POPULATION STRUCTURE OF SIDE BLOTCHED LIZARDS (*UTA STANSBURIANA*) DISPLAYING ADAPTIVE DORSAL COLORATION

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by

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CERTIFICATION OF APPROVAL

I certify that I have read Population structure of side blotched lizards (*Uta stansburiana*) displaying adaptive dorsal coloration by Steven Micheletti, and that in my opinion this work meets the criteria for approving a thesis submitted in partial fulfillment of the requirements for the degree: Master of Science in Biology: Ecology and Systematic Biology at San Francisco State University.

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POPULATION STRUCTURE OF SIDE BLOTCHED LIZARDS (*UTA STANSBURIANA*) DISPLAYING ADAPTIVE DORSAL COLORATION

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We investigated adaptive dorsal coloration of *Uta stansburiana* (Side Blotched Lizard) living on the Cima Lava Field in San Bernardino County, California. We take an experimental approach to ensure that substrate matching is not a plastic trait in this species. We then test a candidate gene, melanocortin-1 receptor (*Mc1r*), to see if it is correlated with dorsal color differences between light and dark individuals. We also use mitochondrial loci (*ND4* and *cytb*) in combination with *Mc1r* to investigate the population structure of this system by means of phylogenetic and population genetic analyses. Results suggest that substrate matching is not a plastic trait in this species; however variation in *Mc1r* is not associated with observed color differences. We also find an extraordinarily high amount of genetic variability in our sample, suggesting a very large population size. Pairwise mismatch analysis and Tajima's D tests are consistent with a recent population expansion. Furthermore, we find genetic differentiation among sub localities, suggesting that abiotic barriers are inhibiting gene flow.

I certify that the Abstract is a correct representation of the content of this thesis.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

1. Introduction

Recently there has been increased focus on the genetic basis of color variation in animals because of the potential for color to be influenced by natural selection for background matching, thermoregulation, and sexual selection (e.g. Rosenblum *et al.* 2004; Uy *et al*. 2009; Hubbard *et a*l. 2010). Reptile coloration has long been studied as an example of adaptive evolution (Cott 1940; Norris and Lowe 1964). However, the color of reptiles can sometimes vary due to social signaling, stress, or active camouflaging (Stuart-Fox 2008). When color variation exists among populations or individuals that is not due to individual responses to external stimuli, it presents the opportunity to evaluate the genetic basis of phenotypic diversity (Cott 1940; Norris and Lowe 1964). For example, Rosenblum (2005) showed that a light/dark polymorphism in three species of lizards was not due to individual plasticity and had a heritable component.

Dorsal color varies among individuals of side-blotched lizards (*Uta stansburiana)* that reside in the Cima Volcanic Field in San Bernardino County, California (Figure 1). This volcanic field, located in the Mojave National Preserve, consists of dark basalt rocks surrounded by lighter granite sands. In *U. stansburiana*, the derived "melanic" phenotype is darker than the ancestral type and is found in individuals that occupy the darker basalt substrate. If the phenotypic variation in this population has an additive genetic basis, this species

can be used to understand the balance between gene flow and adaptive evolution in reptiles. Diurnal reptiles, especially those residing in the desert, experience intense selection for substrate matching (Luke 1989), as dorsal crypsis allows individuals to avoid being detected by highly visual predators (Norris 1965; Kettlewell 1973). Furthermore, studies have demonstrated that darker animals can heat faster and obtain higher body temperatures than lighter animals (Pearson 1977; Forsman 1995). Light coloration may therefore serve to also slow or limit heat gain (Benson 1933). Depending on the strength of natural selection and rates of migration, gene flow may obstruct local adaptation by homogenizing populations subject to different selection pressures (Lenormand 2002, Storfer *et al.* 1999). Alternatively, strong disruptive selection may overwhelm even substantial gene flow and lead to population differentiation (Turrelli et al 2001).

Similar systems have been investigated in the Carrizozo Lava Flow in New Mexico (Rosenblum *et al.* 2007). Several species of desert lizards show a light/dark polymorphism in which dorsal color matches the habitat type on which they reside (Rosenblum 2006). This suggests that natural selection is strong enough to produce a consistent pattern among species despite the fact that the species differ in population structure, historical demography, and ecology. Unlike the Carrizozo Lava Flow, the Cima Lava Field consists of lava outcrops that vary

in size and are separated by a mixture of basalt rock and granite sand. Adjacent to these lava outcrops is a massive soda lake that is thought to act as uninhabitable barrier. This allows for investigation of the effect of the barrier on gene flow, and to compare relatedness between light morphs from the area immediately surrounding the lava flow to light morphs from across the soda lake at the Desert Studies Center.

We investigated this system by first testing whether captive *U. stansburiana* can physiologically substrate match. We examined gene flow and demography by comparing mtDNA sequence among color morphs and geographic localities. Finally, we conducted a preliminary examination of the genetic basis of the color polymorphism by sequencing a candidate locus, Melanocortin 1 receptor (*Mc1r*), that is known to affect color pattern in other species of vertebrates (Rosenblum 2004, Hoekstra *et al.* 2006).

2. Methods

2.1 Sampling

U. stansburiana tail tips (N =121) were collected in San Bernardino County, California. Samples were collected from two main geographic sites; The Cima Volcanic Field and adjacent habitats (N=106), and the Desert Studies Center (N=15) (Appendix 1; Figures 2 and 3). The Cima Volcanic Field was

selected as the main study site due to a large variation in habitat over a small distance. Located in the Mojave National Preserve, the volcanic field includes 52 volcanic cinder cone vents, and extensive basaltic lava flows that cover more than 150 square kilometers of the Mojave Desert (Dohrenwend 1986). This volcanic field ranges from 650 to 1,200 meters in elevation with volcanic cones that range in size from 25 to 155 meters (Dohrenwend 1986). This area consists mostly of dark cinder and basalt rocks that have been aged to be around 7.6 millon years old; however, the estimated last date of eruption is a much recent 10,000 years ago (Wells *et al.* 1985). This recent eruption has created novel dark volcanic substrates that are surrounded by lighter granite rocks and sand. The Desert Studies Center is approximately 23 kilometers west of the Volcanic Field and lacks volcanic rock. The Desert Studies Center site is separated from the Cima Volcanic Field by a massive soda lake consisting of alkaline evaporites, sodium carbonate, and sodium bicarbonate (Ore and Warren 1971). This predominantly dry lake is uninhabitable and potentially acts as a barrier between sites.

Lizards were captured using slip-knot nooses and 0.5 cm of tail tip was removed and preserved in 95% ethanol for DNA analysis. We measured the most prominent dorsal color of each lizard with a Munsell Color Geological Rock-Color Chart (Munsell, North Brunswick, NJ). The Geological Color Chart was

selected to measure dorsal color since lizards rely on substrate (rock) matching for predator avoidance (Luke 1989). Therefore, the Geological Color Chart was used to measure the color of both the lizard's dorsum and the substrate on which they were found. The chart consists of a variety of color chips that classify different colors using three color dimensions: hue, value (lightness), and chroma (color purity). Dorsal temperature was measured with an infrared thermometer, and lizards were photographed against the substrate on which they were first discovered as well as against the Geological Color Chart. Colors were determined at the time of capture and reconfirmed later based on photographs. Lizards were separated into two categories based on their relative color: Light (N=59) and Dark (N=62).

2.2 Tests for Phenotypic Plasticity

6 individuals were collected and maintained in captivity to test whether dorsal color is a plastic trait in these populations (IACUC protocol #A10-002). This sample consisted of 3 light, and 3 dark individuals. Lizards were reared in separate 10-gallon glass aquaria. Aquaria were supplied with white sand, a granite rock for basking, and a granite rock refuge. This substrate best matched an extreme light habitat, like that of the granite sands in the Mojave National Preserve. Lizards were fed crickets *ad libitum* with a 12 hour light/dark schedule.

Heat lamps were used during the day to maintain a temperature of 25-31˚ C. Dorsal colors were recorded using the Munsell Color Geological Rock-Color Chart every week for 52 weeks to measure any color change. Since color is thought to be affected by temperature in most lizard species (Norris, 1965) we accessed the amount of color change based on body temperature. Each collected lizard was measured at four temperature intervals: inactive (16.5-17.5˚ C), room (20 - 21 ˚ C), warm (25.5 - 28.5˚ C), and basking (30.5 - 32˚ C).

2.3 DNA Extraction, Amplification and Sequencing

DNA was extracted using a DNEASY extraction kit (QIAGEN Inc. Valencia, CA) on .5cm tail tissue. Following extraction, the template DNA was amplified using Polymerase Chain Reaction (PCR) for two mitochondrial loci; NADH dehydrogenase subunit 4 (*ND4*) and cytochrome-B (*cytb*), and one nuclear locus; Melanocortin 1 receptor (*Mc1r*) (Appendix 2 and 3). Mitochondrial markers were selected for investigation due to their high sensitivity to population subdivision. In addition, their effective haploidy and lack of recombination (Brown *et al.* 1979) means that mitochondrial DNA sequence data can be used phylogenetically to estimate relationships among alleles without having to deal with heterozygosity and linkage phase. *Mc1r* was selected since it is a G-protein coupled receptor which is a key switch in a signal transduction pathway in

melanin-producing cells (Barsh 1996). *Mc1r* has been studied in natural populations and has been implicated in intraspecific color variation in birds, mammals, and just recently reptiles (Rosenblum *et al.* 2004; Nachman *et al.* 2003; Takeuchi *et al.* 1996). *Mc1r* polymorphisms have been found to be strongly associated with color differences in some desert squamates (Rosenblum *et al.* 2004) making it a candidate gene to investigate in *U. stansburiana* color morphs.

Successfully amplified PCR product was purified using Exonuclease I (EXO), and Thermosensitive Alkaline Phosphatase (FastAP) (Fisher Scientific, Houston, TX). Purified product was sequenced in both directions using Elim Biopharm's DNA sequencing services which use ABI 3730xl Sequencers (Applied Biosystems, Carlsbad, CA). Sequencing primers (Appendix 4) were designed using initial sequence data. Individuals with poor sequence quality, a high occurrence of heterozygosity, or unique sequences were re-sequenced.

2.3 Phylogenetic Analysis

Sequence data were edited and aligned using the computer program Sequencher 3.1.1 (Gene Codes Corporation, Ann Arbor, MI). Sequences were confirmed to be *U. stansburiana* product by using the BlastN program at the NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Mitochondrial haplotypes were identified using MacClade 4.08a (Maddison and Maddison 2010). For the

autosomal *Mc1r*, double peaks present in sequence from both directions were scored as heterozygous. Haplotype phases were inferred using the program PHASE 2.1.1 (Stephens *et al.* 2001).

Haplotype trees for both mitochondrial and nuclear sequence were created using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2005). The substitution model GTR + Γ was used for all datasets. Each run was set for 20 million generations, with sampling at every 1000 generations. Nodes with a posterior probability > 0.90 were considered well supported (Wilcox *et al.* 2002).

2.4 Population Genetic Analysis

Population genetic analyses were performed using Arlequin 3.5 (Schneider *et al.* 2000). For these analyses we defined three different groups of populations. First, analyses were run with all collected samples defined as one entire population (N=121). We defined another group consisting of two populations based on collection site: Cima Lava Field and adjacent habitats (N=106) or Desert Studies Center (N=15). These two localities are separated by the uninhabitable soda lake which potentially splits the population into two. Our last group consisted of two populations based on whether an individual's dorsum was light (N= 59), or dark (N=62). Since color is thought to be an adaptive trait for predator avoidance local selection has the potential to subdivide a population

for loci affecting the trait being selected and loci in linkage disequilibrium with the selected gene.

For each defined group, we used pairwise mismatch distribution to test for recent population size expansion (Rogers and Harpending 1992). Statistical significance was tested using (Harpending 1994) raggedness index (*r*) and sum of squared deviation test (Rogers and Harpending 1992). We estimated per locus diversity as haplotype number and haplotype diversity (*h*, Nei 1987) for all populations and putative subpopulations. Nucleotide diversity was estimated with Watterson's θ (θ, Watterson 1975) and Nei's θ (π, Tajima 1983, Watterson 1975). We calculated Tajima's D (Tajima 1989) to test for departures from neutral expectations.

An analysis of molecular variance (AMOVA; Excoffier *et al.* 1992) was conducted using the different color and locality groups. Φ_{ST} from AMOVA were estimated by computing distance matrices based on pairwise differences with 1000 permutations to determine statistical significance. Finally Jost's D value (D_j, Jost 2008) was calculated using the program Genodive 2.0B20 (Meirmans and Van Tienderen 2004) as an estimator of the degree of differentiation between populations. Jost's D ranges from 0 to 1, and is better than Φ_{ST} at indicating population differentiation when haplotype diversity in the sample is high (Jost 2008).

3. Results

3.1 Adaptive Phenotypic Variation

Lizard color data is displayed in Table 1. Of 121 sampled individuals, 93% matched the color class of the substrate on which they were collected. To ensure that this association was not due to coincidence, a Fisher's exact two-tailed test was performed (Fisher 1922) revealing a p-value < 0.0001. This suggests a significant correlation between habitat type and dorsal color. Based on the Munsell Geological Color Chart, dark males are associated with a primary dorsal color of dark olive black and dark females are associated with an olive gray color; light males are associated with dark yellowish brown whereas light females are associated with light olive gray. In both light and dark lizards, the primary dorsal colors in males are darker than females, suggesting a primary dorsal color sexual dimorphism. Of the 6 captive lizards housed on light substrate, all lived 15 weeks or longer. These lizards showed little to no variation in dorsal color with some exceptions (Figure 4). The first exception was individual 66, which surprisingly darkened in dorsal color after 21 weeks of being captive on a light substrate. Another exception was individual 50 which showed a significant amount of dorsal lightening after shedding, yet darkened again weeks later. The last exception was individual 17, which lightened after shedding yet died five weeks later, making it uncertain whether the color change was permanent or not. Overall,

there is no trend towards a color change to match substrate, suggesting cryptic coloration is not due to plasticity.

3.2 Phylogenetic Analysis

Sequencing of mitochondrial genes produced 1086 basepairs of *cytb*, and 754 basepairs of *ND4* giving a total of 1840 basepairs. 71 polymorphic sites were identified in *cytb* creating 65 haplotypes, and 56 polymorphic sites were identified in *ND4* resulting in 46 Haplotypes. Combining the two genes resulted in a total of 81 haplotypes. We sequenced 701 basepairs of the autosomal gene *Mc1r* with 42 polymorphic sites and 66 haplotypes were estimated using the haplotypes considered most likely by PHASE. Haplotype phylogenies for *Mc1r* and the combined mtDNA data are shown in Figure 5 and Figure 6. There is no phylogenetic clustering of color variants and very little substructuring based on locality, although a few statistically supported clades correspond to groups of individuals collected from the same region.

3.3 Population Genetic Analysis

3.3.1 *Overall Molecular Diversity*

Data for the entire sample of 121 individuals show a substantial amount of molecular variation as shown in Table 2 and Table 3**.** *Cytb* shows the most

variation out of all of the loci. Haplotype diversity is extremely high (*h*=.979) with 65 haplotypes. Sequence diversity is also high (π=.007, θ= 0.016). The pairwise mismatch analysis of *cytb* shows a unimodal distribution (*r*=.011, p=.630; SSD tests agreed with *r* in every case and will not be reported) and Tajima's D is significantly negative (D = -1.869, p=.009). *ND4* also shows similarly high molecular diversity (*h*= 0.948**,** π= 0.006, θ= 0.014) as well as unimodal distribution of pairwise mismatches (*r*= 0.025, p= 0.110) and a negative Tajima's D (D=-1.831, p=0.007).

The combined mitochondrial dataset (*ND4 and cytb)* shows diversity statistics that are consistent with the individual mitochondrial genes (*h*=0.988, π=.006, θ=0.015) as well as a unimodal distribution of pairwise mismatches (*r*=0.004, p=0.81) and significantly negative Tajima's D (D= -1.944, p=.006).

As expected, the autosomal gene *Mc1r* shows less variation than mitochondrial loci yet is still very diverse for a nuclear protein coding locus. Haplotype diversity is extraordinarily high (*h*=.926) with 66 haplotypes in 121 individuals. Sequence diversity is also high (π= 0.004, θ= 0.010). Tests for mismatch distribution show a unimodal distribution (*r*= 0.021, p=.719) and Tajima's D deviates from neutrality (D= -1.699, p=.012). All these values are consistent with those from the mitochondrial loci.

However, because of the high *Mc1r* diversity, PHASE could not assign haplotype identities to all genotypes with high probabilities, and it is possible that the diversity values were inflated because of misassigned haplotypes. In order to be conservative, we reanalyzed the data by examining all possible haplotype designations for each individual with multiple possible genotypes and preferentially choosing the genotype that minimized the overall diversity of the sample. This was done by picking a genotype that involved one of the two most common haplotypes (with frequencies of 0.24 and 0.20, after all genotypes were assigned) even if PHASE estimated higher probabilities for genotypes with rare haplotypes. If none of the possible genotypes contained one of these common haplotypes, genotypes were chosen if they contained a haplotype that was homozygous in a different individual. Two individuals were dropped from the analysis because they could not be assigned genotypes under these criteria. Arlequin analysis shows that this reassignment of haplotypes did not significantly alter the diversity of the *Mc1r* sample. There were 71 unique sequences in the 125 individuals (h=0.898) and nucleotide diversity values were similar to the PHASE predicted data set (π= 0.004, θ= 0.010). Pairwise mismatch and Tajima's D values were also similar (r= 0.018, p=0.809; D=-1.755, p=0.011). This suggests that the high diversity values for this locus are not due to PHASE artifacts.

3.3.2 Population structure and local diversity

If selection for background matching has resulted in reproductive isolation between light and dark lizards, or if geographic barriers have isolated regions of our study area, population structure statistics may reveal differences among these groups even if there is no phylogenetic signal separating them. Given that mitochondrial genes do not undergo recombination and that analysis of the separate mitochondrial genes shows they have similar values for all diversity and population structure statistics, we will only describe the statistics for the combined mtDNA, rather than those for *cytb* and *ND4* separately.

Table 4 and Table 5 show molecular diversity, mismatch distributions, and Tajima's D test for each defined subsample. When comparing putative subpopulations separated by the soda lake, the lava (and adjacent habitats) population shows molecular diversity that is similar to the Desert Studies Center population. Tajima's D is significantly negative for the Lava population and negative but not significant for the Desert Studies Center population, although the smaller sample size (N=15) at the Desert Studies Center may be the reason for the lack of statistical significance. Each comparison also shows a unimodal pairwise mismatch distribution. Population structure statistics for mitochondrial loci (Table 6) for this comparison suggest strong population subdivision (Φ_{ST}= 0.125, p= 0; D_j=.964, p= 0.001). *Mc1r* does show a weak and statistically

significant Φ_{ST} (Φ_{ST} =0.019, p= 0.011), however Jost's D is not statistically significantly different from 0 (D_j = 0.083, p= 0.166). Molecular diversity, mismatch distributions and Tajima's D statistics are compatible with the overall values, which suggests that population subdivision is not responsible for any of the high diversity values for the overall analysis.

When comparing dark and light lizards, population structure analysis of mtDNA indicates weak but statistically significant substructure in mitochondrial loci, but only when the Desert Studies Center individuals are included (Φ_{ST} =0.017, p= 0.016). When the Desert Studies Center animals are removed from the analysis, no significant Φ_{ST} or Jost's D values are found. Although Φ_{ST} and Jost's D values show low levels of differentiation for *Mc1r;* the p-values are just marginally insignificant (Φ_{ST} =0.006, p=0.068; D_j=0.079, p=.066).

4. Discussion

4.1 Physiological Plasticity

Results suggest that phenotypic plasticity cannot explain the dramatic color variation among lizards residing on different substrates. When housed on light substrates, dark lizards did not significantly change color. Rosenblum (2005) similarly found that *Holbrookia maculata* (Common Lesser Eared Lizard) and *Sceloporus undulatus* (Eastern Fence Lizard) vary in color based on substrate

habitat, but show no physiological color change over time when reared on an intermediate colored substrate. We were unable to hatch eggs in captivity; however other studies suggest that captive reared hatchlings of other lizard species in similar systems develop to match maternal coloration, not substrate color (Rosenblum 2005; Norris 1965). Because environmentally induced variation cannot explain patterns of dorsal coloration in *U. stansburiana* it is appropriate to consider the role of natural selection for local substrate matching in shaping observed phenotypic variation.

Tests for phenotypic plasticity due to temperature suggest that *U. stansburiana* do experience plasticity due to temperature; however the darkening response triggered by experimental stimulation varied in intensity for different color morphs. When body temperature was changed from cold to hot we detected negligible color change in darker lava flow lizards but extensive plasticity in lighter lizards. These results are compatible with other studies (Lowe and Norris 1956; Sherbrooke et al. 1994). Studies in diverse taxa have demonstrated that darker animals heat up faster and can reach higher body temperatures than non-melanic conspecifics (Pearson 1977; Forsman 1995). Rates of warming can be important if lizards are more vulnerable to predation early in the day before they can reach a hot enough temperature to be active enough to escape. It may then be advantageous for lizards to be darker during

colder periods of the day to facilitate heat gain and allow optimal body temperatures to be reached more quickly (Norris 1965; Watt 1968). Becoming lighter may reduce heat loads during extremely hot conditions (Benson 1933). The trend that darker lizards have reduced capacity for color change could indicate a trade-off between increased melanin production for substrate matching and decreased ability to aggregate and disperse melanin granules in response to short-term stimuli.

4.2 Genetic Basis of Color

Mc1r did not show any correlation to color based on phylogenetic and population genetic analyses. Rosenblum et al. (2004) found that one amino acid substitution in *Mc1r* is highly associated with lighter color variants of Little Striped Whiptail, *Aspidocelis inornata* and Hokestra et al. (2006) found the same to be true in pocket mice, *Chaetodipus intermedius*. However, many other species showing color variation also show no correlation between color and *Mc1r* (Cheviron 2006). Most polymorphic sites in our *Mc1r* sequences are synonymous base changes, and the non-synonymous base changes (only 5 throughout the sample) are found in too few individuals to correlate with color variation.

Population genetic analysis of *Mc1r* from light and dark lizards found only on the Cima Lava Field and adjacent habitats (without the Desert Studies Center

animals) shows a marginally insignificant low level of differentiation for Φ_{ST} and Jost's D. Mitochondrial genes show no such tendency (Table 6). Because mitochondrial DNA should show more population subdivision than a neutral autosomal locus, these results suggest that there may be some genetic differentiation between colors because of selection operating on genes linked to *Mc1r*.

Overall *Mc1r* does not appear to explain the color polymorphism in *U. stansburiana*. There are many other candidate genes correlated with melanin pigmentation in various taxa that can be investigated in this system. For example, solute carrier family 45 member 2 (*SLC45A2*) is correlated with plumage color in chickens and Japanese quails (Gunnarsson 2007) and sodium/potassium/calcium exchanger 5 *(SLC24A2*) has shown to affect pigmentation in both zebrafish and humans (Lamason et al. 2005). Investigation of other pigmentation genes in reptiles may identify other key genes that show a correlation between color types.

4.3 Molecular Diversity

Both mitochondrial DNA and autosomal DNA show an extraordinary amount of diversity in our sample. Haplotype diversity (*h*) and diversity estimates for π and θ are higher in our limited lava field area than seen in other globally

sampled data sets in other taxa. For example, our nuclear π estimate for the overall sample was close to 0.004, nearly an order of magnitude higher than that observed in humans (π = 0.00075**,** The International SNP Map Working Group 2001). Diversity is higher even when comparing *U. stansburiana* to other taxa that show adaptive color variation on lava flows. For instance dark variants of pocket mice, *Chaetodipus intermedius,* on lava fields in Arizona and New Mexico have a π of about .002 for *Mc1r* (Nachmen et al. 2003). θ estimates for combined populations of beach mice are around .005 for *Mc1r* (Steiner et al. 2009), whereas our *Mc1r* θ estimates are double that (θ= .010). Our diversity estimates are comparable to values found in marine fish samples taken over a global scale. For instance, the Pelagic Wahoo, *Acanthocybium solandri***,** collected from multiple oceans around the world, has *h*=0.918, π= .006 and θ =.006 for *cytb* (Theisen et al. 2008) whereas we found a similar *h*= 0.979, π=.007 and θ =.016 for *cytb* in an area easily small enough to walk across in a day. The Squirrelfish, *Holocentrus ascensionis* also showed similar levels of diversity (*h*=.976 and π= .006) in *cytb* on a global scale (Bowen et al. 2006). The fact that genetic variation in world wide populations of mobile marine fish is similar to genetic variation of our lizards on a single lava field indicates extraordinary variation.

This variation was unlikely due to any sequencing error or artifacts. On top of obtaining sequence in both directions, we resequenced individuals if sequence

data did not have distinct peaks or showed suspicious base changes. Once all the data were acquired we found no unexpected stop codons, and very few non-synonymous base changes. Furthermore, heterozygote base calling was conservative; a position was deemed heterozygous only if the sequence data showed clear double peaks in both directions.

There are a number of mechanisms that could account for the high levels of current genetic variability. One mechanism directly implied by our data is population expansion. Both Tajima's D values and pairwise mismatch distributions show consistent results in the total sample for both mitochondrial and autosomal DNA. A significantly negative Tajima's D means that there is an excess of low frequency polymorphisms (rare variation), consistent with population growth or positive selection. The fact that both a mitochondrial and an autosomal gene show similar patterns suggest that population growth is more likely than selection.

Other studies have identified range expansion in a variety of taxa on different extinct lava fields, with a longer period of time since the last volcanic activities (Vandergast et al. 2004, Gübitz et al. 2005). Rosenblum (2007) identified a similar level of diversity in *S. undulatus* on the Carrizozo lava flow in New Mexico. This suggests that *U. stansburiana* on the Cima Lava field, which is

thought to have last erupted 10,000 years ago, may be undergoing expansion in this novel habitat.

5. Conclusion

Investigation of this system shows a clear distribution of lighter animals on granite and associated rocks and darker animals on dark, basalt rock. Our tests and related studies suggest that this variation in color is not due to phenotypic plasticity. Although the candidate locus *Mc1r* is highly variable in our samples, it does not correlate with the observed color differences. Still this variability, along with the extreme variability seen in our mitochondrial DNA, suggests that this species may be undergoing population expansion and may have a large population size in the Cima Lava Field.

Future studies should investigate similar lava field systems of *U. stansburiana* to determine if this diversity is consistent in other locations. The Pisgah Volcano, also in San Bernardino County, is a younger volcano with a population of *U. stansburiana* that varies in color (Luke 1989). A comparison of these two sites could show how volcanic age is correlated with genetic variation. Other populations of *U. stansburiana* that do not inhabit lava fields should be investigated as well to ensure this variation is not common in this species. Finally other candidate color genes should be sequenced to see if they correlate with

different dorsal color variants. This work contributes to our knowledge of adaptive traits on novel habitats, and will hopefully lead to future studies identifying the underlying mechanisms of the color polymorphism associated with habitats with dramatically contrasting substrate colors.

6. References

Arevalo, E., S. K. Davis, and J. W. Sites. 1994. Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in central Mexico. Syst. Biol. **43**:387–418.

Barsh, G. S. 1996. The genetics of pigmentation: from fancy genes to complex traits. Trends Genet. **12**:299–305.

Benson, S. B. 1933. Concealing coloration among some desert rodents of the southwestern United States. University of California Publications in Zoology **40**:1–20.

Bowen, B. W., A. L. Bass, A. Muss, J. L. Carlin, and D. R. Robertson. 2006 Phylogeography of two Atlantic squirrelfishes (Family Holocentridae): exploring links between pelagic larval duration and population connectivity. Mar Biol **149**:899–913.

Cheviron, Z. A. 2006. Sequence variation in the coding region of the melanocortin-1 receptor gene (*Mc1r*) is not associated with plumage variation in the blue-crowned manakin (*Lepidothrix coronata*). Proc. R. Soc. B **273**:1613–1618.

Corl, A., A. R. Davis, S. R. Kuchta, T. Comendant, and B. Sinervo. 2010. Alternative mating strategies and the evolution of sexual size dimorphism in the side-blotched lizard, *Uta stansburiana*: a population-level comparative analysis. Evolution **64**:79–96.

Cott, H. B. 1940. Adaptive coloration in animals. Methuen and Co., London.

Cushman, S. A., K. S. McKelvey, J. Hayden, and M. K. Schwartz. 2006. Gene Flow in Complex Landscapes: Testing Multiple Hypotheses with Causal Modeling. American Naturalist **168**:486–499.

Dohrenwend, J. C., S. G. Wells, and B. D. Turrin. 1986. Degradation of Quaternary cinder cones in the Cima volcanic field, Mojave Desert, California. Geological Society of America Bulletin **97**: 421–427.

Estoup, A., I. J. Wilson, C. Sullivan, J. M. Cornuet, and C. Moritz. 2001. Inferring population history from microsatellite and enzyme data in serially introduced cane toads, *Bufo marinus*. Genetics **159**:1671–1687.

Excoffier L., Smouse P.E., and J.M Quattro.1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics **131**:479–491.

Fisher, R. A. 1922. On the interpretation of χ^2 from contingency tables, and the calculation of P". Journal of the Royal Statistical Society **85**:87–94.

Forsman, A. 1995. Heating rates and body temperature variation in melanistic and zigzag *Vipera berus*: does colour make a difference? Annales Zoologici Fennici **32**:365–374.

Fu, Y.X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking, and background selection. Genetics **147**:915–925.

Gaggiotti, O. E., S. P. Brooks, W. Amos, and J. Harwood. 2004. Combining demographic, environmental and genetic data to test hypotheses about colonization events in metapopulations. Mol. Ecol. **13**:811–825.

Groombridge, J., C. Jones, M. Bruford, and R. Nichols. 2000. 'Ghost' alleles of the Mauritius kestrel. Nature **403**:616.

Gübitz, T., R.S. Thorpe, and A. Malhotra. 2005. The dynamics of genetic and morphological variation on volcanic islands. Proc. R. Soc. B **272**:751–757.

Gunnarsson, U., A. R. Hellstrom, M. Tixier-Boichard, F. Minvielle, B. Bed'hom, S. Ito, P. Jensen, A. Rattink, A. Vereijken, and L. Andersson. 2007. Mutations in *SLC45A2* cause plumage color variation in chicken and Japanese quail. Genetics **175**:867-877.

Hadly, E. A., U. Ramakrishnan, Y. L. Chan, M. van Tuinen, K. O'Keefe, P. A. Spaeth, and C. J. Conroy. 2004. Genetic response to climatic change: insights from ancient DNA and phylochronology. PLoS Biol. **2**:1600–1609.

Harpending, R. C. 1994. Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. Hum Biol **66**:591–600.

Hoekstra, H. E., Hirschmann, R. J., Bundey, R. A., Insel, P. A,. Crossland, J. P. 2006. A Single Amino Acid Mutation Contributes to Adaptive Beach Mouse Color Pattern. Science: **313**:5783

Hubbard J.K., J.A.C. Uy, M.E. Hauber, H. E. Hoekstra, R. J. Saffran. 2010 Vertebrate pigmentation: from underlying genes to adaptive function. Trends in Genetics. **26**, 231–239.

Huelsenbeck, J. P., and R. Ronquist. 2005. Bayesian analysis of molecular evolution using MrBayes. Pages 183-232 in Statistical Methods in Molecular Evolution (R. Nielsen, ed.). Springer-Verlag.

International SNP Map Working Group. 2001. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. Nature **409**:928–933.

Jost, L. 2008. G_{ST} and its relatives do not measure differentiation. Molecular Ecology **17**:4015-4026.

Kettlewell, B. 1973. The evolution of melanism: the study of a recurring necessity. Clarendon Press, Oxford, U.K.

Lamason, R. L., M. A. Mohideen, J. R. Mest, A. C. Wong, H. L. Norton, M. C. Aros, M. J. Jurynec, X. Mao, V. R. Humphreville, J. E. Humbert, S. Sinha, J. L. Moore, P. Jagadeeswaran, W. Zhao, G. Ning, I. Makalowska, P. M. McKeigue, D. O'donnell, R. Kittles, E. J. Parra, N. J. Mangini, D. J. Grunwald, M. D. Shriver, V. A. Canfield, and K. C. Cheng. 2005. *SLC24A5*, a putative cation exchanger, affects pigmentation in zebrafish and humans. Science **310**:1782–1786.

Lenormand, T. 2002. Gene flow and the limits to natural selection. Trends in Ecology & Evolution **17**:183–189.

Luke, C. A. 1989. Color as a phenotypically plastic character in the side-blotched lizard, *Uta stansburiana*. PhD diss. University of California, Berkeley.

Meirmans, P. G., and P. H. Van Tienderen: 2004. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms, Molecular Ecology Notes **4**:792-794.

Nachman, M. W., H. E. Hoekstra, and S. L. D'Agostino. 2003. The genetic basis of adaptive melanism in pocket mice. Proc. Natl. Acad. Sci. USA **100**:5268–5273.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York, NY, USA.

Norris, K. S. 1965. Color adaptation in desert reptiles and its thermal relationships. Pages 162–226 in W.W. Milstead, ed. Lizard ecology: a symposium. University of Missouri Press, Columbia.

Norris, K. S., and C. H. Lowe. 1964. An analysis of background color-matching in amphibians and reptiles. Ecology **45**:565–580.

Ore, T.H, and C. N. Warren. 1971. Late Pleistocene-Early Holocene Geomorphic History of Lake Mojave, California. Geological Society of America Bulletin **82**:2553–2562.

Pearson, O. O. 1977. The effect of substrate and of skin color on thermoregulation of a lizard. Comparative Biochemistry and Physiology **58**:353–358.

Rogers, A. R., and H. Harpending. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Mol. Biol. Evol. **9**:552-569.

Rosenblum, E. B. 2005. The role of phenotypic plasticity in color variation of Tularosa Basin lizards. Copeia **2005**:586–596.

Rosenblum, E. B. 2006. Convergent Evolution and Divergent Selection: Lizards at the White Sands Ecotone. The American Naturalist **167**: 1–15.

Rosenblum, E. B., H. E. Hoekstra, and M.W. Nachman. 2004. Adaptive reptile color variation and the evolution of the *Mc1r* gene. Evolution **58**:1794–1808.

Rosenblum, E. B., M. Hickerson, C. Moritz. 2007. A multilocus perspective on colonization accompanied by selection and gene flow. Evolution **12**: 2971–2985.

Schneider, S., D. Roessli, and L. Excoffier. 2000. Arlequin: a software for population genetics data analysis. Ver. 2.000. Genetics and Biometry Lab., Dept. of Anthropology, Univ. of Geneva.

Sherbrooke, W. C., A. M. D. L. Castrucci, and M. E. Hadley. 1994. Temperature effects on in vitro skin darkening in the Mountain Spiny Lizard, *Sceloporus jarrovii*: a thermoregulatory adaptation? Physiol. Zool. **67**:659–672.

Steiner C. C, H. Römpler, L. M. Boettger, T. Schöneberg, and H. E. Hoekstra. 2009. The genetic basis of phenotypic convergence in beach mice: similar pigment patterns but different genes. Molecular Biology and Evolution **26**:35–45.

Stephens, M., Smith, N., and Donnelly, P. 2001. A new statistical method for haplotype reconstruction from population data. American Journal of Human Genetics, **68**:978–989.

Storfer, A., J. Cross, V. Rush, and J. Caruso. 1999. Adaptive coloration and gene flow as a constraint to local adaptation in the streamside salamander, *Ambystoma barbouri*. Evolution **53**:889–898.

Stuart-Fox, D., & A. Moussalli. 2008. Selection for social signalling drives the evolution of chameleon color change. Public Library of Science Biology.

Tajima, F. 1983. Evolutionary relationship of DNA sequences in finite populations. Genetics 105: 437–460.

Tajima, F. 1989. The effect of change in population size on DNA polymorphism. Genetics **123**:597–601.

Takeuchi, S., H. Suzuki, S. Hirose, Y. Masafumi, C. Sato, H. Yamamoto, and S. Takahashi. 1996. Molecular cloning and sequence analysis of the chick melanocortin 1-receptor gene. Biochim. Biophys. Acta **1306**:122–126.

Tamura, K. and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol **10**:512–526.

Theisen, T. C., B. W. Bowen, W. Lanier, and J. D. Baldwin. 2008. High connectivity on a global scale in the pelagic wahoo, *Acanthacybrium solandri* (tuna family Scombridae). Molecular Ecology **17**:4233–4247.

Turrelli, M., N. H. Barton, and J. A. Coyne. 2001. Theory and speciation. Trends in Ecology & Evolution **16**:330–343.

Uy, J.A.C.,R.G. Moyle, C. E. Filardi and Z. A. Cheviron. 2009. Difference in plumage color used in species recognition between incipient species is linked to a single amino acid substitution in the Melanocortin-1 Receptor. Am. Nat. **174**:244–254.

Vandergast, A. G., R. G. Gillespie, and G. K. Roderick. 2004. Influence of volcanic activity on the population genetic structure of Hawaiian Tetragnatha spiders: fragmentation, rapid population growth and the potential for accelerated evolution. Molecular Ecology **13**:1729–1743.

Watt, W. B. 1968. Adaptive significance of pigment polymorphisms in *Colias* butterflies. I. Variation of melanin pigment in relation to thermoregulation. Evolution **22**:437–458.

Watterson, G. A. 1975. On the number of segregating sites in genetical models without recombination. Theor. Popul. Biol. **7**: 256–276.

Wells, S. G., J. C. Dohrenwend, L. D. McFadden, B. D. Turrin, and K. D. Mahrer. 1985. Late Cenozoic landscape evolution on lava flow surfaces of the Cima volcanic field, Mojave Desert, California. Geological Society of America Bulletin **96**:1518–1529.

Wilcox, T. P., Zwickl, D. J., Heath, T. A., and D. M. Hillis. 2002. Phylogenetic relationships of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. Molecular Phylogenetics and Evolution **25**:361-371.

Table 1: Munsell Geological Rock-Color Chart Color Calls.

Lizard and substrate colors as determined by the Munsell Rock Chart. Olive black (OB) and olive gray (OG) are associated with dark lizards whereas dark yellowish brown (DYB) and light olive gray (LOG) are associated with light lizards. In some cases the primary dorsal colors fit in between two colors and were assigned two colors. In terms of substrates, brownish gray (BG) is associated with dark basalt rock, whereas medium gray (MG) is associated with lighter granite rocks. Yellowish brown (YB) is associated with soil and tree bark and is also considered light. A lizard is considered to be found matching its substrate if both lizard color class and its substrate color class are the same. Lizards that did not match their substrate are shown in bold. Exceptions to this rule are lizards that had a secondary color that allowed them to match substrates. This generally occurred in lizards that were dark, yet had light spots.

Lizard ID	Lizard Color Class	Substrate Color Class	Lizard Color	Substrate Color
1	Dark	Dark	OB	BG
2	Dark	Dark	OG	BG
3	Dark	Dark	OG	BG
4	Dark	Dark	OG	BG
6	Dark	Dark	OG	BG
7	Dark	Dark	OB	BG
8	Dark	Dark	OB	BG
9	Dark	Dark	OB	BG
10	Dark	Dark	OB	BG
11	Dark	Dark	OB	BG
12	Dark	Dark	OB	BG
13	Dark	Light	OB - DYB	MG
14	**Dark**	**Light**	**OB**	**MG**

Lizard ID	Lizard Color Class	Substrate Color Class	Lizard Color	Substrate Color
15	Dark	Dark	OB	BG
16	Dark	Dark	OG	BG
17	Dark	Dark	OG	BG
18	Dark	Dark	OB	BG
19	Dark	Dark	OB	BG
20	Dark	Dark	OB	BG
22	Dark	Dark	OB	BG
23	Dark	Light	OG -DYB	MG
24	Dark	Dark	OG	BG
25	Dark	Dark	OB	BG
26	Dark	Dark	OG	BG
27	Dark	Dark	OB	BG
28	Dark	Dark	OG	BG
29	Light	Light	DYB	MG
30	Light	Light	DYB	MG
31	Light	Light	LOG	MG
32	Light	Light	LOG	MG
33	Light	Light	DYB	MG
34	Light	Light	DYB	MG
35	Light	Light	LOG	MG
36	Light	Light	LOG	MG
37	Light	Light	DYB	MG
38	Light	Light	LOG	MG
39	Light	Light	LOG	YB
40	Dark	Dark	OG	BG
41	Dark	Dark	OG	BG
42	Dark	Dark	OB	BG
43	Dark	Dark	OB	BG
44	Dark	Dark	OB- OG	BG
45	Dark	Dark	OB	BG
46	Light	Light	DYB - OG	MG
47	Light	Light	DYB	MG
48	Dark	Dark	OB	BG
49	Dark	Dark	OB - OG	BG

Lizard ID	Lizard Color Class	Substrate Color Class	Lizard Color	Substrate Color
50	Dark	Dark	OG	BG
51	Dark	Dark	OG	BG
52	Dark	Dark	OG - DYB	BG
53	Dark	Dark	OG	BG
54	Dark	Dark	OG - DYB	BG
55	Dark	Dark	OB - OG	BG
56	Dark	Dark	OB	BG
57	Dark	Dark	OB - OG	BG
59	**Light**	**Dark**	**LOG - OG**	**BG**
60	Dark	Dark	OB	BG
61	Dark	Light	OB - OG	MG
62	Dark	Dark	OG - DYB	BG
63	**Dark**	**Light**	**OB**	**MG**
64	**Dark**	**Light**	**OB**	**MG**
65	Light	Light	DYB - OG	MG
66	Light	Light	LOG	MG
67	Light	Light	LOG	MG
68	Light	Light	LOG	MG
69	**Dark**	**Light**	**OG**	**MG**
70	Light	Light	DYB	MG
71	Light	Light	DYB	MG
72	Light	Light	LOG	MG
73	Light	Light	DYB	MG
74	Light	Light	DYB	MG
78	Light	Light	LOG	MG
79	Light	Light	DYB	YB
80	Light	Light	DYB	MG
81	Light	Light	DYB - OG	YB
82	Dark	Light	OB - DYB	MG
83	Dark	Light	OB - DYB	MG
84	Dark	Light	OB - DYB	MG
85	Light	Light	LOG	MG
86	Light	Light	LOG	MG
87	Dark	Light	OG - LOG	MG

Lizard ID	Lizard Color Class	Substrate Color Class	Lizard Color	Substrate Color
88	Dark	Dark	OB	BG
89	Light	Light	DYB	MG
90	Light	Light	LOG	MG
91	**Dark**	**Light**	**OG**	**MG**
92	Light	Light	LOG - OG	MG
93	**Dark**	**Light**	**OB**	**MG**
94	Light	Light	LOG	MG
95	Light	Light	DYB	MG
96	Light	Light	DYB	MG
97	Dark	Light	OB - DYB	MG
98	Light	Light	DYB	MG
99	**Light**	**Dark**	**LOG**	**YB**
100	Light	Light	LOG - OG	MG
101	**Dark**	**Light**	**OB**	**MG**
102	Light	Light	DYB	MG
103	Light	Light	DYB	MG
104	Light	Light	DYB - OG	MG
105	Dark	Light	OB - DYB	MG
106	Light	Light	LOG	MG
107	Dark	Light	OB - OG	MG
108	Light	Light	LOG - OG	MG
109	Light	Light	DYB	MG
110	Light	Light	LOG	MG
111	Light	Light	DYB	MG
112	Light	Light	DYB - OG	MG
113	Light	Light	LOG	MG
114	Light	Light	DYB	YB
115	Light	Light	LOG - OG	MG
116	Dark	Dark	OB	YB
117	Light	Light	LOG	MG
118	Light	Light	DYB	MG
119	Light	Light	LOG	MG
120	Light	Light	DYB	MG
121	Light	Light	DYB	MG

Lizard ID	Lizard Color Class	Substrate Color Class	Lizard Color	Substrate Color
122	Dark	Light	OG - LOG	MG
123	Light	Light	LOG - OG	MG
124	Light	Light	LOG - OG	MG
125	Light	Light	LOG	MG
126	Light	Light	LOG	MG

Table 2: Molecular Diversity Summary Statistics
Summary statistics for the two mitochondrial loci and their combined data as well as *Mc1r* using most probable haplotypes (PHASE) and most common haplotypes (see text). Included is the number of base pairs sequenced (bp), number of variable sites, haplotypes, nucleotide diversity (π) , haplotype diversity (*h*) and Watterson's θ (θ).

Locus	bp	N	Variable Sites	Number of Haplotypes	*h*	π	θ
cytb	1086	121	71	65	0.979	0.007	0.016
ND4	754	121	56	46	0.948	0.006	0.014
Combined mtDNA	1840	121	128	81	0.988	0.006	0.015
***Mc1r* (PHASE)**	701	121	42	66	0.926	0.004	0.010
***Mc1r* (common)**	701	121	42	71	0.898	0.004	0.010

Table 3: Mismatch and Neutrality Tests

Tajima's D (D) and raggedness index (*r*) with P-values for each locus. Significant P-values are indicated with an asterisk.

Locus	D	P	*r*	P
cytb	-1.869	0.009*	0.011	0.630
ND4	-1.831	0.007*	0.025	0.110
Combined mtDNA	-1.944	0.006*	0.004	0.810
***Mc1r* (PHASE)**	-1.699	0.012*	0.021	0.719
***Mc1r* (common)**	-1.755	0.011*	0.018	0.811

Table 4: Subsample Molecular Diversity

Molecular diversity values for each subsample for mtDNA and *Mc1r*. Number of Individuals (N), number of variable sites, number of haplotypes, haplotype diversity (*h*), nucleotide diversity (π), and Watterson's θ (θ) are included for each subsample.

mtDNA

Population	N	Variable Sites	Number of Haplotypes	h	π	θ
DSC	15	32	15	0.985	0.004	0.006
Lava	106	116	67	0.985	0.006	0.014
Light	59	100	47	0.991	0.006	0.014
Light (No DSC)	44	81	33	0.985	0.006	0.012
Dark	62	100	48	0.987	0.006	0.014

Mc1r

Population	N	Variable Sites	Number of Haplotypes	h	π	θ
DSC	30	18	17	0.952	0.005	0.006
Lava	212	39	54	0.919	0.004	0.009
Light	118	33	42	0.920	0.004	0.009
Light (No DSC)	88	30	32	0.904	0.004	0.008
Dark	124	31	44	0.921	0.004	0.008

Table 5: Subsample Mismatch and Neutrality Tests
Tajima's D (D) and raggedness index (*r*) with P-values for each subsample. Significant values are indicated with an asterisk

mtDNA

Population	D	P	r	P
DSC	-1.392	0.079	0.017	0.830
Lava	-1.854	0.008*	0.004	0.800
Light	-1.942	0.004*	0.003	0.950
Light (No DSC)	-1.694	0.024*	0.005	0.920
Dark	-1.862	0.009*	0.009	0.340

Mc1r

Population	D	P	r	P
DSC	-0.897	0.196	0.024	0.653
Lava	-0.168	0.012*	0.030	0.738
Light	-1.615	0.026*	0.019	0.820
Light (No DSC)	-1.672	0.018*	0.017	0.870
Dark	-1.526	0.030*	0.022	0.690

Table 6: Population Genetic Differentiation

F_{ST}, Φ_{ST}, and Jost's D (D_j) for each comparison with P-values. Significant values are indicated with an asterisk.

mtDNA

Comparison	F_{ST}	P	Φ_{ST}	P	D_j	P
Light vs. Dark	-0.001	0.577	0.017	0.016*	-0.045	0.561
Light vs. Dark (No DSC)	-0.002	0.792	-0.001	0.460	-0.168	0.756
Lava vs. DSC	0.014	0.012*	0.125	0.000*	0.964	0.001*

Mc1r

Comparison	F_{ST}	P	Φ_{ST}	P	D_j	P
Light vs. Dark	0.008	0.015*	0.006	0.068	0.079	0.066
Light vs. Dark (No DSC)	0.010	0.022*	0.006	0.095	0.076	0.089
Lava vs. DSC	0.020	0.017*	0.019	0.011*	0.079	0.166

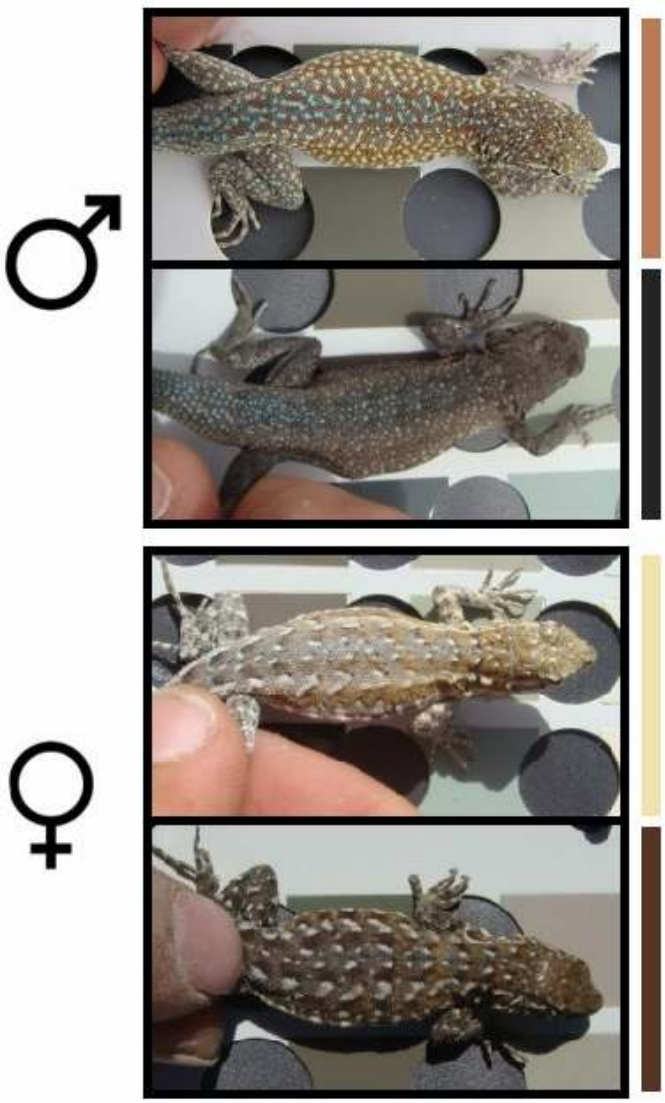

Figure 1: Dorsal Color Variation in *Uta. stansburiana*

Male and female *U. stansburiana* collected from lava (bottom picture) and granite sands (top picture).

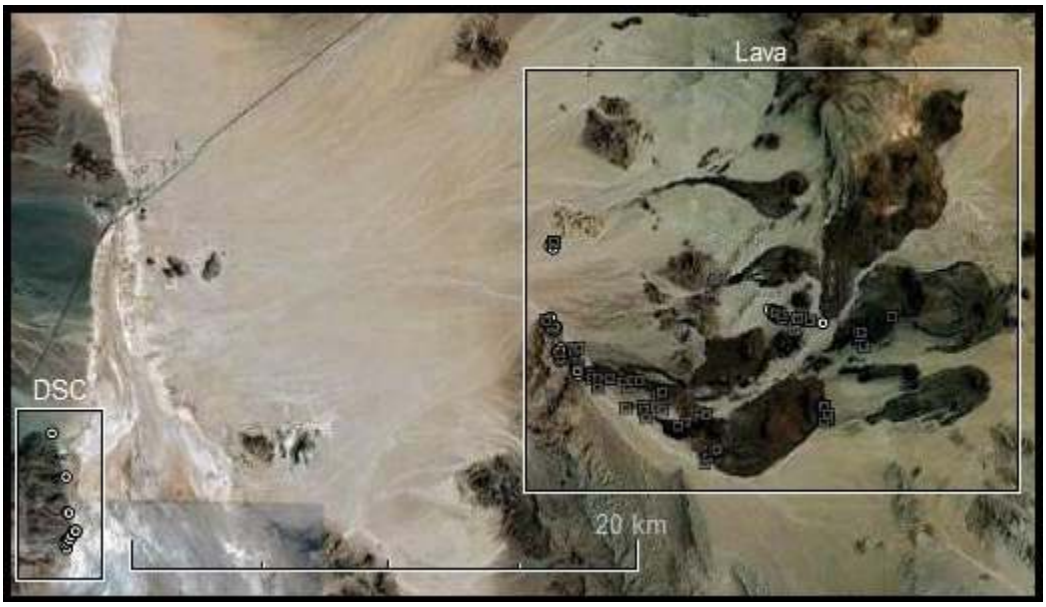

Figure 2: Study Location

Satellite imagery of the Desert Studies Center (DSC), the Cinder Lava Field (Lava) and the soda lake separating the two. Dark squares indicate collection sites of dark individuals and light circles indicate collection sites of light individuals.

Figure 3: Cima Lava Field

Satellite imagery of Cima Lava Field shown with relative collection sites: West Lava, Middle Lava, and South Lava. Dark squares indicate collection sites of dark individuals and light circles indicate collection sites of light individuals.

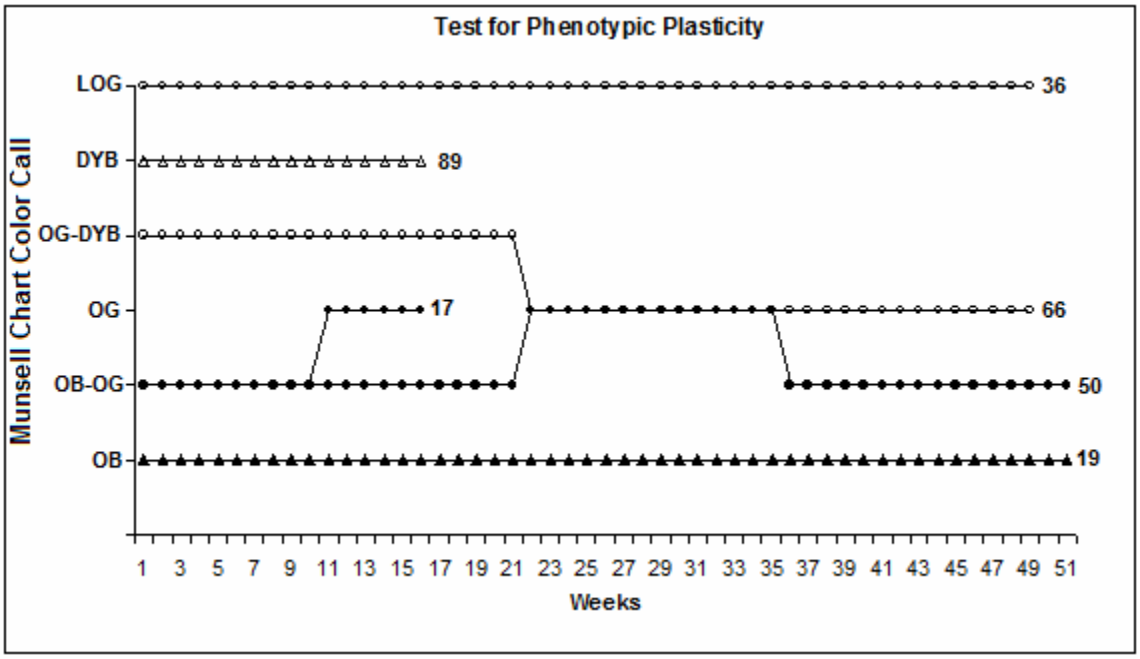

Figure 4: Phenotypic Plasticity of Captive Lizards.

Comparison of lizards that were captive for at least 15 weeks. Colors are based on the Munsell Geological Rock-Color Chart: light olive gray (LOG), dark yellow brown (DYB), olive gray (OG), and olive black (OB). Light called lizards have open symbols; dark lizards have filled in symbols. Males are indicated with triangles; females are indicated with circles.

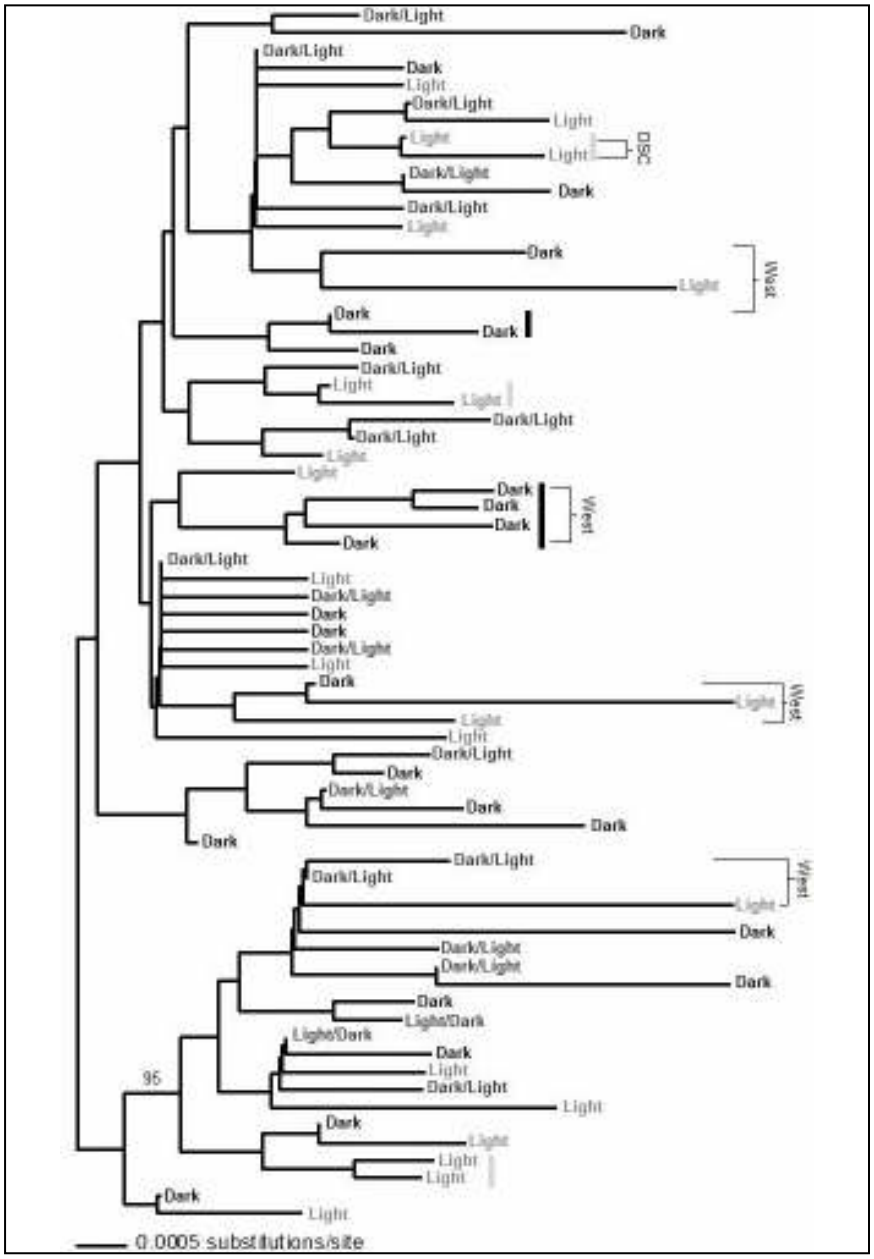

Figure 5: *Mc1r* Haplotype Neighbor Joining Tree

Mc1r haplotype neighbor joining tree with the 66 identified haplotypes**.** Haplotypes are associated with light, dark/light, or dark. Bayesian posterior probabilities greater than 90 are displayed on branches.

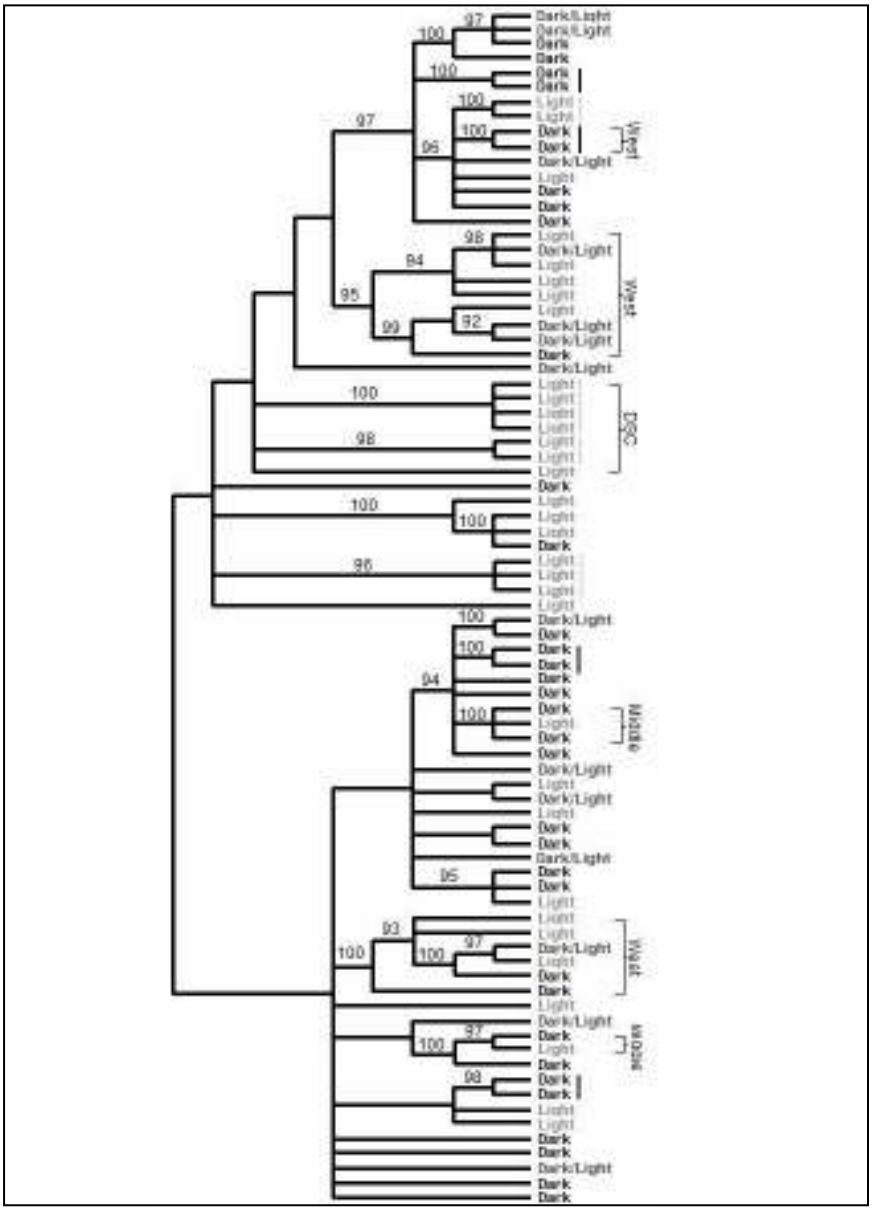

Figure 6: mtDNA Haplotype MrBayes Tree

MrBayes tree for combined mtDNA haplotypes. Posterior probabilities greater than 90 are displayed on branches. Statistically supported clades that correspond to sublocalities are listed. Haplotypes are associated with light, dark/light, or dark.

Appendix 1: Collection Data

Lizard ID	Lizard Color	STV Length (cm)	Sex	Collection Location	GPS North	GPS West	Elevation (m)
1	Dark	4.1	M	S. Lava	35.18666	115.76699	1022
2	Dark	4	F	S. Lava	35.18389	115.76834	1020
3	Dark	4.2	F	W. Lava	35.19930	115.86356	708
4	Dark	3.9	F	W. Lava	35.19827	115.85777	731
6	Dark	3.9	F	W. Lava	35.19854	115.85045	754
7	Dark	5	M	W. Lava	35.19519	115.84063	786
8	Dark	4.3	M	W. Lava	35.19435	115.84007	786
9	Dark	4.8	M	W. Lava	35.18813	115.84016	779
10	Dark	4.3	M	W. Lava	35.18929	115.84853	755
11	Dark	4	M	W. Lava	35.19925	115.86893	680
12	Dark	4	M	W. Lava	35.20052	115.87173	675
13	Dark	4.6	M	W. Lava	35.19927	115.87161	680
14	Dark	4.6	M	W. Lava	35.19683	115.86925	688
15	Dark	4.4	M	W. Lava	35.19601	115.86894	694
16	Dark	4.1	F	W. Lava	35.18874	115.85643	730
17	Dark	4.2	F	W. Lava	35.18806	115.84843	757
18	Dark	NA	M	W. Lava	35.18893	115.84326	772
19	Dark	4.3	M	W. Lava	35.19375	115.84240	770
20	Dark	NA	M	W. Lava	35.19613	115.85579	728
22	Dark	4.5	M	W. Lava	35.20071	115.87244	674
23	Dark	4.4	F	W. Lava	35.18519	115.84745	764
24	Dark	4.2	F	W. Lava	35.18224	115.83325	807
25	Dark	4.7	M	W. Lava	35.18378	115.82997	802
26	Dark	4.4	F	W. Lava	35.18626	115.82051	862
27	Dark	4.3	M	W. Lava	35.18609	115.82127	850
28	Dark	4.2	F	W. Lava	35.18745	115.82337	854
29	Light	NA	M	DSC	35.16284	116.10754	294
30	Light	5.1	M	DSC	35.14280	116.10395	294
31	Light	4.4	F	DSC	35.14300	116.10327	289
32	Light	4.3	F	DSC	35.14300	116.10321	288
33	Light	5	M	DSC	35.14261	116.10317	285
34	Light	4.6	M	DSC	35.14141	116.10528	281

Lizard ID	Lizard Color	STV Length (cm)	Sex	Collection Location	GPS North	GPS West	Elevation (m)
35	Light	2.3	F	DSC	35.13995	116.10594	286
36	Light	4.5	F	DSC	35.14013	116.10629	271
37	Light	5.3	M	DSC	35.14007	116.10670	283
38	Light	NA	F	W. Lava	35.22117	115.89020	600
39	Light	4.4	F	W. Lava	35.22117	115.89020	600
40	Dark	4.7	F	S. Lava	35.17071	115.82032	852
41	Dark	4.4	F	S. Lava	35.17103	115.81965	848
42	Dark	4.6	M	S. Lava	35.17377	115.81586	864
43	Dark	4.5	M	S. Lava	35.17334	115.81672	857
44	Dark	NA	M	S. Lava	35.17303	115.81738	853
45	Dark	4.8	M	S. Lava	35.16899	115.82136	845
46	Light	NA	M	W. Lava	35.21750	115.89040	609
47	Light	4.4	M	W. Lava	35.21753	115.89039	613
48	Dark	4.6	M	S. Lava	35.18483	115.76699	1019
49	Dark	4.6	M	S. Lava	35.18523	115.76686	1019
50	Dark	4.4	F	E. Lava	35.21288	115.75351	1072
51	Dark	4.5	F	E. Lava	35.21624	115.75189	1094
52	Dark	NA	F	E. Lava	35.21620	115.75225	1093
53	Dark	4.6	F	E. Lava	35.21541	115.75241	1082
54	Dark	NA	F	E. Lava	35.21485	115.75280	1084
55	Dark	5	M	E. Lava	35.21479	115.75283	1085
56	Dark	4.9	M	E. Lava	35.21412	115.75315	1080
57	Dark	4.5	M	E. Lava	35.22199	115.73843	1146
59	Light	4.3	F	W. Lava	35.20112	115.87302	671
60	Dark	4.5	M	W. Lava	35.21031	115.87733	647
61	Dark	NA	M	W. Lava	35.20933	115.87958	633
62	Dark	4.2	F	W. Lava	35.21029	115.88094	630
63	Dark	4.3	M	W. Lava	35.24901	115.88827	612
64	Dark	4.5	M	W. Lava	35.24919	115.88850	614
65	Light	NA	M	W. Lava	35.24899	115.88928	620
66	Light	4.8	F	W. Lava	35.24888	115.88941	625
67	Light	4.5	F	W. Lava	35.24845	115.88960	616
68	Light	4.6	F	W. Lava	35.24679	115.88895	612
69	Dark	4.4	F	W. Lava	35.20832	115.88481	639

Lizard ID	Lizard Color	STV Length (cm)	Sex	Collection Location	GPS North	GPS West	Elevation (m)
70	Light	4.6	M	W. Lava	35.20883	115.88577	643
71	Light	4.5	M	W. Lava	35.20848	115.88747	647
72	Light	4.3	F	DSC	35.13663	116.10733	525
73	Light	4.5	M	DSC	35.13835	116.10820	285
74	Light	4.6	M	DSC	35.13882	116.10714	276
78	Light	4.2	F	DSC	35.149727	116.1068	290
79	Light	4.9	M	DSC	35.149584	116.1064	286
80	Light	3.8	M	DSC	35.178772	116.1139	304
81	Light	3.3	M	E. Lava	35.219285	115.7679	1042
82	Dark	4	M	E. Lava	35.220301	115.7750	1047
83	Dark	3.7	M	E. Lava	35.220303	115.7750	1047
84	Dark	4.8	M	E. Lava	35.221665	115.7784	1060
85	Light	3.9	F	E. Lava	35.221244	115.7789	1062
86	Light	3.7	F	E. Lava	35.221119	115.7800	1072
87	Dark	3.6	F	E. Lava	35.221119	115.7800	1071
88	Dark	3.4	M	E. Lava	35.221221	115.7870	1034
89	Light	4.8	M	E. Lava	35.222245	115.7879	1031
90	Light	3.1	F	E. Lava	35.222491	115.7879	1041
91	Dark	3.4	F	E. Lava	35.222662	115.7877	1041
92	Light	5.1	F	E. Lava	35.223628	115.7896	1033
93	Dark	3.9	M	E. Lava	35.22362	115.7896	1032
94	Light	4.1	F	E. Lava	35.223769	115.7897	1032
95	Light	3.5	M	E. Lava	35.22377	115.7897	1032
96	Light	4	M	E. Lava	35.224238	115.7919	1023
97	Dark	3.9	M	E. Lava	35.223338	115.7909	1028
98	Light	4.5	M	E. Lava	35.221942	115.7892	1033
99	Light	3.7	F	E. Lava	35.222465	115.7901	1026
100	Light	3.1	F	E. Lava	35.221647	115.7890	1028
101	Dark	3.7	M	E. Lava	35.216671	115.7816	1058
102	Light	3.6	M	E. Lava	35.216824	115.7794	1053
103	Light	4.6	M	W. Lava	35.200535	115.8776	690
104	Light	3.8	M	W. Lava	35.201636	115.8782	687
105	Dark	3.6	M	W. Lava	35.202096	115.8776	697
106	Light	4.1	F	W. Lava	35.220142	115.8915	616

Lizard ID	Lizard Color	STV Length (cm)	Sex	Collection Location	GPS North	GPS West	Elevation (m)
107	Dark	4.2	M	W. Lava	35.220524	115.8919	619
108	Light	3	F	W. Lava	35.220527	115.8919	618
109	Light	3.6	M	W. Lava	35.220534	115.8919	618
110	Light	3.7	F	W. Lava	35.219962	115.8912	604
111	Light	4.1	M	W. Lava	35.219068	115.8903	607
112	Light	NA	N/A	W. Lava	35.218487	115.8895	610
113	Light	3.6	F	W. Lava	35.217918	115.8881	615
114	Light	3.9	M	W. Lava	35.217917	115.8881	615
115	Light	3.5	F	W. Lava	35.217852	115.8880	616
116	Dark	4.4	M	W. Lava	35.217852	115.8880	616
117	Light	3.5	F	W. Lava	35.217584	115.8874	615
118	Light	4.8	M	W. Lava	35.210237	115.8854	640
119	Light	3.9	F	W. Lava	35.208477	115.8872	654
120	Light	4.3	M	W. Lava	35.209206	115.8871	651
121	Light	NA	M	W. Lava	35.20564	115.8859	652

Appendix 2: Polymerase Chain Reaction Recipes

Mc1r

Stock Reagents	Amount (Per RXN)	Final concentration
Water	15 µl	-
Dream Taq Buffer 10X (20 mM MgCl2)	2.5 µl	1X, 2 mM
Primer 1 (10 µM)	1.6 µl	0.64 µM
Primer 2 (10 µM)	1.6 µl	0.64 µM
dNTPs (10 mM)	2.6 µl	1.04 mM
Fermentas Dream Taq Polymerase (5 U/µl)	0.2 µl	1 U
Template DNA (50 ng/µl)	1.5 µl	3 ng/µl
Total	**25 µl**	

cytb

Stock Reagents	Amount (Per RXN)	Final concentration
Water	11.6 µl	-
Amplitaq 10 X Buffer II	2 µl	1X
Primer 1 (10 µM)	1 µl	0.5 µM
Primer 2 (10 µM)	1 µl	0.5 µM
dNTPs (10 mM)	0.6 µl	0.3 mM
MgCl2 (25 mM)	2.4 µl	3 mM
Fermentas Dream Taq Polymerase (5 U/µl)	0.2 µl	1 U
Template DNA (50 ng/µl)	1.2 µl	3 ng/µl
Total	**20 µl**	

ND4

Stock Reagents	Amount (Per RXN)	Final concentration
Water	12.5 µl	-
Dream Taq Buffer 10X (20 mM MgCl2)	2.1 µl	1.05X, 2.1 mM
Primer 1 (10 µM)	1 µl	0.5 µM
Primer 2 (10 µM)	1 µl	0.5 µM
dNTPs (10 mM)	2 µl	1 mM
Fermentas Dream Taq Polymerase (5 U/µl)	0.15 µl	.75 U
Template DNA (50 ng/µl)	1.25 µl	3 ng/µl
Total	**20 µl**	

Appendix 3: Polymerase Chain Reaction Protocols

Mc1r

Step	Temperature ˚C	Time	Number of Cycles
Initial Denaturation	94˚	4 min	1
Denaturation	94˚	30 sec	25
Annealing	67˚	35 sec	
Extension	72˚	50 sec	
Final Extension	72˚	7 min	1
Refrigeration	10˚	∞	

cytb

Step	Temperature ˚C	Time	Number of Cycles
Initial Denaturation	94˚	4 min	1
Denaturation	94˚	30 sec	25
Annealing	50˚	35 sec	
Extension	72˚	50 sec	
Final Extension	72˚	7 min	1
Refrigeration	10˚	∞	

ND4

Step	Temperature ˚C	Time	Number of Cycles
Initial Denaturation	94˚	4 min	1
Denaturation	94˚	30 sec	25
Annealing	55˚	35 sec	
Extension	72˚	50 sec	
Final Extension	72˚	7 min	1
Refrigeration	10˚	∞	

Appendix 4: PCR and Sequencing Primers

Locus	PCR Primer 1	PCR Primer 2
Mc1r	CAGCAARCCCACAGGTGAG (Rosenblum et al. 2004)	TGGYTCTCTGGCAGATGATG (Rosenblum et al. 2004)
cytb	CCACCGTTGTTATTCAACTAC (Corl et al. 2010)	GGTTTACAAGACCAATGCTTT (Corl et al. 2010)
ND4	CACCTATGACTACCAAAAGCTCATGTAGAA (Arevalo et al. 1994).	CATTACTTTTACTTGGATTTGCACCA (Arevalo et al. 1994).

Locus	Forward Sequencing Primer	Reverse Sequencing Primer
Mc1r	GCCCATCAATGTGACCAAC GATGACGGTTCCATCACCTC	TCAAAGTCCTCCTGAGCT (Rosenblum et al. 2004)
cytb	ACTTTGGCTCTCTTTTAGGAC	ATGATTGAGGCTAGTTGTCCG
ND4	ACTAAAACTTGGGGGATATGG	GCAGTTCTTGGTGTTCAAAAC