

Xenophilic mating preferences among populations of the jumping spider *Habronattus pugillis* Griswold

Eileen A. Hebets^{a,b} and Wayne P. Maddison^c

^aDepartment of Environmental Science, Policy and Management: Division of Insect Biology, University of California at Berkeley, Berkeley, CA 94720, USA, ^bSchool of Biological Sciences, University of Nebraska, Lincoln, NE 68588, USA, and ^cDepartments of Zoology and Botany, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4

Sexual selection is thought to have driven the diversification of courtship behavior and associated ornamentation between geographically isolated populations of the jumping spider *Habronattus pugillis* Griswold. In an attempt to understand the pathways of sexual selection during this diversification, we conducted reciprocal mating trials between two populations of *H. pugillis* (Santa Rita [SR] and Atascosa [AT]) that differ in both male courtship display and secondary sexual ornamentation. Observations of mating frequencies show a xenophilic mating preference in which SR females have a stronger response to AT males than to SR males, while AT females show no difference in mating frequency. These results are not consistent with a coevolutionary process in which male traits and female preferences evolve in concert, positively reinforcing each other. We discuss alternative pathways of sexual selection that may have acted in this system, including the possibility that female preferences and male traits have evolved antagonistically. In addition, we found that SR females spent a higher proportion of time prior to copulation visually attentive to AT males versus SR males. This difference in visual attention prior to copulation was not seen in AT females and may provide insights into our observations of xenophilic mating preference. *Key words*: antagonistic coevolution, attention, diversification, jumping spiders, sexual selection, speciation. [*Behav Ecol* 16:981–988 (2005)]

Understanding the mechanisms underlying patterns of diversification of evolutionary lineages poses a considerable challenge to evolutionary biology because the explanations attempt to cross many levels of organization, translating processes operating among individuals within species into large-scale phylogenetic patterns. Sexual selection driving within-lineage change is one process that has been implicated as an important force in between-lineage diversification. A theoretical basis for this proposal can be seen in models of the evolution of prezygotic isolation, where sexual selection often plays a role (for review see Kirkpatrick and Ravigne, 2002). For example, a Fisherian runaway process of sexual selection can be important in generating trait divergence between closely related populations (Pomiankowski and Iwasa, 1998). Empirical support comes through taxa whose impressive diversifications have been attributed at least in part to sexual selection, including haplochromine cichlids (Seehausen, 2002), birds of paradise (Mitra et al., 1996), and *Laupala* crickets of Hawaii (Mendelson and Shaw, 2005). However, at least some broader compilations of data cast doubt on whether there is a general correlation between clade diversity and sexual selection (Barraclough et al., 1995; Morrow et al., 2003). Further exploration of the relationship between sexual selection and diversification will require both population-level studies of mechanism and studies of a broader range of clades.

One clade promising for studies of sexual selection and diversification is the jumping spider group *Habronattus* (Griswold, 1987; Maddison and Hedin, 2003), consisting of

approximately 100 species with strikingly diverse and complex male courtship ornaments and behaviors (Cutler, 1988; Elias et al., 2003; Elias DO, Hebets EA, Hoy RR, Maddison WP, Mason AC, in preparation; Griswold, 1977, 1987; Maddison and Stratton, 1988; Peckham G and Peckham E, 1889, 1890; Richman, 1977, 1982). The *Habronattus pugillis* complex in particular has apparently undergone a highly localized and possibly rapid diversification among mountain ranges isolated by intervening deserts in the southwestern US and northwestern Mexico (Maddison and McMahon, 2000). Males of *H. pugillis* are characterized by a high degree of phenotypic uniformity within mountain ranges and a high level of differentiation in courtship behavior and sexual ornamentation among mountain ranges (Maddison and McMahon, 2000). Masta and Maddison (2002) showed that rates of divergence in male phenotype exceeded expectations based on a neutral genetic marker, indicating that the divergence is due to selection. The fact that phenotypic differentiation is most pronounced in male secondary sexual ornaments suggests a primary role for sexual selection (Maddison and McMahon, 2000; Masta and Maddison, 2002). Due to the types of population differences, the small spatial scale, and the potentially small temporal scale of this diversification (Maddison and McMahon, 2000), it seems likely that this system is driven by a rapidly diversifying process of sexual selection.

In exploring the diversification of *H. pugillis*, our approach has been to focus our attention on an intermediate level of organization, at the intersection between mechanistic (individual and population) and comparative studies. Comparative approaches for examining processes of sexual selection have been applied to a variety of systems including spiders (Hebets and Uetz, 1999; McClintock and Uetz, 1996), mites (Proctor, 1992), swordtail fish (Basolo, 1990, 1996; Ryan and Wagner, 1987), guppies (Endler and Houde, 1995; Houde and Endler, 1990), frogs (Cocroft and Ryan, 1995; Ryan, 1991; Ryan and

Address correspondence to E.A. Hebets. E-mail: ehebets2@unl.edu.

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Rand, 1993; Ryan et al., 1990), manakins (Prum, 1997), and house finches (Hill, 1994). To the extent that different processes of sexual selection differ in their long-term evolutionary consequences, they would be expected to produce different patterns of interspecific variation in secondary sexual traits, and comparative approaches could provide some power to distinguish among alternative mechanisms. One such approach examines how males and females from different species or populations react to one another as potential mates (Gray and Cade, 2000; Hamilton and Poulin, 1999; Hill, 1994; Houde and Endler, 1990; Jones and Hunter, 1998; Ptacek, 1998). While this same approach is frequently used in studies addressing premating isolation and reinforcement (Bordenstein et al., 2000; Iliadi et al., 2001; Kobayashi et al., 2001; Korol et al., 2000; Shapiro, 2001; Wade et al., 1995), the results are typically not discussed with respect to processes of sexual selection.

Because any single model of sexual selection can yield a broad range of possible predictions (Pizzari and Snook, 2003; Rowe et al., 2003) and because different mechanisms of sexual selection can be acting simultaneously or consecutively in the same system, it appears unlikely that data on interpopulation interactions will be able to distinguish alternative models. Nonetheless, such data can reveal aspects of the pathway that sexual selection takes, such as whether evolution of a male trait is accompanied by increasing the female preference for it. Determining whether or not this positive coevolution has taken place would not necessarily confirm which model of sexual selection has operated. However, it would impose specific constraints on any explanation by sexual selection and would provide insights into the relationship between sexual selection and speciation.

The purpose of this study is to use comparative techniques to explore the evolution of female responses to males in the jumping spider *H. pugillius*. We chose two divergent populations of *H. pugillius* that differ extensively in both male courtship behavior and male secondary sexual morphology. We allowed reciprocal mating opportunities for individuals from both populations and analyzed mating frequencies in order to better understand the nature of sexual selection and the female responses it has generated. In particular, we test whether male traits and female preferences have positively coevolved in the diversification of this species.

MATERIALS AND METHODS

Following a study of 16 populations of *H. pugillius* in southeastern Arizona (Maddison and McMahon, 2000), we chose as our focal groups two divergent populations on nearby mountain ranges, the Santa Rita (SR) and the Atascosa (AT) mountains, based on their having notably distinct behaviors and ornamentation and the ease with which spiders could be collected. Because all *H. pugillius* populations are contemporaneous—none is ancestral—it was not possible to compare ancestral and descendant populations, which would provide better insights into the process of sexual selection. Instead of an actual ancestor, we might seek instead a population with only ancestral traits, but none such has been found in *H. pugillius* (Maddison and McMahon, 2000). Even if the traits of a population match the inferred ancestral traits in many characters, it is difficult or impossible to confirm that all relevant traits are ancestral, especially for traits of female choice. While artificial males might be created bearing inferred ancestral traits (Hebets and Uetz, 2000; McClintock and Uetz, 1996), a considerably more difficult feat would be to invent artificial females with ancestral patterns of choice. Nonetheless, an experimental approach using animals sampled from independently derived populations can offer insights into sexual

selection. Indeed, some of the more intriguing possibilities (e.g., reciprocal susceptibility to independently evolved exploitative traits) may be most likely when both populations show derived traits.

While females from the two focal populations are virtually indistinguishable, males differ in both morphology and courtship behavior as discussed below (Maddison and McMahon, 2000).

SR males

From the front, SR males have a dark brown face with a white horizontal stripe along the bottom (Maddison and McMahon, 2000). They have a thin streak of white scales that extends above their anterior eyes. The anterior-most pair of walking legs also has pendant fringes of setae. The courtship behavior of these males begins with circular rotations of the palpi (modified appendages beside the face) (Maddison and McMahon, 2000). This palpal rotation is unique to SR males and is continued throughout the courtship display. Males remain mostly stationary in location during courtship until the actual approach of the female, which is generally direct. Males occasionally engage in an alternating slow leg wave. The final stages of courtship involve the male holding his first pair of legs above the female and flicking the tips.

AT males

The face of AT males is covered with silver-gray scales, except for the lower lateral portion, which is yellowish with a few dark spots (Maddison and McMahon, 2000). The sides of the carapace are swollen. There is no eye streak above their anterior eyes, as seen in SR males, but the chelicerae are striped. The courtship display of AT males appears more vigorous than that of the SR males. Males approach females rapidly while sidling, which involves moving in large arcs in one direction followed by the other with the first pair of legs held above the ground the entire time (Maddison and McMahon, 2000). AT males have no palpal circling, but similar to the SR males, they have a vigorous leg flicking during the final stages of their courtship.

Experimental design

Mature males and penultimate females were collected from the SR Mountains on 26 and 27 March and 1 April 1998 and 3 March and 9 April 2000. Mature males and penultimate females were collected from the AT Mountains on 26 March and 9 April 1998 and 16 and 27 February, 1, 11, and 24 March, and 18 April 2000. All individuals were collected from the field during the day and brought back to the laboratory where they were individually housed in seven dram polystyrene vials with white polyethylene snap cap lids. They were kept on a constant 12:12 h light:dark cycle and were fed one cabbage looper caterpillar once a week. After females molted to maturity, their age was determined by counting the number of days that had elapsed since their maturation molt. Females ranging in age from 14 to 21 days postmaturation molt were randomly assigned and tested with either males from their own mountain range or males from the foreign mountain range. Most males were collected mature, and thus, their age or mating history could not be determined. All specimens are deposited in a private collection (E.A.H.).

An equal number of females from both populations were assigned males either from the same ("local") or the other ("foreign") population. Each female was tested with up to five different males from the assigned population, each on consecutive days. For each trial, a female was placed in a circular acetate arena approximately 9 cm in diameter with graph

paper lining the bottom. Each female was allowed to acclimate in the arena for approximately 1 min before the male was introduced. Males and females were left in the arena together for up to 15 min during which time all interactions were videotaped. If a male did not court within the first 3 min, he was removed and a new randomly chosen male was introduced. All trials were run between 0900 and 1400 h and were conducted in mid-April through mid-June of 1998 and 2000. Interactions were allowed to proceed until either the 15 min were up or cannibalism or copulation occurred, after which point individuals were removed and placed back into their respective vials. Once a female mated, she was removed from the trial process. Due to insufficient numbers of males, many males were used again after mating, but there was always at least a 5-day rest period.

We chose to conduct our experiments as sequential instead of simultaneous-choice experiments for two reasons: (1) in the field, we feel that it is unlikely that females encounter more than one male simultaneously and thus our design is more relevant to natural interactions, and (2) examining mating responses in this way allows us to measure absolute, not just relative, responses. For species like this in which females encounter males individually, knowledge of absolute responses is more useful. Furthermore, while we can compare absolute responses to infer preferences from simultaneous-choice preference tests, we cannot infer absolute responses, and under some models of sexual selection, only absolute response matters. For instance, a model of antagonistic coevolution predicts that females should evolve to accept enhanced (exploitative) males at lower rates. This decrease in absolute response could be accomplished by a mutation lowering acceptance rates of both enhanced and unenhanced males, or by genetic variation conferring specific resistance to enhanced males. Indeed, the relative response (preference) to different males may be more or less irrelevant as long as the females come to lower their acceptance rates to the enhanced males.

Data analysis

Our data analysis is complicated by the fact that many females were tested more than once. If we include the data from all the trials from each female, we run the risk of pseudoreplication, but if we include only one trial from each female, we discard much of our data. With this in mind, we analyzed our data in several ways.

First, we examined mating frequency during only the first trial for each female, ignoring her subsequent trials. This design is most analogous to a fixed threshold sampling tactic, a one-step decision tactic (Janetos, 1980), or an optimal stopping rule sampling tactic (Dombrovsky and Perrin, 1994; reviewed in Jennions and Petrie, 1997). These designs are probably more relevant to the biology of jumping spiders than other mate sampling tactics (reviewed in Jennions and Petrie, 1997). Because we sought to understand how females from each population react to males from their own versus a foreign population, we conducted separate chi-square tests on each population. The chi-square test was used to determine if females from each population were more likely to mate with local versus foreign males. In a similar analysis, we included only pairs which copulated within 2 min of the initiation of male courtship. In the field, this timescale is likely more realistic because uninterested females can and will jump away immediately (Hebets EA and Elias DO, personal observation). We also conducted analyses on both populations combined.

An analysis including only the first trials for each female represents only a fraction of the information contained in our data, for among females that rejected the first male, some

continued to reject males, while others quickly accepted an alternative male. Thus, in order to make full use of our data while still avoiding the statistical problem of using individual females multiply (pseudoreplication), we counted the number of trials until a female accepted a male of her assigned population as a measure of her readiness to mate with males of that category. This “trial latency score” is simply the trial number on which each female mated, unless she did not mate by the fifth trial, in which case she was given a conservative trial latency score of 6. Each female was only represented once in this statistical analysis. Again, because a common prediction of positive reinforcing selection relates specifically to how females from each population respond to local versus foreign males and because females from each population were run slightly offset in time, separate analyses were conducted for each population. Once trial latency scores for each female were assigned, analyses of variance (ANOVAs) were conducted for each population to look for an effect of male origin (local versus foreign) on “trial latency.” For various reasons (e.g., premature death, escape), some females neither mated nor were used five times, and these females were excluded from the analysis.

Among females that mated, the time from initiation of the trial until mounting by the male was scored as the “copulation latency.” An ANOVA was used on all mating pairs to test for differences in copulation latency between females from each range.

Difference of response differences test

In order to test for positive coevolution between male traits and female response, we compare the strength of female responses to both local and foreign males. We used the trial latency data for this test because it utilized more of our data and represented a more conservative test (see Results). It may initially seem a model of positive coevolution (i.e., reinforcement between male trait and female preference) can be rejected merely by showing that females of one population prefer males of another (e.g., showing that mean trial latency of SR female × AT male is less than mean trial latency SR female × SR male). As discussed further below, positive coevolution within a population allows for the result that females prefer males from a second population; but if so, then females from the second population should prefer their own males, with whom they have coevolved, even more strongly. More precisely, in our study system, positive coevolution predicts that the degree to which AT females respond faster to their own males [(mean trial latency AT × SR) – (mean trial latency AT × AT)] should be greater than the degree to which SR females respond faster to AT males [(mean trial latency SR × SR) – (mean trial latency SR × AT)]. Thus,

$$[MTL(AT \times SR) - MTL(AT \times AT)] > [MTL(SR \times SR) - MTL(SR \times AT)],$$

where $MTL(P \times Q)$ is mean trial latency of female P with male Q. This is algebraically equivalent to the comparable prediction focusing on SR females:

$$[MTL(SR \times AT) - MTL(SR \times SR)] > [MTL(AT \times AT) - MTL(AT \times SR)]$$

as well as to the prediction that trial latency within populations should be lower than that between populations:

$$[MTL(SR \times SR) + MTL(AT \times AT)] < [MTL(AT \times SR) + MTL(SR \times AT)].$$

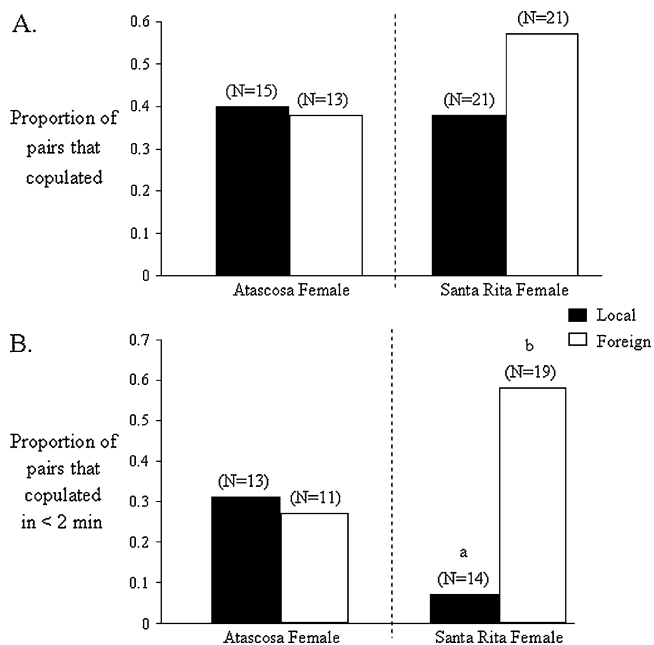


Figure 1

(A) The proportion of females that copulated on their first trial broken down into within-population response (local) and between-population response (foreign). Although not significant, SR females tend to prefer foreign males. (B) The proportion of females that copulated within 2 min of the initiation of male courtship. SR females mate significantly more with foreign versus local males, while AT females show no difference in mating frequency. Different letters indicate significant differences ($p < .05$).

A one-sided z test was used to test the hypothesis of $z \leq 0$, where

$$z = [\text{MTL}(\text{SR} \times \text{SR}) + \text{MTL}(\text{AT} \times \text{AT})] - [\text{MTL}(\text{AT} \times \text{SR}) + \text{MTL}(\text{SR} \times \text{AT})].$$

We use this difference of response differences (DRD) test as the definitive test for positive coevolution and sensory bias (instead of, say, an ANOVA) because this test reflects precisely what the models predict: that this difference of differences will either be 0 or less than 0. Positive coevolution predicts a z of less than 0. In contrast, a simple model of sensory bias predicts a z of exactly 0. The variance of z was calculated as follows:

$$(\text{variance SR} \times \text{SR}) + (\text{variance AT} \times \text{AT}) + (\text{variance SR} \times \text{AT}) + (\text{variance AT} \times \text{SR}),$$

where the variances are weighted by sample size. Thus, for example, $\text{variance AT} \times \text{AT} = S(\text{AT} \times \text{AT})/n(\text{AT} \times \text{AT})$, with S being the sample variance for $\text{AT} \times \text{AT}$ and n being the sample size.

Proximate factors

In order to explore some potential mechanisms underlying the patterns of female choice observed in this study, we scored the videos of pairs that successfully copulated on their first trials (Figure 1A data). Scored behaviors included: number of times a female focused visual attention on the male, duration of male movement (min), and the total duration of time a female spent visually attentive to a courting male (min). Male movement was defined as any time a male moved in location within the arena; this did not include, for example, instances

where males stood stationary and moved only their palpi. Female visual attention was defined as times when a female's anterior median eyes were directly oriented toward a courting male. Females often track male movements by holding the male in the center of their visual field, making this behavior easy to detect. These behaviors were scored prior to copulation. We also standardized the behaviors across pairs by dividing them by the associated latency to copulation. Multiple t tests compared female behaviors between local and foreign males, and a Bonferroni correction was used to adjust the alpha level to $p = .017$.

RESULTS

A total of 70 females (SR = 42 and AT = 28) and 90 males (SR = 43 and AT = 47) were used in a total of 173 trials. Sixty-seven percent of the males were used more than once with the following breakdown: once $N = 31$; twice $N = 20$; three times $N = 15$; four times $N = 16$; five times $N = 5$; six times $N = 1$, and seven times $N = 2$. The individual male had no effect on whether or not the female would mate ($\chi^2 = 0.46$, $p = .5$). AT and SR males did not differ in their average size (AT male mean weight = 13.5 mg, SE = 0.37, $N = 46$; SR male mean weight = 13.43 mg, SE = 0.29, $N = 46$).

Using data from only the first trial for each female, SR females tended to copulate more readily with AT males; but there was no significant difference in mating frequency for either population with local versus foreign males (Figure 1A; SR females $\chi^2 = 1.54$, $p = .2$; AT females $\chi^2 = 0.007$, $p = .93$). In a combined analysis including first trials only for both populations, mating frequency did not depend on mating treatment ($\chi^2 = 2.02$, $p = .57$; Figure 1A). When only pairs which mated in less than 2 min were included, SR females mated significantly more with foreign (AT) males versus local (SR) males (Figure 1B; $\chi^2 = 8.52$, $p = .0035$) while AT females did not distinguish between males (Figure 1B; $\chi^2 = 0.03$, $p = .85$). A combined analysis including pairs that mated in less than 2 min for both populations also reveals that mating frequency depends on mating treatment ($\chi^2 = 8.67$, $p = .034$), and a correspondence analysis shows that SR \times AT pairings result in significantly more matings than any other pairing (Figure 1B).

An analysis using trial latency, or the number of trials each female experienced prior to mating, revealed again that female response to foreign versus local males is significantly different for SR females but not for AT females. Eight AT females and seven SR females were excluded in these comparisons because they had not mated and were not used for a total of five times. SR females had a shorter trial latency with AT males than SR males, indicating a preference for AT males (SR \times SR mean trial latency = 3.0, SE = 0.42, $N = 17$; SR \times AT mean = 1.8, SE = 0.4, $N = 18$; $F_{1,33} = 4.44$, $p = .04$; Figure 2). AT females showed no difference in trial latency (AT \times AT mean = 2.3, SE = 0.67, $N = 10$; AT \times SR mean = 2.9, SE = 0.67, $N = 10$; $F_{1,18} = 0.40$, $p = .54$; Figure 2). In an ANOVA including both populations, trial latency did not depend on mating treatment ($F_{3,51} = 1.48$, $p = .23$). Two females, SR females paired with SR males, refused to mate during each of the five trials. To examine whether they would react differently to AT males, they were presented on subsequent days with AT males. One of the females mated with her first AT male and the other with her second AT male, again suggesting a preference for AT males. Because these females were treated differently, their latency to copulation scores were not included in the copulation latency data (Figure 2).

Using the trial latency data, the DRD test showed that within-population latencies were greater than between-population latencies and thus that AT female preference for AT males

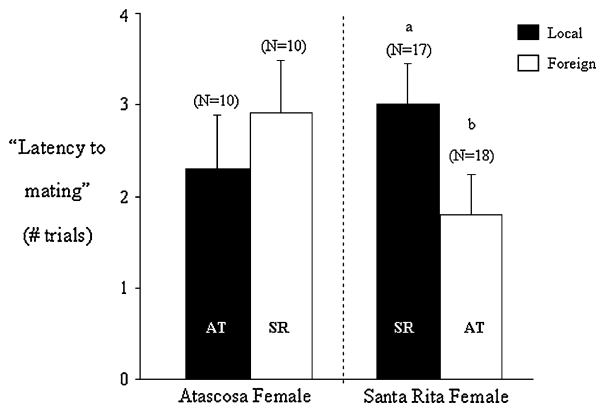


Figure 2 Female response as measured by the trial number in which mating occurred (“trial latency”), broken down into within-population response (local) and between-population response (foreign). SR females had a lower trial latency with foreign males versus local males, indicating a preference for foreign males. AT females showed no difference. Different letters indicate significant differences ($p < .05$).

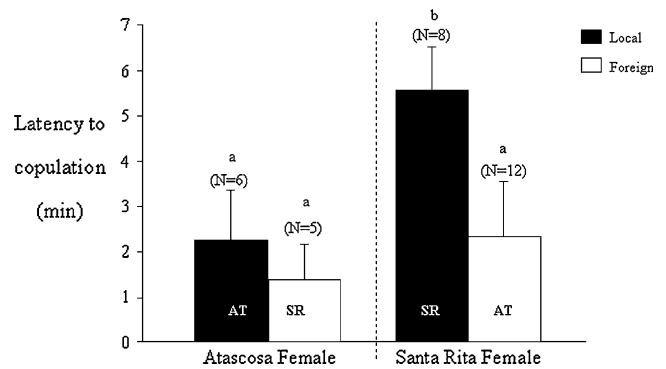


Figure 3 Latency to copulation (in min) broken down into within-population response (local) and between-population response (foreign). SR females have a significantly shorter latency to copulation with foreign males versus local males. AT females show no difference. Different letters indicate significant differences ($p < .05$).

was less than SR female preference for AT males (see Methods) ($z = 0.622$, $SE = 0.304$, $p < .025$). The response differences in the trial latency data were smaller than the response differences observed with the copulation latency <2 min data (Figure 1B), and thus, these data represent a more conservative test.

In analyzing the latency to copulation (in min), SR females took significantly longer to mate with local SR males than with foreign AT males, likewise suggesting a preference for AT males ($F_{1,30} = 15.62$, $p = .0004$, means see Figure 3). AT females did not differ in their latency to copulation between males ($F_{1,14} = 0.15$, $p = .71$, means see Figure 3). In a combined analysis including both populations, latency to copulation did depend on mating treatment ($F_{3,27} = 3.96$, $p = .018$; female origin $F = 1.28$, $p = .27$, male origin $F = 4.29$, $p = .048$, female origin \times male origin $F = 3.94$, $p = .058$). SR \times SR pairs had a significantly longer latency to copulation than any other pair (Figure 3).

Prior to copulation, SR females tend to direct their visual attention toward SR males more frequently than toward AT males, yet this pattern is not significant and disappears when the behavior is compared relative to the mount latency (Table 1). When compared relative to mount latency, AT females tend to direct their visual attention toward SR males more frequently than toward their local AT males, but after the Bonferroni correction, this trend is not significant (Table 1).

When comparing the duration of visual attention prior to copulation, SR females spend more time visually attentive to local SR males compared to AT males (Table 1). Yet, when compared relative to mount latency, SR females spend proportionally more time visually attentive to AT males versus their own local SR males (Table 1). No other behavioral differences were found among females (Table 1). Neither “number of times attentive/mount latency” nor “male movement/mount latency” was dependent on mating treatment in combined analyses (number of times attentive/mount latency, $F_{3,25} = 0.17$, $p = .92$; male movement/mount latency, $F_{3,24} = 0.98$, $p = .42$). However, “prior attention/mount latency” did depend on mating treatment ($F_{3,22} = 5.88$, $p = .004$). Both male origin and an interaction between female and male origin influenced prior attention/mount latency (female origin $F = 2.1$, $p = .16$, male origin $F = 4.59$, $p = .04$, female origin \times male origin $F = 8.89$, $p = .007$). AT males received proportionally

more visual attention than SR males (AT mean = 0.84 ± 0.06 , SR mean = 0.63 ± 0.08). SR females spent proportionally less time visually attentive to local SR males than foreign AT males, and SR females spent proportionally less time visually attentive to their own SR males than did AT females (Figure 4).

DISCUSSION

We conducted reciprocal crosses between two phenotypically divergent populations of the jumping spider *H. pugillis* in order to explore the pathways taken by sexual selection in their diversification. Our data revealed a xenophilic mating preference in which females from one population (SR) had a greater tendency to mate with males from a second population (AT) as compared to their own local SR males, while females from the other population (AT) showed no difference in mating frequency between SR (foreign) and AT (local) males. This xenophilic mating pattern is suggested by the mating frequency data from first trials only (Figure 1A) and is statistically supported when we analyze only pairs that copulated within 2 min (a putatively meaningful cutoff based on field observations). This same statistical pattern is also found in analyses involving both trial latency data as well as the latency to copulation data (Figures 2 and 3). This xenophilic mating pattern is highlighted in the DRD test, which rejects the predictions of a simple positive coevolutionary model. As we will argue below, this does not necessarily rule out entirely positive reinforcing coevolution between male traits and female preferences, but it would impose specific requirements on such a system.

Positive coevolution allows for the possibility that females of one population prefer males of a second population if, for example, males in population 1 are constrained in their elaboration of a trait (e.g., due to predation pressure) or if males from population 2 evolve a particularly attractive trait (e.g., by sensory exploitation). In the first scenario, females from both populations are expected to prefer the more ornamented males, a pattern which is not supported by our results. In the second scenario, females from both populations should be equally susceptible to the newly evolved attractive trait (Jennions and Petrie, 1997). Thus, through a process of positive coevolution, females from population 2 would evolve an enhanced preference for this attractive trait. Females of population 2 then are expected to prefer their own males even more than females from population 1. In other words, under a process of pure positive coevolution, if SR females prefer

Table 1
Summary of behaviors associated with successful copulations from first trials only (means \pm SE)

	AT males	SR males	<i>t</i> Value	<i>p</i> Value
AT females	<i>N</i> = 6	<i>N</i> = 5		
Number of times attentive	1.5 \pm 0.95	3.2 \pm 1.03	-1.2	.26
Number of times attentive/mount latency	0.96 \pm 0.14	1.45 \pm 0.15	-2.4	.04
Male movement (min)	0.43 \pm 0.11	0.16 \pm 0.13	1.57	.15
Male movement (min)/mount latency	0.24 \pm 0.08	0.11 \pm 0.08	1.17	.27
Prior attention	1.5 \pm 0.4	1.1 \pm 0.44	0.68	.52
Prior attention/mount latency	0.76 \pm 0.13	0.85 \pm 0.16	-0.41	.7
SR Females	<i>N</i> = 12	<i>N</i> = 8		
Number of times attentive	1.5 \pm 0.8	4.0 \pm 0.92	-2.02	.06
Number of times attentive/mount latency	1.26 \pm 0.52	1.4 \pm 0.58	-0.18	.86
Male movement (min)	0.25 \pm 0.11	0.42 \pm 0.13	-0.98	.34
Male movement (min)/mount latency	0.19 \pm 0.04	0.12 \pm 0.05	1.07	.3
Prior attention	1.09 \pm 0.23	2.58 \pm 0.3	-3.96	.0014*
Prior attention/mount latency	0.76 \pm 0.1	0.3 \pm 0.12	2.95	.009*

* Indicates significance at $\alpha = 0.017$ (Bonferroni correction).

AT males, then AT females must prefer AT males even more strongly. This hypothesis was rejected by our DRD test, indicating that indeed the preference shown by SR females for AT males is stronger than that shown by AT females, and thus, our results are not consistent with a simple process of pure positive coevolution. Of course, that is not to say that such a process has played no role in this system, only that on its own it cannot explain the observed pattern. Our test is consistent, for instance, with a compound model in which positive reinforcing selection is acting in conjunction with another form of selection or with random drift.

The preceding argument, and our DRD test, implicitly assumes that the two populations are evolving along more or less isolated evolutionary trajectories, with different traits being the focus of selection in the different populations. This appears to be a reasonable assumption for *H. pugillis*, given the strikingly different phenotypes that have been derived in the different populations (Maddison and McMahon, 2000), but it is not what might be expected in general. Evidence in other species suggests that selection acts on similar traits across populations, especially with respect to sexual selection (male coloration: Houde and Endler, 1990; Kwiatkowski and Sullivan, 2002; long chirp call: Simmons et al., 2001). The assumption that populations have independent trajectories permits us to assume that a female's preference enhanced by coevolution is enhanced only to her own male's traits. If the two populations' novel traits and preferences are in similar signaling and sensory modalities, it is possible to imagine scenarios in which the females' evolving enhanced preferences to the traits of local males could coincidentally enhance their preferences to traits of foreign males even more strongly. This coincidental coevolution could yield a significant result in the DRD test even though the process is positive coevolution. Thus, what the DRD test actually rejects is a simple hypothesis of positive male-female coevolution that is exclusive to the local population.

Patterns of foreign-mate preference as seen in this study are certainly not uncommon and have been observed in *Drosophila* (Dobzhansky and Steisinger, 1944; Wu et al., 1995), swordtail fish (Ryan and Wagner, 1987), and sticklebacks (McPhail, 1969). In many of these examples, females from both populations/species prefer males from the same population. For example, a pattern of xenophilia was observed in the mating patterns of *Drosophila prosaltans*, where females from Mexico preferred Brazilian males to their own and Brazilian females

also preferred their own males (Dobzhansky and Steisinger, 1944). In swordtails, females of one species (*Xiphophorus pygmaeus*) prefer to mate with heterospecific (*Xiphophorus nigrensis*) males to their own conspecifics, while *X. nigrensis* females prefer their own males. In this particular example, the female preference seems to relate to the courtship behavior of the *X. nigrensis* males (Ryan and Wagner, 1987). A sensory bias, manifested as the shared preference for full courtship in a common ancestor, was put forth as one potential explanation for this observed heterospecific mating preference (Ryan and Wagner, 1987). All these patterns of foreign-mate preference differ markedly from those presented in this study.

Here, females from both populations did not have higher mating frequencies with males from the same population; instead, the preferred males (AT) were not preferred by their local females. A pattern of mate choice xenophilia similar to that seen in this study was found in a study of female preference functions in poeciliid fish. The strength of female preference for a sword in a genus lacking swords (*Priapella*) was higher than the strength of female preference for a sword in a genus in which swords evolved (*Xiphophorus*) (Basolo, 1998).

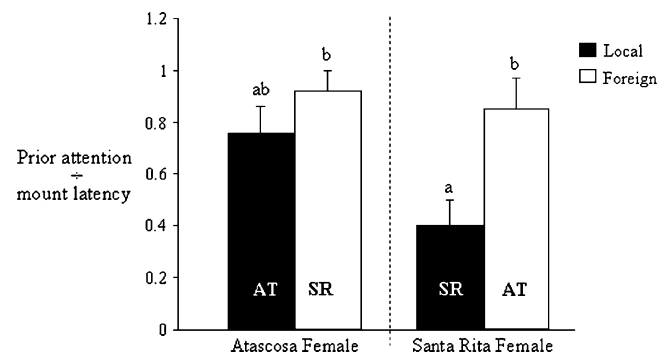


Figure 4

The proportion of time a female spent visually attentive to her future mate prior to copulation (using first trial data only) broken down into within-population response (local) and between-population response (foreign). SR females spent proportionally more of their time visually attentive to foreign males versus local males, while AT females showed no difference. Different letters indicate significant differences ($p < .017$).

In discussing her results, Basolo (1998) mentions a process involving antagonistic coevolution as one potential explanation underlying her observed female preference pattern. More recent studies have also provided empirical evidence of antagonistic coevolution. For example, the coevolution of male seminal signals and female receptors is argued to be driven by sexual conflict in houseflies (Andres and Arnqvist, 2001). In a series of reciprocal mating experiments, Andres and Arnqvist (2001) found that the male's ability to induce oviposition in females was dependent on her genotype. Females of one strain did not respond differently to foreign males (males of different strains), while females from other strains showed the weakest response to local males (Andres and Arnqvist, 2001).

The results observed in this study may also represent an example of antagonistic coevolution. The observed mating pattern of SR female xenophilia and AT female apathy is similar to theoretical predictions of interpopulation crosses involving two populations with varying intensities of sexual conflict (see figure 3b Pizzari and Snook, 2003). Assuming a general resistance, females from the population with more intense conflict should be more resistant to males from both populations as compared to females from the population with less intense conflict (Pizzari and Snook, 2003). Provided that these predictions are valid (but see Rowe et al., 2003), our results would suggest that the AT population is under more intense conflict than the SR population (Figures 1 and 2 compared to figure 3c Pizzari and Snook, 2003). Unfortunately, our data do not permit us to affirm antagonistic coevolution as explaining our data. Without knowledge of the baseline or ancestral mating frequency for this group, it is impossible to discern whether AT females actually have a lower mating frequency with local males than expected or whether SR females are exhibiting an exaggerated mating frequency when paired with AT males. Furthermore, even if we knew such details, they may nonetheless be consistent with many models other than antagonistic coevolution (Rowe et al., 2003).

While details regarding the proximate factors underlying our observed mating patterns cannot be addressed with this study, our results do provide some preliminary insights into factors influencing mate choice among females from different populations. Of the SR females that copulated, those that mated with AT males spent 85% of their time visually attentive to the male prior to copulation, while those that mated with local SR males spent only 40% of their time visually attentive (Figure 4). In contrast, AT females did not significantly differ in the amount of time they spent visually attentive to males from either population. The ability of AT males to retain the visual attention of SR females may be an important factor underlying the xenophilic mating preferences observed in this study; AT males may be taking advantage of some component of the SR female's visual sensory system. Originally, based up the differences in courtship behavior between the two populations (see Methods), we expected that any difference in visual attention would result from differences in male movement during courtship. Surprisingly, the amount of time males spent moving did not differ between populations (Table 1). This difference in attention then does not appear to be related to male courtship movements or to the overall visual locomotory-based signals involved in these courtship displays. Other *Habronattus* species (including *H. pugillis*) produce multimodal (visual plus seismic) signals during courtship (Elias DO, Hebets EA, Hoy RR, in preparation) (Elias et al., 2003; Maddison and Stratton, 1988) and AT males appear to have a more elaborate seismic component to their courtship display than SR males (Elias DO, Hebets EA, Hoy RR, in preparation). The AT male's elaborate seismic component may be involved in focusing/attracting/retaining a female's visual

attention. This type of attention-altering interaction between seismic and visual courtship signals has recently been demonstrated in a wolf spider (Hebets, 2005). Future studies will explore this potential for seismic manipulation of visual attention and will attempt to uncover the proximate factors underlying the xenophilic mating pattern observed between *H. pugillis* from these two populations.

Ambiguity in predictions of interpopulation crosses (Rowe et al., 2003) prevents us from confidently attributing our observations to a particular model of sexual selection. However, we can reject a pure process of positive coevolution of male trait and female preferences, which could have implications for the process of diversification and speciation. Although sexual selection is sometimes implicated in promoting reproductive isolation, antagonistic coevolution between males and females could conceivably do the opposite, promoting hybridization by making females susceptible to male traits with which they have not coevolved (Parker and Partridge, 1998). The xenophilia seen in *H. pugillis* populations allows for the possibility that the very traits in which they are diverging most noticeably might paradoxically promote introgression should the populations come into secondary contact. Indeed, while Masta (2000) did not find support for hybridization in mitochondrial data (Masta, 2000), there are hints of hybridization in the pattern of phenotypic differences among the populations (Maddison and McMahan, 2000).

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REFERENCES

- Andres JA, Arnqvist G, 2001. Genetic divergence of the seminal signal-receptor system in houseflies: the footprints of sexually antagonistic coevolution? *Proc R Soc Lond B Biol Sci* 268:399–405.
- Barraclough TG, Harvey PH, Nee S, 1995. Sexual selection and taxonomic diversity in passerine birds. *Proc R Soc Lond B Biol Sci* 259:211–215.
- Basolo AL, 1990. Female preference predates the evolution of the sword in swordtail fish. *Science* 250:808–810.
- Basolo AL, 1996. The phylogenetic distribution of a female preference. *Syst Biol* 45:290–307.
- Basolo AL, 1998. Evolutionary change in a receiver bias: a comparison of female preference functions. *Proc R Soc Lond B Biol Sci* 265:2223–2228.
- Bordenstein SR, Drapeau MD, Werren JH, 2000. Intraspecific variation in sexual isolation in the jewel wasp *Nasonia*. *Evolution* 54:567–573.
- Cocroft RB, Ryan MJ, 1995. Patterns of advertisement call evolution in toads and chorus frogs. *Anim Behav* 49:283–303.
- Cutler BE, 1988. Courtship behaviour in *Habronattus captiosus* (Araneae: Salticidae). *Gt Lakes Entomol* 21:129–131.
- Dobzhansky T, Steisinger G, 1944. Experiments on sexual isolation in *Drosophila*. *Proc Natl Acad Sci USA* 30:340–355.
- Dombrovsky Y, Perrin N, 1994. On adaptive search and optimal stopping in sequential mate choice. *Am Nat* 144:355–361.
- Elias DO, Mason AC, Maddison WP, Hoy RR, 2003. Seismic signals in a courting male jumping spider (Araneae: Salticidae). *J Exp Biol* 206:4029–4039.
- Ender JA, Houde AE, 1995. Geographic-variation in female preferences for male traits in *Poecilia reticulata*. *Evolution* 49:456–468.
- Gray DA, Cade WH, 2000. Sexual selection and speciation in field crickets. *Proc Natl Acad Sci USA* 97:14449–14454.

- Griswold C, 1977. *Biosystematics of Habronattus in California*. Berkeley, California: University of California.
- Griswold C, 1987. A revision of the jumping spider genus *Habronattus* F.O.P. Cambridge (Araneae: Salticidae) with phenetic and cladistic analyses. *Univ Calif Publ Entomol* 107:1–344.
- Hamilton WJ, Poulin R, 1999. Female preference and male nuptial colouration in the freshwater fish *Gobiomorphus breviceps*: geographic variation among populations. *Can J Zool* 77:463–469.
- Hebets EA, 2005. Attention-altering interaction in the multimodal courtship display of the wolf spider *Schizocosa uetzi*. *Behav Ecol* 16:75–82.
- Hebets EA, Uetz GW, 1999. Female responses to isolated signals from multimodal male courtship displays in the wolf spider genus *Schizocosa* (Araneae: Lycosidae). *Anim Behav* 57:865–872.
- Hebets EA, Uetz GW, 2000. Leg ornamentation and the efficacy of courtship display in four species of wolf spider (Araneae: Lycosidae). *Behav Ecol Sociobiol* 47:280–286.
- Hill GE, 1994. Geographic-variation in male ornamentation and female mate preference in the house finch—a comparative test of models of sexual selection. *Behav Ecol* 5:64–73.
- Houde AE, Endler JA, 1990. Correlated evolution of female mating preferences and male color patterns in the Guppy *Poecilia reticulata*. *Science* 248:1405–1408.
- Iliadi K, Iliadi N, Rashkovetsky E, Minkov I, Nevo E, Korol A, 2001. Sexual and reproductive behaviour of *Drosophila melanogaster* from a microclimatically interslope differentiated population of 'Evolution Canyon' (Mount Carmel, Israel). *Proc R Soc Lond B Biol Sci* 268:2365–2374.
- Janetos AC, 1980. Strategies of female mate choice—a theoretical analysis. *Behav Ecol Sociobiol* 7:107–112.
- Jennions MD, Petrie M, 1997. Variation in mate choice and mating preferences: a review of causes and consequences. *Biol Rev Camb Philos Soc* 72:283–327.
- Jones IL, Hunter FM, 1998. Heterospecific mating preferences for a feather ornament in least auklets. *Behav Ecol* 9:187–192.
- Kirkpatrick M, Ravigne V, 2002. Speciation by natural and sexual selection: models and experiments. *Am Nat* 159:S22–S35.
- Kobayashi A, Hiroki M, Kato Y, 2001. Sexual isolation between two sympatric types of the butterfly *Eurema hecabe* (L.). *J Insect Behav* 14:353–362.
- Korol A, Rashkovetsky E, Iliadi K, Michalak P, Ronin Y, Nevo E, 2000. Nonrandom mating in *Drosophila melanogaster* laboratory populations derived from closely adjacent ecologically contrasting slopes at "Evolution Canyon." *Proc Natl Acad Sci USA* 97:12637–12642.
- Kwiatkowski MA, Sullivan BK, 2002. Geographic variation in sexual selection among populations of an iguanid lizard, *Sauromalus obesus* (=ater). *Evolution* 56:2039–2051.
- Maddison W, Hedin M, 2003. Phylogeny of *Habronattus* jumping spiders (Araneae: Salticidae), with consideration of genital and courtship evolution. *Syst Entomol* 28:1–21.
- Maddison W, McMahon M, 2000. Divergence and reticulation among montane populations of a jumping spider (*Habronattus pugillis* Griswold). *Syst Biol* 49:400–421.
- Maddison WP, Stratton GE, 1988. Sound production and associated morphology in male jumping spiders of the *Habronattus agilis* species group (Araneae, Salticidae). *J Arachnol* 16:199–211.
- Masta SE, 2000. Phylogeography of the jumping spider *Habronattus pugillis* (Araneae: Salticidae): recent vicariance of sky island populations? *Evolution* 54:1699–1711.
- Masta SE, Maddison WP, 2002. Sexual selection driving diversification in jumping spiders. *Proc Natl Acad Sci USA* 99:4442–4447.
- McClintock WJ, Uetz GW, 1996. Female choice and pre-existing bias: visual cues during courtship in two *Schizocosa* wolf spiders (Araneae: Lycosidae). *Anim Behav* 52:167–181.
- McPhail JD, 1969. Predation and evolution of a stickleback (*Gasterosteus*). *J Fish Res Board Can* 26:3183–3208.
- Mendelson TC, Shaw KL, 2005. Sexual behaviour: rapid speciation in an arthropod. *Nature* 433:375–376.
- Mitra S, Landel H, Pruett-Jones S, 1996. Species richness covaries with mating system in birds. *Auk* 113:544–551.
- Morrow EH, Pitcher TE, Arnqvist G, 2003. No evidence that sexual selection is an 'engine of speciation' in birds. *Ecol Lett* 6:228–234.
- Parker GA, Partridge L, 1998. Sexual conflict and speciation. *Philos Trans R Soc Lond B Biol Sci* 353:261–274.
- Peckham G, Peckham E, 1889. Observations on sexual selection in spiders of the family Attidae. *Occas Pap Wis Nat Hist Soc* 1:3–60.
- Peckham G, Peckham E, 1890. Additional observations on sexual selection in spiders of the family Attidae, with some remarks on Mr. Wallace's theory of sexual ornamentation. *Occas Pap Wis Nat Hist Soc* 1:117–151.
- Pizzari T, Snook RR, 2003. Perspective: sexual conflict and sexual selection: chasing away paradigm shifts. *Evolution* 57:1223–1236.
- Pomiankowski A, Iwasa Y, 1998. Runaway ornament diversity caused by Fisherian sexual selection. *Proc Natl Acad Sci USA* 95:5106–5111.
- Proctor HC, 1992. Sensory exploitation and the evolution of male mating-behavior—a cladistic test using water mites (Acari, Parasitengona). *Anim Behav* 44:745–752.
- Prum RO, 1997. Phylogenetic tests of alternative intersexual selection mechanisms: trait macroevolution in a polygynous clade (Aves: Pipridae). *Am Nat* 149:668–692.
- Ptacek MB, 1998. Interspecific mate choice in sailfin and shortfin species of mollies. *Anim Behav* 56:1145–1154.
- Richman DB, 1977. Comparative studies on the mating behaviour and morphology of some species of Pellenes (Araneae—Salticidae). Tucson, Arizona: University of Arizona.
- Richman DB, 1982. Epigamic display in jumping spiders (Araneae, Salticidae) and its use in systematics. *J Arachnol* 10:47–67.
- Rowe L, Cameron E, Day T, 2003. Detecting sexually antagonistic coevolution with population crosses. *Proc R Soc Lond B Biol Sci* 270:2009–2016.
- Ryan MJ, 1991. Sexual selection and communication in frogs. *Trends Ecol Evol* 6:351–355.
- Ryan MJ, Fox JH, Wilczynski W, Rand AS, 1990. Sexual selection for sensory exploitation in the frog *Physalaemus pustulosus*. *Nature* 343:66–67.
- Ryan MJ, Rand AS, 1993. Sexual selection and signal evolution—the ghost of biases past. *Philos Trans R Soc Lond B Biol Sci* 340:187–195.
- Ryan MJ, Wagner WE, 1987. Asymmetries in mating preferences between species—female swordtails prefer heterospecific males. *Science* 236:595–597.
- Seehausen O, 2002. Patterns in fish radiation are compatible with Pleistocene desiccation of Lake Victoria and 14 600 year history for its cichlid species flock. *Proc R Soc Lond B Biol Sci* 269:491–497.
- Shapiro LH, 2001. Asymmetric assortative mating between two hybridizing *Orchelimum* katydid (Orthoptera: Tettigoniidae). *Am Midl Nat* 145:423–427.
- Simmons LW, Zuk M, Rotenberry JT, 2001. Geographic variation in female preference functions and male songs of the field cricket *Teleogryllus oceanicus*. *Evolution* 55:1386–1394.
- Wade MJ, Chang NW, McNaughton M, 1995. Incipient speciation in the flour beetle, *Tribolium confusum*—pre-mating isolation between natural-populations. *Heredity* 75:453–459.
- Wu CI, Hollocher H, Begun DJ, Aquadro CF, Xu YJ, Wu ML, 1995. Sexual isolation in *Drosophila melanogaster*—a possible case of incipient speciation. *Proc Natl Acad Sci USA* 92:2519–2523.