2015. Journal of Arachnology 43:422–424

SHORT COMMUNICATION

Influence of ambient temperature on efficacy of signals produced by female *Schizocosa ocreata* (Hentz, 1844) (Araneae: Lycosidae)

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Abstract. The ambient temperature of an environment has potential to influence many aspects of the behavior and physiology of small-bodied ectotherms, including brush-legged wolf spiders *Schizocosa ocreata* (Hentz, 1844) (Araneae: Lycosidae). Temperature varies significantly, and often unpredictably, in their habitat throughout the spring breeding season, and is known to influence male *Schizocosa* courtship behavior. Currently unknown is what effect fluctuations in ambient temperature alone might have on critical, non-behavioral sexual signals such as female silk and chemical cues. We collected cues from mature, virgin females and subjected each sample to one of three thermal treatments (40°C, 20°C, or -12°C), at constant humidity. We presented treated female cues to mature males and recorded male response across treatment types as a behavioral indicator of signal degradation. There were no significant differences across treatments in the frequency or duration of male behaviors, including critical courtship and exploratory behaviors. Our results suggest that thermally induced degradation of female sexual signals is negligible for this species and likely has little or no influence on male behavior.

Keywords: Wolf spiders, chemical cues, silk, signal degradation

The ambient temperature of an environment has the potential to influence many aspects of the behavior and physiology of small ectotherms. In wolf spiders (Lycosidae), ambient thermal variation has been shown to influence courtship vigor, copulation duration, and reproductive output (Davis 1989; Jiao et al. 2009; Chen et al. 2010). *Schizocosa ocreata* (Hentz, 1844) is a common wolf spider found in the leaf litter of eastern deciduous forests of North America where temperatures vary spatially, diurnally, and seasonally across the spring breeding period (April–June) (Cady 1983; Augspurger 2009, 2013; Roberts unpubl.). When moving through their habitat, female *S. ocreata* deposit silk and chemical cues that provide distinguishing information to males such as species identity, age, and mating status (Roberts & Uetz 2004a,b, 2005). Males of this species respond to appropriate female cues with active courtship and exploratory behavior (Stratton & Uetz 1981; Roberts & Uetz 2004a), and spend a significant amount of time moving through the leaf litter actively seeking hidden females (Cady 1983).

Thermal fluctuations in the environment influence *Schizocosa* male courtship behaviors, where courtship vigor is positively correlated with temperature (Davis 1989). Currently unknown, however, is how ambient temperature may affect other aspects of sexual signaling in this genus, especially with regard to female signals that include silk and/or chemical cues. The possibility exists that some of the variation in temperature-affected behavior by male *Schizocosa rovneri* (Uetz & Dondale 1979) noted by Davis (1989) could be explained by thermal influences on silk-bound female chemicals, the physical structure of the silk, or some interaction of the two. In a study of *Schizocosa malitiosa* (Tullgren 1905), Baruffaldi et al. (2010) found, using male courtship and exploratory responses, that male response declined quickly when female silk and chemical cues were exposed to natural environmental conditions. They concluded that humidity and/or dew was the likely cause of this induced female signal degradation, though they suggested that other untested environmental factors such as sun exposure or ambient temperature could be contributing factors (Baruffaldi et al. 2010). We concur with their findings and sought to specifically rule out the effect of thermal environment on signal degradation. We explored the possibility that ambient thermal environment alone could lead to signal degradation by exposing female *Schizocosa ocreata* silk and chemical cues to thermal extremes and then presenting the treated samples to males. Because males within the genus *Schizocosa* respond reliably to virgin adult female chemical and silk cues with active courtship if such cues are present (Roberts & Uetz 2004a; Roberts & Uetz 2005; Stratton 2005), male behavioral response provides a reliable biological assay of signal quality.

In September and October of 2011, we collected immature spiders at The Dawes Arboretum, Newark, Ohio, USA (39.973863°N, 82.40128°W), and returned them to the lab to be raised to maturity in individual plastic containers (500 ml, round). We fed spiders 2–3 cricket nymphs (*Acheta domestica*) twice weekly, and provided *ad libitum* access to water via moistened, coconut fiber substrate. All individuals used in experiments were between one and four weeks of maturity (post final molt) in order to maximize male response (Roberts & Uetz 2005), and we randomly selected mature female (n = 21) and male (n = 42) *S. ocreata* from the appropriately aged lab population. We modified the methods of Roberts & Uetz (2005) to standardize collection of silk and chemical cues from females. Specifically, we placed each female on a clean disk of filter paper (Fisherbrand, 11 cm dia. round) within a ring of PVC (polyvinyl chloride, 10 cm dia., 5 cm high) and then gently prodded the female with a horse-hair brush until she made 50 circuits around the inner circumference of the ring. Because females naturally deposit dragline silk and chemical cues as they move through the environment (Uetz & Roberts 2002; Foelix 2011), this method prevented females from settling in any one location for an extended period, and provided us with relatively consistent and uniformly distributed cues on each filter paper disk.

At the end of the collection period, we returned each female to her individual container and placed the cue samples in air-tight/moisture-tight plastic bags. Isolating cue samples at constant humidity prior to thermal treatment protected the silk in the samples from excessive

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hydration or desiccation (following cooling or heating, respectively), which are well known to alter mechanical properties of spider dragline silk (e.g., supercontraction, conformational changes). (Guan et al. 2011, 2013), and which may deactivate chemical cues (Baruffaldi et al. 2010). In our behavioral assay (described below), males make direct contact with silk in the cue sample. The sensitivity of males to conformational changes in female silk is unknown and thus we attempted to limit such changes by keeping the cue samples isolated. Prepared samples were always used within 24 hrs of collection. Using a one-way ANOVA design, we randomly assigned each isolated sample to one of three temperature treatments; Hot (40°C), Control (20°C), or Cold (-12°C). We selected maximum and minimum temperature treatments somewhat outside the range of normal variation at the field site during the breeding season to increase the likelihood of inducing and detecting thermal degradation or disruption of the signals (Roberts unpubl.). We held each sample at the appropriate treatment temperature for 60 minutes.

Following the thermal treatment, we kept cues sealed in sample bags, placed them on the lab bench in a single layer, and using results of preliminary experiments as a guideline, allowed them 15 minutes to return to ambient temperature (approx. 20°C) prior to use in a behavioral assay trial. We used an infrared thermometer (Raytek® model: RAYST60XBUS) held 10 cm from the surface of the sample to confirm temperature of the sample prior to use. For each behavioral trial, we removed a sample from a sample bag, cut the filter paper disk in half, and then used each half of a given sample with a different male to control for variation among females (Roberts & Uetz 2005). To start a trial, we cut each filter paper segment in half again (for better fit) and placed both sections into the bottom of a clear plastic box (10 cm x 10 cm x 25 cm). We gently deposited a male onto the sample from above and video recorded the resulting behavior during the five-minute trial. We cleaned the plastic boxes and scissors with 70% ethanol and a clean Kimwipe© between trials, then allowed them to air dry, removing all traces of silk and chemical cues. We scored each recorded trial according to a published ethogram of male *S. ocreata* behaviors (Roberts & Uetz 2004a) using JWatcher (vers 1.0). All behaviors were analyzed for differences in the total number of bouts (frequency over trial) and total duration (total time performing behavior over trial) across treatments using JMP (vers 9, SAS Institute). To meet the assumptions of ANOVA, we log transformed total duration data and square root transformed frequency data (Martin & Bateson 2007). We excluded a single trial from the "Cold" treatment due to a filming error (caused by a power outage during filming) resulting in the following final sample sizes: Cold (n = 13), Control (n = 14); and Hot (n = 14).

We found no significant negative influence of thermal treatment on the total number of behavioral bouts or total duration of behavior of males for any of the recorded behaviors, including critical courtship and exploratory behaviors (Table 1, Fig. 1). All behaviors were performed at rates and durations consistent with responses to untreated female chemical and silk cues found in earlier studies (Roberts & Uetz 2004a, 2005). These results indicate relative thermal stability within the range of natural thermal variation for the cues involved. Any thermally induced signal degradation that may have occurred must have been below male detection thresholds, because the signal remaining after treatment was sufficient to induce normal behavioral responses.

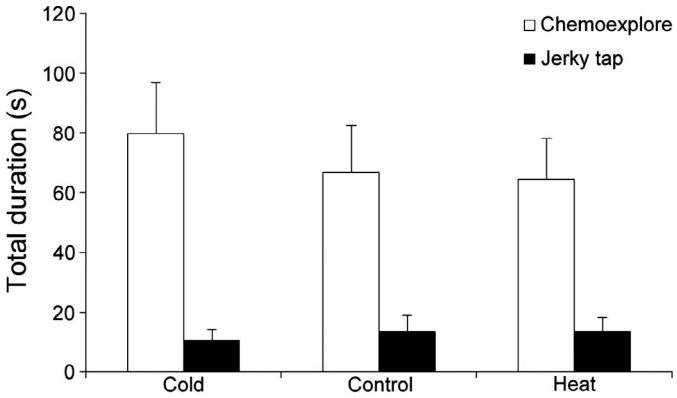

Figure 1.—Mean total duration of bouts (+SE) of Chemoexplore and Jerky Tap behaviors performed in response to female silk and chemical cues across thermal treatment categories.

Thermal treatment of the cue samples in our experiment does not appear to have fundamentally changed the nature of the cues themselves, or the underlying information content. This provides further support for Davis (1989), who demonstrated that thermal environment significantly influenced male courtship behavior in *Schizocosa rovneri*. Male response by temperature was almost certainly due to the physiological effects of ambient temperature on the males (Davis 1989), and not due to some change in quality or information content of the female cues. The apparent thermal stability of signals associated with female *Schizocosa* silk also lends support to the findings of Baruffaldi et al. (2010), who demonstrated a decline in male response to female cues that had been exposed to the natural environment. Variation in ambient temperature of the natural environment probably did not contribute to the inactivation of female signals that led to declining male courtship response over time (Baruffaldi et al. 2010), except in its capacity to contribute to atmospheric condensation/dew formation within the microhabitat. Thermal stability of female cues further confirms that the active signaling chemicals are high molecular weight compounds deposited with silk, as has been previously indicated for *Schizocosa* (Roberts & Uetz 2004a; Baruffaldi et al. 2010), and helps guide future studies of the specific chemical nature of substrate-bound signal compounds in these wolf spiders.

Table 1.—ANOVA results for behaviors of male *Schizocosa ocreata* in response to female cues. Significance indicated at Bonferroni adjusted α=0.007 (*ns* = not significant). Ethogram adapted from Roberts & Uetz (2004a). Jerky tap is active courtship behavior in males and Chemoexplore is active exploratory behavior.

	Total number				Total duration			
Behavior	F	df	*P*		F	df	*P*	
Jerky tap	0.281	2,38	0.757	*ns*	0.096	2,38	0.909	*ns*
Tap	1.671	2,38	0.202	*ns*	1.022	2,38	0.370	*ns*
Leg raise	0.723	2,38	0.492	*ns*	0.646	2,38	0.530	*ns*
Chemoexplore	0.276	2,38	0.760	*ns*	0.190	2,38	0.828	*ns*
Grooming	1.175	2,38	0.320	*ns*	0.715	2,38	0.496	*ns*
Locomotion	2.098	2,38	0.137	*ns*	1.127	2,38	0.335	*ns*
Stationary	1.758	2,38	0.186	*ns*	0.884	2,38	0.422	*ns*

ACKNOWLEDGMENTS

We would like to thank Ryan Bell, Samantha Herrmann, and Bernard Paniccia for their support, help with animal care, and editorial comments. Taxonomic reference citations were taken from The World Spider Catalog (Platnick 2014). Voucher specimens are available upon request from the collections of the corresponding author (JAR) or the Denver Museum of Nature and Science. This work was supported by an Investment in Impact Grant from The Ohio State University at Newark (to JAR) and a Scholarly Activities Grant from The Ohio State University at Newark (to JAR).

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Manuscript received 28 March 2014, revised 9 May 2015.