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Experience leads to preference: experienced females prefer brush-legged males in a population of syntopic wolf spiders

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Experience leads to preference: experienced females prefer brush-legged males in a population of syntopic wolf spiders

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Sexual selection has long been recognized as a potential contributor to the divergence in reproductive characters that ultimately leads to speciation. *Schizocosa ocreata* and *Schizocosa rovneri* wolf spiders embody a classic example of species divergence resulting from such sexual selection, as they are reproductively isolated by courtship behavior alone. Here, we characterize a newly discovered population of wolf spiders in which brush-legged males (sensu *S. ocreata*) and non-ornamented males (sensu *S. rovneri*) are found syntopically. Mitochondrial sequence data (cytochrome oxidase subunit 1) indicate that the 2 male forms are not reciprocally monophyletic. We exposed subadult females from this mixed population to courtship advances from either brush-legged or non-ornamented males. Experienced females mated significantly more with brush-legged males, whereas inexperienced females showed no mating distinction. In essence, we demonstrate that females from this population will differentially choose between males of 2 distinct forms based on prior experience. Specifically, experience leads to a preference for brush-legged males. We also show that brush-legged males are more sexually aggressive than non-ornamented males. This study highlights the importance of prior experience on subsequent mate choice and has potential implications regarding the extent to which experience can influence polymorphism maintenance and/or species divergence and the evolution of secondary sexual traits. *Key words:* mate choice, plasticity, polymorphism, sexual aggression, speciation, subadult experience. *[Behav Ecol 18:1010–1020 (2007)]*

Sexual selection has long been recognized as a contributing cause of divergence in reproductive characteristics and hence of speciation (Lande 1981; West-Eberhard 1983). Elaborate ornamentation and courtship behavior are among the most visible consequences of sexual selection (Darwin 1871). Less evident than these typically male traits are the female preferences that often evolve hand in hand with them. It is these female preferences, however, that are thought to play a predominant role in the evolution of elaborate male traits (Andersson 1994).

Until recently, most studies of female choice have assumed a pure genetic basis. Traditional approaches to understanding the origin and maintenance of female preferences have focused on hypotheses such as Fisherian self-reinforcing selection, selection for direct benefits, selection for species recognition, preexisting female biases, and selection for indicator traits (for overview, see Andersson 1994). However, empirical evidence continues to mount providing insights into the degree to which female mating preferences may be plastic, both among and within individuals and among and within populations. For example, female satin bower birds of varying age use different criteria in making mate choice decisions (Coleman et al. 2004). In the stalk-eyed fly, the strength of female preference for male ornaments is positively associated with female eyespan, a condition-dependent trait (Cotton et al. 2006). Condition-dependent mate choice has also been demonstrated in the black field cricket *Teleogryllus commodus* (Walker) (Hunt et al. 2005). In addition to age and condition, experience has been shown to influence subsequent female mate choice in multiple vertebrate groups (Bakker and Milinski 1991; Brooks and Caithness 1995; Galef and White 1998; Brooks 1999; White and Galef 2000; Dugatkin et al. 2002). Although less common in studies of invertebrates, effects of experience on mate choice and associated behaviors have recently been demonstrated in several invertebrate taxa (damselflies, flies, spiders, and crickets) (Miller and Fincke 1999; van Gossum et al. 2001; Wagner et al. 2001; Hebets 2003; Dukas 2005; Johnson 2005; Fincke et al. 2007).

A variety of methodological approaches are aimed at gaining an understanding of the origin, maintenance, putative variation, and strength of female preferences. For example, many studies involve comparisons among divergent populations (Houde and Endler 1990; Hill 1994; Jones and Hunter 1998; Ptacek 1998; Hamilton and Poulin 1999; Gray and Cade 2000; Hebets and Maddison 2005; Elias et al. 2006) or between closely related species (Stratton and Uetz 1981; Stratton 1983; Stratton and Uetz 1986; Wiernasz and Kingsolver 1992; Fitzpatrick and Gray 2001; Mendelson and Shaw 2002; Saldamando et al. 2005; Gray et al. 2006) as a means to obtain insights into female mate choice. An alternative approach involves the use of artificial male traits displayed back to females from a single population. Several such manipulative studies have greatly advanced our understanding of female choice across various taxa: spiders (Clark and Uetz 1992; McClintock and Uetz 1996; Hebets and Uetz 2000; Hebets 2003), fish (Basolo 1990), frogs (Ryan and Rand 1990; Ryan et al. 1990), and birds (Andersson 1982) to name only a few. Unfortunately, not all systems are amenable to such artificial manipulations—in addition, their relevance to natural interactions are not always straightforward (Fleishman et al. 1998; Hebets et al. 2006).

Here, we take advantage of a system in which both interspecies comparisons as well as artificial manipulations have been previously employed to understand the intricacies of female choice as they relate to the evolution of male courtship displays and associated secondary sexual traits in the sibling

species of wolf spider *Schizocosa ocreata* (Hentz 1844) and *Schizocosa rovneri* (Uetz and Dondale 1979. Although mature males of these 2 species differ greatly in outward appearance, their genitalia as well as the genitalia and general morphology of the females are indistinguishable. In wolf spiders, as in most spiders, the genitalia are generally divergent between species and are often useful as taxonomic characters at the species level. On maturation, male *S. ocreata* possess large tufts of black hairs on the tibiae of their forelegs. *Schizocosa ocreata* males wave these ornamented legs in a courtship dance that incorporates both visual (foreleg waving) and seismic (produced with a stridulatory organ located on the male's pedipalps) signals. In contrast, mature male *S. rovneri* lack conspicuous foreleg ornamentation and possess a mainly seismic courtship display consisting of stridulation in combination with a "body bounce" where males push themselves up off the ground, lift all their legs, and as their body comes down, they hit their chelicerae on the substrate producing an audible seismic signal (Uetz and Denterlein 1979). Through a series of elegant experiments conducted on isolated populations of these 2 species, Stratton and Uetz demonstrated *S. ocreata* and *S. rovneri* to be ethospecies, reproductively isolated by courtship alone (Uetz and Denterlein 1979; Stratton and Uetz 1981; Stratton 1983; Stratton and Uetz 1983; Stratton and Uetz 1986). The courtship behaviors were shown to be heritable and under the control of very few genes or gene complexes (Stratton and Uetz 1986).

The present study capitalizes on the novel discovery of a mixed population of brush-legged (sensu *S. ocreata*) and non-ornamented (sensu *S. rovneri*) *Schizocosa* wolf spiders in northern Mississippi. In this paper (1) we document the presence of a mixed population of *Schizocosa* wolf spiders in which males resembling *S. ocreata* and males resembling *S. rovneri* are found syntopically. We use sequence variation in the mitochondrial gene cytochrome oxidase subunit 1 (COI) to investigate the phylogenetic relationships between individuals resembling both species. (2) Using this mixed population, we describe differences between the male forms in their sexual behavior. (3) We present data suggesting that a female's choice of male form is dependent on her subadult experience. Specifically, prior experience changes adult female mating preferences, resulting in more matings with brush-legged than with non-ornamented males.

MATERIALS AND METHODS

Spider collection and housing

Immature spiders were collected at night on 8 April 2004 and 12–14 April 2006 from a rock substrate at the University of Mississippi's greenhouse (Oxford, MS). In the laboratory, animals were housed in individual, visually isolated 6 × 6 × 8–cm Amac plastic products boxes. They were kept on a 12:12 h light:dark cycle, provided with a constant source of water, fed 2–3 crickets once a week, and checked daily for moults. We recorded maturation dates and ultimate adult male form (brush-legged or non-ornamented) for every individual.

Species determination and phylogenetic analysis

In order to determine whether or not the 2 male forms, or females that actively chose 1 of the 2 male forms, could be separated based on molecular markers, individuals were preserved in 100% EtOH for subsequent DNA extraction on completion of all behavioral trials. Individuals used in the molecular analysis were the same individuals as were used in the behavioral experiments. Digital photographs were taken

of the genitalia of all females. A subsample of 13 specimens was used to assess whether there were 2 reciprocally monophyletic lineages within the population (Table 1). COI was selected as an appropriate phylogenetic marker as it is one of the fastest evolving mitochondrial genes and has been used in Lycosidae to examine inter- and intraspecific relationships (Colgan et al. 2002; Vink and Paterson 2003; Chang et al., 2007). Seven additional *Schizocosa* specimens from other locations in Mississippi were also sequenced: 3 males conforming to *S. ocreata*, 2 males conforming to *S. rovneri*, and 2 females that could be of either species (Table 1). We also included 7 specimens from an Ohio population identified as *S. ocreata* and 6 specimens from a Kentucky population identified as *S. rovneri* (populations previously used for ethospecies studies). Eight *Schizocosa* species were sequenced as outgroup taxa (Table 1) and to provide insight into the relative genetic distances between and within *Schizocosa* species. The 8 outgroup species included 4 specimens each of *Schizocosa uetzi* Stratton 1997, and *Schizocosa stridulans* (Stratton 1984), both thought to be closely related to *S. ocreata* and *S. rovneri* (Stratton 1991; Stratton 1997; Stratton 2005).

DNeasy Tissue Kits (Qiagen, Valencia, CA) were used to extract DNA from 2 legs (usually left legs III and IV) of each specimen. The primers used for polymerase chain reaction (PCR) amplification and sequencing were C1-J-1718-spider (5′-AATCATARGGATATTGGAAC-3′) plus C1-N-2776-spider (5′-GGATAATCAGAATANCGNCGAGG-3′) (Vink et al. 2005). In some instances, the forward primer used was LCO1490 (Folmer et al. 1994). *Ex Taq* DNA polymerase (Takara) was used in the PCR amplifications, which were performed in a Mastercycler (Eppendorf) thermocycler with a cycling profile of 40 cycles of 94 °C denaturation (30 s), 45 °C annealing (30 s), and 72 °C extension (1 min) with an initial denaturation of 3 min and a final extension of 5 min. Excess primers and salts were removed from the resulting double-stranded DNA by using polyethylene glycol/NaCl precipitation. Purified PCR fragments were sequenced in both directions at the Microchemical Core Facility (San Diego State University). Sequence data were deposited in GenBank (http://www.ncbi.nlm.nih.gov/GenBank/) (see Table 1 for accession numbers). Sequences were edited and aligned using Sequencher 4.5 (Gene Codes Corporation). The sequences coded as expected, there were no signs of multiple peaks at any position in the sequencing results and no indication of multiple bands when visualizing the PCR products. Therefore, we are certain that the sequences were of the mitochondrial COI gene and not nontarget nuclear pseudogenes. Bayesian analyses were used to estimate phylogenetic tree topologies with MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003). MrModeltest version 2.2 (Nylander 2005) implemented in PAUP* version 4.0b10 (Swofford 2002) was used to select the model parameters for the Bayesian analyses. Within MrModeltest, the Akaike Information Criterion (see Posada and Buckley 2004) was used for model selection. The 1035 bp of COI sequence data were partitioned by codon position, using the models HKY+I (Hasegawa et al. 1985) for first codon, F81 (Felsenstein 1981) for second codon, and HKY+G (Hasegawa et al. 1985) for third codon. Partitioned analyses were run in MrBayes using the methods of Brandley et al. (2005), and Bayesian analyses were conducted by running 2 simultaneous, completely independent analyses each with 4 heated chains, sampling every 1000th tree. Analyses were run for 10 million generations at which time the average standard deviation of split frequencies had stabilized at approximately 0.0025, which indicated that the 2 tree samples had become increasingly similar. MrBayes was used to construct a majority rule consensus tree, discarding the first 25% of trees generated as burn-in.

Table 1

***Schizocosa* wolf spiders used in molecular analysis**

Species	Specimen code	Sex	Location	GenBank accession number
Schizocosa sp.—mated w/non-ornamented male	c004	Female	USA, MS, Lafeyette County, Greenhouse on UM campus	EF112491
Schizocosa sp.—mated w/brush-legged male	c005	Female	USA, MS, Lafeyette County, Greenhouse on UM campus	EF112492
Schizocosa sp.—mated w/brush-legged male	c009	Female	USA, MS, Lafeyette County, Greenhouse on UM campus	EF112493
Schizocosa sp.—mated w/non-ornamented male	c012	Female	USA, MS, Lafeyette County, Greenhouse on UM campus	EF112494
Schizocosa sp.—mated w/brush-legged male	c016	Female	USA, MS, Lafeyette County, Greenhouse on UM campus	EF112495
Schizocosa sp.—mated w/brush-legged male	c027	Female	USA, MS, Lafeyette County, Greenhouse on UM campus	EF112496
Schizocosa sp.—mated w/brush-legged male	c038	Female	USA, MS, Lafeyette County, Greenhouse on UM campus	EF112497
Schizocosa sp.—mated w/brush-legged male	c045	Female	USA, MS, Lafeyette County, Greenhouse on UM campus	EF112498
Schizocosa sp.—mated w/brush-legged male	c139	Female	USA, MS, Lafeyette County, Greenhouse on UM campus	EF112499
Schizocosa sp.—mated w/non-ornamented male	c155	Female	USA, MS, Lafeyette County, Greenhouse on UM campus	EF112500
Schizocosa sp.—mated w/brush-legged male	c209	Female	USA, MS, Lafeyette County, Greenhouse on UM campus	EF112501
Schizocosa sp.—mated w/non-ornamented male	c343	Female	USA, MS, Lafeyette County, Greenhouse on UM campus	EF112502
Schizocosa sp.—mated w/brush-legged male	c344	Female	USA, MS, Lafeyette County, Greenhouse on UM campus	EF112503
Schizocosa sp.	S1	Female	USA, MS, Washington County, Deciduous woods nr Stoneville	EF112504
Schizocosa sp.	S2	Female	USA, MS, Lafeyette County, Clear Creek	EF112505
? *Schizocosa ocreata* (Hentz 1844)—brush-legged	o1	Male	USA, MS, Penola County, Sardis Reservoir nature trail	EF112506
? *Schizocosa ocreata* (Hentz 1844)—brush-legged	o2	Male	USA, MS, Lafeyette County, 1 mile SW Abbeville	EF112507
? *Schizocosa ocreata* (Hentz 1844)—brush-legged	o3	Male	USA, MS, Lafeyette County, 1 mile SW Abbeville	EF112508
Schizocosa ocreata (Hentz 1844)—brush-legged	o4	Male	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	EF584464
Schizocosa ocreata (Hentz 1844)—brush-legged	o5	Male	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	EF584465
Schizocosa ocreata (Hentz 1844)	o6	Female	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	EF584466
Schizocosa ocreata (Hentz 1844)	o7	Female	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	EF584467
Schizocosa ocreata (Hentz 1844)	o8	Female	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	EF584468
Schizocosa ocreata (Hentz 1844)	o9	Female	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	EF584469
Schizocosa ocreata (Hentz 1844)	o10	Female	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	EF584470
? *Schizocosa rovneri*, Uetz and Dondale, 1979—non-ornamented	r1	Male	USA, MS, Lafeyette County, Clear Creek	EF112509
? *Schizocosa rovneri*, Uetz and Dondale, 1979—non-ornamented	r2	Male	USA, MS, Penola County, Sardis Reservoir nature trail	EF112510
Schizocosa rovneri, Uetz and Dondale, 1979—non-ornamented	r3	Male	USA, KY, Boone County, Sand Run Creek	EF584471
Schizocosa rovneri, Uetz and Dondale, 1979	r4	Female	USA, KY, Boone County, Sand Run Creek	EF584472
Schizocosa rovneri, Uetz and Dondale, 1979	r5	Female	USA, KY, Boone County, Sand Run Creek	EF584473
Schizocosa rovneri, Uetz and Dondale, 1979—non-ornamented	r6	Male	USA, KY, Boone County, Sand Run Creek	EF584474
Schizocosa rovneri, Uetz and Dondale, 1979	r7	Female	USA, KY, Boone County, Sand Run Creek	EF584475
Schizocosa rovneri, Uetz and Dondale, 1979	r8	Female	USA, KY, Boone County, Sand Run Creek	EF584476
Schizocosa bilineata (Emerton 1885)		Male	USA, MS, Lafeyette County, UM field station	EF112511
Schizocosa duplex, Chamberlin, 1925		Male	USA, MS, Penola County, Sardis Reservoir nature trail	EF112512
Schizocosa maxima, Dondale and Redner, 1978		Male	USA, CA, San Diego Country, Jamul	EF112513
Schizocosa mccooki (Montgomery 1904)		Female	USA, CA, San Diego County, Laguna Mountains	EF112514
Schizocosa retrorsa (Banks 1911)		Female	USA, MS, Penola County, Sardis Reservoir nature trail	EF112515
Schizocosa saltatrix (Hentz 1844)		Male	USA, MS, Lafeyette County, "Lonesome 80"	EF112516
Schizocosa stridulans, Stratton, 1984	s1	Male	USA, MS, Penola County, Sardis Reservoir nature trail	EF112517
Schizocosa stridulans, Stratton, 1984	s2	Male	USA, MS, Marshall County, Strawberry Plains Audubon Sanctuary	EF112518
Schizocosa stridulans, Stratton, 1984	s3	Male	USA, MS, Lafeyette County, 1 mile SW Abbeville	EF112519
Schizocosa stridulans, Stratton, 1984	s4	Female	USA, OK, Cleveland County, Lake Thunderbird State Park	EF112520
Schizocosa stridulans, Stratton, 1984	s5	Male	USA, MS, Marshall County, Strawberry Plains Audubon Sanctuary	EF112521
Schizocosa uetzi, Stratton, 1997	u1	Male	USA, MS, Penola County, Sardis Reservoir nature trail	EF112522
Schizocosa uetzi, Stratton, 1997	u2	Female	USA, MS, Lafeyette County, "Lonesome 80"	EF112523
Schizocosa uetzi, Stratton, 1997	u3	Female	USA, MS, Lafeyette County, "Lonesome 80"	EF112524
Schizocosa uetzi, Stratton, 1997	u4	Female	USA, MS, Lafeyette County, "Lonesome 80"	EF112525

Subadult experience

Forty-seven subadult females to be exposed to mature males were randomly assigned to 1 of the 2 male forms: brush-legged males (sensu *S. ocreata*) or non-ornamented males (sensu *S. rovneri*). Exposure treatments were exactly as in Hebets (2003). Briefly, during their penultimate stage (i.e., the life stage immediately prior to maturation), these subadult females were placed in a 8.73 × 8.73 × 11.27–cm Amac plastic product clear box that was lined with a piece of filter paper on which a mature female had remained the night prior. Leaving a mature female on the filter paper overnight allowed for the accumulation of mature female silk and associated phero-mones that elicit mature male courtship displays. After a brief acclimation period, a mature male was introduced into the arena and the 2 individuals were allowed to interact for 30 min. Exposed females were paired with a mature male every 2–3 days until their final maturation moult, resulting in multiple exposures per female. Females were always exposed to the same male form (brush-legged vs. non-ornamented) but never to the same individual male. Courting males always directed their courtship and copulation attempts toward the subadult female, providing her with first-hand experience with courtship advances. We recorded behavioral details of the exposure trials in real time. Behaviors recorded were attempted mounts, forced mounts, and female attacks.

Male behaviors

During an "attempted mount," an actively courting male would approach a female and lift himself high off the ground using mostly his back 3 pairs of legs while his forelegs were held in an arched position. The male would then lunge toward the female in a movement typical of a male mount, seen as the final stage of successful courtship. Because exposure females were not yet sexually mature and thus were incapable of copulating, they typically evaded mounting attempts by darting away quickly. On some occasions, females were not successful in evading the male advance. During such "forced mounts," a male made physical contact with the female and attempted to climb on her in a position typical of copulation. In a few instances, males were able to fully mount the subadult female, but more often, the pair would engage in foreleg grappling which could last up to 15 min, during which time the male continued his attempts to climb on the female while she continued to fight him off.

Adult mate choice

Exposed females

On maturation, the previously exposed females remained iso-lated in their individual cages until their mate choice trials. Adult female mate choice was tested 13–24 days after their final maturation moult, which is within the female's window of receptivity (Norton and Uetz 2005). In adult mate choice trials, females were paired with either the male form to which they had been previously exposed or to the alternate male form. All individuals were weighed immediately prior to mate choice trials. Females were placed in the same size arenas as were used in the exposure trials, and mate choice trials lasted 30 min. During mate choice trials, nonimpregnated filter pa-per lined the bottom of the arena. We scored the following behaviors in real time: presence/absence of copulation, the latency to copulation when present, and the presence/absence of sexual cannibalism. None of the mate choice males had been used previously, and males and females were only used once. On completion of the mate choice trials, females were monitored for egg sac production and hatching.

Unexposed females

Fifty-nine subadult females were collected from the field and maintained in isolated cages until 13–46 days after their final maturation moult. Unexposed females were randomly assigned to one of the male forms (brush-legged vs. non-ornamented) and subjected to mate choice trials in the same manner as were the exposed females (see above).

RESULTS

Species determination and phylogenetic analysis

A total of 132 immature males were collected from Mississippi in early April 2004. In the laboratory, 85 individuals matured into brush-legged males (64%), whereas 47 individuals matured into non-ornamented males (36%). Non-ornamented males matured significantly earlier in the season than brush-legged males (time to maturation from 1 April, brush-legged: mean ± standard error [SE] = 37.5 ± 1.1 days; non-ornamented: mean ± SE = 30.9 ± 1.5 days; Figure 1). Females matured throughout April, May, and into June (Figure 1). Based on comparisons of digital photographs taken of the genitalia (epigynum) of every mature female, we were not able to distinguish among the females from this population.

Ten COI haplotypes occurred among the 20 *Schizocosa* speci-mens from Mississippi, and there was no evidence for reciprocal monophyly linked to brush-legged males or non-ornamented males. There was also no evidence of reciprocal monophyly of the specimens identified as *S. ocreata* from Ohio (o4, o5, o6, o7, o8, o9, o10) and *S. rovneri* from Kentucky (r3, r4, r5, r6, r7, r8). Four of the specimens identified as *S. rovneri* from Kentucky (r3, r4, r5, r6) did form a separate clade to all the other *S. ocreata* and *S. rovneri*, but support for this clade was low (posterior probabil-ity of 0.66); posterior probability values lower than 0.95 indicate low phylogenetic support. Relative branch lengths in the tree are proportional to genetic distance and indicate that COI variation within the clade containing specimens identified as *S. ocreata* and *S. rovneri* is higher than the variation seen within *S. uetzi* and *S. stridulans* but lower than between these closely related species (Figure 2). However, *Schizocosa maxima* Dondale and Redner, 1978, and *Schizocosa mccooki* (Montgomery 1904), 2 clearly separate but closely related species (Dondale and Redner 1978), also have a low genetic distance between them (Figure 2).

Subadult experience

A total of 106 virgin females were run through exposure/mate choice trials: 23 females were exposed to brush-legged males, 24 females were exposed to non-ornamented males, and 59 females were not exposed to any males. Fifty-eight mature males were used for a total of 189 exposures: 28 brush-legged males were used in a total of 96 brush-legged exposures and 30 non-ornamented males were used in a total of 93 non-ornamented exposures. Individual males were used multiple times but never with the same female (2 males used 5 times, 32 males used 4 times, 12 males used 3 times, 3 males used 2 times, and 9 males used once).

Male sexual aggressiveness

An analysis of variance (ANOVA) reveals that subadult females that were exposed to brush-legged males received more attempted mounts on average than those exposed to non-ornamented males (natural logarithm transformation for number of attempted mounts: $F_{(1,46)} = 9.9$, $P = 0.003$; Figure 3a). A contingency analysis reveals that females exposed to brush-legged males were also more likely to have experienced a forced mount than those exposed to non-ornamented males ($\chi^2 = 22.99$, $P < 0.0001$; Figure 3b). Without taking individual males into
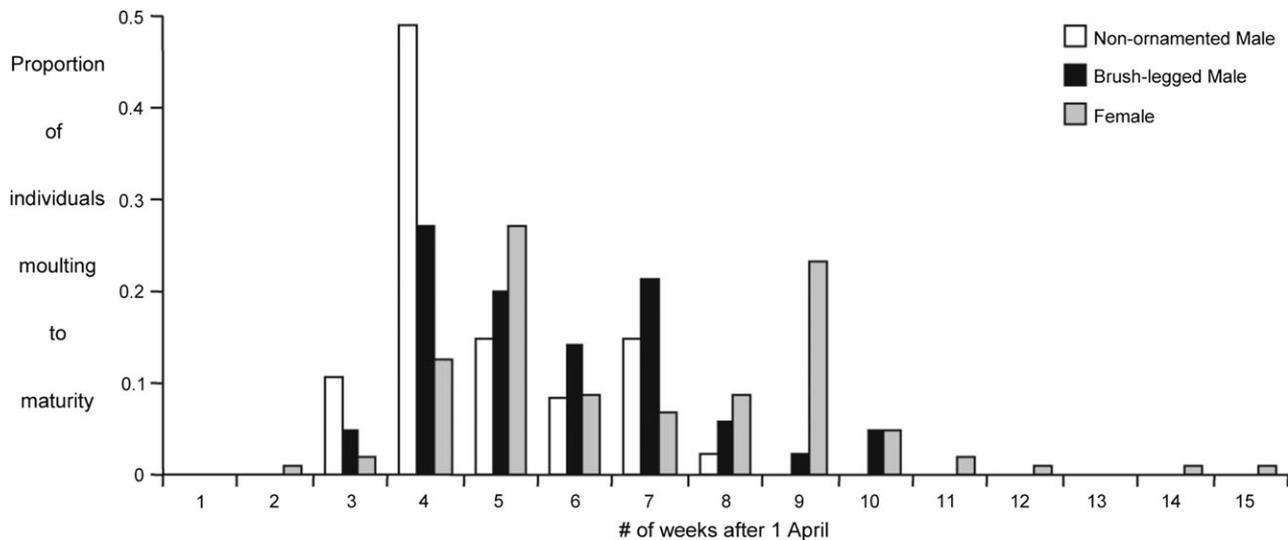
Figure 1
Maturation patterns of males and females over time in 2004. Sixty-four percent of the immature collected males ultimately matured into brush-legged males, whereas 36% matured into non-ornamented males. Bars represent the proportion of the 85 brush-legged males, the 47 non-ornamented males, and the 108 females that matured in the laboratory over time.

consideration, the average number of attempted male mounts was higher in exposure trials with brush-legged males than with non-ornamented males (brush-legged males: $N = 96$ total exposures; non-ornamented males: $N = 93$ total exposures; $F_{(1,187)} = 93.7, P < 0.0001$; Figure 3c) as was the average number of forced mounts ($F_{(1,187)} = 14.6, P = 0.0002$). At the individual level, a brush-legged male was more likely to attempt a mount than a non-ornamented male (brush-legged males: $N = 28$, mean $\pm$ SE $= 10.38 \pm 0.6$; non-ornamented males: $N = 30$, mean $\pm$ SE $= 2.1 \pm 0.58$; $F_{(1,56)} = 95.78, P < 0.0001$). Although brush-legged males were more sexually aggressive than non-ornamented males, females attacked males more often in exposures with non-ornamented males than in exposures with brush-legged males ($F_{(1,187)} = 5.2, P = 0.02$; Figure 3d). At the individual level, non-ornamented males were more likely to receive a female attack than brush-legged males (presence vs. absence of female attack, $N = 58, \chi^2 = 6.3, P = 0.01$); however, the number of attacks did not differ between individual brush-legged versus non-ornamented males ($F_{(1,56)} = 2.5, P = 0.12$).

Male sexual aggressiveness and subsequent female choice
Copulation success varied neither with the number of attempted mounts that a female experienced during her subadult exposure trials (copulate: mean attempted mount = 17, SE = 7.8; no copulate: mean attempted mount = 27.5, SE = 6.3; $F_{(1,52)} = 1.1, P = 0.3$) nor with the number of forced mounts she had previously experienced (copulate: mean forced mount = 0.76, SE = 0.57; no copulate: mean forced mount = 1.27, SE = 0.45; $F_{(1,52)} = 0.49, P = 0.49$). Whether or not a female previously experienced a forced mount did not significantly influence her likelihood to copulate (copulated females: 19% experienced a forced mount; noncopulated females: 30% experienced a forced mount; $\chi^2 = 0.88, P = 0.35$). Furthermore, the total number of exposures a female experienced as a subadult did not vary with copulation success (copulate: mean number of exposures = 3.9, SE = 0.54; no copulate: mean number of exposures = 4, SE = 0.43; $F_{(1,52)} = 1.1, P = 0.3$).

Adult mate choice

In a contingency analysis including all adult mate choice trials, the proportion of pairs that copulated did depend on the exposure/mate choice treatment ($N = 106$, % copulated = unexposed/brushes $N = 29$, 45%; unexposed/non-ornamented $N = 30$, 33%; brushes/brushes $N = 8$, 63%; brushes/non-ornamented $N = 15$, 7%; non-ornamented/brushes $N = 11$, 55%; non-ornamented/non-ornamented $N = 13$, 8%; $\chi^2 = 17.62$, degrees of freedom [df] = 5, $P = 0.0035$). Using a nominal logistic model (JMP 6) to separate out the treatment effects, we found that the presence/absence of exposure did not influence copulation frequency ($\chi^2 = 1.8, P = 0.18$), but the ornamentation of the mate choice male did ($\chi^2 = 13.14, P = 0.0003$), with females copulating more with brush-legged males than with non-ornamented males. In addition, we found a significant interaction between exposure treatment and mate choice male ($\chi^2 = 6.3, P = 0.01$).

In order to explore the interaction between exposure and mate choice male, we conducted contingency analyses on exposed and unexposed females separately. For exposed females, copulation frequency was dependent on exposure/mate choice treatment ($N = 47, \chi^2 = 15.29$, df = 3, $P = 0.0016$). When separating out the effects, we found no influence of exposure male ($\chi^2 = 0.01, P = 0.92$) but a significant influence of mate choice male—females mated more with brush-legged males than with non-ornamented males regardless of their exposure treatment ($\chi^2 = 15.23, P < 0.0001$). For unexposed females, the proportion of pairs that copulated did not depend on the ornamentation of the mate choice male ($N = 59, \chi^2 = 0.82, P = 0.36$), indicating a lack of preference in unexposed females as compared with exposed females.

In 2006, additional sample sizes were added to each of the exposure/mate choice categories by means of another study aimed at exploring the proximate mechanisms underlying the observed differences. We found no year or treatment-by-year effect on our results (year 2004 vs. 2006: $\chi^2 = 0.58, P = 0.45$; treatment: $\chi^2 = 20, P = 0.0013$; treatment $\times$ year: $\chi^2 = 9.8, P = 0.08$), and thus we include the 2006 data here in a combined analysis in order to bolster our sample sizes. As seen previously, a contingency analysis confirms that the proportion of pairs that copulated did depend on the exposure/mate choice treatment ($N = 138, \chi^2 = 21.45$, df = 5, $P = 0.0007$; Figure 4). A nominal logistic model (JMP 6) indicates that the presence/absence of exposure did not influence copulation frequency ($\chi^2 = 0.27, P = 0.61$), but the ornamentation of the
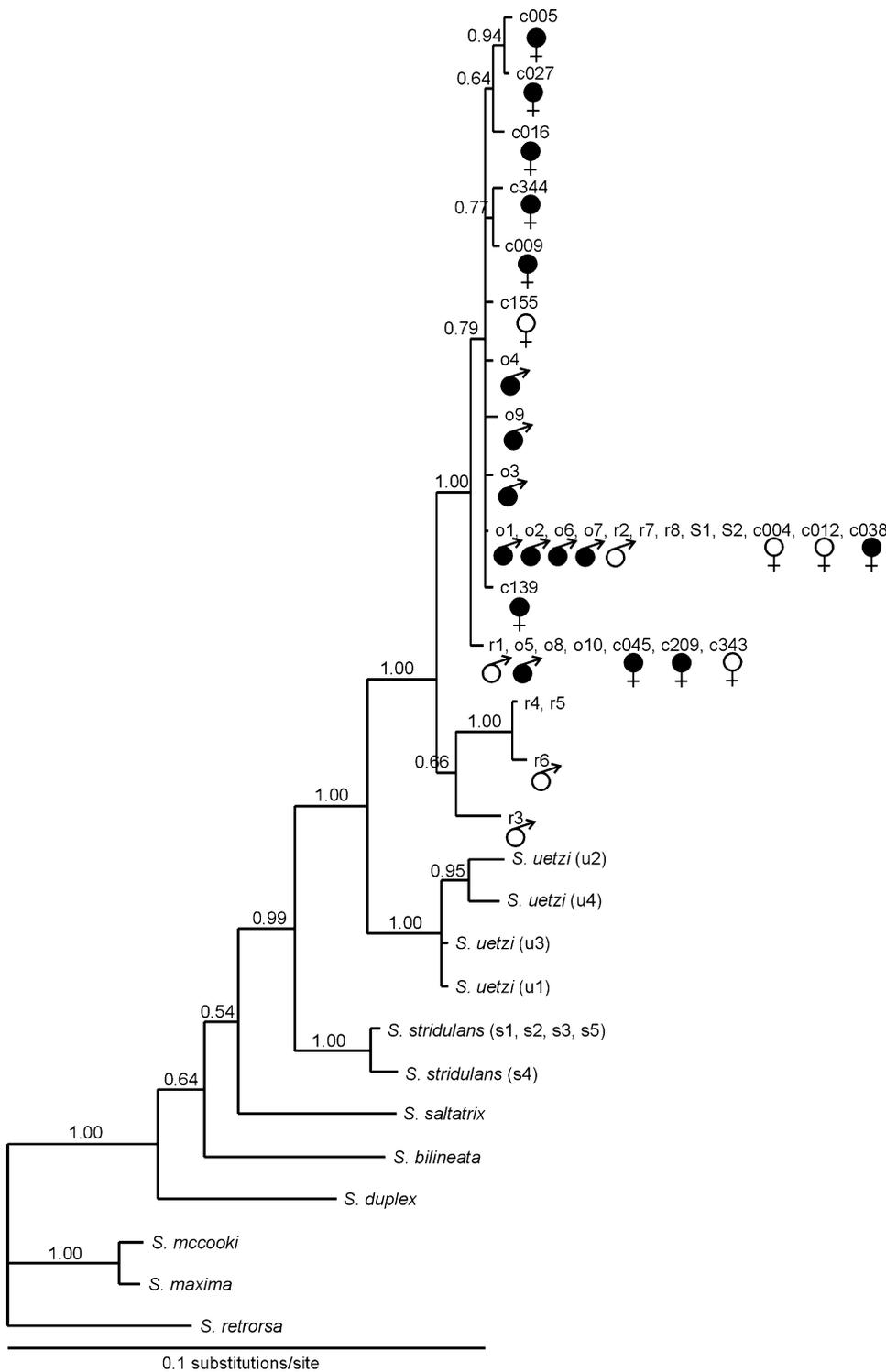
Figure 2
Bayesian phylogram based on COI sequence data. Data are partitioned by codon position, 10 000 000 generations (first 2 500 000 discarded as burn-in), likelihood models first codon HKY+I, second codon F81, third codon HKY+I+G. Values above branches are posterior probabilities. Empty male symbols represent non-ornamented males, filled male symbols represent brush-legged males, empty female symbols represent females that mated with non-ornamented males, and filled female symbols represent females that mated with brush-legged males. Individual numbers beginning with "c" represent the mixed Mississippi population; numbers o4–o10 represent *Schizocosa ocreata* individuals from Ohio and r3–r8 represent *Schizocosa rovneri* individuals from Kentucky. See Table 1 for additional information on individuals.

mate choice male did ($\chi^2 = 13.34$, $P = 0.0003$). Similar to our 2004 analysis, we found an interaction between the exposure treatment and mate choice male ($\chi^2 = 6.51$, $P = 0.01$). For exposed females, copulation frequency was dependent on exposure/mate choice treatment ($N = 65$, $\chi^2 = 20.7$, df = 3, $P = 0.0001$; Figure 4). When separating out the effects, we found no influence of exposure male ($\chi^2 = 0.98$, $P = 0.32$) but a significant influence of mate choice male, with females mating more with brush-legged males than with non-ornamented males regardless of their exposure treatment ($\chi^2 = 18.1$, $P <$

0.0001; Figure 4). For unexposed females, the proportion of pairs that copulated did not depend on the ornamentation of the mate choice male ($N = 73$, $\chi^2 = 0.68$, $P = 0.41$; Figure 4), indicating a lack of preference in unexposed females as compared with exposed females. Because the aim of the 2006 experiment was different from that presented here and thus focused on different data collection, the following analyses only include data from females in 2006, not from males.

We used ANOVA to explore potential differences in males across treatments. We found no difference in male weights

Figure 3
Sexual aggressiveness between brush-legged and non-ornamented male forms. (*a*) Subadult females exposed to brush-legged males experienced more attempted mounts (AM) on average than did females exposed to non-ornamented males. (*b*) More females that were exposed to brush-legged males experienced a forced male mount (FM) than did females that were exposed to non-ornamented males. (*c*) Brush-legged males attempted mounts more frequently than non-ornamented males. (*d*) Females attacked non-ornamented males more frequently than brush-legged males. Statistical details can be found in Results; different letters indicate significant differences ($P < 0.05$).
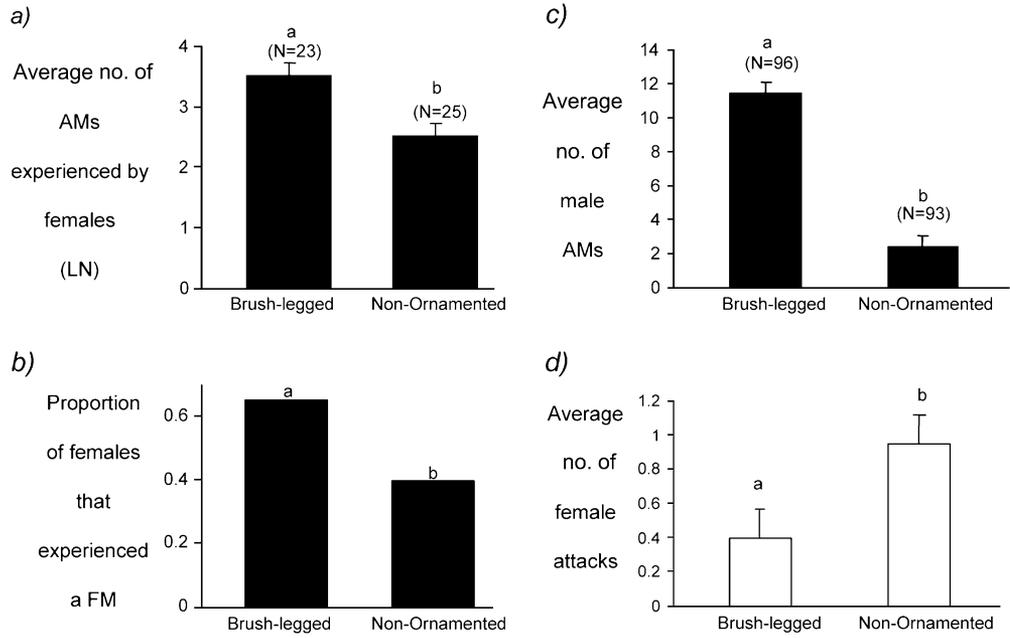
or age across treatments (weight: $F_{(5,101)} = 0.7$; age: $F_{(5,105)} = 0.69$, $P = 0.63$). Furthermore, there was no difference in the weight or age of males that copulated versus those that did not ($F_{(1,105)} = 0.28$, $P = 0.6$; age: $F_{(1,103)} = 2.66$, $P = 0.11$).

Female age was not normally distributed, and we were unable to successfully transform the data. Female age did vary across treatment (Kruskal–Wallis test: $\chi^2 = 56.7$, df = 5, $P < 0.0001$). Due to the logistics of the experiment, exposed females were younger on average than unexposed females (exposed: mean ± SE = 16.72 ± 0.88; unexposed: mean ± SE = 27.24 ± 0.84). However, there was no difference in the age of females that copulated versus those that did not (Kruskal–Wallis test: $\chi^2 = 0.73$, $P = 0.39$). Nonetheless, the observed differences between the mate choice of exposed and unexposed females could be the result of age-specific female mate choice, with younger females preferring brush-legged males. Given this, we would expect to see a difference in average age between females that mated with brush-legged versus non-ornamented males. Contrary to this prediction, of the females

that copulated, there was no significant difference in the average age of females that copulated with brush-legged versus non-ornamented males (one-tailed Kruskal–Wallis test: $\chi^2 = 3$, $P = 0.083$; Figure 5).

Female maturation time was also not normally distributed, and we were unable to successfully transform the data. Again, due to the logistics of the experiment, maturation time for females varied across treatments (Kruskal–Wallis test: $\chi^2 = 57.5$, df = 5, $P < 0.0001$). Unexposed females matured earlier in the season than exposed females (days to maturation from 1 April, unexposed: mean ± SE = 37.15 ± 1.4; exposed: mean ± SE = 54.48 ± 1.5). However, females that copulated with brush-legged versus non-ornamented males did not differ in their time to maturation (days to maturation from 1 April, brush-legged mating: mean ± SE = 46.23 ± 2.6; non-ornamented mating: mean ± SE = 39.6 ± 3.7; one-tailed Kruskal–Wallis test: $\chi^2 = 1.54$, $P = 0.21$; Figure 5).

We used ANOVA to examine the effect of exposure/mate choice treatment on the latency to copulation. The latency to

Figure 4
Proportion of pairs that copulated across the experience/mate choice treatments in 2004 and 2006. Numbers above bars indicate sample sizes per treatment. For unexposed females, the proportion of adult females that copulated did not depend on male form. In contrast, exposed females were more likely to mate with brush-legged males versus non-ornamented males regardless of their exposure treatment. Different letters indicate significant differences ($P < 0.05$).
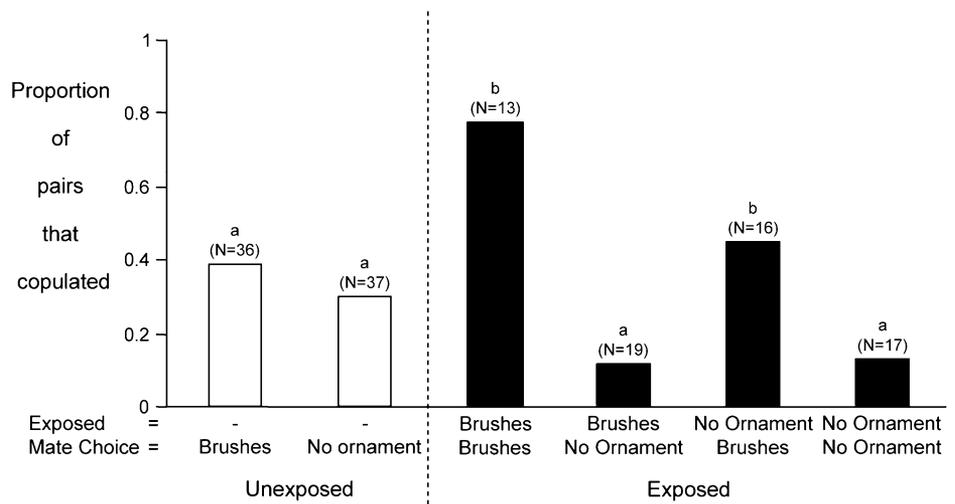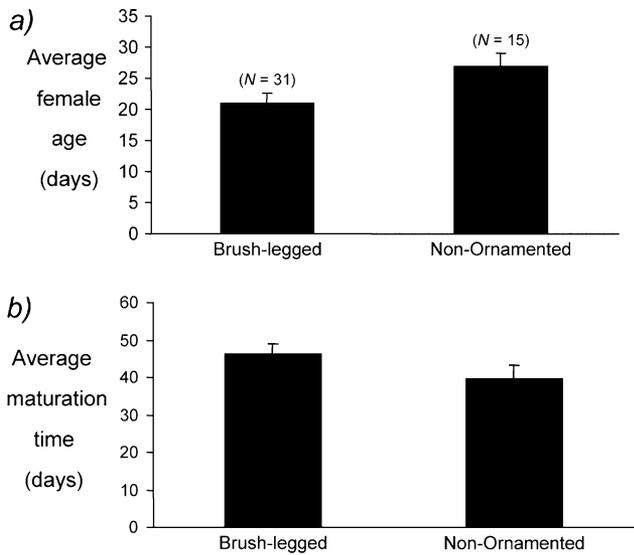
Figure 5
Comparison of the average age and time to maturation for females that copulated with brush-legged versus non-ornamented males in 2004 and 2006. No differences were observed in either average age (days after maturation) or average time to maturation (days to maturation from 1st April).

copulation did not depend on exposure/mate choice treatment ($F_{(5,31)} = 0.49$, $P = 0.78$; Table 2). The proportion of females that produced an egg sac did not depend on exposure/mate choice treatment ($\chi^2 = 1.73$, $P = 0.89$; Table 2), nor did the proportion of egg sacs that hatched ($\chi^2 = 7.39$, $P = 0.19$; Table 2). Although the fitness-related sample sizes in some treatments are small due to the low number of matings, egg sac data from subsequent years confirm that the proportion of females that produced an egg sac, the proportion of egg sacs that hatched, and the overall proportion of matings that resulted in an egg sac hatching did not depend on exposure/mate choice treatment (unexposed/brush-legged, $N = 33$; unexposed/non-ornamented, $N = 17$; brush-legged/brush-legged, $N = 22$; brush-legged/non-ornamented, $N = 4$; non-ornamented/brush-legged, $N = 12$; non-ornamented/non-ornamented, $N = 4$; Hebets EA, unpublished data).

DISCUSSION

This study documents the exciting discovery of a mixed population of *Schizocosa* wolf spiders from northern Mississippi in which 2 male forms exist: brush-legged males resembling in both morphology and courtship behavior the previously described species *S. ocreata* and non-ornamented males resembling in both morphology and courtship behavior the

previously described species *S. rovneri*. Work conducted on more northern isolated populations of these 2 species in the early 1980s clearly demonstrated them to be ethospecies—reproductively isolated by courtship behavior alone (Stratton and Uetz 1981; Stratton 1983; Stratton and Uetz 1986). Here, we provide both molecular and behavioral data to suggest that in this newly discovered southern mixed population, these 2 forms are freely interbreeding. We also document significant differences in the levels of sexual aggression between these male forms with brush-legged males attempting and forcing mounts on subadult females more than non-ornamented males. In addition, using subadult female exposure assays, we demonstrate that experienced females make different mate choice decisions as compared with inexperienced females. Specifically, experienced females mate significantly more with brush-legged males, regardless of the form of their exposure male, whereas inexperienced females mate equally with both male forms. These results suggest that prior experience leads to more discriminating mating preferences, and they imply that experience alone can influence a female's decision to mate with males of 2 distinctly different forms. As females tend to mate only once (Norton and Uetz 2005), this effect of experience could be quite important in determining the frequencies of these 2 male forms in a given population. Although prior work has already demonstrated an influence of early experience on subsequent adult mate choice in a wolf spider (Hebets 2003), this effect was observed within a well-defined species and using artificially manipulated male ornamentation. In contrast, the results we present here take advantage of natural variation in male form and highlight a potential role of prior experience in influencing population composition, which could ultimately have implications for speciation (see discussion below).

In the field, males mature on average a few weeks prior to females (see Figure 1) (Hebets 2003), and there is no known differential habitat use between immature versus mature or male versus female individuals. In addition, population density can be extremely high with more than 3 individuals per $10~\text{cm}^2$ (Hebets EA and Fowler-Finn K, personal observation), making it likely that penultimate females would encounter mature males. Furthermore, mature males will initiate courtship on contact with mature female silk, even the silk of heterospecific females (Roberts and Uetz 2004). The variation in female maturation time incorporates some early maturing females (see Figure 1), and this in addition to our knowledge of the presence of other *Schizocosa* species at the collection site (Hebets EA and Fowler-Finn K, personal observation) make it likely that mature male courtship could be elicited from the presence of female pheromone—resulting in at least some subadult females encountering mature courting males.

Mitochondrial sequence data (COI) indicate that the variation seen among individuals from this mixed population is comparable to the variation seen within 2 other *Schizocosa* species (*S. uetzi* and *S. stridulans*). Our results suggest that

Table 2
Fitness consequences of copulations across exposure treatments

Exposed/mate choice	Latency to copulation (min)	Proportion of matings resulting in an egg sac	Proportion of first egg sacs that hatched
Unexposed/brush-legged	$N = 14$, 7.53 ± 2.24	$N = 14$, 0.78	$N = 11$, 0.7
Unexposed/non-ornamented	$N = 12$, 5.41 ± 2.42	$N = 11$, 0.73	$N = 8$, 0.37
Brush-legged/brush-legged	$N = 10$, 2.54 ± 2.65	$N = 5$, 0.8	$N = 4$, 0.5
Brush-legged/non-ornamented	$N = 3$, 4.52 ± 4.84	$N = 2$, 1	$N = 2$, 0
Nonornamented/brush-legged	$N = 7$, 4.65 ± 3.17	$N = 6$, 0.83	$N = 5$, 0.8
Nonornamented/non-ornamented	$N = 2$, 4.13 ± 5.93	$N = 1$, 1	$N = 1$, 0

the mixed *Schizocosa* population described herein encompasses either a single species with 2 male forms (suggesting a behavioral and phenotypic polymorphism) or 2 species, *S. ocreata* and *S. rovneri*, with incomplete lineage sorting and/or introgression. Currently, we are developing microsatellite markers in order to further explore any putative substructure that may exist in this mixed population or between populations elsewhere in North America identified as *S. ocreata* and *S. rovneri*, including the previously studied northern populations. Data presented herein, however (both molecular and behavioral), support the hypothesis that in this Mississippi population, brush-legged and non-ornamented males are freely interbreeding and that females use prior experience with mature courting males to make subsequent mate choice decisions. At the present time, based on the mitochondrial marker COI, we are hesitant to conclude anything about the previously studied northern populations and are awaiting the results from our microsatellite analyses.

It is important to note that due to the logistics of the experiment, we observed differences in both average age and average time to maturation between exposed and unexposed females, potentially confounding our results. Although neither average age nor maturation time differed between females that copulated versus those that did not copulate, exposed females were younger and matured later on average than unexposed females. Thus, theoretically our mate choice pattern could have resulted from differences in age and maturation time as opposed to differences in subadult experience. However, if this were the case, we would expect to see differences in average age and average time to maturation between females that copulated with brush-legged versus non-ornamented males (e.g., females that copulated with brush-legged males should be younger on average and mature later in the season than those that copulated with non-ornamented males). In fact, we see no such differences (Figure 5) and can rule out the possibility that our observed differences relate solely to age or maturation time. Furthermore, follow-up experiments controlling for age in subsequent years on females from the same population support our findings that experience alters female mating preferences independent of age (Hebets EA, unpublished data). This is not to say that female preference does not vary with age or maturation time, just that any plasticity present related to age or maturation time in this data set cannot alone explain our observed results. In fact, our results do suggest a trend toward younger females preferring brush-legged over non-ornamented males, and research conducted on females of *S. ocreata* (brush-legged) from the isolated population in Ohio has recently demonstrated that female preference for brush size varies with female age (Uetz and Norton 2007). We suspect that under natural conditions, a variety of factors including experience, age, microhabitat, etc. may together dictate whether a female mates with a brush-legged or a non-ornamented male.

Although unlikely based on our molecular data, the possibility remains that there are 2 cryptic female forms within this population and that the differences observed in our mate choice results simply reflect species-specific mate choice differences having nothing to do with prior experience. Based solely on the phenology of female maturation, we might infer that the first peak of females maturing earlier in the season (Figure 1) are *S. rovneri* females, whereas the second peak of females maturing later in the season represent mostly *S. ocreata* females. Following through with this scenario, the later maturing females are mating mostly with conspecific, brush-legged males regardless of their exposure treatment. Early maturing females then should represent *S. rovneri* females and should be mating preferentially with conspecific, non-ornamented males over brush-legged males, a pattern that

our data do not support. In fact, although no significant differences exist, the early maturing unexposed females mated more with brush-legged males than with non-ornamented males, the exact opposite pattern from that predicted above. In addition, as stated earlier, we found no difference in average maturation time between females that mated with brush-legged versus non-ornamented males (Figure 5), further supporting our notion that our observed mate choice differences were not due solely to differences in species-specific maturation time. Nonetheless, if genetic substructure does exist in this population, our results suggest that at the very least, early experience influences subsequent adult mate choice differentially between the 2 female groups, an equally exciting result. Specifically, if 2 distinguishable groups of females exist, we suggest that early experience can influence the mate choice of the early maturing females (potentially *S. rovneri*), although it likely has no influence on the mate choice of the later maturing females (potentially *S. ocreata*).

Given that experience with a mature courting male influences subsequent mate choice, the question remains: why? One could imagine this type of plasticity in mate choice to be adaptive as it could enable females to adjust their mate choice threshold or criteria depending on the available distribution of males (Dukas 2005). For example, if a female did not encounter mature males prior to her own maturation, she may be more likely to subsequently accept the first male she encounters as an adult, regardless of its form. In contrast, a female that had encountered at least 1 male prior to maturation may be more willing to bypass the first male encountered in an attempt to find a more preferred male (e.g., brush-legged form). Alternatively, a female's prior experience with courtship advances could influence her perception of her own attractiveness, thereby influencing her subsequent choosiness. For example, studies in both humans and zebra finches have demonstrated that females that perceive themselves to be more attractive are more selective in choosing mates (Burley and Foster 2006; Little et al. 2001). In our wolf spider system, experiencing courtship advances from mature males may increase a female's self-perception of attractiveness, thereby increasing her subsequent mate choice selectivity as an adult. Demonstrating self-perceived attractiveness in a wolf spider would certainly be an exciting discovery which at this time would require substantial future research.

The next obvious question arising from our results pertains to the putative maintenance of the 2 distinct male forms. Although we cannot currently address how or why non-ornamented males persist in this mixed population, we will briefly discuss a few possibilities. First, although brush-legged males may gain a mating advantage via subadult female experience, not all females will be exposed as subadults, reducing the impact of the brush-legged male advantage. In addition, previous studies suggest that brush-legged males may pay higher costs for their conspicuous ornaments and courtship behavior. For example, using the video playback technique, Pruden and Uetz (2004) demonstrated that a large predatory wolf spider was more likely to attack video stimuli of a *S. ocreata* male (brush-legged males) as compared with a *S. rovneri* male (non-ornamented males). Furthermore, removal of brushes from *S. ocreata* males resulted in significantly reduced predatory responses, suggesting that the brushes in conjunction with the active courtship of *S. ocreata* may increase their detectability to predators, thereby increasing their predation risk (Pruden and Uetz 2004). In essence, brush-legged males may pay higher costs in aspects other than mating as compared with non-ornamented males. Another, nonmutually exclusive possibility is that the male forms have differential mating success in different microhabitats. In the present experiment, all mate choice trials were necessarily conducted in an artificial setting

with filter paper as a substrate. In the field, these 2 male forms are found on both rocks and in deep deciduous leaf litter and the proportion of each male form differs across substrates (Hebets EA and Fowler-Finn K, unpublished data). Previous studies using *S. ocreata* and *S. rovneri* from the northern isolated populations have highlighted the importance of microhabitat characteristics (e.g., substratum type) on male courtship efficacy in these 2 species (Stratton and Uetz 1981; Stratton and Uetz 1983; Scheffer et al. 1996). Preliminary data from our mixed population suggest that substratum type differentially influences copulation frequency between the 2 male forms, with brush-legged males receiving a mating advantage on rocks but not leaf litter (Hebets EA, unpublished data). Thus, the combination of different costs versus benefits on different substrates may help explain the existence of both brush-legged and non-ornamented males in this population.

In summary, this newly discovered mixed population of *Schizocosa* wolf spiders represents a novel and exciting natural system in which we can potentially explore the simultaneous effects of a variety of selective pressures (e.g., substrate type, prior experience, age) on the maintenance of or fixation/extinction of 2 male phenotypes (brush-legged and non-ornamented) previously associated with 2 distinct species (*S. ocreata* and *S. rovneri*, respectively). Results presented here demonstrate that experience alone can influence a female's subsequent mate choice as it relates to these 2 male forms. The implications for these results are far reaching as they suggest that the distribution and behavior of male forms throughout both space and time could significantly influence adult female mate choice patterns, which in turn will alter the subsequent distribution of male forms. If this population does indeed represent a polymorphic species whereas the northern isolated populations represent fully diverged ethospecies, it is tempting to imagine a scenario by which the brush-legged/non-ornamented male polymorphism became fixed in a few northern populations (potentially via effects of prior experience, substrate variability, etc.) and ultimately led to speciation (for reviews on polymorphisms and speciation, see Smith and Skulason 1996; Gray and McKinnon 2006)—a scenario that remains to be tested.

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