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The Complexities of Wolf Spider Communication Exploring Courtship Signal Function in *Rabidosa rabida*

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THE COMPLEXITIES OF WOLF SPIDER COMMUNICATION: EXPLORING COURTSHIP SIGNAL FUNCTION IN *RABIDOSA RABIDA*

by

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A DISSERTATION

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Dustin J. Wilgers, Ph.D.

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Evidence of signal complexity is seemingly pervasive across animal communication systems. Exploring signal function may provide insight into how these displays evolved and are maintained. This dissertation examines the courtship signal function in a grassland wolf spider. *Rabidosa rabida* lives in an extremely complex environment, and males use complex displays incorporating both visual and seismic modalities. Using several approaches I provide insight into the content and efficacy of the various signal components, as well as how variation in these displays influence female mating decisions in isolation and combined.

First, I manipulated male and female body condition using diet quantity manipulations and performed mate choice trials using females of each diet across two different age classes. Female mate choice decisions varied with diet and age. Overall, younger females were choosy, mating more often with good condition males, while older females mated indiscriminately. Next, to determine which signal components may be useful in female mate assessment, I explored the condition-dependence of the signal components and tested their efficacy by performing mate choice trials in environments that differed in modality transmission. Both visual and seismic components are condition-dependent, and

are sufficient to maintain copulation success when detected in isolation. Thus, each signal component may serve as both a content- and efficacy-backup when facing variable sensory environments. Lastly, I manipulated both foreleg ornamentation and the seismic display, and presented them to females both in isolation and combined, to determine if and how variation in each component influences female mating decisions. Females were choosy based on the seismic display alone, and only discriminated males based on foreleg ornamentation when detected along with a seismic signal, suggesting an inter-signal interaction.

Together, these experiments suggest that the sources of selection acting on male *R. rabida* are just as complex as the courtship displays used during mating interactions. The courtship signal components making up the display appear to function by maintaining both copulation success and mate assessment across a variety of environments encountered.

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INTRODUCTION

How can elaborate and often highly conspicuous ornamentation (e.g. coloration, morphology) and behavioral displays (e.g. visual, acoustic) evolve and be maintained despite the obvious costs (e.g. energetic, predation) that reduce their bearer's survival? This question regarding male secondary sexual traits has both baffled and intrigued scientists all the way back to Darwin's time (Darwin 1871). Sexual selection theory suggests that differences in investment into offspring result in males competing to reproduce with females. Females often discriminate between potential mates in order to maximize mate quality, and any trait that enhances a male's ability to reproduce relative to others will increase in frequency. Since females often cannot directly assess mate quality, males must communicate this information to females (reviewed in Andersson 1994).

Signals are the basic building blocks of animal communication. Simply put, signals are packets of energy generated by traits, displays and/or actions that are selected for their adaptive effect on the behavior of the receiver via its sensory-nervous system (definition adapted from Hebets and Papaj 2005). Thus by definition, signal form (i.e. size, color, movement, intensity, etc.) is under simultaneous selection from the receiver's sensory system and the environment to increase detection (i.e. efficacy-based), while being under selection to provide females with information regarding why they should attend/respond to the signal (i.e. content-based; Guilford and Dawkins 1991). There is a variety of information that may be important to females in making mate-choice decisions, including location or some aspect that identifies a male as a suitable mate (e.g. species identification, condition, age; Andersson 1994). In Chapter 4, I review some of the past literature on how body condition influences signal expression, and how a functional approach to studying condition-dependent signals may provide further insight into their evolution and maintenance.

Not only are there numerous sources of selection, but many of these sources are variable in the direction and intensity of selection on male signals, including many examples of variable female mate choice (Jennions and Petrie 1997), and dynamic or variable environments that each have different transmission characteristics (Bro-Jørgensen 2010). Many animal taxa have addressed the issues of detection and information posed by numerous and variable sources of selection by evolving complex signals, which commonly consist of numerous components often spanning multiple sensory modalities (Partan and Marler 1999; Hebets and Papaj 2005; Partan and Marler 2005). Recent theory focused on the evolution and maintenance of complex signals suggest they are one evolutionary answer for males to enhance female detection and elicit appropriate female responses (e.g. acceptance as mate) when facing variation in receivers and environments across mating interactions (Candolin 2003; Hebets and Papaj 2005). The use of multiple components allows responses to different sources of selection by each signal component, resulting in signal components that can either function independently (e.g. efficacy- and content backups, multiple messages; Møller and Pomiankowski 1993; Johnstone 1996) or via interactions with one another (e.g. amplifiers; Hasson 1991), which leaves the composite signal adapted to a variety of circumstances. Thus to fully understand complex courtship signal function, it is imperative to understand: 1) the potential information content of each component, 2) each component's efficacy in eliciting an appropriate female response (e.g. acceptance as mate), 3) how each of the components function in isolation, and 4) how the signal components function in combination (Candolin 2003; Hebets and Papaj 2005; Partan and Marler 2005).

One animal group, spiders, has provided considerable insight into the evolution of multimodal communication (Coleman 2009). Specifically, wolf spiders (family Lycosidae) have been a model system to study the evolution and diversification of reproductive communication systems. Wolf spiders face considerable variability across mating interactions, as their signaling environment is exceptional in both complexity and variability (Elias and Mason In Press), and courting males face strong female choice known to vary with a variety of factors (e.g. age, diet, experience; Hebets 2003; Uetz and Norton 2007; Hebets et al. 2008). The courtship displays witnessed across male wolf spiders are amazing in their diversity, as males vary in presence, degree, and type of foreleg ornamentation (i.e. pigmentation, brushes; Stratton 2005; Framenau and Hebets 2007), the sensory modalities incorporated (e.g. seismic, visual, near-field; Kotiaho et al. 1996; Uetz and Roberts 2002; Rundus et al. 2010), and the overall complexity of the display (Hebets and Uetz 2000; Stratton 2005). Numerous studies have investigated multiple species to determine the function of these courtship displays, focusing on the potential information content (Mappes et al. 1996; Uetz et al. 2002; Hebets et al. 2008; Shamble et al. 2009; Rundus et al.

2011), the efficacy of each modality (Hebets and Uetz 1999; Hebets 2008; Uetz et al. 2009; Rundus et al. 2010; Rundus et al. 2011), and how females respond to variation in these displays (Scheffer et al. 1996; Hebets et al. 2006; Gibson and Uetz 2008; Hebets 2008; Shamble et al. 2009; Hebets et al. 2011).

Males of the wolf spider, *Rabidosa rabida* (Walckenaer) perform complex courtship displays consisting of visual and seismic components. The visual portion of the display consists of pedipalp waves followed by arches and extensions of an ornamented foreleg (Rovner 1968), while the seismic display has multiple parts (i.e. introductory segment, pulse-train) produced via palpal stridulation (Rovner 1967; Rovner 1975). Both of these modalities have been suggested to play a role in conspecific interactions (Rovner 1996) and each is sufficient for female receptivity (Rovner 1967; Rovner 1968). This seminal work has provided important descriptions about the production of these displays and some evidence of their function; however, considerable work must be done in order to fully understand how this complex courtship signal functions during mating interactions. Through a series of experiments in this thesis, I look to explore the variability experienced by male *R. rabida* during mating interactions, and how their complex courtship signals function to maintain copulation success.

In Chapter 1, I investigate one important source of selection on male courtship signals, female choice, and how this type of selection may vary within and across females. In Chapter 2, I explore complex signal function across variable signaling environments by investigating how signal components function to provide females with information on male condition, and how these components function to maintain copulation success when detected both in isolation and in combination. Lastly, in Chapter 3, I explore how variation in each component influences female mate choice decisions when presented to females in isolation and in combination.

Ultimately from these experiments aimed at elucidating signal function in *Rabidosa rabida*, I hope to gain some insight into the evolution and maintenance of this beautiful display.

Plans for Publication of Chapters

I plan to publish Chapter 1 in *Behavioral Ecology and Sociobiology.* This chapter is a revision based on comments back from both the editor and the reviewers. I have published Chapter 2 in a special volume of *Current Zoology* on complex signaling (Wilgers & Hebets 2011). I have submitted to Chapter 3 to *Ethology* for publication. Finally, Chapter 4 is a book chapter that will be submitted for publication in an edited book, *Animal Signaling: Functional and Evolutionary Perspectives*.

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CHAPTER 1

AGE-RELATED FEMALE MATING DECISIONS ARE CONDITION-DEPENDENT IN WOLF SPIDERS

Dustin J. Wilgers

ABSTRACT

Female mating behaviors are known to be sensitive to a variety of individual factors both external and internal to a female. This suite of factors likely interact to influence female mating decisions. By independently manipulating female and male diet in the wolf spider *Rabidosa rabida*, and testing females across age groups, we demonstrate that in addition to its independent effect, female nutritional condition interacts with female age to influence female mating behavior. Overall, high-quantity diet (HD) females were more likely to mate than low-quantity diet (LD) females . Within the LD females, older individuals were more likely to mate than younger individuals, while within HD females, mating probabilities were equal across females of different age classes. With respect to mate choice, only female age influenced the likelihood of mating based on male diet. Young females were choosier, as they were more likely to mate with HD males than LD males; in contrast, older females were equally likely to copulate with males of each diet treatment. In addition, the likelihood of pre-sexual cannibalism was dependent on both female and male diet; high-quantity diet females were more likely to cannibalize than LD females, and attacks were directed towards LD males more often than HD males. We discuss our results in terms of costs versus benefits of female mate choice.

Keywords: variable female choice, sexual selection, *Rabidosa rabida*, interaction, sexual cannibalism

INTRODUCTION

Female mate choice is a product of mating preferences, sampling strategies, and a suite of encounter-specific factors (Wagner 1998). Evidence of intra-specific variation in female mating behavior is widespread (Jennions and Petrie 1997), and is predicted to be an evolutionary response to differential costs and benefits associated with mating decisions (e.g. Pomiankowski 1987; Real 1990; Gibson and Langen 1996; Kokko and Mappes 2005; Cotton et al. 2006). Such variation in female choice can have direct effects on the evolution of male traits (Jennions and Petrie 1997; Widemo and Saether 1999; Coleman et al. 2004), and thus, understanding the factors contributing to this variation may help to explain the diversity of observed male secondary sexual traits (Widemo and Saether 1999).

Female mating behaviors are known to be sensitive to variation in a suite of external variables. For example, studies have documented variation in female mating behaviors in response to their current environment (e.g. Clark et al. 1997; Pfennig 2007; Rundus et al. 2011), time of year (e.g. Backwell and Passmore 1996; Qvanstrom et al. 2000; Milner et al. 2010), and the availability of mates (e.g. Lawrence 1986; Palokangas et al. 1992; Berglund 1993). Females are also sensitive to environmentally induced sampling costs (e.g. Milinski and Bakker 1992), as females searching for and assessing mates under heightened predation risk are known to decrease overall mating activity (Lima and Dill 1990) and alter their mate choice (e.g. Gong and Gibson 1996; Johnson and Basolo 2003).

Variation in factors intrinsic to females, while not as immediately evident as external factors, also has marked influences on female mating behaviors. Internal physiological states can vary both between females and within a female throughout her reproductive life. Some internal variables that are known to influence female mating decisions include female diet/condition (Bakker et al. 1999; Syriatowicz and Brooks 2004; Hunt et al. 2005; Burley and Foster 2006; Fisher and Rosenthal 2006; Hebets et al. 2008; Eraly et al. 2009), female age (Prosser et al. 1997; Kodric-Brown and Nicoletto 2001; Moore and Moore 2001; Coleman et al. 2004; Uetz and Norton 2007), female reproductive state (Lea et al. 2000; Lynch et al. 2005), and even female experience (Dugatkin 1992; Collins 1995; Hughes et al. 1999; White and Galef 2000; Hebets 2003; Dukas 2005; Hebets and Vink 2007; Bailey and Zuk 2008).

As evidenced above, female reproductive behaviors are known to be influenced by a variety of factors, both external and internal to females. Much of this evidence, however, comes from studies assessing single variables in isolation. While isolating single factors can certainly provide insights into female reproductive behavior, taking a broader approach enables one to uncover potential interactions between factors, as well as the relative strengths of a variety of factors simultaneously. Selection on female behavior, such as mating decisions, acts on whole individuals rather than isolated traits, which includes a variety of traits and their interactions (Arnold 1983; Irschick et al. 2008). Thus, taking an integrative approach may provide greater insight into the interactions and relative influences of a variety of dynamic factors naturally facing females during mating decisions. Despite this, only a few studies have yet taken a holistic approach, allowing for the interaction of multiple female intrinsic factors (e.g. Thornhill 1984; Gray 1999; Hingle et al. 2001; Hunt et al. 2005; Mautz and Sakaluk 2008; Judge et al. 2010; Moskalik and Uetz 2011) or allowing for the interaction between extrinsic and intrinsic factors (e.g. Rundus et al. 2010; Rundus et al. 2011).

Wolf spiders (Lycosidae) provide an ideal system to study variability in female mating behaviors due to their mating system and their ease of manipulation. In the wolf spider, *Rabidosa rabida* (Walckenaer), polygamous males use elaborate multimodal courtship displays (Rovner 1967; Rovner 1968) consisting of condition-dependent visual and seismic display components (Wilgers and Hebets 2011) attempting to mate with cannibalistic females, which are mostly monandrous (Wilgers unpub data; also suggested in other wolf spiders, Norton and Uetz 2005; Persons and Uetz 2005). Male *R. rabida* attempt copulations following a female's approach and receptive displays (Rovner 1968; Rovner 1972). Studies on other lycosids have documented intense sexual selection on male displays from female mate choice (reviewed in Uetz and Roberts 2002) and pre-sexual cannibalism (Persons and Uetz 2005). As in other systems, previous studies on wolf spiders have also found female receptivity and mate choice to vary depending on various isolated factors intrinsic to the female, such as food stress/female condition (Hebets et al. 2008; Eraly et al. 2009; Moskalik and Uetz 2011), age and reproductive state (Uetz and Norton 2007) and experience (Hebets 2003; Hebets and Vink 2007). Recently, Moskalik &

Uetz (Moskalik and Uetz 2011) found patterns suggestive of hunger and age interacting to influence female mating decisions in *Schizocosa ocreata*; however, these were not supported statistically.

Here, in the wolf spider *R. rabida*, we explore how age and body condition (manipulated via diet) act independently, as well as potentially interact, to influence female reproductive behavior. Due to potentially different costs and benefits associated with both mate assessment and mating itself for females of different age and condition, we expect to find significant interactions between these factors. For example, females in good body condition can hypothetically better afford to bear the costs associated with both mate assessment and mating, and are thus predicted to be the choosiest group of females (reviewed in Cotton et al. 2006). However, as females age, their degree of choosiness is predicted to decline because of time constraints associated with finding a mate and reproductive senescence (Real 1990). Thus, we expect that choosiness should be most evident in groups of females that can afford the associated mate assessment costs (i.e. young females in good body condition). Similarly, due to costs associated with mate finding and reproductive senescence, we predict that the likelihood of mating for females will vary across groups, increasing with female age and body condition (*e.g* old females in good body condition). We test these predictions by conducting mate choice trials with females of varying age and body condition.

METHODS

Spider Housing and Diet Manipulations

Immature spiders were collected from Lancaster County, NE in 2007 (3 – 12 June) and 2008 (14 June). After collection, spiders were weighed and then housed in individual clear plastic containers (84 mm x 84 mm x 110 mm) with visual barriers to prevent visual contact with neighbors. Containers were housed in a climate-controlled environment (24-27° C; 15:9 L:D cycle). All individuals (males and females) were haphazardly assigned to 1 of 2 diet treatments for the duration of the experiment: 1) High-quantity diet (HD) – spiders were fed 2 body-size matched crickets twice per week, or 2) Low-quantity diet (LD) – spiders were fed 2 body-size matched crickets once every 2 weeks. Crickets, *Acheta domestica*, were supplemented with fish flakes (TetraMin, Blacksburg, VA) and Fluker's cricket feed (Port Allen, LA). All spiders were provided with water ad libitum and were checked for molts every 2-3 days to determine time of maturity. Male seismic and visual signal components were measured after mating trials. Data on the condition-dependence of signal components from these males is published elsewhere (Wilgers and Hebets 2011).

Phenotypic Measurements

To examine whether our diet manipulations influenced spider body size and body condition, we took two separate measures: 1) cephalothorax width (mm), which is fixed at maturation and provides us with a static measure of adult body size, and 2) body weight as measured at the time of the mating trial (mg), which provides a more dynamic measure of size, as it changes with recent foraging history. Cephalothorax width was measured on sacrificed individuals with digital calipers 3 independent times to the nearest 0.1 mm and then averaged across the measurements. For mating trial weight, individuals were weighed to the nearest tenth of a milligram (Ohaus Adventurer Pro AV64) immediately prior to their introduction into the trial arena. We estimated condition, defined as the pool of resources allocated to trait production and maintenance (Rowe and Houle 1996), using the ratio of body weight (mg) / cephalothorax width (mm) as a descriptive index of body condition (Jakob et al. 1996).

Mate Choice Trials

To examine the influences of female age and body condition on female mating behaviors, we separated females of each diet manipulation into 2 age classes to be run in mating trials: 1) young – 12-14 days post maturation molt, and 2) old – 19-22 days post maturation molt (based on Uetz and Norton 2007). Single-choice mating trials were conducted by pairing a single male (M) of a given diet treatment with a female (F) of one diet/age group. The samples sizes for each treatment pairing are as follows: young HDF-HDM: N = 20; old HDF-HDM: N = 21, young HDF-LDM: N = 20, old HDF-LDM: N = 20, young LDF-HDM: N = 18, young LDF-LDM: N = 16, old LDF-HDM: N = 18, old LDF-LDM: N = 9. All females and males were virgins and only used once. Due to differences in maturation dates between the sexes and the diet treatments, we were unable to control for male age, which varied from 7-81 days post maturation in 2007 ($\bar{x}$=31.4 ± 2.2) and 7-60 days post maturation in 2008 ($\bar{x}$=27.6 ± 2.3). Male ages were not different between years (Mann-Whitney test, $Z = 0.47$, $P = 0.64$) or male diet treatments (Mann-Whitney test, $Z = 0.05$, $P = 0.96$). Males were older when paired with LD females versus HD females due to differences in female maturation rates (Mann-Whitney test, $Z = 6.91$, $P < 0.001$), but male age did not differ across female age classes (Mann-Whitney test, $Z = 0.62$, $P = 0.54$). Because of this, male age was included as a random factor in our logistic regression model to test for its influence on the likelihood of copulation (see below).

Both males and females were fed one small cricket (~ ½ the spider's cephalothorax length) 12-24 hrs before the mating trial to control for hunger and mating motivation. Trials were run in clear circular plastic arenas (diameter = 20.2 cm, height = 7.3 cm) surrounded with white walls for visual barriers and lined with filter paper (Whatman #1 185mm). Females were placed in the trial arenas for at least 1 hr to acclimatize and deposit pheromone-laden silk. Arenas were wiped clean with alcohol in between trials to remove any prior chemical cues.

At the start of each trial, females were placed under a clear plastic vial for the introduction of the male. Males were allowed to acclimatize for ~ 1 min prior to lifting the female's vial and starting the trial. Trials lasted 45 min, during which time we observed and recorded live the following behaviors: latency to first courtship, number of courtships, male attempted mounts, female attacks, pre-mating sexual cannibalism, copulation success, and latency to copulation.

Statistical Analyses

Mating trials were run in two separate years (2007: N = 98, 2008: N = 44), however, there were no differences between years in the proportion of mating trials run in each category (Likelihood ratio, χ^2_7 = 7.1, P = 0.41). Thus, we included year as a random effect in our logistic regression to test for differences in copulation frequency across years.

We ran a nominal logistic regression with female diet treatment, female age class, male diet treatment and their interactions (fixed effects) along with male age (random effect) and year (random effect) as predictors for copulation and cannibalism occurrence. If female likelihood to copulate varies with intrinsic factors, we would expect female diet, age class, or interactions between them to significantly predict copulation. Female choosiness would be indicated if male diet treatment was a significant predictor of copulation, and variable female choice would be indicated by any interactions of female intrinsic factors with male diet treatment. To gauge the magnitude of effect of each predictor on the likelihood of copulation or cannibalism in a trial, we calculated the effect sizes (Cramer's φ) for each of our nominal logistic regression models (Nakagawa and Cuthill 2007; es calculator: http://mason.gmu.edu/~dwilsonb/ma.html by D. B. Wilson).

All analyses were performed in JMP v. 6 (SAS Institute Inc., Cary, NC, USA). Data were checked for normality, non-normal data that were unable to be transformed were analyzed non-parametrically. All data are presented as means ± 1 SE.

RESULTS

Effects of Diet Manipulations on Phenotypic Measures

At the time of collection (i.e. prior to diet manipulations), females and males assigned to different diet treatments did not differ in weight (Table 1). When collected, spiders were on average 2.3 molts away from maturity (for both 2007 and 2008). At the time of mating trials, high-quantity diet (HD) males and HD females were consistently heavier, larger (i.e cephalothorax width), and had higher body condition indices (weight/cephalothorax width) than those individuals on the low-quantity diet (LD) treatment (Table 1).

Mate Choice Trials

In total, 142 mating trials were conducted, with 37% ending in copulation and 19% ending in pre-sexual cannibalism. Results of the separate nominal logistic regression models indicated that the frequency of copulation (Overall Model: χ^2_9 = 40.89, $P < 0.001$; Table 2) and cannibalism (Overall Model: χ^2_9 = 40.28, $P < 0.001$; Table 3) were both highly dependent on both female and male treatments.

<u>Likelihood to Copulate-</u> A pair's likelihood to copulate was influenced by female diet and age, as well as an interaction between the two (Table 2). High-quantity diet females were significantly more likely to copulate than LD females (Likelihood ratio, χ^2_1 = 21.2, $P < 0.001$; Figure 1). While the overall model revealed age as a significant predictor of copulation, post-hoc comparisons

between female age classes revealed this difference to be insignificant (Likelihood ratio, χ_1^2 = 2.6, P = 0.11; Figure 1). The influence age of was most evident via its interaction with diet. High-quantity diet females were relatively consistent in their copulation frequencies across the different age classes (Likelihood ratio, χ_1^2 = 0.01, P = 0.92); however, older LD females were more likely to copulate than younger LD females (Likelihood ratio, χ_1^2 = 6.4, P = 0.01; Figure 1).

Mate Choice- In addition to influencing the overall likelihood to mate, our manipulations also influenced female mate choice patterns based on male diet treatments. Overall, HD males did not experience greater copulation success than LD males, instead female mate choice varied with her age as indicated by a significant female age x male diet interaction (Table 2). Younger females were choosier, mating significantly more often with HD males than LD males (Likelihood Ratio, χ_1^2 = 4.53, P = 0.03, Figure 2), while older females mated indiscriminately (Likelihood Ratio, χ_1^2 = 1.19, P = 0.28). No other interactions with male diet were significant (Table 2).

Pre-Sexual Cannibalism- Both female and male diet treatments significantly influenced the likelihood of pre-sexual cannibalism (Table 3). HD females were more likely to cannibalize males (Likelihood ratio, χ_1^2 = 6.2, P = 0.01; Figure 3A), and LD males were more likely to be cannibalized (Likelihood ratio, χ_1^2 = 27.2, P < 0.001; Figure 3B). Female age class and male age had no influence on cannibalism events and none of the interactions were significant (Table 3).

Male Mating Behaviors- While female and male attributes such as age (females only) and diet were manipulated to be different across each female-male pairing, other uncontrolled male-related mating behaviors could have influenced these mating patterns. The latency to first courtship (cube-root transformed) varied across trial pairings (Overall ANOVA Model: $F_{7,119} = 2.58$, $P = 0.02$). Overall, there was no effect of male diet ($F_{1,119} = 0.07$, $P = 0.79$), female diet ($F_{1,119} = 2.52$, $P = 0.11$) or female age class ($F_{1,119} = 3.48$, $P = 0.06$); however, there was a significant interaction between male diet and female age class ($F_{1,119} = 11.43$, $P = 0.001$). HD males tended to court younger females sooner than LD males (HD: $\bar{x} = 164.6 \pm 25.4$ sec; LD: $\bar{x} = 285.2 \pm 57.5$ sec), while LD males tended to court older females sooner than HD males (HD: $\bar{x} = 210.9 \pm 32.6$ sec; LD: $\bar{x} = 113.7 \pm 29.1$ sec). No other interactions were significant ($P > 0.25$). The latency to court (cube root transformed) was found to influence copulation success, with earlier courtship increasing the likelihood to copulate (logistic regression; $\chi^2_1 = 4.3$, $P = 0.04$). However, to see if differential latencies to court influenced copulation differently across pairings, we ran a logistic regression model with courtship latency (cube root transformed) and all possible interactions with female diet treatment, female age class, and male diet treatment as predictors of copulation success, and the overall model was not significant ($\chi^2_8 = 8.47$, $P = 0.39$).

Once males began courting, their courtship rate (# of courtship bouts/sec) strongly influenced their copulation success (when excluding males who were cannibalized before they courted or within 10 seconds of starting; $N = 17$). Males that gained copulations courted ~ 1.8 times more frequently than males that did not copulate (Copulation: $N = 53$, $\bar{x} = 0.061 \pm 0.002$; No Copulation: $N = 72$, $\bar{x} = 0.034 \pm 0.003$; $t_{123} = 7.96$, $P < 0.001$). Male courtship intensity varied based on trial pairing (Overall ANOVA Model: $F_{7,117} = 2.63$, $P = 0.01$), however, males only altered courtship rates based on female diet treatment, courting HD females at higher rates than LD females (HD: $N = 67$, $\bar{x} = 0.052 \pm 0.003$; LD: $N = 58$, $\bar{x} = 0.038 \pm 0.003$; $F_{1,117} = 12.4$, $P < 0.001$). Male diet treatment ($P = 0.44$), female age ($P = 0.46$) and all interactions were not significant. The patterns of this model were robust to the removal of two individuals that courted but were cannibalized within 10 seconds.

The differences in courtship intensity with female diet did not translate to quicker decisions by females, as latency to copulation after first courtship was similar across all age and diet pairings (Kruskal-Wallis test, $\chi^2_6 = 3.85$, $P = 0.70$). Lastly, male mating motivation was similar across pairings, as the number of male attempted mounts was similar across all groups of trials (Kruskal-Wallis test, $\chi^2_7 = 10.3$, $P = 0.17$).

DISCUSSION

Using experimental manipulations of both age and body condition (as manipulated through diet), we found evidence that female *Rabidosa rabida* are sensitive to variation in factors intrinsic to the female and that these factors interact to influence mating decisions. Low-quantity diet (LD) females were generally less likely to copulate than high-quantity diet (HD) females; however, age influenced a female’s likelihood to copulate differently in these two groups.

Older LD females were more likely to copulate than younger LD females, while age did not influence the likelihood to copulate for HD females. Mate choice patterns based on male diet treatments were influenced solely by female age, which failed to support our prediction of female condition influencing mate choice. Younger females were choosier, mating significantly more often with HD males than LD males, while older females mated indiscriminately. In addition to its influence on the likelihood to copulate, female diet treatment also influenced the likelihood to cannibalize, with HD females engaging in more pre-sexual cannibalism than LD females. Low-quantity diet males were also cannibalized more frequently than HD males. Ultimately, our data demonstrate that female age and body condition have independent as well as interacting affects on a variety female mating behaviors.

The majority of our observed mating patterns do not appear to result from different male behaviors across groups. While male latency to court did differ with respect to male diet and female age class, these differences in latency did not influence the likelihood of copulation differently based on female-male pairings. Male courtship rates also varied with female diet treatment, as males courted HD females at higher rates than LD females. HD females were more likely to mate than LD females, and the likelihood of mating increased with courtship rate. The relationship between courtship rate and copulation success has also been found in other lycosids (Kotiaho et al. 1998; Hebets et al. 2011 and references therein). Our observed pattern of HD females being more likely to mate could be a product of differential male courtship rates. Alternatively, these different patterns of male courtship rate could reflect a male's reaction/response to differences in female behavior across the treatment groups. Male *R. rabida* are known to decrease the amount of time between courtship bouts in response to female receptivity displays (Rovner 1967), which would result in higher courtship rates for males paired with receptive females. Thus, rather than female mating decisions being a result of male courtship rate, our observed male courtship patterns could instead reflect female mating decisions. Future experiments measuring male courtship rates to isolated female cues and corresponding female receptivity responses (without individual interaction) will further disentangle this relationship. None-the-less our observed patterns of male courtship cannot account for our observed differences in mating pattern.

Female Likelihood to Copulate

Female body condition, as influenced by diet quantity, independently influenced the likelihood to mate as suggested by the nominal logistic regression model and the magnitude of the effect size. Overall, HD females were more likely to copulate than LD females. Mating can be costly to females due to the production of expensive gametes and mate search/assessment (Alatalo et al. 1988; Slagsvold et al. 1988) and resource limitation can result in tradeoffs among various life history traits, leaving poor-condition females with less to invest in reproduction (e.g. Hunt et al. 2005 and references therein). Indeed, females in poor condition (i.e. food stress, parasitized) are known to reduce mating rates, receptivity, and sampling (e.g. Poulin 1994; Ortigosa and Rowe 2002; Syriatowicz and Brooks 2004; Hunt et al. 2005), but opposite patterns are witnessed in taxa in which males provide nutritional direct benefits (e.g. Gwynne 1990; Bilde et al. 2007; Fox and Moya-Larano 2009). Wolf spiders are terrestrial predators known to frequently be food limited in nature (Wise 2006), which has been found to have direct effects on various aspects of female fecundity (e.g. Reed et al. 2007; Wilder and Rypstra 2008a). Despite this, the effects of female body condition on the likelihood to mate has been mixed, with only one report of an effect of hunger on receptivity (*Schizocosa*: Moskalik and Uetz 2011), and other studies finding either no effect (*Schizocosa*: Hebets et al. 2008; *Pardosa*: Wilder and Rypstra 2008a), or variable population-level responses (*Pirata*: Eraly et al. 2009). Female spiders invest yolk into their eggs both pre- and post-copulation, with post-copulation yolk additions only occurring given sufficient resources (i.e. food; Foelix 1996). Given this, the low copulation rates observed in our low nutrition females may reflect female decisions to allow additional time to find resources.

Unlike body condition, where simply the magnitude of differences between HD and LD females changed with age, the effects of female age on the likelihood to mate were mainly evident through the interaction between age and diet. Age-related female mating decisions were condition-dependent, as female *R. rabida* in good condition maintained high reproductive activity across both age classes, whereas older poor condition females were much more likely to mate than younger poor condition females, who rarely copulated. Selection is thought to favor early female mating to avoid costs associated with time constraints (Bateson and Healy 2005), such as reproductive senescence (Moore and Moore

2001), and the possibility of remaining unmated (Bakker and Milinski 1991), This may be the case in *R. rabida,* where a relatively short one-time mating season (Eason and Whitcomb 1965) amplifies the risk of total fitness loss by remaining unmated, and the likelihood of finding a mate declines over the mating season due to high male mortality (D Wilgers pers. obs.). Across taxa, females are more likely to mate as they age (e.g. Prosser et al. 1997; Mair and Blackwell 1998; Uetz and Norton 2007; but see Judge et al. 2010). Our results suggest that costs associated with time constraints may outweigh those associated with body condition in *R. rabida*. For example, for older females that risk total fitness loss if no other male is encountered, even if in poor body condition, the cost of going unmated may outweigh the potential benefit of delaying mating in an effort to acquire more resources. Our experimental design enabled us to uncover this pattern, potentially reflecting an interesting tradeoff between acquiring sufficient resources to invest in eggs and the fitness costs associated with the likelihood of finding another mate.

Evidence of factors interacting to influence female mating decisions is rare, likely due to the scarcity of studies investigating their effects. Recently Moskalik & Uetz (2011) found potential evidence of age and condition interacting, as they reported differences in female receptivity between young starved and satiated females, but no differences in receptivity based on body condition in older females. The influence of interacting factors on mating decisions have been found in other systems as well, such as in scorpionflies (size & feeding history; Thornhill 1984) and *Teleogryllus* crickets (feeding history & development time;

Hunt et al. 2005), but not in stalk-eyed flies (Hingle et al. 2001) or other cricket species (*Acheta*: Gray 1999; Mautz and Sakaluk 2008; Gryllus: Judge et al. 2010).

Female Choosiness

Female age also influenced female mate choice decisions based on, presumably, a male's body condition. Young females mated more with HD versus LD males, while old females mated indiscriminately. Females are predicted to be choosy given variability in benefits provided by males (Andersson 1994). In fact, several studies on spiders suggest that indirect benefits may be conferred to females by preferred males (Alatalo et al. 1998; Hoefler et al. 2009; Koh et al. 2009), and when resources are available, choosy females have been found to invest more into their offspring (Rundus et al. 2011). Whether male body condition relates to any benefits experienced by the female has yet to be determined. Regardless, *R. rabida* female choice based on male diet history was not consistent, suggesting costs associated with age may outweigh any benefits (if they exist) of mating with high body condition males. When large costs are associated with age (or time in season, see above), virgin females encountering males late in the mating season should mate regardless of male quality, as the likelihood of encountering any male at all, much less a better quality males is a declining probability function (Real 1990; Kokko and Mappes 2005). For the naïve females in our study, the mate density experienced was extremely low which could have influenced the likelihood to accept the first male encountered

(Johnson 2005; Hebets and Vink 2007). Future research should investigate the influence of this potentially interesting interaction between female age and mate density on mate choice. The witnessed reductions in *R. rabida* female choosiness with age suggest that these costs associated with age (i.e. time) potentially counter the benefits of mating with preferred mates to alter mate choice patterns. Similar patterns have been found across a number of taxa (e.g. birds: Alatalo et al. 1982; crustaceans: Backwell and Passmore 1996; fish: Kodric-Brown and Nicoletto 2001; insects: Moore and Moore 2001; spiders: Uetz and Norton 2007). Unfortunately, we were unable to collect data on offspring number or survival as a result of these mating decisions, which have been found to vary with female condition and choice of mate in another wolf spider species (Rundus et al. 2011). Investigating how various factors intrinsic to females interact to influence fitness costs and how these relate to benefits due to choosiness may aid in understanding the mechanisms and consequences underlying the observed variability in female mating decisions.

Female Pre-Sexual Cannibalism

Female, as well as male, body condition also influenced the frequency of pre-sexual cannibalism, with HD females engaging in more pre-sexual cannibalism than LD females and LD males suffering more pre-sexual cannibalisms than HD males. Ultimately, our findings demonstrate that cannibalism events are most likely when including large, HD females or small, LD males. These results are contrary to one hypothesis of pre-sexual cannibalism -

the foraging hypothesis - where cannibalism is hypothesized to be a response to nutritional deficits by females to increase fecundity (Newman and Elgar 1991). The foraging hypothesis has found support in mantids (e.g. Barry et al. 2008) and tarantulas (Rabaneda-Bueno et al. 2008), but only limited support in other spider systems (e.g. Andrade 1998; Johnson 2001; but see: Schneider and Elgar 2002; Johnson and Sih 2005). The patterns of our study follow others on wolf spiders, which suggest that greater degrees of sexual size dimorphism increase the likelihood of cannibalism (Persons and Uetz 2005; Wilder and Rypstra 2008a; Wilder and Rypstra 2008b).

Conclusions

In summary, this study not only demonstrates the variability of female mating behavior based on both age and body condition, but also highlights the importance of investigating variable patterns of female mating decisions while allowing multiple factors to naturally interact. While our experimental design does not allow us to disentangle the underlying mechanisms responsible for the observed variation in mating patterns, regardless of the mechanisms involved, selection from female reproductive behavior in this system (copulation vs. cannibalism) on males is extremely varied and female state-dependent. Mating encounters in sexually cannibalistic species are often typified as females choosing between a mate and a meal, which has obvious and extreme consequences on male fitness (Elgar 1992; Persons and Uetz 2005). The fact that larger, well-fed females are the most cannibalistic, coupled with age-dependent choosiness, suggests that young females in good condition appear to be a strong source of selection on courting males. Males able to copulate with these females will gain further fitness advantages through increased egg/offspring production by larger females (Reed and Nicholas 2008). Further investigations into how variation in male courtship display components influence mate choice decisions of females at different states (i.e. condition, age, etc.) will shed light on how this source of selection has influenced the evolution of male ornamentation and courtship displays.

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TABLES

Table 1. Effects of diet quantity manipulations on body measures of male and female *R. rabida* across two separate years.

		Males (*N*)			**Females (*N*)**		
Year	**Body Measure**	**LD**	**HD**	***P*-Value[a]**	**LD**	**HD**	***P*-Value[a]**
2007	Initial Mass (mg)	110.8 ± 8.0 (49)	115.7 ± 8.0 (49)	0.74	100.8 ± 7.2 (38)	119.4 ±7.1 (60)	0.15
	CW (mm)	4.28 ± 0.06 (48)	4.85 ± 0.07 (46)	***< 0.001***	4.31 ± 0.05 (38)	5.23 ± 0.08 (60)	***< 0.001***
	Trial Mass (mg)	187.2 ± 7.0 (49)	268.9 ± 9.2 (49)	***< 0.001***	213.5 ± 7.5 (36)	401.0 ± 13.1 (60)	***< 0.001***
	Trial Condition[b]	43.2 ± 1.1 (48)	55.1 ± 1.3 (46)	***< 0.001***	49.4 ± 1.1 (38)	75.4 ± 1.6 (60)	***< 0.001***
2008	Initial Mass (mg)	43.4 ± 1.5 (16)	47.4 ± 2.4 (28)	0.81	48.4 ± 2.9 (23)	50.4 ± 3.4 (21)	0.62
	CW (mm)	3.20 ± 0.05 (16)	4.27 ± 0.07 (28)	***< 0.001***	3.26 ± 0.05 (23)	4.53 ± 0.08 (20)	***< 0.001***
	Trial Mass (mg)	81.7 ± 3.2 (16)	182.9 ± 7.6 (28)	***< 0.001***	99.3 ± 3.7 (23)	260.0 ± 11.1 (21)	***< 0.001***
	Trial Condition[b]	25.4 ± 0.8 (16)	42.3 ± 1.2 (28)	***< 0.001***	30.3 ± 0.9 (23)	56.6 ± 1.8 (20)	***< 0.001***

- Means ± SE reported for each body measure

[a] P-values reported from Mann-Whitney tests on differences between diet treatments

[b] Condition calculated as ratio of body mass at time of trial (mg) / cephalothorax width (CW; mm)

Table 2. Table of effects for nominal logistic regression model to predict copulation success in *R. rabida*.

Source	**df**	χ^2	***P*-value**	ϕ **(CI)**[a]
Year	1	1.1	0.30	0.09 (-0.08 - 0.25)
F Diet	1	12.5	***<0.001***	0.30 (0.14 - 0.44)
F Age	1	7.2	***0.007***	0.23 (0.06 - 0.38)
M Diet	1	1.9	0.17	0.12 (-0.05 - 0.27)
M Age	1	0.2	0.68	0.04 (-0.13 - 0.20)
F Diet*F Age	1	6.5	***0.01***	0.21 (0.05 - 0.37)
F Diet*M Diet	1	0.1	0.73	0.03 (-0.14 - 0.19)
F Age*M Diet	1	7.2	***0.007***	0.23 (0.06 - 0.38)
F Diet*F Age*M Diet	1	2.6	0.11	0.14 (-0.03 - 0.29)

-Female age was treated as a nominal variable (young, old), while male age was treated as continuous

[a] Cramer's phi and confidence intervals (CI) calculated as effect size estimate for each predictor in the model

Table 3. Table of effects for nominal logistic regression model to predict cannibalism frequencies in *R. rabida*.

Source	**df**	χ^2	***P*-value**	ϕ **(CI)**[a]
Year	1	2.9	0.09	0.14 (-0.02 - 0.3)
F Diet	1	9.3	***0.002***	0.26 (0.09 - 0.41)
F Age	1	0	1.0	0.0004 (-0.16 - 0.16)
M Diet	1	16.4	**<0.001**	0.34 (0.18 - 0.48)
M Age	1	2.7	0.10	0.14 (-0.03 - 0.30)
F Diet*F Age	1	0	0.99	0.0004 (-0.16 - 0.16)
F Diet*M Diet	1	1.6	0.2	0.1 (-0.06 - 0.27)
F Age*M Diet	1	0	0.99	0.0005 (-0.16 - 0.16)
F Diet*F Age*M Diet	1	0	1.0	0.0001 (-0.16 - 0.16)

-Female age was treated as a nomial variable (young, old), while male age was treated as continuous

[a] Cramer's phi and confidence intervals (CI) calculated as effect size estimate for each predictor in the model

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FIGURES

Figure 1. Influence of female age and diet on a female's likelihood to copulate in *Rabidosa rabida.* Female diet treatments indicated by HD (high-quantity diet) and LD (low-quantity diet). Asterisks indicate significant differences between groups under line (Post-hoc likelihood ratio tests, $P < 0.05$).

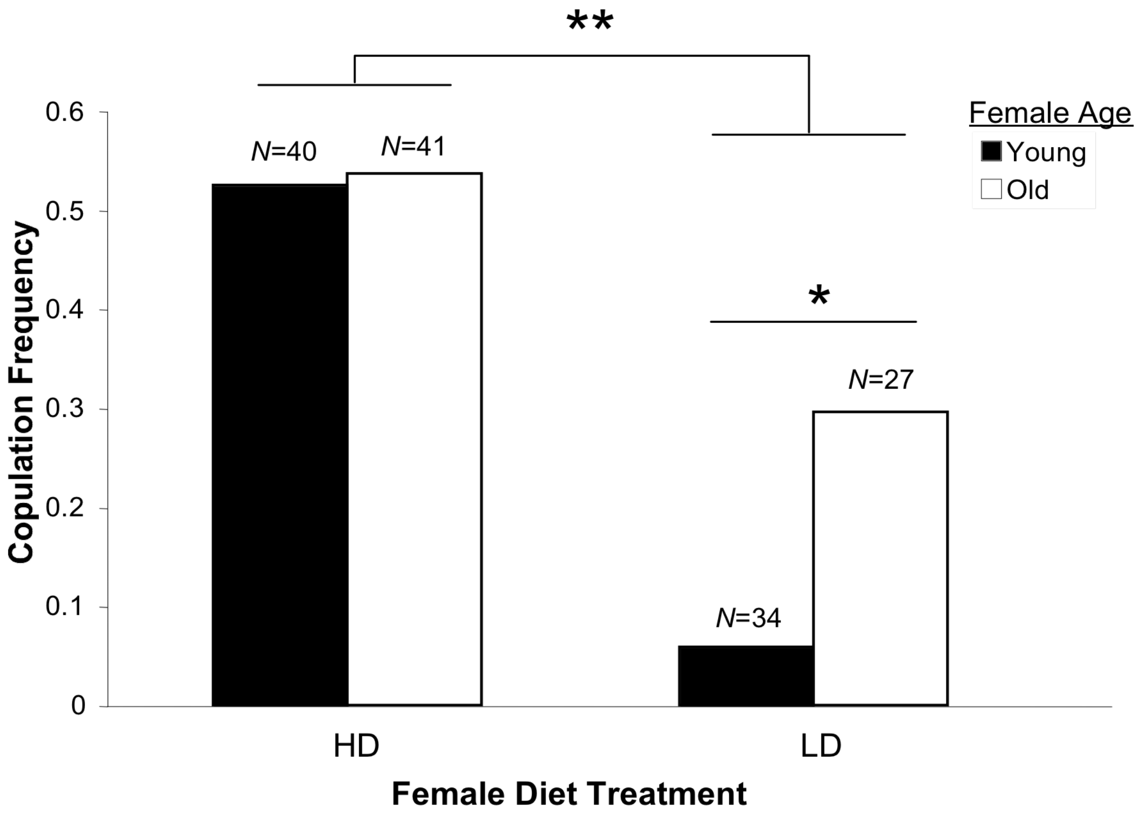


Figure 2. Influence of female age on female mate choice in *R. rabida*. Male diet treatments are shown by different bar colors (High-quantity diet = HD, low-quantity diet = LD). Asterisks indicate significant differences between groups under line (Post-hoc likelihood ratio tests, *P* < 0.05).

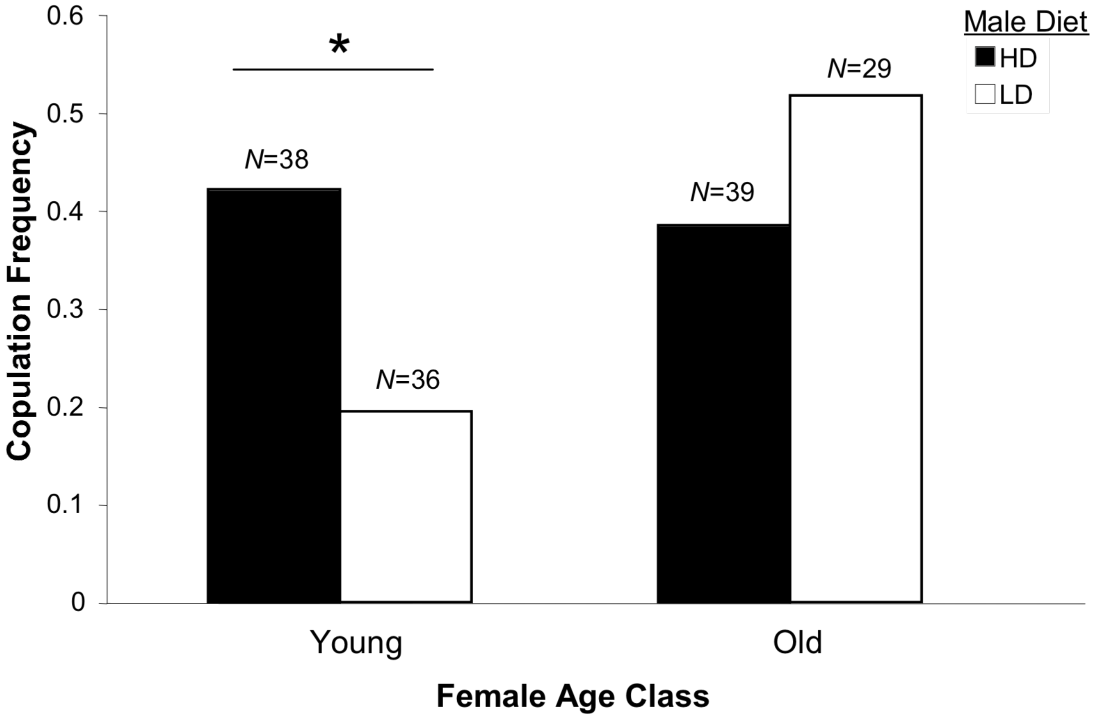
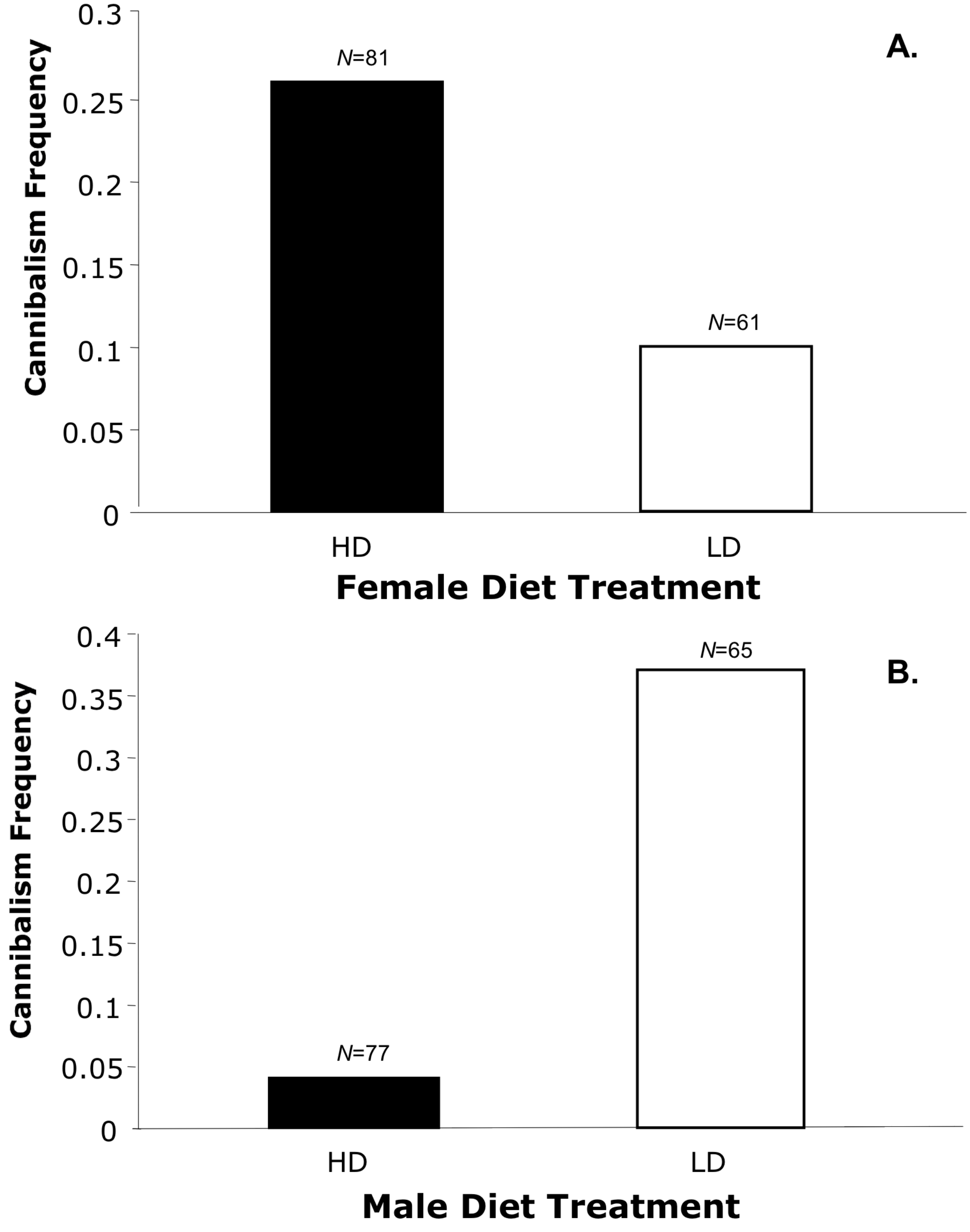


Figure 3. Likelihood of cannibalism during mating trials as a function of diet history of A) females, and B) males. Diet quantity treatments indicated by HD (high-quantity diet) and LN (low-quantity diet).



CHAPTER 2

COMPLEX COURTSHIP DISPLAYS FACILITATE MALE REPRODUCTIVE SUCCESS AND PLASTICITY IN SIGNALING ACROSS VARIABLE ENVIRONMENTS

Dustin J. Wilgers

ABSTRACT

Effective signal transmission is essential for communication. In environments where signal transmission is highly variable, signalers may utilize complex signals, which incorporate multiple components and modalities, to maintain effective communication. Male *Rabidosa rabida* wolf spiders produce complex courtship signals, consisting of both visual and seismic components. We test the hypothesis that the complex signaling of *R. rabida* contributes to male reproductive success in variable signaling environments. We first examined the condition-dependence of ornamentation (a presumed visual signal) and seismic signal components and found that both may provide potentially redundant information on foraging history. Next, we assessed reproductive success across manipulated signaling environments that varied in the effectiveness of visual and/or seismic signal transmission. In environments where only one signal could be successfully transmitted (*e.g.* visual or seismic), pairs were able to successfully copulate. Additionally, we found that males altered their courtship display depending on the current signaling environment. Specifically, males reduced their use of a visual display component in signaling environments where visual signal transmission was ablated. Incorporating signals in multiple modalities not only enables *R. rabida* males to maintain copulation success across variable signaling environments, but it also enables males to adjust their composite courtship display to current signaling conditions.

Keywords: communication, multimodal, signal evolution, condition-dependent, Lycosidae, *Rabidosa rabida,* efficacy back-up, redundant signals

INTRODUCTION

The environment is an important source of selection on both signalers and receivers and has had a considerable influence on the evolution of reproductive communication (Endler, 1992; 1993; Boughman, 2002). While the messages conveyed in signals are often important in influencing receiver decisions, in order to be effective, these messages must transmit through the environment and remain recognizable to the receiver (Guilford and Dawkins, 1991). Across taxa and encompassing multiple signaling modalities, the environment has been suggested to shape male mating signals for maximizing detection and discrimination by receiving females (e.g. Ryan and Wilczynski, 1991; Boughman, 2001; Elias et al., 2004; Leal and Fleishman, 2004; Cokl et al., 2005; Cocroft et al., 2006; Seehausen et al., 2008; Elias et al., 2010). While this signal-environment match benefits males in homogenous environments, many males regularly encounter variable environments. Often, this variability favors selective signaling behavior, where male courtship displays tend to occur in environments that maximize signaling efficacy (e.g. Endler, 1991; Endler and Thery, 1996; Andersson et al., 1998; Kotiaho et al., 2000; Heindl and Winkler, 2003; McNett and Cocroft, 2008). While selective signaling may be favored by reducing unnecessary costs when signals are unlikely to be recognizable, it also limits potential reproductive activity and associated benefits, which could have important fitness consequences. Thus, for males encountering multiple signaling environments, selection may favor male displays that transmit information effectively in multiple environments (Hebets et al., 2008a).

Males faced with variable environments could use a generalized signal that would transmit (often sub-optimally) across several environments (e.g. Hebets et al., 2008a; Milner et al., 2008). Use of such a generalized signal might lead to some males modifying signals behaviorally (e.g. amplitude, duration, velocity) to increase detection probabilities (e.g. Patricelli and Blickley, 2006; Ord et al., 2007; Peters et al., 2007). Alternatively, males could use displays that incorporate multiple signals or related components across different sensory modalities (i.e. complex displays). In this scenario, selection from different sources (i.e. environments) could act separately on various signal components to optimize the efficacy of information transfer across a multitude of signaling environments (reviewed in Candolin, 2003; Hebets and Papaj, 2005). When detection across variable environments is an issue, selection may act on signal form to enhance transmission, while maintaining similar information content. Thus, selection may lead to the evolution of complex signaling incorporating redundant information across signal components, (Møller and Pomiankowski, 1993; Johnstone, 1995), enabling both accurate and consistent female assessment regardless of the environment (Candolin, 2003; Partan and Marler, 2005).

Complex displays are common across the animal kingdom and numerous hypotheses exist regarding their function (see reviews in Partan and Marler, 1999; Rowe, 1999; Candolin, 2003; Hebets and Papaj, 2005; Partan and Marler, 2005). Recent empirical as well as theoretical work highlights the role of variable and dynamic environments in the evolution of signal complexity (e.g. Candolin, 2003; Peters and Evans, 2003; Ord and Martins, 2006; Peters et al., 2008; Bro-Jørgensen, 2009; Heuschele et al., 2009). Work on spiders has provided considerable insight into the evolution of multimodal signals used in mate choice (Coleman, 2009). In particular, wolf spiders (Araneae: Lycosidae) are known for their incredible diversity and complexity of male courtship displays. Male courtship varies in the number of modalities incorporated into displays (e.g. seismic, visual, near-field; Kotiaho et al., 1996; Uetz and Roberts, 2002; Stratton, 2005; Rundus et al., 2010), in the degree of ornamentation (review of genus *Schizocosa* Stratton, 2005; Framenau and Hebets, 2007), and in the complexity of the visual display (Hebets and Uetz, 2000; Stratton, 2005). Various aspects of these courtship displays have been posited to enhance signal efficacy across signaling environments (Scheffer et al., 1996; Hebets and Uetz, 1999; 2000; Uetz et al., 2009), as signaling environments for spiders are exceptional in their complexity and variability (Elias and Mason, In Press).

The wolf spider, *Rabidosa rabida* (Walckenaer), is particularly well suited to studies exploring the influence of the signaling environment on reproductive behavior. Male *R. rabida* perform complex courtship displays consisting of multiple components across different sensory modalities. The visual portion of *R. rabida* courtship involves palpal rotations and leg-waves, consisting of arches and extensions of darkly pigmented forelegs (Kaston, 1936; Rovner, 1968). Additionally, males produce a seismic component, which is transmitted through the substrate via palpal stridulation (Rovner, 1967; 1975). The substrate for *R. rabida* consists of grassland plants, as they are found above the ground in the dense vegetation of open grasslands (Kuenzler, 1958). In addition to the heterogeneity of the seismic signaling substrate, individuals are reproductively active during both day and night (D. Wilgers pers. obs.; Rovner, 1967), resulting in a highly variable visual signaling environment as well. Previous work on *R. rabida* has suggested that not only do both the visual and seismic courtship components play a role in conspecific interactions (Rovner, 1996), but that each signal modality is sufficient for female receptivity, potentially allowing males to maintain reproductive behavior at any time of day (Rovner, 1967; 1968). This previous work predominantly focused on female receptivity displays. Here, we look to extend this work by first assessing the potential information content, or condition-dependence, of components in these signaling modalities. We next examine male copulation success across signaling environments that vary in their transmission efficacies. Finally, we examine male courtship behavior across these same signaling environments to determine whether complex signaling facilitates plasticity in male courtship behavior.

METHODS

Condition-dependence of Signal Components

We collected immature male spiders from Lancaster County, NE in 2007 (3-11 June) and 2008 (14 June). Individuals were housed in a climate controlled environment (24-27°C, 15:9 L:D cycle) in individual plastic containers (84 mm x 84 mm x 110 mm) with visual barriers. Individuals were immediately placed on an assigned diet treatment. As in previous wolf spider studies (e.g. Hebets et al., 2008b; Shamble et al., 2009; Rundus et al., In Press), we used diet manipulations to investigate the condition-dependence of components of visual and seismic signals. Briefly, upon collection, all males were randomly assigned to 1 of 2 diet treatments for the duration of the experiment: 1) high quantity diet (HD) – spiders were fed 2 body-size matched crickets, *Acheta domestica*, twice per week, or 2) low quantity diet (LD) – spiders were fed 2 body-size matched crickets once every 2 weeks. All crickets were supplemented with fish flakes (TetraMin, Blacksburg, VA) and Fluker's cricket feed (Port Allen, LA). Spiders were provided with water ad libitum. Individuals were checked for molts every 2-3 days to determine their time of maturation. To assess the efficacy of our diet manipulations, males were weighed within 2 days of their molt to maturation. After preservation, their cephalothorax width (mm) was measured 3 independent times using digital calipers, and averaged for analyses.

Foreleg Color Analysis - We analyzed the foreleg coloration, a male secondary sexual trait that both appears and is fixed at maturation, of males raised on the different diet manipulations described above (2007: HD: N = 58, LD: N = 51; 2008: HD: N = 24, LD: N = 17). Additionally, we analyzed the foreleg coloration of males caught as adults in the field in 2008 (4 August; N = 27).

After sacrificing, male right forelegs were removed and frozen at -80° C. Prior to image capture, forelegs were allowed to thaw. Forelegs were placed on a clear microscope stage and illuminated from above using a 150-watt Lumina dual fiber optic light (Chiu Technical Corporation, Kings Park, NY, USA). The lateral side of each foreleg was photographed using a stereoscope (Leica MZ16, Bannockburn, IL, USA) and a Spot Flex digital camera (Model 15.2 64 MP, Diagnostic Instruments, Inc. Sterling Heights, MI, USA), under a 1.0x objective and 1.2x camera coupler. Images were imported onto a desktop computer using Image Pro Discovery v. 5.1 (MediaCybernetics, Inc., Silver Spring, MD). All foreleg images (diet manipulation and field caught) were captured on the same day, using the same settings and light levels, enabling direct comparisons among individuals.

Foreleg coloration was quantified using identical methods to previous studies working with foreleg color in wolf spiders (Shamble et al., 2009; Rundus et al., In Press). Briefly, images of each leg were imported into Adobe Photoshop CS2 and changed to grayscale. We analyzed the entire femur, patella, tibia, metatarsus, and tarsus of all forelegs and the tibia segment on leg II (for males in 2008) for mean segment image intensity (i.e. darkness, ‘K’ a numerical value where lower scores indicate darker images; 0= black → 255= white). Each measurement was taken once. Since foreleg coloration is highly correlated between all segments (Spearman’s correlation, all $P < 0.001$), we calculated the mean image intensity across all foreleg segments for our analyses.

Sexually selected ornaments are expected to show a greater degree of condition dependence than similar non-sexually selected traits (Cotton et al., 2004), and thus we would expect to see greater degrees of difference in darkness between the diet treatments for leg segments that are presumably under sexual selection. To test this, we compared the tibia segments from the second pair of legs (non-ornamented) between males reared on the same diet manipulations in 2008. Analyses were performed in JMP v. 6 (SAS Institute Inc., Cary, NC, USA). Non-normal data were analyzed using non-parametric tests.

<u>Seismic Analysis</u> - We analyzed the seismic component of male courtship displays for a subset of males raised on diet manipulations in 2007 (N = 15 / diet treatment). Male age ranged from 36-92 days ($\bar{x}$=65.9), and there were no differences in age between diet treatments (t_{28} = 0.51, P = 0.61).

To control for pseudoreplication and to reduce any potential influence of individual female silk cues on male courtship behaviors, we provided males with multiple female cues simultaneously. Prior to each recording, 4 mature virgin female spiders were allowed to deposit silk on a piece of filter paper (Whatman #1 185mm) for one hour each. Given the density of spiders at our collection location, males are highly likely to encounter silk cues from numerous females (D Wilgers, pers. obs.). Silk from mature females is known to elicit courtship in the absence of a female (Tietjen, 1979). The filter paper from which we recorded seismic signals was suspended 2.5 cm above the floor on a circular ring of acoustic foam with rubber footings. A 0.5cm x 0.5cm piece of retroreflective tape (3M Diamond Grade, 3M, Saint Paul, MN, USA) was placed in the center of the filter to increase the signal strength of the vibrometer. A transparent acetate wall was attached to a ring of high-density acoustic foam and placed on top of the filter paper to prevent the spiders from escaping. A female spider was placed on the outside of, and not in contact with, the recording arena – providing males with a visual stimulus only. While a female visual stimulus is not necessary to elicit male courtship, we added this component based on prior observations suggesting that males court longer with a visual stimulus of a female (D. Wilgers pers. obs.). A single female was present in a small confined space (5 cm diameter plastic vial), limiting their movements, outside of all test male arenas and thus, their presence is unlikely to explain any of our observed patterns.

Males were recorded in a soundproof chamber (50cm x 37cm x 43cm) lined with loaded vinyl PSA and soundproof foam (Super Soundproofing Co., San Marcos, CA, USA) placed on a vibration isolation table (Minus K 50BM-8C, Minus K Technology, Inglewood, CA, USA). Trials were illuminated in the enclosure with a Vita-lite full spectrum fluorescent bulb (Duro-Test Lighting Inc., Philadelphia, PA, USA) and filmed using a Logitech Webcam Pro 9000 (Logitech, Fremont, CA, USA). Seismic recordings were made using a laser vibrometer (Polytec PDV100), set for a peak velocity measurement range of ± 20mm/s, with a low pass filter at 22kHz, and at a 24bit 48kSa/s sample rate. Digital output from the vibrometer was recorded on an Apple iMac in Quicktime Pro, where it was synchronized with the video recordings. All vibration recordings were exported from Quicktime Pro as uncompressed AIFF files at 44.1 kHz sampling rate with 16-bit mono encoding.

At the beginning of trials, males were placed directly in the recording arena where we recorded up to five minutes of courtship (range 6-13 courtship bouts, $\bar{x}$=10.3). The total number of courtship bouts recorded and analyzed for males in each diet treatment were similar (Mann-Whitney test, Z =0.95, P = 0.34). The seismic display of *R. rabida* consists of two components, a series of introductory bursts of pulses, followed by a distinct pulse-train, consisting of more rapid pulses increasing in frequency and amplitude until the end of the bout (Figure 1; see description by Rovner 1967). Using Raven Pro (v 1.3, Cornell Laboratory of Ornithology, Ithaca, NY), we blindly (with respect to diet treatment) analyzed all courtship bouts within a trial for the following parameters: 1) duration of introductory segment (sec), 2) pulse-train length (sec), 3) inter-bout interval (time between end of previous pulse-train and next introductory pulse; sec), 4) beginning pulse-train amplitude (dB, quantified as mean power over 100 ms), 5) maximum pulse-train amplitude (dB, quantified as mean power over 100 ms), 6) number of introductory pulse bursts, and 7) number of pulse bursts in pulse-train. Introductory segments and pulse-trains were both identified visually (from waveform) and by ear. The start of the introductory pulse segments were marked by the beginning of pulse bursts, while the beginning of pulse-trains were identified when pulse frequency and amplitude increased (Rovner, 1967). To control for differential attenuation of signal amplitude due to distance of the spider from the laser, we analyzed amplitude change by calculating the difference in beginning and maximum amplitudes within each separate pulse-train. We analyzed all courtship bouts performed in a trial and used the average (across all bouts) for each of the seismic display parameters in our statistical analysis. To test for differences in seismic display parameters based upon our diet treatments, we used a logistic regression with male diet as a response variable and the means of each parameter as predictor variables.

Reproductive Success across Signaling Environments

We collected immature spiders (N = 194) from the same site in Lancaster County, NE in 2008 (9-12 July). Spiders were brought back to the laboratory and housed in individual plastic containers (84 mm x 84 mm x 110 mm) with visual barriers. All individuals were housed in a climate controlled room at 27°C under a 15:9 Light:Dark cycle. Spiders were fed 3 body-size matched crickets, *Acheta domestica*, once per week and provided water ad libitum. Crickets were supplemented with fish flakes (TetraMin, Blacksburg, VA) and Fluker's cricket feed (Port Allen, LA). We checked spiders 2-3 times per week for molts to determine the day of maturation.

We used a fully crossed 2 x 2 experimental design with respect to the signaling environment, in which we independently manipulated the visual (V) and seismic (S) environment by performing trials in the light (V+) versus dark (V-) and on filter paper (S+) versus granite substratum (S-). Thus, we performed single choice mating trials (1 female and 1 male) in 1 of 4 environments (V+/S+, V+/S-, V-/S+, V-/S-) that differed in their signal transmission. Light trials (V+) were performed under illumination from 2 full spectrum Vita-Lite 30-watt fluorescent bulbs (Duro-Test Lighting Inc., Philadelphia, PA, USA), while dark trials (V-) were performed in complete darkness with observations of the trial aided by infrared night vision goggles (Rigel 3200, Rigel Optics Inc., Washougal, WA, USA) and an infrared illuminator (Supercircuits IR20, Supercircuits, Austin, TX, USA). Spectral sensitivity data on wolf spiders, as well as the wandering spider, *Cupiennius salei*, provide no indication that these spiders can detect the IR wavelengths emitted by the illuminator (~ 850nm; Devoe et al., 1969; Devoe, 1972; Barth, 2002). Seismic present trials (S+) were run using a filter paper substrate (Whatman #1 185mm), while seismic absent trials (S-) were run using bottomless arenas placed on granite slabs, which like other types of rock are effective at ablating seismic signals of spiders (D. Wilgers, pers. observation; Elias et al., 2004). All trials were performed in circular plastic arenas (diameter = 20.2 cm, height = 7.3 cm) surrounded with white walls for visual barriers.

Females were 12-14 days post maturation when used in trials, the age at which females are most discriminating (Uetz & Norton 2007; D. Wilgers, unpublished data). Male age ranged from 11-20 days post maturation and was similar across signaling environments (Kruskal-Wallis, $\chi^2_3 = 1.39$, $P = 0.71$). All individuals were used only once. Approximately 24 hrs prior to trials, males and females were given a small (~ 1/2 body size) cricket to standardize hunger levels and minimize the probability of pre-mating sexual cannibalism. All individuals were weighed (Ohaus Adventurer Pro AV64 Pine Brook, NJ, USA) just prior to their introduction to the arena; both female and male weights were similar across environments (Kruskal-Wallis tests, both $P > 0.31$). Females were placed in the mating arena at least 1 hr prior to their trial to acclimatize and deposit pheromone-laden silk. For introduction of the male, females were placed under a clear plastic vial. Males were allowed to acclimatize for ~ 1 min, and then the female's vial was lifted and the trial commenced. Trials lasted for 45 min, during which time we observed the following behaviors: latency to the first courtship, # of courtship bouts, male attempted mounts, female attacks, copulation, latency to copulation, cannibalism, and latency to cannibalism. After observing several trials, we noticed variability in the number of male courtship bouts that incorporated a leg-wave and began recording whether each courtship included a leg-wave. Since we did not begin recording this behavior at the beginning of the experiment, we have a reduced sample size for this variable.

To test the influence of the signaling environment on copulation success, we used a nominal logistic regression model with the presence/absence of both the visual and signaling environments as predictor variables for copulation success. All statistics were performed in JMP v. 6 (SAS Institute Inc., Cary, NC, USA). Non-normal data were analyzed using non-parametric tests. All results are reported as means ± 1 SE.

RESULTS

Condition-dependence of Signal Components

At time of collection in both years, males were similar in mass (Table 1). Upon maturation, HD males were significantly larger (cephalothorax width) and in better body condition than LD males (mass (g)/cephalothorax width (mm); Table 1).

Foreleg Coloration- Male foreleg coloration was significantly influenced by diet quantity treatment, as HD males had overall darker forelegs than LD males (Mann-Whitney test, $Z = 8.5$, $P < 0.001$; Figure 2a). Males placed on the same diet quantity manipulations in 2008 showed no differences in darkness of the tibia segments on the non-ornamented second leg (HD: $N = 24$, $\bar{x} = 106.3 \pm 1.9$; LD:

$N = 15$, $\bar{x} = 111.7 \pm 3.5$; $t_{37} = 1.5$, $P = 0.15$). However, the overall darkness of the forelegs was again found to be significantly different (HD: $N = 20$, $\bar{x} = 40.2 \pm 1.0$; LD: $N = 14$, $\bar{x} = 69.9 \pm 2.6$; Mann-Whitney test, $Z = 4.9$, $P < 0.001$). Measurements from mature males caught from the field revealed a significant negative correlation between body size and foreleg darkness (Spearman's correlation, $\rho = -0.74$ $P < 0.001$; Figure 2b), identical to the pattern observed in our diet manipulations.

Seismic Component- Seismic display parameters within each bout significantly varied with diet (Overall Model: $\chi^2_5 = 21.2$, $P < 0.001$; Table 2). The length of time spent exhibiting each of the major seismic components differed between diet groups - LD males had longer introductory segment durations than HD males, while HD males produced longer pulse-trains (Figure 1, Table 2). We found no differences between diet treatments in other display variables, such as number of pulse bursts per second in either the introductory segment or pulse-train, or the relative increase in amplitude within each pulse-train bout (Table 2). Males spent equal amounts of time signaling, as the overall length of each seismic bout and the time between bouts did not differ between HD and LD males (bout length: $t_{28} = 0.87$, $P = 0.39$; inter-bout interval: Mann-Whitney test, $Z = 0.75$, $P = 0.46$).

Reproductive Success across Signaling Environments

A total of 97 male-female pairs were run in the variable signaling environments. Copulations occurred in 27% of trials, while the probability of cannibalism during trials was 13%, but was equally distributed across treatments

(Likelihood Ratio, χ^2_3 = 0.2, *P* = 0.98). In five trials cannibalism occurred before males began courtship. For those trials in which males courted (*N* = 92), mating frequency was highly dependent on the signaling environment (Overall Model: χ^2_3 = 22.9, *P* < 0.001; Figure 3). Copulation frequencies were influenced by the presence/absence of both the visual and seismic signals, however there was no interaction between the two (visual: χ^2_1 = 3.97, *P* = 0.046; seismic: χ^2_1 = 21.98, *P* < 0.001; visual X seismic: χ^2_1 = 2.02, *P* = 0.16). While copulation success was significantly reduced when either modality was removed, the presence/absence of the seismic signal had a greater relative impact on copulation frequencies compared to the visual environment (Figure 3), as copulation frequencies in seismic-only trials (V-/S+) were significantly greater than visual-only trials (V+/S-; Likelihood ratio, χ^2_1 = 5.31, *P* = 0.02). Copulation frequencies in trials with both modalities present (V+/S+) were significantly greater than in trials with only visual (V+/S-: Likelihood ratio, χ^2_1 = 9.72, *P* = 0.002), but not when compared to seismic only (V-/S+; Likelihood ratio, χ^2_1 = 0.73, *P* = 0.39). No copulations occurred when both signal modalities were removed (V-/S-), which was significantly less than trials with only the seismic modality present (Likelihood ratio, χ^2_1 = 12.46, *P* < 0.001), but not statistically less than trials with only the visual modality present (Likelihood ratio, χ^2_1 = 3.27, *P* = 0.07).

Across treatments, male motivation to mate appeared similar. The latency to a male's first courtship (Kruskal-Wallis, χ^2_3 = 3.55, *P* = 0.31) and attempted mounts of the female (Kruskal-Wallis, χ^2_3 = 2.57, *P* = 0.46) did not differ across treatments. However, male courtship rate (# courtship bouts / sec) was dependent on the signaling environment (Kruskal-Wallis, χ^2_3 = 17.27, $P < 0.001$; Figure 4a). Post-hoc pairwise comparisons using Mann-Whitney tests ($P < 0.05$) revealed that males courted at higher rates in the V+/S+ trials than either of the dark treatments, and males courted at higher rates in all signaling environments compared to trials run in the absence of both modalities (Figure 4a). However, despite these differences in courtship effort, males gained copulations just as fast regardless of the signaling environment, as the time from first courtship to copulation did not differ (Kruskal-Wallis, χ^2_2 = 1.56, $P = 0.46$).

Interestingly, we found that males altered the composition of their complex display depending on the signaling environment (Kruskal-Wallis, χ^2_3 = 26.95, $P <$ 0.001; Figure 4b). Males significantly decreased the proportion of courtship bouts that incorporated a leg-wave in treatments run in complete darkness (V-/S+ and V-/S-), while the presence/absence of the seismic modality had no influence on the number of leg-waves incorporated into courtship displays (Figure 4; post-hoc pairwise comparisons using Mann-Whitney tests, $P < 0.05$).

DISCUSSION

The multimodal courtship display of male *Rabidosa rabida* is well suited for communication in signaling environments that vary in modality-specific transmission. Both foreleg ornamentation and the seismic display are condition-dependent, and reflect past foraging history. As such, these signals have the potential to convey similar information, and potentially act as redundant components or signals (Møller and Pomiankowski, 1993). Additionally, visual and seismic signals in isolation are each sufficient to maintain male reproductive success, supporting a back-up function of complex signaling, where each component backs up the other in the face of environmental variability (Candolin, 2003; Hebets and Papaj, 2005). The potential redundancy of information and sufficiency of each signal modality for mating would make it possible for males to maintain reproductive success irrespective of variation in the signaling environment. Furthermore, our results highlight an additional advantage to complex signaling – flexibility in the composition of courtship displays – which could enable males to adjust the composite make-up of courtship displays depending upon current signaling conditions. Together, our results are consistent with the hypothesis that the signaling environment of *R. rabida* has played a major role in the evolution of their complex courtship display.

In *R. rabida* courtship displays, we found display components of each modality, the visual foreleg coloration and the seismic courtship signal, to be condition-dependent. Similar condition-dependent display components have been found across several lycosids (e.g. Kotiaho, 2000; Uetz et al., 2002; Gibson and Uetz, 2008; Hebets et al., 2008b; Shamble et al., 2009; Rundus et al., In Press). With respect to the seismic courtship signal specifically, our results show that males vary in the structure of their seismic display depending upon their condition. HD males produced longer pulse-trains while LD males had longer introductory segments. Our observed differences in amplitude and frequency of pulses between the introductory and pulse-train components suggest that the pulse-train is likely more costly to produce. One possibility is that only males in good condition can afford this lengthy display; and that poor condition males may instead invest more time in a less costly signal component (e.g. introductory segment). Although both quantified displays components (foreleg coloration and seismic signal) were shown to be condition-dependent, our diet manipulations involved a sustained diet treatment that included both juvenile and adult life stages. It remains possible, therefore, that juvenile versus adult foraging efficacy influence visual and seismic components differently, leaving the possibility that different signal components convey different information (i.e. multiple messages; Møller and Pomiankowski, 1993). Additionally, numerous other display components exist in *R. rabida* courtship (*e.g.* pedipalpal color and movement and foreleg movement), which were not measured in this study. Future work focusing on both receiver responses to isolated display components, as well as the incorporation of additional display components, are essential to fully understand complex signal function in this species.

In *R. rabida*, we found that copulation success is maintained with only one modality present, and that both visual and seismic signals play a role in *R. rabida* mating success, corroborating previous studies on this species (Rovner, 1967; 1968; 1996). While we found the seismic signal to be relatively more important in female mate choice decisions, a result that has been found across multiple species of *Schizocosa* wolf spiders (Scheffer et al., 1996; Hebets and Uetz, 1999; Hebets, 2005; 2008; Rundus et al., In Press; but see Rundus et al. 2010), copulation success was maintained, albeit at lower levels, with just the visual signal present. The maintenance of reproductive success regardless of signal transmission suggests environmental variability could have selected for complex signal components in *R. rabida* to function as efficacy backups.

Interestingly, we found that male *R. rabida* courtship rate and display composition is flexible and responsive to the signaling environment. Males tended to court females at the highest rates in environments where both modalities were transmitted. Males also reduced the incorporation of one visual component, the foreleg wave, when courting in an environment that did not transmit visual signals. Dynamic courtship signaling via the alteration of display rates, intensity, or signaling location have been found in response to receivers (e.g. Patricelli et al., 2002; Dukas, 2008; Sullivan-Beckers and Hebets, In Review) as well as to the signaling environment (e.g. Reynolds, 1993; Brumm, 2004; Ord et al., 2007). However, fewer studies have demonstrated that signalers will alter display type or composition based upon the signaling environment (e.g. Jackson, 1992; Taylor and Jackson, 1999; Taylor et al., 2005; Grafe and Wanger, 2007; Peters et al., 2007). Dynamic flexibility in displays in response to environmental variability has been shown to be adaptive by maintaining signal detectability (Ord et al. 2010). In addition, given the variety of costs associated with signaling (e.g. metabolic, predation; Andersson, 1994; Bradbury and Vehrencamp, 1998), flexibility in complex signal composition in response to each context (e.g. environment, predator proximity) could limit costs by reducing production of unnecessary or costly components while continuing production of others and maintaining (at some level) the associated benefits. In *R. rabida*, the production of the foreleg wave may be costly, as we have evidence that LD males incorporate significantly fewer foreleg waves in their displays (Wilgers unpub. data). Alternatively, visual feedback from females may be important in eliciting the full production of *R. rabida* complex displays. Consistent with this alternative, previous studies suggest that male *R. rabida* reduce activity and remain stationary under reduced illumination (Frings, 1941; Rovner 1991), indicating detection of females in proximity as a possible release mechanism resulting in the greater occurrences of full courtship displays. Female visual feedback has also been previously found to influence inter-courtship intervals and the brevity of the male visual displays in *R. rabida* (Rovner, 1967; 1968), which could also help to explain the observed increased courtship rates of males in the light. These explanations are not mutually exclusive and all may play a role in the flexible composition of courtship displays we witnessed across signaling environments in *R. rabida*.

In nature, given the complex signaling environment of *R. rabida*, females may frequently find themselves the recipients of courtship signals via only one sensory modality. Here we provide evidence that both the visual signal and the seismic signal can potentially convey redundant information regarding a male's condition, and that both of these modalities are important during mate choice decisions in *R. rabida*. Our results suggest that these signals may act as both content and efficacy backup signals, which seems adaptive given the natural history of *R. rabida*. Additionally, when faced with environmental variability in the efficacy of modality-specific signal transmission, multimodal signals may afford males the flexibility to reduce costs associated with the production of ineffective signals by removing them from their repertoire and relying upon signals for which transmission remains effective. Large-scale comparisons of environmental and corresponding signal complexities across taxa may shed light on the role of signaling environment on the evolution of signal complexity.

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TABLES

Table 1. Effects of diet quantity manipulations on body measures of male *R. rabida*.

		Male Diet Treatment (*N*)		
Year	**Body Measure**	**LD**	**HD**	***P*-Value[a]**
2007	Initial Mass (mg)	110.1 ± 8.0 (51)	113.4 ± 7.0 (58)	0.78
	CW (mm)	4.31 ± 0.06 (48)	4.87 ± 0.06 (53)	***< 0.001***
	Condition[b]	43.6 ± 1.2 (48)	54.1 ± 1.2 (53)	***< 0.001***
2008	Initial Mass (mg)	42.3 ± 1.7 (17)	47.6 ± 2.6 (24)	0.43
	CW (mm)	3.18 ± 0.05 (16)	4.27 ± 0.07 (24)	***< 0.001***
	Condition[b]	23.4 ± 0.6 (16)	42.6 ± 1.3 (24)	***< 0.001***

-Means ± SE shown for each parameter

[a] P-values reported from Mann-Whitney tests on differences between diet treatments

[b] Condition calculated as ratio of body mass at maturation (mg) / cephalothorax width (mm)

Table 2. Variability in seismic display parameters between high and low quantity diet males of *R. rabida.*

Signal Parameter	**HD (*N*=15)**	**LD (*N*=15)**	χ^2[a]	***P*-value**
Introductory Segment Duration (sec)	4.30 ± 0.23	5.24 ± 0.47	13.4	< 0.001
Pulse-train Duration (sec)	3.57 ± 0.15	3.20 ± 0.12	11.9	< 0.001
Introductory Pulse Bursts / Sec	2.06 ± 0.10	2.14 ± 0.13	0.01	0.91
Pulse-train Pulse Bursts / Sec	4.56 ± 0.24	4.41 ± 0.12	2.99	0.08
Pulse-train Amplitude Increase (dB)	12.22 ± 0.51	10.52 ± 0.60	2.16	0.14

-Means ± SE shown for each parameter

[a] Chi-Square values from a logistic regression model incorporating all signal parameters to predict male diet treatment (Overall Logistic Regression Model: P < 0.001)

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FIGURES

Figure 1. A) Waveform of *R. rabida* seismic display showing various parameters measured for influence of diet manipulation. B) Magnified pulse-train showing discrete pulse bursts, consisting of individual pulses previously described as each individual waveform spike (Rovner 1967). The magnified portion does not represent the entire section indicated but is a shorter segment blown up for detail.

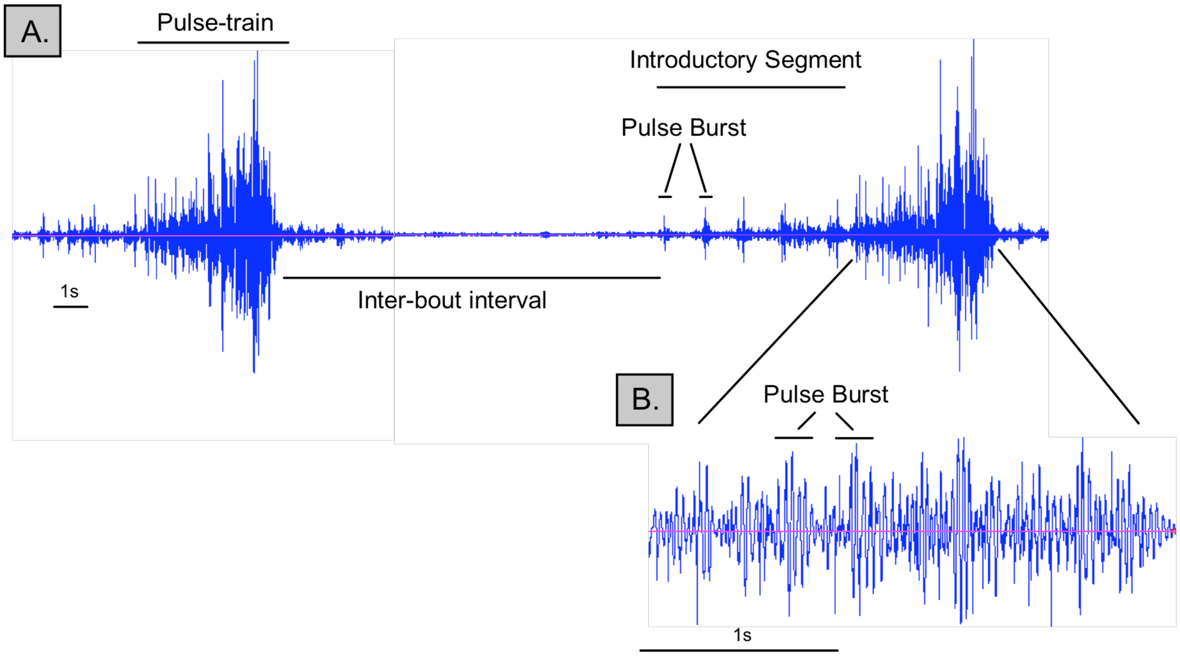


Figure 2. Variability in *R. rabida* foreleg coloration. A) Across males varying in juvenile diet via diet-quantity manipulations (HD: *N* = 58; LD: *N* = 51). Arrows below $\bar{x}$ denote means for HD and LD males. Lower 'K' values indicate darker legs. Gray-scale foreleg pictures under x-axis provide an example of the extremes witnessed in foreleg darkness. B) Across mature males caught in the field varying naturally in size (*N* = 27; Spearman's correlation, $\rho = -0.74$, $P < 0.001$).

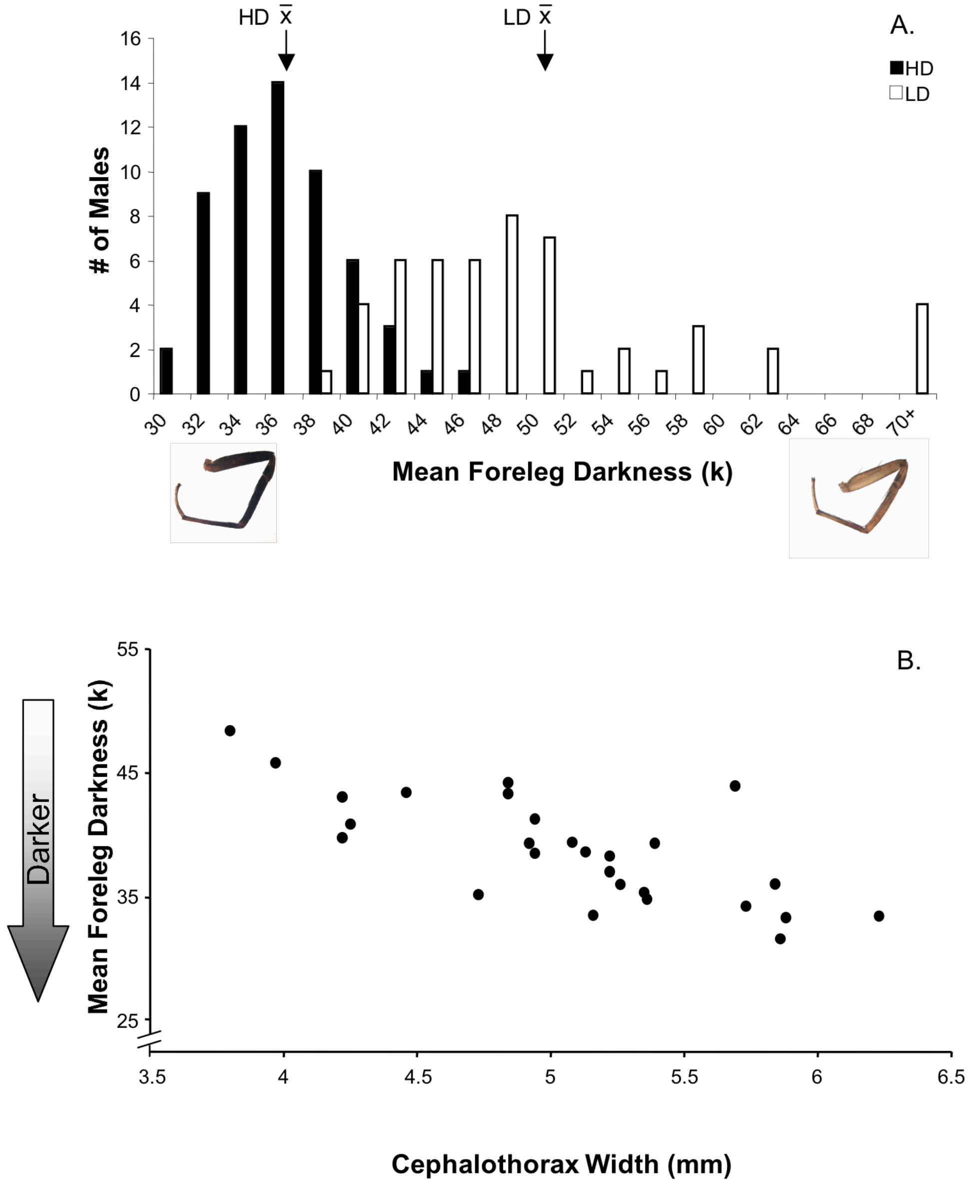


Figure 3. Influence of signaling environment on copulation frequencies in *R. rabida*. Male-female pairs were placed in mating trials in the presence (+) or absence (-) of the visual (V) or seismic (S) signal.

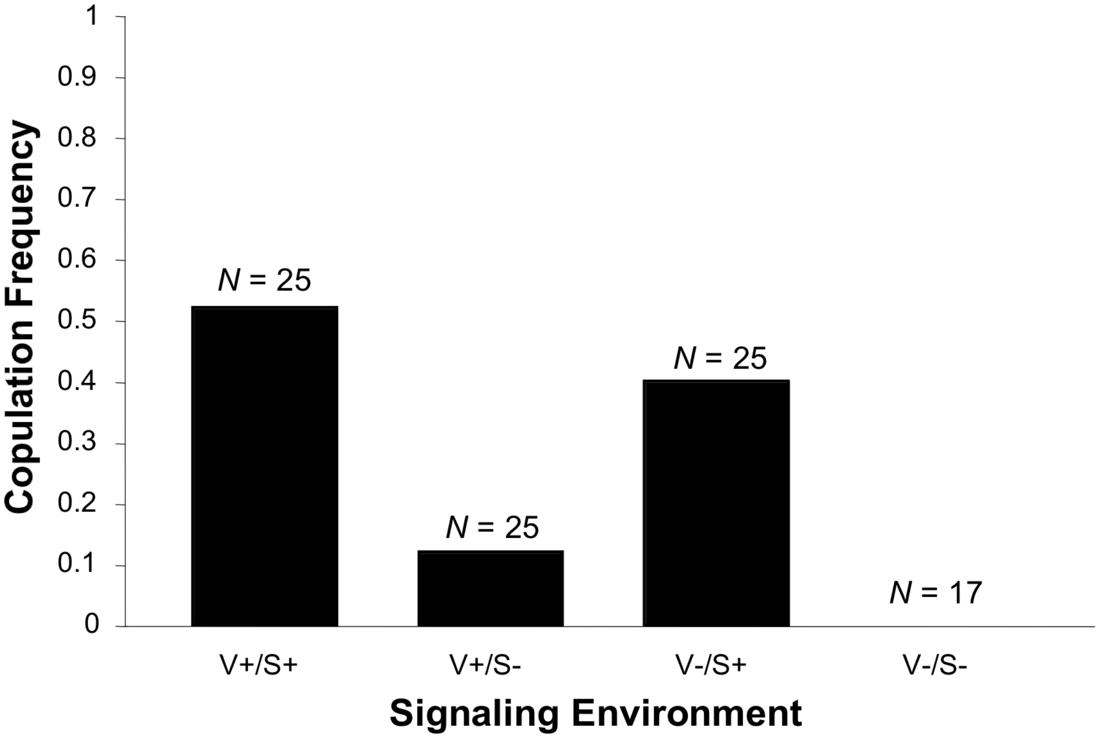
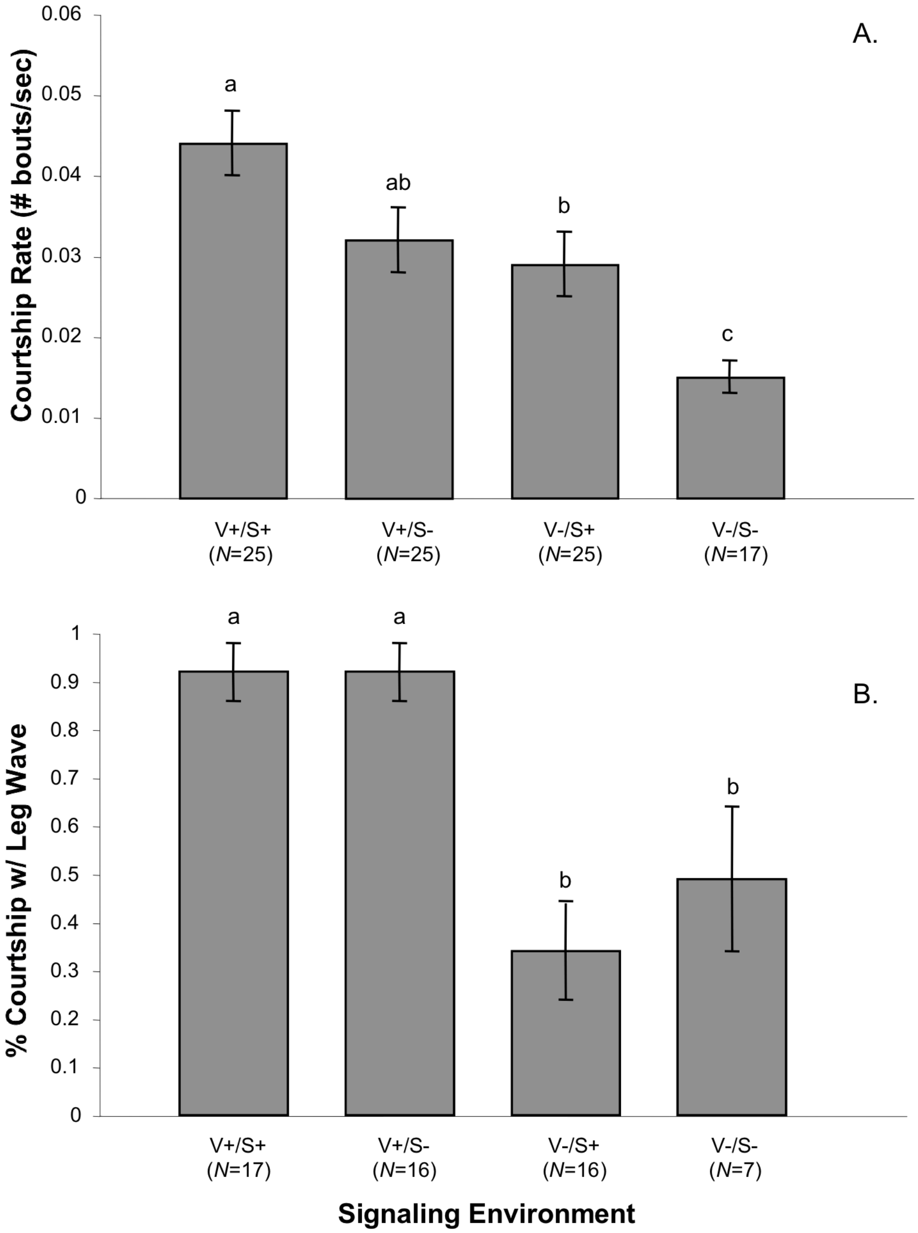


Figure 4. Influence of the signaling environment on *R. rabida* male courtship A) rate, and B) display composition. Male-female pairs were placed in mating trials in the presence (+) or absence (-) of the visual (V) or seismic (S) signal. Letters denote significant differences detected by post-hoc pairwise comparisons using Mann-Whitney tests, $P < 0.05$.



CHAPTER 3

FEMALE CHOOSINESS IS DRIVEN BY SEISMIC SIGNALING IN A MULTIMODAL SIGNALING WOLF SPIDER

Dustin J. Wilgers

ABSTRACT

Complex courtship signals can be dissected into distinct components that can either function indepedently or via interactions with one another. Male *Rabidosa rabida* wolf spiders use courtship displays that couple waving a ornamented foreleg with a seismic display. While previous studies suggest that female *R. rabida* are choosy, and that both the visual and seismic modalities are important in mating interactions, it remains unclear how variation in each component influences female mate choice decisions. To investigate this, we ran two separate experiments in which we manipulated (i) male diet, to induce variation in the seismic courtship signal, and (ii) male foreleg color, to artificially induce variation in visual ornamentation. Females were paired with males in environments that allowed the detection of just the manipulated signal component (*e.g.* seismic signal only and visual signal only). Variability in the seismic signal alone influenced female mate choice, but variability in visual ornamentation alone did not. In a third experiment, we allowed the two signal components to interact by artificially manipulating visual ornamentation and performing mate choice trials in the presence of seismic signaling. When females were able to detect both signal components, females discriminated among males with versus without ornamentation. Thus, female mating decisions differed when presented with variable male ornamentation in isolation versus when detected as part of a composite display. Together, these results suggest that the seismic signal of male *R. rabida* is integral for female choosiness, and that the components of the courtship display interact to influence female mate choice decisions.

Keywords: complex signal, interaction, mate choice, *Rabidosa rabida*

INTRODUCTION

Male courtship displays commonly consist of multiple components, often across multiple sensory modalities (reviews in Hebets and Papaj 2005; Partan and Marler 2005), and females are known to use multiple cues in mate choice decisions (Candolin 2003). Individually, these cues can increase mate detection, mate assessment, and/or receiver memory (Rowe 1999), but little is known about if and/or how multiple components might interact. Until recently, the vast majority of studies have assumed that display components function independently to increase female assessment or detection of male displays, without allowing for the possibility of interactions among display components (Møller and Pomiankowski 1993; Johnstone 1996; but see Hebets and Papaj 2005).

Inter-signal interactions, where the presence of one signal component alters the type, probability, or latency of a female's response to another component, are potentially widespread among animals (reviews in Candolin 2003; Hebets and Papaj 2005). Additional components could function to provide information (e.g. species identification, sex, condition, location) that alters the receiver's reliance on sequential signals (Patricelli et al. 2003; Leonard and Hedrick 2010) or to modify the context of the interaction, resulting in different interpretations of signal content and responses (Hughes 1996). Additionally, one signal could alter the efficacy of other display components by alerting females to the presence of sequential signals (Grafe and Wanger 2007), or by altering a female's attention to a second signal (Hebets 2005). The presence of morphological traits may act as amplifiers by making other morphological or behavioral traits more conspicuous (e.g. amplifier; Hasson 1991), which has been a suggested function of ornamentation associated with a variety of motor displays (Hebets and Uetz 2000; Smith et al. 2009; Byers et al. 2010). Due to the variety of ways complex display components can interact, female mate preferences based on single components could be obscured or enhanced when experimentally tested with other display components. Thus, to fully understand complex signal function, it is essential to understand how variation in each display component influences female mate choice decisions both in isolation and in combination (Partan and Marler 1999; Candolin 2003; Partan and Marler 2005).

In wolf spiders (Araneae: Lycosidae), females are known to base mating decisions on complex male courtship displays consisting of multiple components (*e.g.* foreleg ornamentation, leg-wave, seismic displays), produced both sequentially and simultaneously across multiple modalities (i.e. visual, seismic; Uetz and Roberts 2002). Foreleg ornamentation and courtship displays are species-specific (examples in Schizocosa: Stratton 2005; Framenau and Hebets 2007), and in some instances, differences in the seismic signal are known to maintain species boundaries (Stratton and Uetz 1981). In addition to potentially indicating species identity, seismic displays have been found to reflect a male's diet (Kotiaho 2000; Rundus et al. 2011), which in turn can influence female mate choice decisions (Kotiaho et al. 1996; Gibson and Uetz 2008; Rundus et al. 2011). Males of species with active visual displays typically have ornamented forelegs (e.g. pigmentation, brushes), the expression of which is also condition-dependent (Uetz et al. 2002; Shamble et al. 2009; Rundus et al. 2011). Evidence that foreleg ornamentation is a direct target of selection via female choice is mixed. Examples of ornament variation in isolation influencing mate choice is rare (Hebets and Uetz 2000), and while several studies have found evidence of ornamentation influencing copulation success when presented with a seismic signal (McClintock and Uetz 1996; Scheffer et al. 1996; Persons and Uetz 2005), others have failed to find any relationship (Hebets et al. 2006; Hebets 2008; Shamble et al. 2009; Rundus et al. 2011). Several studies suggest that ornamentation may influence female mate decisions via interactions with other display components. In *Schizocosa uetzi,* females pay attention to the degree of ornamentation only when the seismic signal is present (Hebets 2005). Previous studies suggest that ornamentation interacts with the foreleg waving display to amplify these conspicuous movements (Hebets and Uetz 2000), and may reduce male reliance on other dynamic traits, such as courtship rate, to maintain reproductive success (Hebets et al. 2011). From these past studies, it is clear that mate choice in wolf spiders may be just as complex as the displays they are based on, and hence, inclusive studies allowing components to interact are essential for understanding the factors contributing to the evolution and maintenance of their complex displays.

In the wolf spider, *Rabidosa rabida* (Walckenaer), males court females using complex courtship displays consisting of visual signals, including palpal rotations and waving of a darkly pigmented foreleg (a secondary sexual ornament that both appears at and is fixed at maturation; Foelix 1996), as well as seismic signals, produced via palpal stridulation (Kaston 1936; Rovner 1967; Rovner 1968). Both signaling modalities play a role in female mate choice decisions (Rovner 1967; Rovner 1968; Wilgers and Hebets 2011), and young females are known to be choosy, mating significantly more often with large males in good body condition (Wilgers and Hebets In Review). However, in *R. rabida* males, multiple traits and courtship display components are nutrition-dependent (e.g. body size, body condition, foreleg pigmentation, seismic signal; Wilgers and Hebets 2011), and thus, it is unclear as to which traits or display components females may use to assess males during mating interactions. Here we independently manipulated the seismic signal and the foreleg ornamentation of courting *R. rabida* males, and presented these components to females both in isolation and in combination to investigate whether variation in each display component influences female mate choice decisions.

METHODS

Experiment 1: Influence of Seismic Signal

Our first experiment explored the relationship between a male's seismic signal and copulation success. Previous work has demonstrated that the seismic display of male *R. rabida* is nutrition-dependent (Wilgers and Hebets 2011). As such, we used previously established diet manipulation methods to induce variation in the seismic display (see below), and asked whether variation in male seismic displays influences the likelihood of copulation. Mating trials were conducted in signaling environments that successfully propagated seismic signals only (see below).

We collected immature spiders in Lancaster County, NE in 2010, (15-24 June). Spiders were housed individually in a climate-controlled environment (~ 27°C) in individual plastic containers (84 mm x 84 mm x 110 mm) that visually isolated them from their neighbors. Because it is not possible to sex immature *R. rabida*, upon collection, all spiders were randomly assigned to 1 of 2 diet treatments for the duration of the experiment: 1) high quantity diet (HD) – spiders were fed 2 body-size matched crickets, *Acheta domestica*, twice per week, or 2) low quantity diet (LD) – spiders were fed 2 body-size matched crickets once every 2 weeks. Males from these two diet manipulations were used in the subsequent experiment. However, since we were interested in the influence of male variation, and not female variation, on mating success, only females from the HD manipulation were used. All crickets were supplemented with fish flakes (TetraMin, Blacksburg, VA) and Fluker's cricket feed (Port Allen, LA). Individuals were checked for molts 2-3 times per week to determine maturity.

For each mate choice trial, we randomly paired a HD naïve, virgin female with a single naïve virgin male (HD or LD) in a circular plastic mating arena (diameter = 20.2 cm, height = 7.3 cm) lined with filter paper substrate (Whatman # 1 185mm). Trials were conducted in complete darkness to eliminate visual signal transmission. Observations of trials were aided by infrared night vision goggles (Rigel 3200, Rigel Optics Inc., Washougal, WA, USA) and an infrared illuminator (Supercircuits IR20, Supercircuits, Austin, TX, USA). Spectral sensitivity data on wolf spiders provide no indication that these spiders can detect the IR wavelengths emitted by the illuminator (~ 850nm; Devoe et al. 1969; Devoe 1972).

Prior to mating trials (12-24 hours), both males and females were fed 1 small cricket (~ ½ cephalothorax length) to standardize hunger levels and minimize cannibalism events. All individuals were weighed just prior to their introduction into the mate-choice arena. Females were introduced into the arena at least 1 hr prior to the start of a trial to acclimatize and to deposit pheromone-laden silk. During introduction of the male into the arena, females were placed under a clear plastic vial. Males were allowed to acclimatize for ~ 1 min, after which the vial was lifted and the trial commenced. Trials lasted 45 minutes, during which we recorded the following behaviors live: latency to first courtship, # of courtship bouts, female attacks, male attempted mounts, copulation, latency to copulation, cannibalism, and latency to cannibalism. During two HD male trials, courtship latency was not recorded, resulting in the sample size discrepancies below for courtship latency and courtship rate. Mate-choice arenas were cleaned with alcohol after each trial to remove any potential chemical cues. Females and males were only used once in trials.

All females were 12-15 days post maturation ($\bar{x}$ = 13.3 ± 0.1 days) when used in mating trials, the age at which females are most discriminating (Wilgers and Hebets In Review). Male age ranged from 6-39 days post-maturation ($\bar{x}$ = 14.2 ± 0.9 days). Both female and male ages did not differ across diet treatments (Mann-Whitney test, females: $Z = 1.09$, $P = 0.28$; males: $Z = 1.36$, $P = 0.17$).

Experiment 2: Influence of Foreleg Ornamentation

Our second experiment explored the relationship between a male's foreleg ornamentation, and copulation success. To do so, we artificially altered male foreleg ornamentation (presence vs. absence) and performed mate choice trials in arenas that allowed for the successful propagation of the visual display only (see below). Our aim was to manipulate only the presence/absence of the ornamental component of the visual display, not the associated leg movements, and thus, we used artificial manipulations instead of diet manipulations.

We collected immature spiders from Lancaster County, NE in 2009 (30 June-12 July). Spiders were housed as in Experiment 1. In contrast to Experiment 1, spiders were not placed on diet manipulations, but instead, all spiders were fed 3 crickets per week and provided with water ad libitum. Individuals were checked for molts 2-3 times per week to determine maturity.

We manipulated the presence/absence of foreleg ornamentation using acrylic paint (Anita's All Purpose Acrylic Craft Paint, Clarkston, GA). Manipulations were achieved by painting both forelegs of a male either: 1) black (i.e. ornament present; 11002 Black), or 2) brown (i.e. ornament absent; 11044 Coffee). For both 1) and 2), the paint fully covered the natural ornamentation of the male's foreleg. While seemingly extreme, this variation appears naturally. In food-stressed males, pigmentation is often lacking and forelegs resemble the other walking legs (Wilgers pers. obs.). To paint the forelegs, males were placed into a Ziploc bag with a cut corner. In attempt to escape, males naturally stick the forelegs out of the cut corner, where they were gently restrained and painted the appropriate color with a cotton-swab. Each male was painted the morning of its mating trial (~ 4 hours prior).

For each mating trial, we randomly paired a naïve, virgin female with a naïve virgin male of one foreleg treatment (present vs. absent) in a circular mating arena with plastic walls and a granite floor (diameter = 20.2 cm, height = 7.3 cm). The granite floor effectively ablates seismic signal propagation (D Wilgers pers. obs; Elias et al. 2004). Trials were performed under illumination from 2 full spectrum Vita-Lite 30-watt fluorescent bulbs (Duro-Test Lighting Inc., Philadelphia, PA, USA). Mating trials were conducted exactly as seen in Experiment 1 (see above).

Female age ranged from 12-16 days post maturation ($\bar{x}$ = 13.7 ± 0.2 days), while male age ranged from 12-31 days post-maturation ($\bar{x}$ = 19.8 ± 1.1 days). Both female and male ages did not differ across foreleg treatments (Mann-Whitney test, females: $Z = 0.06$, $P = 0.95$; males: $Z = 0.66$, $P = 0.51$). Females paired with males of each foreleg treatment were similar in mass (present: N = 19, $\bar{x}$ = 236.3 ± 16.4 mg; absent: N = 19, $\bar{x}$ = 249.0 ± 13.5 mg; Mann-Whitney, $Z = 0.72$, $P = 0.47$). Males in each foreleg treatment group were similar in weight (present: N = 19, $\bar{x}$ = 168.3 ± 9.1 mg; absent: N = 19, $\bar{x}$ = 166.2 ± 10.0 mg; $t_{36} = 0.16$, $P = 0.88$), size (present: N = 19, $\bar{x}$ = 4.48 ± 0.07 mm; absent: N = 19, $\bar{x}$ =

4.52 ± 0.11 mm; $t_{32} = 0.31$, $P = 0.75$), and condition (present: $N = 19$, $\bar{x} = 37.2 \pm 1.6$; absent: $N = 19$, $\bar{x} = 36.3 \pm 1.5$; Mann-Whitney test, $Z = 0.96$, $P = 0.34$).

Experiment 3: Interaction between Ornamentation and Seismic Signal

Our third experiment explored the potential for the seismic signal to affect the influence of the visual ornamentation in female *R. rabida* mate choice. Such an inter-signal interaction has been demonstrated in the wolf spider *Schizocosa uetzi*, where variation in ornamentation is known to influence female receptivity only when detected in conjunction with the seismic signal (Hebets 2005). To investigate whether a similar inter-signal interaction occurs in *R. rabida,* we manipulated male foreleg ornamentation (presence vs. absence) and performed mate choice trials in arenas where females could detect both visual and seismic signals.

We collected immature spiders from Lancaster County, NE in 2008 (14 June-12 July). Spiders were housed and maintained identically to the experiment above (experiment 2). Male forelegs were manipulated using the same protocol as in Experiment 2 (see above). Males were painted ~ 24 hours in advance of mating trials; spiders were checked the next morning and paint was re-applied if any paint had been groomed off.

We performed single-choice mating trials in arenas allowing successful propagation of both visual and seismic signals. In contrast to Experiment 2, our circular mating arenas were lined with filter paper substrate (Whatman # 1

185mm) instead of granite, in order to allow seismic signal propagation. All other aspects of the arena and mating trial were identical to those of Experiment 2.

All females were 12-15 days post maturation ($\bar{x}$ = 12.9 ± 0.1 days) when used in mating trials. Male age ranged from 8-30 days post-maturation ($\bar{x}$ = 19.1 ± 1.0 days). Both female and male ages did not differ across foreleg treatments (females: Mann-Whitney test, $Z = 0.29$, $P = 0.77$; males: $t_{32} = 0.53$, $P = 0.60$). Females paired with males of each foreleg treatment were similar in mass (present: $N = 17$, $\bar{x}$ =233.3 ± 7.0 mg; absent: $N = 17$, $\bar{x}$ = 235.9 ± 13.0 mg; Mann-Whitney, $Z = 0.14$, $P = 0.89$). Males in each foreleg treatment group were similar in weight (present: $N = 17$, $\bar{x}$ = 199.5 ± 11.2 mg; absent: $N = 17$, $\bar{x}$ = 204.0 ± 11.9 mg; Mann-Whitney, $Z = 0.0$, $P = 1.0$), size (cephalothorax width; present: $N = 17$, $\bar{x}$ = 5.1 ± 0.1 mm; absent: $N = 17$, $\bar{x}$ = 5.09 ± 0.1 mm; t_{32} = 0.03, $P = 0.97$), and condition (weight (mg)/cephalothorax width (mm); present: N = 17, $\bar{x}$ = 38.8 ± 1.5; absent: $N = 17$, $\bar{x}$ = 39.7 ± 1.6; Mann-Whitney test, Z = 0.17, $P = 0.86$).

Statistical Analyses

Non-normal data were analyzed using non-parametric tests. In all experiments, some males were cannibalized prior to courtship (Experiment 1: $N = 3$; Experiment 2: $N = 4$; Experiment 3: $N = 3$). All statistical tests and conclusions were robust to the removal of these males, thus, we report analyses including all data points. We used likelihood ratio chi-square tests for each experiment to test whether copulation success for males was independent of our experimental manipulation (Experiment 1: male diet HD/LD; Experiments 2 and 3: ornamentation presence/absence). To gauge the magnitude of the effect of each component on female mate choice decisions, we calculated the effect size (Cramer's φ) of our manipulation on copulation frequency for each experiment (Nakagawa and Cuthill 2007; es calculator: http://mason.gmu.edu/~dwilsonb/ma.html by D. B. Wilson). All statistics were performed in JMP v. 6 (SAS Institute Inc., Cary, NC, USA). All results are reported as means ± 1 SE.

RESULTS

Experiment 1: Influence of Seismic Signal

Our diet manipulations were successful at diverging males in each treatment based on weight (HD: $N = 25$, $\bar{x} = 199.7 \pm 7.9$ mg; LD: $N = 16$, $\bar{x} = 120.1 \pm 7.7$ mg; $t_{39} = 6.84$, $P < 0.001$), size (HD: $N = 25$, $\bar{x} = 4.65 \pm 0.07$ mm; LD: $N = 16$, $\bar{x} = 4.02 \pm 0.09$ mm; $t_{39} = 5.66$, $P < 0.001$), and our condition index (weight (mg)/cephalothorax width (mm); HD: $N = 25$, $\bar{x} = 42.6 \pm 1.2$; LD: $N = 16$, $\bar{x} = 29.5 \pm 1.4$; $t_{39} = 7.12$, $P < 0.001$). Due to differences in maturation times between HD and LD males, despite all females being raised on a HD treatment, females paired with HD males were smaller ($N = 25$, $\bar{x} = 253.8 \pm 11.3$ mg) than females paired with LD males ($N = 16$, $\bar{x} = 364.9 \pm 18.0$; Mann-Whitney test, $Z = 4.45$, $P < 0.001$).

We performed 41 mating trials in the dark (25 HD males, 16 LD males), with copulation occurring in 49% of trials and pre-sexual cannibalism occurring in

22% of trials. Male diet influenced the likelihood of a male to copulate, as females were more likely to mate with high-quantity diet (HD) males compared to low-quantity diet (LD) males (Likelihood ratio, χ_1^2 = 6.15, *P* = 0.01; φ = 0.39, 95% CI: 0.09-0.62; Fig. 1). Not only were HD males more likely to copulate, they also tended to gain copulations faster after courtship was initiated (HD: *N* = 15, $\bar{x}$ = 631.2 ± 120.1 sec; LD: *N* = 4, $\bar{x}$ = 1414.3 ± 412.8), but this difference was insignificant (Mann-Whitney test, *Z* = 1.75, *P* = 0.08). Of the 22% of trials in which males were cannibalized, LD males were cannibalized more often than HD males (HD = 8%, LD = 44%; Likelihood ratio, χ_1^2 = 7.29, *P* = 0.007).

The diet manipulations did not appear to influence mating motivation, as males of each diet treatment behaved similarly during mating trials. Both HD and LD males had similar latencies to initial courtship (HD: *N* = 22, $\bar{x}$ = 163.0 ± 27.5 sec; LD: *N* = 14, $\bar{x}$ = 183.6 ± 58.1; Mann-Whitney test, *Z* = 0.68, *P* = 0.50), courtship rates (HD: *N* = 22, $\bar{x}$ = 0.043 ± 0.004 bouts/sec; LD: *N* = 14, $\bar{x}$ = 0.049 ± 0.008 bouts/sec; t_{34} = 0.76, *P* = 0.45), and number of attempted mounts of the female did not differ between the male groups (HD: *N* = 25, $\bar{x}$ = 0.32 ± 0.11; LD: *N* = 16, $\bar{x}$ = 0.56 ± 0.44; Mann-Whitney test, *Z* = 0.55, *P* = 0.58).

Experiment 2: Influence of Foreleg Ornamentation

A total of 38 male-female pairs were run in mate-choice trials (*N* =19 per foreleg treatment), with copulations occurring in 32% of trials and pre-sexual cannibalism in 26% of trials. Male foreleg treatment did not influence copulation frequency (Likelihood ratio, χ_1^2 = 0.49, *P* = 0.48; φ = 0.11, 95% CI: -0.20-0.41; Fig. 2A).

Females mated regardless of male ornamentation, and took similar amounts of time to copulate after males of each treatment began to court (Mann-Whitney test, $Z = 0.16$, $P = 0.87$). Cannibalism events were independent of male foreleg treatment (Likelihood ratio, $\chi_1^2 = 0.55$, $P = 0.46$).

Male traits (e.g. size, condition) were controlled for and not different between the groups (see methods). Males in each foreleg ornamentation treatment group (present vs. absent) behaved similarly and appeared equally motivated to mate, as all trial behaviors were similar: latency to court (present: $N = 17$, $\bar{x} = 136.4 \pm 45.8$ sec; absent: $N = 16$, $\bar{x} = 202.6 \pm 73.3$ sec; Mann-Whitney test, $Z = 1.26$, $P = 0.21$), courtship rate (present: $N = 17$, $\bar{x} = 0.037 \pm 0.006$ bouts/sec; absent: $N = 16$, $\bar{x} = 0.034 \pm 0.005$ bouts/sec; $t_{31} = 0.40$, $P = 0.69$), and attempted mounts (present: $N = 19$, $\bar{x} = 0.26 \pm 0.15$; absent: $N = 19$, $\bar{x} = 0.58 \pm 0.53$; Mann-Whitney test, $Z = 0.42$, $P = 0.67$).

Experiment 3: Interaction between Ornamentation and Seismic Signal

A total of 34 female-male pairs were run in mate-choice trials ($N = 17$ per foreleg treatment) with copulations occurring in 41% of trials and pre-sexual cannibalism in 21% of trials. Contrary to when the seismic signal was absent (Experiment 2), male foreleg treatment influenced copulation frequency, as females mated more often with ornamented males when compared with males lacking ornamentation (Likelihood Ratio, $\chi_1^2 = 4.49$, $P = 0.03$; $\varphi = 0.36$, 95% CI: 0.03-0.62; Fig. 2B). The latency to copulation after a male's first courtship bout was not different between male treatment groups (t_{12} = 0.6, P = 0.57). Male foreleg treatment did not influence the likelihood of cannibalism (Likelihood Ratio, χ^2_1 = 0.18, P = 0.67).

Male traits (e.g. size, body condition) were again controlled for and not different between the groups (see methods). Males in each foreleg ornamentation treatment group (present vs. absent) behaved similarly and appeared motivated to mate, as indicated by no differences in the latency to court (present: N = 16, $\bar{x}$ = 102.6 ± 22.0 sec; absent: N = 15, $\bar{x}$ = 187.1 ± 100.3 sec; Mann-Whitney test, Z = 0.22, P = 0.83), courtship rate (present: N = 16, $\bar{x}$ = 0.048 ± 0.006 bouts/sec; absent: N = 15, $\bar{x}$ = 0.036 ± 0.007 bouts/sec; t_{29} = 1.19, P = 0.24), and attempted mounts of the female (present: N = 17, $\bar{x}$ = 0.12 ± 0.08; absent: N = 17, $\bar{x}$ = 0.29 ± 0.19; Mann-Whitney test, Z = 0.5, P = 0.61).

DISCUSSION

During mating interactions, male *R. rabida* use complex courtship displays consisting of multiple components to convince females of their suitability as a mate. Here, in two separate experiments, we presented female *R. rabida* with experimentally manipulated, isolated courtship components – males expressing variable seismic signals (HD vs. LD males) and males expressing variable foreleg ornamentation (present vs. absent). We followed these modality-isolation experiments with a third experiment in which we experimentally manipulated foreleg ornamentation (present vs. absent), but allowed males to successfully transmit their seismic signal – resulting in a complete composite courtship display. Our results demonstrate that female *R. rabida* discriminate among potential mates based on variation in both seismic and visual courtship components, and that these components interact to influence mating decisions. In the absence of visual courtship components, females appear to discriminate among males based upon seismic signals only – mating with HD males more than LD males. In contrast, in the absence of seismic signals, females did not appear to discriminate among males with and without foreleg ornamentation. However, when signals from the two modalities were allowed to interact, females mated more with males possessing ornamentation versus those lacking ornamentation. This study provides yet another example of a signaling system in which signal components interact, and further highlights the need for studies examining composite displays when exploring the evolution and function of complex signals (e.g. Basolo and Trainor 2002).

When presented with the transmission of only the seismic signal of a male's courtship display, female *R. rabida* were discriminating in their mating decisions, mating more often with larger, good condition males than smaller, poor condition males. Previous work on this species had established the importance of seismic signaling in *R. rabida* courtship, as mating frequencies were significantly reduced in the absence of seismic signal transmission (Wilgers and Hebets 2011). The importance of seismic signaling in mating success and/or in eliciting female receptivity is well established among numerous wolf spider species (Hebets and Uetz 1999; Uetz and Roberts 2002; Hebets et al. 2006; Hebets 2008; Rundus et al. 2011), and variation in male seismic displays has been found to influence female mate choice decisions in several species (Kotiaho et al. 1996;

Gibson and Uetz 2008; Rundus et al. 2011). In *R. rabida,* several body traits (size, weight) and seismic signal parameters (introductory segment, pulse train) are known to differ between males raised on high nutrient versus low nutrient diets (Wilgers and Hebets 2011). As trials in experiment 1 were run in complete darkness, it is presumably the differences in the seismic signal that females attend to when making mate choice decisions.

In contrast to variation in the seismic signal in isolation, variation in the presence/absence of visual foreleg ornamentation did not influence copulation frequency. Interestingly, this finding is not uncommon across studies of ornamented wolf spiders. Despite the presence of seemingly conspicuous visual ornamentation in numerous wolf spider species, and despite the frequently demonstrated condition-dependence of foreleg ornamentation (Uetz et al. 2002; Hebets et al. 2008; Shamble et al. 2009; Rundus et al. 2010; Wilgers and Hebets 2011), multiple studies have found no direct effect of male ornamentation on male mating success (Hebets et al. 2006; Shamble et al. 2009; Hebets et al. 2011; Rundus et al. 2011). In *S. stridulans*, although previous experiments suggested that male mating success was independent of foreleg ornamentation (Hebets 2008), it was recently discovered that foreleg ornamentation may influence male mating success through its interaction with courtship rate (Hebets et al. 2011). Similarly, in *R. rabida*, foreleg ornamentation appears to influence mating success through its interaction with seismic signaling.

In the presence of successful seismic signal transmission, the presence/absence of foreleg ornamentation does appear to influence copulation frequency - ornamented males achieved more matings than those lacking ornamentation in the presence of seismic signaling (Experiment 3). Production of the *R. rabida* courtship displays begins with an introductory seismic signal, prior to the movement of the ornamented foreleg (Wilgers pers. obs.), while the majority of the seismic display (*i.e.* the pulse train) occurs simultaneously with foreleg waves (Rovner 1968). Given the sequential production of seismic followed by seismic plus visual display components, it is possible that the seismic signal alerts females to the production of sequentially produced foreleg waves, ultimately altering their probability of detection (i.e. alerting) or enhancing their accuracy of assessment (i.e. attention-altering; Hebets and Papaj 2005) of the male's visual signal. Such a function is proposed for the acoustic call that precedes the leg wave in foot-flagging frogs, *Staurois guttatus* (Grafe and Wanger 2007). In the complex signaling environment of *R. rabida*, females are commonly found higher in the vegetation than males (Wilgers unpublished data), placing them out of visual contact from a stationary courting male. In such a scenario, females could be alerted to the presence of a courting male beneath them through the seismic signal, which could initiate a female's approach and further assessment. Detection of the seismic signal may also alter a female's attention, influencing the female's filtering mechanism to enhance male assessment and discrimination based on foreleg ornamentation. Additional cues in different sensory modalities have been shown to enhance cue discrimination and comprehension in humans (Sumby and Pollack 1954; Spence et al. 1998; Macaluso et al. 2000); and an attention-altering function has been suggested in the wolf spider *S. uetzi*, where the presence of a seismic signal similarly enhances discrimination of male foreleg ornamentation (Hebets 2005). In addition to these previously proposed efficacy-based interactions, the seismic signal might provide contextual information (e.g. species identification) used to help interpret variation in foreleg ornamentation. Placing stimuli into a mating context may help females focus on other aspects of the courtship display to further assess potential male quality. In snapping shrimp, *Alpheus heterochaelis*, a chemical signal identifies the sex of an approaching conspecific and mitigates an appropriate response to an open-chela display, which facilitates pairing or an agonistic defense of their burrow (Hughes 1996).

Ultimately, we show that while both the seismic signal and foreleg ornamentation influence female mate choice decisions, the seismic signal is integral in maintaining female choosiness. Females were able to discriminate males based on nutritional condition with seismic signals alone, but not with ornamentation alone. Instead females required the combination of a seismic signal, albeit with no differential information on male foraging history, along with foreleg ornamentation. The interaction between a static (i.e. foreleg ornamentation) and dynamic (i.e. seismic) courtship signal allows these components to fluctuate at different rates. While female integration and use of both modalities may provide females with a more complete history of a male’s foraging success, they may potentially be confronted with conflicting information over different temporal scales (Candolin 2003). In fact, static and dynamic traits have been found to influence a female’s ability to detect and discriminate among males in the guppy, *Poecilia reticulata* (Kodric-Brown and Nicoletto 2001). Future research should investigate how potentially conflicting information in *R. rabida* influences female mate choice decisions, which may provide insight into the window of male foraging history to which females pay the most attention.

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FIGURES

Figure 1. *Rabidosa rabida* female mate choice based on seismic signal alone. Mating trials were conducted in complete darkness, where males varied in diet history. Copulation frequencies significantly differed between male diet treatments (Likelihood ratio, $P = 0.01$).

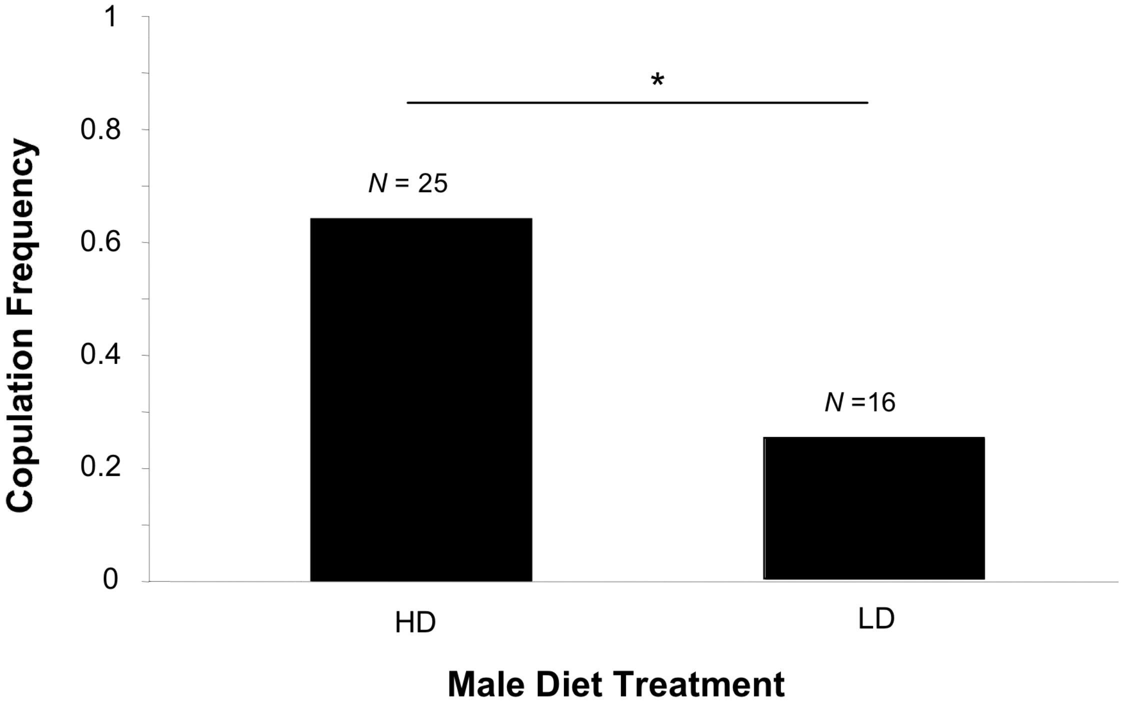
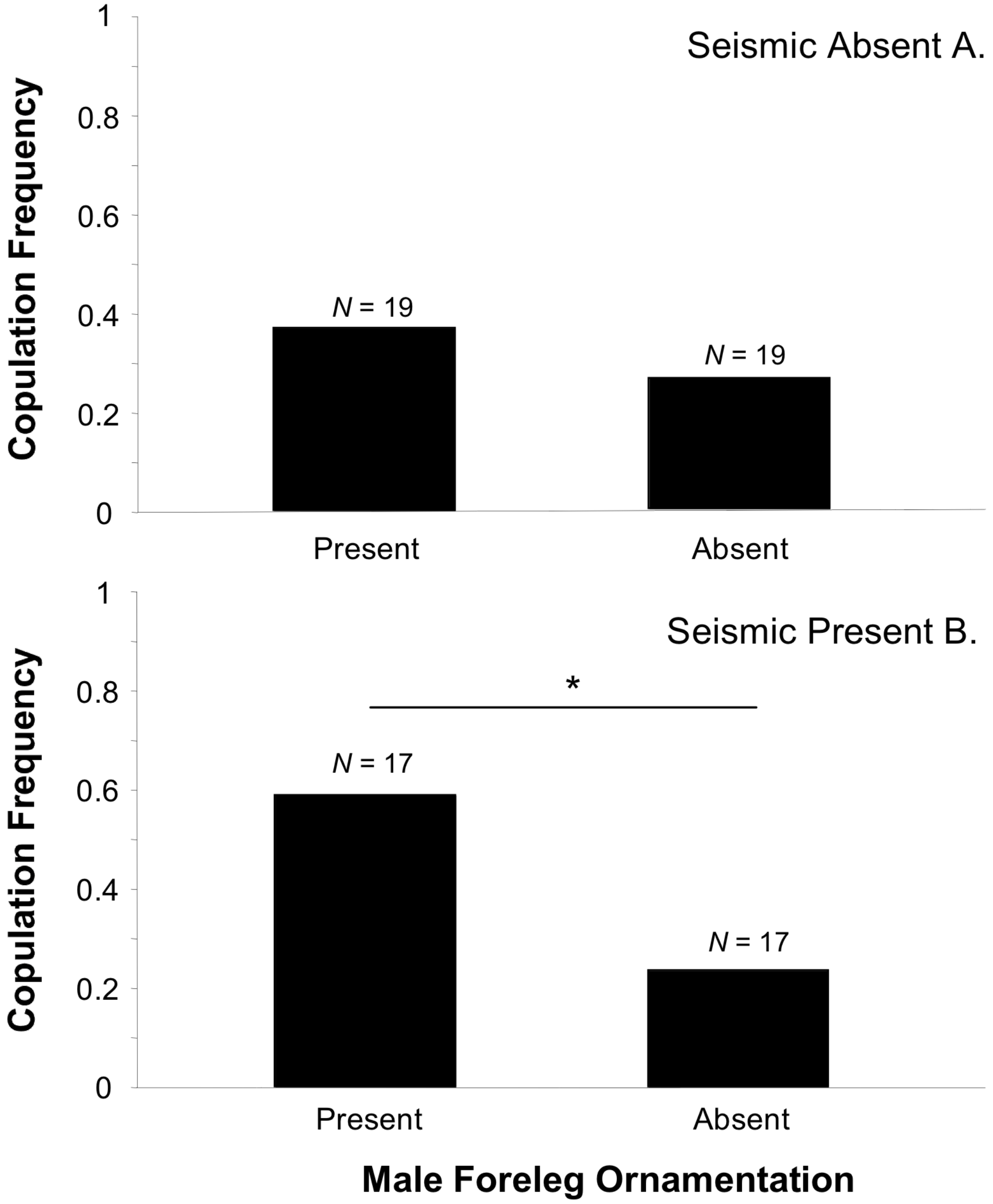


Figure 2. *Rabidosa rabida* female mate choice based on variation in foreleg ornamentation A) alone, or (B) in the presence of the seismic signal. Mating trials were conducted in the light on either on granite (seismic absent) or filter paper (seismic present). Copulation frequency depended on the foreleg ornamentation when the seismic signal was present (Likelihood ratio, $P = 0.03$) but not when the seismic signal was absent (Likelihood ratio, $P = 0.49$).



CHAPTER 4

FUNCTIONAL APPROACH TO CONDITION

Dustin J. Wilgers

ABSTRACT

Animal signals are commonly found to be condition-dependent, recognized as a positive correlation between signal expression and a proxy of individual condition. The level of expression of these condition-dependent signals has the potential to provide females with information on the male's ability to acquire and allocate resources, which is assumed to relate to his health, vigor and viability. Despite its widespread use and sizeable literature base, the term condition remains somewhat enigmatic. We begin this chapter with a broad discussion of 'condition', highlighting that it encompasses the resources used during development to create structures, the resources used in the normal functioning of an individual, as well as the resources currently available for allocation to various fitness-related functions. Since the pool of available resources is constantly changing, scientists typically focus on current energy reserves and estimate these using proxies – the most common of which is body condition. More detailed estimates of energy reserves are sometimes acquired through direct measures of carbohydrates, fats, and/or proteins. Numerous studies, incorporating a range of taxonomic groups, have demonstrated links between body condition and animal performance, with several examples relating to reproductive performance (*e.g.* courtship displays). Yet few of these studies examine either the details of available energy reserves or the genetic basis of body condition. Additionally, while significant evidence exists demonstrating that in systems with condition-dependent signaling, females prefer males with higher levels of signal expression, the link between these mate choice decisions and

female fitness benefits frequently remain elusive. We suggest that a more proximate approach will ultimately facilitate our understanding of the relevant sources of selection influencing the evolution of condition-dependent signaling. Specifically, we advocate for more of a focus on (i) the specifics of available energy reserves (*i.e.* carbohydrates, fats, and proteins), with a concentration on how they are utilized throughout an individual's life in relevant reproductive-related tasks, (ii) the genetic basis of resource acquisition and allocation, and (iii) the direct and indirect benefits females receive from mate decisions based upon condition-dependent signal expression.

INTRODUCTION

Animal signaling is commonly thought to be costly, as it is energetically expensive, may attract predators, etc. (reviews in Zuk and Kolluru 1998; Kotiaho 2001), and, the costs associated with signaling are predicted to increase with signal expression (*e.g.* size, amplitude, intensity; Johnstone 1997). Due to these costs, signaler condition, which is hypothesized to be a reflection of a signaler's genetic quality, is expected to influence the level of signal expression one can afford (Zahavi 1975), resulting in a positive correlation between signaler condition and signal expression – *i.e.* condition-dependent signaling (Zahavi 1977; West-Eberhard 1979; Andersson 1982; Nur and Hasson 1984; Zeh and Zeh 1988; von Schantz et al. 1999). In mating systems with condition-dependent courtship signaling, it is expected that females could indirectly assess a potential mate's quality by examining courtship signal expression, and such a scenario is predicted to result in stable female preferences for exaggerated male secondary sexual traits (Andersson 1986; Heywood 1989; Hoelzer 1989; Grafen 1990; Iwasa et al. 1991; Iwasa and Pomiankowski 1999).

Despite the wealth of theoretical and empirical work on condition-dependent signaling, the term condition itself is somewhat enigmatic. An individual's condition is a theoretical construct associated with the acquisition and allocation of nutritional resources. This acquisition and allocation of resources (*i.e.* condition) is assumed to be related to an individual's health, vigor, and viability (Andersson 1982; Nur and Hasson 1984; Zeh and Zeh 1988). Condition is often thought of as polygenic in nature, capturing much of the additive genetic variance responsible for viability spanning numerous loci across the genome (Andersson 1982; Rowe and Houle 1996); however, like other quantitative traits, condition is also influenced by the environment as well as by interactions between an individual's genotype and the environment (Hunt et al. 2004b).

A widely accepted working definition of condition is provided by Rowe and Houle (1996) - a pool of resources acquired from the environment, which is available for allocation to various fitness-related traits (see Figure 1). Under this definition, an individual's condition, which sums numerous processes throughout its lifespan, is constantly fluctuating, as resources are allocated to different functions, resulting in a reduced available resource pool until an individual is able to acquire new resources (Figure 1). This broad definition of condition incorporates information on the resources available throughout an individual's life - it encompasses the resources used during development to create structures (including those used for resource acquisition), the resources used in the normal functioning of an individual (*i.e.* its physiology), as well as the resources currently available in an individual’s energy stores (Figure 1). As mentioned previously, the quantity of available resources that can be allocated to each of these above-mentioned functions (*i.e.* condition in a broad sense) is constantly changing and is a product, in part, of a variety of traits and decisions influencing resource acquisition and resource allocation strategies (Figure 1). These traits and strategies responsible for resource acquisition and allocation are influenced by both an individual’s environment (*e.g.* presence/absence of predators, food abundance, etc.) and its genotype (*e.g.* heritable traits related to foraging ability, digestion, learning, etc.). In summary, condition encompasses the complex interactions between numerous factors, including those related to resource acquisition and allocation and their relationships with an individual’s genotype and environment (Figure 1), making it difficult, if not impossible, to quantify.

PRACTICAL APPROACHES TO CONDITION

Proxies of Current Energy Reserves

Scientists interested in condition-dependent signaling typically consider an individual’s current condition, or current energy stores; but even this narrower definition proves difficult to quantify. As such, proxies are commonly utilized, with the most frequent proxy being body condition. A variety of indices have been used to estimate body condition, or current energy reserves (see Table 1). Some studies use measures of over-all body weight, or measure the physical size of certain body parts that are assumed to be indicative of energy reserves (*e.g.* shape of abdomen in birds; Owren 1981). While potentially easy to quantify, these absolute measurements are confounded with body size, which may give little or no information about differences in current energy reserves (Piersma and Davidson 1991). Instead these measures could reflect larger quantities of non-energy related compounds (*e.g.* water, bone; Tomkins et al. 2004), which interestingly could instead be indicative of past body condition and resource allocation (*e.g.* to body size).

The vast majority of indices used attempt to control for body size by investigating the relationship between a dynamic body variable thought to represent energy reserves and a static, or less dynamic, estimate of overall body size (Jakob et al. 1996). Commonly, scientists use body weight or volume, measures that are known to change rapidly with resource acquisition, and control for body size using the length/width of skeletal (or exoskeletal in invertebrates) structures that are either fixed during certain life stages (*e.g.* thorax/cephalothorax fixed in between molts in insects/arachnids respectively), or remain effectively static over the time period of interest (e.g. limb/bone length in vertebrates). The methods used to control for body size in body condition indices vary tremendously, and are the topic of much debate (*e.g.* Jakob et al. 1996; Garcia-Berthou 2001). Here, we will simply highlight a few of the more common body condition indices and direct our readers to the relevant literature regarding the issues associated with each.

The simplest body condition index is the ratio index, which is calculated as body weight, or volume, divided by a linear measure of body size. Ratio indices provide a good descriptive index that is comparable across groups or populations and has been used consistently in the literature (Table 1). However, the ratio index is often not independent of body size, which limits conclusions on body condition alone (for criticisms see Blem 1984; Ranta et al. 1994; Jakob et al. 1996).

Additional methods to quantify body condition separate the effects of energy reserves and body size by incorporating size measures as a covariate in ANCOVA models, and analyzing variation in body weight or other dynamic measures (Garcia-Berthou 2001). Alternatively, researchers use the residuals from a linear regression of body weight against some linear measure of body size. The use of residuals has become increasingly common over the past two decades (reviewed in Green 2001), in part due to their straightforward interpretation: positive residuals indicate an individual in higher body condition than the average for that population, while negative residuals indicate a lower than average body condition (Jakob et al. 1996). Kotiaho (1999), however, cautions the interpretation of residual units due to relative differences across individuals varying in size (when untransformed) or to changing the allometric relationship between weight (estimate of fat content) and size (when transformed). The use of residuals also limits comparisons across heterogeneous groups (*e.g.* populations; Jakob et al. 1996), and depending on the study organism, the calculation of residuals likely violates key statistical assumptions (*e.g.* linear relationships, independence, etc; Green 2001). Ultimately, the choice of body condition index should reflect both realistic and biologically relevant specifics surrounding both the study and the organism. It is important to keep in mind that the decision as to which index to use is non-trivial, as results and conclusions often vary widely based on the chosen index (Bolger and Connolly 1989; Jakob et al. 1996; Moya-Larano et al. 2008).

Regardless of the index chosen, an individual's body condition is assumed to be a good proxy for the current resources available for allocation to fitness-related traits (see Condition and Animal Performance), and is often used as a proxy of individual fitness itself. None-the-less, more direct measurements relating to the specifics of an individual's energy reserves are possible and often preferable. For example, variation in an individual's resource storage across life stages and seasons, in it's reproductive stage, and in it's current behavioral activities will largely influence the types of energy stores in the body (Tomkins et al. 2004) - making the availability of certain types of energy reserves more or less important for critical resource allocation across different taxa and potentially across different life stages. Additionally, measures of different types of energy reserves do not necessarily correlate with one another and can even provide contradictory results (*e.g.* Blanckenhorn and Hosken 2003). As such, a more detailed approach incorporating the specifics of energy reserves may prove to be extremely important for our understanding of the evolution of condition-dependent signaling.

Acquisition of Energy Reserves

Condition is intricately tied to nutrition and the acquisition of resources. Individuals vary in the rates at which they can acquire resources, and the rate of nutrient intake is a function of numerous potentially interacting factors. To be more explicit, foraging rates may vary with environmental factors such as food availability, reproductive season, age, or predator abundances (among others), as well as with genetic factors at least partly responsible for feeding morphology, foraging behavior, or digestive efficiency (among others). Regardless of the underlying determinants of nutritional uptake, any variation among individuals will translate into differences in energy stores available for allocation to various fitness-enhancing traits and processes (i.e. body condition). Given that different animals, and even different developmental stages of a single animal, may have different nutritional requirements and may require different essential elements, the black box approach commonly used to estimate body condition provides little information as to the mechanisms underlying condition, and how they may differ across taxa (Lailvaux and Irschick 2006). Thus, a working knowledge of an organism's nutritional requirements and energy reserves across their lifespan seems invaluable for addressing questions of resource allocation tradeoffs and condition-dependent signaling.

Direct Measures of Current Energy Reserves

Newly acquired resources are processed and are either made available for relatively immediate use or are stored in some form. This pool of current energy resources is composed of a variety of compounds, all serving potentially different functions. Upon the intake of new resources, organisms convert the ingested organic matter into three main groups of compounds: carbohydrates, fats, and proteins.

Also known as saccharides, carbohydrates are organic compounds consisting of carbon, hydrogen, and oxygen. Monosaccharides like glucose are used to fuel metabolism, which may be important for energetically demanding behavioral displays or in highly active organisms. If not used immediately, these simple carbohydrates are converted to energy storage compounds, such as starch and glycogen. These polysaccharides are often stored in liver and muscle tissue and can be quickly mobilized as an energy source and used in either aerobic or anaerobic metabolism. The size and hydrophilic nature of glycogen imposes large constraints on the amount of energy able to be stored in this form, and thus a vast majority of excess carbohydrates are broken down to form acetyl-CoA, which eventually is used to synthesize more compact long-term energy storage, like fats (*i.e.* fatty acids, triglycerides).

Consisting of chains of carbon and hydrogen atoms with a carboxylic acid group at one end (fatty acids) bonded to a backbone structure, fats serve both metabolic and structural functions. They are likely an important energetic reserve for animals where feeding is limited for long periods of time. Importantly, fat stores can also be accumulated in one life stage for use in another. Additionally, fats are useful for delivering other important resources, such as carotenoids, which are known to perform a variety of physiological functions that benefit numerous systems, including nervous, digestive, endocrine, and more (reviewed in Olson and Owens 1998).

Proteins are chains of amino acids consisting of carbon, hydrogen, oxygen, nitrogen and other important atoms. The amino acids required for protein synthesis can either be consumed (*i.e.* essential amino acid) or be synthesized in the body (*i.e.* non-essential amino acid) from other compounds and elements, of which nitrogen is an essential element. Proteins serve important developmental functions. A number of amino acids, both essential (*e.g.* 10 common essential for animals) and non-essential (*e.g.* proline, asparagine) have been found to be necessary for normal development in a variety of animals (*e.g.* Eagle 1959; House 1961; Dadd 1978). Proteins also serve as another common long-term energy storage molecule. However, the efficiency and extent to which proteins can be effectively digested by certain taxa may vary (*e.g.* insects; Chapman 1998). Unlike fats, there is no specialized store for proteins, and thus energy must come from catabolism of both structural and functional organs (*e.g.* muscles, digestive organs) where excessive depletion can harm animal performance (Jenni and Jenni-Eiermann 1998). Because of this, the relative protein contribution to the energy budget is only about 5% in migrating birds (Jenni and Jenni-Eiermann 1998), and protein catabolism for metabolic energy is typically only utilized when other energy stores are depleted, (king penguins: Robin et al. 1988; green sea turtles: Jessop et al. 2004), suggesting that this energy store may be a last resort for some animals.

Given differences in their accessibility, their storage, and their potential use, detailed knowledge of the abundance of an individual's energy resources (*e.g.* quantification of carbohydrates, fats, and/or proteins), when they are stored, and how they are allocated to various life history traits may be critical for understanding targets of selection. While the proxies of body condition we highlight above (*Proxies of Current Energy Reserves*) have at times been found to be good predictors of specific energy reserves, such as fat content (Sibly et al. 1987; Schulte-Hostedde et al. 2001; Cattet et al. 2002; Ardia 2005; Schulte-Hostedde et al. 2005) variation in these proxies may also reflect variation in other compounds (*e.g.* water; Schulte-Hostedde et al. 2001; Schulte-Hostedde et al. 2005). Additionally, using proxies of body condition does not provide information about the ratios of specific energy reserves, which may be important for illuminating resource allocation trade-offs in particular animal groups. In a following section, we discuss the relationship between various measures of energy reserves (both body condition indices and direct measures of carbohydrates, fats, and proteins) and animal performance, highlighting studies in which detailed knowledge of energy stores has been crucial.

Manipulating Energy Reserves

An examination of the natural variation observed among individuals is often a first step towards determining whether a relationship exists between an individual's current condition, or current energy reserves, and its expression of signals and displays related to reproductive success (*i.e.* putatively sexually selected traits).

To do this, individuals are typically collected from the wild, where both their genotype and their environment (and interactions between the two) have presumably led to variation in current energy reserves. Upon collection, signal/display expression is quantified and correlated with some proxy of condition (*e.g.* body condition index, parasite load, etc.). Numerous studies have used this approach to correlate current proxies of condition and signal expression (*e.g.* Hoglund et al. 1992; Buchholz 1995; Thompson et al. 1997; Doucet and Montgomerie 2003). While such an approach is informative on some level, it provides no information about the relative influence of genotype versus environment. Thus, often in conjunction with field-based correlations between proxies of condition and signal expression, scientists employ more controlled environmental manipulations to experimentally alter current body condition and examine corresponding changes in signal expression.

Manipulating nutrition (quantity and quality), density, parasite load, temperature, etc. can lead to measurable differences among treatment groups in various proxies associated with condition (reviewed in Cotton et al. 2004). Regardless of the manipulation, treatments commonly represent two extremes (*e.g.* high vs. low nutrient levels, parasitized vs. unparasitized). Such an approach aims to generate a significant difference in the chosen proxy of current condition between the two groups, ultimately increasing the power to detect a relationship between current condition and signal expression. Using such extreme manipulations, however, removes much of the potentially relevant middle of the population distribution in phenotype response. As such, Cotton et al. (2004) suggest the use of a broader set of manipulations, providing a more comprehensive assessment of the relationship between various proxies of condition and signal expression. Unfortunately, increasing variation in the number, breadth, and timing of manipulations introduces problems associated with sample size and statistical power.

The majority of studies of condition-dependent signaling thus far have utilized phenotypic manipulations across a random sampling of individuals. Additionally, various studies have incorporated genetic controls across experimental treatments, using split brood/full sib designs (*e.g.* Kodric-Brown 1989; Houde and Torio 1992; Birkhead et al. 1998; Grether 2000). Such approaches can either swamp out or eliminate potential variation among individuals in underlying genetic quality, and thus do not allow variation in condition to manifest solely as a result of additive genetic variation among individuals (Cotton et al. 2004). Given that many hypotheses relating to the evolution of condition-dependent signal expression (*e.g.* various indicator mechanisms) assume phenotypic quality/viability (*i.e.* condition) to be heritable (*e.g.* Andersson 1982; Hamilton and Zuk 1982; Iwasa et al. 1991; Iwasa and Pomiankowski 1999), future studies are needed which focus more on the genetic basis of condition (Tomkins et al. 2004).

Surprisingly few studies of condition and animal signaling have incorporated genetics into their experimental design. To explicitly test for genetic effects on condition and corresponding signal expression, a few studies have either included discrete genetic lines as independent variables into the experimental design, or manipulated the genotype directly. Representation of distinct genotypes as independent variables in experiments can be done through either collecting individuals from distinct populations known to be reproductively isolated (reduced or no gene flow; *e.g.* Grether 2000), or through large-scale laboratory rearing of genetically distinct family lines and separating full siblings (or sometimes half siblings) between environmental stressor manipulations (*e.g.* Wagner and Hoback 1999). While lab-reared genetic lines are extremely work-intensive, this removes the assumption of independence between populations, which could be problematic in highly mobile species. However, caution should be taken with lab-reared genetic lines as well, as looking for genetic variance in traits associated with resource acquisition under lab conditions relaxes dependence on underlying genetic variance due to benign conditions (Hunt et al. 2004b), resulting in potentially different conclusions in the field versus in the lab (Hine et al. 2004). This issue magnifies the importance of selecting relevant biological manipulations (number and type) in attempting to more realistically simulate natural conditions. One interesting genetic manipulation is via inbreeding depression to induce genetic stress on individuals (Sheridan and Pomiankowski 1997; Van Oosterhout et al. 2003). Inbreeding depression has been suggested (but not explicitly tested) in the guppy, *Poecilia reticulata* to influence carotenoid signal expression and courtship activity through potentially impairing health or fitness of individuals carrying excessive loads of deleterious mutations (Van Oosterhout et al. 2003).

Studies aiming to experimentally manipulate condition and subsequently measure corresponding signal expression become increasingly complex by incorporating the realistic and relevant sources of variation (genetic and environmental) experienced by each organism in their natural environment. Complex studies incorporating genetic variation into their design, which are sorely needed, necessitate both large sample sizes, and study organisms that can be maintained in the laboratory. In the review by Cotton et al. (2004; Table 1), of the studies on condition-dependent sexual traits that incorporated a genetic component testing for an effect of genotype (not just a control), only 33% (3/9) of the species were vertebrates, with one species well-known in labs, the guppy, *Poecilia reticulata*. Sadly, this limits our complete understanding of condition-dependent signaling to a relatively small subset of systems that meet this requirement, leaving us to fill in the pieces that we can with less amenable animal groups.

CONDITION AND ANIMAL PERFORMANCE

Allocation of energy reserves - The pool of current energy reserves that an individual possesses is expected to directly relate to its ability to afford costly activities, such as the development of elaborate morphological characters and the production of behavioral displays that may enhance reproductive success, and/or the engagement in other behaviors and physiological processes that may enhance survival. As such, variation in this pool of resources should translate into variation in animal performance, where performance is defined as an organism's ability to conduct various ecologically relevant tasks related to survival (*e.g.* foraging, running speed, overall endurance, etc.) and/or reproduction (*e.g.* fighting ability, courtship displays, etc.; see Lailvaux and Irschick 2006; Irschick et al. 2008), the main focus of this chapter.

As discussed previously, body condition indices represent a holistic approach to measuring energy reserves, and are best considered as estimates of current overall energy reserves, reflecting variation in a variety of energy-related compounds (*i.e.* carbohydrates, fats, and protein) along with other compounds (*e.g.* water; Tomkins et al. 2004). This holistic approach to estimating energy reserves has been found to correlate with various fitness measures - individuals in better body condition survive better (*e.g.* Naef-Daenzer et al. 2001; Shine et al. 2001; Murray 2002; Morrison et al. 2007), and have overall higher reproductive success (*e.g.* Chastel et al. 1995; Dobson and Michener 1995; Otronen 1995; Wauters and Dhondt 1995).

In analyses involving more detailed examinations of energy reserves, direct links between available carbohydrates and animal performance have also been documented. For example, in the broadtailed hummingbird (*Selaphorus platycercus)*, 20–60 minutes after resuming feeding following a fast, individuals switch from primarily metabolizing fats, to oxidizing mainly carbohydrates (Welch et al. 2006). Similarly, up to 78% of the fuel required for hovering flight in the nectarivorous bat, *Glossophaga sorincina,* was shown to come from recently ingested carbohydrates (Welch et al. 2008). For animals that engage in energetically demanding behavioral displays, we might expect a direct link between display performance and exogenous sugar uptake, making the direct measurement of carbohydrates potentially extremely informative. In addition to behavioral displays, ingested carbohydrates are an important energy source for sex pheromone expression in the cockroach, *Nauphoeta cinerea*, which increases male attractiveness to females (South et al. 2011). Interestingly, when given a choice, males consumed diets with higher carbohydrate content, and this increased carbohydrate intake resulted in greater accumulations of lipids, which are known precursors of cockroach sex pheromone synthesis (South et al. 2011).

In addition to the immediate use of carbohydrates, glycogen stores are also known to be important in energetically demanding behavioral displays. For example, in the mosquito, *Anopheles feeborni*, males feed on nectar during the night, and store the acquired carbohydrates as glycogen for use during swarming flight the next day (Yuval et al. 1994). In fiddler crabs, males wave a single large claw during courtship displays to attract females. One of the primary stores of energy in decapod crustaceans is glycogen, which is readily mobilized into blood glucose for ATP synthesis during fights and leg-waving displays. Matsumasa and Murai (2005) found that males who waved their legs more frequently had higher lactate levels, a byproduct of glucose catabolism, in their bloodstream. Their results also suggest that variation in blood glucose levels (i.e. measure of condition) enhanced vigorous leg-waving activities, and that the increased levels of the lactate byproduct may be detrimental, resulting in reduced waving frequency. Glycogen is also a known energy source for frogs during metabolically demanding calling activity (Bevier 1997), while glycogen depletion was not found to correlate with calling rate (Schwartz et al. 1995; Bevier 1997), this could be due to males conserving energy to allow calling activity throughout the entire period of female activity (Schwartz et al. 1995) or the combined use of another known energy substrate for frogs, lipids (Bevier 1997).

With respect to fat reserves, large stores of body fat are frequently accumulated prior to instances requiring long periods of fasting (*e.g.* hibernation, reproduction), and evidence across taxa indicate both survival and fecundity increase with fat reserves (Elowe and Dodge 1989; Atkinson and Ramsay 1995; Vleck and Vleck 2002). Additionally, fat stores accumulated during early developmental stages have been found to dramatically influence subsequent life-stages. For example, accumulation of fat stores by juveniles has been found to be crucial for terrestrial survival post-metamorphosis in amphibians (Scott et al. 2007) and for reproductive success in damselflies (Plaistow and Siva-Jothy 1996). Studies on birds also provide evidence that body condition during juvenile stages can have dramatic affects on adult body condition (*e.g.* fat reserves), and that these fat reserves are extremely important for survival during strenuous activities, such as migration (Merila and Svensson 1997). Given the above-demonstrated relationships between early life fat storage and later life performance, fats provide an excellent example of the need for a more inclusive (incorporating more than simply current body condition indices) and detailed (quantifying fats vs. carbohydrates or proteins) examination of an animal’s energy reserves. In birds, flight is the most energetically demanding activity per unit time (Blem 2000). Long-distance migrations require tremendous energetic reserves from multiple sources (*e.g.* glycogen, fats, proteins). In several species, when fat reserves are near depletion (< 5-10%), protein catabolism increases (Schwilch et al. 2002), primarily in the breast and leg muscles (Bauchinger and Biebach 2001). However, the relative utilization of these compounds differs across birds and has been found to be a function of their diet (Gannes 2001), suggesting a direct link between the types of resources acquired and those used during performance.

Proteins are commonly used in the development of a variety of structures, including bird feathers, which are produced by keratins. In dark-eyed juncos, *Junco hyemalis*, on protein-enriched diets, birds had faster feather growth rates along with larger and brighter white plumage ornaments on the tail, which are produced through structural coloration, suggesting the intake of specific energetic compounds (i.e. diet quality) can result in condition-dependent expression (McGlothlin et al. 2007). Similar results have been found in house sparrows, *Passer domesticus*, where male house sparrows on protein-enriched diets have large white wing bars (Poston et al. 2005). In addition, male house sparrows had brighter (i.e. not as black) but not bigger melanin-based black bibs when fed diets lacking melanin-precursors compared to males fed normal diets (Poston et al. 2005).

Trade-offs in Resource Allocation – The allocation of resources from a finite pool (although the pool is rarely finite as currently available resources are frequently used for addition nutrient acquisition – *e.g.* to sustain foraging) is expected to result in trade-offs in resource allocation, such that allocating resources to one trait reduces the available resources for allocation to other traits (Rowe and Houle 1996; Zera and Harshman 2001). An individual’s optimal resource allocation among traits aims to maximize overall fitness and depends on both the strength of selection from a variety of sources, as well as the amount of available resources (Rowe & Houle 1996). For example, in reproductive systems exemplified by strong female choice, signalers might maximize fitness by allocating greater resources to secondary sexual traits (*e.g.* ornamentation, displays, etc.), or other traits under selection via females, thereby gaining increased reproductive success, even at a cost to other life history traits, such as potentially decreased longevity. For example, male *Hygrolycosa rubrofasciata* wolf spiders engage in intense sexual displays by drumming the substrate with their abdomens. Drumming has been shown to be both condition-dependent and energetically expensive - drumming males increase their metabolic rate 22-fold over resting rates (Kotiaho et al. 1998). Males vary naturally in their drumming rates and females prefer to mate with males that produce high drumming rates (Kotiaho et al. 1996). Mappes et al. (1996) investigated the tradeoff between this costly display and viability by inducing some males to court at higher rates. Males induced to court at higher rates suffered greater mortality and lost more weight over the trial than did males that courted at lower rates, suggesting that allocation of energy reserves to courtship reduced their availability for allocation to survival. Additionally, within the high courting group, males that maintained high courtship levels survived better, suggesting variation in an individual’s ability to afford the high energetic costs associated with the display. This tradeoff has also been found in the field cricket, *Teleogryllus commodus*, in which high body condition males invest so many resources to their sexual displays that they tend to die younger than lower condition males (Hunt et al. 2004a). However, for individuals with fewer energy reserves, it might be necessary to allocate more resources to basic survival requirements, making investment in reproductive traits relatively more costly. Individuals in good body condition have greater resource pools and thus are expected to be able to afford greater levels of reproductive trait expression while simultaneously experiencing greater viability (*e.g.* immunocompetence, survival; Jennions et al. 2001). However, as increased body condition corresponds with larger resource pools, even traits not under sexual selection are expected to increase with the size of the resource pools – making it imperative to examine the degree of condition-dependence (*e.g.* the rate at which traits change) between those traits that are or are not under sexual selection (Cotton et al. 2004). It is important to note, however, that condition-dependent signal expression need not imply current sexual selection and vice versa (*i.e.* a lack of condition-dependent signal expression need not imply a current lack of sexual selection).

Signaling systems in which there is a working knowledge of the resources utilized during costly signal expression, and how these resources are used in other fitness-related functions, can facilitate our understanding of the details surrounding the selection and subsequent evolution of condition-dependent signaling. To illustrate this, we turn to carotenoids. Carotenoid pigments (e.g.

carotenes, xanthophylls) are resources that cannot be synthesized by animals; instead they must be acquired through consumption of carotenoid rich food items (often plants, algae). Carotenoids are important in immune system function, serving as antioxidants and free-radical absorbers that generally boost the immunity of individuals and potentially reduce the risks of cancer and parasitism (reviews in Lozano 1994; Shykoff and Widmer 1996; Lozano 2001). They have also been found to be a photo-protectant, and to benefit various tissues (*e.g.* eye) by absorbing harmful short-wavelengths (example in Japanese Quail; Thomson et al. 2002). Carotenoid levels in the body are limited, and traits or processes using carotenoids are dependent on an individual's foraging ability and assimilation efficiency, both of which are known to vary (Hill 2002; McGraw 2006a). Given that carotenoids can be a limiting resource (*e.g.* if carotenoid-rich food is limiting in the environment; Grether et al. 1999), there are clear benefits to individuals to allocate carotenoid resources to non-signaling functions. None-the-less, carotenoid-based coloration is found in numerous taxa (*e.g.* birds, fish, amphibians, reptiles, insects, mollusks, crustaceans; Matsuno 2001; McGraw et al. 2005), producing some of the most brilliant coloration in the animal kingdom (e.g. yellows, reds, oranges, greens, purples, blues; Olson and Owens 1998).

Studies relating to the evolution of condition-dependent sexual signals incorporating carotenoids are numerous and encompass a substantial portion of the literature on condition-dependent sexual signaling (Cotton et al. 2004; McGraw 2006a). The general hypothesis is that only those individuals in good nutritional condition (*i.e.* high reserves of carotenoids) and of overall good general health can afford to allocate greater resources to a colorful display (*e.g.* (McGraw and Hill 2000; McGraw and Ardia 2003; Saks et al. 2003; Mougeot et al. 2007). In mating contexts, females that pay attention to the size or intensity/saturation of the carotenoid coloration may receive some information about relative male quality, and should favor those males with larger or brighter carotenoid displays. In fact, this is what we see in a variety of taxa: females predominantly prefer brighter males with more coloration (*e.g.* guppies: Endler 1983; house finches: Hill 1990; three-spined sticklebacks: Bakker and Mundwiler 1994). By understanding the physiology underlying signal expression, we gain further insights into traits that compete for limited resources within the body and the precise tradeoffs that result. Carotenoids provide just one such example, but others certainly exist (*e.g.* melanins; McGraw 2006b).

As we hope to have highlighted above, knowledge of essential nutrients and their potential allocation could lead to elegant hypotheses regarding traits under strong sexual selection. As condition is influenced by both the genotype and the environment, we might expect, for example, that females pay more attention to traits that incorporate essential, but hard to obtain resources, which may reflect aspects of male quality rather than their environment. Such a hypothesis was tested by Grether (2000) on carotenoid coloration in the guppy, *Poecilia reticulata*, across a gradient of carotenoid availabilities. They found no support for female preferences corresponding with nutrient limitation. However, other limiting nutrients or energetic compounds may provide support for this proximate hypothesis explaining female preferences for condition-dependent male displays.

Time Scales for Resource Allocation – Prior to turning our attention to potential benefits of attending to condition-dependent signals, we would like to highlight again the dynamic nature of resource allocation (see Figure 1) and the potential for individuals to allocate resources differently over time. It is imperative to remember that the condition-dependence of signals involved in reproductive behavior can reflect the pool of available resources at different time scales (Johnstone 1995), making current measures of body condition of questionable relevance in certain situations. For example, condition-dependent morphological traits associated with signaling can potentially provide receivers with information about the resource pool available to an individual throughout the development of a particular structure (*e.g.* deer antlers; Clutton-Brock et al. 1982; Suttie and Kay 1983; horns in beetles: Emlen 1994; spider coloration; Shamble et al. 2009; Rundus et al. 2011; Taylor et al. 2011). Alternatively, morphological traits such as carotenoid coloration may reflect more recent, or even current resource pools (*e.g.* Grether 2000; Rosen and Tarvin 2006). To complicate matters further, many behavioral displays (e.g. acoustic and vibratory song, body movements) require intense motor performance, which is known to raise metabolic rates considerably, and thus require substantial immediate energy reserves (putatively reflected in current measures of body condition). Such elaborate displays may require complex structures (*e.g.* muscles) and motor skills acquired during development (Byers et al. 2010), and thus reflect resource pools available at earlier life stages. Ultimately, the production of costly displays can provide information about both current energy reserves (Mappes et al. 1996; Hoefler et al. 2008) as well as energy reserves available during development (Nowicki et al. 2002); thus, knowing the relative influence of each may be important for understanding the selection pressures associated with condition-dependent signal evolution.

CONDITION AND MATE CHOICE

Thus far, this chapter has been focused upon the relationship between signaler condition and signal expression. Generally, higher levels of signal expression are greater afforded by individuals in better condition; and numerous studies incorporating a variety of taxonomic groups have found signal expression to correlate strongly with various proxies of a signaler's body condition – making these signals condition-dependent. We have also discussed how allocating resources to signaling frequently comes at the expense of allocating resources to other traits associated with survival (*e.g.* Blount 2004; Hunt et al. 2004a; Guerra and Pollack 2007). These costs raise questions about the selection pressures responsible for the evolution and maintenance of condition-dependent signaling. Selection imposed by choosy females (*i.e.* female choice) is frequently touted as an explanation for the evolution of condition-dependent signaling (Bondurianski 2007). There is consistent evidence that females prefer to mate with males that exhibit displays that are more elaborate (e.g. larger, brighter, louder, etc.; reviews in Andersson 1994; Johnstone 1995; but see Griffith et al. 1999; Lebas and Marshall 2001; Shamble et al. 2009 for examples of no evidence of female choice). For these choosy females, mate choice can be costly (e.g. Alatalo et al. 1988; Rowe 1994), and thus, females attending to information in male condition-dependent courtship displays must receive some benefit(s) via their preferences in order to counter the costs associated with being choosy. The next section of this chapter will focus upon the direct and indirect benefits female might gain by using condition-dependent signal expression to direct their mate choice decisions.

Choosy females mating with preferred males could gain increased fitness benefits directly through increased paternal care, increased nutritional resources, better quality territories, or reduced parasitism risk (among others). Such direct benefits from chosen males are expected to reflect their energy reserves and resource allocation – with preferred males being able to allocate more resources to reproductive traits. This creates a clear prediction – there should be a positive correlation between a signaler's condition and the direct benefits conveyed to females. Indeed, condition-dependent signals have been found to correlate with paternal care (*e.g.* birds; Hill 1991; Senar et al. 2002), with the quantity of sperm transferred (*e.g.* guppies; Matthews et al. 1997; crickets; Wagner and Harper 2003) and with the production of nutritional benefits, such as spermatophores (*e.g.* crickets; Wagner and Harper 2003) and nuptial gifts (*e.g.* fireflies; Crastley 2004). The frequent ability to quantify the direct benefits females receive and to then relate them to proxies of signaler condition makes direct benefits a compelling, and readily testable, hypothesis regarding condition-dependent female mate choice.

Choosy females are also expected to benefit indirectly if preferred mates pass ‘good genes’ to their offspring that increase their fitness. Evidence for underlying heritable additive genetic variance for body condition is slowly accumulating (Merila 1996; Merila and Svensson 1997; Sheldon et al. 1997; Grether 2000; Kotiaho et al. 2001; Merila et al. 2001; Blanckenhorn and Hosken 2003), as are examples demonstrating that condition-dependent signals covary with genetic variance (*i.e.* genic capture; e.g. David et al. 2000; Brandt and Greenfield 2004; Parker and Garant 2004; Missoweit et al. 2008). Additionally, there is some evidence that the additive genetic variance in sexually selected traits results in viability benefits to offspring (Moller and Alatalo 1999), although the effects may be relatively minor (Alatalo et al. 1998; Moller and Alatalo 1999). Regardless of the magnitude, offspring from highly ornamented males in good body condition have been found to experience benefits in a variety of fitness-related traits, where offspring feed at higher rates (tree frogs: Doty and Welch 2001) survive better (guppies: Evans et al. 2004), are in better body condition (collared flycatchers: Sheldon et al. 1997), have increased resistance to parasites (sticklebacks: Barber et al. 2001), and have overall better performance during development (tree frogs: Welch et al. 1998). However, these measurements fall short of evidencing true fitness benefits to females, which should be measured minimally by the number of offspring that each offspring produces (*i.e.* grandchildren; Hunt et al. 2004b).

Similar to the previously highlighted variation in proxies used for estimating male condition (see Table 1), scientists also use a variety of different proxies for estimating female and offspring fitness, including condition itself, making it difficult to obtain an over-all picture of the evidence for female's receiving fitness benefits from condition-dependent mate choice decisions. Furthermore, while examples do exist that suggest benefits, both direct and indirect, to females for condition-dependent mate choice, the evidence is surprisingly sparse (especially for indirect benefits) and the current viewpoint may be exaggerated due to publication bias towards positive results (Kotiaho and Puurtinen 2007). We suggest that the relative scarcity of examples may reflect an insufficient approach to condition-dependent signal evolution. For example, proxies of current body condition may not accurately reflect resources relevant to female fitness, as it may be the case that the details about energy reserves that are important to females and/or reflective of energy reserves available at a different time scale (*e.g.* during juvenile development). Additionally, the relative influences of the environmental component, additive genetic variance, and their interactions on body condition and corresponding offspring fitness needs to be more firmly established, as signal reliability especially in condition-dependent signaling systems with proposed indirect benefits are hypothesized to be compromised (Greenfield and Rodriguez 2004; Hunt et al. 2004b). As we hope to have highlighted throughout, we advocate for a more proximate, functional approach to understanding condition-dependent signal evolution.

SUMMARY

Evidence of condition-dependent signals is ubiquitous. Such signals are hypothesized to be the result of selection via choosy females and are thought to have had important effects on the evolution of animal communication systems (Iwasa et al. 1991). However, the ambiguous nature of condition makes it currently un-measurable, causing scientists to rely on brief snapshots of body condition to estimate an individual's current energetic reserves. The variety of proxies used to estimate body condition both across and within taxa has allowed considerable progress in advancing our understanding of ultimate explanations of animal signaling. However, using proxies of body condition as a proxy for fitness may cloud this relationship, making patterns enigmatic, thus we feel a more proximate approach is now in order. We argue that a focus on the physiological basis of condition, on the processes underlying resource allocation, and on the relationship between these and measures of whole organism performance will provide a more complete understanding of underlying mechanisms resulting in a signal's condition-dependence (Lailvaux and Irschick 2006). Additionally, more proximate approaches to understanding the relationship between condition-dependent signal expression and female mating decisions, and the putative associated fitness benefits, will improve our understanding of selection pressures that might influence the evolution of condition-dependent signaling. Focusing on more proximate physiological underpinnings of what condition means and how it directly influences signal expression and female fitness benefits lays the

foundation for future comparisons across taxa that share similar mechanisms, which may illuminate interesting broad scale patterns.

Table 1. Examples of proxies used to estimate body condition across taxa

Proxy	Measure	Group	Citation
Estimates of Reserves via Absolute Body Measures			
Body Size	Wing Size	Insects	Hooper et al. 1999, David et al. 2000, Blanckenhorn & Hosken 2003, Van Homrigh et al. 2007
	Shape of Abdomen	Birds	Owren 1981
	Pectoral Muscle Size	Birds	Perez-Rodriguez et al. 2006
Mass		Birds	Gonzalez et al. 1999, Hill 2000, McGraw & Hill 2000, Johnsen et al. 2003, Perez-Rodriguez et al. 2006
		Insects	Kotiaho et al. 2001, Kotiaho 2002, Rantala et. al. 2003, Scheuber et al. 2003a
		Spiders	Mappes et al. 1996, Rundus et al. 2011
Volume		Birds	Sibly et al. 1987
Growth Rate		Birds	Zuk et al. 1990, Keyser & Hill 1999
Trait Assymetry		Birds	McGraw et al. 2002
Estimates of Reserves Controlling for Body Size			
Relative Weight		Fish	Neumann & Flammang 1997
Density	Weight / Volume	Spiders	Moya-Larano et al. 2008
Ratio	Weight / Fixed Body Measure	Amphibians	Arntzen et al. 1999
		Fish	Tonn et al. 1989, Greenstreet 1992, Candolin 2000
		Lizards	van Berkum et al. 1989, van Marken Lichtenbelt et al. 1993

Table 1. Continued.

Proxy	Measure	Group	Citation
		Birds	Moller 1987, Evans & McMahon 1987,
		Spiders	Shamble et al. 2009, Wilgers & Hebets 2011
	Dynamic Body Part / Static Body Part	Spiders	Anderson 1974, Watson 1990, Jakob 1991
Slope Adjusted Ratio	Weight / (Body Size)^slope[1]	Fish	Kulling & Milinski 1992, Nicoletto 1993
		Insects	Pierce et al. 1985, Juliano 1986, Baker 1989
Residual	Weight / Body Size	Birds	Andersson 1992, Carranza & Hidalgo 1993, Hamer & Furness 1993, Schluter & Gustafsson 1993, Veiga 1993, Qvanstrom 1999, Weatherhead et al. 1999, Merila et al. 2001, Moller & Petrie 2002, Doucet & Montgomerie 2003, Sarasola et al. 2004, Ardia 2005 Bize et al. 2006
		Crustaceans	Jennions & Backwell 1998
		Insects	Marden & Rollins 1994, Wagner & Hoback 1999, Gray & Eckhardt 2001, Holzer et al. 2003, Scheuber et al. 2003b
		Mammals	Dobson 1992, Dobson & Michener 1995, Woodroffe 1995, Dobson et. al 1999, Fisher 1999, Schulte-Hostedde 2001, Blackwell 2002
		Amphibians	Murphy 1994, Judge & Brooks 2001,
		Reptiles	Dunlap & Mathies 1993, Weatherhead et al. 1995, Keller et al. 1997, Cuadrado 1998, Shine et al. 2001
		Spiders	Uetz et al. 2002, Hoefler et al. 2008, Wilder & Rypstra 2008, Lomborg & Toft 2009, Wilgers et al. 2009, Taylor et al. 2011
ANCOVA	Weight with Body Size Covariate	Birds	Torok et al. 2003, Parker & Grant 2004
		Spiders	Lomborg & Toft 2009, Wilder & Rypstra 2008
PCA	Factor Loading with Size and Mass	Birds	Bize et al. 2006

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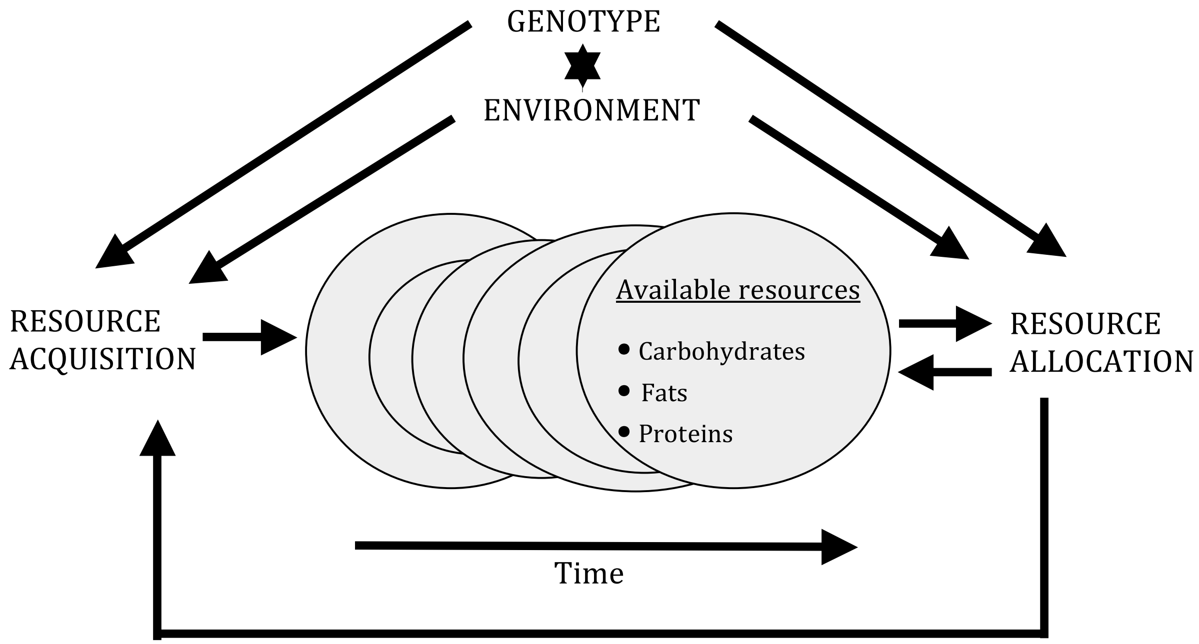
FIGURES

Figure 1. A diagrammatic representation of the complex interactions between an individual's genotype and environment and their interactions with resource acquisition, available resource pool, and resource allocation. Resource acquisition and/or resource allocation can vary over the lifespan of individuals, resulting in different sizes of resource pools (i.e. condition) over time.



SYNTHESIS AND FUTURE DIRECTIONS

Male *Rabidosa rabida* use complex courtship displays directed at choosy and cannibalistic females in order to gain reproductive success. From these studies presented in this thesis, it is clear that two of the sources of selection on these displays (female choice, environment) are extremely variable, and result in mating patterns that are just as complex as the displays used during these interactions.

In Chapter 1, we found female mate choice decisions vary with female body condition and age. Specifically, we suggest that younger good condition females are likely the strongest source of selection on males, and these females were choosy based on differences in male body condition. In Chapter 4 we suggest body condition may provide important information regarding fitness benefits conveyed to females. In Chapter 2, we provided evidence that multiple male courtship signal components may provide females with information on body condition. Both signal modalities were found to be used in female mating decisions, and while the seismic signal appears to be more important, copulation success is maintained when either signal modality is detected in isolation. Given the complex and variable daytimes and environments that *R. rabida* mating interactions occur in, these potential content- and efficacy-backups could help maintain reproductive success regardless of when are where males and females interact. While reproductive success for males may be maintained regardless of mating circumstance, in Chapter 3, I show that the seismic signal is integral to maintain female choosiness. Female assessment of male variation in both the seismic signal and ornamentation only occurs when females are able to detect a seismic signal, regardless of whether the seismic signal has any additional information on male condition or not. Thus, the complex courtship displays of male *R. rabida* appear to maintain both copulation success and female assessment across a variety of circumstances encountered during mating interactions, which likely favored the evolution of signal complexity.

This thesis has been largely focused on the selection acting on male courtship displays. While this approach has provided considerable insight into this system, it largely ignores selection on females to maintain their mating preferences. While we show that male body condition influences female mate choice decisions, and we discussed how various costs associated intrinsic factors have likely influenced mating decisions, we were unable to measure any correlated benefits associated with female choice. Future studies will be focused on a more functional approach to condition suggested in Chapter 4, and how that corresponds with direct or indirect benefits to females. This future work will provide considerable insight into the maintenance of female preferences for male displays.

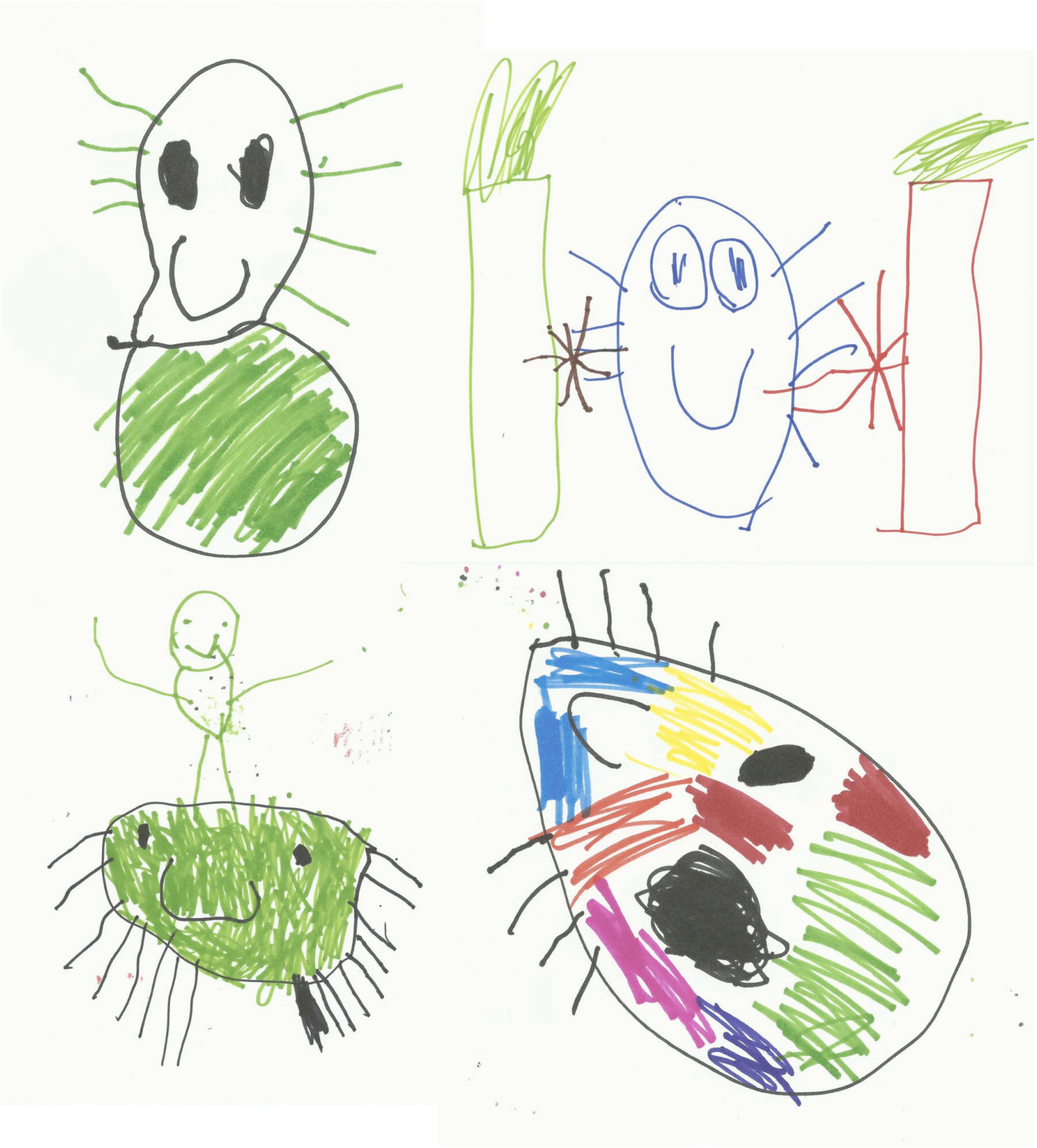
APPENDIX – **A collection of my favorite artwork by Noah done in my office.**

Drawing 1. Darwin with a finch on the HMS Beagle in the Galapagos Islands.

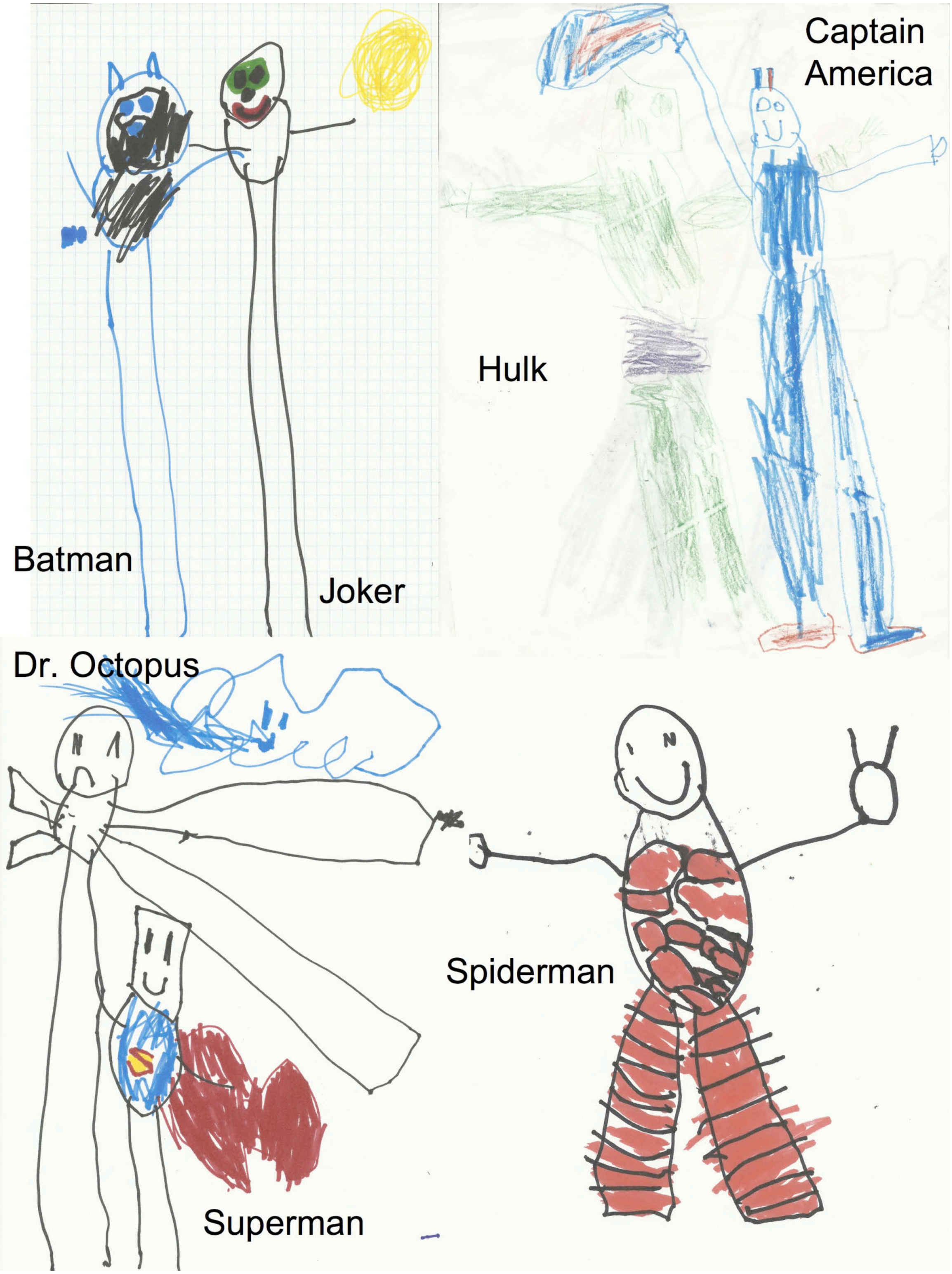
Drawing 2. Noah depicts Hominid evolution.

Drawing 3. Dad at work with his coffee.

Drawing 4. Various spiders drawings.



Drawing 5. A collage of Noah's favorite Super Heroes.



Drawing 6. A pirate finds Treasure Island.