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Preference for Local Mates in a Recently Diverged Population of the Lesser Earless Lizard (*Holbrookia maculata*) at White Sands

ERICA BREE ROSENBLUM[1]

Museum of Vertebrate Zoology, University of California, Berkeley, California 94720, USA

ABSTRACT.—**When divergent selection favors different phenotypes in different environments, a preference for resident mates can help ensure that offspring are well suited to local conditions, thus increasing an individual's lifetime fitness. Here I use both field and laboratory experiments to investigate the potential for mate choice in a unique color morph of the Common Lesser Earless Lizard, *Holbrookia maculata*, which inhabits the gypsum dune fields of White Sands in southern New Mexico. I demonstrate that light-colored *H. maculata*, which have evolved within the last 6,000 years, can discriminate between potential mates. In the field, simultaneous choice experiments revealed that White Sands males in their natural habitat courted females from White Sands sooner than females from a nearby population occupying dark substrate. The use of paint treatments to reverse female dorsal coloration showed that male choice was not based on dorsal coloration alone, if at all. The only variable measured in field trials that correlated strongly with male courtship behavior was female display activity. Resident females displayed in more trials than nonresident females, and males preferentially courted actively displaying females suggesting that behavioral cues may be important in this system. In the lab, sequential choice experiments on an intermediate substrate color showed that preference for local mates was reciprocal, with males of both color morphs preferentially addressing courtship behavior to potential mates from their same locality. By integrating field and lab experiments, I demonstrate that male preference for local mates in the recently diverged White Sands population is robust to experimental design.**

Although mate choice is common for many groups of vertebrates, (e.g., Andersson, 1994; Ptacek, 2000), choosiness appears to be less widespread for squamates (lizards and snakes) (Tokarz, 1995). A number of studies have looked for and failed to find any evidence for mate choice in this group (e.g., Olsson, 2001; Lailvaux and Irshick, 2006). However, even without direct parental care, costs associated with reproduction for female squamates can be high because of increased predation and compromised overall body condition (Angilletta and Sears, 2000; Miles et al., 2000; Webb, 2004). Although female choice is certainly not as ubiquitous for squamates as for other vertebrates, theoretical (e.g., Alonzo and Sinervo, 2001) and empirical studies (e.g., Hews, 1990; Lopez et al., 2003; Hamilton and Sullivan, 2005) have suggested a role for female choice in at least some squamate species. Male squamates also may suffer a higher price during the breeding season than previously considered because of energetic costs and predation exposure associated with competitive and territorial behavior (Marler and Moore, 1989; Aldridge and Brown, 1995; Bonnet et al., 1999). A handful of studies have demonstrated that male lizards and snakes can discriminate among potential mates (e.g., Olsson, 1993; Orrell and Jenssen, 2002; Shine et al., 2004), but again evidence for male choice in this group is limited.

For those squamate species that do exhibit mate choice, a variety of cues have been shown to influence mating decisions. Potential mates may be assessed by visual cues (e.g., coloration or body size), behavioral displays, olfactory signals, and territory quality (e.g., Hews, 1990; Censky, 1997; Martin and Lopez, 2000). Given the diverse repertoire of signals used, squamates represent a potentially rich group in which to study the conditions that promote the use of specific mating cues. Therefore, it is necessary to identify systems in which a priori predictions can be made about which signals are likely important in particular populations.

The rapid divergence of two distinct color morphs of the Common Lesser Earless Lizard, *Holbrookia maculata,* in southern New Mexico provides an opportunity to evaluate the potential for mate discrimination in recently diverged lizard populations. White Sands is a dramatic and geologically recent landscape of stark white hydrous calcium sulfate (gypsum) dunes deposited during the last 6,000 years (Kocurek et al., 2007; S. G. Fryberger, unpubl. data). The 275 square miles of gypsum contrast dramatically with the dark colors of adjacent substrate. A

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[1] Present address: Department of Biological Sciences, University of Idaho, Moscow, Idaho 83844, USA; E-mail: rosenblum@uidaho.edu

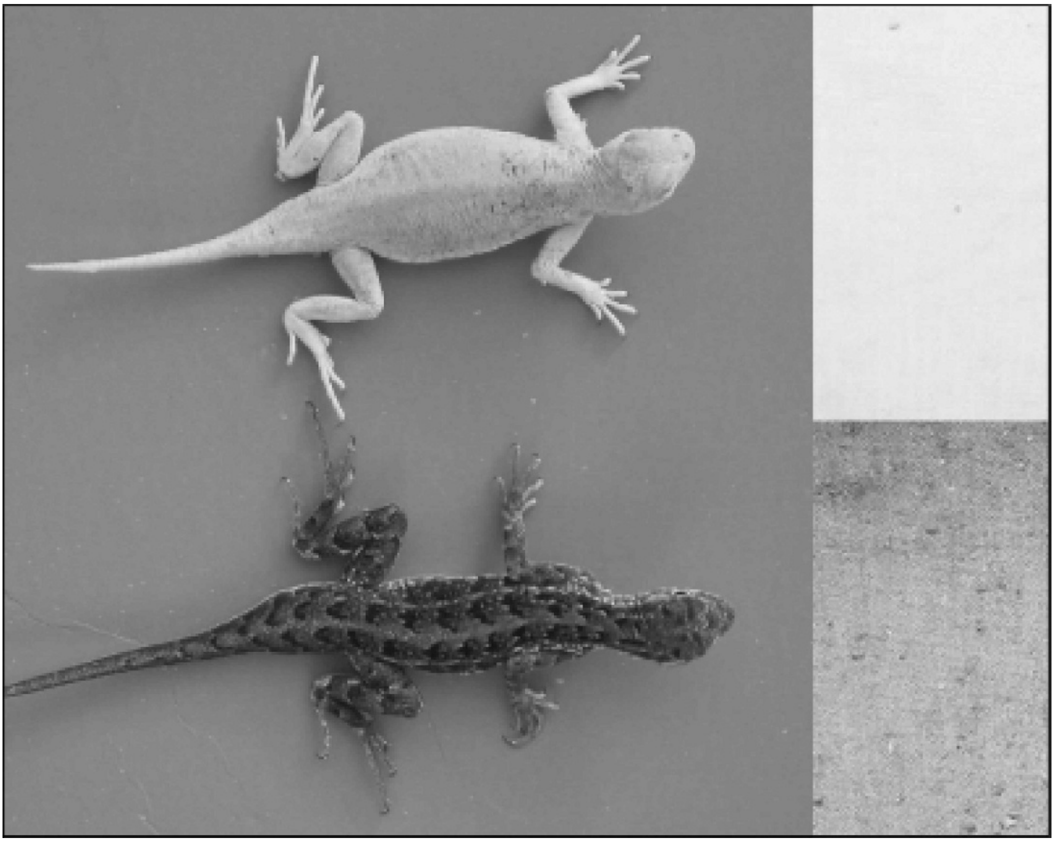

FIG. 1. Photographs of divergent *Holbrookia maculata* color morphs. The light color morph (female shown) that inhabits white sand habitat is depicted above the dark color morph (male shown) that inhabits dark soil habitat. The divergent color morphs are shown together on a neutral background for direct comparison. Photographs of typical substrate for each color morph are shown to the right.

light color morph of *H. maculata* inhabits the gypsum dunes, and a dark color morph is found in the surrounding habitat matrix and the rest of the species' range (Smith, 1943; Hager, 2002; Rosenblum, 2006; Fig. 1). Light and dark forms of *H. maculata* are significantly different in dorsal coloration (Rosenblum, 2006), and common-garden rearing experiments have demonstrated that this color variation is not a consequence of phenotypic plasticity (Rosenblum, 2005).

The importance of assortative mating based on coloration is potentially high in *H. maculata* because of the strong selection pressure on small diurnal lizards for substrate matching. Visual predators in New Mexico, such as the Loggerhead Shrike, *Lanius ludovicianus*, preferentially prey on poorly background-matched prey (Kaufman, 1973; Luke, 1989). Therefore, dorsal color variation in *H. maculata* is likely to be an adaptation for crypsis. Because lifetime fitness depends not only on an individual's survival but also on offspring survival, mating decisions should maximize the probability that offspring will be well adapted to their environment. If progeny from pairings between color-mismatched lizards exhibit reduced viability, lizards would accrue a fitness benefit from choosing like-colored mates. Further, if assortative mating was historically important in reducing gene flow among divergent *H. maculata* color morphs, a preference for like-colored mates is predicted.

Choosing the correct mate is especially important when reproductive resources are limited. In *H. maculata*, females invest in a single clutch of 3–6 eggs per season (Dixon, 1967; Droge et al., 1982; Jones and Ballinger, 1987), requiring both pre- and postcopulation energetic costs. Although postcopulation investment is lower for males, territorial and courtship behaviors are energetically demanding and may increase conspicuousness to predators. Therefore, males may also benefit from directing time, energy, and reproductive resources toward appropriate mates. In addition to having a reason to be choosy, *H.*

maculata may also have a way for individuals to be choosy. Breeding bouts in this species contain stereotyped courtship behaviors with active displays by both males and females (Hager, 2001; pers. obs.) providing an opportunity for mate selection based on differences in dorsal coloration and other visual or behavioral cues.

It is possible that males and/or females could be choosy in this system; however, in practice, male choice can be evaluated more directly than female choice in *H. maculata*. Social behavior in iguanid lizards is fairly stereotyped (Carpenter and Ferguson, 1977). However, similar behaviors can be used in different contexts (e.g., Martins, 1993; Decourcy and Jenssen, 1994). For example, a number of behaviors that can occur during *H. maculata* courtship interactions (e.g., head bobbing [both sexes], leg flexion for ''push-ups'' [males], cloacal display [females]) are not unambiguous courtship behaviors because similar behaviors are used for advertisement and aggression displays (Clarke, 1965). Male *H. maculata* exhibit certain behaviors that are not used in interactions other than courtship and, thus, represent unambiguous precopulatory courtship behaviors (e.g., biting female dorsonuchal skin or hindquarters and mounting or attempted copulation [Clarke, 1965]). Although male courtship behaviors in *H. maculata* can be stringently defined, female precopulatory behaviors cannot be as clearly dissected. Although some female behaviors such as back arching and biting are considered unambiguous aggression/rejection displays (Carpenter, 1967), behaviors that may be used to signal receptivity or solicitation are also used in other contexts (Clarke, 1965). Therefore, although I present data below on both male and female behaviors, I only attempt to draw direct inference regarding the potential for male choice in this system.

The overall objective of this study is to evaluate mating preferences in dark and light color morphs of *H. maculata* to better understand the potential for (1) assortative mating across the White Sands ecotone and (2) dorsal color variation as a mechanism for identifying local mates. I specifically test the potential of dorsal coloration to serve as a cue for mate recognition because divergent selection can lead rapidly to reproductive isolation when traits that are important for defining an organism's ecological niche are also used for choosing mates (Kirkpatrick and Ravigne, 2002; Gavrilets, 2003; Servedio, 2004). First, I use simultaneous choice trials in *H. maculata*'s natural habitat at White Sands National Monument (WSNM) to evaluate mating preferences in the unique gypsum dune environment. In these field trials, I test the importance of dorsal coloration in mate recognition by manipulating the color of females with a paint treatment. All field trials were conducted at WSNM because the primary purpose of this study is to test whether rapid adaptation of light dorsal coloration is associated with evolution of mating preferences. Differences between study sites (i.e., lower population densities of *H. maculata*, higher densities of predators and competitors, and increased habitat complexity at nearby dark soil sites) preclude conducting reciprocal field trials. Therefore, I employ sequential choice trials in a laboratory setting to examine mating preferences of both color morphs in a uniform substrate environment and to ensure results of field trials are robust to experimental design.

MATERIALS AND METHODS

Animals Used.—Only reproductively mature individuals were used in experiments (minimum size at maturity for New Mexico populations is 44 and 45 mm snout–vent length [SVL] in males and females, respectively [Gennaro, 1974]). Males were at least 47 mm SVL (mean SVL of 55.6 and 57.2 mm and mean mass of 5.6 g and 6.6 g in lab and field trials, respectively), and females were at least 49 mm SVL (mean SVL of 54.1 mm and 54.5 mm and mean mass of 5.6 g and 5.7 g in lab and field trials, respectively). Experimental trials were conducted in May and June, the peak of the breeding season. New Mexico populations of *H. maculata* are reproductively active during this period (enlarged male testes and mature spermatozoa for males and gravid condition for females from May through July [Gehlbach, 1965; Dixon, 1967]). Once females are gravid, male courtship intensity decreases, and females tend to reject male advances (Hager, 2001). Therefore, gravid females were excluded from the study by (1) conducting trials early in the breeding season before most females were gravid, (2) using females with low-intensity nuptial coloration (correlated with receptivity) rather than high intensity nuptial coloration (correlated with gravidity; Hager, 2001), and (3) palpating for eggs and excluding detectibly gravid females. During the experimental period, lizards were housed in small groups in indoor cages on an intermediately colored sandy substrate. Captive lizards were provided with food, water, and basking lights to mimic natural light cycles. After use in the experiments, lizards were released at the GPS coordinates of their capture site or accessioned as specimens to the Museum of Vertebrate Zoology, University of California, Berkeley.

Field Trials: Experimental Design.—Ten light-colored females were collected on the gypsum dunes at White Sands National Monument

(WSNM), Otero County, New Mexico, and nine dark-colored females were collected on the dark soils at Jornada Long-Term Ecological Research Station (JLTER), Doña Ana County, New Mexico, between 12 May and 17 June 2002. Female lizards were caught by noose and transported to research facilities in Alamogordo, New Mexico, and there were no differences in handling between females from WSNM and JLTER. Females from opposing habitats were paired based on similarity in size and intensity of nuptial coloration. Most matched pairs showed $<5\%$ difference in SVL and mass, and all were within 13%. Most females were kept in the same pairing throughout the experiment, but three females from each habitat were assigned to more than one partner during the experimental period. A total of 14 female pairs were used, and each individual female was used in an average of four trials (range 1–8 trials).

The dorsal surfaces of half the female pairs (determined by a coin toss) were painted such that WSNM females appeared dark and JLTER females appeared light. Paint methods have been used with success in previous studies of reptile mate choice in *H. maculata* (Hager, 2001) and other species (Cooper, 1986; Cooper and Burns, 1987; Watkins, 1997) without affecting individual behavior. For this study, watercolor paint was used to obtain a visual match to the overall dorsal color field of each morph, and then darker dorsal patterning was added. Paint was reapplied between trials as necessary. Therefore, painted females had a ''true color'' (i.e., color before paint treatment) and a ''presentation color'' (i.e., color after paint treatment). I used an Ocean Optics USB2000 spectrometer for an objective assessment of the accuracy of paint treatments in the spectral range visible to squamates and their avian predators (300–700 nm; following Rosenblum, 2006). Representative samples were assessed for similarity in brightness (area under the spectral curve [AUC]) because differences in brightness explain 90% of the variation between WSNM and JLTER lizards (Rosenblum, 2006). Paint treatments were remarkably accurate with similarly low AUC for the JLTER lizard (4,025) and the WSNM lizard painted dark (4,203) and similarly high AUC for the WSNM lizard (14,686) and the JLTER lizard painted light (14,899). Preliminary trials indicated that there were no detectible effects of paint treatment on behavior; females were active whether or not they were painted, and males courted both painted and unpainted females.

Forty field trials were conducted on the gypsum dune fields of WSNM between 5 June and 17 June 2002 when male lizards were found active in their territories, most between 800 and 1200 h ($N = 32$) and fewer between 1300 and 1830 h ($N = 8$). All trials were conducted on natural gypsum substrate in natural light conditions. The following test procedure was used with 40 independent males. An active male was captured by noose, its mass and SVL were recorded, and its right rear toe was marked to ensure a unique male was used in each trial. Simultaneously, one pair of females was installed within several meters of the male's point of capture, either painted or unpainted as determined by a coin toss. Each female was tethered around the waist with a separate 1-m long string, which was then tied to a common stake in the ground. Therefore, females could behave freely within a circle of radius 1 m without escaping. Similar experiments in *H. maculata* and other squamates have demonstrated that tethering does not affect the social behavior of the tethered lizards or of the free-roaming lizards to which they are presented (e.g., Cooper, 1984; Cooper and Burns, 1987; Hager, 2001). Once the pair of females was installed, the male was held facing the females at a 1-m distance for 30 sec to make sure that all trials began with males oriented in the same direction at the same distance from the females. The male was then released, untethered. Males were captured and measured prior to trials to ensure that trials were initiated consistently and because recapture of untethered males was not guaranteed. Despite brief handling, males were motivated and exhibited courtship behavior (see Results).

Trials were observed from a distance of 10 m. Behavior of all subjects was recorded for 5 min. The length of field trials was determined by preliminary experiments in which I found that when trials ran longer than 5 min, the displays of experimental lizards often attracted nonexperimental lizards that interrupted trials by competing for mating opportunities. Additionally, tethering limits the ability of females to behaviorally thermoregulate, and shorter trials minimized heat-stress on females. Finally, in most trials, courtship behavior was initiated rapidly, so 5-min trials were sufficient to record male preference.

Following Clarke (1965), I considered only the following male behaviors to be unambiguous precopulatory courtship behaviors: biting female dorsonuchal skin or hindquarters and mounting or attempted copulation (Clarke, 1965). These behaviors are not used in behavioral interactions other than courtship and were clearly directed toward a particular female. Although additional male behaviors, such as ''shudder bobs'' (rapid volley of head bobs) have been used as reliable indicators of male courtship in previous studies of this species

(Hager, 2001), in this experiment, because two females were tethered to a common stake, shutterbobs could not always be unequivocally assigned toward a particular female. Because many female precopulatory behaviors are also used in other contexts, I present only whether females were actively displaying in each trial and do not attempt to draw additional inference regarding the information content of these displays.

Field Trials: Data Analysis.—Field data were summarized in binary codes. Male choice was recorded as the first female to which unambiguous courtship behavior (i.e., directly approached and nipped or mounted) was exhibited. Females were scored as active or not active based on whether they exhibited any nonaggressive display behavior. Four trials were excluded because of intrusion by other lizards or by visitors to WSNM, leaving a total of 36 trials for analysis. Multiway contingency tables were used to evaluate the association between female locality, female presentation color, and female display activity on male preference. Female ''presentation color'' was nested within female locality. For example, females could ''present'' light if they were from WSNM and unpainted or if they were from JLTER and painted. If tests of mutual dependence were significant, tests of partial dependence were conducted, and if those were significant, simple Chi-square tests were performed. These analyses were used to address three related questions. First, I asked whether male choice was correlated with female locality and female presentation color. Second, I asked whether male choice was associated with female display activity. Third, I asked whether display activity levels were similar for WSNM and JLTER females. Bonferroni corrections were used to determine significance when multiple post hoc tests were performed. In addition to the multiway contingency analyses described above, simple Chi-square tests were used to evaluate whether males or females tended to initiate behavioral interactions and to evaluate the specific prediction that males may court behaviorally active WSNM females more often than behaviorally active JLTER females. To be conservative, Yates' correction was employed for all Chi-square tests with only one degree of freedom if expected frequencies less than five were observed.

Because body size can be an important factor in squamate mate choice (e.g., Censky, 1997; Olsson, 1993), I evaluated whether male choice might have be influenced by female body size. I used a General Linear Model (GLM) with a binary indicator of male choice as the dependent variable, female locality as a categorical predictor variable, and female SVL as a continuous predictor variable. I also used a GLM to confirm that individual effects due to female use in multiple trials did not exert a detectable influence on results.

Lab Trials: Experimental Design.—Four males and four females were collected from each habitat type (WSNM and JLTER) between 15 May and 25 May 2003 and divided into two replicated blocks for lab trials. Each block consisted of two light males, two dark males, two light females, and two dark females. Within blocks, each lizard was exposed to all individuals of the opposite sex in a randomized order; thus, each individual was used in a total of four trials.

Thirty-two lab trials were conducted between 1500 and 1800 h from 26–29 May 2003. Trials were performed in a circular open-air arena with a 1-m radius. Sand of intermediate color was used as a substrate and was changed between each trial to remove any scent deposition between trials. Arena walls were painted to obtain a visual match to the substrate color. For each trial, one male and one female were held facing each other at opposite ends of the arena for 30 sec to ensure that all trials were initiated with animals oriented in the same direction and at the same distance apart. Lizards were then released and interaction trials were videotaped from above. The constraints on field trial length were not present for laboratory trials; hence, 10-min trials were used. The onset and duration of courtship behaviors for both males and females were later scored from video footage using Spectator GO! software (BIOBSERVE).

Lab Trials: Data Analysis.—Four variables were analyzed from lab trials: male latency to courtship; total male courtship time; female latency to display; and total female display time. Latency to courtship (male) or display (female) was defined as the time from the initiation of the trial until the first courtship/display behavior for a given individual. Total courtship (male) or display (female) time was defined as the cumulative time during a 10-min trial that a given individual was engaged in courtship/display behavior. Because individuals were used in multiple trials, data points were not independent; thus, comparison of mean interaction times for similar and dissimilar color morphs was not appropriate. Therefore, I conducted analyses based on individual preference (male) or display (female) ranks. Each lizard met four potential mates, two from WSNM and two from JLTER. For each variable, a rank preference/display score from one to four was assigned for each potential mate.

To evaluate the prediction that lizards preferred potential mates with like color morphology (i.e., local mates), I tested whether the single most highly ranked potential mate had a color morphology that matched that of the chooser using Fisher's Exact Test (FET). Additionally, I tested whether the mean rank for dark versus light potential mates was higher using nonparametric Mann-Whitney *U*-tests.

To evaluate whether behavior was influenced by body size, I used a GLM with preference/display rank as the dependent variable, locality as a categorical predictor variable, and SVL as a continuous predictor variable. I also used a GLM to evaluate the effect of using individuals in multiple lab trials.

RESULTS

Field Trials.—Behavioral data indicated that lizards were motivated in the experimental context. Males exhibited unmistakable courtship behavior in 53% of trials (19 of 36). At least one female was actively displaying in 81% of trials (29 of 36). Copulations occurred in five of 36 trials. Paint treatment was confirmed to have no significant effect on male courtship behavior or on female display activity; males were equally likely to exhibit courtship behavior to females whether they were painted or unpainted ($X^2{}_1 = 2.79$, $P = 0.1$), and females were equally likely to exhibit display behavior whether or not they were painted (Chi-square test: $X^2{}_1 = 0.51$, $P = 0.48$). Also, I confirmed that using individual females in multiple trials did not bias results; there was no significant relationship between male choice and female identity ($F_{13,24} = 2.00$, $P = 0.094$).

Multiway contingency analyses revealed a significant relationship between male choice, female locality, and female ''presentation color'' ($X^2{}_4 = 16.32$, $P < 0.005$). Post hoc chi-square tests showed that males courted females based on their locality but not their presentation color. WSNM males displayed toward and interacted with WSNM females (true color = light) significantly more than with JLTER females (true color = dark) ($X^2{}_1 = 12.74$, $P < 0.0005$, significant following Bonferroni correction for multiple tests; Fig. 2). Males did not preferentially court females ''presenting'' light versus females ''presenting'' dark ($X^2{}_1 = 0.95$, $P = 0.33$, Fig. 2). In other words, WSNM males courted WSNM females first in 79% of trials (15 of 19) in which courtship behavior was observed, even if these females were painted dark.

Multiway contingency analyses also revealed a significant relationship between male choice, female behavior, and female locality ($X^2{}_7 = 34.23$, $P < 0.001$). Post hoc Chi-square tests showed differences in display activity for WSNM and JLTER females. When presented with WSNM males, WSNM females were significantly more likely to display than JLTER females ($X^2{}_1 = 12.74$, $P < 0.0005$, significant following Bonferroni correction; Fig. 2). WSNM females displayed in 78% of trials (28 of 36); JLTER females displayed in only 36% of trials (13 of 36). Post hoc Chi-square tests also indicated that male choice was correlated with female display behavior. Males were more likely to participate in trials that contained at least one actively displaying female ($X^2{}_1 = 4.25$, $P = 0.04$) although this result was not significant following a Bonferroni correction for multiple tests. Furthermore, males were significantly more likely to court actively displaying females ($X^2{}_1 = 5.73$, $P = 0.017$, statistically significant following Bonferroni correction; Fig. 2). Males courted displaying females in 84% of trials (16 of 19) in which courtship behavior was observed. Additionally, females were significantly more likely to display prior to males ($X^2{}_1 = 21.60$, $P < 0.0001$). Of the trials in which displays were observed, female subjects displayed prior to males in 80% (24 of 30).

Even given the predilection for males to court displaying females, males disproportionately courted displaying WSNM females over JLTER females, a statistically significant result using Yates' corrected Chi-square ($X^2{}_1 = 5.48$, $P = 0.02$). Males courted WSNM females in 14 of the 17 trials in which WSNM females displayed (82%), whereas they only courted JLTER females in two of the eight trials in which JLTER females displayed (25%). This test was formulated to evaluate the specific prediction that males would choose displaying WSNM females over JLTER females and was not a post hoc test from the multiway contingency analyses. Therefore, no Bonferroni was necessary, and the result was statistically significant. Because this test contained expected frequencies less than five, I also performed a Fisher's Exact Test (FET), which is valid even for small samples, and a similar level of statistical significance was obtained ($P = 0.01$)

Although few copulations were observed, patterns associated with male intromission bear mention. In 36 trials, only five copulations were observed with three nonresident females. It is interesting that copulations only occurred with nonresident females; however, the trend was not statistically significant even before applying the necessary, more stringent Bonferonni correction ($X^2{}_1 = 3.69$, $P = 0.06$). Because of the small number of copulations and, thus, low statistical power, I do not draw any additional inference from this trend.

Finally, male choice in field trials was not strongly correlated with body size of potential female mates. Using a GLM, male choice was

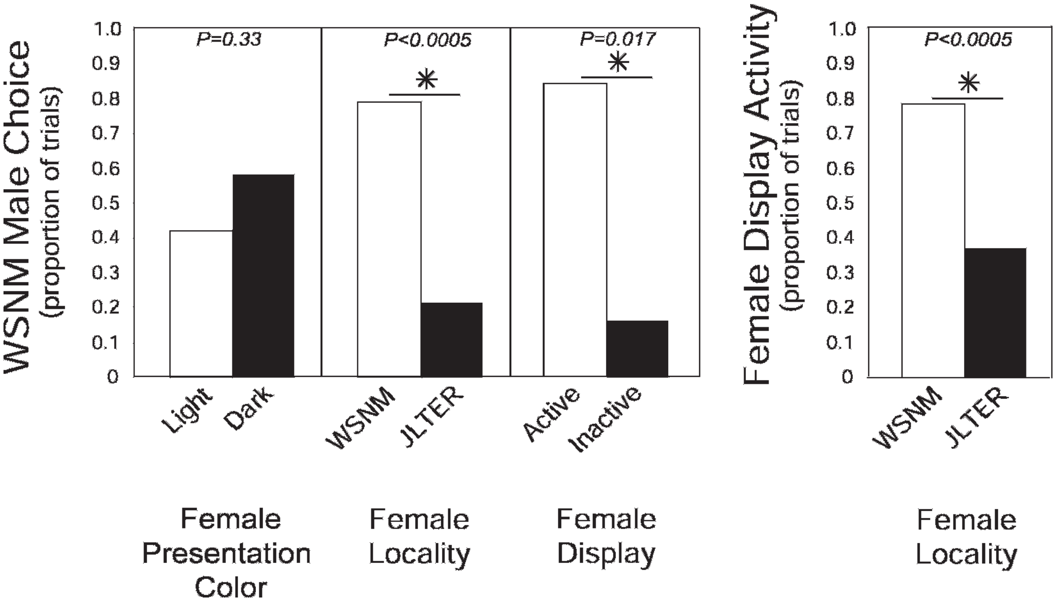

FIG. 2. Results from simultaneous choice field experiments in which females from White Sands National Monument (WSNM) and Jornada Long-Term Ecological Research Station (JLTER) were presented to males from WSNM. Relationships between male choice and female presentation color, female locality, and female display activity are presented for all trials in which males exhibited choice ($N = 19$ for all tests in the left panel). Relationship between female locality and display activity is also shown on the right panel ($N = 36$). Asterisks indicate statistically significant comparisons.

again shown to be significantly associated with female locality ($F_{1,35} = 11.14$, $P = 0.002$) but not SVL ($F_{1,35} = 0.10$, $P = 0.752$).

Lab Trials.—Lizards in laboratory trials were also motivated; courtship behavior was observed in 97% of trials (31 of 32), and attempted copulations were observed in 28% of trials. Again, I confirmed that individual effects did not exert a strong influence on results. No particular female received significantly higher or lower preference ranks than others for total time of male courtship ($F_{7,24} = 0.86$, $P = 0.553$) or latency until male courtship ($F_{7,24} = 0.86$, $P = 0.551$). All males were not equal for total time of female display ($F_{7,24} = 3.16$, $P = 0.016$) and latency until female display ($F_{7,24} = 2.53$, $P = 0.042$). However, post hoc tests showed that only one of eight experimental males received significantly higher rankings than others, an inequality that was not strong enough to obscure the general pattern of female display behavior presented below.

Both males and females tended to interact sooner and longer with potential mates of their same color morphology. Average male latency until interaction was 66 sec when paired with a female of similar color morphology and 90 sec when paired with a female of opposing color morphology. Mean total time of male courtship when paired with same versus opposing colored females was 158 and 98 sec, respectively. Average female latency to display was 64 sec when paired with a male of similar color morphology and was 94 sec when paired with a male of opposing color morphology. Mean total time of female display when paired with same versus opposing colored males was 189 and 134 sec, respectively.

Potential mates with similar color morphology had on average higher ranks than those of opposing color morphology for total time spent interacting and for latency until interaction (Fig. 3). This trend was statistically significant for both males and females for total courtship/display time but not for latency until courtship/display. The preference ranks for similar colored females were significantly higher than those for opposing colored females based on total time of male courtship ($N_1 = N_2 = 16$, $U = 64.00$, $P = 0.016$). The preference ranks were also higher for similar colored females based on latency until male courtship, but this result was not statistically significant ($N_1 = N_2 = 16$, $U = 91.50$, $P = 0.1689$). For females, the ranks for similar colored males were significantly higher than those for opposing colored males based on total time of female display activity ($N_1 = N_2 = 16$, $U = 75.00$, $P = 0.046$). Ranks based on latency until female display were not statistically significant ($N_1 = N_2 = 16$, $U = 96.00$, $P = 0.228$).

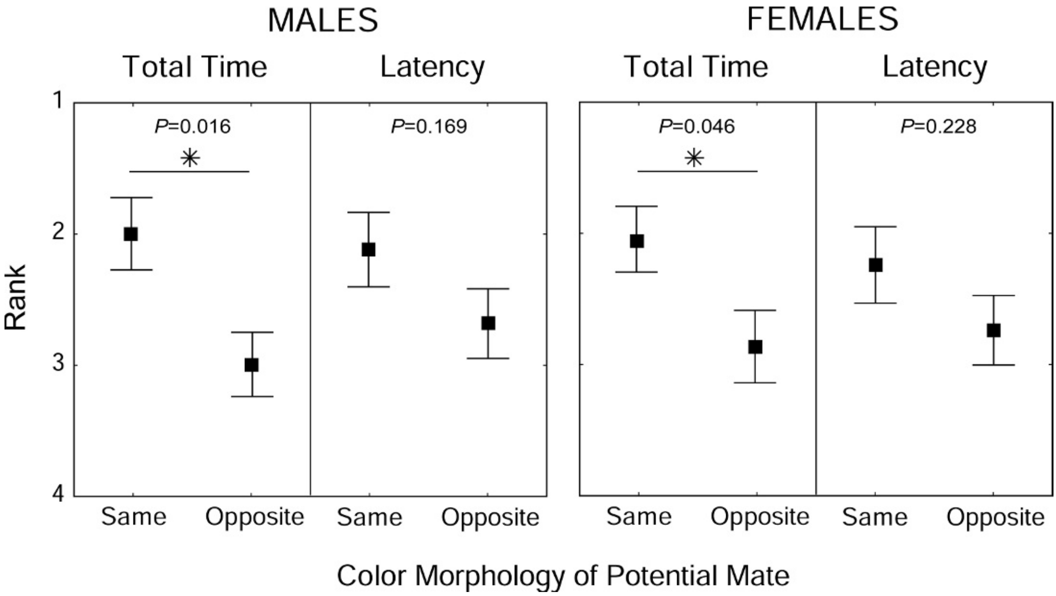

FIG. 3. Results from sequential choice laboratory experiments in which reciprocal trials were conducted with WSNM and JLTER lizards. Mean rank and standard error of the mean are given for potential mates of similar and opposing color morphologies (rank score 1 for the potential mate receiving most rapid/frequent interaction). Ranks for total time spent interacting and latency until interaction are shown separately for male courtship and female display activity. Asterisks indicate statistically significant comparisons ($N_1 = N_2 = 16$ for all tests).

For both males and females, the potential mate with the single highest ranking was generally of the same color morphology. Based on total time spent courting, the most preferred potential mate for a significant proportion of males (88%, seven of eight) was a female of like color morphology ($P = 0.005$). Based on latency until interaction, the trend observed for male preference was in the predicted direction but not statistically significant (five of eight, $P = 0.1431$). For the majority of females (75%, six of eight) the potential mate eliciting the most display activity was a male of like color morphology, although this trend was not statistically significant ($P = 0.066$ for both total time spent displaying and latency until display).

Preference/display rankings in laboratory trials were not correlated with body size of potential mates for any comparison. GLMs for total time spent courting revealed that both male and female preference/display ranks were significantly associated with potential mate locality ($F_{1,29} = 7.26$, $P = 0.012$ and $F_{1,29} = 4.76$, $P = 0.037$, respectively) but not body size ($F_{1,29} = 0.04$, $P = 0.844$ and $F_{1,29} = 0.50$, $P = 0.487$, respectively). Results for latency until courtship/display, consistent with Mann-Whitney *U*-tests, did not show a significant relationship between male or female ranks and potential mate locality ($F_{1,29} = 2.18$, $P = 0.150$ and $F_{1,29} = 1.59$, $P = 0.217$, respectively) but again demonstrated that preference/display ranks were not associated with body size ($F_{1,29} = 3.12$, $P = 0.087$ and $F_{1,29} = 1.31$, $P = 0.262$, respectively). Finally, males generally initiated interactions in laboratory trials with male displays occurring prior to female displays in 81% of trials (26 of 32; $X^2{}_1 = 25.0$, $P \leq 0.0001$).

DISCUSSION

I evaluated the potential for mate choice in *H. maculata* because the White Sands population appeared a priori to have a reason to be choosy (i.e., potentially strong natural selection against color mismatched offspring) and a way to be choosy (i.e., distinctive light dorsal color morphology). In fact, as would be predicted for locally adapted populations, a preference for resident mates was observed for divergent color morphs of the Lesser Earless Lizard. Male lizards inhabiting the gypsum dunes of White Sands discriminated among potential mates and preferentially displayed courtship behavior to local females. In the vast majority of field trials in which courtship behavior was observed, the first bout of male courtship was directed toward the resident female. Similarly in lab trials, males preferentially courted local females. However, contrary to initial predictions, males in this study did not make courtship decisions solely, if at all, based on female dorsal coloration. In field trials, White Sands males preferentially courted White Sands females even when these females were painted dark. Therefore, males used cues other than dorsal coloration to discriminate between white sand and dark soil females.

Although data presented here do not fully elucidate the mechanism by which male *H. maculata* from White Sands discriminated among potential mates, they suggest that behavioral cues may have played an important role. In field trials, male choice was highly correlated with female behavior. Males were significantly more likely to participate in trials if females were actively displaying and were significantly more likely to court females exhibiting display behavior. Although I have drawn no conclusions about the social meaning of female displays in this study, the correlation between male preference and female behavior remains strong. Other studies in lizards have shown that more vigorously displaying lizards can attract more mates (e.g., Greenberg and Noble, 1944; Ruby, 1981). In field trials, female displays occurred prior to male courtship in the majority (80%) of all behavioral interactions, providing an opportunity for males to assess some aspect of female behavior such as display intensity or signal morphology. Further, males courted displaying resident females more often than displaying nonresident females, suggesting that males were not merely choosing females exhibiting an ''acceptance'' signal. Head-bob and push-up behaviors in Phrynosomatid lizards are evolutionarily highly labile (Ferguson, 1971; Martins et al., 1998); hence, rapid population-level divergence in some aspect of female *H. maculata* displays (i.e., amplitude or number of repetitions of the head-bob display) is certainly plausible.

The relative importance of female signaling behavior to male mate choice also may depend on the social context of courtship interactions. For example, in simultaneous choice field trials (two females paired with one male), females initiated the majority (80%) of courtship interactions. However, in sequential choice laboratory trials (one female paired with one male), males initiated the majority (81%) of courtship interactions. Although this discordance could represent a difference between behavior in the field and in the lab, it might also indicate a stronger influence of female behavior on male choice when males are choosing between competing females. It is plausible that, when multiple females are present, males are choosier and attempt to assess female receptivity before initiating courtship or that females display more actively when in the presence of a potential competitor. These alternative explanations for observed patterns require future studies explicitly testing the effect of social context on courtship behavior.

There are a number of additional signals that are also thought to influence mating decisions in some species of lizards (Tokarz, 1995). Many of these, such as olfactory cues, may play an important role in mate choice but were not tested directly in this study. However the data presented here do relate to the potential influence of several visual signals besides dorsal coloration. First, body size was not associated with preference in this study. Second, although variation in female dorsal coloration was not correlated with male choice, other color cues aside from dorsal coloration may influence mate choice in *H. maculata*. For example, the use of high intensity throat patches for sexual signaling has been documented for both sexes in some lizard species (e.g., Cooper and Burns, 1987; Cooper and Greenberg, 1992; LeBas and Marshall, 2000; Weiss, 2006), and there is evidence in *H. maculata* that adult females from white sand and dark soil populations exhibit differences in throat and neck coloration during the breeding season (Hager, 2002; unpubl. data). Because female nuptial coloration is used in *H. maculata* to indicate receptivity to potential mates (Hager, 2001), population-level differentiation in these breeding colors may affect mate recognition. In the experiments presented here, female dorsal coloration was altered with a paint treatment, but throat color was not and thus provided a potential signal for males to discriminate between resident and nonresident females. The effect of population differences in female breeding coloration can be tested directly with male choice experiments that directly manipulate female nuptial color patches.

Finally, it is important to address the potential relationship between male and female choice in this system. The laboratory experiments presented here provide evidence that males exhibit preference for like-colored females even before they assess female display behavior (i.e., in 81% of lab trials, males exhibited courtship behavior before females displayed). However, in field trials, it is more difficult to fully disentangle male from female choice because male preference was correlated not only with female locality but also with female behavior (i.e., females displayed first in 80% of trials and males preferentially courted displaying females). The association between male and female behavior, and the fact that in both lab and field trials females consistently displayed more toward like-colored males, raises the possibility that females in this system also are choosy. Mate choice has been previously documented for both sexes in species of Phrynosomatid lizards, with female choice in *Urosaurus ornatus* (Hamilton and Sullivan, 2005) and *Uta palmeri* (Hews, 1990) and male choice in *Holbrookia propinqua* (Cooper, 1985), but little is known about the potential for male and female choice to occur simultaneously in lizard species. Mutual mate-choice models (Johnstone et al., 1996; Kokko and Monaghan, 2001) have generally been applied to species with high levels of parental care (e.g., birds [Jones and Hunter, 1993; Romero-Pujante et al., 2002] and fish [Kraak and Bakker, 1998; Sandvik et al., 2000]), but may merit consideration in squamates, particularly for species in which reproduction may be costly for both sexes (e.g., as a result of high levels of resource allocation to gamete production, mate-guarding behavior, ornamentation, local adaptation).

Overall, this study suggests a correlated response in mating preferences for populations of *H. maculata* that have undergone adaptive divergence in response to a geologically recent change in selection regime. This result is noteworthy for two reasons. First, evidence for mate choice in lizards is sparse. Whether few squamate species are choosy or whether choice has been understudied in this group remains to be determined. Identifying squamate species for which the benefits of being choosy may outweigh the costs is important for testing predictions regarding the conditions in which mate choice is expected in this group. Second, theoretical models suggest that the rapid development of reproductive isolation is increasingly plausible when divergent natural selection is coupled with a preference for local mates (Kirkpatrick and Ravigne, 2002; Gavrilets, 2003; Servedio, 2004), and evidence for this interaction between natural and sexual selection is beginning to accumulate in other vertebrates (e.g., birds: Podos and Nowicki, 2004; fish: McKinnon et al., 2004; Vines and Schluter, 2006). Although dorsal coloration, per se, does not appear to be a strong cue for mate choice in this system, color morphs that have diverged within the last 6,000 years are able to discriminate between local and nonlocal mates. Whether or not traits used for mate choice are mechanistically correlated with those important for survival at White Sands, the potential importance of both natural and sexual selection in this system provides a window into the role of divergent selection in population differentiation.

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