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"SAME SAME BUT DIFFERENT": REPLICATED ECOLOGICAL SPECIATION AT WHITE SANDS

Erica Bree Rosenblum[1,2] and Luke J. Harmon[1]

[1]*Department of Biological Sciences, University of Idaho, Moscow ID 83844-3051*

[2]*E-mail: rosenblum@uidaho.edu*

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Understanding the factors that promote or inhibit species formation remains a central focus in evolutionary biology. It has been difficult to make generalities about the process of *ecological speciation* in particular given that each example is somewhat idiosyncratic. Here we use a case study of replicated ecological speciation in the same selective environment to assess factors that account for similarities and differences across taxa in progress towards ecological speciation. We study three different species of lizards on the gypsum sand dunes of White Sands, New Mexico, and present evidence that all three fulfill the essential factors for ecological speciation. We use multilocus nuclear data to show that progress toward ecological speciation is unequal across the three species. We also use morphometric data to show that traits other than color are likely under selection and that selection at White Sands is both strong and multifarious. Finally, we implicate geographic context to explain difference in progress toward speciation in the three species. We suggest that evaluating cases from the natural world that are "same same but different" can reveal the mechanisms of ecological speciation.

KEY WORDS: **Adaptation, gene flow, genetic clustering, lizards, speciation by selection.**

The phrase "same same but different" is a common colloquial expression in Thailand with analogs in many cultures around the world. This phrase captures the idea that things can be mostly similar but still different in some important way. It is common in evolutionary biology to contrast "same" and "different" as mutually exclusive alternatives. For example, in molecular studies, research in the last decade has focused on determining whether the molecular mechanisms underlying adaptation are the same or different in different species (Colosimo et al. 2005; Hoekstra et al. 2006; Arendt and Reznick 2007; Chan et al. 2010; Rosenblum et al. 2010). However, there are many examples in which the answer is not "same or different" but "same and different." For example, the same gene can influence convergently evolved phenotypes in different species but do so by different functional mechanisms (e.g., Protas et al. 2006; Storz et al. 2009; Rosenblum et al. 2010). Thus we learn about evolutionary generalities from shared similarities and mechanistic details from key differences (Langerhans and DeWitt 2004).

"The same same but different" paradigm is particularly enlightening for the study of speciation. Speciation remains one of the profound mysteries in evolutionary biology and has resisted generality for over 100 years despite focused research on the topic (Coyne and Orr 2004). *Ecological* speciation—when speciation is driven by divergent selection between environments (Rundle and Nosil 2005; Schluter 2009)—is particularly tricky because ecological speciation can happen rapidly and does not always proceed to completion. Even when the necessary conditions for ecological speciation are met in nature, progress toward—or completion of—ecological speciation is not guaranteed (Gavrilets 2004; Hendry 2009). Empirical studies have uncovered a full range of situations, from nearly complete admixture to nearly complete isolation, associated with conditions favorable to ecological selection in the wild (Schluter 1996; Funk 1998; Wu 2001; Dres and Mallet 2002; Crespo et al. 2005; Barluenga et al. 2006; Savolainen et al. 2006; Vines and Schluter 2006; Ryan et al. 2007; Mallet 2008; Berner et al. 2009; Brelsford and Irwin 2009; Hendry et al. 2009; Chamberlain et al. 2009). To determine the factors that promote or inhibit ecological speciation it is necessary to study natural replicates of ecological speciation in progress (e.g., phytophagous insects [Stireman et al. 2005]; stickleback fish [Berner et al.

2009]). Focusing on what is "same same" and what is "different" across repeated speciation events in a common environment can help understand and predict general patterns of speciation in the wild.

There are two *essential factors* that form the core of all models of ecological speciation (reviewed in Gavrilets 2004; Bolnick and Fitzpatrick 2007). First, ecological speciation requires divergent selection—different ecological conditions that favor distinct phenotypes and heritable variation for these phenotypes (Schluter 2001, 2009). Second, ecological speciation requires a correlation between mating and ecology due to mate choice, geographic structure, or both (Gavrilets 2004). These essential factors provide the necessary pre-conditions for ecological speciation and may help explain patterns that are shared across taxa. However, there are also a number of *promoting factors* that affect the potential for completion of ecological speciation and may differ among species and environments. For example, strong or multifarious selection (Slatkin 1982; Doebeli and Dieckmann 2003; Fry 2003; Gavrilets 2004; Nosil and Sandoval 2008; Nosil et al. 2009a; Thibert-Plante and Hendry 2009; de León et al. 2010), "magic traits" that affect both ecology and mating (Maynard Smith 1966; Rice 1984; Rice and Hostert 1993; Dieckmann and Doebeli 1999; Kondrashov and Kondrashov 1999; Schluter 2001; Via 2001; Kirkpatrick and Ravigne 2002; Gavrilets 2004), and geographic structure (Endler 1977; Gavrilets et al. 2000a; Doebeli and Dieckmann 2003; Gavrilets 2004) can all promote ecological speciation.

One of the best places for studying replicated ecological speciation is White Sands, New Mexico. The stark white gypsum dunes at White Sands represent a dynamic backdrop for observing ecological speciation in action. The substrate color of the white sand habitat contrasts dramatically with that of the surrounding Chihuahuan desert dark soil habitat (Fig. 1), providing a novel selection environment for crypsis (Dice 1947; Kaufman 1973; Luke 1989; Kiltie 1992; Reed and Janzen 1999). Any response of the local fauna to the White Sands environment has occurred very rapidly as the bulk of the gypsum deposition has occurred within the last 2000–5000 years (Kocurek et al. 2007). Three lizard species exhibit blanched forms on the gypsum dunes that contrast with dark forms in the rest of their ranges (Smith 1943; Lowe and Norris 1956; Dixon 1967). These three species, *Aspidoscelis inornata* (Little Striped Whiptail), *Sceloporus undulatus* (Eastern Fence Lizard), and *Holbrookia maculata* (Common Lesser Earless Lizard) are independent lineages evolving convergently in a shared environment.

Previous research has focused on adaptation in this system but the White Sands species also represent replicate examples of parapatric ecological speciation in action. The White Sands system is well suited for considering ecological speciation because of its young age and natural replication. Models suggest that, once ecological speciation is initiated, the expected time to completion is quite short, on the order of hundreds to thousands of generations (Hendry et al. 2000, 2007; Gavrilets 2003). The young age of the gypsum dunes allows us to study the process of ecological speciation on an appropriate time scale. Additionally, the three species of lizards considered here are quite distantly related (Reeder and Wiens 1996; Wiens et al. 2010). This is in contrast to most other studies of replicated ecological speciation (e.g., Nosil et al. 2002; Vines and Schluter 2006; Nosil and Sandoval 2008; Berner et al. 2009), which have focused on very closely related lineages in geographically separate but similar environments (but see Stireman et al. 2005). Distantly related species are more likely to differ in ways that promote or inhibit ecological speciation. Thus studying independent evolutionary replicates in a shared environment can be particularly important for understanding both essential and promoting factors.

There are five main lines of evidence that White Sands lizards are examples of speciation in progress. First, differences in dorsal coloration between white sand and dark soil habitats are driven by strong divergent selection (Rosenblum 2006). Second, mitochondrial data suggest some genetic clustering by habitat despite recent divergence and/or ongoing gene flow (Rosenblum 2006; Rosenblum et al. 2007b). Third, white sand and dark soil habitats are parapatric with an abrupt transition, providing geographic structure associated with habitat differences. Fourth, lizard coloration may function as a "magic trait," a trait affecting

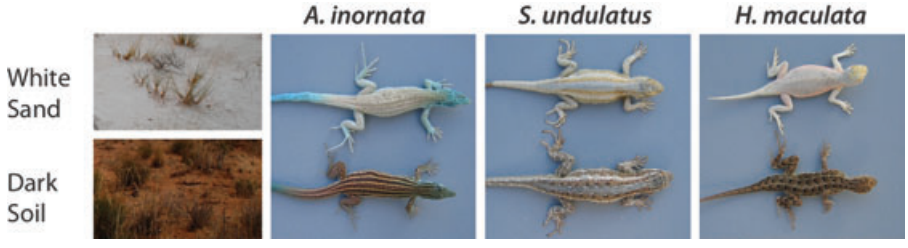

Figure 1. Representative samples of the three lizard species (*A. inornata*, *S. undulatus*, *and H. maculata*) inhabiting White Sands (top row) compared to conspecifics in surrounding dark soil habitat (bottom row). Typical substrate colors are shown on the left. Modified from Rosenblum (2006).

both ecological fitness and mating (Gavrilets 2004). Changes in melanin production affect not only dorsal coloration but also color patches used for sexual signaling and mate choice (Robertson and Rosenblum 2009). Therefore, color change may provide a direct link between natural and sexual selection at White Sands that could accelerate the process of ecological speciation (Robertson and Rosenblum 2009). Fifth, behavioral experiments have shown discrimination between local and non-local mates and competitors in two of the White Sands species (Rosenblum 2008; Robertson and Rosenblum 2010) indicating that some of the early stages of reproductive isolation may already be established.

Here we bring diverse data to bear on the question of progress toward ecological speciation at White Sands. We have three main goals: (1) to demonstrate that all three species of lizards in White Sands satisfy the essential requirements for ecological speciation, (2) to quantify the progress that each species has made toward the completion of speciation, and (3) to seek explanations for variation in this progress across the three species. We find that the species are "same same but different"—all three species exhibit evidence of ecological speciation in progress, but to varying degrees. We use these similarities and differences to explain progress toward speciation at White Sands.

Methods

We present a combination of genetic and phenotypic data to understand the dynamics of ecological speciation at White Sands. For each species, we sampled individuals from white sand and dark soil habitat. Even though we sampled multiple localities within both habitat types, here we focus only on the differences that are most relevant for ecological speciation: the differences between the two habitats (for more details about variation within habitats see Rosenblum 2005, 2006; Rosenblum et al. 2007b). For one set of analyses we also included individuals from the "ecotone," the narrow transition zone between the two habitats. Sample sizes from each habitat for each dataset are presented in the online supplement.

GENETIC DATA

We used anonymous multilocus nuclear markers to assess genotypic clustering across habitats for all three species. For *S. undulatus* we present an expansion of a previously published dataset containing 19 anonymous nuclear loci with 207 single nucleotide polymorphisms (SNPs; Rosenblum et al. 2006; 2007a). The expanded *S. undulatus* nuclear dataset contains 67 total individuals. For *A. inornata* and *H. maculata*, we present new previously unpublished amplified fragment length polymorphism (AFLP) datasets. The complete *A. inornata* AFLP dataset contains 47 variable bands for 46 individuals. The complete *H. maculata* AFLP dataset contains 49 variable bands for 47 individuals. Our analyses are comparable across different types of nuclear data in different species (i.e., SNPs vs. AFLPs) because we compare within- to between-habitat genetic variation and have sufficient (but not saturated) levels of variation in all species.

For *H. maculata* and *A. inornata*, we generated AFLP datasets. Whole genomic DNA was digested with the restriction enzymes MseI and EcoRI and ligated with the respective adaptors with T4 DNA ligase. After overnight incubation at room temperature, the products were amplified by PCR using MseI and EcoRI primers with the following temperature profile: 94°C for 2 min (initial denaturation), 20 cycles of 94°C for 30 sec (denaturation), 56°C for 30 sec (annealing), 72°C for 1 min (extension), and then 10 min at 72°C (final extension). The products from this reaction were then selectively amplified using 10 MseI primers with trinucleotide extensions on the 3′ ends. The MseI primer was: 5′–GAC TGC GTA CCA ATT C NNN–3′, with NNN being one of the following: CGC, CGG, CGA, CGT, CAG, CAT, CAC, CTC, CAA, or CTA. Each MseI primer was paired with a labeled, selective EcoRI primer with a TGA extension on the 3′ end: 5′–GAC TGC GTA CCA ATT C TGA–3′. The temperature profile for the selective amplification was: 94°C for 2 min (initial denaturation), then 12 cycles of 94°C for 30 sec (denaturation), initially 65°C for 30 sec then reduced by 0.7°C per cycle (annealing), 72°C for 1 min (extension). This was followed by 23 cycles of 94°C for 30 sec (denaturation), 56°C for 30 sec (annealing), 72°C for 1 min (extension), and finally 72°C for 10 min (final extension). The selectively amplified products were diluted with TE buffer (two parts buffer to one part reaction), then mixed with formamide and TAMRA (Applied Biosystems, Foster City, CA) and run on a polyacrylamide gel on an ABI Prism 377 DNA sequencer (Applied Biosystems). The gels were tracked and analyzed on GeneScan (Applied Biosystems). The sequence files were then imported to Genographer (Benham et al. 1999) for analysis. Bands ranging from 50 to 400 bp were scored as present or absent for each individual. Ambiguities were treated as missing data, and bands with ambiguous states for more than 15% of the individuals were not analyzed.

We also compared new multilocus nuclear data to mitochondrial data for all three species published previously (Rosenblum 2006) and added additional samples following the protocol from Rosenblum (2006). The mitochondrial data included approximately 800 bp of the mitochondrial ND4 gene (and associated tRNA) for 41 *A. inornata*, 82 *S. undulatus*, 64 *H. maculata* (with 17, 54, and 49 variable sites for each species, respectively).

MORPHOLOGICAL DATA

We used measurements of body size and shape to assess phenotypic change across habitats for all three species. We measured weight, snout–vent length (SVL), foreleg, hindleg, longest toe length, head width, head depth, snout-parietal distance and

pelvic width for 41 *A. inornata*, 64 *S. undulatus*, and 55 *H. maculata*.

We also compared new morphometric data to previously published data on dorsal coloration (Rosenblum 2006; Robertson and Rosenblum 2009) and added additional samples following the protocol from Rosenblum (2006). Colors were quantified using an Ocean Optics spectrophotometer (USB 2000) for a total of 66 *A. inornata*, 116 *S. undulatus*, and 85 *H. maculata*.

STATISTICAL ANALYSES

We first analyzed the genetic data to compare the level of progress toward speciation in the three species. We calculated Fst values for nuclear and mtDNA separately. In each case, we calculated Fst between white sand and dark soil habitats and estimated confidence intervals by permuting haplotypes among habitat types in Arlequin version 3.5 (Excoffier and Lischer 2010). For mitochondrial data we also calculated nucleotide diversity in Arlequin and constructed haplotype networks for each species using statistical parsimony implemented in TCS version 1.21 (Clement et al. 2000).

We used Bayesian assignment tests (Pritchard et al. 2000) to evaluate the level of genetic clustering in nuclear DNA between white sand and dark soil habitats of each lizard species. We used the program STRUCTURE (version 2.3; Pritchard et al. 2000) to calculate the posterior probability that individuals belong to each of k clusters assuming linkage equilibrium and HWE across multiple, unlinked loci. We used prior distributions for population assignments based on sampling locations, which allows structure to be detected at lower levels of divergence without bias (Hubisz et al. 2009). We modeled AFLPs as dominant markers following Falush et al. (2003, 2007). For each value of k we carried out five runs, each with a burn-in of 1 million generations and 2 million generations to calculate posterior probabilities. We then compared clustering results and average maximum likelihood values at five possible numbers of genetic demes ($k = 1$ through $k = 5$) using the Δk method to select the most appropriate number of clusters needed to fit our data (Evanno et al. 2005). We then used CLUMPP (Jakobsson and Rosenberg 2007) to create assignment profiles for each species at that value of k.

To more directly compare levels of structure across datasets, we developed a custom clustering method to analyze all datasets. This clustering method allowed us to generate results from the molecular and phenotypic data that were directly comparable. For the molecular datasets, our clustering analyses involved three steps: coding genetic data as quantitative characters, carrying out a principal components analysis (PCA) on these quantitative characters, and performing k-means clustering (Lloyd 1982) on the PCA axes. We first isolated the variable sites from each molecular dataset. We then coded each variable site with quantitative codes representing diploid genotypes: 1 for the homozygote of the most common allele, 0 for heterozygotes, and -1 for the homozygote of the rare allele. The few instances in which variable sites had more than two alleles were excluded (11 SNP sites from the *S. undulatus* nuclear dataset). Mitochondrial alleles were coded as 1 for the common allele and -1 for the rare allele. The molecular datasets contained some missing information because not all variable sites could be scored for all individuals. Because of this, we used non-linear iterative partial least squares (NIPALS) algorithm (Wold 1966), a method of PCA that can be used for incomplete datasets, as implemented in the R package pcaMethods (Stacklies et al. 2007). For consistency, we retained the first five PCA axes from this analysis, which was generally enough to explain ~99% of the variation in the data. Finally, we conducted a k-means clustering analysis (Lloyd 1982) on these data. Because we were strictly interested in differences between white sand and dark soil habitat, we used a value of $k = 2$ for each analysis. We reran each k-means analysis from 10 random starting points to ensure convergence to a stable solution. We compared the membership of the two inferred groups to the true identity of the white sand and dark soil lizards, scoring individuals as "correct" if they were in a cluster with most of the other lizards from their habitat and "incorrect" otherwise. We then compared the number of "incorrect" classifications across datasets.

For the morphometric data, we first compared body size for each species between white sand and dark soil habitats using analysis of variance (ANOVA). Then, we removed the effect of body size using linear regression. For each species, we regressed each ln-transformed character on ln-transformed body size, measured as SVL. We retained the residuals from this analysis as our body shape axes. We then conducted a principal component analysis on the correlation matrix of these residuals, again retaining the first five axes. These five axes were used for the k-means clustering analysis with $k = 2$, as mentioned previously.

For the color data, we analyzed the visual spectrum (wavelengths between 300 and 700 nm), following Rosenblum (2006). We conducted a principal components analysis on the covariance matrix of these data, again retaining the first 5 axes. We then conducted a k-means analysis, as described previously, on these dorsal color PC axes.

Finally, we conducted a discriminant analysis to determine the status of ecotonal individuals of each species for mtDNA, nucDNA, color, and body shape. These analyses differ from the k-means clustering analyses because for the discriminant analysis we use a priori information about group membership for white sand and dark soil lizards to define our discriminant axes, which were then used to classify the ecotone individuals. For each dataset, we conducted a discriminant function analysis comparing white sand and dark soil individuals of each species for each type of data. We used the first five PC axes for each analysis. We then applied the resulting discriminant function to the ecotone

individuals, calculating the posterior probability that each ecotone individual was a part of the white sand or dark soil group. We classified any individuals with less than 95% posterior probability of membership in either group as "unassigned." Statistical analyses were conducted in R version 2.9.2 (R Development Core Team 2009).

Results

Here we present comparisons among the three White Sands lizards for nuclear (Fig. 2), mitochondrial (Fig. 3), and morphological (Fig. 4) datasets. We then integrate across diverse data types (Figs. 5 and 6) to compare progress toward speciation in the three species.

GENETIC DATA

STRUCTURE analyses of multilocus nuclear data revealed distinct patterns of genetic clustering across species between white sand and dark soil habitats (Fig. 2). In all cases, likelihoods either peaked or reached a plateau at k = 2, so we used this value for all remaining analyses. For *H. maculata*, white sand and dark soil individuals were clearly assigned to separate groups. For *S. undulatus*, there was some genetic clustering but without a clear relationship to habitat. For *A. inornata*, nuclear data showed no structure. Estimates of Fst based on multilocus nuclear data were consistent with STRUCTURE results and showed stronger genetic structure between habitats for *H. maculata* than for *A. inornata* and *S. undulatus* (Table 1). K-means analysis showed similar results to the Fst analysis, with *H. maculata* more distinct than *S. undulatus*, which was in turn easier to classify correctly than *A. inornata* (Fig. 5). Overall the nuclear data showed the strongest clustering between habitats for *H. maculata*.

Haplotype networks showed no mitochondrial structure in *A. inornata* with shared haplotypes between white sand and dark soil habitats (Fig. 3). In contrast, no haplotypes were shared across habitats in *S. undulatus* and *H. maculata*, and both species exhibited more mitochondrial structure than *A. inornata*. For example,

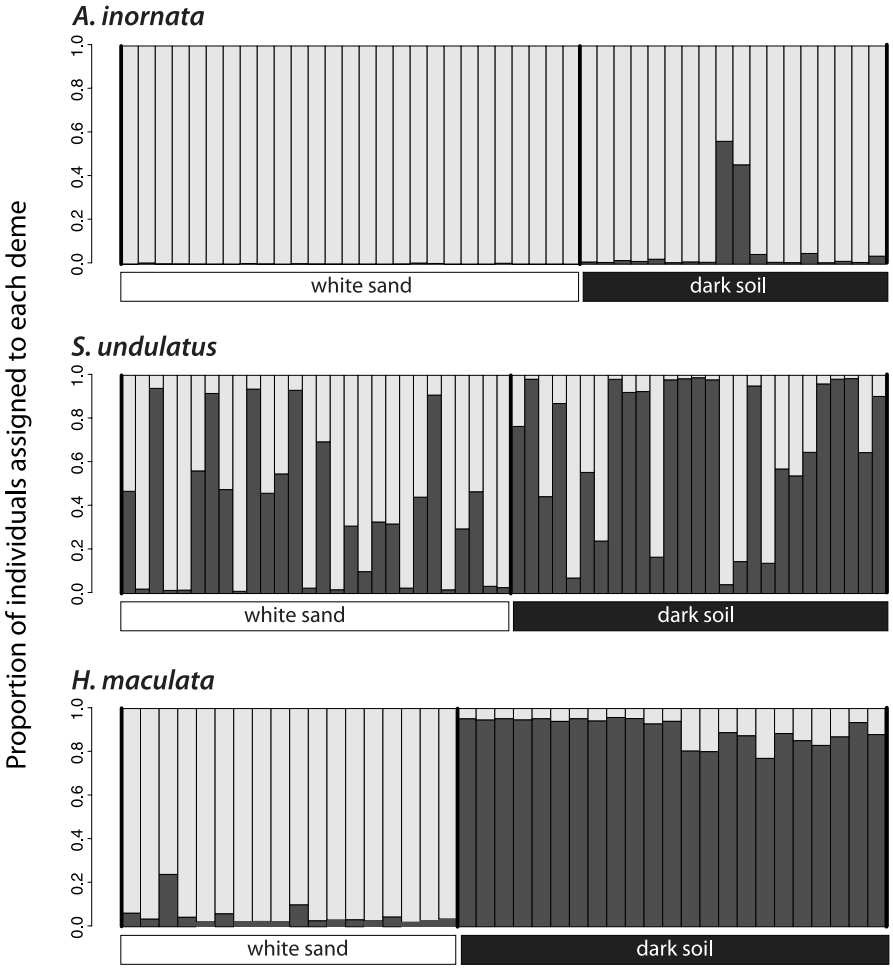

Figure 2. STRUCTURE plots based on multilocus nuclear data for the three focal species. Each vertical bar represents a single individual. Bar colors represent posterior probabilities of identity to inferred genotypic cluster. Individuals from dark soil and white sand habitat are indicated by horizontal black and white bars, respectively.

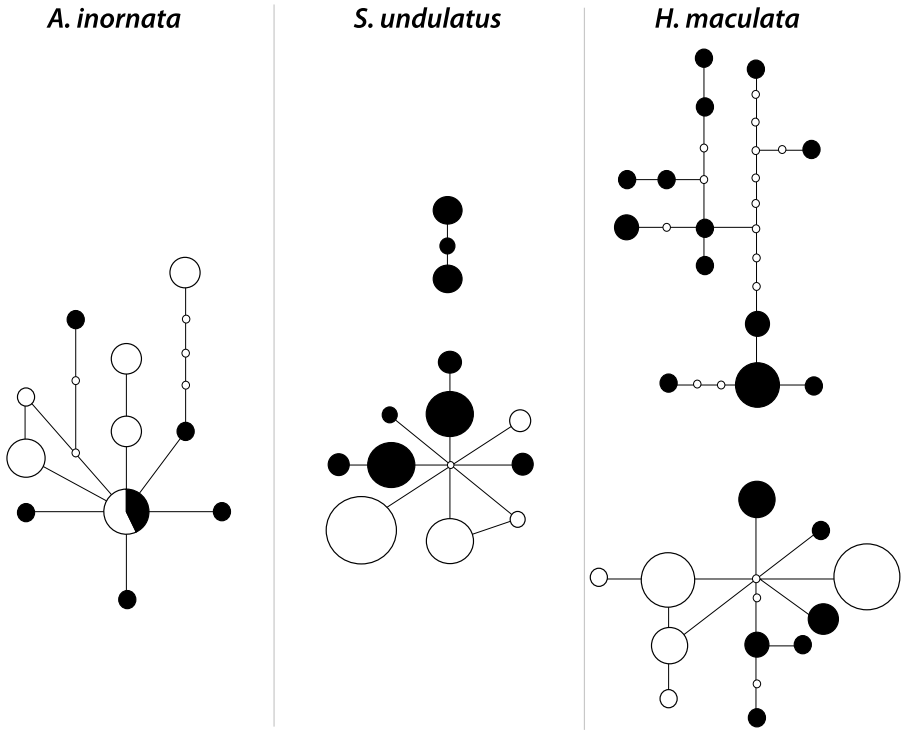

Figure 3. Mitochondrial haplotype networks for the three focal species. Each circle represents a single mitochondrial haplotype, and each connecting line (or small circle) represents one mutational step. White and black circles represent samples from white sand and dark soil habitats, respectively. Circle size is proportional to number of individuals with each haplotype.

in both *H. maculata* and *S. undulatus*, the most geographically disparate population was not connected to the white sand cluster (note two separate clusters in Fig. 3). Estimates of Fst based on mitochondrial data showed much less genetic structure between habitats for *A. inornata* than *S. undulatus* and *H. maculata* (Table 1). Likewise, results of the k-means analysis showed higher concordance between genetic and habitat groups for *S. undulatus* and *H. maculata* than for *A. inornata* (Fig. 5). Overall, the mitochondrial data showed that *A. inornata* had little genetic structure between habitats whereas *S. undulatus* and *H. maculata* had greater (and comparable) clustering between white sand and dark soil habitats.

MORPHOLOGICAL DATA

White sand lizards exhibit important differences in body shape compared to their dark soil counterparts. First, one of the three species differs in size (Table 2, Fig. 4). *A. inornata* was significantly larger in white sand compared to dark soil habitat, whereas *S. undulatus* and *H. maculata* showed no significant differences in body size across habitats (although there was a trend of white sand individuals being slightly smaller in these species). Second, we removed the effect of size to better understand changes in shape (Table 2, Fig. 4). In the full model including all three species, there were significant differences in body shape across species and across habitats. Multivariate analysis of variances (MANOVAs) on each species independently confirmed that white sand and dark soil individuals were significantly different from each other in body shape. Again, there was also an interaction between species and habitat, indicating that the direction and/or magnitude of body shape change varied across species. PC1 primarily explains species differences, with larger values corresponding to relatively shorter legs and smaller heads. In general, *S. undulatus* and *H. maculata* had longer legs and larger heads than *A. inornata*. Differences between white sand and dark soil individuals were primarily explained by PC2. In fact, all three white sand species exhibited the same direction of change along PC2. In all three species, white sand individuals had relatively larger heads (both longer and deeper) and longer toes. Although the direction of change was similar across all species, the magnitude varied by species. White sand and dark soil *A. inornata* and *H. maculata* were quite diverged in body shape whereas *S. undulatus* was the most similar across habitats in body shape. K-means analysis showed good concordance between groupings based on body shape and those based on habitat for *A. inornata* and *H. maculata* and weak concordance for *S. undulatus* (Fig. 5). Overall, the morphometric data showed strong differences between dark soil and white sand *A. inornata* and *H. maculata*, and weak differences for *S. undulatus*.

Individuals from white sand and dark soil habitats also differed dramatically in dorsal coloration. In the full model including

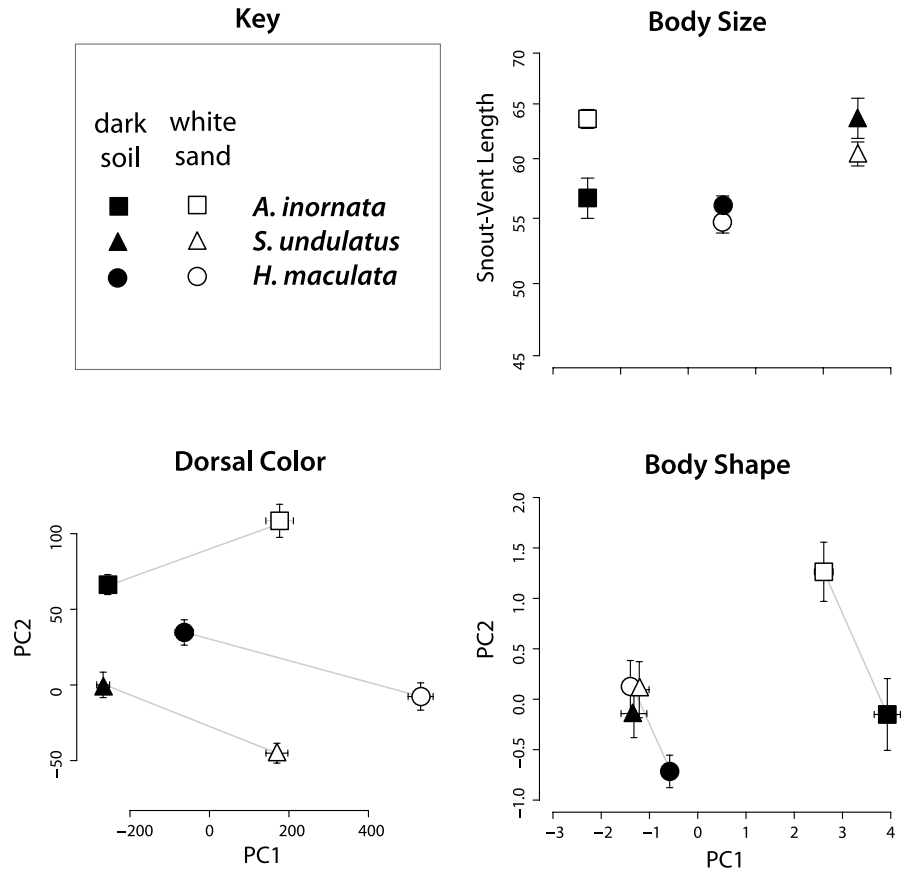

Figure 4. **Morphological divergence between dark soil and white sand lizards in dorsal color, body size, and body shape. For body size we plot mean SVL with standard error bars for each species (note that X axis is arbitrary in this panel). For dorsal color and body shape we plot PC1 and PC2 with standard errors for each species (note that in some cases error bars are smaller than the population symbols). For color, PC1 is related to brightness and PC2 to both chroma and hue (see Supporting Information). These two axes explain 95% and 3.6% of the variation in color, respectively. For body shape, larger values of PC1 signify wider heads and smaller legs whereas larger values of PC2 are associated with longer and deeper heads and longer toes; these two axes explain 53.8% and 17.7% of the variation, respectively.**

all three species, there were significant differences in color across species and between white sand and dark soil habitats (Table 2, Fig. 4). MANOVAs on each species independently confirmed that white sand and dark soil individuals were significantly different from each other in color in each species. All three species exhibited the largest degree of change along PC1; as expected all three species were brighter in color at White Sands (PC1 represents brightness). There was also an interaction between species and habitat, indicating that the direction and/or magnitude of change varied across species. Differences among species were primarily explained by difference along PC2, which is influenced by both chroma and hue (see online Supporting Information). As expected, the three species were different from each other in chroma and/or hue. K-means clustering analysis showed a very

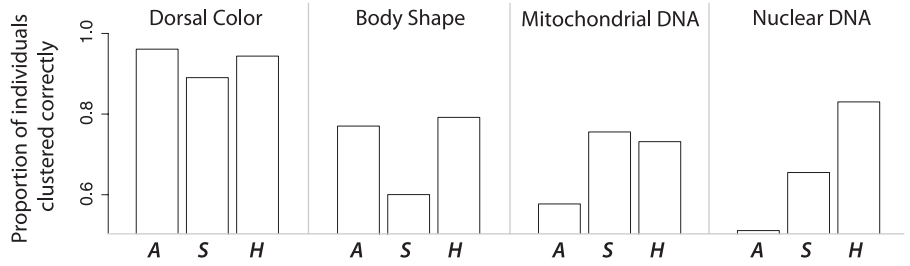

Figure 5. **K-means clustering analysis (K = 2) showing proportion of individuals that were grouped correctly for each species and each data type. Species are abbreviated with generic initial (*A = A. inornata*, *S = S. undulatus*, *H = H. maculata*).**

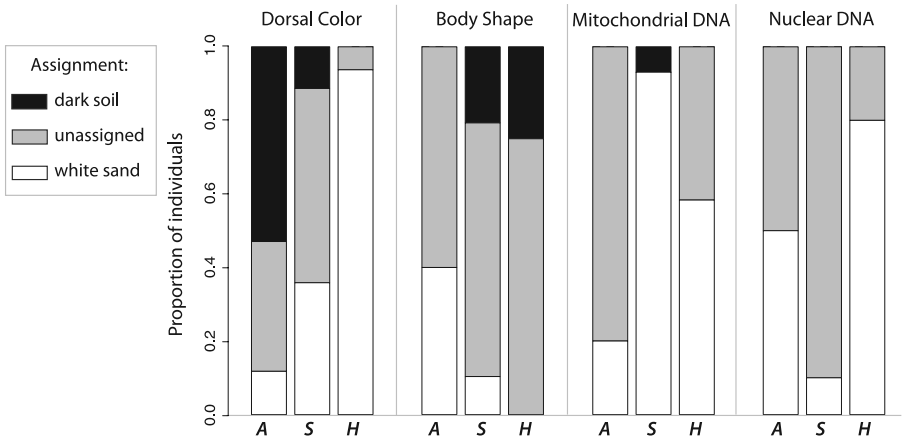

Figure 6. Discriminant function analysis of ecotonal individuals showing proportion of individuals assigned as white sand or dark soil for each species and each data type. Individuals all posterior probabilities less than 95% are shown as "unassigned." Species are abbreviated with generic initial (*A = A. inornata*, *S = S. undulatus*, *H = H. maculata*).

strong concordance between groups based on color phenotype and groups based on habitat classification (Fig. 5). Overall, the color data show clearly that white sand and dark soil individuals differ strongly in dorsal color across habitats.

CLUSTERING ANALYSIS ACROSS DATASETS

Across all three species, lizards from white sand and dark soil habitats had different average dorsal color, body size, and body shape. However, k-means clustering analysis, which does not use a priori information about group membership, varied in the ability to recover groups that correspond to white sand and dark soil habitats (Fig. 5). The most consistent clustering used dorsal coloration, which classified most individuals that came from the same habitat together (overall 93% of individuals were classified with others from the same habitat). K-means clustering using body shape was less effective, but still placed individuals in a cluster with others from the same habitat most of the time (overall 72%). The k-means analysis using body shape also confirmed that *A. inornata* and *H. maculata* were more distinct across habitats morphologically than *S. undulatus*. The mitochondrial and nuclear datasets showed similar amounts of overall clustering (mtDNA overall 71%; nucDNA overall 68%). The clustering analyses based on molecular data also confirmed that there was little or no genetic structure across habitats in *A. inornata* compared to moderate levels in *S. undulatus* and *H. maculata*. The nuclear data also suggested that *H. maculata* had the highest levels of genetic structure across species.

ECOTONE ANALYSIS ACROSS DATASETS

Discriminant function analysis was able to classify most of the individuals from white sand and dark soils to the correct population using either dorsal color, body shape, nDNA, or mtDNA (see online supplement). Classification of ecotone individuals also differed dramatically across species and data types (Fig. 6). For dorsal color, most *H. maculata* ecotonal individuals were very light in color and were classified as white sand. *S. undulatus* ecotonal individuals ranged widely in color with many individuals unclassified. Finally, *A. inornata* ecotonal individuals mostly clustered with dark soil individuals, although some were light or unclassified. The clustering of ecotonal individuals reported here are consistent with previous analyses (Rosenblum 2006; ecotonal individuals generally light in *H. maculata*, intermediate in *S. undulatus* and dark in *A. inornata*). For body shape, most ecotonal individuals for all three species were unclassified, suggesting that ecotonal individuals were generally intermediate morphologically. Genetic patterns at the ecotone for both *A. maculata* and *H. maculata* were consistent across molecular datasets. For both mtDNA and nucDNA, ecotone *A. inornata*

Table 1. Fst for mitochondrial and nuclear data and mitochondrial nucleotide diversity for dark soil (DS) and white sand (WS). All Fsts are significantly different from zero. Note that nucleotide diversity could not be calculated from nuclear AFLP data.

Species	Mitochondrial Fst	Nuclear Fst	Mitochondrial DS Nucleotide Diversity	Mitochondrial WS Nucleotide Diversity
A. inornata	0.11	0.05	0.0025	0.0034
S. undulatus	0.44	0.04	0.0196	0.0058
H. maculata	0.40	0.19	0.0156	0.0022

Table 2. Tests of differences in body size, body shape, and dorsal coloration across white sand and dark soil populations of three species. ANOVAs were conducted for body size and MANOVAs for body shape and dorsal color. "Overall" tests were two-way tests with three species, two habitats, and the interaction between species and habitat included. Asterisks indicate level of statistical significance (*P<0.05, **P<0.01, ***P<0.001).

Test	Effect	Body size	Body shape	Dorsal color
Overall	Species	$F_{2,98}$=17.9***	Wilks' λ=0.03	Wilks' λ=0.13***
	Habitat type	$F_{1,98}$=0.05	Wilks' λ=0.59***	Wilks' λ=0.18***
	Species ∗ Habitat type	$F_{2,98}$=8.8***	Wilks' λ=0.58***	Wilks' λ=0.49***
A. inornata	Habitat type	$F_{1,24}$=15.0***	Wilks' λ=0.24***	Wilks' λ=0.21***
S. undulatus	Habitat type	$F_{1,33}$=2.2	Wilks' λ=0.57*	Wilks' λ=0.13***
H. maculata	Habitat type	$F_{1,41}$=1.2	Wilks' λ=0.36***	Wilks' λ=0.15***

were usually unclassified, but sometimes clustered with white sand individuals. Ecotone *H. maculata* were usually classified with white sand individuals, but some were unclassified. By contrast, genetic patterns across the ecotone were discordant for *S. undulatus.* Many ecotonal *S. undulatus* clustered with white sand individuals according to mtDNA but were unclassified according to nucDNA. These results for *S. undulatus* were consistent with observations previously mentioned showing greater genetic differentiation between individuals in white sand and dark soil habitats using mtDNA than nucDNA.

Discussion

"The evolution of the entire White Sands dune fauna is in need of careful study. This small fauna is certainly one of the most unique in North America. It is possible that intrinsic reproductive isolation of White Sands populations from those surrounding the dune mass may be an actuality. If this is the case, the animals are true species. As there are no geographic barriers involved, such a discovery would provide a concrete example of ecological speciation . . . "— Lowe and Norris (1956)

Fifty years after this prescient passage above, we now have strong evidence for the early stages of ecological speciation in White Sands lizards. In particular, all three species share key essential factors that set the stage for ecological speciation. However, there are also striking differences in the degree of progress toward ecological speciation of white sand lizards. Given that the white sand lizards inhabit the same environment with the same conditions for ecological species, why has there been unequal progress? We examine the factors that are shared and unique across white sand species to reveal "same same" generalities and "but different" mechanisms.

"SAME SAME": STRONG AND MULTIFARIOUS SELECTION AT WHITE SANDS

Both essential factors for ecological speciation are present in all three species at White Sands. The light color of the gypsum substrate promotes strong divergent selection between white sand and dark soil adapted populations, and is evidenced by the rapid convergent evolution of blanched morphology in all three species (Figs. 1 and 4; see also Rosenblum 2006). Our new results show that divergent selection across habitats is not only strong but also multifarious, potentially promoting ecological speciation in all three species. There are strikingly parallel shifts in body shape across species (i.e., Figs. 1 and 4). All three species have evolved longer toes and broader heads at White Sands, which could be associated with changes in habitat structure. White sand habitat has less vegetation, different plant species, different substrates, different food resources and fewer competitors and predators compared to dark soil habitat (Lowe and Norris 1956; Dixon 1967). These ecological differences likely place different functional demands on lizard morphology. For example, lizard limb length and head shape affect ability to run on sand and exploit different food resources, respectively (Luke 1989; Anderson et al. 2008). Overall, parallel changes in body shape suggest that divergent selection acts in a predictable way on traits other than color across habitats.

"BUT DIFFERENT": PROGRESS TOWARD ECOLOGICAL SPECIATION

When the essential factors are present, why do some taxa fail to progress toward speciation? Even when conditions are right, ecological speciation only sometimes leads to stable, reproductively isolated new species (Dieckmann and Doebeli 1999; Gavrilets 2004; Stireman et al. 2005; Barluenga et al. 2006; Savolainen et al. 2006; Ryan et al. 2007; Chamberlain et al. 2009; Hendry 2009; Schluter 2009). Ecological speciation often fails to progress because the essential factors are necessary, but not sufficient, for ecological speciation (Gavrilets 2004).

To quantify the degree of progress toward ecological speciation, we measured genetic clustering (Mallet 1995; Feder 1998). Genetic clustering is a reasonable approach for young incipient species in which other diagnostic criteria for species are unlikely to be met (e.g., amount of pre- or post-mating reproductive isolation, mitochondrial monophyly) at the short timescale

considered here (Bulgin et al. 2003; Funk and Omland 2003; Chamberlain et al. 2009; Ballentine and Greenberg 2010; Weisrock et al. 2010). Under all models of ecological speciation the degree of genetic clustering increases over time with progress toward speciation (Mallet 1995; Nosil et al. 2009b). The association between the selective environment and neutral genetic markers strongly suggests ecological barriers to gene flow (Funk et al. 2006; Nosil et al. 2008; Räsänen and Hendry 2008).

In fact, there is evidence for uneven progress toward ecological speciation in the three White Sands lizards. Multilocus nuclear data indicate a rank order of genetic clustering across the White Sands ecotone for the three species (Figs. 2 and 5, Table 1), concordant with mitochondrial results. *A. inornata* exhibited little to no genetic clustering between white sand and dark soil habitats. *S. undulatus* showed intermediate and somewhat discordant patterns, with relatively strong clustering of mtDNA haplotypes, but weaker clustering of nuclear genotypes. *H. maculata* had consistently strong genetic clustering by habitat. Overall the genetic data suggest that *H. maculata* is well on its way toward completing speciation, *S. undulatus* has made incomplete progress toward speciation and *A. inornata* is failing to speciate. Uneven progress toward ecological speciation indicates important differences across the three focal species at White Sands. It is striking that three species can colonize the same novel environment and adapt in the same way but still make uneven progress toward speciation. The differences among species hold the key to understanding what factors promote or inhibit ecological speciation (Stireman et al. 2005).

"SAME SAME BUT DIFFERENT": PROMOTING FACTORS FOR ECOLOGICAL SPECIATION

We compare three promoting factors across species to explain differences in progress toward speciation. First, ecological speciation will be fastest when divergent selection is strong or involves multiple ecological and/or genetic axes (Nosil and Sandoval 2008; Nosil et al. 2009a). At White Sands, all three species exhibit *strong and multifarious selection* as evidenced by convergent evolution of multiple traits (e.g., color and body shape; Figs. 4 and 5, Table 2). However there are important differences across species in the magnitude of changes of color and body shape. For color, *H. maculata* is the best substrate matched (Rosenblum 2006), the most different from its ancestral coloration (Fig. 4), and the only species for which ecotonal animals were assigned to the white sand cluster (Fig. 6). By contrast *A. inornata* and *S. undulatus* are less substrate matched (Rosenblum 2006) and ecotonal animals were either more variable in color or more similar to dark soil populations (Fig. 6). These differences in coloration may correspond with difference in strength of selection across species. For example, *H. maculata* uses extremely exposed microhabitats (Hager 2000) and is a slow runner whereas *S. undulatus* and *A. inornata* may be less vulnerable to predation due to association with vegetated microhabitat (*S. undulatus,* Hager 2000) or fast running speed (*A. inornata,* Punzo 2007). For body shape, all three species have evolved longer legs and broader heads at White Sands, which could be associated with running on sand and changes in diet (Luke 1989; Anderson et al. 2008). However, the magnitude of morphometric changes vary across species. *A. inornata* is the only species that differed in body size. Additionally, white sand *H. maculata* and *A. inornata* are entirely distinct from their dark soil counterparts in body shape, whereas *S. undulatus* shows distinct but overlapping patterns of body shape. Overall patterns of morphological selection cannot explain differences in genetic clustering among species. Although *H. maculata,* the species with the most dramatic changes in color, shows the most genetic clustering, the species with the most dramatic changes in body size and shape, *A. inornata*, actually exhibits the weakest signal of genetic isolation at White Sands. Selection thus appears to be both strong and multifarious at White Sands but it cannot explain observed differences between species in their progress toward speciation.

Second, traits that have pleiotropic effects on both ecology and mating can promote ecological speciation (Maynard Smith 1966; Rice 1984; Rice and Hostert 1993; Dieckmann and Doebeli 1999; Kondrashov and Kondrashov 1999; Schluter 2001; Via 2001; Kirkpatrick and Ravigne 2002; Gavrilets 2004). At White Sands, color can potentially serve as a *magic trait* in all three species. In white sand lizards, selection on dorsal coloration for crypsis can have a by-product effect on sexual signaling. Because melanin underlies all other cell layers in the dermal chromatophore unit (Bagnara and Hadley 1973), changes in melanin production can also change color patches that appear blue (due to iridophore reflectance) or orange (due to xanthophore pigments). All three white sand species have color patches used for sexual signaling, and in all three species these patches differ in color between white sand and desert scrub populations (Robertson and Rosenblum 2009). Thus, melanin production could mechanistically link natural and sexual selection for White Sands lizards. However, the genetic architecture of adaptation differs across species in important ways. A combination of association studies and functional assays have established that single amino acid replacements in a gene of large effect, the Melanocortin-1 receptor gene (*Mc1r*), are responsible for blanched phenotypes in white sand *A. inornata* and *S. undulatus* (Rosenblum et al. 2004, 2010). *Mc1r* also may play a role in *H. maculata* but the functional mechanism has yet to be determined (Rosenblum et al. 2010). Although the same gene is implicated in evolution of melanin production in at least two of the three White Sand lizards, there are differences among species that may make this trait more or less "magic." First, the functional details of *Mc1r* disruption differ across species: the *Mc1r* mutation leading

to blanched coloration is recessive in *A. inornata* but dominant in *S. undulatus* (Rosenblum et al. 2010). Differences in dominance should affect the visibility of the blanched allele to selection and the permitted direction of migration, both important aspects of ecological speciation models (Hendry 2004). Second, the importance of color for sexual selection may differ across species. For example iguanian lizards (like *S. udulatus* and *H. maculata*) tend to be more territorial and more visually oriented in their mating displays than teiid lizards (like *A. inornata*) (Huey and Pianka 1981). It is clear that *Mc1r* has the potential to be a magic gene in any or all of the White Sand lizards, but there is no evidence that differences in genetic architecture of color or differences in strength of sexual selection across species can explain differential progress toward speciation.

Third, geographic structure is a simple mechanism to reduce the amount of gene flow and promote divergence between populations (Endler 1977; Gavrilets et al. 2000a; Doebeli and Dieckmann 2003; Gavrilets 2004). At White Sands, all three species exhibit some *geographic structure* given the parapatric distribution of white sand and dark soil habitats. However, there are strong differences across species in degree of geographic structure. *H. maculata* is a habitat specialist with small isolated populations separated by larger areas of unsuitable habitat (Gennaro 1972; Rosenblum 2006). In particular, it has a much patchier distribution across the White Sands ecotone than either of the other two species. *S. undulatus* has a more continuous distribution, but with some geographic structure. This could be due to *S. undulatus* having relatively small home ranges and limited dispersal across populations (Haenel et al. 2003). *A. inornata*, on the other hand is continuously distributed across the white sand ecotone and exhibits no population structure across mountains that are dispersal barriers for other taxa in the Chihuahuan Desert. This species is an active forager that likely has high dispersal rates (Persons 2005). The differences in population connectivity are consistent with molecular data. We see the highest degree of population structure for *H. maculata* both within and across habitats, conflicting evidence for genetic clustering for *S. undulatus* and nearly complete panmixis for *A. inornata.* Additional evidence for the importance of geography is the status of individuals at the ecotone for nuclear markers, which are the most appropriate measure of population cohesiveness (Nosil et al. 2008). Ecotonal *H. maculata* are more often clustered with white sand individuals for nuclear genotype than *A. inornata* or *S. undulatus* (Fig. 6). Thus geographic conditions are consistent with accelerated progress toward speciation in *H. maculata*, intermediate progress in *S. undulatus* and failure to speciate in *A. inornata*. Our results are concordant with models of ecological speciation, which suggest that species with highly subdivided ranges are most likely to undergo parapatric speciation (Gavrilets et al. 2000b), and empirical results, which show an important role for geography in the formation of ecological species (e.g., Moritz et al. 2000; Rice and Hostert 1993; Grahame et al. 2006; Panova et al. 2006; Quesada et al. 2007; Nosil 2008; Seehausen et al. 2008; Berner et al. 2009; Hendry et al. 2009; Mallet et al. 2009). Of the three promoting factors, geographic context is the best candidate for explaining differential progress toward speciation in white sand lizards.

There are additional factors that could help explain differences among species. The most important to consider is the potential for differences in colonization time. Two observations suggest that differences in colonization time cannot entirely account for patterns in our data. First, all else being equal, we would expect the species with the lowest dispersal ability to be the latest colonist and thus the least diverged. However, this is an unlikely explanation given the limited dispersal ability of the most genetically diverged species *H. maculata*. Second, if differences in colonization time were important, we would expect parallel patterns of trait divergence across species, with the earliest colonist the most diverged across all data types. But conversely, we found that degree of divergence across habitats varies depending on what trait is considered (Fig. 5). Future work could consider whether other factors (e.g., cost of choosiness [Bolnick 2004; Bürger et al. 2006], selection against immigrants [Gavrilets et al. 2000b; Hendry et al. 2001; Bolnick 2004; Hendry 2004; Nosil 2008], reinforcement [Servedio and Noor 2003; Bolnick and Fitzpatrick 2007; Ortiz-Barrientos et al. 2009]) can help explain differential progress toward speciation in this system. Further direct measures of reproductive isolation between habitats will be necessary to fully address possible mechanisms of ecological speciation at White Sands.

Our results lend strong empirical support for the interaction between geography and natural selection in ecological speciation (Sobel et al. 2010). For example, in studies of ecological speciation in lake-stream pairs of threespine stickleback, differential progress toward speciation was attributed to differences in the strength of selection and the geographic patterns of dispersal (Berner et al. 2009; see also Hendry et al. 2009). The interaction between natural selection and geographic structure may influence speciation across a broad range of taxonomic groups (e.g., *Drosophila*: Rice and Hostert 2003; molluscs: Panova et al. 2006; walking sticks: Nosil 2008; cichlids: Seehausen et al. 2008; stickleback fish: Berner et al. 2009). One could argue that neither geography nor selection alone are typically enough to promote the formation of new species; it is only when geography and selection act in concert that speciation often proceeds to completion.

CONCLUSION

The three species of white sand lizards illustrate the value of having a "same same but different" perspective in evolutionary biology. The blanched forms share a set of essential factors that set the stage for ecological speciation—and yet the species have

made unequal progress toward completing ecological speciation. We argue that these differences shed light on the factors that promote ecological speciation. The importance of geography has often received less attention in studies of ecological speciation, but we find that differences in geographic structure across species is the best explanation for unequal progress toward speciation by selection. We suggest that evaluating cases from the natural world that exhibit this characteristic of "same same but different" can shed the most light on ecological speciation.

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Supporting Information

The following supporting information is available for this article:

Figure S1. Plot of loadings for PC1 and PC2 on lizard coloration. There is one arrow for each measured wavelength. Each arrow represents the loadings for PC1 (x) and PC2 (y) at that wavelength. Arrows are colored from short wavelengths (~300 nm, white) to long wavelengths (~700 nm, black).

Table S1. Sample sizes by habitat for each data set.

Table S2. Results of discriminant function analyses of lizards from white sands and dark soil populations. The numbers in the table represent the proportion of individuals correctly classified to their own population.

Table S3. Loadings for PCA analysis on body shape. Variables represent residuals from body size.

Supporting Information may be found in the online version of this article.