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The complexities of female mate choice and male polymorphisms: Elucidating the role of genetics, age, and mate-choice copying

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Abstract Genetic, life history, and environmental factors dictate patterns of variation in sexual traits within and across populations, and thus the action and outcome of sexual selection. This study explores patterns of inheritance, diet, age, and mate-choice copying on the expression of male sexual signals and associated female mate choice in a phenotypically diverse group of *Schizocosa* wolf spiders. Focal spiders exhibit one of two male phenotypes: 'ornamented' males possess large black brushes on their forelegs, and 'non-ornamented' males possess no brushes. Using a quantitative genetics breeding design in a mixed population of ornamented/non-ornamented males, we found a strong genetic basis to male phenotype and female choice. We also found that some ornamented males produced some sons with large brushes and others with barely visible brushes. Results of diet manipulations and behavioral mating trials showed no influence of diet on male phenotype or female mate choice. Age post maturation, however, influenced mate choice, with younger females being more likely to mate with ornamented males. A mate-choice copying experiment found that, following observations of another female's mate choice/copulation, virgin mature females tended to match the mate choice (ornamented vs. non-ornamented males) of the females they observed. Finally, analyses of genetic variation across phenotypically pure (only one male phenotype present) vs. mixed (both phenotypes present) populations revealed genetic distinction between phenotypes in phenotypically-pure populations, but no distinctionin phenotypically-mixed populations. The difference in patterns of genetic differentiation and mating across geographic locations suggests a complex network of factors contributing to the outcome of sexual selection [*Current Zoology* 61 (6): 1015–1035, 2015].

Keywords Male polymorphism, Assortative mating, Speciation, Heritability, *Schizocosa*

Sexually-selected traits—e.g., signals and preferences—are one of the most variable aspects of phenotype across populations and species (West-Eberhard, 1983; Eberhard, 1985; Andersson, 1994; Wells and Henry, 1998; Coyne and Orr, 2004; Mendelson and Shaw, 2005; Cocroft et al., 2008). They exhibit rapid rates of diversification and elaboration, and often play a key role in the formation of reproductive isolation (e.g., Gray and Cade, 2000; Boughman, 2001; Masta and Maddison, 2002; Boughman et al., 2005; Svensson et al., 2006; Boul et al., 2007; Seehausen et al., 2008; Funk et al., 2009; Sota and Tanabe, 2010). Divergence in sexually-selected traits, however, does not always correspond with genetic differentiation; signals and preferences frequently exhibit high levels of variation within populations resulting from various intrinsic and environmental factors (Andersson, 1982; West-Eberhard, 1983; Jennions and Petrie, 1997; Cotton et al., 2004; Hunt et al., 2005; Cotton et al., 2006; Safran et al., 2013; Miller and Svensson, 2014; Morehouse, 2014; West-Eberhard, 2014). By influencing the expression of—and therefore patterns of variation in—sexual traits, factors such as life history stage, resource availability, and social experience can affect patterns of mating, and therefore the action and consequences of sexual selection.

The extent to which environmental factors influence the expression of sexual traits will depend, in part, on the genetic basis, or heritability, of those traits. Signals and preferences often have significant heritability (Bakker and Pomiankowski, 1995; Chenoweth and Blows, 2006; Chenoweth and McGuigan, 2010; Roff and Fairbairn, 2014; Fowler-Finn and Rodríguez, 2015). When this heritability is high, the extent to which environmental factors influence the expression of a plastic sex-

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ual trait is limited (Chenoweth and McGuigan, 2010). For example, when heritability in a trait is high, environmental inputs such as diet may only have a small effect on the expression of that trait. However, when heritability is low, diet may have a large influence on the expression of traits. In fact, diet is known to influence the expression of male sexual traits by affecting morph phenotype in condition-dependent polymorphisms (Cade, 1980; Plaistow et al., 2004), the degree of expression of a trait such as brightness or size of an ornament (Andersson, 1982; Cotton et al., 2004; Morehouse, 2014), and mating tactic (Wilgers et al., 2009). Diet is similarly known to influence female mating behavior by influencing the degree of selectivity, likelihood of mating, or preferred male phenotype (for reviews: Jennions and Petrie, 1997; Cotton et al., 2006). Diet could influence patterns of mating within and across populations due to its influence on plastic sexual traits. However, the degree to which it might do so will be determined in part by the relative role of genetics and environment on trait expression.

The expression of sexual traits can also vary with life history traits like age. Theory predicts that female choice should correspond negatively with reproductive potential, being weaker when reproductive potential is low (Parker, 1983). Particularly, as a female ages, her reproductive potential often decreases and she is expected to be less picky in mate choice. This pattern is seen across several taxa, with younger females often being choosier/more selective (e.g., wolf spiders, Mautz and Sakaluk, 2008; Wilgers and Hebets, 2011) or more likely to choose more ornamented males (guppies, Kodric-Brown and Nicoletto, 2001; wolf spiders, Uetz and Norton, 2007). Given the plethora of recent examples highlighting the importance of female age in reproductive behavior, studies addressing the action of sexual selection on trait evolution would be remiss not to incorporate an assessment of age.

Finally, variation in the social environment may influence sexual trait expression, as mate choice is ultimately the outcome of a mate preference expressed within the context of interactions with potential mates and other conspecifics (Rodríguez et al., 2013, Miller and Svensson, 2014). Choice can vary as a result of direct interactions with others (e.g., Hebets, 2003); it can also vary based on observing interactions of others (Hebets and Sullivan-Beckers, 2010). For example, in mate-choice copying, females base their choice of male phenotype on observations of mating females (e.g., Dugatkin, 1992; Witte and Massmann, 2003; Godin et al., 2005; Mery et al., 2009; Whitte et al., 2015). Such copying can vary with the identity of the acting female (Amlacher and Dugatkin, 2005; Vukomanovic and Rodd, 2007), or even life stage—i.e. juvenile versus adult—in which the learning occurs (Hebets and Sullivan-Beckers, 2010; Verzijden et al., 2012). Mate-choice copying can generate temporal and spatial differences in the sexual traits favored by choice (Miller and Svensson, 2014; Whitte et al., 2015), and has a variety of consequences for within- and among- population patterns of variation, ranging from the maintenance of phenotypic variation within populations (Fowler-Finn and Rodríguez, 2012; Verzijden et al., 2012; Rodríguez et al., 2013) to reinforcement and speciation (Verzijden and ten Cate, 2007; Servedio et al., 2009; Verzijden et al., 2012; Servedio and Dukas, 2013). Mate-choice copying can also increase rates of divergence among divergent populations (Dukas, 2013), or even slow divergence by reducing the evolution of further assortative mating (Servedio and Dukas, 2013).

Understanding the contributions of this myriad of factors influencing sexual selection can be quite difficult. An ideal situation for exploration would be one in which individuals show discrete variation in sexually selected traits. This study focuses on one such natural system – a group of *Schizocosa* wolf spiders – and combines a quantitative genetics breeding design, diet manipulations, mate-choice copying trials, and microsatellite genetic analyses to explore observed phenotypic variation in sexual traits. The spiders of focus exhibit variation in male phenotype and female choice both within and across populations. Specifically, males of this group are either ornamented (sensu *S. ocreata*) or non-ornamented (sensu *S. rovneri*). The two male phenotypes are virtually identical prior to maturation, when they lack secondary sexual traits; even upon maturation, their genitalic and basic body characters are indistinguishable (Uetz and Dondale, 1979). The morphological and behavioral sexual display traits acquired upon maturation, however, are quite distinct. Ornamented males have large prominent brushes of black hairs on their foreleg tibiae and their courtship display is very active, involving both visual components (waving of the forelegs, tapping on the substrate, and a 'jerky' walk) and multi-component vibratory signals (Stratton and Uetz, 1981). In contrast, non-ornamented males lack foreleg ornamentation and have a primarily stationary courtship display involving a percussive body bounce that produces a vibratory signal when they strike their body against the substrate (Uetz and Denterlein, 1979; Stratton and Uetz, 1981). Females associated with ornamented versus non-ornamented males are phenotypically indistinguishable from one another (Uetz and Denterlein, 1979; Uetz and Dondale, 1979).

These spiders show intriguing patterns of variation in male phenotype and female mate choice across their geographic distribution. This study focuses on two sets of populations. The first involves phenotypically- pure populations in the Ohio Valley where spiders are found in populations of either solely ornamented or solely non-ornamented males (Uetz and Denterlein, 1979). The second is a phenotypically-mixed population in Mississippi where both male phenotypes are present (Hebets and Vink, 2007). In the Ohio Valley, a strong genetic basis of male phenotype and female choice has already been established: females show strong choice for males matching their population of origin (Uetz and Denterlein, 1979; Stratton and Uetz, 1981; Stratton and Uetz, 1986). Offspring from copulations forced between an individual from a phenotypically-pure ornamented population with an individual from a phenotypically- pure non-ornamented population produce behaviorally sterile females that will not mate with any male phenotype, as well as males of intermediate sexual displays that are not attractive to any female (Stratton and Uetz, 1986). Nothing is currently known about the genetic basis of male phenotype or female choice in the phenotypically-mixed population in Mississippi, however.

In the phenotypically-pure populations (Ohio Valley), diet is known to influence signal expression within the ornamented male phenotype—both the vibratory component of the courtship display (Gibson and Uetz, 2008) and ornament size (Uetz et al., 2002) vary with diet. Similarly, diet influences the expression of secondary sexual traits in the mixed population (Mississippi). Males reared on a high quantity diet from a subadult stage to maturation tend to have larger foreleg brushes than those raised on a low quantity diet (Hebets et al., 2008). Also, females reared on high quantity diets mate preferentially with males raised on high quantity diets (Hebets et al., 2008). In this latter study, individuals were raised from a stage late in development, and females were provided a choice of high versus low diet males of a single male phenotype (high- versus low-diet ornamented, or high- versus low-diet non-ornamented). To date, nothing is known about how diet from an early age might influence male phenotype development (ornamented or non-ornamented) or a female's choice *between* male phenotypes in this mixed population.

Studies from the phenotypically pure (Ohio Valley) and phenotypically-mixed (Mississippi) populations also suggest differences in the patterns of plasticity in mate choice in response to exposure to different social environments. Experience with the courtship of either ornamented or non-ornamented males does not disrupt the pattern of strong assortative mating in spiders from phenotypically-pure populations, where females are unlikely to encounter males of a phenotype that differs from their population of origin (Rutledge and Uetz, 2014). Within one of the phenotypically-pure ornamented populations, females exhibit a stronger preference for larger-brushed males if they encounter mature males during juvenile stages (Stoffer and Uetz, 2015). Plasticity described thus far in the phenotypically-mixed Mississippi population shows a different pattern: experience as a juvenile with courting males of either male phenotype leads a female to be more likely to mate with an ornamented male (Hebets and Vink, 2007). Encounter rates in the mixed population of focus can be quite high, with densities reaching 3 individuals per 100 cm^2 (Fowler-Finn and Hebets, 2011); thus, females are likely to experience male courtship and mating in the field (Hebets and Vink, 2007; Deng et al., 2014). Furthermore, mathematical modeling suggests that variable sexual selection on male phenotype due to sub-adult imprinting and habitat heterogeneity can contribute to the maintenance of the two male phenotypes in this mixed Mississippi population (Deng et al., 2014). Currently, however, nothing is known about how learning at the adult stage could influence mate choice and the potential maintenance of the two male phenotypes.

The difference in the distribution of male phenotypes and patterns of female choice between the phenotypically-pure (Ohio Valley) and phenotypically-mixed (Mississippi) populations suggest that the action and consequences of sexual selection may vary geographically in this group of wolf spiders. To explore this system further, we had three major components to this study, the first two of which focused exclusively on the phenotypically-mixed (Mississippi) population. First, we examined the influence of genetics and diet on the expression of adult male phenotype, and the influence of genetics, diet, and age on adult female mate choice using a quantitative genetics breeding design in combination with mating experiments. Second, we examined the role of learning via mate-choice copying to see whether mate choice varies with adult social experience. Our third aim focused on differences between the phenotypically-pure vs. phenotypically-mixed populations. To determine if genetic distinction existed between phenotypes or not, we compared patterns of correspondence between phenotypic and genetic variation in the Ohio Valley and Mississippi populations. Examination of these patterns allowed us to identify potential consequences of sexual selection, in terms of assortative mating, in these two regions (Fig. 1).

Fig. 1 Maps indicating the collection localities of ornamented and non-ornamented spiders throughout their range
Insets show collection localities of the focal phenotypically pure localities in the Ohio Valley (indicated with light green and the number 1 for the non-ornamented locatlity, and light blue and the numbers 2 and 3 for the ornamented localities) and the phenotypically-mixed locality in Mississippi (green and blue square with the numbers 4 and 5) as well as other nearby localities of *S. nr. ocreata* and *S. nr. rovneri* (indicated by the black dots).

1 Materials and Methods

1.1 Variation in male phenotype and female choice

1.1.1 Male sexual signals: Patterns of inheritance and diet

We tested patterns of inheritance and the influence of diet on the expression of male phenotype using offspring from a classic quantitative genetics breeding design. Thus, the paternal phenotype of all individuals was known. The parents were wild-caught individuals that we collected as subadults from the grounds of the University of Mississippi greenhouse in Oxford, MS (Lafayette Co, USA) in the spring of 2008. We paired 17 unique ornamented males with a total of 21 females, and 31 unique non-ornamented males with a total of 34 females (i.e., 3 ornamented and 3 non-ornamented males sired offspring with two different females). We reared the offspring in individual plastic deli dishes, which were visually isolated from each other, and filled with 0.5 cm of plaster of paris to maintain humidity. The spiders were maintained on a 12:12 hr L:D cycle at 23 ± 2°C and provided water *ad libitum*. We fed each offspring several springtails a week for the first two weeks, then either one fruit fly or one pinhead cricket subsequently until their third molt. At the third molt, we randomly split individuals from each family into high-diet and low-diet treatments. For the remainder of their lifetime (~5 molts), we fed high diet spiders 2 crickets approximating their body size once a week, and low diet spiders 1 cricket approximating their body size every other week.

Upon maturation, we determined the phenotype of each male offspring as: no brush (no visible dark hairs apparent on the forelegs), partial brushes (some hairs present), and full brushes (hairs clearly present, forming a full brush).

1.1.2 Female choice: Patterns of inheritance, diet, and age

We tested for patterns of inheritance of female mate choice as well as age-dependence and diet-dependence of choice in the mixed Mississippi population using the offspring in the quantitative genetics breeding design above. Once females matured, we conducted two-choice and one-choice trials with females of different ages and diets. Throughout, we will refer to females with ornamented fathers as ‘ornamented females’ and females with non-ornamented fathers as ‘non-ornamented females’.

For both two- and one-choice trials, we used circular arenas made of clear plastic with 7.5 cm walls (Amac Plastic Products; Westbrook, ME). Each arena had a filter paper substrate, which has been shown to effectively transmit the vibratory signals of *Schizocosa* spiders (e.g. Hebets, 2005; Sullivan-Beckers and Hebets, 2011). We covered the walls of the arena with paper printed with an image of natural leaves, and placed a 6 × 6 cm folded piece of filter paper (A-frame) in the center of the arena to provide shelter. In between trials we changed the filter paper and cleaned the arenas with

95% Ethanol to remove any chemical cues. We housed each female in the arena for 12 hours prior to her trial, during which time she deposited pheromone-laden silk that stimulates male courtship. It has been previously demonstrated that males in the Ohio Valley will court females from ornamented and non-ornamented populations with equal vigor (Roberts and Uetz, 2004), and thus the identity of the female should not influence male courtship effort. We weighed both female and male(s) immediately prior to the start of each trial, and then placed the test female into the arena and allowed her to acclimate for 2 minutes. Trials commenced when the male(s) were placed in the arena, and ended when either mating occurred or 45 minutes had elapsed.

Two-choice mating trials – We conducted all two-choice trials in 20-cm diameter arenas. We simultaneously presented females with a male that matched her paternal phenotype and a male that did not (i.e., one ornamented and one non-ornamented male). We tested 71 females in total – 39 ornamented females (19 high diet; 20 low diet), and 32 non-ornamented females (14 high diet; 18 low diet). Males paired with females were of the same diet treatment as the female (i.e., high diet males with high diet females; low diet males with low diet females). For each trial, we age-matched the ornamented male and non-ornamented male to each other by ± 2 days post maturation, but did not age match them to females.

One-choice mating trials – Our two-choice trials resulted in patterns consistent with complete assortative mating (i.e., ornamented females mated with ornamented males and vice versa; see Results). Thus, we conducted one-choice mating trials to increase the opportunity to document non-assortative mating by removing the potential confound of male-male competition presented by two-choice trials. We used 13-cm diameter arenas where a single female and a single male were allowed to interact for 45 minutes. Females were always initially paired with a male that was the opposite phenotype of her father. If a female did not mate in the first trial, we rested her for 5 minutes and then allowed her to interact with a male that matched her paternal phenotype for 30 minutes. This second step allowed us to determine whether a female chose not to mate because she was not attracted to a given male or whether she had a lack of motivation to mate altogether. We conducted the one-choice trials towards the conclusion of the two-choice trials and as a result had limited availability of females and males that had not already been used in the two-choice trials. We tested 13 females: 5 ornamented females (all low diet) and 8 non-ornamented females (5 high diet; 3 low diet). The 25 males we used for the one-choice trials ranged in age from 8–35 days post-maturation. We were unable to match male age within a given trial, but there was no difference in age between ornamented and non-ornamented males across trials (t_{28} = 1.13, P = 0.27). We also re-used 3 males that had not mated in a previous trial.

Statistical analyses – For both the two- and one-choice trials, we looked at the incidence of a female mating with the same versus different phenotype as her paternal phenotype. We then examined patterns of mating across paternal phenotype, diet, age, and family ID for the two-choice trials using two different analyses. First, we used a nominal logistic regression to examine variation in the likelihood of mating. The dependent variable was whether a female mated or not, and the independent variables were paternal phenotype, diet, and age. We tested multiple females from some families, and thus included family ID as a random effect. Second, we used a nominal logistic regression to examine patterns of mating among only those females who mated. The phenotype with which a female mated was the dependent variable. Paternal phenotype, diet, age, and family ID nested within paternal phenotype (to account for families sired by the same male) were the independent variables.

1.1.3 Female choice: Mate-choice copying

We tested whether learning influenced the expression of female mate choice by conducting a mate-choice copying experiment in which there were three distinct groups of females. The first comprised virgin females that had no opportunity to observe other females interacting with potential mates – these were the 'no exposure' females. The remaining groups were given the opportunity to observe either one or two different stages of reproductive interactions: (i) courtship and copulation – which we refer to as 'courtship-exposed' or (ii) copulation only – which we refer to as 'copulation-exposed'. Courtship-exposed females observed up to 30 minutes of interaction between an actor trio: an actor female, an ornamented male, and a non-ornamented male. Copulation-exposed females observed 30 minutes of copulation between an actor female and her chosen male phenotype-either an ornamented or non-ornamented male. These two categories represent potential interactions females may observe in the field. Mating trials with courtship-exposed and copulation-exposed females immediately followed their observation period (details to follow). Each actor female (unexposed female) was

ultimately observed by two observer females: (1) a courtship-exposed and (2) a copulation-exposed female. All individuals were field-collected as subadults from the grounds of the University of Mississippi greenhouse in Oxford, MS (Lafayette Co, USA) in the spring of 2008. They matured in the laboratory, ensuring their virgin status. Given that these individuals were collected from the field, however, we lack information on any female's paternal phenotype.

Prior to the start of all trials, we placed the actor female (unexposed female) in the mating arena with a filter paper substrate and six leaves for an hour, during which time she deposited pheromone-laden silk. The six leaves were subsequently used in pairs as a stimulus for male courtship during the subsequent mating trials (i) actor/unexposed female, (ii) courtship-exposed female, and (iii) copulation-exposed female. Following the hour pheromone deposition period, all but two leaves were removed from the mating arena and set aside until the appropriate trial (courtship-exposed mating trial or copulation-exposed mating trial). For courtship-exposed female observation trials, the observer female (age-matched to the actor female) was placed inside the mating arena in a small (~1.5 cm diameter) clear acetate barrier so that she could observe (visual and vibratory exposure) all female-male interactions, but could not physically interact with the actor trio. At the start of the trial, the actor female was reintroduced into the arena outside of the acetate barrier, followed by the simultaneous release of two aged-matched (± four days) males—one ornamented and one non-ornamented—into opposite ends of the arena. The actor female was allowed to interact with the two males simultaneously for 30 minutes or until copulation occurred, during which time the courtship-exposed female could observe. Immediately following a successful copulation we removed both the non-copulating male and the courtship-exposed female from the arena and placed an acetate barrier around the copulating pair. The copulation-exposed female was then added to the arena with the copulating pair so that she could observe the pair for 30 minutes.

Upon removing the courtship-exposed female from her observation arena, the exposed female was immediately (within 5 minutes) run through a two-choice mating trial with a novel ornamented and non-ornamented male. Protocol for these mating trials mimicked those of the actor mating trials. Similarly, copulation-exposed females were also immediately (within 5 minutes) run through a two- choice mating trial with a novel ornamented and non- ornamented male after their observation period. In both the courtship- and copulation-exposed mating trials, we placed two of the original six leaves that were laden with the actor female's silk to the arena to provide cues for male courtship.

For all mating trials—no exposure, courtship-exposed, copulation-exposed—we noted whether a female mated or not, and, if she did, with which male phenotype she mated. For the courtship-exposed and copulation-exposed trials we noted whether one or both observer females mated; if both mated, whether they mated with the same male type as each other; and, if they mated with the same or different male phenotype as the actor female.

Statistics—Age was a confounding factor in the Hebets and Vink (2007) study that showed an influence of juvenile experience on subsequent adult female mate choice, and age influenced mate choice in our initial set of mating experiments (see Results). Thus, we compared the ages of females across the three treatments (unexposed, courtship-exposed, copulation-exposed) and the outcome of the trial (mated with ornamented, mated with non-ornamented, no mating). We also included age as a variable in all following analyses.

The first set of analyses we ran included unexposed (actor), courtship-exposed, and copulation-exposed trials, and examined how patterns of mating varied among these three treatment groups. We first tested how the likelihood to mate varied with treatment and age. We used a nominal logistic model with whether or not a female mated as dependent variable. The independent variables were treatment (unexposed, courtship-exposed, copulation-exposed), female age, and the treatment × female age interaction. For those females who mated, we tested whether the phenotype with which a female mated varied with these same factors. To do so, we used a nominal logistic regression with mated phenotype (ornamented versus non-ornamented) as the dependent variable, and female age, and the treatment × female age interaction as the independent variables.

The second set of analyses included only exposed females, and looked at patterns of mating in courtship- versus copulation-exposed females. We used a nominal logistic regression to test if the likelihood for a female to mate varied with her treatment (courtship- versus copulation-exposed), age, and the phenotype observed mating. We then used a nominal logistic regression to test if the phenotype with which a female mated depended on treatment (courtship- versus copulation-exposed), age, and the phenotype observed mating.

The third set of analyses pooled the courtship- and

copulation-exposed females that mated (we found no differences in patterns of mating in the above analyses, see Results), and zeroed in on the specific factors determining the phenotype with which a female mated. This set of analyses allowed us to determine whether or not mate-choice copying occurred. If no mate-choice copying is occurring, the incidence of mating with the same vs. different phenotype as the actor female should not differ from a random 50:50 frequency. Because all females were age-matched, we did not include age in this analysis. To test this null hypothesis, we compared the distribution of choice of observer females (i.e., same versus different than actor), with the null expectation of mating 50:50 using a χ^2 analysis. Second, if mate-choice copying is occurring, we would predict that the phenotype with which an exposed female mates will match the phenotype she observed copulating. To test this prediction, we used a nominal logistic regression with mated phenotype as the response variable, and the following independent variables: male phenotype observed copulating, female age, and the male phenotype × female age interaction. Finally, we determined whether the likelihood of matching the observed phenotype differed with whether a female saw an ornamented versus non-ornamented male mate. To do so, we used a nominal logistic regression with the dependent variable: same/different as the observed phenotype, and the independent variables: male phenotype observed copulating, female age, and the male phenotype × female age interaction. A significant interaction term indicates a difference in the rate of copying that depends on whether a female was exposed to an ornamented versus non-ornamented male.

1.2 Patterns of genetic variation

The results from the behavioral experiments suggested strong assortative mating, but also the potential for mate copying to influence patterns of mating (See Results, section 2.1.1–2.1.3). Our next step then, was to use a molecular approach to determine the consequences for the above patterns on population genetic structure. Given that our results and those of Hebets and Vink (2007) (where experience appears to influence choice) contrast with those of Rutledge and Uetz (2014) (where experience appears not to influence choice), we were particularly interested to determine whether there are different patterns of correspondence between genotypic and phenotypic variation across phenotypically-pure vs. phenotypically- mixed populations.

Specimen collection – We collected mature *Schizocosa* spiders from numerous populations between April 2005 and May 2008 (Fig. 1; Online Appendix). Our efforts were primarily concentrated on populations used in previous studies characterizing female choice. In the Ohio Valley, we collected (i) non-ornamented males from a phenotypically-pure population in Kentucky (Giles Conrad Park, Boone County, KY; Fig. 1; Online Appendix); (ii) ornamented males collected from a phenotypically-pure population in Ohio (the Cincinnati Nature Center Rowe Woods, Clermont County, OH; Fig. 1; Online Appendix); (iii) ornamented males from a phenotypically-pure population in Kentucky (Devou Park, Kenton County, KY). The first two populations correspond to those populations in the Ohio River Valley used in prior studies establishing strong assortative mate choice (Stratton and Uetz, 1981, 1983, 1986). The third was included to determine whether phenotype or the potential barrier created by the Mississippi River influenced patterns of genetic variation. The three collection sites were within 35–50 km of one another (Fig. 1).

In Mississippi, we collected at the University of Mississippi Campus Greenhouse (Lafayette County, MS; Fig. 1; Online Appendix): (iv) non-ornamented males from the phenotypically-mixed population; and (v) ornamented males from the same phenotypically-mixed population. We greatly expanded upon previous sampling from this population (Hebets and Vink, 2007).

We stored two legs (usually right legs III and IV) from each individual in 100% ethanol at -20 °C for subsequent DNA extraction. Female *Schizocosa* associated with ornamented and non-ornamented males are morphologically identical, so only adult males were collected to ensure proper identification. We extracted total DNA from the two legs of each specimen using DNeasy Tissue Kits (Qiagen, Valencia, CA) and ZR Genomic DNA II Kit (Zymo, Orange, CA).

Mitochondrial sequence data—We determined if ornamented and non-ornamented males formed a monophyletic group, suggestive of either a lack of divergence or very recent divergence, by examining sequence variation at a portion (1,200 bp) of cytochrome oxidase subunit I (COI). This marker has been examined in previous studies of intra- and inter-species studies of wolf spiders (Colgan et al., 2002; Vink and Paterson, 2003; Chang et al., 2007; Hebets and Vink, 2007; Hebets et al., 2013). We examined a total of 25 individuals from each of (i–v) above. We also generated sequences for 26 spiders from 24 additional collecting localities, and obtained 31 sequences from GenBank (Online Appendix). These additional sequences included ornamented and non-ornamented males, and 13 other species in the ge-

nus *Schizocosa* (Online Appendix). All species in the *S. ocreata* clade (the major clade in the genus containing ornamented and non-ornamented males) were represented along with additional outgroups in the genus.

We used the primers C1-J-1718-spider and CI-N-2776-spider (Vink et al., 2005) to amplify a ~1,200 bp region of COI via polymerase chain sequencing reaction (PCR). We performed all sequencing reactions in a Mastercycler (Eppendorf) thermal cycler. We used the following cycling parameters: 35 cycles of 94°C denaturation (30 s), 48°C annealing (30 s), and 72°C extension (60 s), with an initial 94°C denaturation (3 min), and 72°C final extension (5 min). We purified PCR products using either ExoSap or QIAGEN PCR purification kit (Qiagen, Valecia, CA). We sequenced the purified product in both directions at the High Throughput Genomics Unit at the University of Washington or Idaho State University Molecular Research Core Facility. We edited sequences in ContigExpress (Vector NTI suite, Informax), aligned them in BioEdit (Hall, 1999) and confirmed them manually by visual inspection. The amplified DNA sequences coded as expected, and aligned with additional coding sequences of *Schizocosa* previously deposited in GenBank, thus verifying that the fragments we amplified were of the coding region found in the mitochondria and not nuclear pseudogene copies.

We merged identical sequence haplotypes using TCS1.21 (Clement et al., 2000) before performing phylogenetic analyses. We used Akaike information criterion (Posada and Buckley, 2004) in MrModeltest version 2.3 (Nylander, 2008) implemented in PAUP* version 4.0b10 (Swofford, 2002) to select the best model of nucleotide evolution and estimate the parameters for the chosen model. The model of evolution selected for the data was a special case of the general time reversible (GTR) model (Taveré, 1986) with among-site rate heterogeneity (GTR+Γ). We implemented Bayesian inference of phylogeny in MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003) using GTR model of evolution with the gamma distribution of rate heterogeneity of 1.5197. We ran two independent analyses, each with four heated chains, sampling every 5,000th tree, for 2.5× 10^6 generations at which point the average standard deviation of split frequencies had dropped below 0.008, indicating convergence. We used MrBayes to construct majority rule consensus trees, discarding the first 25% of trees as burn-in.

We calculated the number of haplotypes, haplotype diversity (h) and nucleotide diversity (π) for the Ohio Valley and mixed Mississippi localities in DnaSP version 4.90 (Rozas et al., 2003).

Multi-locus microsatellite data — We examined fine-scale population structure of the five focal groups using variation in microsatellites. We generated multi-locus genotypes at 13 microsatellite loci (see Supplemental Material) for 292 individuals. These individuals included 136 from the three focal groups in the phenotypically-pure Ohio Valley localities – 49 ornamented males from OH, 44 non-ornamented males from KY, and 43 ornamented males from KY. The remaining 139 individuals were from the phenotypically-mixed Mississippi locality – 40 ornamented males and 99 non-ornamented males.

We amplified 13 microsatellite loci using polymerase chain reaction (for marker development and characterization, see Supplemental Material). DNA fragment analysis was performed at the University of Illinois-Urbana-Champaign Biotechnology Center on the ABI Prism 3730xl Analyzer and size calling was performed manually using GeneMapper v3.7 (Applied Biosystems).

We calculated allelic diversity, and levels of observed and expected heterozygosities in Genepop v 3.4 (Raymond and Rousset, 1995; Rousset, 2008). We tested for linkage disequilibrium between all pairs of loci within and among localities in Genepop v 3.4. Due to the large number of pairwise comparisons among markers, we took a step-up false discovery rate (FDR) method (Benjamini and Hochberg, 1995) to increase the power of our significance testing and control for type II errors. We also calculated F_{ST} values among the five focal groups of individuals in Arlequin (Excoffier et al., 2005). All analyses in Genepop were performed with the following parameters: dememorization = 10,000, batches = 1,000, iterations per batch = 10,000.

Hardy-Weinberg expectations – We calculated F_{IS} (Weir and Cockerham, 1984) using the Markov Chain method implemented in Genepop. If ornamented and non-ornamented males represent genetically distinct groups, we predicted that we would find Hardy-Weinberg disequilibrium in the pooled data set within each geographic region with no deviation from Hardy-Weinberg expectations when the data set for each phenotype is analyzed separately.

STRUCTURE analyses – We utilized a Bayesian clustering algorithm implemented in the program STRUCTURE (Version 2.2; Pritchard et al., 2000) to infer population structure. We ran 20 simulations for each putative number of genetic clusters (K = 1–10). For each simulation, we ran 500,000 replicates of the

MCMC following a burn-in period of 500,000 replicates. We used a model of admixture, and allowed allele frequencies to be correlated among subpopulations. In cases where population structure is potentially subtle, these parameters are thought to provide the best resolution (Falush et al., 2003). To determine the most likely number of genetic clusters, we evaluated the magnitude of change in Ln (P) between each K and determined the largest change in ΔK using the program Structure Harvester (Evanno et al., 2005). We used values of q, the proportion of an individual's sampled genome that is characteristic of each genetic cluster to assign individuals to genetic clusters. Values of $q > 0.7$ indicated unambiguous assignment of individuals to a given cluster. Values of $q < 0.7$ for all clusters indicated ambiguous assignments. We determined the percentage correct unambiguous assignments to genetic clusters as the proportion of individuals of a given phenotype that were unambiguously assigned to the genetic cluster corresponding to their phenotype. Using these same criteria, an incorrect assignment was an unambiguous assignment to a genetic cluster that corresponded to a different phenotype.

The ΔK method identifies large-scale population genetic structure, but further analyses are often needed to detect substructure (Evanno et al., 2005). Thus, we reran structure analyses for the genetic cluster containing the non-ornamented males from the Ohio Valley, and the two male phenotypes from Mississippi.

2 Results

2.1 Variation in choice and signals

2.1.1 Male sexual signals: Patterns of inheritance and diet

The number of total offspring born in each family was 54.8 ± 3.0 (mean ± *SE*). Of these, the number of male offspring surviving to adulthood was 7.8 ± 0.6 (mean ± *SE*). The majority of males sired offspring that matched their phenotype (84%, n = 55 total clutches). However, one non-ornamented male sired one ornamented offspring (3%; n = 31 non-ornamented sires). Also, five ornamented males sired clutches with primarily large-brushed males, but also sired one or more male offspring with either no brushes or extremely reduced brushes (29%; n = 17 total ornamented sires). Of the three ornamented males that sired two clutches (with different females), two sired purely ornamented offspring and one sired both non-ornamented and ornamented males (Fig.2).

While we observed that not all sons matched the phenotypes of their fathers, phenotype did not vary with diet (paternal phenotype $\chi^2 = 455.5$, $P < 0.0001$, diet $\chi^2 = 2.4$, $P = 0.29$). While the ornamented offspring sired by the non-ornamented father was reared on a high diet, the non-ornamented offspring sired by ornamented fathers were on a mix of diets: there were 3 reduced/absent brushes in the high diet treatment group, and 4 in the low diet treatment group.

2.1.2 Female choice: Patterns of inheritance, diet, and age

All females that mated in the two-choice trials (n = 44; 25 ornamented females, 19 non-ornamented females) and one-choice trials (n = 8: 3 ornamented females, 5 non-ornamented females) mated with a male matching their paternal phenotype. Family ID influenced the overall likelihood of mating (Table 1). Neither diet nor age influenced choice, but paternal phenotype did (Table 2).

2.1.3 Female choice: Mate-choice copying

Results for the first set of analyses including unexposed and exposed females were as follows. The likelihood of mating did not vary with treatment (unexposed, courtship-exposed or copulation-exposed) (Fig. 3) or age (Treatment: $\chi^2_{1,3} = 0.2$, $P = 0.55$; Age: $\chi^2_{1,3} = 0.1$, $P =$

Fig. 2 Phenotypes of the *Schizocosa* offspring sired by males of known phenotype (ornamented or non-ornamented) from the quantitative genetics breeding design

The bars indicate the proportion of clutch that had no brushes, partial brushes, and full brushes.

0.12; Treatment × age: $\chi^2_{1,3}$ = 1.6, P = 0.90). For those trials in which a female mated, the likelihood of mating with an ornamented versus non-ornamented male did not vary with treatment, but did vary with age (Fig. 4) (Treatment: $\chi^2_{2,5}$ = 0.3, P = 0.85; Age: $\chi^2_{1,5}$ = 15.0, P = 0.0001; Treatment × age: $\chi^2_{2,5}$ = 0.6, P = 0.74). See Table 3 for the female ages and trial outcome for each treatment.

Table 1 The effects of paternal phenotype, diet, age, and family ID on the overall likelihood for *Schiozocosa* females to mate when presented simultaneously with an ornamented and non-ornamented male

Factor	*df*	χ^2	*P*
Paternal phenotype	1	0.0	1.0
Female diet	1	0.0	0.4445
Female age	1	0.3	0.5955
Family ID (nested within paternal phenotype)	29	53.7	**0.0035**

Significant *P*-values are highlighted in bold.

Table 2 The effects of paternal phenotype, diet, age, and family ID on whether a *Schiozocosa* female mated with an ornamented versus non-ornamented male in two-choice trials

Factor	*df*	χ^2	*P*
Paternal phenotype	1	47.4	< **0.0001**
Female diet	1	0.0	1.0
Female age	1	0.0	1.0
Family ID (nested in paternal phenotype)	22	0.0	1.0

Significant *P*-values are highlighted in bold.

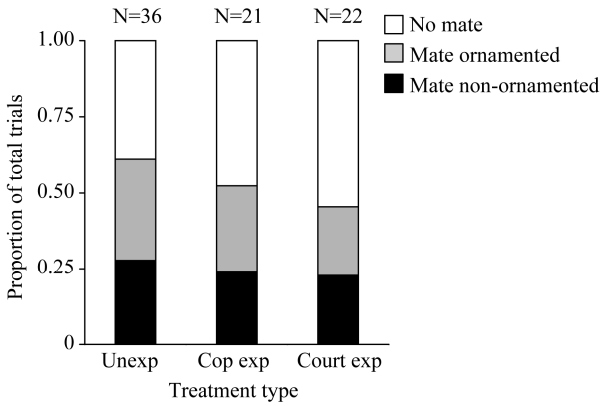

Fig. 3 The outcome of two-choice mating trials for female *Schizocosa* wolf spiders that were unexposed, courtship-exposed, or copulation-exposed

Females mated with an ornamented male, non-ornamented male, or not at all.

Results for the second set of analyses included only exposed females, and compared patterns of mating between courtship- versus copulation-exposed females. First, we found that the likelihood to mate did not depend on treatment (courtship- versus copulation-exposed), age, or the phenotype a female observed mating (Treatment: $\chi^2_{1,3}$ = 0.3, P = 0.61; Age: $\chi^2_{1,3}$ = 0.1, P = 0.74; Phenotype observed: $\chi^2_{1,3}$ = 1.6, P = 0.21). Of those females who mated, whether a female mated with an ornamented or non-ornamented male did not depend on treatment or age, but did vary with the male phenotype she observed mating previously (Table 4).

Results for the third set of analyses included courtship- and copulation-exposed females pooled. First, we compared patterns of mating of these exposed females (same vs. different as compared to actor) to patterns expected by chance (50:50). We note that we had 17 unexposed females that mated, and we exposed 33 females (one less copulation-exposed than courtship-exposed female). In every case in which both the courtship-exposed and copulation-exposed females mated, they mated with the same phenotype as each other (n = 6 pairs). Of the exposed females, 21 mated in subsequent trials, and 15 of these mated with the same phenotype as the actor (71%; Fig.5). This 71% was significantly greater than the 50% null expectations χ^2 = 3.98, P = 0.046. Second, we found that the phenotype with which a female mated depended upon the phenotype she ob-

Table 3 Mean ages (days post-maturation) for females from the three treatments and three mating outcomes (mated with an ornamented male, non-ornamented male, or no mating)

Treatment	Ornamented	Non-ornamented	No mating
Unexposed	10.6 ± 2.1	22.2 ± 2.1	19.5 ± 1.8
Copulation-exposed	13.2 ± 3.0	21.0 ± 2.7	20.8 ± 2.1
Courtship-exposed	15.0 ± 3.6	21.4 ± 3.6	20.3 ± 2.3

Table 4 For female *Schizocosa* wolf spiders who were exposed to courtship and/or copulation and subsequently mated: The phenotype with which a female mates as a function of her treatment (courtship- versus copulation-exposed), her age, and the phenotype she observed mating

Factor	*df*	χ^2	*P*
Treatment	1,3	0.1	0.8171
Age	1,3	1.5	0.2174
Phenotype observed	1,3	4.8	**0.0288**

Significant *P*-values are highlighted in bold.

served mating, her age, and their interaction (Table 5). The effect of phenotype observed indicates that the female mated more frequently with the male phenotype she observed copulating. The effect of age was due to the fact that older females mated more often with non-ornamented males (Fig. 4). Further, the significant age × phenotype observed interaction term indicates a difference in the likelihood of mating with the male phenotype observed that depended on age, with age making a difference only in the group of females exposed to non-ornamented males (mean ± *SE*, exposure phenotype/mated phenotype: ornamented/ornamented 14.0 ± 2.5; ornamented/non-ornamented 17.0 ± 4.4; non-ornamented/ornamented 15 ± 4.3; non-ornamented/non-ornamented 22.8 ± 1.5). Finally, whether an exposed female mated with the same or different phenotype as the actor depended on the male phenotype she observed mating (Table 6). Females that observed ornamented males mating were more likely to mate with ornamented males; but, females that observed non-ornamented males were as likely to mate with ornamented males as they were to mate with non-ornamented males (Fig. 5).

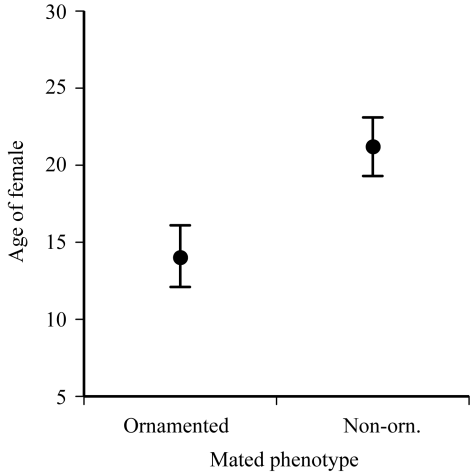

Fig. 4 Ages of female *Schizocosa* wolf spiders that mated with either an ornamented or non-ornamented male in two-choice mating trials

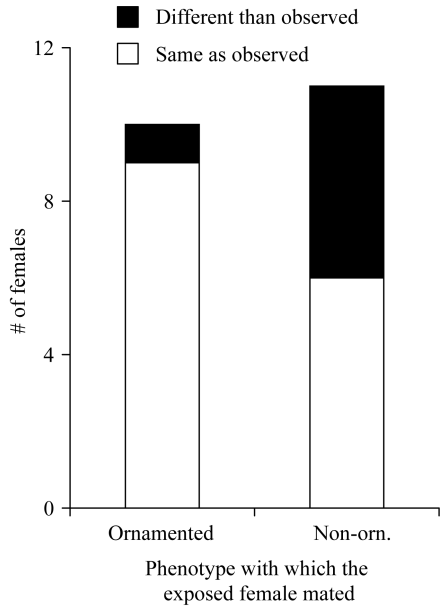

Fig. 5 Phenotype with which courtship-exposed and copulation-exposed female *Schizocosa* wolf spiders mated in two-choice mating trials with ornamented and non-ornamented males

A female mated with either the same or different phenotype to which she was exposed.

2.2 Patterns of genetic variation

Mitochondrial sequence data — Ornamented and non-ornamented males from all localities sampled formed a monophyletic clade relative to the rest of the genus (Fig. 6). We found no evidence for reciprocal monophyly or any delineation between ornamented and non-ornamented individuals, nor between southern localities (including the phenotypically-mixed Mississippi localities) and northern localities (including the phenotypically-pure localities in the Ohio Valley; Fig. 6). In contrast, the most closely related species to ornamented and non-ornamented males (*S. ocreata* clade: *S. uetzi*, *S. stridulans*, *S. crassipes*, *S. floridana*) are reciprocally monophyletic from their most closely related species (Fig. 6). Genetic diversity was comparable among geographic localities (Ohio Valley and Mississippi; Table 7).

Table 5 For female *Schizocosa* wolf spiders who were exposed (courtship- and copulation-exposed females pooled) and subsequently mated: The phenotype with which a female mates as a function of the phenotype she observed mating, her age, and their interaction

Factor	*df*	χ^2	*P*
Phenotype observed	1,3	5.2	**0.0232**
Age	1,3	6.7	**0.0097**
Phen obs. × age	1,3	5.2	0.0224

Significant *P*-values are highlighted in bold.

Table 6 For female *Schizocosa* wolf spiders who were exposed (courtship- and copulation-exposed females pooled) and subsequently mated: Whether a female mated with the same versus different phenotype as the male she observed mating as a function of the observed phenotype, age, and their interaction

Factor	*df*	χ^2	*P*
Phenotype observed	1,3	1.8	0.1824
Age	1,3	5.2	0.0224
Phen obs. × age	1,3	6.7	**0.0097**

Significant *P*-values are highlighted in bold.

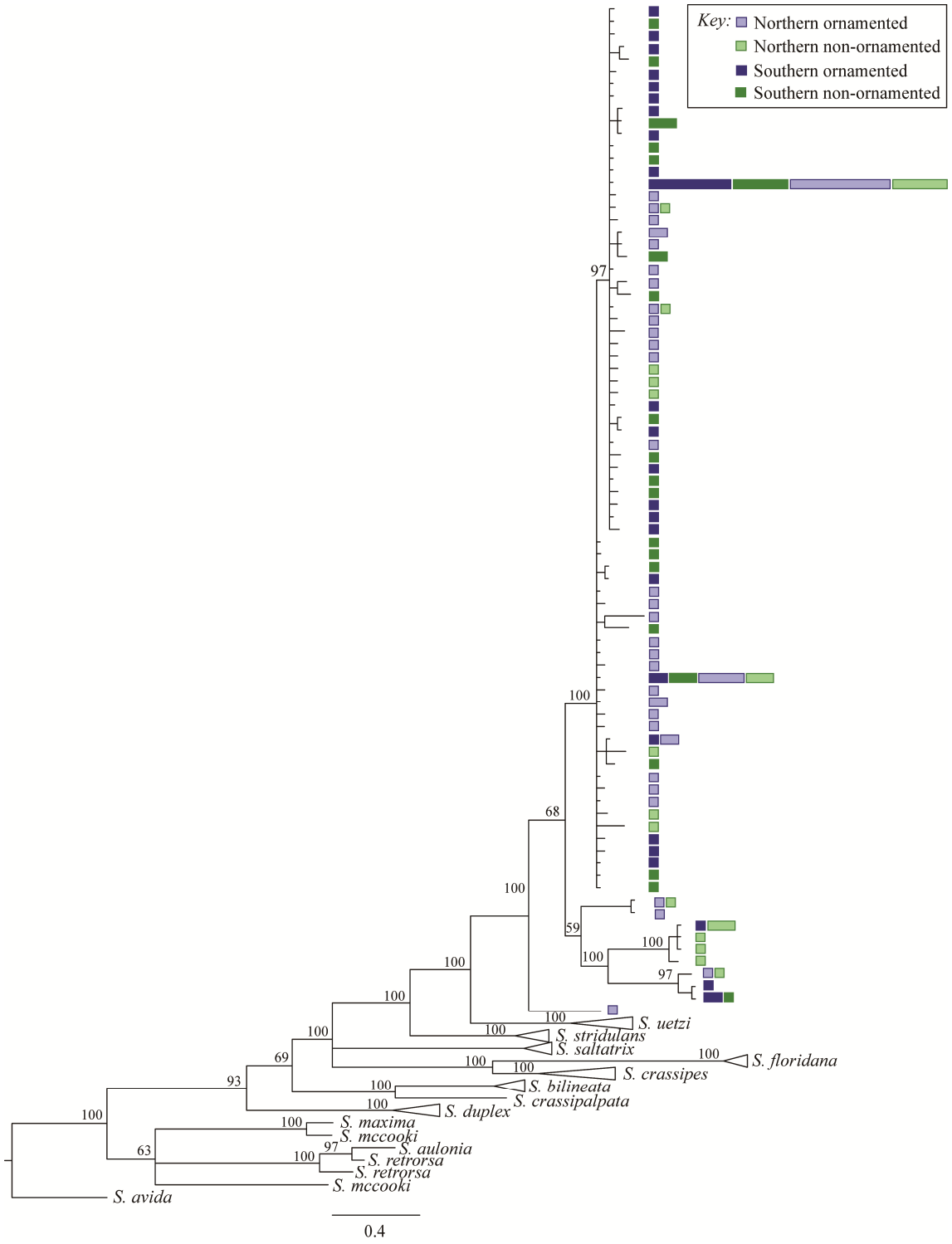

Fig. 6 Bayesian consensus tree based on unique mitochondrial (COI) haplotypes for ornamented and non-ornamented spiders across the collection localities as well as additional species in the genus *Schizocosa* (collapsed into single branch tips)

The individuals represented by each unique haplotype is indicated to the right of the phylogeny, with blue colors indicating individuals with brushes, and green indicated individuals without brushes. Branch lengths are proportional to the expected number of substitutions per site, indicated by the scale bar. Posterior probabilities represented with numbers at each node. Accession numbers for sequence data deposited in genbank: KT963556-KT9637.

Multi-locus microsatellite data— Of the 13 microsatellite loci that we tested, one pair was in significant linkage disequilibrium across localities (C101 and B2), and so we removed one (B2) from further statistical analysis. We found no further evidence of linkage disequilibrium between pairs of loci within each of the five

focal groups of individuals for the remaining 12 markers. Four markers (D12, D104, C107, D107) showed particularly high levels of FIS across groups, potentially indicative of the presence of null alleles or some other factor confounding analyses, so we removed these markers from further analyses (see Supplemental Material for analyses with these five markers). Thus, our final analyses included eight of the original 13 microsatellite markers. We obtained multi-locus genotypes for 296 individuals – of these, we had high amplification success for 271 individuals (92% of these amplified for at least seven of eight loci)—we included these 271 individuals in further analyses. Levels of F_{ST} among the five focal groups varied from 0.01–0.04 (Table 8).

In the phenotypically-pure localities, deviations from Hardy-Weinberg equilibrium did not correspond to male phenotype; those loci where the observed homozygosity did not meet the expected values for the pooled data also exhibited homozygote excess within one or more localities (Table 9). In the phenotypically-mixed locality, deviations from Hardy-Weinberg equilibrium also did not correspond to male phenotypes; further, those loci exhibiting homozygote excess in the pooled sample also exhibited homozygote excess within either one or both male phenotypes (Table 10).

Table 7 Genetic diversity in mitochondrial sequences (COI) in the ornamented and non-ornamented *Schizocosa* from phenotypically-pure localities (Ohio Valley), and the phenotypically-mixed locality (Mississippi)

Region	*n*	H	h (mean ± SE)	π, (mean ± SE)
Ohio Valley	75	31	0.887 ± 0.003	0.00727 ± 0.00013
Mississippi	50	20	0.868 ± 0.005	0.00564 ± 0.00018

n = number of individuals; H = number of haplotypes, h = haplotype diversity, π = nucleotide diversity.

Table 8 Pairwise F_{ST} values between the 5 focal groups of ornamented and non-ornamented *Schizocosa* wolf spiders: Three phenotypically-pure localities from the Ohio Valley and a phenotypically-mixed locality in Mississippi (comprised of a group of ornamented individuals and a group of non-ornamented individuals)

	Ohio Valley localities			Mississippi locality	
	Non-orn (KY)	Orn (OH)	Orn (KY)	Non-orn	Orn
Non-orn (KY)	--				
Orn (OH)	0.035	--			
Orn (KY)	0.031	0.008	--		
Non-orn (MS)	0.037	0.031	0.023	--	
Orn (MS)	0.027	0.019	0.015	0.009	--

All values were non-significant.

Table 9 Measures of genetic diversity derived from multi-locus microsatellite genotypes for *Schizocosa* individuals from the Ohio Valley localities. A. All individuals (ornamented, *n* = 94) and non-ornamented (*n* = 47) *Schizocosa*. B. Non-ornamented individuals from Giles Conrad Park (KY; *n* = 47). C. Ornamented individuals from Rowe Woods (OH; *n* = 50). D. Ornamented individuals from Devou Park (KY; *n* = 44)

A. Ohio Valley: Ornamented and non-ornamented

Locus	*n*	N_a	H_e	H_o	F_{IS} (WandC)	*P*
A3	137	14	0.57	0.23	0.45	*< 0.0001*
D4	135	28	0.27	0.07	0.22	*< 0.0001*
C104	137	6	0.66	0.36	0.48	*< 0.0001*
D6	139	10	0.50	0.33	0.25	*< 0.0001*
A4	138	25	0.18	0.09	0.10	0.1201
C116	139	35	0.09	0.10	-0.02	0.6217
C101	140	13	0.33	0.25	0.08	*< 0.0001*
C12	140	13	0.33	0.25	0.08	0.0199

B. Ohio Valley: Kentucky non-ornamented

Locus	*n*	N_a	H_e	H_o	F_{IS} (WandC)	*P*
A3	43	9	0.47	0.35	0.31	*< 0.0001*
D4	41	22	0.93	0.80	0.34	*< 0.0001*
C104	42	5	0.57	0.17	0.27	0.0768
D6	44	7	0.75	0.64	0.15	0.0777
A4	44	18	0.86	0.75	0.04	0.4904
C116	42	20	0.91	0.86	-0.06	0.4080
C101	43	9	0.51	0.53	0.12	0.0862
C12	44	8	0.70	0.68	0.03	0.3686

C. Ohio Valley: Cincinnati Nature Center (OH) ornamented

Locus	*n*	N_a	H_e	H_o	F_{IS} (WandC)	*P*
A3	49	11	0.82	0.57	0.31	*< 0.0001*
D4	48	22	0.92	0.60	0.34	*< 0.0001*
C104	49	5	0.65	0.49	0.27	0.0768
D6	48	9	0.58	0.50	0.15	0.0777
A4	47	16	0.87	0.85	0.01	0.4904
C116	49	23	0.90	0.96	-0.06	0.4080
C101	48	13	0.73	0.65	0.12	0.0862
C12	49	10	0.78	0.76	0.03	0.3686

D. Ohio Valley: Devou park (KY) ornamented

Locus	*N*	N_a	H_e	H_o	F_{IS} (WandC)	*P*
A3	41	9	0.80	0.34	0.60	*< 0.0001*
D4	41	22	0.93	0.80	0.14	0.0076
C104	41	5	0.63	0.32	0.51	*< 0.0001*
D6	42	7	0.52	0.36	0.34	0.0030
A4	42	18	0.90	0.88	0.03	0.5548
C116	42	20	0.86	0.93	-0.07	0.8774
C101	42	8	0.74	0.69	0.09	0.1668
C12	42	8	0.71	0.62	0.16	0.0602

Shown are the number of individuals analyzed per locus (note that not all loci amplified for all individuals), the number of alleles (N_a), expected heterozygosities (H_e), observed heterozygosities (H_o), F_{IS} and *P*-values from Hardy-Weinberg test for homozygote excess (Weir and Cockerham, 1984). Italicized *P*-values are those remaining significant after a sequential Bonferroni correction.

Table 10 Measures of genetic diversity derived from multi-locus microsatellite genotypes for *Schizocosa* individuals from the phenotypically-mixed locality in Mississippi. A. All individuals (ornamented, *n* = 48; non-ornamented, *n* = 108). B. Ornamented males only. C. Non-ornamented males only

A. Mississippi: Ornamented and non-ornamented

Locus	*n*	N_a	H_e	H_o	F_{IS} (WandC)	*P*
A3	145	19	0.41	0.12	0.31	< **0.0001**
D4	150	38	0.49	0.05	0.44	< **0.0001**
C104	155	12	0.48	0.35	0.26	< **0.0001**
D6	117	10	0.54	0.33	0.34	0.0009
A4	145	21	0.40	0.12	0.34	< **0.0001**
C116	156	25	0.19	0.09	0.11	0.0283
C101	144	13	0.40	0.39	-0.01	0.4550
C12	144	13	0.40	0.39	-0.01	0.1019

B. Mississippi: Ornamented

Locus	*n*	N_a	H_e	H_o	F_{IS} (WandC)	*P*
A3	33	10	0.82	0.42	0.23	< **0.0001**
D4	38	26	0.92	0.45	0.39	0.0010
C104	39	7	0.62	0.49	0.28	< **0.0001**
D6	34	7	0.62	0.47	0.26	0.0671
A4	39	12	0.85	0.87	0.47	< **0.0001**
C116	39	17	0.90	0.77	0.09	0.1043
C101	33	7	0.36	0.36	-0.03	0.4511
C12	36	7	0.61	0.56	-0.06	0.6271

C. Mississippi: Non-ornamented

Locus	*n*	N_a	H_e	H_o	F_{IS} (WandC)	*P*
A3	94	18	0.88	0.68	0.49	< **0.0001**
D4	96	29	0.95	0.57	0.52	< **0.0001**
C104	96	9	0.65	0.49	0.23	0.0134
D6	73	8	0.66	0.51	0.29	0.0193
A4	90	17	0.84	0.46	-0.03	0.1966
C116	97	18	0.90	0.84	0.16	0.0546
C101	90	8	0.41	0.42	0.02	0.8314
C12	91	9	0.58	0.63	0.12	0.0710

Shown are the number of individuals analyzed per locus (n; note that not all loci amplified for all individuals), the number of alleles (N_a), expected heterozygosities (H_e), observed heterozygosities (H_o), F_{IS} and P-values from Hardy-Weinberg test for homozygote excess. Italicized P-values are those remaining significant after a sequential Bonferroni correction.

STRUCTURE analyses – The Δ*K* method indicated that K = 2 was the most likely number of populations for the total sample, which had a mean ln(*p*) = -8145. Visual inspection of STRUCTURE output for both K = 2 and K = 3 indicates differentiation between the non-ornamented and ornamented males in the phenotypically-pure population, but not between ornamented and non-ornamented males from the phenotypically-mixed population (Fig. 7 A, B). When K = 3 was forced on the entire sample, we found high rates of unambiguous assignment to the associated genetic clusters for the phenotypically pure populations (78% unambiguous assignment to the pure non-ornamented cluster for the non-ornamented males in the Ohio Valley; 92% and 95% to the pure ornamented cluster for the two sets of ornamented males in the Ohio Valley). The rate of unambiguous assignment to its own cluster was lower for the Mississippi population (44% for the ornamented males, 64% for non-ornamented males).

Inspection of the STRUCTURE output for K = 2 for the analyses of the non-ornamented males in the Ohio Valley and both male phenotypes from Mississippi suggest differentiation between the disparate geographic locations, but not between phenotypes in the mixed population (Fig. 7 C). When K = 2 was forced on the subpopulation containing the non-ornamented males from the Ohio Valley and both male phenotypes from Mississippi, we found high rates of unambiguous assignment of the non-ornamented males from the Ohio Valley to their respective cluster (80%). We found lower levels of unambiguous assignment of males from Mississippi to their own cluster (60% for ornamented, 46% for non- ornamented), but also low levels of assignment to the other cluster (20% for ornamented, 38% for non-ornamented).

3 Discussion

We found that a complex set of factors likely determines variation in the action and outcome of sexual selection in a group of ornamented and non-ornamented wolf spiders. Using a quantitative genetics breeding experiment, we found a strong genetic basis to male phenotype and mate choice in the phenotypically-mixed population in Mississippi, as has been found previously in the phenotypically-pure populations in the Ohio Valley (Stratton, 1983, Stratton and Uetz, 1986). We found no support that diet influences either male phenotype or mate choice in the mixed population. However, younger females tended to mate with ornamented males, and mate-choice copying likely influences patterns of mating. Examining the correspondence between genetic and phenotypic variation across both locations, we find genetic distinction between male phenotypes in the Ohio Valley where learning does not influence choice of ornamented versus non-ornamented males. However, we find no genetic distinction between male phenotypes in the mixed population in Mississippi where learning appears to play a role in mate choice.

We found a strong genetic basis on male phenotype in the mixed Mississippi population. Males tended to have the same phenotype as their father, except in a few

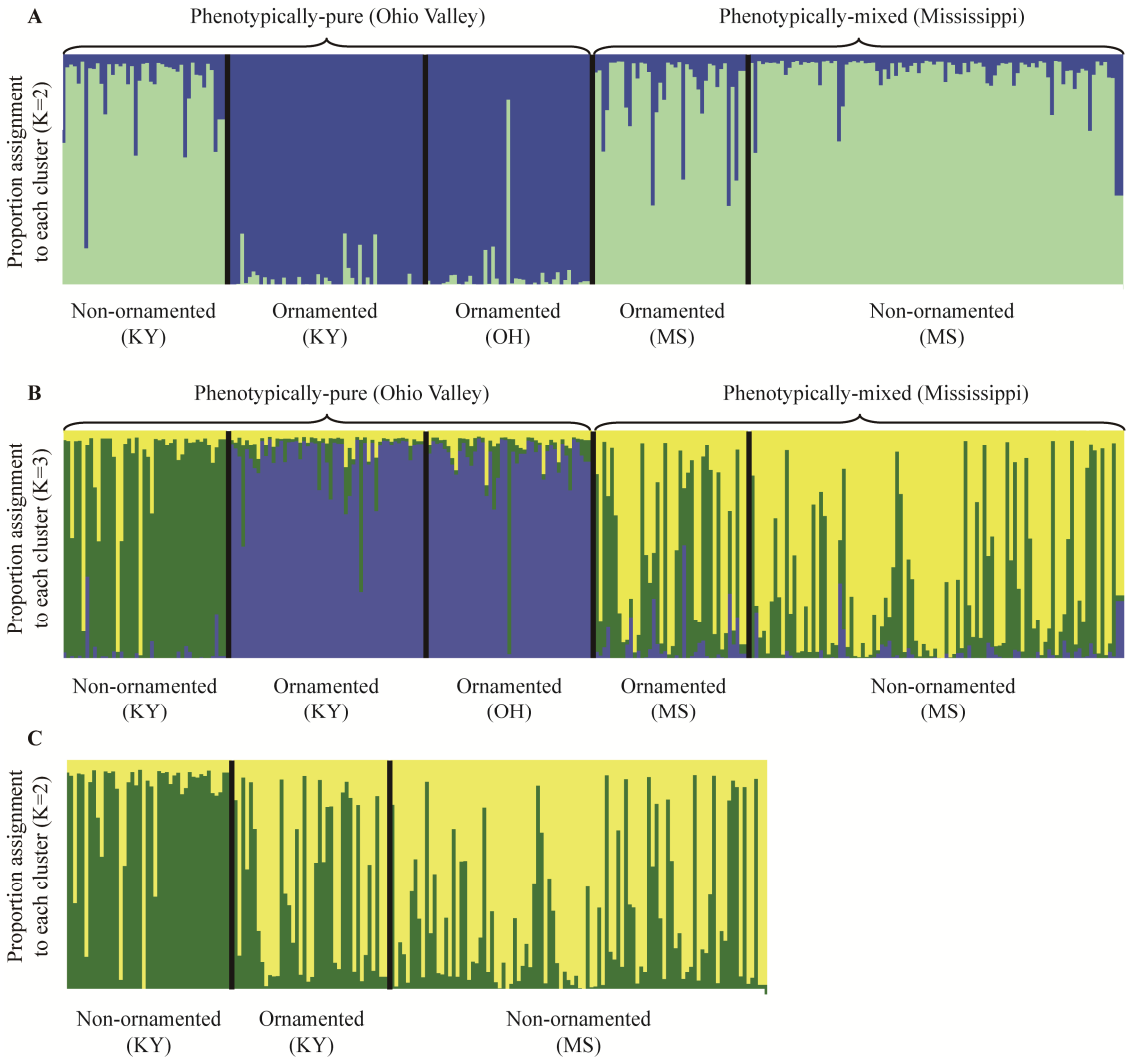

Fig. 7 Representation of the population structure of ornamented and non-ornamented male *Schizocosa* (from phenotypically-pure localities in the Ohio Valley and a phenotypically-mixed locality in Mississippi) generated by the program STRUCTURE

A. The selected number of populations is set to K = 2. Each vertical bar represents a single individual and the proportion of assignment to each genetic cluster is equal to the fraction of color in each bar (blue and light green). **B**. The selected number of populations is set to K = 3, the proportion of assignment to each genetic cluster is equal to the fraction of color in each bar (blue, green, and yellow). **C**. Population structure of the Ohio Valley non-ornamented with Mississippi ornamented and non-ornamented with the selected number of populations set to K = 3. The proportion of assignment to each genetic cluster is equal to the fraction of color in each bar (green and yellow).

notable cases where some families show male offspring exhibiting no brushes, partial brushes and full brushes. The partial brushes resemble hybrid phenotypes resulting from forced copulation among populations in the Ohio Valley (Stratton, 1983, Stratton and Lowrie, 1984). We found no variation in male phenotype across diet treatments, and so we interpret variation in brush size in some families as an indication of mating among historically ornamented and non-ornamented lineages.

We also found a strong genetic basis for female mate choice in the mixed Mississippi population. Females from our breeding experiment—where mate-choice learning was not possible—mated with males that matched their father's phenotype. While male-male competition may factor into mating outcome in the two-choice trials, previous work suggests little influence of male-male competition on the outcome of mating trials (Scheffer et al., 1996). Additionally, our one-choice trials showed the same pattern of assortative mating as our two-choice trials. We also found genetic variation in the likelihood of a female to mate, with some families being more likely to copulate than others. The potential underlying causes of this variation are many, including variation in: the motivation to mate, female mate selectivity, and

responses females elicit from males. Genetic variation in mate choice behavior is commonly assumed in many models of sexual selection (Kokko et al., 2002), and has been detected in a number of case studies (Chenoweth and Blows, 2006; Prokuda and Roff, 2014; Fowler-Finn and Rodríguez, In Press). It can play an important role in patterns of variation in traits (Roff and Fairbairn, 2014), and thus should be considered as a potential factor contributing to genetic and phenotypic variation observed in this group of *Schizocosa* wolf spiders. Furthermore, if females that are more likely to mate copy are also overall more likely to mate, the influence of learning could have a stronger effect on patterns of choice within the population.

Even when mate choice has a strong genetic basis, associations between genetic and phenotypic variation can be disrupted by environmentally-induced plasticity (Verzijden et al., 2012), and also potentially by life-history based changes in choice. While we found no plasticity in female choice due to diet, choice did vary with age. Younger females tended to mate more frequently with ornamented males. Furthermore, the influence of being exposed to a mating male (i.e. mate-choice copying) also appeared to depend on age. Thus, age could play a significant role not only in terms of choice over a female's lifetime, but also in the strength of the influence of social experience, influencing patterns of mating in complex ways.

The social context in which mate selection takes places can have profound implications for the action and outcome of sexual selection (West-Eberhard, 1983, West-Eberhard, 2014), not the least of which is the opportunity for mate choice copying to result in patterns of choice that differ from genetically-based preferences (Whitte et al., 2015). Social context varies dramatically between the Ohio Valley location where females encounter either only ornamented males or only non-ornamented males, and the Mississippi location where females encounter both male phenotypes and population densities can reach three individuals/100 cm^2 (Fowler-Finn and Hebets, 2011). Interestingly, we have evidence that learning from social experience at the juvenile stages influences patterns of female choice only in the Mississippi location (Hebets and Vink, 2007; Rutledge and Uetz, 2014). Here, we show that social experience during the adult stage can also affect mate choice in the form of mate-choice copying in the Mississippi location. Even when we accounted for the influence of age, we still found an effect of the phenotype a female observed mating on her mate choice decisions. Furthermore, similar to Hebets and Vink (2007), the phenotype of male with which a female had experience affected patterns of mating. Females that observed ornamented males mating were more likely to mate with ornamented males, but females who observed non-ornamented males were equally likely to mate with either phenotype. Given that there is variation in brush size within the population, it would be interesting to explore how the size of brushes influences the magnitude of this effect.

The difference in the effect of learning on mate choice between the Ohio Valley and Mississippi locations could evolve as a result of variation between the locations in the costs of mating with males that deviate from a female's paternal phenotype, encounter rates of different male phenotypes, or a plethora of other factors. Regardless of how it evolves, the consequences of mate-choice learning can be profound. While learning generally is thought to increase rates of divergence among populations (Dukas, 2013), our results suggest that learning may contribute to a weakening of assortative mating indicated by a lack of genetic distinction between phenotypes (this study; Deng et al., 2014). Given the patterns of genetic and phenotypic variation we observed in the Ohio Valley and Mississippi, it is even possible that learning contributes to the maintenance of genetic variation in Mississippi. Interestingly, our results suggest that social experience may eventually lead to the fixation of ornamented male phenotypes in the Mississippi population. While there have been small fluctuations in the proportion of each phenotype in the Mississippi location, five years of data show ornamented males remaining at a proportion of ~60% in the population (Deng et al. 2014; Fowler-Finn pers. Obs.), but this is a process that likely takes many generations. Furthermore, the apparent advantage that social experience confers to ornamented males may be balanced by an advantage of non-ornamented males with older females.

Another potential social factor that could influence patterns of reproductive success in the phenotypically-mixed population is multiple male mountings. Insemination by both male phenotypes is not likely to occur in the Ohio Valley, where populations contain either purely ornamented or purely non-ornamented males. However, in two-choice mating trials using individuals from the Mississippi location, we have witnessed numerous instances of an ornamented and a non-ornamented male simultaneously mounted on a female and attempting to mate (Hebets, pers. obs.). Future work is necessary to

determine whether both males are able to successfully transfer sperm and fertilize eggs. However, given that females tend to mate only once during their lifetime (Norton and Uetz, 2005), any incidence of multiple fertilizations could reduce the effect of mate choice decisions by females.

Habitat heterogeneity provides another potential factor that could influence the patterns of genetic and phenotypic variation observed. For example, in the Ohio Valley, the two male phenotypes occupy different microhabitats that are fairly homogeneous for a given population, whereas in the Mississippi location, the habitat is quite heterogeneous, with each male phenotype having a mating advantage depending on substrate (Hebets, unpublished data). Habitat heterogeneity can influence the maintenance of multiple phenotypes within a population (Chunco et al., 2007), and modeling in the Deng et al. (2014) study shows that a combination of habitat heterogeneity and social experience can lead to the persistence of the two male phenotypes in the Mississippi population.

Our genetic data suggest a very recent evolution of any population-level differences in patterns of variation in male phenotype and mate choice, and supports the sister species status of ornamented and non-ornamented *Schizocosa* in the Ohio Valley (Stratton and Uetz, 1981; Stratton and Uetz, 1983; Stratton and Uetz, 1986). This evidence comes from a lack of genetic structure using a mitochondrial marker, very low levels of F_{ST} among populations, and genetic structure corresponding to phenotype only among the phenotypically-pure populations using the more quickly-evolving microsatellite markers. Weak distinction among some locations may be due to recent divergence or high gene flow, but also a lack of power with the microsatellite markers. We cannot be certain of the origin of the differences in patterns of phenotypic and genetic variation across the Ohio Valley and Mississippi locations. However, we do know that variation in the composition of phenotypes across environments can increase the speed at which speciation can occur (McLean and Stuart-Fox, 2014), and we do observe genetic distinction between the Mississippi and Ohio Valley locations. Therefore, this group of wolf spiders we studied may provide a prime example of a polymorphism that becomes fixed for different phenotypes across populations, leading to rapid speciation (West-Eberhard, 1986; Corl et al., 2010). Finally, any processes contributing to genetic and phenotypic differentiation, as well as variation in patterns across populations, is likely to be influenced by a variety of genetic, life history, ecological and social factors, as well as complex interactions arising among them.

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Supplemental Material

Development and characterization of novel microsatellite markers for *Schizocosa* wolf spiders

We extracted whole genome DNA from the legs of *S. ocreata* and *S. rovneri* collected from 10 individuals from seven localities (Table 1) using the DNeasy kit (Qiagen). Genetic Identification Services (http://www.genetic-id-services.com/; Chatsworth, CA) pooled DNA fragments of 350–700 base pairs long to construct libraries enriched for the repeats ATG-, CAG-, TACA-, and TAGA-. Bacterial cultures were produced by ligating *Schizocosa ocreata* genomic DNA fragments enriched for each of the four motifs into the *Bam H1* (GGATCC) cut site of pUC19 plasmid (forward primer 5′- AGG AAA CAG CTA TGA CCA TG -3′; reverse primer 5′- ACG ACG TTG TAA AAC GAC GG -3′; annealing temperature of 57°C).

The recombinant plasmids were electroporated into *E. coli* strain DH5. These colonies were screened for successful transformations using bluo-gal/IPTG/ampicillin LB (BIA-LB). Plasmid DNA from successful clones was purified using using Millipore MultiScreen MAFB NOB Plates (http://www.millipore.com/publications.nsf/docs/TN004). Plasmids of successful clones were sequenced using Amersham's DYEnamic™ ET Terminator Cycle Sequencing Kit (Amersham Biosciences P/N US81050), followed by electrophoresis on an Applied BioSystems Model 377 DNA Sequencer. After identifying appropriate microsatellites, PCR primers for the flanking regions were designed in DesignerPCR, version 1.03 (Research Genetics, Inc.).

For initial screening, a total of 40 unlabeled primers were tested for polymorphism on seven individuals from five localities (Table 1) by PCR amplification and visualization on 3% agarose gels stained with ethidium bromide. Cycling parameters were: 94°C initial denaturation (3 min); 35 cycles of: 94°C denaturation (30 s), 48°C annealing (30 s), and 72°C extension (60 s); 72°C final extension (5 min). We identified potentially suitable primer pairs that yielded polymorphic fragment lengths across the screening individuals. We converted to a 4-dye system and assayed loci in 304 individuals from 4 populations for primer performance and variation among individuals. Fragment analysis of fluorescent PCR product was performed on an ABI 3730xl Analyzer and manually sized using GeneMapper version 3.7 (Applied Biosystems).

Testing yielded 13 polymorphic loci that produced at most two alleles per individual. All but one pair of loci segregated independently (B2 with C101). The number of alleles per locus ranged from 12–86 Online Appendix. We com-

puted observed and expected heterozygosity using Genepop v 3.4. (Raymond and Rousset, 1995). Tests for departure from HWE showed evidence of high inbreeding in a number of loci Online Appendix. We sequenced one ore more individuals for each locus, and deposited sequences in GenBank (accession numbers KT954050-KT954095).

Supplemental Table 1. Characterization and variability of 13 microsatellite loci in 4 populations of the wolf spiders *S. ocreata* and *S. rovneri.*

Marker name	Primer sequences (5′-3′)	Motif	Annealing temp.	No. of alleles	Allele size range	Ho	He
Schiz_A3	F: GCA TTG AGC CCA AAC TAT C R: CGA-AAA-TAA-GCA-CCC-TAA-CTG	(CAT)2-8	57.4°	20	164-191	0.51	0.84
Schiz_D12	F: CCC-CAA-CTT-CAT-TTA-TCT-GG R: GGT-GTG-TTC-ATC-AAT-TTC-TTT-G	(AC)4-14(TA)4-19 (TAGA)5-22(GA)6-8	57.4°	86	187-375	0.64	0.97
Schiz_D4	F: GAG-TGG-TGA-AGT-TTG-ACA-TAA R: CTT-AAA-AGC-ACC-TTG-AAC-TG	(TAGA)8-9	58.6°	43	152-226	0.61	0.95
Schiz_C104	F: AAA-CGG-CTA-AGT-CTT-TTG-GG R: TGA-ACC-GCT-TTG-GAA-ATG	(TACA)8	57.4°	12	170-196	0.43	0.65
Schiz_D6	F: TTA-GCA-GAT-TTT-TGG-TTA-CGA-C R: GCC-CCG-CTC-TAT-TAC-TTG	(TCTA)4-13	57.4°	13	230-272	0.48	0.68
Schiz_A4	F: GGC-AAG-GCT-TTA-CAA-GGA-C R: GCT-TTT-TTG-GCT-CTT-CAG-TG	(GAT)5-10(GTT)1-6	57.4°	33	223-306	0.71	0.91
Schiz_C107	F: TTT-AGA-GTT-ATA-CCC-CTC-AGT-G R: TAT-GGC-TAG-TTT-AGT-CGT-GAA	(CATA/G)5-23	58.6°	22	219-311	0.46	0.85
Schiz_D107	F: TCC-CAC-TCT-CTT-AAC-TGA-AAT-C R: ATC-TGC-AAA-GGT-GAA-TCT-TAT	(TAGA)9(TAGA)5 (GA)12	58.6°	78	124-302	0.77	0.98
Schiz_C116	F: GCG-ACA-TTC-ATT-ACC-GAA-AC R: GGT-TCC-AGA-ACG-AAT-ACG-C	(GTAT)4-7(AT)2-12	57.4°	40	259-327	0.86	0.91
Schiz_B2	F: AAT-GGC-AAT-AAT-AAC-GGG-GTA R: AAA-TCG-CCG-AGG-TCA-TCT	(AAC)5AGC(AAC)3	57.4°	18	212-256	0.65	0.66
Schiz_C12	F: AAA-CGA-AAA-TGC-CCT-AAA-GTC R: GGA-AAT-GGG-AGT-TTT-GGA-G	(TACA)5	57.4°	17	254-322	0.63	0.69
Schiz_D104	F: TAA-AGG-CCG-TGA-ATT-TTA-CTC R: CAG-AAG-ACC-GGA-TAT-GAA-CTA-G	(CTAT)10	56.8°	19	186-258	0.31	0.81
Schiz_C101	F: AGC-ACG-CAA-CAA-CAG-CAG R: ATG-CCG-GAT-CAA-GAC-CTG	(TGTA)6	58.6°	21	166-204	0.51	0.7

Online Appendix: *Schizocosa* specimens used for phylogenetic analyses (Fig. 6).

Species	Specimen code	Location	GenBank accession number
Schizocosa sp.--ornamented	001_011_brush	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963556
Schizocosacrassipes	007_001_05_crassipesCCNA	USA, MS, Wilkinson County, Clark Creek Natural Area	KT963562
Schizocosacrassipes	007_030_05_crassipesCCNA	USA, MS, Wilkinson County, Clark Creek Natural Area	KT963563
Schizocosastridulans	007_037_05_stridCCNA	USA, MS, Wilkinson County, Clark Creek Natural Area	KT963564
Schizocosacrassipes	008_005_05_crassipes_natchez	USA, MS, Adams County, Natchez State Park	KT963565
Schizocosacrassipes	008_1_002_06_crassipes_natchez	USA, MS, Adams County, Natchez State Park	KT963566
Schizocosastridulans	022_026_05_amys_stridulans	USA, MS, Lafayette County, Molly Barr Road and Park Boulevard	KT963574
Schizocosacrassipes	024_001_06_S_crass_legion	USA, MS, Winston County, Legion State Park	KT963576
Schizocosauetzi	026_001_06_gumsprLA_uetzi	USA, LA, Winn County, Gum Springs campgound	KT963578
Shizocosa non-ornamented	004_041_06_tobytuby_non	USA, MS, Lafayette County, "Toby Tuby"	KT963559
Schizocosa sp.--ornamented	002_1_001_06_sardis_brush	USA, MS, Penola County, Sardis Reservoir	KT963557
Schizocosa sp.--non-ornamented	004_034_06_tobytuby_non	USA, MS, Lafayette County, "Toby Tuby"	KT963558
Schizocosa sp.--ornamented	005_054_06_hurrland_brush	USA, MS, Lafayette County, Hurricane Landing	KT963560
Schizocosa sp.--ornamented	006_006_05_hurrland_brush	USA, MS, Lafayette County, Hurricane Landing	KT963561
Schizocosa sp.--ornamented	009_001_05_natchez_brush	USA, MS, Adams County, Natchez State Park	KT963567
Schizocosa sp.--ornamented	014_005_06_Clarcko_brush	USA, MS, Clarke County, Clarcko State Park	KT963568
Schizocosa sp.--ornamented	017_001_05_grahamlake_brush	USA, MS, Lafayette County, Graham Lake	KT963569
Schizocosa sp.--ornamented	018_002_05_grahamlake_brush	USA, MS, Lafayette County, Graham Lake	KT963570
Schizocosa sp.--non-ornamented	020_001_05_bagleybott_non	USA, MS, Lafayette County, Baggley Bottoms	KT963571
Schizocosa sp.--non-ornamented	021_006_05_strawpl_non	USA, MS, Marshall County, Strawberry Plains Audubon Sanctuary	KT963572
Schizocosa sp.--non-ornamented	022_001_05_amys_non	USA, MS, Lafayette County, Molly Barr Road and Park Boulevard	KT963573
Schizocosa sp.--non-ornamented	023_005_05_ecru_non	USA, MS, Pontotoc County, Ecru woods	KT963575
Schizocosa sp.--ornamented	025_001_06_vicksburg_brush	USA, MS, Warren County, Vicksburg	KT963577
Schizocosaocreata (Hentz 1844)--ornamented	030_002	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	KT963579
Schizocosaocreata (Hentz 1844)--ornamented	030_003	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	KT963580
Schizocosaocreata (Hentz 1844)--ornamented	030_004	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	KT963581
Schizocosaocreata (Hentz 1844)--ornamented	030_006	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	KT963582
Schizocosaocreata (Hentz 1844)--ornamented	030_008	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	KT963583
Schizocosaocreata (Hentz 1844)--ornamented	030_011	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	KT963584
Schizocosaocreata (Hentz 1844)--ornamented	030_012	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	KT963585
Schizocosaocreata (Hentz 1844)--ornamented	030_013	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	KT963586
Schizocosaocreata (Hentz 1844)--ornamented	030_014	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	KT963587
Schizocosaocreata (Hentz 1844)--ornamented	030_015	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	KT963588
Schizocosaocreata (Hentz 1844)--ornamented	030_016	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	KT963589
Schizocosaocreata (Hentz 1844)--ornamented	030_017	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	KT963590
Schizocosaocreata (Hentz 1844)--ornamented	030_018	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	KT963591
Schizocosaocreata (Hentz 1844)--ornamented	030_019	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	KT963592
Schizocosaocreata (Hentz 1844)--ornamented	030_020	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	KT963593

Continued Table

Species	Specimen code	Location	GenBank accession number
Schizocosaocreata (Hentz 1844)--ornamented	030_022	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	KT963594
Schizocosaocreata (Hentz 1844)--ornamented	030_023	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	KT963595
Schizocosaocreata (Hentz 1844)--ornamented	030_027	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	KT963596
Schizocosaocreata (Hentz 1844)--ornamented	030_028	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	KT963597
Schizocosaocreata (Hentz 1844)--ornamented	030_030	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	KT963598
Schizocosaocreata (Hentz 1844)--ornamented	030_035	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	KT963599
Schizocosaocreata (Hentz 1844)--ornamented	030_038	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	KT963600
Schizocosaocreata (Hentz 1844)--ornamented	030_039	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	KT963601
Schizocosaocreata (Hentz 1844)--ornamented	030_043	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	KT963602
Schizocosaocreata (Hentz 1844)--ornamented	030_046	USA, OH, Clermont County, Rowe Woods, Cincinnati Nature Center	KT963603
? Schizocosaocreata (Hentz 1844)--ornamented	031_001	USA, KY, Kenton County, Devou Park	KT963604
? Schizocosaocreata (Hentz 1844)--ornamented	031_002	USA, KY, Kenton County, Devou Park	KT963605
? Schizocosaocreata (Hentz 1844)--ornamented	031_003	USA, KY, Kenton County, Devou Park	KT963606
? Schizocosaocreata (Hentz 1844)--ornamented	031_004	USA, KY, Kenton County, Devou Park	KT963607
? Schizocosaocreata (Hentz 1844)--ornamented	031_005	USA, KY, Kenton County, Devou Park	KT963608
? Schizocosaocreata (Hentz 1844)--ornamented	031_006	USA, KY, Kenton County, Devou Park	KT963609
? Schizocosaocreata (Hentz 1844)--ornamented	031_008	USA, KY, Kenton County, Devou Park	KT963610
? Schizocosaocreata (Hentz 1844)--ornamented	031_009	USA, KY, Kenton County, Devou Park	KT963611
? Schizocosaocreata (Hentz 1844)--ornamented	031_010	USA, KY, Kenton County, Devou Park	KT963612
? Schizocosaocreata (Hentz 1844)--ornamented	031_011	USA, KY, Kenton County, Devou Park	KT963613
? Schizocosaocreata (Hentz 1844)--ornamented	031_012	USA, KY, Kenton County, Devou Park	KT963614
? Schizocosaocreata (Hentz 1844)--ornamented	031_013	USA, KY, Kenton County, Devou Park	KT963615
? Schizocosaocreata (Hentz 1844)--ornamented	031_014	USA, KY, Kenton County, Devou Park	KT963616
? Schizocosaocreata (Hentz 1844)--ornamented	031_016	USA, KY, Kenton County, Devou Park	KT963617
? Schizocosaocreata (Hentz 1844)--ornamented	031_017	USA, KY, Kenton County, Devou Park	KT963618
? Schizocosaocreata (Hentz 1844)--ornamented	031_018	USA, KY, Kenton County, Devou Park	KT963619
? Schizocosaocreata (Hentz 1844)--ornamented	031_019	USA, KY, Kenton County, Devou Park	KT963620
? Schizocosaocreata (Hentz 1844)--ornamented	031_024	USA, KY, Kenton County, Devou Park	KT963621
? Schizocosaocreata (Hentz 1844)--ornamented	031_026	USA, KY, Kenton County, Devou Park	KT963622
? Schizocosaocreata (Hentz 1844)--ornamented	031_027	USA, KY, Kenton County, Devou Park	KT963623
? Schizocosaocreata (Hentz 1844)--ornamented	031_032	USA, KY, Kenton County, Devou Park	KT963624
? Schizocosaocreata (Hentz 1844)--ornamented	031_035	USA, KY, Kenton County, Devou Park	KT963625
? Schizocosaocreata (Hentz 1844)--ornamented	031_036	USA, KY, Kenton County, Devou Park	KT963626
? Schizocosaocreata (Hentz 1844)--ornamented	031_042	USA, KY, Kenton County, Devou Park	KT963627
? Schizocosaocreata (Hentz 1844)--ornamented	031_044	USA, KY, Kenton County, Devou Park	KT963628
Schizocosarovneri, Uetz and Dondale, 1979	032_002_c	USA, KY, Boone County, Giles Conrad Park	KT963629
Schizocosarovneri, Uetz and Dondale, 1979	032_005	USA, KY, Boone County, Giles Conrad Park	KT963630
Schizocosarovneri, Uetz and Dondale, 1979	032_006	USA, KY, Boone County, Giles Conrad Park	KT963631
Schizocosarovneri, Uetz and Dondale, 1979	032_008	USA, KY, Boone County, Giles Conrad Park	KT963632
Schizocosarovneri, Uetz and Dondale, 1979	032_009	USA, KY, Boone County, Giles Conrad Park	KT963633
Schizocosarovneri, Uetz and Dondale, 1979	032_011	USA, KY, Boone County, Giles Conrad Park	KT963634
Schizocosarovneri, Uetz and Dondale, 1979	032_012	USA, KY, Boone County, Giles Conrad Park	KT963635

Continued Table

Species	Specimen code	Location	GenBank accession number
Schizocosarovneri, Uetz and Dondale, 1979	032_014	USA, KY, Boone County, Giles Conrad Park	KT963636
Schizocosarovneri, Uetz and Dondale, 1979	032_015	USA, KY, Boone County, Giles Conrad Park	KT963637
Schizocosarovneri, Uetz and Dondale, 1979	032_018	USA, KY, Boone County, Giles Conrad Park	KT963638
Schizocosarovneri, Uetz and Dondale, 1979	032_022	USA, KY, Boone County, Giles Conrad Park	KT963639
Schizocosarovneri, Uetz and Dondale, 1979	032_025	USA, KY, Boone County, Giles Conrad Park	KT963640
Schizocosarovneri, Uetz and Dondale, 1979	032_026	USA, KY, Boone County, Giles Conrad Park	KT963641
Schizocosarovneri, Uetz and Dondale, 1979	032_028	USA, KY, Boone County, Giles Conrad Park	KT963642
Schizocosarovneri, Uetz and Dondale, 1979	032_029	USA, KY, Boone County, Giles Conrad Park	KT963643
Schizocosarovneri, Uetz and Dondale, 1979	032_030	USA, KY, Boone County, Giles Conrad Park	KT963644
Schizocosarovneri, Uetz and Dondale, 1979	032_031	USA, KY, Boone County, Giles Conrad Park	KT963645
Schizocosarovneri, Uetz and Dondale, 1979	032_032	USA, KY, Boone County, Giles Conrad Park	KT963646
Schizocosarovneri, Uetz and Dondale, 1979	032_033	USA, KY, Boone County, Giles Conrad Park	KT963647
Schizocosarovneri, Uetz and Dondale, 1979	032_034	USA, KY, Boone County, Giles Conrad Park	KT963648
Schizocosarovneri, Uetz and Dondale, 1979	032_035	USA, KY, Boone County, Giles Conrad Park	KT963649
Schizocosarovneri, Uetz and Dondale, 1979	032_037	USA, KY, Boone County, Giles Conrad Park	KT963650
Schizocosarovneri, Uetz and Dondale, 1979	032_039	USA, KY, Boone County, Giles Conrad Park	KT963651
Schizocosarovneri, Uetz and Dondale, 1979	032_041	USA, KY, Boone County, Giles Conrad Park	KT963652
Schizocosarovneri, Uetz and Dondale, 1979	032_045	USA, KY, Boone County, Giles Conrad Park	KT963653
Schizocosa sp.--non-ornamented	o007_05e_non	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963654
Schizocosa sp.--non-ornamented	o010_05_3_non	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963655
Schizocosa sp.--non-ornamented	o011_05_non	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963656
Schizocosa sp.--ornamented	o015_05_2_non	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963657
Schizocosa sp.--ornamented	o022_05e_brush	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963658
Schizocosa sp.--ornamented	o028_05_3_brush	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963659
Schizocosa sp.--non-ornamented	o030_05_2_non	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963660
Schizocosa sp.--non-ornamented	o035_05_2_non	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963661
Schizocosa sp.--non-ornamented	o038_05e_non	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963662
Schizocosa sp.--ornamented	o041_05_1_brush	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963663
Schizocosa sp.--ornamented	o042_05_4_brush	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963664
Schizocosa sp.--non-ornamented	o043_05_2_non	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963665
Schizocosa sp.--non-ornamented	o044_06_non	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963666
Schizocosa sp.--non-ornamented	o046_05_1_non	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963667
Schizocosa sp.--non-ornamented	o049_05_4_non	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963668
Schizocosa sp.--ornamented	o050_05_brush	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963669
Schizocosa sp.--ornamented	o059_05e_brush	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963670
Schizocosa sp.--non-ornamented	o060_05_3_non	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963671
Schizocosa sp.--non-ornamented	o060_05_4_non	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963672
Schizocosa sp.--non-ornamented	o063_05e_non	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963673
Schizocosa sp.--ornamented	o075_05_4_brush	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963674

Continued Table

Species	Specimen code	Location	GenBank accession number
Schizocosa sp.--non-ornamented	o091_05u_non	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963675
Schizocosa sp.--ornamented	o116_06_brush	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963676
? Schizocosaocreata, (Hentz 1844)--ornamented	ON_034_06	USA, NE, Lancaster County, Wilderness Park	KT963677
Schizocosa sp.--non-ornamented	p4_11c_non	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963678
Schizocosa sp.--non-ornamented	p4_11d_non	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963679
Schizocosa sp.--ornamented	p4_a1_brush	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963680
Schizocosa sp.--ornamented	p4_b2_brush	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963681
Schizocosa sp.--ornamented	p4_b3_brush	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963682
Schizocosa sp.--ornamented	p4_b4_brush	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963683
Schizocosa sp.--ornamented	p4_b7_brush	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963684
Schizocosa sp.--ornamented	p4_b8_brush	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963685
Schizocosa sp.--ornamented	p4_c1_brush	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963686
Schizocosa sp.--ornamented	p4_c4_brush	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963687
Schizocosa sp.--non-ornamented	p4_c6_non	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963688
Schizocosa sp.--non-ornamented	p4_d1_non	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963689
Schizocosa sp.--non-ornamented	p4_d2_non	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963690
Schizocosa sp.--ornamented	p4_d3_brush	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963691
Schizocosa sp.--ornamented	p4_d4_brush1	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963692
Schizocosa sp.--non-ornamented	p4_d6_non	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963693
Schizocosa sp.--non-ornamented	p4_e2_non	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963694
Schizocosa sp.--ornamented	p4_e3_brush	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963695
Schizocosa sp.--ornamented	p4_e4_brush1	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963696
Schizocosa sp.--non-ornamented	p4_e6_non	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963697
Schizocosa sp.--non-ornamented	p4_f2_non	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963698
Schizocosa sp.--ornamented	p4_f3_brush	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963699
Schizocosa sp.--ornamented	p4_g3_brush	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963700
Schizocosa sp.--ornamented	p4_h1_brush	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963701
Schizocosa sp.--non-ornamented	p4_h2_brush	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963702
? Schizocsaocreata(Hentz 1844)--ornamented	S_ocreata_o1	USA, MS, Penola County, Sardis Reservoir nature trail	EF112506
? Schizocsaocreata(Hentz 1844)--ornamented	S_ocreata_o2	USA, MS, Lafayette County, 1 mile SW Abbeville	EF112507
? Schizocsaocreata(Hentz 1844)--ornamented	S_ocreata_o3	USA, MS, Lafayette County, 1 mile SW Abbeville	EF112508
*? Schizocsarovneri*Uetz and Dondale 1979--non-ornamented	S_roverni_r1	USA, MS, Lafayette County, Clear Creek	EF112509
*? Schizocsarovneri*Uetz and Dondale 1979--non-ornamented	S_rovneri_r2	USA, MS, Penola County, Sardis Reservoir nature trail	EF112510
Schizocosa sp.--ornamented	p4_h4_brush	USA, MS, Lafayette County, grounds of UM Campus Greenhouse	KT963703
Schizocosabilineata (Emerton, 1885)	S_bilineata	USA, MS, Lafayette County, UM field station	EF112511
Schizocosa duplex (Chamberlin, 1925)	S_duplex	USA, MS, Penola County, Sardis Reservoir nature trail	EF112512

Continued Table

Species	Specimen code	Location	GenBank accession number
*Schizocosa maxima*Dondale and Redner, 1978	S_maxima	USA, CA, San Diego County, Jamul	EF112513
Schizocosamccooki(Montgomery 1904)	S_mccooki	USA, CA, San Diego County, Laguna Mountains	EF112514
Schizocosaretrorsa(Banks 1911)	S_retrorsa	USA, MS, Penola County, Sardis Reservoir	EF112515
Schizocosasaltatrix(Hentz 1844)	S_saltatrix	USA, MS, Lafayette County, "Lonesome 80"	EF112516
*Schizocosastridulans*Stratton 1984	s1	USA, MS, Penola County, Sardis Reservoir	EF112517
*Schizocosastridulans*Stratton 1984	s2	USA, MS, Marshall County, Strawberry Plains Audubon Sanctuary	EF112518
*Schizocosastridulans*Stratton 1984	s3	USA, MS, Lafeyette County, 1 mile SW Abbeville	EF112519
*Schizocosastridulans*Stratton 1984	s4	USA, OK, Cleveland County, Lake Thunderbird State Park	EF112520
*Schizocosastridulans*Stratton 1984	s5	USA, MS, Marshall County, Strawberry Plains Audubon Sanctuary	EF112521
*Schizocosauetzi*Stratton 1997	u1	USA, MS, Penola County, Sardis Reservoir nature trail	EF112522
*Schizocosauetzi*Stratton 1997	u2	USA, MS, Lafeyette County, "Lonsesome 80"	EF112523
*Schizocosauetzi*Stratton 1997	u3	USA, MS, Lafeyette County, "Lonsesome 80"	EF112524
*Schizocosauetzi*Stratton 1997	u4	USA, MS, Lafeyette County, "Lonsesome 80"	EF112525
*Schizocosaauloniа*Dondale 1969	S_aulonia	USA, KS, Montgomery County, Elk City Lake St Park	JX870624
Schizocosaavida (Walckenaer 1837)	S_avida	USA, NE, Lancaster County	JS870625
Schizocosabilineata(Emerton 1885)	S_bilineata	USA, OH, Licking County, Ohio State University-Newark	JX870626
*Schizocosacrassipalpata*Roewer 1951	S_crassipalpata	USA, OH, Summit County, Akron	JX870627
Schizocosamccooki(Montgomery 1904)	S_mccooki	USA, CO, Douglas County, Roxborough	JX870631
Schizocosaretrorsa(Banks 1911)	S_retrorsa	USA, MS, Marshall County	JX870632
Schizocosasaltatrix(Hentz 1844)	S_saltatrix	USA, MS, Lafeyette County	EF112523
Schizocosa duplex Chamberlin 1925	S_duplex_JX…	USA, MS, Penola County, Sardis Reservoir	JX870629
Schizocosafloridana(Hentz 1844)	S_florid_JX…	USA, FL, Alchua County	JX970630

Online Appendix: List of individuals sharing unique mitochondrial sequence haplotypes (COI) represented on the *Schizocosa* phylogeny (Fig. 6).

Individual represented on phylogeny	Individuals with matching haplotypes
030_003	030_012, 030_018, 030_030, _030_035, _030_038, 030_043, 031_010, 031_012, 031_027, 031_044, 032_009, 032_012, 032_022, 032_028, 032_030, 032_041, o028_05_3_brush, o030_05_2_non, o035_05_2_non, o042_05_4_brush, o044_06_non, o049_05_4_non, o059_05e_brush, o060_05_3_non, o091_05u_non, p4_a1_brush, p4_b8_brush, p4_c1_brush, p4_e3_brush, p4_f3_brush, p4_g3_brush
030_006	032_006
030_014	032_014
030_019	031_008
030_023	030_027, 031_003, 031_017, 031_026, 032_005, 032_029, 032_034, o022_05e_brush, p4_c6_non, p4_d3_brush, p4_e2_non, p4_h2_non
030_039	031_016
031_002	031_036, p4_b4_brush
031_005	032_031
031_006	032_033
032_008	032_011, 032_018, p4_b3_brush
o007_05e_non	o063_05e_non, p4_d1_non
o010_05_3_non	o011_05_non
o015_05_2_non	o075_05_4_brush, o116_06_brush
S-ocr_o1_sar_EF112508	S_rovneri_r2_Sar_EF112510
S_stridulans_s2_EF112518	S_stridulans_s3_EF112519, S_stridulans_s5_EF112521