

3-2015

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Rosenthal, Malcolm F. and Hebets, Eileen A., "Temporal patterns of nutrition dependence in secondary sexual traits and their varying impacts on male mating success" (2015). *Eileen Hebets Publications*. 68.

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Temporal patterns of nutrition dependence in secondary sexual traits and their varying impacts on male mating success

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Abstract

Variation in the quantity of nutrients ingested over an individual's lifetime is likely to differentially affect distinct male secondary sexual traits and courtship signals, potentially providing females with information about a male's past and present foraging history. We hypothesize that female choice is thus influenced by a male's lifetime foraging history. To test this, we manipulated the quantity of nutrients (i.e. prey items) available to male wolf spiders, *Schizocosa stridulans*, using a fully crossed 2×2 design with low versus high prey quantity across juvenile and adult life stages, and assessed the impact of these diet treatments on male foreleg pigmentation, courtship rate and mating success. We found foreleg pigmentation to be dependent upon both juvenile and adult diet, with increased nutrition dependence of pigmented versus unpigmented leg segments. Despite this, the degree of foreleg pigmentation did not predict mating success. In contrast, courtship rate was not nutrient dependent, yet strongly predicted mating success. Finally, we found a significant interaction between juvenile diet, adult diet and courtship rate on mating success. Males that experienced a diet switch (low juvenile to high adult, LH; high juvenile to low adult, HL) exhibited no relationship between courtship rate and mating success, while those that experienced a consistent diet (LL; HH) showed increased mating success with increased courtship rates. Our results suggest that nutrition dependence of secondary sexual traits is not necessarily a predictor of their role in mating success and that female mate choice is the result of complex interactions between multiple male traits.

Keywords: body condition courtship display lifetime foraging history mate choice mating success nutrition-dependent trait *Schizocosa stridulans* secondary sexual trait wolf spider

Within a single season, individuals may experience rapid shifts in food availability. For males, especially those with multicomponent (or multimodal) mating displays, this variability may have serious implications on future mating success. Not only can components of a male's courtship display vary in their sensitivity to changes in food intake, but the timescales over which these components respond can also be variable. Because of this, multicomponent courtship displays have the potential to convey information concerning the nutritional history of an individual over a variety of timescales. Understanding how such fluctuations are reflected in male secondary sexual traits and how these traits, in turn, influence mating success is therefore of significant interest for sexual selection and communication research.

Shifts in food availability can occur for many reasons. For example, the density of small arthropods, a common prey type for numerous predators, is affected by rainfall (Shultz, Lensing, & Wise, 2006; Staley et al., 2007) and temperature (Bale et al., 2002; Pearce-Higgins, Dennis, Whittingham, & Yalden, 2010), both of which may change unexpectedly. Additionally, for ectothermic predators,

rate (and potentially efficiency) of foraging is positively correlated with temperature (Kruse, Toft, & Sunderland, 2008). Therefore, short-term temperature fluctuations may affect foraging success even if they do not directly affect prey density. Evidence supports the notion that the frequency of extreme environmental or climatic fluctuations, as well as the degree of variability in seasonal weather, is increasing (Coumou & Rahmstorf, 2012; Easterling et al., 2000; Schär et al., 2004; Yeh et al., 2009), suggesting that the likelihood that populations will experience unexpected or stochastic fluctuations in prey availability may also be on the rise, further emphasizing our need to understand the effect of variability in food availability on sexual signaling systems.

Fluctuations in diet can have various effects on male mating displays, depending on when and if display components are diet sensitive. On the one hand, morphological traits are often mostly impacted by juvenile diet. Many characters become fixed after production (e.g. avian plumage) or after maturation, but nutrition intake prior to maturation can affect the resources available to produce pigmentation or ornamentation (Ohlsson, Smith, Raberg, &

Hasselquist, 2002; Taylor, Clark, & McGraw, 2011; Uetz, Papke, & Kilinc, 2002). On the other hand, energetically demanding behaviors such as elaborate courtship displays may be more strongly affected by adult (or current) diet, which has an impact on available energetic resources (Brown, 2011; Droney, 1996, 1998; Dussourd, Harvis, Meiwald, & Eisner, 1991; Kolluru & Grether, 2004; Wedell, 1994). However, these trends in dietary life-history effects on behavioral and morphological traits are far from universal. Juvenile diet may affect adult behavior by having an impact on neurological development (Nowicki, Hasselquist, Bensch, & Peters, 2000; e.g. the developmental stress hypothesis, reviewed in Spencer & MacDougall-Shackleton, 2011) or by influencing the development of morphological traits associated with the production of the behavioral display (e.g. Scheuber, Jacot, & Brinkhof, 2003). Similarly, morphological traits such as ornaments can be affected by current diet if they require maintenance such as preening or cleaning (e.g. Griggio, Hoi, & Pilastro, 2010), or if they contain vascularized tissue (e.g. goldfinch bill color, Rosenthal, Murphy, Darling, & Tarvin, 2012; booby foot color, Torres & Velando, 2003). Traits that reflect a male's dietary life history can provide discriminating females with information about his quality as a mate. In fact, female preferences for honest displays may lead to the evolution of signal components that are more sensitive to changes in condition (i.e. heightened condition dependence; see Grafen, 1990; Iwasa & Pomiankowski, 1994; Pomiankowski, 1987). In complex courtship displays that encompass multiple distinct components (e.g. morphological traits and dynamic movement displays), one might predict different display components to be influenced by foraging rates at different life stages, thus revealing different patterns of condition dependence, potentially providing females with information about the lifetime foraging history of a male.

We test the hypothesis that distinct components of the complex courtship display of *Schizocosa stridulans* wolf spiders demonstrate different temporal patterns of nutrient dependence and that variable nutrient intake across life stages will influence a male's reproductive success. *Schizocosa* wolf spiders provide an excellent system for examining temporal patterns of nutrient-dependent secondary sexual traits and their impact on male mating success, as males exhibit a wide range of multicomponent and multimodal courtship behaviors (reviewed in Hebets, Vink, Sullivan-Beckers, & Rosenthal, 2013). Complex courtship displays often comprise pigmented legs that are waved or tapped to produce dynamic visual displays. The foreleg pigmentation is absent prior to a male's maturation and, in some species, is influenced by juvenile diet (*Schizocosa ocreata*: Hebets, Wesson, & Shamble, 2008; Uetz et al., 2002; *Schizocosa uetzi*: Shamble, Wilgers, Swoboda, & Hebets, 2009; *Schizocosa floridana*: Rosenthal & Hebets, 2012). In addition to foreleg pigmentation, males also produce a substrate-transmitted acoustic "song," which is usually both necessary and sufficient to elicit copulations (for review, see Hebets et al., 2013; but see Stafstrom & Hebets, 2013). Female mate choice behavior in wolf spiders is as complex as the male's display. For example, in *Rabidosa rabida*, female mating decisions may be age or condition dependent (Wilgers & Hebets, 2012), and in both *Rabidosa* and *Schizocosa* species, the visual and vibratory components of the male's display can interact to affect the likelihood of copulation (Hebets, Stafstrom, Rodriguez, & Wilgers, 2011; Stafstrom & Hebets, 2013; Wilgers & Hebets, 2012). Components of complex display can also vary in their temporal patterns of nutrition dependence, which can ultimately influence male mating success. Prior research using *S. floridana* demonstrated that male foreleg darkness was influenced by juvenile diet whereas adult body condition (measured as body mass/carapace width) was influenced by adult diet (Rosenthal & Hebets, 2012). Interestingly, in this species, diet was found to interact with courtship rate to affect cop-

ulation success; courtship rate was a strong predictor of copulation success for all males except those that switched from a high-quantity diet before maturation to a low-quantity diet after maturation (HL), suggesting that variability experienced by a male may have an impact on females' assessment of his display.

Here, we build on previous studies by examining the potential temporal patterns of nutrition dependence in secondary sexual traits (pigmentation and courtship rate) and body condition in the conspicuously dimorphic wolf spider *S. stridulans*, a forest floor predator found in the southeastern United States. Mature males possess dark pigmentation on the first pair of walking legs (forelegs), which covers both the tibia and patella and can extend as far as halfway up the femur (Stratton, 1991). The foreleg tibiae also have short brushes of black hair (Stratton, 2005), further enhancing the visual contrast between the pigmented and unpigmented leg segments. During courtship, males tap their first pair of legs repeatedly and vigorously in front of the female (Stratton, 1991). This display is accompanied by a "song" consisting of the vibrations produced by the leg taps, as well as vibratory components produced through abdominal tremulation and palpal stridulation (Elias, Lee, Hebets, & Mason, 2006). As in many other *Schizocosa* species, courtship rate (measured as leg taps/min) is a strong predictor of copulation success in *S. stridulans* (Hebets et al., 2011), and it is likely that leg taps correlate with bouts of vibratory signaling. Despite the presence of conspicuous pigmentation, however, previous studies suggest that it is the vibratory component that is necessary and sufficient to elicit copulations (Hebets, 2008; Hebets et al., 2011). Nevertheless, foreleg pigmentation has been found to have an impact on female assessment of male courtship; increased pigmentation is suggested to reduce the strength of female preference for higher courtship rates, allowing more pigmented males to gain copulations at lower rates (Hebets et al., 2011). Given the putative interaction between courtship rate and foreleg pigmentation in *S. stridulans*, we sought to explore the potential temporal pattern of nutrition dependence in these traits and their role in male mating success.

We used a 2×2 full factorial design of low versus high nutrient/food availability during juvenile and adult life stages in male *S. stridulans* to (1) assess the temporal patterns of nutrition dependence in two male secondary sexual traits (foreleg pigmentation and courtship rate) as well as body condition, (2) determine which components of the male's display predict mating success, and (3) explore the potential interactions of diet and courtship rate on male mating success. Based upon prior research in this and other *Schizocosa* species, we predicted that the degree of tibial pigmentation would be strongly affected by juvenile diet, that courtship rate would be unaffected by diet and that body condition would be influenced by adult diet or the interaction between juvenile and adult diet. We further predicted that tibial pigmentation acts as an indicator trait, and that it would be more sensitive to diet than overall body pigmentation. In terms of mating success, we predicted condition-dependent mating success. Additionally, given the previous evidence for the possibility of an interaction between diet and courtship rate (Rosenthal & Hebets, 2012), we predicted that courtship rate would be the strongest predictor of copulation success in all males except those experiencing a high juvenile/low adult food treatment.

Methods

Spiders and Diet Manipulation

Subadult *S. stridulans* (~4 weeks pre-maturation; $N = 1431$) were collected at night on 3–5 April 2011 in Lafayette County, MS, U.S.A.

Spiders were transported to the University of Nebraska–Lincoln and individually housed in clear plastic containers 6 × 6 cm and 8 cm high (Amac Plastic Products, Petaluma, CA, U.S.A.), visually isolated with opaque tape. For both housing and trials, spiders were maintained in a facility at 25 °C under a 12:12 h light:dark cycle and were provided with *ad libitum* water.

To assess the independent and interacting effects of juvenile and adult nutrient quantity (i.e. food availability) on male phenotype and mating success, we randomly assigned males to diet treatments following a fully crossed 2 × 2 design of nutrient quantity (low quantity, L, versus high quantity, H) and life stage (juvenile versus adult). This resulted in four diet treatments (HH, HL, LH, LL). Spiders on a high-quantity diet were fed two crickets (1/8 inch, 0.3 cm, in length) twice per week, and spiders on a low-quantity diet were fed two crickets (1/8 inch, 0.3 cm, in length) once every 2 weeks. Most crickets were consumed immediately (personal observation), and live crickets, or dead uneaten crickets, were rarely found at subsequent feedings. Thus, while our methods only manipulated access to different quantities of prey, we are confident that this resulted in different realized nutritional intake. To standardize female condition, which could potentially affect female mate choice decision making, females used in mating trials were fed a high-quantity diet both prior to and following maturation. Crickets were purchased from Ghann's Cricket Farm (Augusta, GA, U.S.A.) and fed Ghann's Hi-Calcium Cricket Diet in the laboratory for at least 2 days prior to use as a food source.

We chose to begin diet treatments at the subadult stage for several reasons. First, previous studies in related species of wolf spider have shown that subadult males placed on different diets show different phenotype expression as adults (Rosenthal & Hebets, 2012; Rundus, Sullivan-Beckers, Wilgers, & Hebets, 2011). Additionally, since juvenile diet affects maturation time (Rosenthal & Hebets, 2012; Rundus et al., 2011), restricting the juvenile diet duration enabled us to run all trials within a reasonable time frame. Individuals were placed into diet treatments 23 ± 4 days prior to maturation.

Mating Trials

We ran a total of 143 pairs (143 males: 56 HH, 57 HL, 23 LH, 7 LL; 143 females: all HH) in mating trials in which a single male and female were allowed to interact, and male courtship behavior and copulation success were recorded. Males were run through courtship trials 23 ± 3 days after maturation and were paired with females from a similar age range. We ran mating trials in circular plastic arenas measuring 12.5 cm in diameter (Pioneer Plastics, Dixon, KY, U.S.A.). The bottom of the arena was lined with a piece of Whatman number 1 filter paper (12.5 cm diameter; Schleicher and Schuell, Keene, NH, U.S.A.), and the sides were wrapped in white paper.

We weighed both males and females immediately prior to trials. We then placed females into individual arenas and allowed them to deposit silk on the filter paper for 1 h. Female silk contains pheromones that excite the male to begin courting (Kaston, 1936; Roberts & Uetz, 2005; Rovner, 1968). Following the female acclimation period, we introduced one male in each arena under an inverted glass vial with an opaque tape barrier at the top, which visually isolated males and females until the vial was removed. We allowed males to acclimate (and potentially sample female silk pheromones) for 3 min, and then removed the vials.

We simultaneously recorded four trials from overhead with a Sony DCR-HC65 Handycam. The four arenas were visually isolated from one another by their opaque sides, and acoustically isolated by being placed on separate vibration-damping granite plates, with space between each of the four plates. Recordings began immedi-

ately following removal of the male's acclimation vial and continued for 30 min, or until copulation occurred.

Sample sizes in the LH and LL treatments were low because juvenile low-diet individuals experienced heightened mortality and delayed maturation compared with high-diet individuals. Of 695 juvenile low-diet individuals, 215 died prior to maturation, whereas only 11 of 697 juvenile high-diet individuals died prior to maturation. Additionally, only 30% of juvenile low-diet individuals ($N = 203$) matured within the time frame (i.e. the time period during which mature females of an appropriate age were available) of the study compared with 85% ($N = 591$) of juvenile high-diet individuals. Finally, whereas the sex ratio was fairly even for juvenile high-diet males, only 28 of the 133 LH individuals and 8 of the 70 LL individuals that matured were males. Additional mortality occurred during the 3-week period between maturation and trials, ultimately leading to the smaller sample sizes for LH and LL males.

Following trials, males and females were killed through freezing, and then preserved in individual vials containing 70% ethanol.

Diet and Secondary Sexual Traits

We quantified foreleg pigmentation and body condition in males from all 143 pairs (56 HH, 57 HL, 23 LH, 7 LL). We measured male courtship rate and copulation success in 118 pairs (48 HH, 49 HL, 16 LH, 5 LL); trials in which the male failed to court or in which the male or the female retreated under the filter paper for more than 30 s were excluded.

To quantify foreleg pigmentation we removed the right first walking leg from every preserved male, dried it for 24 h and photographed it with a SPOT Flex digital camera (Model 15.2 64MP, Diagnostic Instruments, Sterling Heights, MI, U.S.A.) mounted on a Leica DM4000 B microscope. Microscope and camera set-up followed the methods of Shamble et al., (2009). We imported the resulting photographs into Adobe Photoshop CS2 and converted them to greyscale. The tibia was selected using the polygonal lasso tool, and recorded mean darkness of the leg on a scale of 0–255, where 0 is black and 255 is white.

To assess whether the pigmentation of the foreleg tibia, a presumed ornament, exhibits a greater degree of nutrition dependence than unpigmented segment, we compared the darkness of the pigmented tibia to the darkness of the unpigmented regions of the metatarsus. Because pigmentation in *S. stridulans* often extends part way (covering at most the proximal one-fifth of the segment) onto the metatarsus, we measured the metatarsus using the Photoshop ruler tool, and then selected only the distal two-thirds with the polygonal lasso tool before recording darkness as before.

We calculated male body condition as the ratio of body mass (g) to carapace width (mm). This measure of body condition combines a static measure of body size (carapace width) with a more dynamic measurement (mass) to provide a metric of the energetic reserves available to an individual. We measured body mass immediately prior to trials. We measured carapace width (at the widest point of the prosoma) on preserved males using digital calipers.

We scored courtship rate from the recordings using JWATChER (version 1.0; Blumstein, Daniel, & Evans, 2006). We recorded every instance of male leg tapping and calculated male courtship rate as the number of leg taps/min (total leg taps/duration of trial). We also scored tapes for instances of forced mounts by the males, cannibalization of the male by the female and female attacks.

We assessed the influence of diet on phenotype using standard least squares analyses of the effects of juvenile diet, adult diet and their interaction on tibial/metatarsal pigmentation, male body condition and courtship rate. We used a square-root transformation of courtship rate in these analyses to meet the

assumption of normality. We also assessed the effect of diet on metatarsal pigmentation in order to test the degree of nutrition dependence of the tibial pigmentation. We ran a standard least squares analysis to assess the effect of diet on the difference in darkness between the two leg segments (|tibial pigmentation – metatarsal pigmentation|), under the assumption that a significant effect of diet on that difference would imply increased nutrition dependence of the pigmented segment relative to the unpigmented segment. Additionally, we assessed the condition dependence of tibial pigmentation and courtship rate by testing the correlation of courtship rate and pigmentation with body condition.

Secondary Sexual Traits, Diet and Mating Success

We identified predictors of mating success with a nominal logistic regression analysis containing tibial pigmentation, male body condition and courtship rate as independent variables. Additionally, given the potential for diet to affect male attractiveness or behavior in ways other than those measured in this study, we assessed the direct effects of diet on copulation success and latency to copulate.

Interacting Traits and Mating Success

Given the lack of evidence of an effect of diet on copulation success (see Results), and given the findings of Rosenthal and Hebets (2012), where juvenile and adult diet were found to interact with courtship rate to influence copulation success, we assessed the possibility that diet might indirectly affect the attractiveness of the male sexual display. We did this using a nominal logistic regression model containing the independent variables of courtship rate, juvenile diet, adult diet and all two- and three-way interactions. We chose courtship rate for this analysis because it is the only putative signal whose role in mate choice is supported by our findings, and because it is not affected by diet (see Results). Males raised on juvenile low-quantity diets (LH and LL) took longer than juvenile high-quantity diet males (HH, HL) to mature. Because of this, females paired with juvenile low-quantity diet males were significantly older than females paired with juvenile high-quantity diet males (ANOVA: $F_{1,104} = 15.9762$, $P = 0.0001$). Therefore, we also included female age in this model as a random effect.

Results

Diet and Secondary Sexual Traits

The model testing the effect of diet on tibial pigmentation was significant ($F_{3,142} = 37.616$, $P < 0.0001$). Juvenile diet ($F_{1,142} = 107.827$, $P < 0.0001$), adult diet ($F_{1,142} = 30.852$, $P < 0.0001$) and their interaction ($F_{1,142} = 13.946$, $P = 0.0003$) all had an effect on leg darkness. Juvenile high-diet males were darker than juvenile low-diet males, and LH males were darker than LL males (Figure 1a). The model testing the effect of diet on metatarsal pigmentation was also significant ($F_{3,141} = 10.863$, $P < 0.0001$). Juvenile diet ($F_{1,141} = 29.549$, $P < 0.0001$), adult diet ($F_{1,141} = 11.775$, $P = 0.0008$) and their interaction ($F_{1,141} = 4.145$, $P = 0.044$) were, again, all significant (Figure 1b). The model testing the effect of diet on the degree of difference in pigmentation between the tibia and the metatarsus was nearly significant ($F_{3,141} = 2.479$, $P = 0.064$). Within this model, juvenile diet significantly predicted the degree of pigmentation difference ($F_{1,141} = 7.325$, $P = 0.008$), but neither adult diet ($F_{1,141} = 0.937$, $P = 0.335$) nor the interaction of adult and juvenile diet ($F_{1,141} = 0.848$, $P = 0.359$) did (Figure 1c).

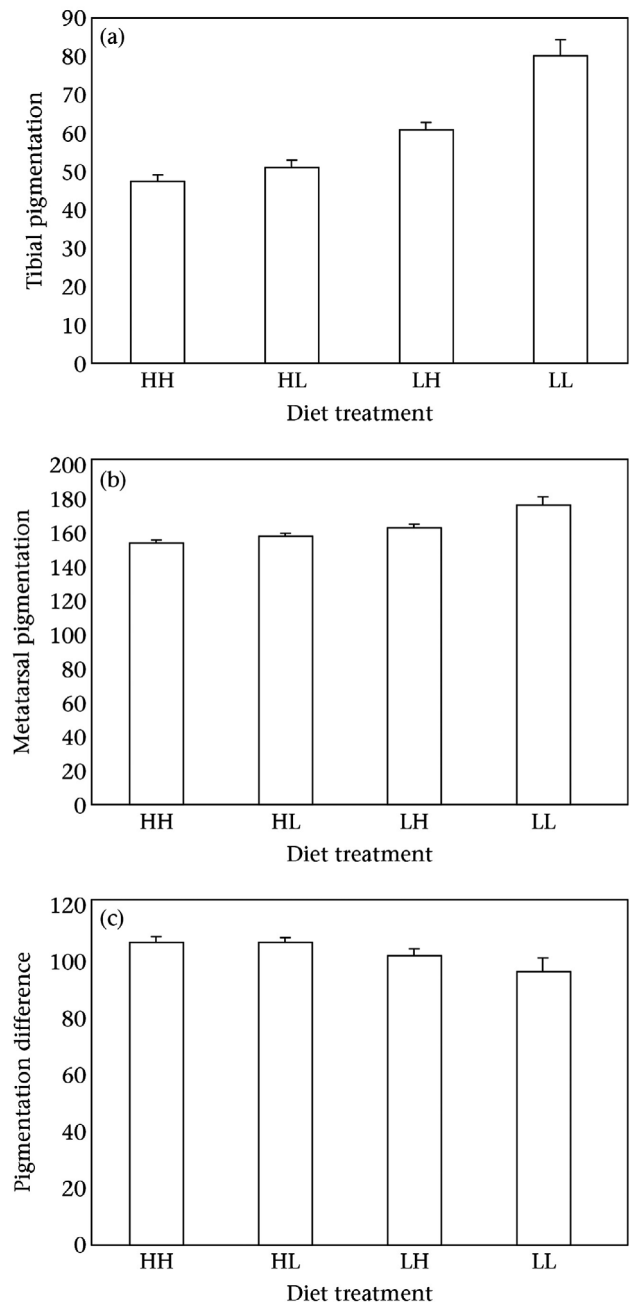


Figure 1. Mean values of (a) tibial pigmentation, (b) metatarsal pigmentation and (c) the difference of tibial–metatarsal pigmentation for male wolf spiders in all four diet treatments (HH, HL, LH, LL; high-quantity (H) versus low-quantity (L) diet in juvenile and adult life stages, where HH refers to males fed the high-quantity diet in both life stages). For (a) and (b), smaller numbers indicate darker individuals. For (c), larger numbers indicate greater difference, which is usually indicative of darker tibiae.

The model testing the effect of diet on body condition was significant ($F_{3,143} = 45.534$, $P < 0.0001$). Body condition was significantly affected by juvenile diet ($F_{1,143} = 121.884$, $P < 0.0001$), but not by adult diet ($F_{1,143} = 2.147$, $P = 0.145$) or the interaction of juvenile and adult diet ($F_{1,143} = 0.723$, $P = 0.397$). Juvenile high-diet males were in better condition than juvenile low-diet males (Figure 2).

The model testing the effect of diet on courtship rate was not significant (overall model: $F_{3,119} = 1.024$, $P = 0.385$), suggesting that courtship rate is not affected by diet.

Additionally, correlation analyses demonstrated a significant correlation between leg darkness and body condition (Pearson

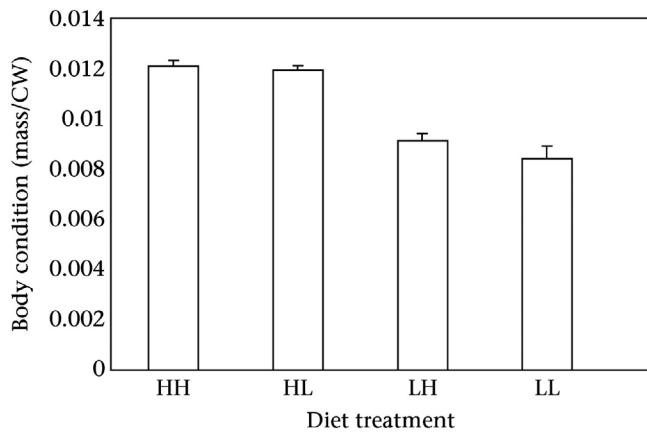


Figure 2. Mean values of body condition (body mass/carapace width) for male wolf spiders in all four diet treatments (abbreviations as in Figure 1). Larger numbers indicate better body condition.

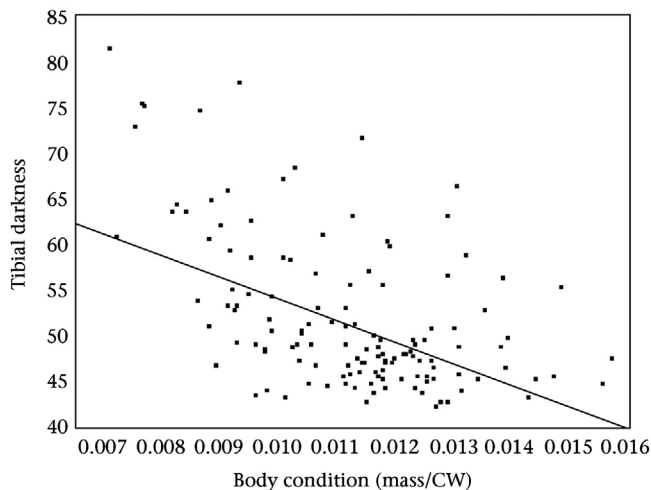


Figure 3. Correlation of body condition (body mass/carapace width) and tibial darkness in male wolf spiders. For body condition, larger numbers indicate better condition. For tibial darkness, smaller numbers indicate darker legs.

correlation: $r_{103} = 0.505$, $P < 0.0001$; Figure 3), but no correlation between courtship rate and body condition ($r_{104} = 0.167$, $P = 0.087$).

Finally, given the heightened mortality experienced by juvenile low-diet males, we assessed the possibility that differences in condition and tibial pigmentation between juvenile low-diet males and juvenile high-diet males are driven by differing selection intensities, rather than a direct effect of diet. If this were the case, it would be predicted that the variances of these traits would be significantly smaller for juvenile low-diet males. We found no difference in the amount of variance in body condition between the two diet treatments ($F_{1,139} = 0.066$, $P = 0.797$). Variances were unequal between the two diet treatments for tibial pigmentation ($F_{1,139} = 17.390$, $P = 0.0001$); however, the variance was higher for juvenile low-diet males. Furthermore, much of the variance in juvenile low-diet leg pigmentation and condition fell outside the range of juvenile high-diet individuals, suggesting that these findings are not the result of increased selection strength due to the restricted diet.

Secondary Sexual Traits, Diet and Mating Success

The nominal logistic regression model containing tibial pigmentation, body condition and courtship rate as independent vari-

ables was significant (chi-square test: $\chi^2_3 = 14.751$, $P < 0.002$). Tibial pigmentation did not significantly predict copulation success ($\chi^2_1 = 2.313$, $P = 0.128$), but both body condition ($\chi^2_1 = 7.215$, $P = 0.007$; Figure 4) and courtship rate ($\chi^2_1 = 6.262$, $P = < 0.012$; Figure 5) did. Males that were in better body condition and that courted at a higher rate were more likely to acquire a mating.

On average, roughly 75% of males copulated in all four treatments (HH = 40/48, HL = 42/49, LH = 11/16, LL = 2/5). Although the model testing the effect of diet on likelihood to copulate was nearly significant (Fisher's exact test: $P = 0.0502$), this tendency was driven by the LL treatment, which was the only group in which the number of males copulating was less than the number of males not copulating. Removing this group from the analysis erased the tendency (Fisher's exact test: $P = 0.271$). Additionally, for pairs that did copulate, there was no significant effect of diet treatment on latency to copulation ($\chi^2_3 = 0.530$, $P = 0.663$), but there was an effect of courtship rate on latency to copulation ($F_{1,119} = 39.362$, $P < 0.0001$), with males that courted more intensely copulating sooner.

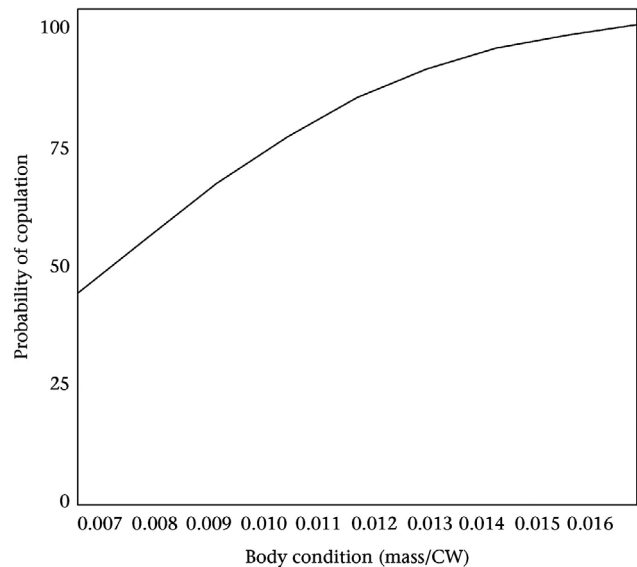


Figure 4. Relationship between body condition (body mass/carapace width) and probability of copulation in male wolf spiders. Larger numbers indicate better body condition.

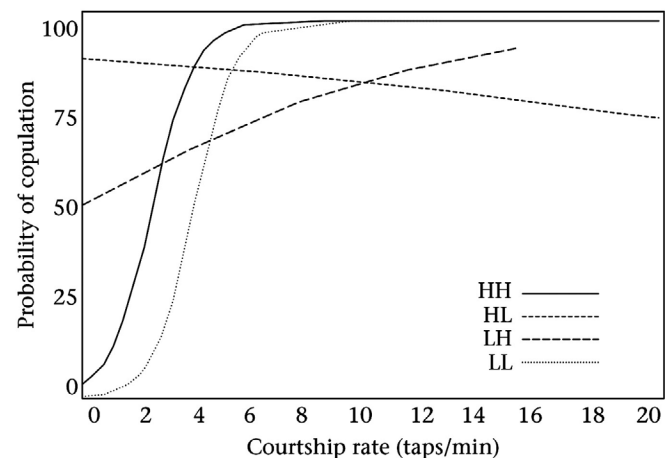


Figure 5. Relationship between courtship rate and probability of copulation for male wolf spiders in the four diet treatments (abbreviations as in Figure 1). The significant three-way interaction of juvenile diet, adult diet and courtship rate can be seen in the difference between constant-diet (HH and LL) and variable-diet (HL and LH) males.

Table 1. Table of effects for nominal logistic regression examining copulation success in male wolf spiders, *S. stridulans*, with the independent variables of juvenile diet, adult diet and courtship rate

Source	χ^2_1	P
Court rate	11.073	0.0009
Juvenile diet	0.127	0.721
Adult diet	0.038	0.846
Female age	2.575	0.0109
Court rate \times juvenile diet	0.020	0.886
Court rate \times adult diet	0.0001	0.991
Juvenile diet \times adult diet	0.985	0.321
Court rate \times juvenile diet \times adult diet	10.262	0.0014

Interacting Traits and Mating Success

The nominal logistic regression model containing juvenile diet, adult diet and courtship rate as independent variables was highly significant ($\chi^2_8 = 33.914$, $P = <0.0001$), and both courtship rate and the three-way interaction of juvenile diet, adult diet and courtship rate significantly predicted copulation success (Table 1). To visually examine this interaction, we graphed the relationship between courtship rate and likelihood of copulation for the four diet treatments (HH, HL, LH, LL; Figure 5).

For the constant-diet males (HH and LL), the steep regression curves suggest a significant effect of courtship rate on copulation success. For HH males, courtship rate significantly predicted copulation success ($\chi^2_1 = 23.275$, $P < 0.0001$), and the relationship was nearly significant for LL males ($\chi^2_1 = 3.698$, $P = 0.055$). Note that the sample size for LL males was very small ($N = 5$, 2 copulating, 3 not copulating), potentially contributing to the nonsignificance of this result. However, despite sample size limitations, LL males showed a nearly significant predictive relationship between courtship rate and copulation success, a tendency that matches the findings of Rosenthal and Hebets (2012). For the variable-diet males (HL and LH), the shallow curves indicate no relationship between courtship rate and copulation success. When tested singly, courtship rate did not predict copulation success in either HL males ($\chi^2_1 = 0.047$, $P = 0.829$) or LH males ($\chi^2_1 = 1.362$, $P = 0.243$).

Female age, which was included as a random effect in the nominal logistic regression, also significantly predicted copulation success, with the likelihood of copulation decreasing as female age increased.

Only one female attack (on an HL male) and three cannibalization events (one HH male, two LL males) were observed. No forced mounts were observed. Given the rarity of these events, no further analyses were run.

Discussion

In the wolf spider *S. stridulans*, nutritional intake affects different components of the male's sexual display to varying degrees, and females exhibit preferences for a combination of diet-sensitive and diet-insensitive characters. As predicted, tibial pigmentation exhibits heightened sensitivity to juvenile diet compared with unpigmented leg segments but does not predict mating success. In contrast, body condition, which is also influenced by juvenile diet and was positively correlated with foreleg pigmentation, does influence mating success, with males in better body condition more likely to achieve a mating. Courtship rate, a trait unaffected by diet at any developmental stage studied, was also a good predictor of mating success. Finally, while the diet treatments did not affect male mat-

ing success directly (all four groups of males had similar probabilities of mating), they did affect copulation success through a complex interaction with courtship rate. Courtship rate did not predict mating success for males that experienced a switch in prey availability (and thus nutritional intake), regardless of the direction of the switch (HL and LH), but strongly predicted mating success for males experiencing consistent nutritional intake (HH and LL).

The nutrition dependence of foreleg pigmentation has been documented in a number of wolf spider species, including several species of *Schizocosa* (i.e. Hebets et al., 2008; Rosenthal & Hebets, 2012; Shamble et al., 2009; Uetz et al., 2002; see Introduction). The dark leg coloration in wolf spiders is due to both the deposition of pigment granules in the hypodermis tissue directly under the cuticle (Holl, 1987; Seligy, 1972) and the presence of dark tibial brushes. These structures are produced during the molt to maturity, and it has been assumed that coloration is insensitive to diet after maturation. Yet in our present study, the darkness of the tibial coloration was affected by both juvenile and adult diet. This could be due to differential grooming or maintenance, or possibly to the deposition or loss of pigment following maturation. Importantly, we found an enhanced effect of juvenile diet on tibial pigmentation compared with metatarsal pigmentation. The heightened sensitivity of pigmented leg segments to the effects of diet is expected if the nutrition dependence of the tibial pigmentation has been sexually selected. This is of particular interest as, although there are a number of studies describing correlations between degree of ornamentation and various components of male condition or quality, strong evidence for heightened nutrition or condition dependence is still not widespread (Cotton, Fowler, & Pomiankowski, 2004; Hill, 2011; Prum, 2010).

Despite the observed heightened condition dependence, there is no evidence of a direct relationship between tibial pigmentation and male mating success in *S. stridulans*. One possible explanation for this disconnect is that the condition-dependent expression of foreleg pigmentation has been selected for in a context other than mate choice: for example, male-male competition. Given their relatively high densities (>700 collected at one site in a single night), it is likely that males in the field encounter one another, and may even simultaneously court a female. Unfortunately, there are currently no data to support or reject this hypothesis. Alternatively, and we suggest more likely, selection for pigmentation in *S. stridulans* may be the result of its indirect effect on female assessment of courtship rate, easing female reliance on courtship rate for assessing males with darker ornaments (Hebets et al., 2011). Regardless, heightened condition dependence of foreleg pigmentation does not directly relate to increased mating, suggesting that the strongest predictor of male mating success in *S. stridulans* is a trait that is insensitive to dietary effects.

As a metric intended to reflect the energetic reserves of an individual male, we expected that body condition would be most strongly affected by adult diet, especially given the potential for body mass to change significantly in a short time. Alternatively, given that carapace width is affected by juvenile diet, we imagined that juvenile and adult diet might have interacted to predict body condition. In contrast to our expectations, body condition was significantly affected only by juvenile diet, yet unlike the degree of tibial pigmentation, body condition significantly predicted copulation success. Given that body condition is a metric rather than a trait, however, it is not clear how it is perceived by females, but females are likely assessing other cues related to body condition.

Male courtship rate, although highly predictive of mating success, was not affected by a male's nutritional intake at either the juvenile or adult life stage. This is despite the fact that courtship

behavior is metabolically costly (Kotiaho et al., 1998) and that the intensity of the display (at least across species) is correlated with the degree of metabolic rate increase (Cady, Delaney, & Uetz, 2011). This pattern, although not intuitive, is well established in several other wolf spider species (Hebets et al., 2008; Rosenthal & Hebets, 2012). The lack of correlation between juvenile or adult diet and courtship rate suggests that it may be genetically determined.

Although courtship rate itself is extremely important in determining *S. stridulans* male mating success, we also find an interaction between courtship rate and male nutritional history. Specifically, courtship rate predicted copulation success for males raised on constant diets (HH, LL), but did not predict copulation success for males raised on variable diets (HL, LH). We expected this relationship to be missing in the HL males, but not necessarily in the LH males. This latter finding is not particularly surprising, however, because prior work in this species has already demonstrated a clear relationship between the degree of foreleg pigmentation (which is influenced by juvenile diet) and male courtship rate (Hebets et al., 2011). Irrespective of this, these results mirror the findings of Rosenthal and Hebets (2012), who used similar methods to assess the effect of food variability on courtship and copulation success in the related wolf spider *S. floridana*. The similar findings from two studies in two related species supports the notion that this trend is not an aberration, and that consistency/inconsistency of prey intake can have a significant impact on a male's mating success, specifically through an influence on the relationship between courtship rate and mating success.

A mechanistic explanation for the distinct pattern of a positive relationship between courtship rate and mating success for HH and LL males, but no relationship for HL or LH males, is not clear, but there are several possible explanations. For example, a shift in prey intake by males during development may affect the female's ability to accurately assess their display. Courtship is an energetically costly behavior (Kotiaho et al., 1998), and a male's ability to sustain rapid courtship may be a signal of his vigor (e.g. motor performance; Byers, Hebets, & Podos, 2010). Because the energetic costs of courtship vary with male body size (Kotiaho et al., 1998), females may need to assess courtship rate in the context of a male's size in order to accurately gauge his degree of effort. It is possible that the variable diet produces a male phenotype that prevents females from being able to accurately assess a male's size, or leads to inaccurate assessments. Additionally, females may use their own foraging history as a benchmark for male assessment. Since all females in this study were raised on a consistent diet (HH), their assessment of males with similar diet histories may have been eased. In the future, it would be interesting to see whether the mating patterns of variable-diet males (HL or LH) would differ if we used females that were similarly raised on variable diets (HL or LH).

Despite our inability to provide an explanation for the relationship between courtship rate and consistency/inconsistency of prey intake on male mating success, our findings have interesting implications, especially if courtship rate is related to mating benefits. Our results first suggest that a female's perception of, and response to, a single component of the male's display (such as courtship rate) is a complex process that takes into account multiple other sources of information. Second, our results may be relevant to the relationship between nutrition-dependent, or -independent, secondary sexual traits and female benefits, a relationship that has implications for the strength of sexual selection. If, for example, females receive benefits by mating with males expressing particular courtship rates, a putative disruption of a female's ability to as-

sess this accurately, as might occur in variable food environments, could have clear consequences for the choosing females.

To date, ours is one of only a few studies to provide evidence that changes in food availability experienced by males can affect their mating success without directly affecting display components (e.g. Rosenthal & Hebets, 2012), although it is known that female preference for male traits fluctuates over time (e.g. Chaine & Lyon, 2008) and that, more broadly, the form and strength of sexual selection probably fluctuates across ecological contexts (for review, see Miller & Svensson, 2014). Our results suggest that sexual signals may reside within a "phenotypic environment" in which the form of traits (both those related and those unrelated to mating) affects the perception of and response to a focal trait. This is similar to genetics, where the function of a gene in isolation may be known, but the actual effect of that gene is in part mediated by the "genetic environment." Studies like ours, which aim to examine the effects of environmental variability on traits and behavior, highlight the need to consider the possibility that the function of signaling traits may be variable and may function indirectly.

In summary, we find that diet across life stages affects some, but not all, traits associated with mating success. The fact that both body condition and courtship rate predict copulation success but are not correlated with each other suggests that females are assessing multiple independent aspects of the male during his courtship. We also find that heightened nutrition dependence does not predict whether a trait will be used in mate attraction, and that a male's dietary life history can affect female assessment of display components, even if those components are not directly affected by diet. Our findings highlight the complex interactions that probably typify female assessment of most multicomponent courtship displays, and urge for caution when assuming a relationship between nutrition-dependent traits and mating success. Through manipulative studies such as this, we are generating a picture of the temporal patterns of nutrition dependence of secondary sexual traits and their role in male mating success, and this information will ultimately facilitate our understanding of complex signal evolution and function in this, and other, systems.

Acknowledgments — We thank Mitch Bern, Laura Sullivan-Beckers and Dustin Wilgers for their help in collecting, and Mitch Bern, Rachel Bond, Marie-Claire Chelini, Matthew Hansen and Sarah Potts for their help with spider maintenance. Marie-Claire Chelini provided additional assistance in running courtship trials. We also thank members of the Hebets, Basolo and Wagner lab groups for reading drafts of this manuscript. This work was supported by a National Science Foundation CAREER grant to E.A.H. (I.O.S-0934990).

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