males ($G^2=6.38$, df=4, $p=0.1725$; Figure 10). Encounters involving both lab-reared males (full table: $G^2=30.38$, df=4, $p<0.0001$; by mating status: $G^2=27.64$, df=1, $p<0.0001$; Figure 11) and field-reared males (full table: $G^2=13.48$, df=4, $p=0.0092$; by mating status: $G^2=13.04$, df=1, $p=0.0003$; Figure 11) were more likely to end in copulation when females were unmated, regardless of female feeding status.

Latency to begin courtship was significantly lower for lab-reared males when paired with virgin females than when paired with mated females (whole model test: $X^2=11.5025$, df=3, $p=0.0093$; by mating status: $X^2=10.465$, df=1, $p=0.0012$; Figure 12b), while virgin females paired with lab-reared males displayed receptivity significantly sooner than mated females (whole model test: $X^2=24.4816$, df=3, $p<0.0001$; by mating status: $X^2=22.737$, df=1, $p<0.0001$; Figure 13b). For lab-reared males, latency to achieve copulation was also significantly lower when paired with virgin females (whole model test: $X^2=33.0329$, df=3, $p<0.0001$; by mating status: $X^2=28.4606$, df=1, $p<0.0001$; Figure 14b). For field-reared males, there were no differences in courtship latency across female treatment ($X^2=1.7021$, df=3, $p=0.6365$; Figure 12a). Likewise, there were no differences in receptivity latency for females paired with field-reared males ($X^2=5.0248$, df=3, $p=0.17$; Figure 13a), though pairings involving virgin females
did proceed to copulation significantly sooner (whole model test: $X^2=13.5317$, df=3, $p=0.0036$; by mating status: $X^2=13.4726$, df=1, $p=0.0002$; Figure 14a).

For male courtship behaviors in live mating, the full model MANOVA was significant (Wilks’ Lambda $F_{21,411}=2.2699$, $p=0.0012$) and there was a significant influence of male rearing (Wilks’ Lambda $F_{3,143}=6.4970$, $p=0.0004$) and female mating status (Wilks’ Lambda $F_{3,143}=4.0090$, $p=0.0089$), with a significant interaction of male rearing and female mating status (Wilks’ Lambda $F_{3,143}=3.5787$, $p=0.0155$). Mean number of cheliceral strikes per courtship bout was again significantly influenced (with Bonferroni corrected alpha of 0.017) by male rearing and female mating status (ANOVA whole model: $F=3.9974$, df=7, $p=0.0005$; by male rearing: $F=12.0549$, df=1, $p=0.0007$; by mating status: $F=9.8755$, df=1, $p=0.002$; Figure 15). Mean rate of courtship bouts (ANOVA $F=0.7224$, df=7, $p=0.6532$; Figure 16) and cheliceral strikes (ANOVA $F=2.3398$, df=7, $p=0.0272$; Figure 17) were not significantly influenced by any factor at the adjusted alpha. The mean rate of female receptivity displays was significantly higher in virgin females (ANOVA whole model: $F=2.6921$, df=7, $p=0.0119$; by mating status: $F=10.6977$, df=1, $p=0.0013$; Figure 18).

**Discussion**

Though female state does not always influence male mating behavior, both female mating status and feeding status can have effects on male behavior and male mate choice under different circumstances. This variability suggests that *Schizocosa ocreata* may engage in a more complex preference system than previously reported. However, when female state was found to have an effect on male behavior, female mating status was always a significant contribution to the variation observed. Variation in male behavior due to female feeding status was observed less frequently. Furthermore, there were striking differences in the patterns of discrimination.
exhibited by males from both rearing conditions across the contexts tested. Males collected as mature adults were highly selective when exposed to only female silk, but showed no discrimination in any measured behavioral metric when paired with live females. Conversely, lab-reared males were less selective when only female silk was present and displayed their greatest level of discrimination when in a live encounter. Female behaviors varied across male rearing condition as well, but with mating status being the only large scale influence on receptivity.

In the field, males actively search for females and engage in trail-following behavior once silk has been located and before engaging in courtship (Cady 1983; Bell and Roberts 2017). When exposed to only female silk in the limited space of a trial arena, males are unable to use this strategy to locate the female that produced the cues in question, preventing them from forming a complete assessment of the potential costs and benefits of courtship. Many previous studies have demonstrated that female silk cues are sufficient to stimulate courtship in male *S. ocreata*, but the presence of silk and silk-bound chemical cues alone may not necessarily indicate a female is in the immediate area or in a position suitable to receive male courtship signals. Therefore, in circumstances where a male is not able to locate a female beyond the chemical signals which suggest she is nearby, it may be advantageous to be more selective. Not only would it be energetically costly for a male to court when there is less probability of a female receiving his signals, but the dark forelegs tufts and rapid motion associated with male courtship in *S. ocreata* greatly increase the probability of detection by nearby predators (Roberts et al 2007; Pruden and Uetz 2003; Clark et al 2016). Males that have experience courting in the presence of predators or in the complex environment of a deciduous leaf-litter habitat might therefore be more likely to exhibit mate choice under these conditions, consistent with the data.
observed in the field-reared trials from Part II where field males lowered their within-bout courtship vigor towards mated and food-deprived females. Why lab-reared males did not respond similarly to differences in female state is unclear. Past work has demonstrated that naïve, lab-reared males can discriminate between mated and non-mated females and display a clear preference for the latter (Roberts and Uetz 2005), but this was not observed in the same manner. Lab-reared males in this study showed the same level of within-bout courtship vigor toward all female treatments when exposed to silk alone, though they were significantly slower to begin courtship and reduced the total number of courtship bouts when the silk was deposited by a previously-mated female, which may suggest that males were still displaying some preference for virgin females. The differences between these results and past results may be due to a number of factors requiring further study, but the large range of variation in male behavior observed within this study alone justifies further research into how male mate choice may be expressed in this species.

Female behavior was examined under two contexts – with and without a male present – to determine a baseline response to combining states and to observe any changes brought about by male choice and behavior. Under playback conditions, with no male present, all females were equally receptive. Again, laboratory experiments – especially playback – cannot model interactions in a natural setting with complete accuracy. From a female’s point of view, the artificial males used in playback studies represent males displaying extremely persistent courtship, which may indicate an aspect of male quality that females are more likely to respond to regardless of state.

Live mating trials provide an opportunity to examine the real costs and benefits of mate choice strategies inferred from experiments with isolated individuals, as well as modifications to
these strategies that arise from changes in context. In contrast to their behavior in silk-only experiments, lab-reared males demonstrated a clear preference for virgin females in these live mating trials, matched by an equal pattern of receptivity in their female partners. This suggests that in the simplest mating system, without interaction of additional abiotic or biotic factors, female mating status drives both female reproductive behavior and male mate choice. However, in live mating trials involving field-reared males, with the possible influence of additional factors inherent in development and maturation under field conditions, the influence of female mating status largely disappears in the behavior of both sexes. Field-reared males show no form of discrimination based on any female state and females likewise show few differences in behavior toward those males. As female behavior has been shown to depend on female state in this species (see Introduction) and does indeed follow this rule in pairings involving lab-reared males who do display mate choice, this suggests that the lack of mate choice exhibited by field-reared males may be driving a similar reduction in mate discrimination in females.

An increase in reproductive effort on the part of males – as signified by lack of mate choice – can be indicative of alternative mating tactics or terminal investment. Alternative male mating tactics have been observed in spiders due to several factors including age, condition, and mating history (Gaskett et al 2004; Wilgers et al 2009). Though field-reared males were used at the same post-maturity age as lab-reared males, a side effect of laboratory conditions is that spiders collected earlier in the year will mature sooner than those in nature and, as a result, all lab-reared males matured and were used in trials several weeks before males began maturing in the field. In terms of absolute age - from egg sac emergence to trial date - field males were older on average than lab-reared males and are possibly already mated. The benefits of being choosy may be greatest when naïve – i.e., when males have yet to secure a successful mating and are yet to
fertilize any eggs – and diminished after males have already mated, as every additional copulation represents additional fitness benefits. In taxa where there is inherent risk of cannibalism in each interaction, multiple matings may not be guaranteed and it would advantageous for males to choose a female with a high probability of being receptive. However, this benefit is not constant and as the breeding season comes to an end, the number of females that have not mated is theoretically reduced and competition among males increases. Therefore, over a male’s lifetime, selectivity may become maladaptive and therefore being less choosy might result in higher overall lifetime fitness, as males would then have a greater probability of additional reproductive success due to the possibility of females submitting to secondary mating.

Confirming that the differences in male behavior observed are the result of change in reproductive tactics would require extensive additional research. If this were the case, the ultimate benefit for field-reared males would be a higher probability of copulation with mated females, but this was not found in this study. However, other age-related changes in behavior have been observed in male *S. ocreata* and congenerics (Nickley et al 2016; Rundus et al 2015) and it is possible that different results would be observed under actual field conditions. It is unknown at this time what constitutes “field experience” or what differences there are in development and life history between field-reared and lab-reared males. Selective pressures over the course of the field season from factors such as predation may be biasing the results obtained in this study by eliminating lower quality males and males more likely to display mate preference or elicit differential female response. Furthermore, there is likely significant variation in rearing environments within field-reared males across space – in terms of microhabitat – and across time – from season to season and year to year. Future studies may further demonstrate the plasticity of this species as environmental conditions change with increased urbanization and
climate effects. Unfortunately, until comprehensive studies have been done examining the effects of different biotic and abiotic factors on *S. ocreata* development, the “field” will remain a black-box and an unknown and this uncertainty makes it difficult to directly compare lab-reared and field-reared spiders and draw any strong conclusions about what is contributing to the variance observed.

However, differences within field-reared males can still be informative regarding the selective pressures faced by males in natural environments. For example, as in previous studies, male sensitivity or choice with regards to female feeding history was only observed in males with previous courtship and female experience – in this case the presumed experience that comes with life under field conditions. This suggests that either the ability to detect cues relating to female feeding state is experience dependent or the benefit of responding to these cues is variable. Although there is evidence of behavioral effects of starvation in females, these effects are not always present, which in turn suggests that starvation may have graded or asynchronous effects in males and females. For instance, one possibility is that females may produce cues indicating starvation before displaying corresponding behavioral effects, which would increase the variability in benefits obtained by discriminatory males. Although statistically significant effects of female feeding status and interactions between the two states were rare, visual inspection of the trends in data frequently show the lowest attractiveness and lowest receptivity in mated, food-deprived females. It is therefore likely that future studies may see increased influence of female feeding history by increasing the starvation burden to a point where its effects become more clear.

Tests for multi-axis preference with feeding and mating variation in other taxa have yielded a broad spectrum of results. In the Western black widow, *Latrodectus hesperus*, a strong
preference was observed for well-fed non-mated females (MacLeod and Andrade 2014). Regardless of male type or environment, changes in female feeding status and female mating status resulted in identical decreases in female attractiveness, where a starved female was just as attractive as a mated female or a mated starved female. This was interpreted to indicate an equal importance of sperm competition and female fecundity (or behavioral changes brought about by starvation) in male mate choice in this species (MacLeod and Andrade 2014). In the Chinese mantis *Tenodera aridifolia sinensis*, males were discriminatory based on both female feeding status and female mating status as well. However, these effects were distinct, with feeding status only resulting in a difference between virgins. Once mated, female *T. aridifolia sinensis* were equally attractive regardless of hunger state (Lelito and Brown 2008). In the common cricket *Acheta domesticus*, males showed no preference related to feeding state and body size and preferentially associated with cues from virgin females (Assis et al., 2016). Again, in the orb-weaving spider *Argiope bruennichi*, males discriminated largely based on female mating history regardless of fecundity (Schulte et al 2010). Based on the results of this study and previous studies, female mating history and associated sperm competition seem to be predominant factors in male mate choice, with additional factors becoming relevant and integrated into that choice based on the organism’s life style traits.

In summary, this study reinforces the complexity of the mating system of *Schizocosa ocreata* by highlighting the variation displayed by both males and females across contexts, physiological state, life history, and experience. The primary goal of this work was to compare the effects of female mating and feeding status on male behavior and to determine which has a greater influence on behavior. Evidence now suggests that female mating status is the main factor driving mate choice in both males and females, although it is evident that the hereto
understudied variation in male experience may also have a significant influence across contexts of interest. Additionally, some aspects of male courtship behavior and female response may show a greater or lesser influence of female state - possibly suggesting different costs, benefits, or meanings of each display. Although the majority of sexual selection research has focused on how female mate choice exerts selective pressure on the evolution of male traits, there is a growing body of work examining how male mate choice can influence the evolution of female traits as well (Clutton-Brock 2007). The strong male preference observed herein for virgin females may therefore help inform future work examining the evolution of female chemical signaling and behavior.
References


Figures:

Figure 1: The percent of female *Schizocosa ocreata* responding with receptivity displays to video/vibratory playback.
Figure 2: A survivorship curve representing the latency of female *Schizocosa ocreata* to respond with receptivity displays to video/vibratory playback.
Figure 3: The mean number of receptivity displays performed by female *Schizocosa ocreata* in response to video/vibratory playback. Treatments connected by a solid line are not significantly different.
Figure 4: The percent of male *Schizocosa ocreata* responding to female silk with courtship.
Figure 5: Survivorship curves representing the latency to engage in courtship for male *Schizocosa ocreata* when exposed to female silk. Plot A represents field-reared males and Plot B represents lab-reared males.
Figure 6: The mean number of cheliceral strikes performed by male *Schizocosa ocreata* per courtship bout when exposed to female silk. Different letters above each bar represent significant differences.
Figure 7: The mean number of courtship bouts performed by male *Schizocosa ocreata* when exposed to female silk. Different letters above each bar represent significant differences.
Figure 8: The mean number of cheliceral strikes performed by male *Schizocosa ocreata* when exposed to female silk. Treatments connected by a solid line are not significantly different.
Figure 9: The percent of male *Schizocosa ocreata* responding with courtship to a live female.
Figure 10: The percent of female *Schizocosa ocreata* responding to male courtship with receptivity displays.
Figure 11: The percent of live mating trials that ended in successful copulation.
Figure 12: Survivorship curves representing the latency to engage in courtship for male *Schizocosa ocreata* in live mating trials. Plot A represents field-reared males and Plot B represents lab-reared males.
Figure 13: Survivorship curves representing the latency to display receptivity for female *Schizocosa ocreata* in live mating trials. Plot A represents field-reared males and Plot B represents lab-reared males.
Figure 14: Survivorship curves representing the latency for a live mating trial to result in copulation in *Schizocosa ocreata*. Plot A represents field-reared males and Plot B represents lab-reared males.
Figure 15: The mean number of cheliceral strikes performed by male *Schizocosa ocreata* per courtship bout in a live mating trial. Different letters above each bar represent significant differences.
Figure 16: The mean number of courtship bouts per second performed by male *Schizocosa ocreata* in a live mating trial. Treatments connected by a solid line are not significantly different.
Figure 17: The mean number of cheliceral strikes per second performed by male *Schizocosa ocreata* in a live mating trial. Treatments connected by a solid line are not significantly different.
Figure 16: The mean number of receptivity displays performed by female *Schizocosa ocreata* in live mating trials. Different letters above each bar represent significant differences.