Current Zoology 59 (1): 20–30, 2013

Asymmetrical mate preference in recently adapted White Sands and black lava populations of *Sceloporus undulatus*

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Abstract Speciation can proceed rapidly when natural and sexual selection act in concert. For example speciation can be accelerated when traits that confer a selective advantage in a particular habitat also influence mate preference. Studying parallel but evolutionarily independent instances of ecological divergence can illuminate the interaction between natural and sexual selection during speciation. Locally adapted populations of the eastern fence lizard *Sceloporus undulatus* have recently evolved in three different habitats in the Chihuahuan desert: blanched color morphs occur on the gypsum dunes of White Sands, melanic color morphs occur on the Carrizozo lava flow, and brown color morphs occur in the surrounding desert scrubland. In addition to differences in cryptic dorsal coloration, populations also differ in the size and color of ventral patches used for social signaling. This system therefore provides an opportunity to investigate the interplay of natural and sexual selection during rapid ecological speciation. We used mate preference experiments to determine whether locally adapted populations may exhibit the early stages of behavioral reproductive isolation. We observed an asymmetrical mate preference in this system; White Sands males preferentially courted local females, while males from dark soils and black lava populations did not exhibit a preference for local mates. We also found that female behavior and ventral patch phenotype were associated with male courtship. Our results suggest that the observed preference for local mates evolved at White Sands, and we discuss the possible link between local adaptation and traits involved in mate preference in this system [*Current Zoology* 59 (1): 20–30, 2013].

Keywords Eastern fence lizard, Magic trait, Ecological speciation, Sexual signaling, Courtship, Mate preference asymmetry

Understanding the interaction between natural and sexual selection during ecological divergence is a central goal of speciation research (Ritchie, 2007; Maan and Seehausen, 2011). For populations undergoing divergent selection, mate preference can accelerate reproductive isolation (e.g., Lande, 1981; Barraclough et al., 1995; Seehausen et al., 1997; Price, 1998, Boughman et al., 2005). In fact, speciation can proceed rapidly when traits subject to divergent natural selection have pleiotropic effects that cause assortative mating (Maynard Smith, 1966; Gavrilets, 2004). A recent review suggested that traits linking ecology and mating may not be rare in natural populations (Servedio et al., 2011), and there are a number of empirical examples of traits that pleiotropically affect adaptation and reproductive isolation in animal populations [e.g., beak morphology in Darwin's finches (Podos and Nowicki, 2004), body size in stickleback fish (Nagel and Schluter, 1998), color pattern in *Heliconius* butterflies (Jiggins et al., 2001)]. It is now important to determine how ubiquitous the interaction is between natural and sexual selection during rapid ecological divergence.

Ecologically distinct populations of the eastern fence lizard *Sceloporus undulatus* in the Chihuahuan desert of New Mexico represent an ideal system to study the interplay of local adaptation and mate preference. Locally adapted morphs of *S. undulatus* occur in three dramatically different habitats in the Chihuahuan desert. Blanched color morphs occur at White Sands, a habitat with white gypsum substrate. Melanic color morphs occur at the Carrizozo lava flow, a habitat composed of black basalt deposits. Brown morphs are found in the surrounding Chihuahuan "dark soils" scrubland, a habitat characterized by brown substrate. Dark soils populations of *S. undulatus* are ancestral to White Sands and black lava populations (Rosenblum et al., 2007). Both White Sands and the Carrizozo lava flow are geologically recent formations of approximately equal size that

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Received Apr. 4, 2012; accepted Oct. 28, 2012.

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were likely colonized by *S. undulatus* less than 6,000 years ago (Kocurek et al., 2007; Fryberger, unpublished data).

The parallel and rapid evolution of cryptic *S. undulatus* ecotypes in White Sands and black lava habitats allows us to evaluate the links between natural and sexual selection in independent but comparable natural evolutionary experiments. Color is a key trait promoting ecological divergence in this system as it plays a role in both predator avoidance and intraspecific interactions (Robertson and Rosenblum, 2009). Dorsal coloration is important for reptile crypsis and often evolves rapidly in habitats with different colored substrates. Substrate-matching coloration for *S. undulatus* in the Chihuahuan Desert is presumably an adaptation for avoidance of visually hunting predators such as the greater roadrunner *Geococcyx californianus* and the loggerhead shrike *Lanius ludovicianus* (Rosenblum, 2006). *Sceloporus* lizards also have bright blue ventral color patches which are used for intraspecific communication (Cooper and Burns, 1987). Both ventral patch size and color vary across *S. undulatus* populations and may be used as cues for population recognition (Robertson and Rosenblum, 2009, 2010). Natural and sexual selection on color may be mechanistically linked in this system because the color of both dorsal and ventral patches is largely determined by the density and distribution of melanin in the skin (Bagnara and Hadley, 1973). Therefore changes in melanin production due to natural selection for substrate-matching can have a by-product effect on ventral color patches, potentially impacting mate preference and playing a role in reproductive isolation in this system.

In addition to population differences in coloration, there is evidence that the focal *S. undulatus* populations are in the early stages of ecological speciation (Rosenblum and Harmon, 2011). White Sands and dark soils populations differ not only in dorsal and ventral coloration but also in other ecologically important morphological (e.g., body shape) and behavioral (e.g., territorial and anti-predator response) traits (Robertson and Rosenblum, 2010; Robertson et al., 2011; Rosenblum and Harmon, 2011). Corresponding ecological studies have not yet compared black lava and dark soils populations, but genetic data suggest some degree of isolation among all three populations (Rosenblum et al., 2007).

An important and unanswered question in this system is whether locally adapted populations exhibit behavioral reproductive isolating mechanisms, a critical component in accelerating the process of ecological speciation. Therefore, we tested whether different *S. undulatus* ecotypes exhibit a preference for local mates. Using sequential behavior experiments in males' natural territories, we evaluated mate preferences in one ancestral and two derived populations with independent evolutionary histories. Specifically, we asked whether White Sands, dark soils, and black lava *S. undulatus* males preferentially courted local (i.e., ecologically similar) versus foreign (i.e., ecologically distinct) females and determined the extent to which preferences were symmetrical across populations.

1 Materials and Methods

1.1 Data Collection

We conducted mate preference experiments in the field with *S. undulatus*. During the breeding season, adult *S. undulatus* males are highly territorial, and several females may be found within a single male's territory at one time (Haenel et al., 2003). Some long-term association between males and females has been observed (Ferguson, 1970), but males will also court novel females (Cooper and Burns, 1987; Haenel et al., 2003). Individual courtship interactions are generally brief, so behaviors can be scored in relatively short trials in a natural context. We conducted trials from May to July 2010 during the peak of the local *S. undulatus* breeding season (Vinegar, 1975; Smith and John-Alder, 1999) and during the hours of 07:30–12:00 when males are active. All males in the study were reproductively mature (mean SVL = 5.8 cm). Each male was presented sequentially with one local and one foreign female. This resulted in four trial categories: 1) White Sands males presented with White Sands vs. dark soils females, 2) dark soils males presented with dark soils vs. white sand females, 3) dark soils males presented with dark soils vs. black lava females, and 4) black lava males presented with black lava vs. dark soils females (Fig. 1). We used these focal populations because White Sands and black lava populations represent independent and recently evolved distinct ecotypes. There were two trial categories for dark soils males because the dark soils population is ancestral to populations in both novel habitats (Rosenblum et al., 2007). In each trial category we conducted between 19 and 33 trials for a total of 96 trials (Fig. 1). We observed male behavior in at least 16 trials per trial category (Fig. 1).

Prior to behavioral trials, we captured females from the three habitats by hand or noose. We used 18, 20, and 12 reproductively mature females from White Sands, dark soils, and black lava habitats respectively. Previous

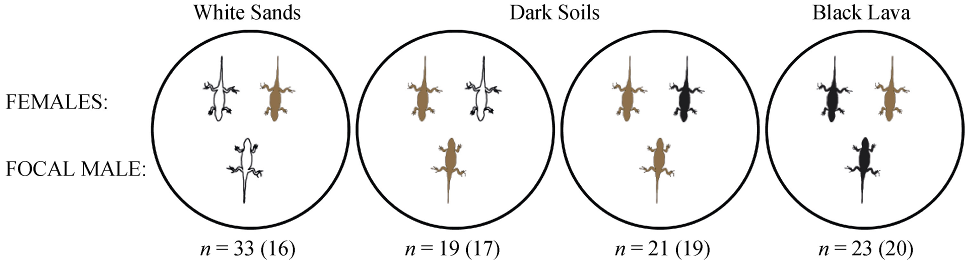

Fig. 1 Staged arena encounters occurred in the natural territory of White Sands (white), dark soils (grey), and black lava (black) focal males

We used a sequential mate preference design to examine the response of each focal male to both local and foreign females. The number of total trials conducted for each category is provided with the number of trials with male behavior in parentheses.

studies of iguanid lizards have shown that males exhibit differences in courtship and territorial behavior towards familiar conspecifics (e.g., Tokarz, 1992; Whiting, 1999). We therefore collected females from a number of different locations within each habitat to ensure that male courtship response did not merely reflect familiarity with neighboring individuals. We weighed and measured all females, and took measurements of their dorsal and ventral coloration using a StellarNet EPP2000Cs spectrometer (StellarNet, Tampa, Florida; UV-VIS range of 280–900 mm) with a deuterium and tungsten/halogen light source (SL4-DT) and a reflectance probe (R600-8-UV-VIS-SR) fitted with a 45 degree angle tip (RTIP45). In addition, we took digital photographs of female ventral surfaces. Each female was used in an average of 4 trials (90% of the females were used in 1–6 trials, although a few females were used in 8–11 trials). Females were never used in more than 3 trials per day. During the experimental period, females were housed individually in small cages with 12 hour light cycles and fed *ad libitum*. After experimental trials females were released at their point of capture.

The test procedure was as follows. For each trial we captured a male in his natural territory by hand or noose. We immediately placed the male in a circular behavioral arena in his territory and allowed him to acclimate for 5 minutes. The arena was made of metal flashing (diameter = 0.85 m; height = 0.35 m) and the inside was painted light brown to eliminate reflectance. The arena was easy to transport and erect quickly, so the same arena was used for all trials. The male was then presented sequentially with two females (one local and one foreign). Females used in trials were size-matched by snout-vent length and their presentation order was randomized. We introduced the first female to the arena by hand via a small hole in the sand at a point in the arena directly opposite of the male's location and in his line of sight. We scored behavioral interactions for 5 minutes (see below). Next we removed the female by noose and the male was allowed to rest for 5 minutes. We then introduced the second female in the same manner and scored behavioral interactions for 5 minutes.

We recorded each five-minute trial using a digital video camera (Canon FS11, Canon, Lake Success, NY, USA). We recorded male and female behavior in the field and subsequently rescored and verified behavioral observations from the videos. Although some *S. undulatus* behaviors are used in multiple contexts, male precopulatory courtship behavior in this species is highly stereotyped (Cooper and Burns, 1987; Martins et al., 2005). For males, we recorded the following courtship behaviors: pushups (i.e., leg flexion moving the entire body towards and away from the ground), head bobs (i.e., up-down movements of the head), shudderbobs (i.e., multiple rapid up-down movements of the head), tongue flicks, nips to the female tail and neck, mounts, and copulation attempts. We summarized the male data by calculating the latency to first courtship behavior and the total time spent courting. For females, we recorded the following behaviors: sidlehops (i.e., sideways hopping with back arched), pushups, lateral flattening, approaches to the male, and attempts to escape from the male. We summarized the female data by scoring whether or not each behavior was performed and by calculating the time spent in the two most common behaviors (i.e., pushups and sidlehops). Detailed descriptions of all quantified behaviors can be found in Greenberg 1977.

1.2 Statistical Analysis

To understand the dynamics of mate preference in this system we used a series of nonparametric categorical analyses because our behavior data were not normally distributed. Our categorical analyses accounted for the paired nature of the behavior trials (e.g., a single male was presented sequentially with two females). For each pair of trials we assigned a preference for the focal male based on which female was courted faster (shorter latency) and which female was courted longer (longer total time in courtship). We then tested for associations between male preference and female characteristics (detailed below) using binomial tests. Statistical tests were performed for each trial category shown in Figure 1. Statistical analyses were conducted in R (Vers. 0.95.262, R Core Development Team, 2011) and JMP (Vers. 9, SAS 2011).

First, we asked whether males preferentially courted local vs. foreign females. Specifically, for each trial category we used binomial tests to determine whether local or foreign females elicited shorter latency and longer total time in courtship (removing trial pairs where there was no male courtship towards either female). If males prefer local females we would expect shorter latency until male courtship and longer total time in courtship for local females compared with foreign females. Additionally, to determine whether the proportion of males that preferred to court local females differed from random expectation, we calculated pairwise (PTI) and global (I_{PTI}) indicators of sexual isolation using the program JMating (Vers. 1.0.8, Carvajal-Rodriguez and Rolan-Alvarez, 2006). PTI is the observed number of trials where local mates were preferred, divided by the number of trials where (assuming random mating) we would expect local mates to be preferred. I_{PTI} is the joint isolation index calculated from PTI coefficients (Rolan-Alvarez and Caballero 2000). For both PTI and I_{PTI}, average test statistics, standard deviations, and one-tailed probabilities of rejecting the null hypothesis were determined by resampling 10,000 times both for the observed and for the expected frequencies of pairs (Carvajal-Rodriguez and Rolan-Alvarez, 2006).

Second, we asked whether male courtship behavior was correlated with any female behaviors. For each pair of trials, we determined which female spent more or less time performing the behaviors described above (i.e., sidlehops, pushups, lateral flattening, approaches to the male, and attempts to escape from the male). We then used binomial tests to determine whether males preferentially courted females that spent more or less time displaying each of these behaviors. To determine if there were corresponding population differences in female behavior, we used Pearson's chi-square tests to investigate the relationship between female population and whether or not females engaged in behaviors described above.

Third, we asked about the timing of male and female behavior in the trials, which may indicate whether males assess female behavior during courtship interactions. For each focal male we calculated an average latency to courtship (in seconds). We then used Kruskal-Wallis tests to determine whether latency until male courtship was similar for focal males from different populations. We also used binomial tests to determine whether males or females behaved first more often in trials.

Fourth, we asked about phenotypic differences in female coloration across populations and whether female color was correlated with male behavior. To quantify divergence in coloration across populations we used Endler's segmentation method (Endler, 1990) to measure hue, chroma, and brightness over the complete visible spectrum (400–700 nm). In addition, we measured the size of female ventral color patches as the ratio of ventral patch area to the total area of the ventral surface from photographs using ImageJ (NIH 2010). We log transformed patch size data because it was not normally distributed. To characterize female color at a multivariate level, we performed a MANOVA comparing hue, chroma, brightness, and patch size among ecologically distinct populations. We then used one-way ANOVAs to compare each aspect of color separately among female populations. To test for associations between female color and male behavior, for each pair of trials we determined which female had higher or lower values of hue, chroma, brightness, and patch size. We then performed binomial tests to determine whether males preferentially courted females based on these different aspects of color phenotype.

Our non-parametric approach is warranted by the violation of normality in our behavioral data. But one limitation of this approach is the inability to integrate all of the data in a single model. Therefore we conducted one parametric analysis to incorporate multivariate measures of female color and behavior into linear models explaining male preference. Specifically, we performed principal components analyses with female behavioral data (average time spent doing sidlehops and pushups during trials) and female color data (hue, chroma, brightness, and patch size). We then used linear models to test the effects of male source population, female color, and female behavior on male courtship response. We also evaluated the interactions between male source population and each color and behavior variable. We included male identity as a random effect. The model therefore contained the following explanatory variables: male population, male identity, female color PC 1 (corresponding to dorsal hue and brightness), female color PC 2 (corresponding to ventral patch hue, brightness, and size), female behavior PC 1 (corresponding to average time performing sidlehops), female behavior PC 2 (corresponding to average time performing pushups), and the interaction between male population and each female PC. Given the violation of normality, we focus our data interpretation on the non-parametric tests, but cautiously consider the added insights from the linear models.

2 Results

Focal male and female lizards engaged in precopulatory behavior in the majority of our trials. Of 96 total trials, we observed male courtship behavior in 72 trials and female behavior in 74 trials. There were copulation attempts in 11 trials (5 of these towards local females and 6 towards foreign females, Fisher's exact test, $P = 0.73$). In general, we did not observe any order effects (i.e., male behavior did not depend on female presentation order, binomial tests, all $P > 0.05$). There was one exception whereby dark soils males presented with local vs. black lava females exhibited shorter latency until courtship for the second female presented (binomial test, $n = 19$, $P = 0.03$). We also did not observe any effect of female identity on male courtship behavior (i.e., no individual female elicited a disproportionately strong courtship response compared with all other females, Wilcoxon rank-sum tests, all $P > 0.05$).

White Sands focal males were the only males in our study to exhibit differences in precopulatory behaviors toward local vs. foreign females. White Sands males exhibited shorter latency until courtship for local females (i.e., they courted White Sands females more quickly than they courted dark soils females) (binomial test, $n = 16$, $P = 0.04$) (Fig. 2). White Sands males also exhibited longer total time in courtship when presented with local females (binomial test, $n = 16$, $P = 0.01$) (Fig. 2). Neither dark soils nor black lava males exhibited differences in courtship behavior for local vs. foreign females for any metric (i.e., binomial tests of latency until courtship and total time in courtship, all $P > 0.05$).

Measures of sexual isolation using the program JMating were also consistent with the results presented above. Global measures of sexual isolation were significant for total time in courtship for pairings between White Sands and dark soils lizards ($I_{\text{PTI}} = 0.38$, $P = 0.03$). Pairwise indices suggested that White Sands males preferentially courted White Sands females more often than expected by chance (PTI = 1.62, $P = 0.04$) and dark soils females less often than expected by chance (PTI = 0.37, $P = 0.01$). Pairwise indices of isolation were not significant for trials where dark soils males were presented with local (PTI = 1.05, $P = 0.87$) or White Sands (PTI = 0.94, $P = 0.81$) females, indicating asymmetric sexual isolation. In addition, Global I_{PTI} and pairwise PTI were non-significant (all $P > 0.05$) for trials with black lava and dark soils lizards, indicating a lack of sexual isolation between these ecologically distinct populations.

Male behavior was correlated with female behavior in some of the trial categories. Most notably, White Sands males exhibited longer total time in courtship when paired with females that sidlehopped (Table 1). Additionally, we observed a nonsignificant trend where White Sands males exhibited shorter latency for females that sidlehopped (Table 1). Males did not exhibit differences in courtship correlated with any of the other female behaviors quantified in our study (binomial tests, all $P > 0.05$).

Females did not exhibit population level differences in most of the behaviors quantified in our study (i.e., sidlehops, lateral flattening, approaches to the male, and attempts to escape from the male, all $P > 0.05$). The lack of population differences in sidlehop behavior is particularly important because male behavior was correlated with female sidlehop behavior in some trials. The only behavior that did show population differences was female pushups; females from White Sands engaged in pushup behavior less than either dark soils or black lava females (Table 2).

Whether or not males had an opportunity to assess female behavior may depend on if males initiated courtship before or after females displayed. Latency to courtship was significantly different for different categories of focal males (Kruskal-Wallis test, $n = 96$, H(2) = 21.64, $P < 0.01$). Mean latency to courtship was longer for White Sands males than for black lava males (Wilcoxon rank-sum test, $n = 56$, W = 290, $P < 0.01$), and was also longer for White Sands males than for dark soils males (Wilcoxon rank-sum test, $n = 73$, W = 157.5, $P < 0.01$). Further, in trials with White Sands males there was no significant difference between whether males or females behaved first (males behaved first in 17 trials while females behaved first in 8) (binomial test, $P = 0.12$). In contrast, males initiated courtship behavior before females in trials with both black lava males (males behaved first in 29 trials while females behaved first in 6) (binomial test, $P < 0.01$) and dark soils males

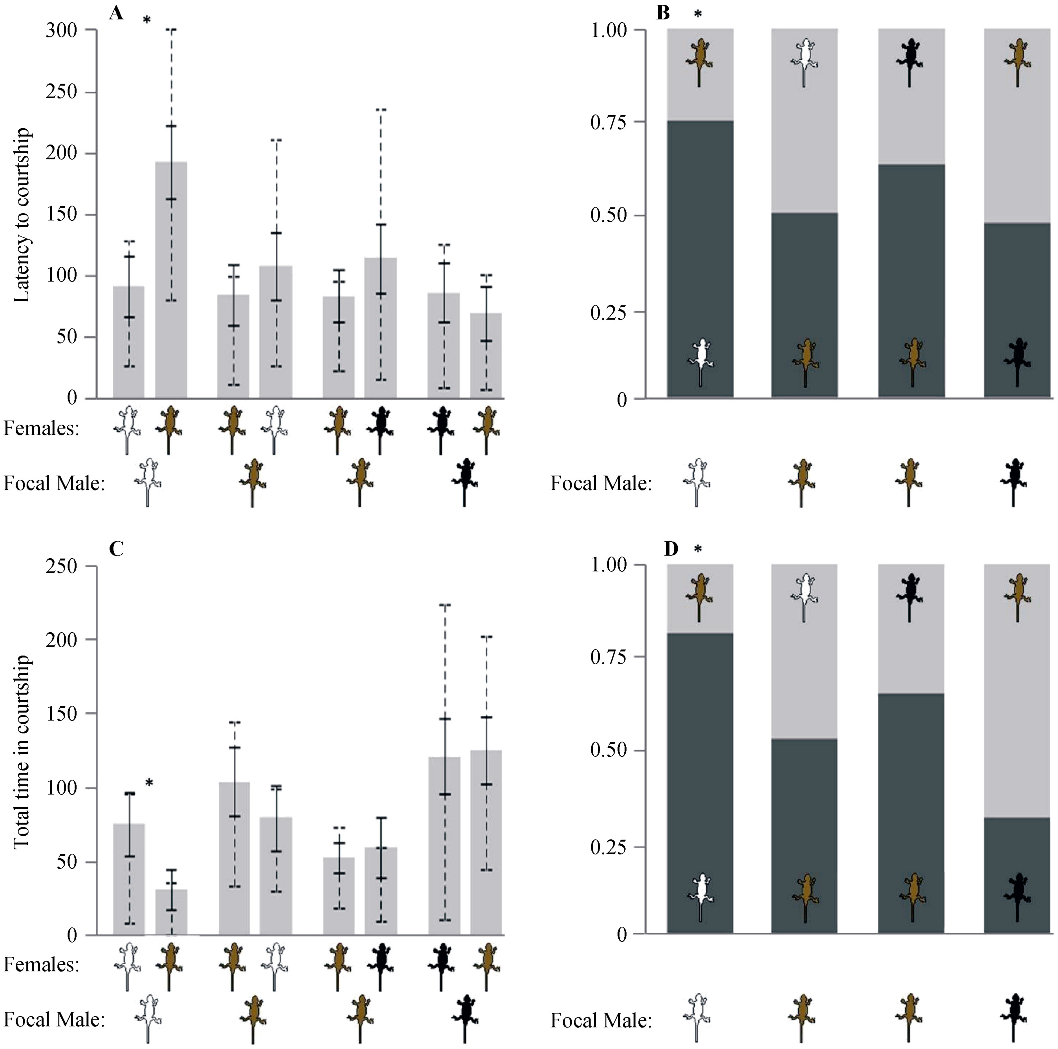

Fig. 2 Male courtship response to ecologically distinct females in each trial category

“*”indicates a significant ($P < 0.05$) difference in courtship response towards local and foreign females. **A**. Mean latency to courtship in seconds by focal males in response to local and foreign females. Error bars with solid lines display standard errors, and error bars with dashed lines display interquartile ranges. **B**. Proportion of trial pairs where focal males displayed shorter latency to courtship for local and foreign females. Dark grey bars represent trials with shorter latency for local females, and light grey bars represent trials with shorter latency for foreign females. **C**. Mean total time in courtship in seconds by focal males in response to local and foreign females. Error bars with solid lines display standard errors, and error bars with dashed lines display interquartile ranges. **D**. Proportion of trial pairs where focal males displayed longer total time in courtship for local and foreign females. Dark grey bars represent trials with longer time in courtship for local females, and light grey bars represent trials with longer time in courtship for foreign females.

Table 1 Effects of female behavior and female color on male courtship response for each trial category (White Sands focal males presented with local and dark soils females, dark soils focal males presented with local and White Sands females, dark soils focal males presented with local and black lava females, and black lava focal males presented with local and dark soils females)

		Latency to Courtship					Total Time in Courtship				
		Female Behavior		Female Coloration			Female Behavior		Female Coloration		
Focal Male	Females	Sidlehop	Pushup	Dorsal Brightness	Patch Brightness	Patch Size	Sidlehop	Pushup	Dorsal Brightness	Patch Brightness	Patch Size
Sands	Sands, Soil	0.06	1.00	0.27	0.58	**0.02**	**0.01**	1.00	0.09	1.00	0.09
Soil	Soil, Sands	1.00	0.22	1.00	1.00	1.00	1.00	0.69	1.00	1.00	0.63
Soil	Soil, Lava	0.73	0.12	0.81	0.48	0.24	0.51	1.00	0.65	0.34	0.36
Lava	Lava, Soil	1.00	0.51	0.61	1.00	1.00	1.00	0.75	0.45	0.21	0.45

For each set of trials we determined focal male preference based on which female was courted faster (shorter latency) and which female was courted longer (longer total time in courtship). We then tested for associations between male preference and female behavior (time spent performing sidlehops and pushups) and female color (ventral patch size, ventral patch brightness, and dorsal brightness) using binomial tests. Significant results ($P < 0.05$) in bold. Focal male and female populations are indicated by the following abbreviations: sands (White Sands), soil (dark soils), and lava (black lava).

Table 2 Population level differences in female behavior and female coloration

	Female Behavior		Female Dorsal Coloration			Female Ventral Coloration			
Females Compared	Sidlehop	Pushup	Dorsal Hue	Dorsal Chroma	Dorsal Brightness	Patch Hue	Patch Chroma	Patch Brightness	Patch Size
All Populations	2.02: 0.36	13.55: **<0.01**	24.34: **<0.01**	35.64: **<0.01**	80.68: **<0.01**	2.15: 0.13	3.51: **0.04**	8.95: <**0.01**	10.67: **<0.01**
Pairwise Differences	Sands≈Soil≈ Lava	Sands<Soil, Lava	Sands>Soil> Lava	Sands<Soil, Lava	Sands>Soil> Lava	Sands≈ Soil≈Lava	Sands>Lava Soil≈Sands, Lava	Sands<Soil, Lava	Soil<Sands, Lava

We used Pearson's chi-square tests to determine whether sidlehop and pushup behavior varied among female populations. We also used ANOVAs to determine whether dorsal and ventral hue, chroma, and brightness, as well as ventral patch size, varied among female populations. For traits that varied among female populations, we performed post-hoc tests to determine which pairs of populations differed. All results are reported as test statistic: *P*-value. Significant results ($P < 0.05$) in bold. Female populations are indicated by the following abbreviations: sands (White Sands), soil (dark soils), and lava (black lava).

(males behaved first in 57 trials while females behaved first in 9) (binomial test, $P < 0.01$).

Females from distinct populations differed in overall color phenotype, which included dorsal and ventral hue, chroma, and brightness, as well as ventral patch size (MANOVA, $df = 2$, $F = 8.95$, $P < 0.01$). Specifically, White Sands, dark soils, and black lava females differed in dorsal hue, dorsal chroma, and dorsal brightness (Table 2). Females from ecologically distinct populations also differed in ventral patch chroma and ventral patch brightness (but not ventral patch hue, Table 2). Finally, female ventral patch size varied across populations (Table 2).

Male courtship behavior was associated with female patch size in trials with White Sands and dark soils focal male categories. White Sands males exhibited shorter latency to courtship for females with large ventral patches (i.e., White Sands females), and we also observed a nonsignificant trend where White Sands males exhibited longer total time in courtship for females with large ventral patches (Table 1). Male behavior did not vary with respect to any other aspect of female color measured (i.e., dorsal and ventral hue, chroma, and brightness) (binomial tests, all $P > 0.05$).

Linear models (incorporating male population, male individual, female color, female behavior, and interactions between male population and each female variable) showed that male courtship response was predicted by the interaction between female color phenotype and male source population. In the linear models, male source population had a significant effect on male latency to courtship (ANOVA, $df = 2$, $F = 3.12$, $P = 0.04$) and total time in courtship (ANOVA, $df = 2$, $F = 4.93$, $P < 0.01$). The interaction between male population and female color PC 2 (ventral patch color and size) was also significant for male latency to courtship (ANOVA, $df = 2$, $F = 4.69$, $P = 0.01$) and total time in courtship (ANOVA, $df = 2$, $F = 4.20$, $P = 0.02$). The interaction effect is explained by the fact that White Sands males preferentially courted females with greater values of color PC 2, while dark soils and black lava males did not.

3 Discussion

We investigated mate preference in lizard populations undergoing rapid ecological divergence. Specifically, we asked whether male *S. undulatus* from ecologically distinct populations preferentially courted local females. We compared male preference in one ancestral (dark soils) and two derived (White Sands and black lava) populations. We found that White Sands males preferentially courted local females while dark soils and black lava males did not exhibit differences in courtship behavior based on female locality (Fig. 2). The observed preference asymmetry suggests the evolution of preference at White Sands. We also found that White Sands male preference was associated with several aspects of female morphology and behavior (Table 1). Below, we discuss the possible mechanisms involved in mate preference in this system and the evolutionary implications of the observed preference asymmetry across populations.

3.1 Mating cues

Determining the cues used to identify local mates is important for understanding mechanisms of sexual selection. Reptile courtship interactions can involve a number of different signaling modalities (e.g., visual, chemical, tactile), and manipulative experiments are necessary to test the importance of specific cues and their multimodal interactions (Tokarz, 1995). Our mate preference trials did not directly test cues males could use to identify local females. However, male behavior in our experiment was associated with several aspects of female behavior and morphology, providing hypotheses for cues that influence male mate preference in this system.

Female ventral patches are likely one of the most important cues for mate preference in this system. Previous studies have demonstrated that ventral patches are an important mating cue in *S. undulatus* by showing that manipulations of female ventral patch size alter male behavioral response (Cooper and Burns, 1987). White Sands and dark soils females exhibit dramatic differences in dorsal coloration, ventral patch coloration, and ventral patch size (Table 2). Further, White Sands male behavior was significantly correlated with female patch size (Table 1). In fact, male courtship response was best predicted by the interaction of male population and female ventral patch phenotype. In the White Sands system, color appears to be involved in both adaptation (because cryptic dorsal coloration is important for avoiding predators) and incipient reproductive isolation (because female ventral patch phenotype predicts male courtship response). Color is often the target of both natural and sexual selection, and there are a number of well-studied examples in other systems of color pleiotropically affecting both adaptation and reproductive isolation [e.g., in walking-stick insects (Jiggins et al., 2001), butterflies (Fordyce et al., 2002), monkeyflowers (Bradshaw and Schemske, 2003), coral reef fish (Puebla et al., 2007), and poison-dart frogs (Reynolds and Fitzpatrick, 2007)]. Therefore further studies are warranted at White Sands to understand the specific effect of female coloration on male mate preference and to evaluate the potential for color to act as a "magic trait" (sensu Servedio et al., 2011) in this system.

We found that White Sands male courtship was associated with aspects of female behavior, indicating that females may actively influence mate preference in this system with solicitation and/or rejection displays. White Sands males were the only males that exhibited a preference for local females, and White Sands males exhibited a delayed courtship response relative to dark soil and black lava males (i.e., White Sands males had a longer average latency until courtship than other males and their courtship often occurred after females displayed). Thus White Sands males may have been better able to evaluate female signals during the beginning of staged behavioral interactions. In addition, White Sands male total time in courtship was associated with female sidlehop behavior. The context dependence of female sidlehop behavior is poorly understood in lizards, and additional work is needed to understand the significance of female sidlehops in *S. undulatus* courtship interactions (Greenberg, 1977; Kelso and Martins, 2007). Our data cannot disentangle whether female sidlehop behavior functions as a trigger for male courtship or a response to it. It is important to note that although females that sidlehopped were courted more extensively by White Sands males, sidlehop behavior did not vary between White Sands and dark soils females. Therefore, female behavior alone cannot explain White Sands male preference but may complement other cues.

Our study focused primarily on male preference, but it is also important to consider the potential for female choice. There are examples of both male and female mate choice in lizards (e.g., Hews, 1990; Tokarz, 1992; Olsson, 1993). We focused on male preference in this study because male *S. undulatus* courtship displays are highly stereotyped, and males display specific behaviors that occur only in a courtship context (Cooper and Burns, 1987; Martins et al., 2005). Although females are also behaviorally active during courtship, it is more difficult to ascribe female preference because many behaviors are used in multiple contexts (Cooper and Burns, 1987; Martins et al., 2005). Future research should more explicitly consider the contribution of female choice in the White Sands system and should explore the expected consequences for reproductive isolation of single-sex versus mutual mate choice.

3.2 Mate preference asymmetry

Our results suggest an asymmetry in sexual isolation and male mating preferences across our focal *S. undulatus* populations (i.e., White Sands males preferred local females while dark soils and black lava males showed no preference). Previous studies with model organisms have found that asymmetrical sexual isolation may occur during population divergence and can occur in either direction (i.e., either the ancestral or the derived population can show a larger degree of isolation) (Kaneshiro, 1976; Watanabe and Kawanishi, 1979). In these studies, mate preference and population divergence in sexually selected traits are frequently required for isolation asymmetry to evolve (Kaneshiro, 1980). Empirical studies have detected isolation asymmetry in nature through observations of mate preference and copulation attempts between different populations in multiple diverse taxa [e.g., wasps (Bordenstein et al., 2000), snakes (Shine et al., 2002), salamanders (Arnold et al., 1996), and fish (McPhail, 1969)].

Our results suggest that preference has evolved at White Sands given that only White Sands (but not dark soils or black lava) males exhibited a preference for local mates. It is possible that preference for local mates could prevent maladaptive hybridization and the production of poorly background matched offspring. But why would mate preference be found in the derived White Sands population and not the ancestral dark soils population? Mate preference may be more important in the derived population than the ancestral population in a "mainland-island" system with local adaptation (Watanabe and Kawanishi, 1979; Kirkpatrick and Servedio, 1999). A greater degree of gene flow is expected to occur from regions of high to low population density, which can inhibit small peripheral populations from evolving to their local ecological optima (Garcia-Ramos and Kirkpatrick, 1997). White Sands is a small "habitat island" surrounded by dark soils populations, so the swamping effects of gene flow are expected to be more pronounced from dark soils into White Sands populations than the reverse. The evolution of mate preference could therefore have facilitated local adaptation in the White Sands population. Although demographic processes like migration can facilitate the evolution of asymmetrical preference for local mates, there are alternative ways for isolation asymmetry to arise between ecologically distinct populations, e.g., differences across habitats in the strength of sexual selection on certain traits (Gerhardt, 2005; Cocroft et al., 2010). Therefore further work is needed to understand the demographic backdrop and the dynamics of natural and sexual selection at White Sands.

It is important to raise several caveats about the observed mate preference asymmetry and its implications for adaptive evolution of White Sands *S. undulatus*. Some studies suggest that mating asymmetries may be transitory phenomena observed at intermediate stages of divergence (Arnold et al., 1996). Other studies have demonstrated that mating asymmetry may inhibit speciation because, in the context of reinforcement, reproductive isolation is more likely to evolve when gene flow occurs in both directions (Servedio and Kirkpatrick, 1997). However, these caveats may not be particularly relevant to the White Sands system. White Sands is a geologically young formation and *S. undulatus* across the ecotone are in the early stages of divergence. Given the recent timeframe for divergence, the observed asymmetry is unlikely to be a transitory phenomenon attributable to intermediate stages of divergence. In addition, theoretical predictions predicated on reinforcement models may not be applicable given that divergence across the White Sands ecotone has occurred in parapatry (rather than allopatry and subsequent recontact). Further work is needed to reconcile theoretical predictions and empirical results in specific case studies like White Sands.

The preference we observed for local mates in one derived population (White Sands) was not exhibited by another derived population (black lava). One possible explanation for this observation is that color may be a more direct link between naturally and sexually selected traits in the White Sands population compared to the black lava population. The genetic basis of the derived White Sands phenotype is controlled by the melanocortin-1 receptor gene (*Mc1r*; Rosenblum et al., 2010), while the melanic lava flow phenotype is not due to a mutation at *Mc1r* (Rosenblum et al., 2004). It is possible that the genetic architecture of the melanic phenotype (which remains to be determined) or the melanic phenotype itself provides less of an opportunity for sexual selection. For example, the difference in ventral patch size is larger between dark soils and White Sands females than between dark soils and black lava females. Thus if patch size is a phenotypic cue used to inform mate choice, it is possible that White Sands males could more easily discriminate local vs. foreign females than black lava males. Finally, it is possible that black lava or dark soils males do have subtle preferences that were not detected using our metrics or with the geography of our sampling. For example, we used dark soils lizards from a nearby (but allopatric) locality where we could reliably sample large numbers of lizards. Conducting trials with additional populations would be important to confirm a lack of preference for local females in parapatric dark soils and black lava populations.

3.3 Summary

We examined male mate preference in multiple populations undergoing rapid ecological divergence. We found evidence for mate preference at White Sands, whereby males favored local females. Preference for local mates was associated with female ventral patch phenotype, which may indicate that color is playing a role pleiotropically in both adaptation and reproductive isolation in this system. The finding of mate preference in White Sands *S. undulatus* provides behavioral evidence that White Sands lizards are undergoing the early stages of ecological speciation (Rosenblum and Harmon, 2011). Rosenblum (2008) previously demonstrated a preference for local mates in another species with a white form at White Sands, *Holbrookia maculata* (Rosenblum, 2008). That two White Sands species exhibit a preference for local mates after only several thousand years of divergence suggests that sexual selection may play a key role even in the early stages of ecological divergence. However, our results also suggest that mate preference does not necessarily evolve in a predictable manner in cases of ecological divergence. We detected a preference for local mates in only one of the two derived populations we investigated, indicating that migration-selection balance may be sufficient to maintain the adaptive phenotype in the absence of behavioral isolation. It would be fruitful to compare the degree of genetic isolation for the two derived populations relative to the ancestral population to determine whether the evolution of mate preference at White Sands is associated with accelerated speciation. Lastly, we observed a mate preference asymmetry, whereby the ancestral dark soils population did not exhibit a preference for local mates. Mate preference asymmetry has been observed in other taxa, but additional work is needed to understand how isolation asymmetries may promote or hinder speciation. White Sands represents a fruitful system to further study the interaction between local adaptation, mate preference, and isolation asymmetry during ecological divergence.

Acknowledgements We thank Alex Krone, Simone Des-Roches, and Jack Torresdal for assistance with field work, Emilia Martins, Saul Nava, and Diana Hews for input on experimental design, Luke Harmon for consultation on data analysis, and David Bustos, Doug Burkett, John Anderson, and Eddie Garcia for facilitating our research in the field. We thank White Sands National Monument, White Sands Missile Range, Jornada Long Term Ecological Research Station, and New Mexico Department of Game and Fish for permits. All experiments were conducted in accordance to approved animal care and use protocols. This study was funded by an NSF CAREER grant (DEB-1054062) to EBR. We also acknowledge the NSF BEACON Center for the Study of Evolution in Action (DBI-0939454) and the Sigma Xi GIAR program.

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