Testing maintenance of phenotypic diversity in a North American skink (*Plestiodon gilberti)*

Background. Gene flow is a homogenizing evolutionary force[1]. Nonetheless, intraspecific phenotypic diversity is common in nature. Hybrid zones between reproductively compatible but phenotypically divergent lineages provide natural experiments examining maintenance of diversity in the face of gene flow[3]. The clinal phenotypic variations between clades of the North American scincid lizard *Plestiodon gilberti*[7] are a putative example of a hybrid tension zone, where phenotypic diversity is maintained by selection against hybrids, and the width of the zone is dependent on prezygotic and postzygotic barriers to reproduction[2].

Study Species. Mitochondrial and nuclear DNA evidence indicate three *P. gilberti* clades have independently evolved[8] over the past 12 million years[7] in California, with two juvenile tail color phenotypes: a blue-tailed clade Sierran clade, a pink-tailed southwestern clade, and a second pink-tailed clade in the Inyo Mountains[7,8]. Bright tail coloration in juvenile lizards is an anti-predatory visual decoy complimenting tail autotomy[9]. Putative tail color intergrades, which are characteristically more muted in color, may maladaptively cease to draw predator attention to the tail[10]. Intergrades are present at a southern contact zone between Sierran and southwestern clades, and molecular data have identified a second contact zone to the northeast between the Sierran and Inyo clades[7]. Additionally, differences in body size impose a strong interspecific prezygotic reproductive barrier with *P. gilberti*'s closest relative, *P. skiltonianus*[11], and may act as an additional reproductive barrier between *P. gilberti* clades. Sierran and southwestern clades converge in size enough to engage in successful copulation at the first contact zone[7,11] (AFLP data suggest isolation-by-distance introgression[7]), but the Sierran and Inyo clades at the second contact zone differ in mean body size[8].

Hypotheses. My research seeks to address the ***strength*** and ***type*** of selection that maintains the tail color polymorphism between clades of *P. gilberti* skinks. I predict that at both contact zones, tail color polymorphism is maintained by strong selection against maladapted tail color intergrades. At the second contact zone, I predict that an additional mechanical prezygotic barrier, due to greater body size divergence, also contributes to color polymorphism maintenance.

Methods. ***Field Collection.*** I will use ASIH funding for a field trip to sample up to 10 populations (minimum of 10 individual/population) along a transect spanning each *P. gilberti* lineage contact zone[12,13], with decreased geographic distance between sampled populations near the contact zone border. I will measure tail color with a spectrophotometer to characterize the width of tail color clines, measure adult snout-vent length (SVL) to characterize potential body size differences between clades, and take tail tip biopsies for next generation genotyping. To obtain diagnostic alleles for each *P. gilberti* clade, I will sample broadly across allopatric regions of each clade's range[14]; tissue samples from allopatric regions are already available from collaborators in California and museum collections.

Laboratory Work. I will use next-generation restriction site associated (RAD) sequencing to conduct broad genomic sampling of single nucleotide polymorphisms (SNPs) in collected *P. gilberti* contact zone individuals. I will pool individuals by population and perform standard RADseq library preparation[15]. RAD sequencing allows selection of rare diagnostic SNPs per *P. gilberti* clade from tens of thousands of candidate SNPs[16]. Diagnostic SNPs reduce confounding effects of genetic drift and incomplete lineage sorting on estimates of allelic introgression across the contact zone[3,17].

Data Analyses. I will estimate the ***strength*** of selection (*s*) against color intergrades using the tail color cline width, and the dispersal distance[2] of *P. gilberti* calculated via estimates of linkage disequilibrium among loci[18]. Strong selection suggests the maladaptive nature of tail color intergrades at the contact zones, but alone is insufficient to explain polymorphism maintenance. Using the R package INTROGRESS[12], I will determine ***type*** of selection against hybrid individuals using genomic cline analysis[12]. Genomic cline analysis measures the probability of a diagnostic SNP's degree of introgression as a function of that individual's hybrid index[19] (the measured overall genome-wide admixture in that individual). If individuals across the contact zones possess low hybrid indices, this suggests a prezygotic barrier; higher hybrid indices suggest postzygotic barriers[20]. The degree and frequency of diagnostic SNP introgression informs the type of postzygotic barrier present[3]. If diagnostic SNPs are rarely underintrogressed compared to the rest of the genome, this indicates incompatible loci associated with tail color maintenance (i.e. selection against tail color intergrades)[3]; or, if underintrogressed diagnostic SNPs are more common, these loci likely reduce intrinsic hybrid fitness (e.g. underdominance due to genomic incompatibilities)[3].

Data Interpretation. At the first contact zone, I expect a narrow tail color cline width, high hybrid indices, and rare SNP underintrogression due to selection against tail color intergrades and uninhibited crossing between Sierran and southwestern clades. At the second contact zone, I also expect a narrow tail color cline due to selection against tail color intergrades, but lower hybrid indices due to incompatible body size creating a prezygotic barrier to reproduction.

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