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Juvenile experience and adult female mating preferences in two closely related *Schizocosa* species

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Abstract. Social experience is well-known to influence female mate preference in vertebrate animals, but such effects have been studied less in invertebrates. Studies have documented flexibility in female mate choice in the wolf spider genus *Schizocosa* as a result of juvenile female experience with courting adult males. Here we investigate whether juvenile exposure to male courtship influences adult female species-level mate recognition in the wolf spider *Schizocosa ocreata* (Hentz 1844) and its sympatric sibling species *S. rovneri* (Uetz & Dondale 1979). Because these species overlap in range, contact between them is probable as interspecies hybrids are occasionally found in nature. Juvenile females were exposed multiple times to conspecific or heterospecific male courtship. Upon maturing, each exposed female was paired with an adult male of the same or different species to which it had been previously exposed, and was observed to determine receptivity and willingness to copulate. Results suggest that juvenile experience plays only a minor role (if any) in development of female mate recognition at the level of species, because the type of juvenile experience (conspecific vs. heterospecific) did not significantly affect mating outcome for either species. However, some context-specific effects of experience were observed, because the amount of juvenile exposure to adult male courtship affected adult receptivity of females in both species in different ways. In *S. ocreata*, the overall amount of juvenile experience (rather than type) influenced adult female receptivity and aggression toward heterospecific males; females with more exposures were initially more receptive and less aggressive to heterospecifics. In *S. rovneri*, neither type nor amount of juvenile exposure had significant effects on female receptivity towards conspecific males, although females exposed to heterospecific male courtship as juveniles were initially less receptive towards conspecific males than were unexposed females. While these results confirm earlier findings of behavioral reproductive isolation at the species level, they differ from some other studies, raising intriguing questions about varying degrees of behavioral and genetic isolation in different geographical populations of these two species.

Keywords: Mate choice, social experience, sexual imprinting, behavioral plasticity, Lycosidae

Recent interest in the mechanisms that lead to speciation has revealed that experience and learning can have important roles in maintaining reproductive barriers between closely related species (Panhuis et al. 2001; Mendelson & Shaw 2012). This is especially important for sympatric species where morphological and ecological divergence is minimal and/or where environments are variable (Dukas 2009; Bailey & Zuk 2009; Mendelson & Shaw 2012; Verzijden et al. 2012). Selection pressures that maintain species barriers are usually considered to be intense, as mating between species often has costly fitness consequences, such as sterile or inviable offspring and reduced reproductive output (Stratton & Uetz 1986; Maheshwari & Barbash 2011). In many animals, evolution of species recognition mechanisms appears to have been driven by female preferences for male traits and/or courtship behaviors (Endler & Houde 1995; Wagner 1998). Mate preferences are then reinforced by fitness benefits of selectivity (mate quality) as well as fitness costs of recognition errors (hybrid incompatibility, sterility) (Pfennig 1998). However, the extent to which female preferences influence the direction of evolution of male traits (and ultimately speciation) may depend on the level of plasticity in female choice behavior (Wagner 1998; Kodric-Brown & Nicoletto 2001; Coleman et al. 2004; Uetz & Norton 2007; Mendelson & Shaw 2012; Verzijden et al. 2012). In particular, social experience during juvenile development (e.g., sexual imprinting) can influence mate preferences and result in recognition errors at adulthood (Hebets 2003; Hebets & Vink 2007; Bailey & Zuk 2009; Kozak et al. 2011).

Although evidence that social experience can affect female mate preferences comes primarily from studies of vertebrate animals, behaviors of invertebrates—including mate preference—can be influenced by experience as well (e.g., Jackson & Wilcox 1993; Punzo 2000, 2004; Wagner et al. 2001; Johnson 2005; Dukas 2006, 2008, 2009; Fowler-Finn & Rodriguez 2012a, 2012b; Rodriguez et al. 2013). For example, Hebets (2003) found that adult females of the wolf spider *Schizocosa uetzi* Stratton 1997 mated more often with males possessing familiar phenotypes to which they were exposed as juveniles. Likewise, Hebets and Vink (2007) found that juvenile experience influences adult female mate preference for brush-legged males in a potentially interbreeding population of two wolf spider species (both brush-legged and no brushes). While these studies suggest that invertebrate mating preferences may be more flexible than previously assumed, and that 'hard-wired', genetically-directed threshold responses to specific stimuli (Parri et al. 1997; Wagner et al. 2001; Hebets 2003) can be modified with experience, other studies with related wolf spider species have suggested that mate recognition at the species level is not influenced by experience (Hebets 2007).

Although speciation is most often attributed to geographic isolation, the occurrence of behavioral reproductive isolating mechanisms in closely related sympatric species suggests that behavioral barriers are sufficient to restrict gene flow and result in speciation (Stratton & Uetz 1981, 1983, 1986; but see Mendelson & Shaw 2012). However, mate choice plasticity in response to experience (especially with novel phenotypes, as in

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the case of wolf spiders) can influence both the directionality of preference and the degree of behavioral isolation (Verzijden et al. 2012). In this study, we investigated how juvenile experience influences adult female mate recognition, using two well-studied sympatric wolf spider species within the genus *Schizocosa* Chamberlin 1904 (Araneae: Lycosidae). Female *S. ocreata* (Hentz 1844) and *S. rovneri* (Uetz & Dondale 1979) are morphologically indistinguishable, but male *S. rovneri* lack the tufts of bristles on their forelegs that are characteristic of mature male *S. ocreata.* Courtship displays also differ dramatically between the two species. *Schizocosa ocreata* male courtship is multimodal with visual (leg tapping and leg waving) and seismic components (substrate-borne vibration and/or stridulation), whereas *S. rovneri* male courtship is primarily unimodal and is made up of patterned seismic vibrations/stridulation only. Females distinguish between conspecific and heterospecific males on the basis of male courtship displays and male traits (Uetz & Denterlein 1979; Stratton & Uetz 1981, 1983; Uetz 2000). Males, however, court in response to female silk (and pheromones) of either species equally (Roberts & Uetz 2004). Thus, reproductive isolation is presumably maintained via female preference.

On rare occasions, apparent hybrids of these two species have been collected from the field, suggesting that a breakdown in behavioral species barriers does occur, perhaps due to constraints on sensory modes (e.g., limited vibration transmission or restricted visual line-of-sight) in complex litter environments (Scheffer et al. 1996; Uetz 2000; Uetz et al. 2013). The existence of hybrids in nature may also imply that female preference/mate recognition is not entirely genetically-based, and that exposure to courting males early in the mating season (males mature earlier than females) might influence female mate choice. Recently, a mixed and presumed inter-breeding population from Mississippi was discovered, with male morphs resembling *S. ocreata* and *S. rovneri,* but with no genetic distinction between morphs (Hebets & Vink 2007; Fowler-Finn 2009). Moreover, in the Mississippi (MS) population, juvenile exposure to males increased female preference for the brush-legged morph (Hebets & Vink 2007). Because of this, the MS population, representing a phylogenetic cluster distinct from our Ohio (OH) and Kentucky (KY) populations of *S. ocreata* and *S. rovneri* (respectively), has raised questions about juvenile experience and behavioral reproductive isolation in these species. Here we test the hypothesis that sub-adult exposure to male courtship of heterospecific vs. conspecific males influences adult mate recognition by female *S. ocreata* and *S. rovneri* from Ohio and northern Kentucky populations known to exhibit behavioral reproductive isolation (Uetz & Denterlein 1979; Stratton & Uetz 1981, 1986).

METHODS

Study species.—*S. ocreata* and *S. rovneri* are common ground-dwelling wolf spiders that occur in the leaf litter of deciduous forests in the eastern United States. When raised in isolation, both female *S. ocreata* and *S. rovneri* exhibit mate recognition based on species-specific male traits (secondary sexual characteristics and courtship displays). Nonetheless, it is probable that in the field, females are exposed to male courtship (of both species, where species co-occur) multiple times prior to maturity, as males begin to mature before females, occur in high densities and are engaged in near constant courtship activity in response to pheromones contained within the silk "draglines" laid down by mature females.

General methods.—Studies of these two species were conducted in different years, although there is no indication from long-term studies in our lab that differences between years would affect species behavior differences. *Schizocosa ocreata* were collected during spring of 2005 as sub-adults from the Cincinnati Nature Center Rowe Woods (Clermont Co., OH; 39° 07′30.31″ N, 84 ° 15′55.90″ W) where *S. rovneri* do not occur. In 2009, *S. rovneri* were reared in the laboratory from egg sacs produced by females collected as adults from the Ohio River flood plain at Sand Run Creek (Boone Co., KY; 39 ° 06′43.75″ N, 84 ° 46′58.22 W) during the spring of the previous year (2008). All spiders were housed individually in opaque plastic containers (10-cm diam. "deli dish" food containers) and kept under a 13/11 h light/dark cycle at approximately 25° C, and constant relative humidity. Spiders were fed one to two 10-day old crickets (*Acheta domesticus*) twice a week. To control for effects of hunger, female spiders were also fed one cricket the day before a trial in addition to regularly scheduled feedings (if on different days).

To examine how sub-adult male courtship influences adult female mate recognition and/or mate preferences, we conducted two-stage experiments in which (1) females first gained experience with male courtship during their penultimate life stage (one molt prior to maturity), and (2) their receptivity to male courtship was measured as adults. Juvenile females were exposed multiple times to courting adult heterospecific or conspecific males prior to maturity. Once females were mature, we recorded their behavioral responses to an adult male of the same or different species to which they had been exposed as juveniles. As a control condition, a group of females were raised to maturity in isolation and tested at adulthood without prior exposure to males.

Juvenile exposure.—Upon reaching their penultimate instar, juvenile female *S. ocreata* ($n = 87$) and *S. rovneri* ($n = 78$) were randomly assigned to one of three exposure treatment groups: 1) conspecific male exposure (*S. ocreata* females $n = 27$; *S. rovneri* females $n = 23$); 2) heterospecific male exposure (*S. ocreata* females $n = 35$; *S. rovneri* females $n = 26$); and 3) control – no exposure (*S. ocreata* female $n = 25$; *S. rovneri* female $n = 29$). Although an effort to create equal sample sizes across treatment groups was initially made, both populations experienced parasitism and/or mortality leading to decreased and uneven sample sizes.

For *S. ocreata* female exposure trials, juvenile females were individually placed into an arena consisting of a transparent, plastic, open-bottom box (9.5 × 9.5 × 9.8 cm L×W×H) adjacent to an adult courting conspecific/heterospecific male (depending on treatment group) (Fig. 1A) allowing females to gain experience with both substrate-borne seismic courtship cues (through the shared posterboard substrate) as well as visual cues. For *S. rovneri* exposure trials, juvenile females were placed in open-bottom clear plastic (acetate) cylinders (diameter: 6.4 cm, height: 5.7 cm), surrounded by a larger plastic cylinder (diameter: 15.2 cm, height: 7 cm) in which the male was placed (Fig. 1B). The cylindrical apparatuses were used for the later experiments because they kept the spiders better contained

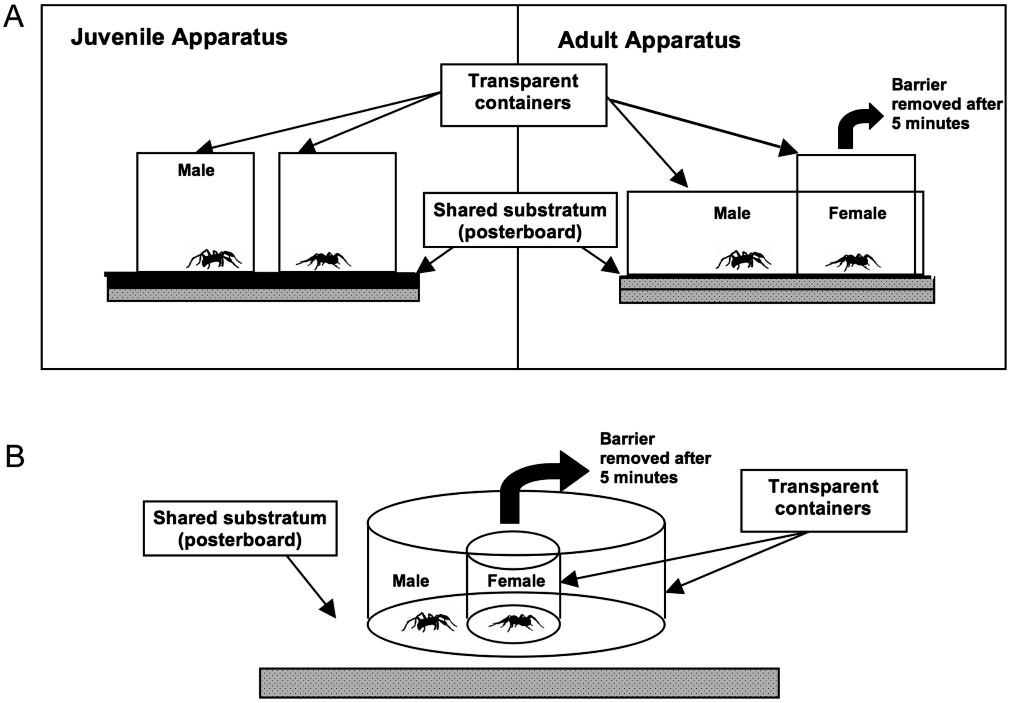

Figure 1.—Experimental apparatuses. (A) Apparatus used for *S. ocreata* trials. Juvenile exposure apparatus: penultimate females and adult males were placed onto a shared substrate known to conduct seismic signals (posterboard) adjacent to each other in open-bottom, transparent boxes (9.5 × 9.5 × 9.8 cm, L×W×H); adult trial apparatus: adult males and females were placed together in a rectangular arena (19.5 × 12.5 cm). The female was physically separated from the male for the first 5 minutes of the 10-minute trial in the same type of open-bottom boxed as used in the juvenile trials. (B) Apparatus used for *S. rovneri* trials. The same apparatus was used for juvenile exposure and adult trials, but in adult trials, the inner plastic open-bottomed circle that contained the female was removed after 5 minutes.

(spiders occasionally climbed the corners of the rectangular containers) and allowed males to circle females while courting, increasing total exposure. Males and females were kept physically isolated from one another during this phase of the experiment to control for variation in male chemical cues (cuticular or silk-borne). Juvenile females were exposed to male courtship for 30 minutes every other day until they matured, as in the experiment of Hebets (2003) with *S. uetzi.*

Males were used multiple times for exposure trials, but no female was paired twice with the same male. To ensure that multiple usages did not affect male courtship vigor, males were used for one exposure trial per day. To induce male courtship during the trial periods, silk (and pheromones thereon) of a mature conspecific female (at least 10 days post-maturity; Uetz & Norton 2007) were deposited on the male portion of the trial substrate overnight (~12 hours). Males of both species court in response to the presence of adult female silk (and the pheromones thereon), even in the absence of the female visual stimulus (Stratton & Uetz 1986).

Adult mate recognition trials.—In the second stage of the experiment, females from all three treatment groups were assigned at random to one of two adult treatment groups: 1) conspecific male (*S. ocreata* females $n = 41$; *S. rovneri* females $n = 40$); 2) heterospecific male (*S. ocreata* females $n = 46$; *S. rovneri* females $n = 38$). Testing began 7–14 days following each female's final molt to standardize receptivity level (Norton & Uetz 2005; Uetz 2000). Based on their assigned treatment group, females were randomly paired with either an adult heterospecific or conspecific male. Female *S. ocreata* were placed in a rectangular test arena (19.5 cm × 12.5 cm; Fig. 1A) and female *S. rovneri* were placed in the same cylindrical apparatus used for juvenile exposure (Fig. 1B). During the first five minutes of every trial the male and female were separated by a clear barrier so that female receptivity could be measured without tactile and/or chemical stimuli from the male. At the end of five minutes the clear barrier was removed and interactions between the male and female were observed for an additional five minutes to determine whether the pairing would result in copulation. Trials were videotaped for later analysis of female behavior.

Females of both species perform identical stereotypic behaviors that indicate receptivity and/or willingness to copulate (Uetz & Denterlein 1979; Scheffer et al. 1996; Delaney 1997; Uetz & Norton 2007) including a slow pivot (90–360 degree slow turn or turns in place), tandem leg extend (the extension of both pairs of legs I and II together anteriorly while lowering cephalothorax towards substrate and raising abdomen slightly), and settle (the lowering of cephalothorax to the substrate while keeping the abdomen slightly lifted). Because females of both species behave aggressively towards males if unreceptive or if a mate is unsuitable, all cases of cannibalism and female aggression (lunging) towards the male were also recorded. A composite receptivity score (sum of receptivity displays minus lunges—as in Uetz & Roberts 2002; Uetz & Norton 2007) was also calculated for each female. Because total trial length varied depending on whether or not

Table 1.—Results of a two-way ANOVA on the effects of juvenile and adult treatment on female composite receptivity rates in *S. ocreata* before and after removal of the transparent barrier between the male and the female. Asterisks indicate significant effects (P < 0.05).

	Factor	*df*	Sum of squares	*F*-ratio	*P*
Before barrier removal	Model	5	0.2144	14.2081	< 0.0001*
	Juvenile treatment	2	0.0010	0.1656	0.8477
	Adult treatment	1	0.2116	70.1150	< 0.0001*
	Juv. Treat. × adult Treat.	2	0.0005	0.0893	0.9146
After barrier removal	Model	5	0.1120	27.5989	< 0.0001*
	Juvenile treatment	2	0.0174	2.1448	0.1238
	Adult treatment	1	0.5123	126.2252	< 0.0001*
	Juv. Treat. × adult Treat.	2	0.0173	2.1371	0.1247

mating occurred, a composite receptivity rate was calculated (composite receptivity / trial length in seconds) and used for analyses (as in Rutledge et al. 2010).

Statistical analyses.—Data for the two species were analyzed separately. All composite receptivity rate data were log-transformed to improve normality. Trials in which mating did not occur and males courted for less than 10% of the total trial time (1 minute) were excluded from analysis. In all, one trial from the *S. ocreata* data set and two trials from the *S. rovneri* data set were excluded for this reason. To test whether juvenile and/or adult treatments had an effect on female mate preferences, female receptivity data were analyzed using a 2-way ANOVA with composite receptivity rate (referred to as "female receptivity" from here on) as the dependent variable and juvenile treatment group and adult treatment group as independent variables. Female receptive and aggressive behaviors that occurred prior to the removal of the clear barrier were analyzed separately from those that occurred following the removal of the barrier. Also, because Hebets (2003) found that amount of juvenile experience influenced female mate preference and/or willingness to mate with a male possessing a novel phenotype, in addition to type of experience, female receptivity and female aggression towards heterospecific versus conspecific males were tested with respect to amount of exposure. Male size and courtship vigor were factored into the statistical model; however, neither variable explained a significant portion of the variation in female receptivity and they were thus removed from the final model.

RESULTS

Adult treatment (heterospecific/conspecific) for both species most strongly explained variance in both female receptivity (Tables 1 & 2) and the presence/absence of copulation (*S. ocreata*: $\chi^2_1 = 68.4$, $P < 0.0001$; *S. rovneri*: $\chi^2_1 = 4.39$; $P = 0.036$). Exposed and unexposed females of both species were significantly more receptive to conspecific males than to heterospecific males (t-test: *S. ocreata*: $t_{50} = 11.156$, $P < 0.0001$; *S. rovneri*: $t_{50} = 6.232$, $P < 0.0001$; Fig. 2). Receptivity towards heterospecific males did not differ between exposed and unexposed females in either species (t-test: *S. rovneri*: $t_{56} = 0.8407$, $P = 0.4062$; *S. ocreata*: $t_{58} = 1.7086$, $P = 0.0946$). With two notable exceptions, mating did not occur between heterospecific individuals, nor did experience (type or amount) seem to influence mating success in conspecific pairings. Heterospecific mating occurred one time in each species. In both cases, the females were exposed to heterospecific male courtship as juveniles five or six times prior to maturation. Because female *S. ocreata* and *S. rovneri* are morphologically identical, to ensure that both matings were true hybridization events (and not the result of experimenter or collection error), the females were allowed to lay egg sacs and the resulting offspring were reared to adulthood. Upon maturation, male offspring were examined morphologically and behaviorally for hybrid indicator traits (reduced leg tufts relative to those found on *S. ocreata* males, and courtship behavior that is a mixture of both species; see Stratton & Uetz 1986 for a full description). In both cases, hybridization was verified.

Type of juvenile experience did not affect mating outcome for either species (*S. ocreata*: Likelihood Ratio: $\chi^2 = 1.956$, $P = 0.3760$; *S. rovneri*: Likelihood Ratio: $\chi^2 = 0.850$, $P = 0.6539$). However, in *S. rovneri* (but not *S. ocreata*), type of juvenile experience (heterospecific, conspecific, or none) explained a significant portion of the variation in adult female receptivity rates before the barrier between the male and female was removed (Table 1), but not after. A multiple comparisons analysis revealed that during the first five minutes of trials (while the male and female remained physically separated from each other) female *S. rovneri* that

Table 2.—Results of a two-way ANOVA on the effects of juvenile and adult treatment on female composite receptivity rates in *S. rovneri*, before and after removal of the transparent barrier between the male and the female. Asterisks indicate significant effects (*P* < 0.05).

	Factor	*df*	Sum of squares	*F*-ratio	*P*
Before barrier removal	Model	5	0.0435	9.4417	< 0.0001*
	Juvenile treatment	2	0.0083	4.5251	0.0142*
	Adult treatment	1	0..0265	28.7442	< 0.0001*
	Juv. Treat. × adult Treat.	2	0.0054	2.9332	0.0598
After barrier removal	Model	5	0.0038	7.7845	< 0.0001*
	Juvenile treatment	2	0.0005	0.5506	0.5791
	Adult treatment	1	0.0178	36.5962	< 0.0001*
	Juv. Treat. × adult Treat.	2	0.0001	0.1384	0.8710

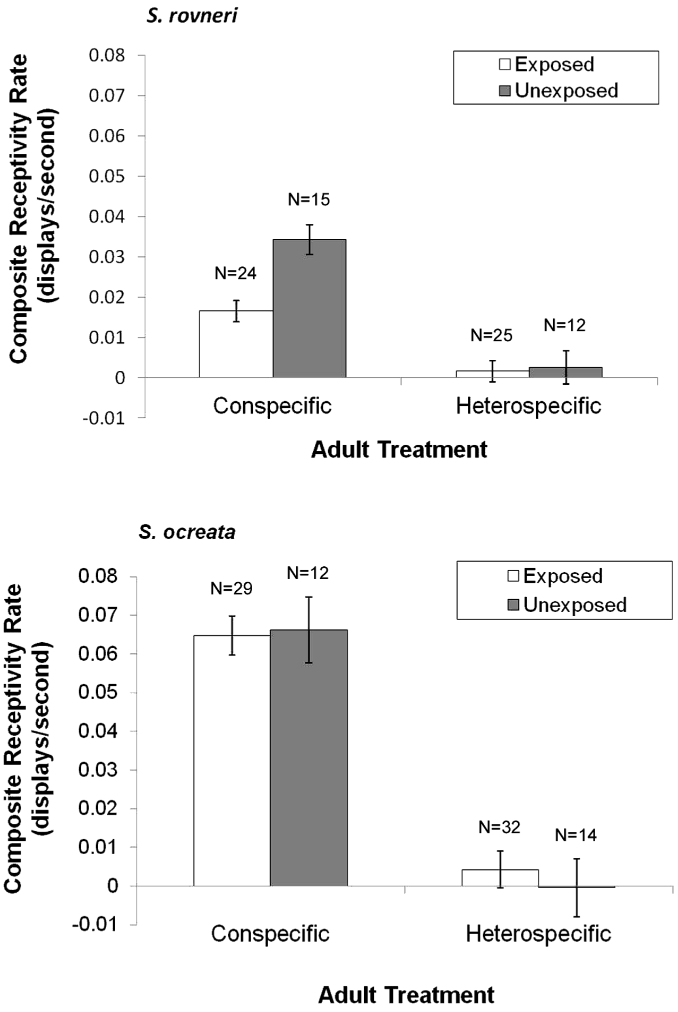

Figure 2.—Effect of female exposure on total (entire trial) composite receptivity rate in response to heterospecific or conspecific males. Error bars indicate standard error about the mean.

were exposed as juveniles to heterospecifics were significantly less receptive to conspecifics than unexposed females (ANOVA: $F = 4.512$, df = 2, $P = 0.0178$; means were compared via a Tukey HSD analysis). Receptivity towards conspecifics did not differ significantly between females exposed to conspecifics and females exposed to heterospecifics.

Adult female aggression towards adult male conspecifics vs. heterospecifics differed between species (Fig. 3). *S. ocreata* females were significantly more aggressive towards heterospecific males both before and after the barrier between the spiders was removed (t-tests: $t_{60} = 2.1508$, $P = 0.0343$ and $t_{60} = 4.5867$, $P < 0.0001$, respectively). However, aggression by adult female *S. rovneri* towards conspecific and heterospecific males was not significantly different before or after the barrier between the spiders was removed (t-tests: $t_{38} = -0.1190$, $P = 0.9056$ and $t_{36} = 0.5020$, $P = 0.6171$, respectively). Additionally, adult female *S. ocreata* were significantly more aggressive as a species towards heterospecific males after the barrier was removed than were female *S. rovneri* (t-test: $t_{83} = -1.8254$, $P = 0.0105$; Fig. 3).

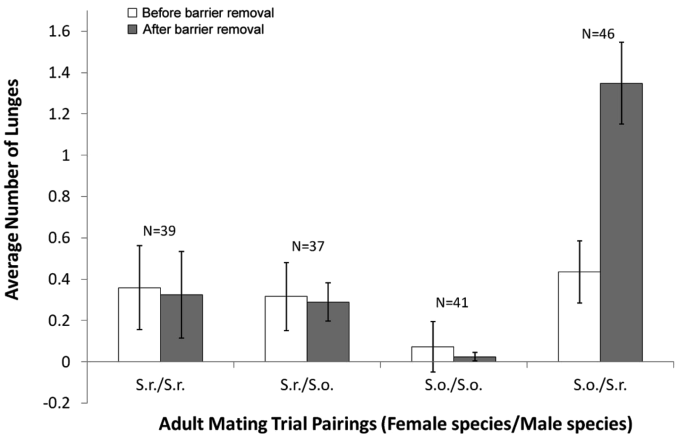

Figure 3.—Comparison of aggression between species towards heterospecific and conspecific males (S.o. = *S. ocreata*, S.r. = *S. rovneri*) before and after the barrier between the male and female was removed. Error bars show standard error about the mean.

Amount of juvenile exposure to adult male courtship affected adult receptivity of females in both species, but in different ways, and only before the barrier was removed. In *S. ocreata,* females who received the most juvenile exposure to male courtship (of either species) showed increased receptivity to heterospecific males prior to the removal of the barrier ($R^2 = 0.0973$, $P = 0.0348$). In *S. rovneri*, females with the highest number of juvenile exposures with male courtship (of either species) were significantly less receptive than females with fewer juvenile exposures ($R^2 = 0.2553$, $P = 0.0010$). These effects were independent of treatment group, as the amount of juvenile exposure did not have an effect on adult female receptivity towards conspecific or heterospecific males in either species (before or after barrier removal) with respect to treatment group (Table 3). In addition, there was a significant correlation between the number of juvenile exposures in combination with type of exposure on aggression towards males by female *S. ocreata* (but not female *S. rovneri*). Female *S. ocreata* that received higher numbers of exposures to conspecific male courtship as juveniles were less aggressive towards conspecific males after the barrier was removed than females who had received fewer exposures ($R^2 = 0.0551$, $n = 15$, $P = 0.0136$). Exposure to heterospecific male courtship did not have a detectable effect on aggression in *S. ocreata*.

DISCUSSION

Taken together, results of this study suggest that experience plays a minor role at best in species recognition for these behaviorally isolated sibling species, as the type of juvenile experience did not influence adult female mate choice. Our results differ from those of another nearly identical study conducted recently by Hebets & Vink (2007) using a population from Mississippi (MS) which has been suggested to be a mixed (freely-interbreeding) syntopic population with male morphs resembling *S. ocreata* and *S. rovneri*. In that experiment, juvenile females from the mixed population were repeatedly exposed to adult male courtship of either a tufted (*S. ocreata*) or non-tufted (*S. rovneri*) male, as in the study described here. In contrast to our finding, Hebets & Vink (2007) report that juvenile experience with male courtship of either male type led to preference for tufted males (*S. ocreata*) by females. Although our species populations were specifically chosen to be non-

Table 3.—Relationships between amount of juvenile exposure and female receptivity rates by treatment group for *S. ocreata* and *S. rovneri*.

			Before		After	
Female species	Treatment (Juv./adult)	*N*	R^2	*P*	R^2	*P*
S. ocreata	*S. ocreata*/ *S. ocreata*	12	0.0023	0.8829	0.0002	0.9632
	S. ocreata/ *S. rovneri*	15	0.0075	0.7596	0.2044	0.0907
	S. rovneri/ *S. ocreata*	17	0.0296	0.5091	0.0613	0.3381
	S. rovneri/ *S. rovneri*	17	0.1651	0.1056	0.0543	0.3680
S. rovneri	*S. rovneri*/ *S. rovneri*	11	0.1198	0.2971	0.2763	0.0968
	S. rovneri/ *S. ocreata*	12	0.0003	0.9554	0.0010	0.9228
	S. ocreata/ *S. rovneri*	13	0.1480	0.1943	0.1220	0.2421
	S. ocreata/ *S. ocreata*	13	0.0098	0.7472	0.0002	0.9684

overlapping, differences in these two studies raise intriguing questions about behavioral and genetic isolation in different geographical populations of these two species.

Recent studies by Fowler-Finn (2009) have revealed that the MS population forms a distinct genetic group from the northern (OH/KY) populations of the two species used in this study. Interbreeding between *S. ocreata* and *S. rovneri* occurs in nature in our study populations in Ohio and Kentucky (i.e., hybrids are occasionally collected from the field), and can be forced in the lab (Stratton & Uetz 1986). In cross-species hybrids, inheritance of male traits in these two species results in males that exhibit distinct intermediate morphology (reduced male tufts) and a mixture of parental species courtship behavior (Stratton & Uetz 1986). This does not seem to be the case in the Mississippi population, as males originating from a single egg sac of the same mother show varying degrees of these traits ranging from very small to large and robust brushes of tibial bristles (Fowler-Finn, pers.-comm.), or that hybrids with the characteristics described above for forced *ocreata*/*rovneri* matings (Stratton & Uetz 1986) are uncommon. Differences observed in the effects of experience on female mate choice would suggest geographic behavioral divergence across a latitudinal gradient, with a higher degree of behavioral reproductive isolation in northern populations. This is supported by comparison of microsatellite DNA, which found that that the MS population forms a distinct genetic group from the OH/KY populations of either phenotype (Fowler-Finn 2009). It is also possible that since these two species have cryptic females, some mating along species lines may go undetected within a mixed population. Consequently, it remains uncertain how and why juvenile exposure to male courtship can have such different impacts on female preferences in different geographic populations.

In the study presented here, *S. rovneri* females exposed to heterospecific male courtship as juveniles were initially (prior to barrier removal) less receptive towards conspecific males than unexposed females. This finding could indicate that juvenile experience with male courtship leads females of this species to be more cautious or even choosier about their mate decisions (Stoffer and Uetz, unpubl.), which may ultimately serve to reinforce species isolation. Because this effect was observed only prior to the removal of the separation barrier, this may also provide evidence that physical interaction between the male and the female is important for species identification in *S. rovneri*. These results support other recent findings involving the effects of juvenile experience with different artificial conspecific male phenotypes on adult female mate preferences for those phenotypes in *S. rovneri* (Rutledge et al. 2010). Rutledge et al. (2010) found that females of this species were less receptive as adults to males possessing phenotypes to which they had been exposed to as juveniles, and found evidence that chemical cues within the environment during juvenile experience and adult mate choice may ultimately influence mating decisions.

Although the same effects of experience type on female receptivity were *not* observed in *S. ocreata*, females who were exposed repeatedly to conspecific male courtship as juveniles showed decreased aggression towards conspecific males vs. females with less or no exposure. In addition, aggression toward heterospecific males by *S. ocreata* females was higher overall regardless of exposure than for female *S. rovneri* (both before and after the females and males were allowed to physically interact). One interpretation of these findings might be that female *S. ocreata* have a stronger innate recognition template for species identification than *S. rovneri*, which is reinforced rather than modified by experience. Given that these species exhibit considerable differences in the level of signaling complexity (Hebets et al. 2013) it may be that *S. ocreata* has more specific signaling criteria to be met before mating can occur. It is also plausible that because one difference between the methods utilized here and those employed by Hebets (2003) is the manner in which females were exposed to males, that physical contact with male cuticular chemical cues might play an important role in female mating decisions and agonistic behavior. In this study, females were kept physically isolated from males during exposure and thus were not exposed to male tactile or chemical cues prior to maturity, whereas in Hebets' study, juvenile females had the opportunity to physically interact with adult males during exposure.

The ability of female *S. ocreata* and *S. rovneri* to correctly identify potential (conspecific) mates has important fitness consequences, as the reproductive costs of choosing to mate with a heterospecific male are potentially high (Stratton & Uetz 1986). Visual and seismic signals are known to be important in species recognition of *S. ocreata* and *S. rovneri* (Hebets & Uetz 1999; Uetz 2000; Uetz & Roberts 2002), thus it might be maladaptive for females to exhibit too much plasticity in preference for male visual and/or seismic courtship. Previous studies have suggested the hypothesis that the foreleg tufts and multimodal courtship of *S. ocreata* males are adaptations that help males overcome the visual and seismic barriers to communication that result from the complex leaf litter environment in which they live (Stratton & Uetz 1986; Scheffer et al. 1996; Uetz et al. 2009, 2013). The most recent phylogeny of the genus *Schizocosa* suggests that *S. rovneri* males have secondarily lost leg tufts (considered to be a secondary sexual characteristic) and multimodal courtship (Stratton 2005; Hebets et al. 2013). Nonetheless, both female *S. ocreata* and *S. rovneri* exhibit preferences for male foreleg ornamentation (a possible pre-existing bias in *S. rovneri*) (McClintock & Uetz 1996; Scheffer et al. 1996), suggesting that factors apart from sexual selection have driven the loss of secondary male traits in *S. rovneri*.

Although these species are sympatric throughout a large portion of their ranges, populations of *S. rovneri* tend to be most dense in leaf litter along flood plains. Though the litter does not differ dramatically in terms of the types of leaves found in these habitats, generally the floodplain leaf litter has less vertical structure than forest floor leaf litter, and is cemented together by mud, creating a contiguous substrate across the floor of the habitat (Scheffer et al. 1996). These differences have significant impacts on the transmission and reception of seismic and visual signals (Scheffer et al. 1996; Uetz et al. 2013) and are likely to have played a role in the evolution of male morphology and behavior of both species. The enhanced visual courtship displays produced by the addition of leg tufts, as seen in *S. ocreata,* may improve a male's chances of communicating effectively in the complex leaf litter and thus reduce his risk of predation by females. However, in the floodplain leaf litter, males are likely to perform the bulk of their courtship on the upper surface of the leaf litter, and having such high contrast visual characteristics may ultimately make them overly conspicuous to predators. If true, then natural selection would favor males with reduced or absent visual traits, not because females lack a preference for them, but because they are the males that would survive to mate. Nonetheless, reduction in male courtship signals may ultimately hinder a female's ability to assess species identity of a potential mate. As a consequence, this might have led females to become more hesitant about accepting and/or attacking males, since costs of mistaken identity (accepting a heterospecific male as a mate) are severe, but costs of not accepting a male may limit reproductive options.

In some cases it seems that juvenile experience may help to inform female expectations about mate availability at maturity which may lead to shifts in female choosiness and receptivity (Stoffer & Uetz, unpubl.). In a different study conducted by Johnson (2005), in which juvenile female *Dolomedes triton* (Walckenaer 1837) (fishing spiders) were raised in the presence or absence of adult male chemical, visual and seismic cues, experience had a significant impact on female mating decisions and rates of cannibalism, as virgin females, exposed to male cues prior to maturation, were more likely to cannibalize males than naïve females. In this study, repeated juvenile exposure to heterospecific males led *S. rovneri* females to be less receptive to conspecific males than unexposed females. It is possible that this exposure to heterospecific males led to modified expectations about the frequency distribution of conspecific mates, leading females to be more cautious (and ultimately less receptive) about accepting males as mates.

From the growing body of literature on the effects of experience on female mate choice in spiders as well as other invertebrate animals, it is clear that plasticity in female mate choice is higher than previously expected. However, experience can have unpredictable effects that appear to be largely species-specific, and often depend on the timing and type of experience (Verzijden et al. 2012). For example, in *S. uetzi* it was shown that exposure to artificially modified males as juveniles led to female preference for the familiar artificial male phenotype to which they had been previously exposed (Hebets 2003). However, exposure to male courtship from a closely related species (*S. stridulans* Stratton 1984) did not result in any shifts in female preferences for heterospecific males (Hebets 2007). In *S. rovneri,* females that were exposed to artificial male phenotypes were shown to discriminate against familiar visual and chemical conspecific male phenotypes (Rutledge et al. 2010). However, as reported here, experience with heterospecific males had limited effects on female mate choice in *S. rovneri*. Ultimately, future research concerning plasticity of female mate preferences in wolf spiders and other taxa should consider how multiple sensory cues (e.g., chemical and/or tactile cues) might influence female mate choice, which may allow more insight about the role of social factors and sexual selection in species divergence.

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