Convergent Evolution and Divergent Selection: Lizards at the White Sands Ecotone

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ABSTRACT: Ecological transition zones, where organismal phenotypes result from a delicate balance between selection and migration, highlight the interplay of local adaptation and gene flow. Here, I study the response of an entire species assemblage to natural selection across a common ecotone. Three lizard species, distributed along a dramatic environmental gradient in substrate color, display convergent adaptation of blanched coloration on the gypsum dunes of White Sands National Monument. I investigate the role of gene flow in modulating phenotypic response to selection by quantifying color variation and genetic variation across the ecotone. I find species differences in degree of background matching and in genetic connectivity of populations across the ecotone. Differences among species in phenotypic response to selection scale precisely to levels of genetic isolation. Species with higher levels of gene flow across the ecotone exhibit less dramatic responses to selection. Results also reveal a strong signal of ecologically mediated divergence for White Sands lizards. For all species, phenotypic variation is better explained by habitat similarity than genetic similarity. Convergent evolution of blanched coloration at White Sands clearly reflects the action of strong divergent selection; however, adaptive response appears to be modulated by gene flow and demographic history and can be predicted by divergence-with-gene-flow models.

Keywords: color variation, local adaptation, gene flow, *Holbrookia maculata*, *Sceloporus undulatus*, *Aspidoscelis inornata*.

Ecological transition zones provide a dynamic opportunity to understand the balance between gene flow and adaptive evolution in natural systems. Depending on the strength of natural selection and rates of migration, gene flow may hinder local adaptation by homogenizing populations subject to different selection pressures (Slatkin 1987; Kirkpatrick and Barton 1997; Storfer et al. 1999; Lenormand 2002). Alternatively, divergent selection may overwhelm even substantial gene flow and lead to population differentiation (Turrelli et al. 2001; Doebeli and Dieckmann 2003). Population-level divergence in the face of countervailing gene flow has been documented in diverse taxa (e.g., plants [Pressoir and Berthaud 2004], flies [Michalak et al. 2001], fish [Saint-Laurent et al. 2003], lizards [Schneider et al. 1999], and birds [Smith et al. 2001]). Although these studies have been focused primarily at the population level, recent work also suggests a role for ecological divergence in reproductive isolation and speciation (Greenberg et al. 2003; McKinnon et al. 2004).

Most empirical studies of divergent selection across ecological transition zones have focused on single species (Smith et al. 1997; Ogden and Thorpe 2002). However, different species may exhibit different phenotypic responses to the same environmental gradient, especially if the relationship between selection and gene flow varies among species. When exposed to selection across an ecotone, theoretical models predict that phenotypic divergence should scale with genetic isolation (Endler 1977; Orr and Smith 1998). For example, species with lower levels of gene flow between divergent habitats (i.e., low intrinsic dispersal capability or patchy population distribution) are expected to exhibit greater phenotypic differentiation. Studying multiple species distributed along the same environmental gradient therefore provides a comparative framework to test the hypothesis that species with reduced population connectivity exhibit stronger phenotypic responses to divergent selection.

The White Sands National Monument of southern New Mexico provides a unique setting in which to examine the response of multiple species to natural selection across a common environmental gradient. White Sands is a distinctive landscape of stark white hydrous calcium sulfate (gypsum) dunes. The 275 square miles of gypsum contrast dramatically with the dark colors of adjacent substrate. The deposition of white substrate represents a geologically

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recent change in selective environment. The Tularosa Basin was engulfed by an inland lake at the last glacial maximum, and estimates suggest that much of the dune sedimentation is as young as 2,000 years old (S. Fryberger, unpublished manuscript). Therefore, any evolutionary response in local fauna to this post-Pleistocene formation has occurred relatively rapidly. There are no physical barriers separating the white sands from the surrounding dark soils, and many species have continuous distributions across the ecotone. Therefore, gene flow between different substrate environments may modulate organismal response to natural selection at White Sands.

There has been dramatic convergence in dorsal color morphology by all of the lizard species that inhabit White Sands (fig. 1). *Holbrookia maculata* (common lesser earless lizard), *Sceloporus undulatus* (eastern fence lizard), and *Aspidoscelis inornata* (little striped whiptail, formerly *Cnemidophorus inornatus*; Reeder et al. 2002) exhibit blanched forms on the gypsum dunes and dark forms in the surrounding habitat matrix and the rest of their ranges (Smith 1943; Lowe and Norris 1956; Dixon 1967; Hager 2001*b*). Breeding experiments with *H. maculata* and *S. undulatus* (Rosenblum 2005) and candidate gene studies with *A. inornata* (Rosenblum et al. 2004) provide evidence that dorsal color variation in this system has a genetic basis and is thus visible to natural selection.

The blanched coloration of lizards at White Sands is hypothesized to be an adaptation for crypsis. The importance of substrate matching in diminishing visibility from avian predators has been demonstrated for a variety of taxa (Dice 1947; Kiltie 1992; Reed and Janzen 1999). Small diurnal lizards at White Sands likely receive similar benefits from cryptic coloration. For example, studies in both lizards (Luke 1989) and small mammals (Kaufman 1973) have documented the importance of background matching for avoiding predation by the loggerhead shrike, *Lanius ludovicianus*, a known predator of lizards at White Sands (Reid and Fulbright 1981; E. B. Rosenblum, personal observation). The available evidence is also inconsistent with the alternative hypothesis that light coloration at White Sands is a thermoregulatory adaptation. A number of studies have demonstrated that more melanic animals can heat faster and obtain higher body temperatures than less melanic conspecifics (Pearson 1977; Forsman 1995). Light coloration may therefore serve to slow or limit heat gain (Benson 1933). However, ambient and substrate temperatures at White Sands are actually lower than in surrounding dark soil habitats (Hager 2000; E. B. Rosenblum, unpublished data), so selection pressure to reduce heat loads would not be predicted on the gypsum dunes.

If the relationship between selection and migration is important at White Sands, the effect of divergent selection may be modulated by ecological and distributional differences among species. Observational data indicate that *S. undulatus* and *A. inornata* are continuously distributed across the White Sands ecotone while *H. maculata* is more patchily distributed at the ecotone and in surrounding dark soil habitat (Dixon 1967; E. B. Rosenblum, personal observation). Differences in vagility, microhabitat association, and foraging mode among the three species (Dixon and Medica 1966; Dixon 1967; Jones and Droge 1980; Degenhardt et al. 1996; Hager 2001*a*) also ultimately affect the genetic connectivity of populations. Because of its patchy distribution, gene flow between dark soil and white sand habitats is likely reduced for *H. maculata* relative to *S. undulatus* and *A. inornata*. Divergence-with-gene-flow models therefore predict that phenotypic differentiation among populations should be greater for *H. maculata*.

In this study, I ask how the complete lizard fauna at White Sands has responded to natural selection across a common ecotone. To understand phenotypic response to selection, I quantify patterns of lizard color variation in three substrate environments (i.e., white sand, dark soil, ecotone). To understand current and historical levels of population connectivity, I evaluate patterns of genetic variation across the ecotone. I then integrate phenotypic and molecular data to examine the interaction between gene flow and natural selection and to explore the evidence for ecologically mediated divergence at White Sands.

Methods

Sampling

Collection locality information is presented in figure 1 and table 1. For each species, three habitat categories were sampled in the Chihuahuan Desert of southern New Mexico. First, "white sand" habitat is defined as pure gypsum substrate. This landscape consists of high barren dunes separated by low interdune areas dominated by rabbitbrush (*Chrysothamnus pulchellus*), yucca (*Yucca elata*), Mormon tea (*Ephedra torrreyana*), sand verbena (*Phyla incise*), and a variety of grasses (*Oryzopsis* sp., *Sporobolus* sp.). Two dune localities were sampled for all species (populations A and B). Second, "dark soil" refers to habitat with brown adobe substrate, typical of the Chihuahuan Desert region. These grasslands and scrublands are dominated by yucca (*Yucca elata)*, mesquite (*Prosopis glandulosa*), cactus (*Opuntia* sp.), and grama grasses (*Bouteloua* sp.). Six dark soil localities were sampled to capture variation both within the Tularosa Basin (populations G–J) and just west of the San Andres Mountains (populations E and F). Because of differences in habitat requirements, not all species were sampled in all dark soil localities; however, sampling for each species occurred over the same spatial scale. Third, "ecotone" habitat is defined as areas

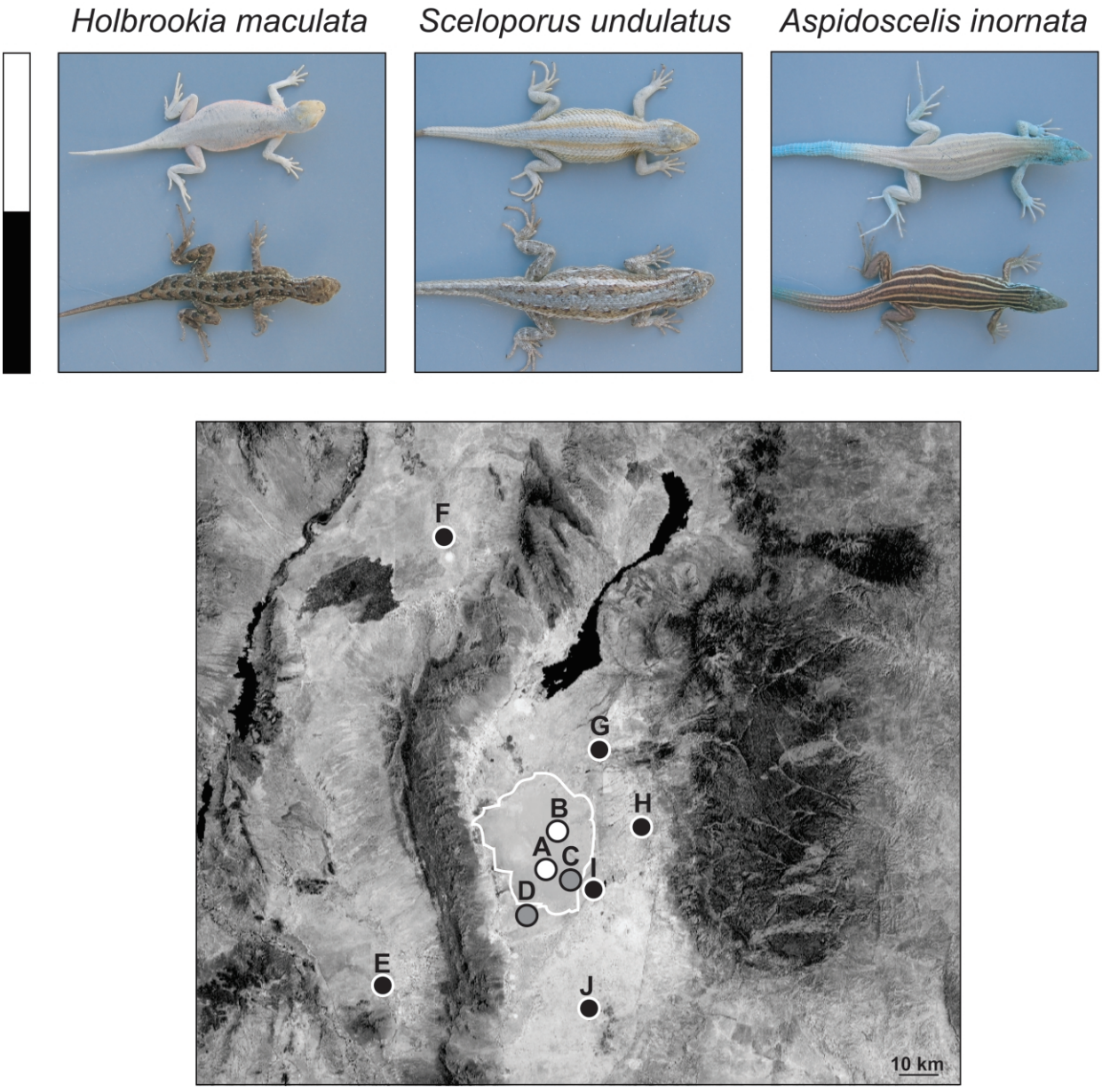


Figure 1: Aerial photograph of the Tularosa Basin of southern New Mexico with photographs of lizards from different substrate color environments. The blanched color morphs found at White Sands (indicated by the white bar) are compared to the wild-type color morphs found throughout the rest of the species' ranges (indicated by the black bar). On the map, black, white, and gray circles represent collecting localities with dark soil, white sand, and ecotonal habitats, respectively. Population letter designations are used throughout and correspond to the following geographic localities: *A*, Alkali Flats, White Sands National Monument; *B*, Range Road 10, White Sands Missile Range; *C*, Big Dunes, White Sands National Monument; *D*, Observatory, White Sands Missile Range; *E*, Experimental Range, Jornada Long-Term Ecological Research Station; *F*, Mockingbird Pass, White Sands Missile Range; *G*, Rita Site, White Sands Missile Range; *H*, Taylor Draw, Three Rivers; *I*, Visitor's Center, White Sands National Monument; *J*, Otero Mesa, Fort Bliss.

of transition from dark soil to white sand substrate. These narrow bands of habitat are found either at the margin of the large dune system or at small satellite dunes just removed from the primary dune field. The movement of the dunes over time and the temporal layering of gypsum deposition cause greater variability in substrate color in ecotonal areas. These transition zones are extremely restricted, and the boundary of the white sand is abrupt and

Table 1: Taxa surveyed and sampling design

Species	White sand	Ecotone	Dark soil
Holbrookia maculata (lesser earless lizard)	All white sand populations (19); populations A (12), B (7)	All ecotone populations (12); populations C (10), D (2)	All dark soil populations (34); populations E (10), F (10), H (10), J (4)
Sceloporus undulatus (eastern fence lizard)	All white sand populations (20); populations A (13), B (7)	All ecotone populations (20); populations C (11), D (9)	All dark soil populations (25); populations E (8), F (2), G (9), I (6)
Aspidoscelis inornata (little striped whiptail)	All white sand populations (14); populations A (11), B (3)	All ecotone populations (14); populations C (12), D (2)	All dark soil populations (13); populations E (10), F (2), G (1)

Note: Population letter designations refer to map in fig. 1. Collection locality details are provided in table A1 in the online edition. Number of lizards sampled from a population is given in parentheses.

easily defined. Lizards caught at the ecotone were generally only meters from the edge of the dune field. Two ecotone localities were sampled for all species (populations C and D).

Distances from dark soil to white sand populations varied among species. Dark soil populations of both *Sceloporus undulatus* and *Aspidoscelis inornata* occur essentially parapatric to the gypsum dune fields, while the closest dark soil population of *Holbrookia maculata* was found farther away. Dark soil populations were sampled 35–64 km away from the white sands for *H. maculata*, 10–85 km away for *S. undulatus*, and 10–64 km away for *A. inornata*. The effect of *H. maculata*'s patchy distribution on analyses is discussed below. For each species, 12–34 adult individuals per habitat type were sampled. When multiple localities were sampled per habitat, roughly 10 individuals per locality were collected, although samples are smaller for several localities. Samples were generally made up of a balanced number of males and females. A subset of lizards was kept as vouchers and deposited in the Museum of Vertebrate Zoology at the University of California, Berkeley. All other lizards were released at collection sites once spectrophotometric data and tissue samples were taken. Sample numbers and locality information for voucher and tissue accessions are provided in table A1, available in the online edition of the *American Naturalist*. Genotypic and phenotypic data were collected for all individuals except for eight *H. maculata* that were included in the molecular analyses but for which no color measurements were made.

Quantifying Color Variation

Because environmental effects on reptile coloration are well documented (Waring 1963; Norris 1965; Sherbrooke et al. 1994; Nery and Castrucci 1997), it is essential to understand the capacity for color change in the focal species. Prior experiments have evaluated the potential for both physiological (rapid) and ontogenetic color change in *H. maculata*, *S. undulatus*, and *A. inornata*. These studies found that color change within morphs when held at different temperatures and on different substrate colors was minimal compared to the dramatic differences between morphs (Smith 1943; Bundy 1955; Lowe and Norris 1956; Rosenblum 2005). Common-garden breeding experiments (Rosenblum 2005) also indicate that environmental effects cannot explain the blanched coloration of lizards at White Sands. To control for the slight darkening response that can occur for lizards at colder temperatures, all color recordings were obtained at approximately 30°C. Lizards were held on an intermediate substrate before making color measurements.

Lizard dorsal body coloration was characterized by taking spectrophotometric readings along the dorsal midline. Spectral readings were highly consistent within individuals. However, to account for any intra-individual measurement variation, three readings along the dorsal midline were averaged: between the front limbs, at the center of the body, and between the hind limbs. The color of four to six substrate samples from each habitat type was also quantified.

Spectrophotometric readings were taken with an Ocean Optics USB 2000 spectrophotometer with a dual deuterium/tungsten halogen light source. A custom-made probe holder was used to orient the probe at 45° and 1 cm away from the dorsal body surface. Each spectral reading was taken in reference to a white standard and consisted of percent transmission recordings at 0.3-nm intervals. The mean of every 10 points along the spectra was taken to create 3-nm bins, reducing the number of variables from ~2,000 to ~200. Readings from 300 to 700 nm, the spectral range visible to squamates and their avian predators (Ben-

nett and Cuthill 1994; Ellingson et al. 1995; Fleishman et al. 1997; Cuthill et al. 1999), were used for analysis.

Although there are multiple analytical methods available for spectrophotometric data (Endler 1990; Grill and Rush 2000; Thorpe 2002), there are several reasons why principal components analysis (PCA) is preferred for the data set presented here. First, PCA is appropriate when little is known about the visual system of focal species and their predators because no assumptions are made about how organisms perceive different segments of the spectrum. Second, this method provides information about specific aspects of coloration. Color is composed of three components: brightness describes light transmission intensity, chroma describes color purity, and hue describes the wavelength of maximum slope (Endler 1990). Empirical findings show that principal component 1 (PC1) corresponds to brightness while PC2 and PC3 generally contain information about chroma and hue (Grill and Rush 2000). I corroborated this finding in my data set by comparing results from PCA with a more direct estimation of brightness: area under the spectral curve (AUC). In the analyses of dorsal coloration presented here, PC1 explains over 84% of the variance in all data sets and returns results nearly identical to calculations based on AUC. Therefore, PC1 scores are an accurate quantification of the brightness aspect of color and describe most of the color variation observed in Tularosa Basin lizards. Finally, PCA allows for visualization of data in multivariate color space that univariate techniques such as AUC do not.

Three PCAs were performed on spectrophotometric data. First, substrate comparisons were used to document color environment in white sand, dark soil, and ecotonal habitats. Second, comparisons among species were used to determine whether *H. maculata*, *S. undulatus*, and *A. inornata* exhibit different degrees of local adaptation to the white sand environment. For interspecific comparisons, only white sand and dark soil individuals were included. Third, comparisons within species were used to document patterns of phenotypic variation across the ecotone. For intraspecific comparisons, individuals from all three habitats were included. For each analysis, principal component factor scores (FS1, FS2, FS3) were analyzed with a MANOVA design. All analyses were performed with individuals grouped by habitat (white sand, dark soil, and ecotone). If a MANOVA was significant, univariate tests were performed for FS1, FS2, and FS3 to determine which aspect of color explained most of the observed differences among lizards. If an ANOVA on FS1, FS2, or FS3 was significant, post hoc Tukey HSD tests were used to determine which groups occupied significantly different regions of color space. Finally, to better understand relative levels of local adaptation among species, the average phenotypic distance along PC1 between white sand and dark soil animals was calculated for each species. Statistical analyses were executed in Statistica (StatSoft).

Characterizing Genetic Variation

To determine levels of historical isolation between white sand and dark soil populations, the mitochondrial ND4 gene and associated tRNAs were sequenced. Primers modified from Arevalo et al. (1994) were used for polymerase chain reaction and sequencing (ND4: 5′-CAC CTA TGA CTA CCA AAA GCT CAT GTA GAA GC-3′ and LEU: 5′-CAT TAC TTT TAC TTG GAT TTG CAC CA-3′). Forward and reverse sequencing were conducted on an ABI 3730 (Applied Biosystems). Sequences were aligned and edited in Sequencher (GeneCodes). For *H. maculata*, 812 bp were sequenced for 65 individuals, for *S. undulatus* 813 bp for 65 individuals, and for *A. inornata* 793 bp for 41 individuals. Higher levels of ND4 variation were found for *H. maculata* and *S. undulatus* than for *A. inornata*, but variation was observed for all species (*H. maculata*, 51 variable sites and 24 haplotypes; *S. undulatus*, 55 variable sites and 17 haplotypes; *A. inornata*, 17 variable sites and 14 haplotypes). All mitochondrial sequences have been deposited in Genbank (accession nos. DQ113953–DQ114121).

Intraspecific relationships were reconstructed using a maximum likelihood algorithm implemented in PAUP* 4.0 (Swofford 2003). Appropriate models of evolution and likelihood parameters were determined with MODELTEST (Posada and Crandall 1998). The HKY+G model was used for *H. maculata* and *A. inornata*, and the TrN+I+G was used for *S. undulatus*. Nodal support was determined by bootstrap analysis. For *H. maculata* and *S. undulatus*, conspecific individuals from Arizona were used as outgroups for tree rooting, and for *A. inornata* the closely related *Aspidoscelis uniparens* was used as an outgroup.

Levels of population subdivision within species were computed using Tamura and Nei molecular distances (Tamura and Nei 1993) implemented in Arlequin (Schneider et al. 2000). A hierarchical analysis of molecular variance (AMOVA) was conducted with populations nested within the three habitat types. Confidence intervals for the global Φ_{ST} from AMOVA were estimated by permuting haplotypes among populations and among habitat groups. To explore the effect of sampling design on inferences of population subdivision, Φ_{ST} was also calculated with a reduced sample in *S. undulatus* and *A. inornata*. Because dark soil populations of *H. maculata* were not found parapatric to White Sands, analyses excluding population I (fig. 1) for *S. undulatus* and *A. inornata* provide a more direct comparison of levels of genetic differentiation among species. Pairwise population comparisons were also conducted. For all Φ_{ST} analyses, 1,000 permutations were performed to determine statistical significance.

To understand whether patterns revealed by Φ_{ST} are explained by ongoing or historical population dynamics, two additional analyses were performed. First, levels of gene flow between divergent color morphs were estimated using the program IM (Hey and Nielsen 2004). IM uses a Markov chain Monte Carlo method to estimate the posterior probability of *m*, the migration rate between two populations per gene per generation scaled by mutation rate. With a known mutation rate, *m* can be used to obtain the population migration rate, Nm. Here I ask whether migration rates between populations inhabiting divergent habitats are significantly greater than 0; all results are presented as *m*, not Nm. For each species, migration rates were estimated between white sand populations and the geographically nearest dark soil population with adequate sample size (population H for *H. maculata*, population I for *S. undulatus*, population E for *A. inornata*; fig. 1). Maximum times for population splitting were set based on the known geological history of White Sands. To explore the effects of unequal sample sizes on inferences using IM, analyses were also conducted with a subsample of white sand individuals. Runs using full and reduced data sets returned similar point estimates for migration rates, so results from full analyses are presented here. Because IM simultaneously estimates migration rate and divergence time, it is difficult to differentiate current and historical migration, especially for populations with shallow divergence times. Therefore, to further explore the possibility that recent common ancestry (i.e., population expansion) could explain observed genetic patterns, historical population size changes were estimated using FLUCTUATE (ver. 1.4; Kuhner et al. 1998). FLUCTUATE uses Metropolis-Hastings sampling to calculate *g*, population growth rate per generation scaled by mutation rate. For each species, population growth was estimated using all available samples. For *S. undulatus* and *H. maculata*, the species for which the assumption of panmixis is likely violated at this spatial scale, population growth was also estimated using only white sand samples. Significance levels were the same regardless of sampling scheme, so results with all samples are reported here. For IM and FLUCTUATE analyses, likelihood ratio tests were used to determine whether $m = 0$ and $g = 0$ could be rejected, and statistical significance was approximated using the χ^2 distribution.

Comparing Phenotypic and Genotypic Variation

To determine whether patterns of phenotypic and genotypic variation were concordant across the White Sands ecotone, I conducted hypothesis-driven matrix correspondence tests (MCTs). MCTs can be a powerful tool in understanding the association among distance metrics for a variety of data types (Smouse et al. 1986; Thorpe et al. 1996; Storz 2002). In the simplest case, the association between two matrices (i.e., dependent and independent variables) can be evaluated using randomization procedures. If there are multiple potentially explanatory variables, partial MCTs can be used to test the association between a dependent variable and several independent variables simultaneously. In this case, partial regressions are conducted to understand the correlation between two matrices while controlling for the effect of a third matrix. Because partial MCTs may be misleading when spatial autocorrelations of the dependent variables are important (Raufaste and Rousset 2001; Castellano and Balletto 2002; Rousset 2002)—a problem not encountered with pairwise MCTs—it is informative to compare results of both pairwise and partial tests.

For each species, three matrices were generated based on pairwise population comparisons. The first matrix described phenotypic variation in dorsal brightness and was generated using absolute values of linear distances along PC1. The second matrix described genetic differentiation within species and was composed of pairwise estimates of linearized F_{ST}. The third matrix, the hypothesis matrix, established expectations for the relationship between phenotypic and genotypic variation among different habitat types. Phenotypic distance was predicted to be highest for comparisons between the most dissimilar habitats (white sand vs. dark soil), lowest for within-habitat comparisons, and intermediate for comparisons involving ecotonal habitat.

Both partial and pairwise MCTs were conducted for each species. Partial MCTs were used to test for significant correspondence between the phenotypic matrix and the hypothesis matrix while controlling for the genotypic matrix. This method effectively "removes" the component of population-level phenotypic divergence that would be expected because of observed levels of genetic subdivision. Pairwise MCTs were used to test for significant correspondence between all pairwise combinations of the phenotypic, genotypic, and hypothesis matrices. Statistical significance for all MCTs was assessed with permutation tests, and Bonferroni corrections were used to adjust significance levels for multiple comparisons conducted with pairwise MCTs. Results were consistent whether analyses were conducted with partial or pairwise tests.

In general, phenotypic and genotypic matrices were constructed from data from identical sets of individuals. However, only genotypic data were available for eight *H. maculata* from dark soil populations; the phenotypic matrix for this population therefore contained fewer individuals. To ensure that results were robust to the inclusion of populations with small sample sizes, MCTs were conducted both with and without the several populations with fewer

than five individuals. Results of analyses were the same regardless of whether these populations were included; results of tests with all data points are presented here.

Results

Adaptive Phenotypic Variation

Substrates from dark soil, white sand, and ecotonal habitats were all statistically distinguishable in color space (fig. 2; table 2). The brightness aspect of color (PC1) explained 98% of the variation in substrate color. The white sand of the gypsum dunes was significantly brighter than any other substrate measured from the surrounding region. As expected, ecotonal substrates were intermediate in brightness between white sand and dark soil and showed a larger variance in brightness. PC2 explained less than 2% of the variation in color, but differences among substrates were also observed on this axis, with ecotonal substrates appearing unique. No differences among groups were observed on PC3.

Interspecific comparisons revealed convergent evolution of blanched coloration but also species differences in degree of background matching (fig. 3; table 2). Similar to the partitioning of variation in substrate color, brightness explained the bulk of variation in lizard coloration (87% for interspecific comparison). In the dark soil habitat, all three species exhibited dark color and occupied similar regions of color space. Dark soil populations of *Holbrookia maculata* were brighter than those of *Sceloporus undulatus*, but otherwise dark soil populations were similar in color across species. In the white sand habitat, light color morphs of all three species were significantly brighter than conspecifics in dark soil habitat. However, the three species showed varying degrees of background matching in the white sand environment. The blanched morph of *H. maculata* was significantly brighter than that of either *S. undulatus* or *Aspidoscelis inornata*. Although *H. maculata* was brightest in both white sand and dark soil habitats, the greatest phenotypic distance between different color morphs was also observed in this species (fig. 3). The difference between mean factor scores on PC1 for white sand and dark soil animals was greater for *H. maculata* (19.46, SE = 1.48) than for *S. undulatus* (14.61, SE = 1.47) or *A. inornata* (13.83, SE = 1.89). A significant difference in dorsal coloration was also observed on PC2 (accounting for 8% of observed variation) and was explained primarily by differences among species. Differences on PC3 explained 4% of variation and did not elucidate any obvious intra- or interspecific patterns.

Intraspecific comparisons revealed differences among species in phenotypic patterns observed at the ecotone (fig. 4; table 2). Again, PC1 (brightness) explained the majority of observed variation (more than 84% in all comparisons). For *H. maculata*, ecotonal animals were statistically indistinguishable in dorsal brightness from white sand animals. For *S. undulatus*, ecotonal samples comprised a third, statistically distinguishable group intermediate in brightness between those from dark soil and white sand habitats. In *A. inornata*, ecotonal populations were also intermediate in brightness but were statistically indistinguishable from dark soil conspecifics. Differences among morphs along PC2 and PC3 were detectable only in *S. undulatus* and explained only a small proportion of total variation in color. For *S. undulatus* PC2 (9% of total variation), ecotonal animals were grouped with dark soil animals to the exclusion of white sand animals, and for PC3 (<2% of total variation), dark soil and white sand animals were grouped together to the exclusion of ecotonal animals. Because PC1 explained the vast majority of the color variation in all analyses and because it was not clear what the relative contributions of chroma and hue were to PC2 and PC3, subsequent discussion focuses primarily on PC1 (brightness).

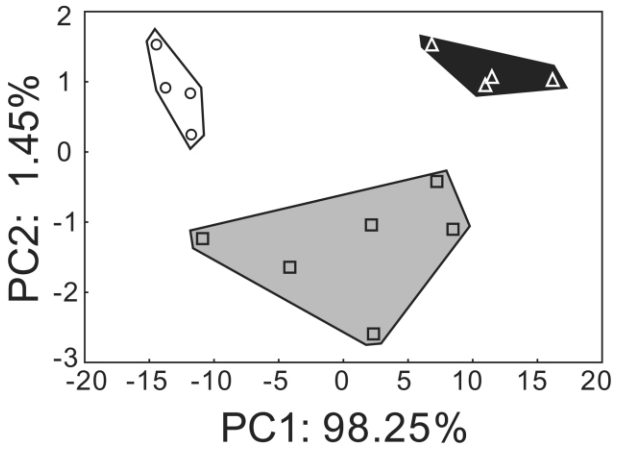


Figure 2: Substrate color. For principal components analysis (PCA) of spectrophotometric data, PC1 (representing brightness) is plotted against PC2. Values on each axis indicate the percentage of variation explained by that factor. Black, white, and gray polygons enclose substrate measurements from dark soil, white sand, and ecotone habitats, respectively.

Genetic Structure

Patterns of mitochondrial geographic structure differed among species (fig. 5). *Holbrookia maculata* was the most highly structured at this spatial scale, and clades generally represented geographically defined populations. A major phylogeographic split was observed across the San Andres Mountains; populations east of the San Andres Mountains were clearly differentiated from populations E and F west

Table 2: Variation in substrate coloration and lizard dorsal coloration among different habitats: results from principal components analysis (PCA) and MANOVA

Species	Variation explained by PC axis (%)	df	*F*	*P*	Distinguishable groups[a]
Substrate		6, 18	14.42	<.00001	
PC1 (brightness)	**98.25**	**2, 11**	**20.77**	**.0002**	**(DS) (E) (WS)**
PC2	1.45	2, 11	27.67	.0001	(DS + WS) (E)
PC3	.26	2, 11	.17	.845	NS
All species		15, 301	39.01	<.000001	
PC1 (brightness)	**86.75**	**5, 111**	**76.08**	**<.00001**	**See text**
PC2	7.78	5, 111	7.34	.00001	See text
PC3	4.12	5, 111	42.02	.0001	See text
Holbrookia maculata		6, 104	26.55	<.000001	
PC1 (brightness)	**89.47**	**2, 54**	**100.66**	**<.00001**	**(DS) (E + WS)**
PC2	7.36	2, 54	2.13	.128	NS
PC3	2.02	2, 54	1.00	.376	NS
Sceloporus undulatus		6, 120	21.68	<.000001	
PC1 (brightness)	**88.88**	**2, 62**	**48.19**	**<.00001**	**(DS) (E) (WS)**
PC2	8.74	2, 62	6.27	.003	(DS + E) (WS)
PC3	1.51	2, 62	3.69	.031	(DS + WS) (E)
Aspidoscelis inornata		6, 72	15.86	<.000001	
PC1 (brightness)	**84.82**	**2, 38**	**31.13**	**<.00001**	**(DS + E) (WS)**
PC2	11.30	2, 38	4.67	.015	(DS + E) (WS)
PC3	2.02	2, 38	.44	.645	NS

Note: For each analysis, results from the full MANOVA are shown first, and results from post hoc tests on PC1, PC2, and PC3 are shown below. The percentage of variation explained by each PC is given. Results for brightness (PC1), which explain most of observed variance in color, appear in bold. Degrees of freedom, *F* statistics, and *P* values from ANOVA on PCA factor scores are provided.

[a] Groups distinguishable with post hoc Tukey HSD tests are enclosed in separate parentheses; "NS" indicates an ANOVA with no significant differences among groups. Habitat group abbreviations: DS = dark soil; WS = white sand; E = ecotone.

of the San Andres Mountains. Within the Tularosa Basin, dark soil forms were basal; however, the dark soil population H was nested within the clade containing white sand individuals. In *S. undulatus*, organisms from similar habitats did not group together, and white sand individuals did not form a monophyletic group. In this species, there were only two clades that corresponded to geographic groupings (populations F and G). Other than these two groups, animals did not tend to cluster with others from the same locality. For *A. inornata*, the tree revealed a nearly panmictic population at this spatial scale. There was no substructuring of individuals from different habitat types, and white sand animals were dispersed throughout the tree.

Patterns of population subdivision, as revealed by AMOVA, enabled the three species to be ranked by degree of genetic differentiation among populations. Global estimates of Φ_{ST} were high for *H. maculata* (0.83), intermediate for *S. undulatus* (0.54), and low for *A. inornata* (0.09). When Φ_{ST} was estimated excluding dark soil populations of *S. undulatus* and *A. inornata* adjacent to White Sands, this pattern remained the same (for *S. undulatus*, $\Phi_{ST} = 0.64$, and for *A. inornata*, $\Phi_{ST} = 0.09$). Levels of population subdivision were significantly different from 0 in *H. maculata* and *S. undulatus* but not in *A. inornata*. Results from pairwise Φ_{ST} comparisons were also consistent with the observation of less genetic differentiation among populations of *A. inornata* than in the other two species. For *H. maculata* and *S. undulatus*, 71% and 75% of populations were statistically differentiated, respectively, while for *A. inornata* only 24% of pairwise population comparisons showed significant differentiation.

Comparisons of Φ_{ST} involving ecotonal populations also suggested that levels of genetic connectivity across the ecotone varied among species. For *H. maculata*, ecotonal populations were genetically similar to white sands populations ($\Phi_{ST} = 0.01$) but were highly differentiated from dark soil populations ($\Phi_{ST} = 0.86$). For *S. undulatus*, ecotonal populations were differentiated from both white sand ($\Phi_{ST} = 0.20$) and dark soil populations ($\Phi_{ST} = 0.55$). For *A. inornata*, ecotonal populations exhibited low levels of differentiation when compared to either white sand ($\Phi_{ST} = 0.06$) or dark soil ($\Phi_{ST} = 0.07$) populations.

Estimates of migration and population growth were used to better understand the demographic processes responsible for observed patterns of population structure.

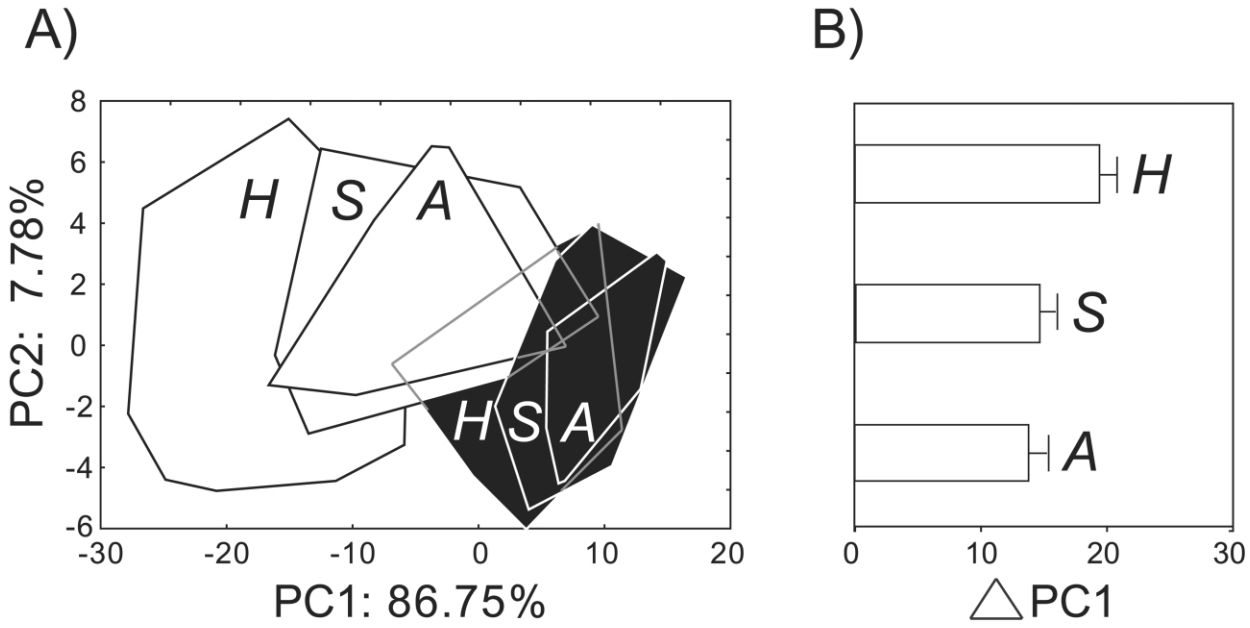


Figure 3: Interspecific comparison of dorsal coloration. Species are indicated by generic initial: *H* = *Holbrookia maculata*; *S* = *Sceloporus undulatus*; *A* = *Aspidoscelis inornata*. *A*, Black polygons enclose all data points within species from dark soil habitats and white polygons enclose all data points within species from white sand habitats. *B*, Mean difference (and standard error of the mean) between white sand and dark soil individuals along PC1 for each species.

Migration rates were measured between white sand populations and their geographically closest dark soil neighbors. For *H. maculata*, the species exhibiting the highest levels of population differentiation, no gene flow was detected between white sand animals and the nearest dark soil population (population H; fig. 1; $m_1 = 0$, likelihood ratio [LR] = 0, $P = 1$; $m_2 = 0$, LR = 0, $P = 1$). For *S. undulatus*, the species with an intermediate level of genetic structure, estimates of migration between white sand and the nearest dark soil population (population I; fig. 1) were positive and asymmetric ($m_1 = 1.62$, LR = 4.60, $P = .0320$; $m_2 = 2.60$, LR = 2.41, $P = .1206$), with higher numbers of migrants estimated moving from dark soil to white sand than the reverse ($m_2 > m_1$). Although the point estimate for m_2 was larger than that for m_1 in *S. undulatus*, only m_1 was statistically significant. Although not statistically significant, the *P* value associated with m_2 was low, and previous work has shown that likelihood ratio tests may be conservative when applied to migration estimates (Nielsen and Wakeley 2001). Therefore, it is reasonable to

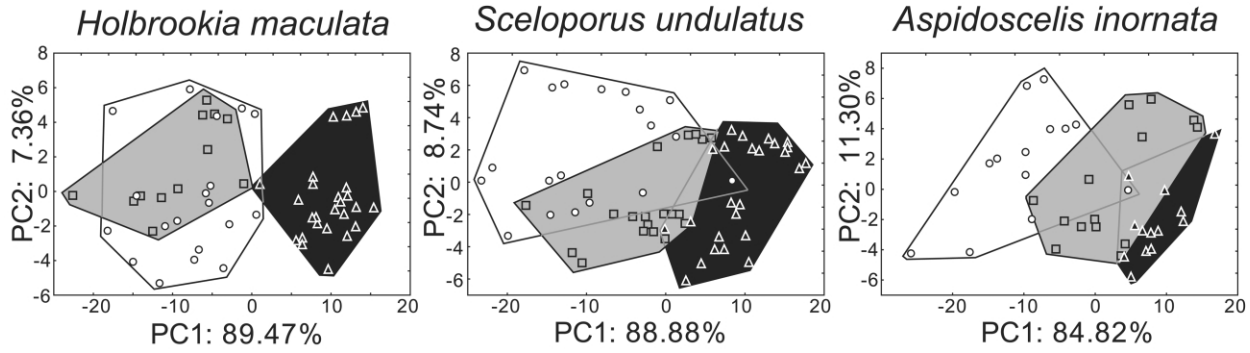
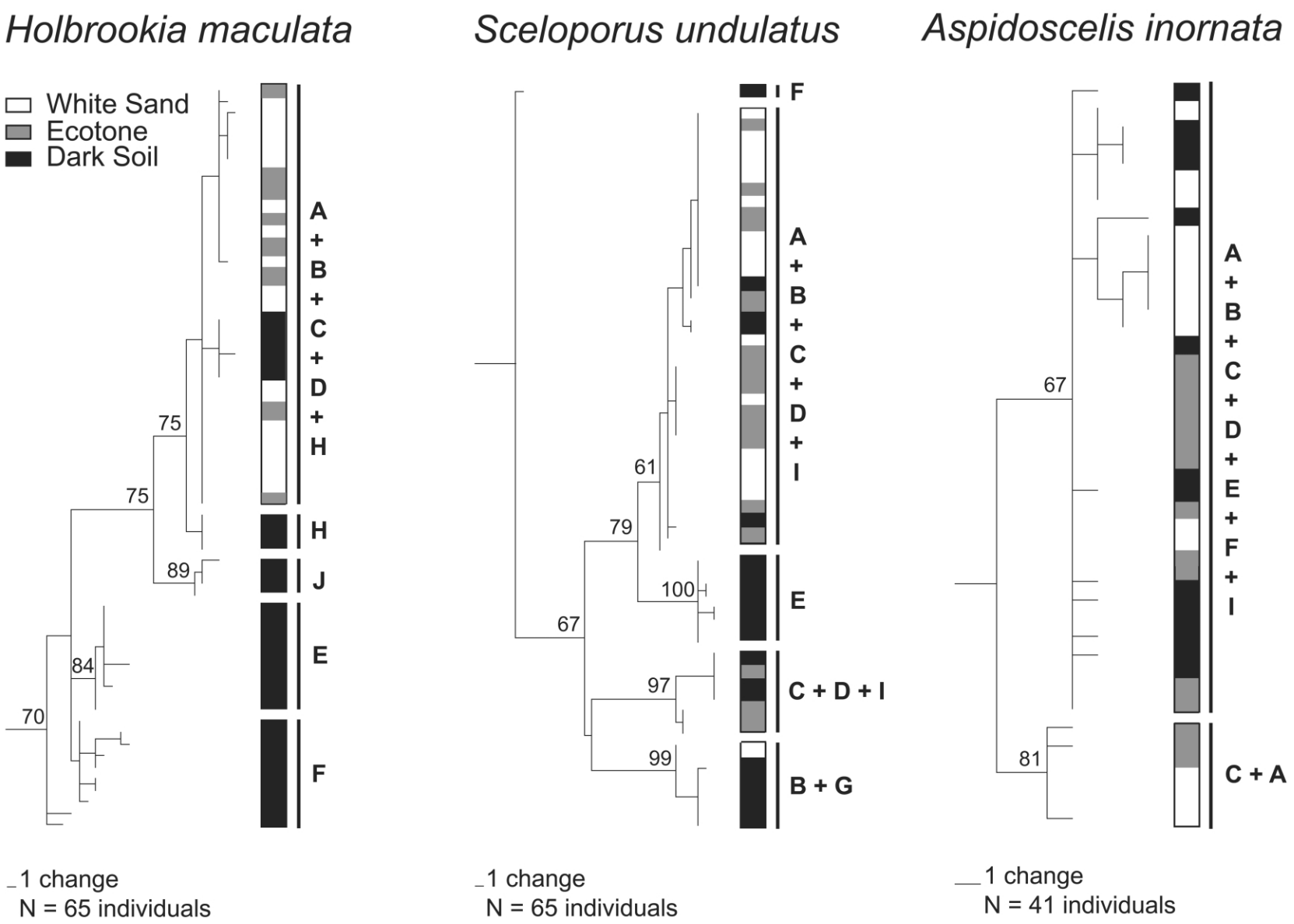


Figure 4: Intraspecific comparisons of dorsal coloration. Black, white, and gray polygons enclose data points from dark soil, white sand, and ecotone lizards, respectively.


Holbrookia maculata
White Sand
Ecotone
Dark Soil
A + B + C + D + H
H
J
E
F
75
75
89
84
70
1 change
N = 65 individuals
Sceloporus undulatus
F
A + B + C + D + I
E
C + D + I
B + G
61
79
100
67
97
99
1 change
N = 65 individuals
Aspidoscelis inornata
A + B + C + D + E + F + I
C + A
67
81
1 change
N = 41 individuals

consider scenarios involving symmetrical gene flow across the ecotone for *S. undulatus*. For *A. inornata*, the species with little genetic differentiation among populations, the point estimate for migration from white sand populations to dark soil population *E* was positive, but $m = 0$ could not be rejected ($m_1 = 3.44$, LR = 0.45, $P = .5023$; $m_2 = 0.02$, LR = 0.08, $P = .7773$). Overall, the probability of ongoing migration between divergent color morphs was low for *H. maculata*, high for *S. undulatus*, and ambiguous for *A. inornata*. To explore a possible explanation besides ongoing gene flow for the low observed Φ_{ST} in *A. inornata*, population growth rates were measured for all species. For *H. maculata* and *S. undulatus*, no significant changes in population size were detected (*H. maculata* $g = 100.27$, LR = 1.54, $P = .2137$; *S. undulatus* $g = -55.33$, LR = 1.58, $P = .2085$). However, for *A. inornata*, a strong signature of population growth was observed ($g = 1{,}301.32$, LR = 12.76, $P = .0004$), suggesting that genetic similarity among populations is likely due to historical population expansion rather than ongoing gene flow in this species.

Ecological Divergence: Comparing Phenotypic and Genetic Variation

The prediction that phenotypic divergence should be highest among dissimilar habitats and lowest among similar habitats was tested with MCTs. Genotype, phenotype, and hypothesis matrices are provided in figure B1, available in the online edition of the *American Naturalist*; results from MCTs are shown in table 3. For partial MCTs, the null hypothesis was strongly rejected for each species, indicating that there was more phenotypic variation among different habitat types than expected on the basis of observed genetic variation (linearized F_{ST}). For pairwise MCTs, correspondence between phenotypic distance and hypothesis matrices was strong and highly significant in all species, corroborating the ranking of phenotypic differentiation that was predicted from an ecological divergence model. Correlations between phenotypic and genetic distance matrices were weak and not statistically significant after correction for multiple comparisons, revealing that populations were highly divergent in color morphology without commensurate differentiation at the mitochondrial locus. There was little correlation between genetic distance and hypothesis matrices, indicating that genetic variation was not partitioned corresponding to habitat boundaries. Overall, MCTs indicated that color morphology correlated with habitat similarity but not genetic similarity for White Sands lizards.

Discussion

Divergent Selection and Gene Flow

Although all lizards inhabiting the white sand environment exhibit convergent evolution of blanched coloration, the strength and geographic pattern of phenotypic response to selection vary among species. In the white sand habitat, there are species differences in the degree of background matching to the gypsum environment (fig. 3; table 2). Most notably, *Holbrookia maculata* is significantly brighter in color and more highly substrate matched than either *Sceloporus undulatus* or *Aspidoscelis inornata*. Not only is *H. maculata* more highly background matched at White Sands than the other two species, this species shows the greatest phenotypic change between dark soil and white sand forms (fig. 3). The blanched coloration of *H. maculata* therefore represents a dramatic adaptation to the gypsum environment, not merely a tendency for this species to be brighter than *S. undulatus* and *A. inornata* on all substrates. In the ecotonal habitat, where the balance between selection and migration is expected to be the most dynamic, each species also displays a unique phenotypic pattern (fig. 4; table 2). In *H. maculata*, the dorsal color of ecotonal animals is indistinguishable from that of white sand animals. In *S. undulatus*, ecotonal individuals form a statistically distinct group, intermediate in color between white sand and dark soil forms. In *A. inornata*, ecotonal populations are indistinguishable in color from dark soil animals. Overall, the strongest phenotypic response to selection is observed in *H. maculata*, an intermediate response occurs in *S. undulatus*, and the weakest response is seen in *A. inornata*.

The strength of phenotypic response to selection across the White Sands gradient scales exactly with levels of population structure (table 4). *Holbrookia maculata*, the species exhibiting the most dramatic phenotypic response to the white sand environment, also exhibits the greatest degree of genetic differentiation among populations ($\Phi_{ST} = 0.82$). *Sceloporus undulatus*, the species showing an intermediate phenotypic response, also shows an intermediate degree of population structure ($\Phi_{ST} = 0.54$). *Aspidoscelis inornata*, the species with the weakest phenotypic response at the ecotone, is nearly panmictic across the

Figure 5: Maximum likelihood reconstructions of historical relationships within species with individuals as operational taxonomic units. Colored bars indicate the habitat from which animals were collected: black, white, and gray bars represent dark soil, white sand, and ecotonal habitats, respectively. Population letter designations refer to the locality map in figure 1. Support levels are indicated only for major nodes with bootstrap support above 50.

Table 3: Correlation coefficients and *P* values for matrix correspondence tests (MCTs)

Species and matrix correspondence comparison	Correlation	*P*
Holbrookia maculata:		
Phenotype + hypothesis given genotype	**.539**	**.007***
Phenotype + hypothesis	.518	.013*
Genotype + hypothesis	.078	.355
Phenotype + genotype	.426	.031
Sceloporus undulatus:		
Phenotype + hypothesis given genotype	**.780**	**.002***
Phenotype + hypothesis	.720	.008*
Genotype + hypothesis	.000	.182
Phenotype + genotype	.074	.355
Aspidoscelis inornata:		
Phenotype + hypothesis given genotype	**.916**	**<.001***
Phenotype + hypothesis	.860	.002*
Genotype + hypothesis	.291	.224
Phenotype + genotype	.087	.358

Note: Results from partial MCTs appear in bold, with results from pairwise MCTs below. Data matrices are provided in fig. B1 and include pairwise population comparisons of phenotypic distance (linear distance along PC1), genetic distance (linearized F_{ST}), and hypothesized divergence (based on habitat similarity).

* Significant results after Bonferroni correction for multiple comparisons.

Tularosa Basin ($\Phi_{ST} = 0.09$). Inferences about population structure were not driven by differences among species in sampling distance between white sand and dark soil populations; the rank order of Φ_{ST} was identical even when dark soil populations collected adjacent to the dunes were excluded for *S. undulatus* and *A. inornata*.

Because Φ_{ST} may be affected by dispersal rate and dispersal history, genetic similarity may result from ongoing migration or recent common ancestry (i.e., population expansion). Coalescent analyses indicate that differences in population structure among species reflect underlying differences in both colonization history and ongoing gene flow. In *H. maculata*, extremely high levels of genetic structure can be explained by fairly stable, isolated populations through time. Phylogenetic breaks are consistent with landscape features in this species, indicating a strong geographic signal to the partitioning of genetic variance. In addition, no signatures of ongoing gene flow or population size change were recovered for *H. maculata*. Reduced contact across the White Sands ecotone for *H. maculata* may therefore be due to lower intrinsic dispersal ability or a more patchy spatial distribution of populations. For *S. undulatus*, intermediate levels of population structure can be explained by retained ancestral polymorphism and likely ongoing gene flow. Moderate levels of migration between dark soil and white sand populations were inferred for *S. undulatus*, indicating that ongoing gene flow may still connect populations with divergent color morphologies. No signature of population expansion was detected for this species. In *A. inornata*, extremely low levels of genetic structure can best be explained by recent colonization of the white sand region. In this species, a strong pattern of population expansion was observed, and migration rates were not significantly different from 0. Although patterns of population differentiation as inferred from mitochondrial DNA provide a biologically meaningful comparison among species, additional data are necessary to better estimate current and historical levels of gene flow among populations. Other studies have shown that nuclear and mitochondrial markers can yield substantially different estimates for demographic parameters (see, e.g., Hey and Nielsen 2004). Particularly for model-based analyses, results inferred from a single marker often have broad confidence intervals and may not lead to robust reconstructions of population history. Future work in this system will use multilocus nuclear data to further explore the underlying processes responsible for observed patterns of genetic variation.

Whether levels of genetic isolation are explained by current or historical interaction among populations, patterns observed strongly support the prediction of divergence-with-gene-flow models that phenotypic differentiation should increase with genetic isolation between habitats (table 4). In the White Sands system, the species exhibiting reduced genetic exchange across the ecotone, *H. maculata*, does show greater phenotypic divergence between white sand and dark soil forms. The two species for which recent genetic contact between divergent morphs was inferred, *S. undulatus* and *A. inornata*, exhibit decreased substrate matching on the gypsum dunes. Species differences in coloration at the ecotone are also strongly correlated with patterns of gene flow. The light color of ecotonal animals in *H. maculata* is commensurate with high levels of gene flow between ecotone and white sand populations and low levels of genetic connectivity between ecotone and dark soil populations. The intermediate color of ecotonal individuals in *S. undulatus* is consistent with indications that gene flow may occur in both directions across the ecotone for this species. Finally, the dark color of ecotonal populations in *A. inornata* may reflect the recent expansion of dark soil populations into white sand habitat. Observations are thus consistent with divergence-with-gene-flow models and suggest dispersal as a fundamental parameter in understanding phenotypic patterns across ecological transition zones.

Although differences in population connectivity across the White Sands ecotone are sufficient to explain the variation in phenotypic response among species, several al-

Table 4: Support for divergence-with-gene-flow models: phenotypic divergence at White Sands scales with genetic isolation

	Holbrookia maculata	*Sceloporus undulatus*	*Aspidoscelis inornata*
Phenotypic response across the ecotone	Strong: white sand populations highly substrate matched; ecotonal populations indistinguishable from white sand populations	Moderate: white sand populations moderately substrate matched; ecotonal populations intermediate between white sand and dark soil populations	Weak: white sand populations moderately substrate matched; ecotonal populations indistinguishable from dark soil populations
Genetic isolation among populations	Strong: high levels of population structure ($\Phi_{ST} = .82$); no evidence of population size changes or ongoing gene flow	Moderate: moderate levels of population structure ($\Phi_{ST} = .54$); no evidence of population size changes but data suggest ongoing gene flow	Weak: low levels of population structure ($\Phi_{ST} = .09$); strong evidence of population expansion but not of ongoing gene flow

ternative explanations must be considered. First, populations of *H. maculata* could be more highly adapted to the white sand environment if natural selection is stronger on this species for ecological reasons, such as foraging mode or microhabitat association. A previous study comparing activity patterns between *H. maculata* and *S. undulatus* at White Sands found that *H. maculata* spent more time in open areas and was less closely associated with vegetation than *S. undulatus* (Hager 2001*a*). Quantitative data on microhabitat association are not currently available for *A. inornata*, but this species is found both under vegetation and in more open areas (Degenhardt et al. 1996). Therefore, it is plausible that *H. maculata* is more visible to predators and that selection pressure for substrate matching is higher in this species. Second, intermediately colored *S. undulatus* could be locally adapted to the intermediate substrate color at the margin of the dune field. However, in contrast to the large expanse of pure gypsum habitat, the band of intermediately colored ecotonal substrate is extremely narrow, often only meters wide. Given the likelihood of gene flow across the ecotone in this species and the restricted area of the ecotone, natural selection would need to be implausibly strong to provide an adaptive explanation for maintenance of intermediate color morphs. Third, the three species may show different responses to selection if genetic or developmental constraints limit the potential for *S. undulatus* or *A. inornata* to obtain the dramatically blanched coloration of *H. maculata*. However, candidate gene studies have identified a single gene of large effect associated with color variation in White Sands populations of *A. inornata* (Rosenblum et al. 2004). Therefore, predictions based on genetic architecture would suggest that potential response to selection in *A. inornata* would be high, not low. Although further study is necessary to reject these alternatives, the relationship between population structure and phenotypic variation remains the strongest current explanation for observed patterns.

Convergent Evolution and Ecologically Mediated Divergence

Hypothesis-driven tests reveal the importance of ecological factors in shaping phenotypic diversity at the White Sands ecotone. Data support the predicted relationship between dorsal color and substrate color: phenotypic divergence increases with habitat dissimilarity for all species. Moreover, patterns of dorsal coloration are better explained by habitat variation than by genetic variation. Both MCTs and phylogenetic analyses indicate that color variation cannot be explained by neutral processes alone and underscore the importance of divergent selection in shaping phenotypic patterns for lizards at White Sands.

The strong signal of ecologically mediated divergence at White Sands is particularly compelling given the adaptive and historical differences observed among species. The three species vary in the strength of their phenotypic response to selection and in their demographic histories (table 4). Convergence of blanched coloration in the entire guild of lizards at White Sands suggests that natural selection is sufficiently strong to produce a concordant pattern among species despite species-specific idiosyncrasies in population structure, historical demography, and ecology. The strength of natural selection at White Sands is also suggested by the conspicuous absence of ecologically similar species from the dunes. A more diverse lizard assemblage is found in dark soil habitats adjacent to White Sands, but only three species, all with blanched coloration, inhabit the gypsum environment.

The results from this study reinforce a growing body of evidence for the importance of ecologically mediated divergence (see, e.g., Kingsolver et al. 2001; Schluter 2001;

Rieseberg et al. 2002). Although patterns observed in White Sands lizards suggest the action of strong divergent selection, they also underscore the importance of gene flow in modulating this response. Empirical studies of community assemblages provide a powerful tool for extending our understanding of the role of adaptive evolution in shaping the phenotypic diversity observed in natural populations.

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