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Landscape Genetics of the Endangered California Tiger Salamander (Ambystoma californiense) in the Los Vaqueros Watershed

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LANDSCAPE GENETICS OF THE ENDANGERED CALIFORNIA TIGER SALAMANDER (*AMBYSTOMA CALIFORNIENSE)* IN THE LOS VAQUEROS WATERSHED

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By

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ABSTRACT

Genetic diversity is essential for maintaining healthy biological systems, and dispersal-mediated gene flow increases genetic diversity by introducing new alleles to a gene pool. Populations that frequently experience gene flow will show characteristic similarities in allele frequencies. Measuring genetic diversity among many populations across a landscape has proven to be a powerful approach for assessing the ways that habitat discontinuities may affect patterns of gene flow, and in turn, influence allele frequencies and population dynamics. This study examines the landscape-level population genetic structure of the California tiger salamander, *Ambystoma californiense*, within the Los Vaqueros Watershed of Contra Costa County, California. I investigated correlations between genetic structure and landscape features using larvae collected from 16 ponds and genotyped at 12 microsatellite loci. The data indicate that gene flow is occurring among the sampled populations and that pairwise interpond distance is negatively correlated with gene flow. Furthermore, the analysis suggests a pattern of population stratification developing across the watershed, representing a landscape-mediated mechanism of genetic isolation. The results from this investigation will help address the conservation requirements for *A. californiense*, while also contributing to the body of knowledge needed for the effective conservation of similar species.

Keywords: amphibian, conservation, gene flow, genetic diversity, isolation by distance, metapopulation, microsatellite

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CHAPTER 1

INTRODUCTION

In recent years, scientists have reported an increase in the rate of population declines and extinctions of plant and animal species (Ricciardi and Rasmussen 1999; Singh 2002; McCallum 2007; Barnosky et al. 2011). Of all the taxa affected by this modern biodiversity crisis, the most severely affected has been the class Amphibia, with nearly a third of all amphibian species now threatened by population decline (Baillie et al. 2004). A number of factors are thought to be responsible for these population declines, including climate change (Thomas 2004), pesticides (Sparling et al. 2001), introduced species (Fisher and Shaffer 1996), disease (Weldon et al. 2004), and ultraviolet-B radiation (Blaustein et al. 1998). Each of these factors likely contribute to various degrees for different species, but researchers have consistently indicated that the modification and fragmentation of natural habitats is the single most pervasive factor contributing to global amphibian declines (Alford and Richards 1999; Cushman 2006; McKinney 2008).

This apparent sensitivity to environmental change has been attributed to many intrinsic factors (*i.e.*, life history and physiological characteristics) associated with the amphibians, such as their reliance on moist microhabitats for cutaneous respiration, aquatic habitats for reproduction, and their limited capacity for long-distance terrestrial movements through inhospitable habitat (Sinsch 1990). These intrinsic factors contribute to make amphibians considerably more vulnerable to the effects of human activity and

fragmented habitats. However, these characteristics also directly shape the structure of amphibian populations across a landscape. For many organisms, defining a population can be difficult; but pond-breeding amphibians have population structures that can easily be modeled as a centralized breeding site and an associated upland terrestrial habitat (Marsh and Trenham 2001; Johnson et al. 2007).

When a group of spatially separated populations are able to exchange individuals through immigration and emigration dynamics, we can collectively consider them to be a *metapopulation* (Gilpin and Hanski 1991; Hanski 1998). The dynamics of metapopulations are typically viewed in an "extinction-recolonization" framework, where individual populations (or demes) can appear and disappear over time as habitat suitability and resource availability changes. Populations that occupy high quality habitats are called sources, because they typically have high reproductive outputs and produce an excess of individuals. These extra individuals disperse away from the source population and into other populations that might only persist with the addition of these new individuals (*i.e.*, sinks), or patches of suitable habitat that had formerly experienced an extinction event. These source-sink dynamics decrease the probability of metapopulation (or landscape-level) extinction and contribute to the regional persistence of a species even as individual populations temporarily go extinct. This model offers many important insights for conservation managers, because the source-sink model explains population viability over spatiotemporal scales and addresses how populations can respond to changing habitats. Understanding the interface between an organism and

its environment is critical when planning conservation efforts and models such as these will help to elucidate the species-specific requirements for effectively preserving the global biodiversity.

In the metapopulation framework, the movement of individuals between populations is of critical importance to the probability of metapopulation persistence and therefore of great interest to population ecologists and conservation biologists. However, these disciplines have traditionally relied on direct measurements of biological diversity and distribution (Koenig et al. 1996). These direct measurements are typically obtained through methods such as visual observation, quadrat sampling, capture-recapture field techniques, or radiotelemetry (Chao 1987). Techniques such as these have relatively narrow study timeframes and can often become costly with regard to labor and resource expenditures (Colbert 1995). Implementation of these techniques may prove to be inefficient, if not impossible, for organisms whose life expectancies are too short to allow for recapture or for individuals too small for a non-invasive telemetry tagging.

Additionally, traditional methods of direct observation may provide an incomplete picture of the behavior an organism exhibits with regards to its environment (Guisan et al. 2005). Ecological field methods such as radiotelemetry have undoubtedly proven useful for certain purposes, such as identifying the home range of an individual or quantifying their relative utilization of different microhabitats. But an important ecological variable that direct measurements cannot account for is reproductive success, especially when this success follows dispersal events. This is a critically important

variable, especially within the metapopulation framework, as a group of individual populations are only held together as a cohesive evolutionary unit by the gene flow contributions of individuals who successfully disperse to and breed in populations other than those of their parents.

The discipline of landscape genetics offers a novel framework in which to study the patterns of gene flow in relation to the environment (Storfer et al. 2007; Balkenhol et al. 2009). Landscape genetics uses allelic frequency data as a replacement or supplement to traditional methods of investigating population dynamics (Pritchard and Rosenberg 1999; Guillot et al 2005; Roughgarden 2014). By incorporating a number of tools from traditional ecology, modern molecular biology and geographic information systems, landscape genetics is able to provide a comprehensive picture of spatial genetic patterns as they relate to various habitat features (Manel et al. 2003). Landscape genetics studies have effectively been used in this manner to identify wildlife corridors, which are the pathways essential for facilitating gene flow between spatially discrete populations.

Although the conceptual foundation for landscape genetics can be traced back to the papers of American geneticist Sewall Wright (1931), the full potential of this discipline was only realized with the development of polymerase chain reaction (PCR) by Kary Mullis in 1983 and the subsequent technical advances in biotechnology and modern genomics (Petren 2013). The identification of microsatellites as a molecular marker further expanded the potential of landscape genetics (Toonen 2006). Microsatellites are short stretches of a consecutively repeated nucleotide sequence, such as $(ATCA)_n$, where

ATCA represents a tetranucleotide sequence being repeated in tandem for *n* number of times. Most importantly, microsatellites are highly polymorphic and co-dominant, allowing some loci to exist as more than 30 different alleles. This multi-allelic variability is attributed to the high rate of mutation in these regions during meiosis, and their location within non-coding regions of the genome means there are no selective forces acting to reduce the frequency of mutant alleles in populations (Schlötterer 2000). These characteristics make microsatellites an appealing molecular marker, and they have now been used in various studies to quantify rates of population connectivity and dispersal, construct phylogenetic trees and pedigrees, and answer questions about the relatedness of different species or conspecific population segments (Jarne and Lagoda 1996).

In the face of growing human populations it is increasingly important that we build a complete understanding of how anthropogenic environmental changes affect patterns of biodiversity. This study utilizes multilocus microsatellite genotype data within a landscape genetics framework in order to assess the structure of salamander populations across a recently modified, heterogeneous landscape. I hypothesize that the construction of a large reservoir has influenced the genetic structure of the surrounding salamander populations and has led to increased isolation among pairs of breeding ponds. The results from this project will not only shed light on the ways that salamander populations interact with one another, but it will also help determine what environmental/anthropogenic factors are responsible for facilitating or inhibiting these patterns of interaction.

CHAPTER 2

MATERIALS AND METHODS

Study Species

The California tiger salamander, *Ambystoma californiense* (Fig. 1), is an imperiled species of amphibian, with three distinct population segments (DPSs; Fig. 2). The northern 'Sonoma DPS' and southern 'Santa Barbara DPS' are listed as 'Endangered', and the Central Valley DPS is listed as 'Threatened' under the US Endangered Species Act (USFWS 2004). In spite of legislation and ongoing conservation efforts, the California tiger salamander is still largely threatened by urbanization and the resultant fragmentation or loss of their native habitats (Barry and Shaffer 1994).

Ambystoma californiense populations can be found throughout the grasslands and foothills of California's central valley (Storer 1925). This region has a characteristic mediterranean climate with cool, rainy winters and dry, warm summers (Holland and Jain 1981). In conjunction with this pattern of precipitation, the highly impermeable and clay-rich soil profile of California's central valley allows for seasonal rainfall to accumulate into standing deposits of water called vernal pools (Barbour et al. 2007), which are essential to the reproductive life history of local amphibian species.

During the hot and arid summer months, adults are seldom observed as they take refuge underground, typically in the burrows of California ground squirrels (*Spermophilus beecheyi*) or pocket gophers (*Thomomys bottae*) (Trenham and Shaffer

2005). Following the first heavy rains of late autumn/early winter, adults migrate from their upland burrows in search of small ephemeral pool breeding sites (Searcy and Shaffer 2008, Cook et al. 2006). After an explosive period of migration, reproduction, and oviposition, the adults return to their upland foraging habitats. Larvae hatch in approximately two weeks, forage and grow in the aquatic environment, and metamorphose into terrestrial juveniles in early spring, anywhere from 3 to 6 months after oviposition (Holland et al. 1990). Juveniles then leave their natal pond in search of a suitable terrestrial habitat, where they will spend several years before maturing and either return to their natal pond, or disperse to find a different suitable breeding habitat.

Study Landscape

This study focuses on 16 breeding ponds and the surrounding landscape of the Los Vaqueros Watershed (37º48'49.7"N, 121º43'52.2"W; Fig. 3) in Contra Costa County, California, within the Central Valley DPS (Fig. 2). The Los Vaqueros Watershed is an ideal landscape for assessing the effects of anthropogenic activity on salamander populations, especially with regard to environmental modifications and habitat fragmentation. The freshwater reservoir at the center of this landscape was constructed by the Contra Costa County Water District over the course of four years, beginning in September of 1994 and reaching completion in May of 1998. The construction project for the Los Vaqueros Reservoir was initially opposed by many citizens and researchers on the grounds that habitat disturbances from the construction process, in addition to the

reservoir itself, would have detrimental effects on the environment and organisms living in the region. The Contra Costa County Water District made an up-front commitment to preserving the environment and wildlife, both during the construction project and thereafter. Throughout the building process, drift fences and pitfall traps were employed to intercept any *A. californiense* individuals moving towards areas of construction activity. Salamanders were retrieved from the traps each day and transported to safer areas of the landscape. In addition to the 1,400-acre reservoir, the Contra Costa County Water District also secured nearly 20,000 acres of the surrounding landscape to designate as protected wildlife habitats, all of which comprise the Los Vaqueros Watershed (Hunt 1999). In recognition of these efforts made by the Contra Costa County Water District, the American Society of Civil Engineers named the Los Vaqueros dam and reservoir project as the 1999 Outstanding Civil Engineering Achievement (Rogers et al. 2004).

Sampling and Extraction

Larvae were collected by Jeff Alvarez (Permit SCP-000040 & TE-027427) in 2010, sampling 16 breeding pools surrounding the reservoir within the Los Vaqueros Watershed, with GPS coordinates recorded for each location. At each breeding pool, *A. californiense* larvae were captured and tissue samples (~1cm tail tips) were taken. This is a standard practice for non-lethal sampling of amphibian tissue, and has been shown to have little adverse effect on *A. californiense* individuals (Polich et al. 2013). Excised tail tissues were placed into a pond-specific vial (50mL) containing 95% ethanol, and all

larvae were released after adequate sampling. Collected tissue samples were brought back to the lab for processing, where individual tissues were transferred from pond-specific jars into 2.0mL cryogenic vials filled with fresh ethanol. Each tube was then assigned a unique sample identification number and stored at -80C in order to create a complete tissue repository for future reference. In the process of transferring tissue samples to their individual cryogenic vials, a small sample of tail tissue between 25-50mg was excised from each individual for DNA isolation and placed into a separate 1.5mL microcentrifuge tube containing 300µL of cell lysis solution and appropriately labeled with the individual's unique identification number.

Tissue samples were manually disrupted with stainless steel dissecting scissors. Dissection scissors were rinsed in ethanol and flame-sterilized between the processing of each individual. Following tissue disruption, 1.5µL of Proteinase K (20mg/mL) was added to the resultant mixture of cell lysis solution and macerated tissue. Each tube was vortexed gently to ensure homogeneity and then placed in a heat block to incubate for 3-6 hours at 55ºC. Once cell lysis was complete, tubes were removed from the heat block and treated with 0.6µL of enzyme RNAse A (10mg/mL) to digest all RNA and leave only genomic DNA. Tubes were then incubated at 37ºC for 30-45 minutes, after which the samples were removed from heat and allowed to cool to room temperature. Upon reaching room temperature, 100µL of protein precipitation solution was added to the cell lysate solution. Each tube was then vortexed vigorously before being centrifuged for 5 minutes at 13,000 RPM. After centrifugation, the precipitated proteins formed a solid

pellet at the bottom of the tube. The supernatant, still containing the genomic DNA of interest, was carefully transferred to a new 1.5mL tube with a micropipette. The tube containing the protein-pellet was then discarded. To precipitate the DNA from solution, 300µL of 100% isopropyl alcohol was added to solution and mixed gently via inversion. Centrifugation was performed at 13,000 RPM for 5 minutes in order to pellet the genomic DNA. After centrifugation, the isopropyl alcohol was decanted and the DNA pellet was washed with 300µL of 70% ethanol via inversion. Centrifugation was repeated for 5 minutes at 13,000 rpm and the ethanol was poured off. The remaining DNA pellet was allowed to air-dry for 8-12 hours. Once dry, the DNA pellet was rehydrated in 200µL of Tris-EDTA buffer.

The final concentration of genomic DNA was quantified using a NanoDrop© NC-1000 Spectrophotometer. Successfully extracted DNA isolates were stored at -20ºC. Samples with concentrations less than 10ng/µL were discarded and DNA isolation was repeated, with new tissue being obtained from the sample repository.

PCR and Fragment Analysis

Polymerase chain reaction (PCR) was utilized for the amplification of microsatellite DNA alleles from template DNA. Twelve microsatellite loci specific to *A. californiense* were selected for use in this study from those previously identified and characterized by Savage (2008; Table 1). All of these loci exhibit a tetranucleotide repeat motif.

PCR reaction recipes were as follows: 0.2µL forward primer (10µM), 0.2µL reverse primer (10µM), 0.2µL $MgCl_2$ (25mM), 2.0µL template DNA (25 ng/µL), 2.6µL of Nuclease-free H_2O, and 5µL of Promega GoTaq MasterMix (2X) for a total reaction volume of 10µL. Thermocycler parameters were: 95.0ºC initial denaturation for 2 minutes, followed by 35 cycles of: 94.0ºC denaturation for 30 seconds, 58.0ºC annealing for 60 seconds, and 72.0ºC extension for 60 seconds. Following the completion of 35 amplification cycles, a final annealing stage of 72.0ºC was held for 3 minutes, followed by an immediate refrigeration hold at 4.0ºC.

PCR product was visualized on agarose gels at 1% concentration in 40mL of TBE stained with 1µL of ethidium bromide (10mg/mL). If amplification was successful, PCR was repeated using fluorescent primers for downstream fragment analysis. Fluorescently labeled PCR primers were designed for microsatellite fragment analysis. Forward primers were designed to serve as reporter molecules through the addition of a 5' fluorescent tag. Primers were tagged with one of four different dyes; NED (yellow), PET (red), FAM (blue), or VIC (green). This allowed for multiple loci to be analyzed simultaneously during capillary electrophoresis. In addition, loci with the same fluorescent label were designed to have non-overlapping size ranges, allowing for 5 or more loci to be analyzed in a single run. This effectively reduced many of the time and cost restraints that are typically associated with sample analysis.

Fluorescently-amplified PCR products were pooled and fragment sizes were determined for each individual at Western Kentucky University's Biotechnology Center

using an ABI 3130 automated sequencer. Capillary electrophoresis results from the sequencer were imported into GeneMapper (Applied Biosystems, Inc.) for electropherogram analysis and genotype scoring. Electropherogram peaks were scored according to the guide published by Selkoe and Toonen (2006). Loci or individuals with inconsistent amplification (<75% success) were re-run or omitted from the dataset and all subsequent statistical analyses.

Data Analysis

I utilized Micro-Checker v2.2.3 (Van oosterhout et al. 2004) to detect the potential for null alleles in the multilocus dataset. Null alleles arise from PCR failing to amplify one allele of a locus due to errors during PCR. This results in a dataset with an underestimated level of heterozygosity and skewed allele frequencies. If only one allele is successfully amplified during PCR, the genotype is erroneously scored as a homozygote based on the usual assumption that both alleles amplified without error and are simply the same size. The electropherogram peaks of a true homozygote overlap and are indistinguishable as two separate allele products.

GenAlEx 6.5 (Peakall and Smouse, 2012) was used to analyze and manipulate the dataset inside of Microsoft Excel. Using GenAlEx, I was able to graph and compare allele frequencies according to population origin or locus, calculate levels of heterozygosity, and generate F-statistics (F_{ST}, F_{IS}).

Population assignments were obtained from STRUCTURE 2.3.3 (Pritchard et al 2000), which uses multilocus genotype data to assign individuals to "clusters", or hypothetical breeding populations (K). The assignment probability of individuals results from a Markov Chain Monte Carlo (MCMC) simulation modeled after Bayesian statistical inference. I used STRUCTURE to iteratively test for all possible numbers of breeding clusters starting at K=1, which would represent a panmictic population, up to K=16, which would represent all sampling locations existing as discreet highly genetically isolated populations. Each K value simulation was repeated 5 times, with a burn-in period of 100,000 MCMC repetitions and 1,000,000 MCMC repetitions after burn-in. The analysis method utilized was "admixture based on pre-defined populations" with breeding pond of origin used as prior information.

Using the program STRUCTURE HARVESTER I identified the most likely number of genetic population clusters. The likelihood value from all five simulations for a given K value was averaged and graphed as a log-transformed value, including the standard deviation amongst simulations. To ensure proper identification of the most likely K value, the Evanno et al. (2005) ΔK method was also used to assess the most likely number of breeding clusters.

GPS coordinates for each sampling site were included in the multilocus dataset. Using GenAlEx, I obtained a matrix of pairwise geographic distances. From the mulilocus allele frequencies, I was also able to generate a matrix of pairwise fixation index values (F_{ST}) for all sampling sites. F_{ST} values are a comparison of the genetic

variability between subpopulations as compared to the total genetic variability of all subpopulations combined. This value can range from 0 to 1, where a value of 1 would suggest complete genetic isolation and a value of 0 would indicate complete panmixia. By combining the two matrices I was able to employ a mantel test in order to evaluate potential relationships between genetic distance (F_{ST}) and geographic distance.

CHAPTER 3

RESULTS

The final data set included 216 individuals from 16 distinct breeding ponds. Of the 12 microsatellite loci adapted for this study, three were discarded due to inconsistent amplification across multiple populations. Successfully amplified fragments ranged in size from 96 to 204 base pairs, and the effective number of alleles per locus varied between 5 and 13, with an average of 9.8 alleles per locus (Table 2).

Overall, populations consistently showed high levels of genetic diversity, with a mean observed heterozygosity (H_o) of 0.589 (Table 2). Micro-Checker indicated the possibility for null alleles to be present in two of the remaining nine loci. Locus B148 was flagged as showing signs of a null allele in six of the sixteen populations. Locus D032 was also flagged for showing signs of a null allele, though only in two populations. These loci were retained in the final dataset despite the possibility of null alleles being present, as a number of studies have demonstrated little to no significant difference between the accuracy of assignment tests (such as those utilized by STRUCTURE) when null alleles are removed/included (Hauser et al. 2006; Carlsson 2008).

Pairwise Fst values ranged from 0.019 to 0.192 (Table 3), indicating low to moderate differentiation among sites. The mantel test performed on combined matrices of pairwise geographic and genetic (F_{ST}) distances indicated a negative correlation existing

between geographic distance and genetic similarity (Fig. 4), indicating that dispersal and successful gene flow decreases as pairwise interpond distance increases.

Likelihood analysis results obtained from STRUCTURE HARVESTER suggest that two distinct breeding clusters likely exist on the Los Vaqueros Watershed (Fig. 5). Assignment probability values generated during STRUCTURE simulations for K=2, K=3, and K=4 (Fig. 6) were imported to R (R Development Core Team 2013) and graphed as pie charts for each breeding site, then plotted at their appropriate GPS locations to better visualizes the distribution of the inferred clusters (Fig. 7).

CHAPTER 4

DISCUSSION

Gene flow is an essential process for metapopulation health and cohesion (Petit and Excoffier 2009). This study utilized microsatellite markers in order to evaluate the spatial patterns of gene flow among populations of *A. californiense* across the Los Vaqueros Watershed. These patterns of gene flow are largely influenced by environmental features that either restrict or facilitate the dispersal of individuals. Thus, the underlying genetic structure of a metapopulation can attest to the functional connectivity and regional persistence of a species. The impedance of gene flow across a landscape can prevent the spread of novel genetic diversity (*i.e.*, mutations) and prevent the recovery of populations that suffer from reduced genetic diversity due to genetic drift. Subsequently, populations are isolated which leads to greater levels of adaptive (via selection) or non-adaptive (via drift) evolutionary divergence.

The analysis indicates that gene flow is occurring among the sampled populations and that pairwise interpond distance is negatively correlated with gene flow. These findings are in agreement with those reported in another study conducted in Solano County, California, which found that nearly one-third of first time breeding California tiger salamanders had dispersed to and been collected at ponds other than their natal ponds (Trenham et al. 2001). High rates of juvenile interpond dispersal may be facilitating the high levels of genetic diversity across the landscape, but the spatial

distribution of breeding ponds also has a direct impact on the rates of gene flow, as the comparison of genetic versus geographic distance indicated sufficient evidence of isolation by distance.

The data also indicate that gene flow is not equivalent among all pairs of populations. In other words, the STRUCTURE results do not indicate that a single panmictic population exists across the watershed. Rather, the assignment probability values generated by STRUCTURE suggest that two distinct breeding clusters have formed across the study landscape. With the reservoir included as a point of reference, a pattern of genetic isolation can be seen developing across the landscape, particularly between ponds that are located to the north or south side of the reservoir. The plots in Figure 5 indicate some support exists for the hypothesis that K=3 in this system (because the likelihood value is high and the error is low across replicates), with the major difference being a lone pond comprising a group distinct from the other two clusters of populations. This site remains distinct at higher estimates of K (Fig. 6), and may represent an interesting location to focus future investigations regarding the effects of fine scale habitat variation and population demographic history on genetic structure in this landscape (see below).

The pattern of genetic structure observed is potentially in agreement with my expectations, that the Los Vaqueros Reservoir would influence the rates of gene flow occurring across the watershed, however future work will be required to determine if this pattern existed prior to the formation of the reservoir, or if, as I predicted, the reservoir

has influenced the genetic structure of this metapopulation since its construction twenty years ago. It is possible that factors such as elevation or microhabitat variation existed prior to the construction of the reservoir that have influenced the genetic structure of this landscape. Unfortunately, samples were not collected before the reservoir was constructed, so no direct comparison of genetic patterns can be made, but using GIS data to compare the interpond corridors before and after the reservoir could allow for empirical testing of the fit between the observed pattern of genetic structure and the two alternative (pre- and post-reservoir) landscape configurations. This next step is critically important in drawing conclusions from these data because it remains to be seen how rapidly microsatellite allele frequencies might have changed over the relatively short time span since the creation of the reservoir in 1994. We can assume that the last twenty years have seen between 7 and 10 generations of California salamanders on this landscape, and the potential for large changes in allele frequencies exists. Particularly if population sizes fluctuate dramtically, and the effects of genetic drift are high. Additional, analyses regarding effective population sizes and past population bottlenecks would also be important in drawing firm conclusions from these data.

Patterns of gene flow may also be influenced by the commensalistic relationship the California tiger salamander has with populations of small burrowing mammals, such as the California ground squirrel. Ground squirrel burrows are typically constructed in open habitats such as fields, pastures, and grasslands. The burrows built by these squirrels are the primary habitat for *A. californiense*. Therefore, the location of ground squirrel

populations and their burrows will directly influence the patterns of terrestrial habitat utilization of *A. californiense.*

In addition to these burrows being used as habitats, they are also used as temporary refuge sites from predation and dessication, especially by the slower moving juveniles who emerge from ponds during the dry summer months. The protection afforded by these burrows is important in facilitating long-distance migration and dispersal of both adults and juvenile *A. californiense*. Thus, it's possible that the presence or absence of these burrows can directly affect the degree of variability seen in the distribution and abundance of local *A. californiense* populations. Quantifying the ground squirrel burrow microhabitat available to salamanders is difficult and beyond the scope of this study, but should be noted as a potential contributor to the patterns of gene flow observed in this study.

Conclusions

In this metapopulation system in the Los Vaqueros Watershed, interpond dispersal and gene flow seem to be occurring relatively often. This suggests that these populations have a reduced extinction risk relative to ponds that are more isolated. However, more work remains to be done to characterize the health of these populations, such as calculations of effective population size. The results of this study also suggest that the populations on this landscape have been bisected by the constructed reservoir. Whether the reservoir itself or other, older landscape features have had stronger influences on the contemporary

patterns of genetic diversity cannot be determined until additional GIS data are collected and analyzed. If the segregation of the two genetic clusters can be demonstrated to be the result of the reservoir, land managers might consider facilitating gene flow across the watershed to maintain connectivity where it once existed.

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APPENDIX OF FIGURES

Table 1. Twelve microsatellite markers adapted for this study from those identified and characterized by Savage (2008) for *A. californiense*.

Locus	Repeat Motif	Number of Alleles	GenBank Accession #
AcalB126	ATGG	7	EU442375
AcalB136	GGAT	3	EU442376
AcalB148	ATCC	9	EU442378
AcalD001	TATC	9	EU442379
AcalD012	TTTC	7	EU442380
AcalD017	GATA	9	EU442381
AcalD032	GATA	6	EU442385
AcalD065	TAGA	11	EU442388
AcalD071	ATCT	8	EU442389
AcalD088	AGAT	9	EU442392
AcalD102	CTAT	5	EU442394
AcalD108	ATCT	9	EU442395

Table 2. List of the nine microsatellite loci retained in the final dataset, including allelic frequency/polymorphism data as observed in the Los Vaqueros Watershed populations.

Locus	Minimum Allele Size	Maximum Allele Size	H_o	Number of Alleles	Populations w/ private alleles
*Acal*B126	133	173	0.659	11	1
*Acal*B136	150	178	0.617	8	2
*Acal*B148*	122	182	0.280	13	1
*Acal*D001	114	158	0.527	12	4
*Acal*D017	156	204	0.710	9	0
*Acal*D032*	99	151	0.686	13	1
*Acal*D071	113	137	0.579	7	0
*Acal*D102	102	118	0.537	5	0
*Acal*D108	96	152	0.706	10	1

*Possible null alleles present

Table 3. Matrix containing pairwise Fst values (above the diagonal) and Euclidian geographic distances in km (below the diagonal), for 16 populations sampled across the Los Vaqueros Watershed.

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	--	0.029	0.034	0.046	0.038	0.019	0.091	0.115	0.065	0.043	0.086	0.034	0.038	0.091	0.048	0.063
2	6.07	--	0.041	0.072	0.045	0.030	0.109	0.126	0.086	0.057	0.093	0.047	0.042	0.097	0.056	0.073
3	8.22	6.51	--	0.047	0.038	0.030	0.116	0.117	0.072	0.049	0.064	0.031	0.028	0.082	0.066	0.056
4	1.89	4.84	6.36	--	0.048	0.053	0.131	0.152	0.096	0.069	0.097	0.060	0.039	0.136	0.087	0.108
5	8.91	5.6	2.1	7.02	--	0.028	0.104	0.119	0.095	0.053	0.061	0.061	0.037	0.121	0.076	0.094
6	7.87	3.74	3.37	6.06	1.89	--	0.094	0.110	0.075	0.047	0.069	0.041	0.027	0.075	0.044	0.071
7	7.4	3.71	3.04	5.57	1.92	0.57	--	0.164	0.162	0.146	0.177	0.134	0.118	0.192	0.145	0.155
8	5.29	1.11	7.18	4.34	6.5	4.71	4.58	--	0.142	0.136	0.180	0.125	0.101	0.170	0.144	0.147
9	3.35	6.56	10.83	4.7	11.02	9.57	9.23	5.47	--	0.052	0.107	0.042	0.079	0.093	0.075	0.037
10	1.98	7.48	10.2	3.86	10.85	9.72	9.28	6.55	2.46	--	0.062	0.021	0.059	0.070	0.040	0.044
11	4.05	6.4	4.81	2.68	6.2	5.99	5.42	6.3	7.27	5.9	--	0.065	0.088	0.123	0.111	0.096
12	2.82	8.64	10.88	4.68	11.7	10.69	10.22	7.74	3.49	1.22	6.33	--	0.048	0.052	0.046	0.037
13	9.22	6.04	1.97	7.33	0.45	2.34	2.35	6.94	11.4	11.17	6.36	12	--	0.100	0.063	0.071
14	7.39	3.05	3.84	5.63	2.56	0.7	0.8	4.01	8.94	9.2	5.84	10.2	3.01	--	0.067	0.079
15	3.45	5.42	10.13	4.34	10.14	8.6	8.3	4.33	1.2	3.24	7.01	4.4	10.54	7.95	--	0.069
16	2.02	7.97	8.97	3.3	10.04	9.29	8.77	7.27	4.71	2.39	4.25	2.24	10.29	8.9	5.17	--

Figure 1. Adult California Tiger Salamander.

Figure 2. Range of the California Tiger Salamander in the Great Central Valley of California (in purple). Gold boxes represent individual locality records, and arrows denote the three distinct population segments delineated by the USFWS, and the Los Vaqueros study site.

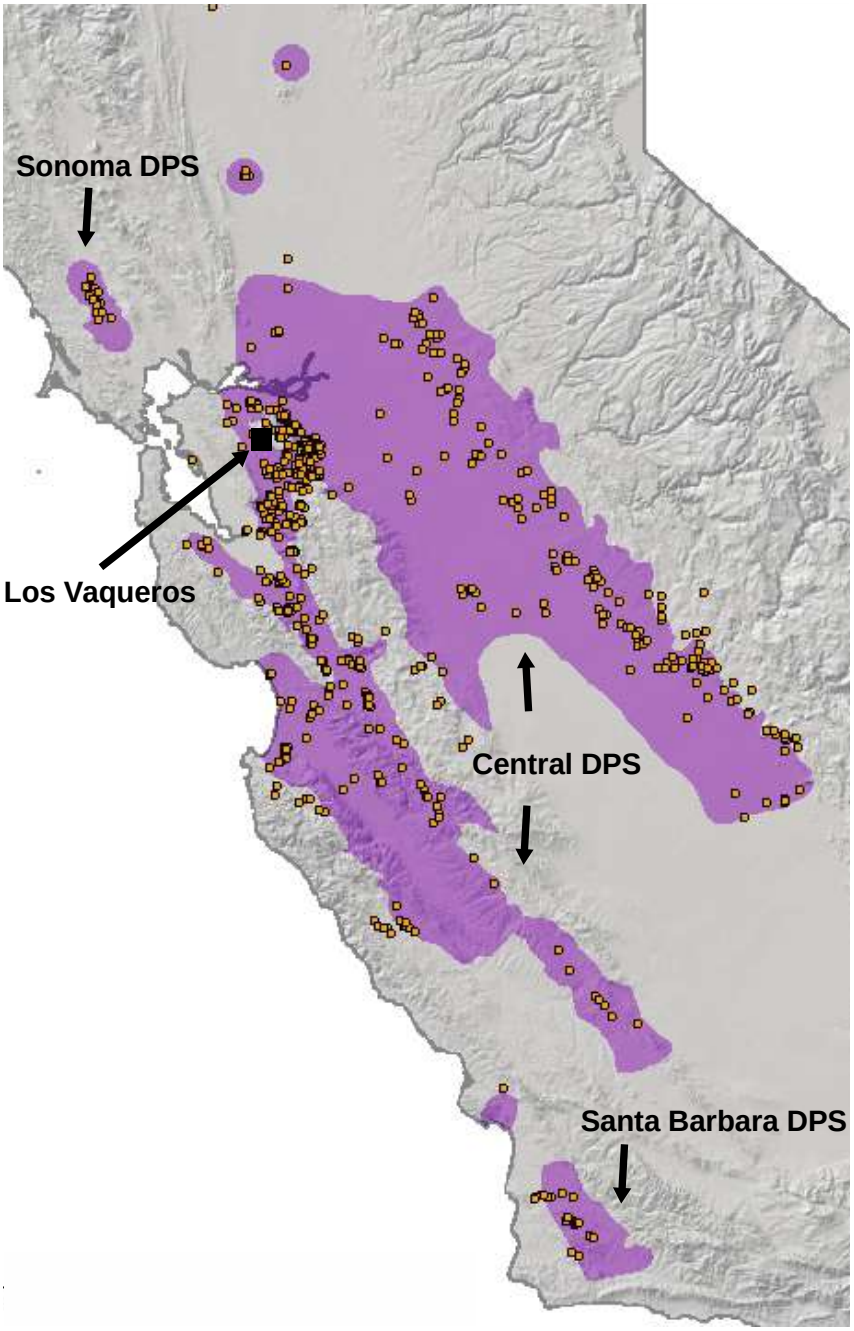


Figure 3. Study ponds in the uplands adjacent to the Los Vaqueros Reservoir. Image from Google Earth.

Figure 4. Isolation by distance (IBD) scatterplot comparing pairwise Fst values and log-transformed geographic distances (in km).

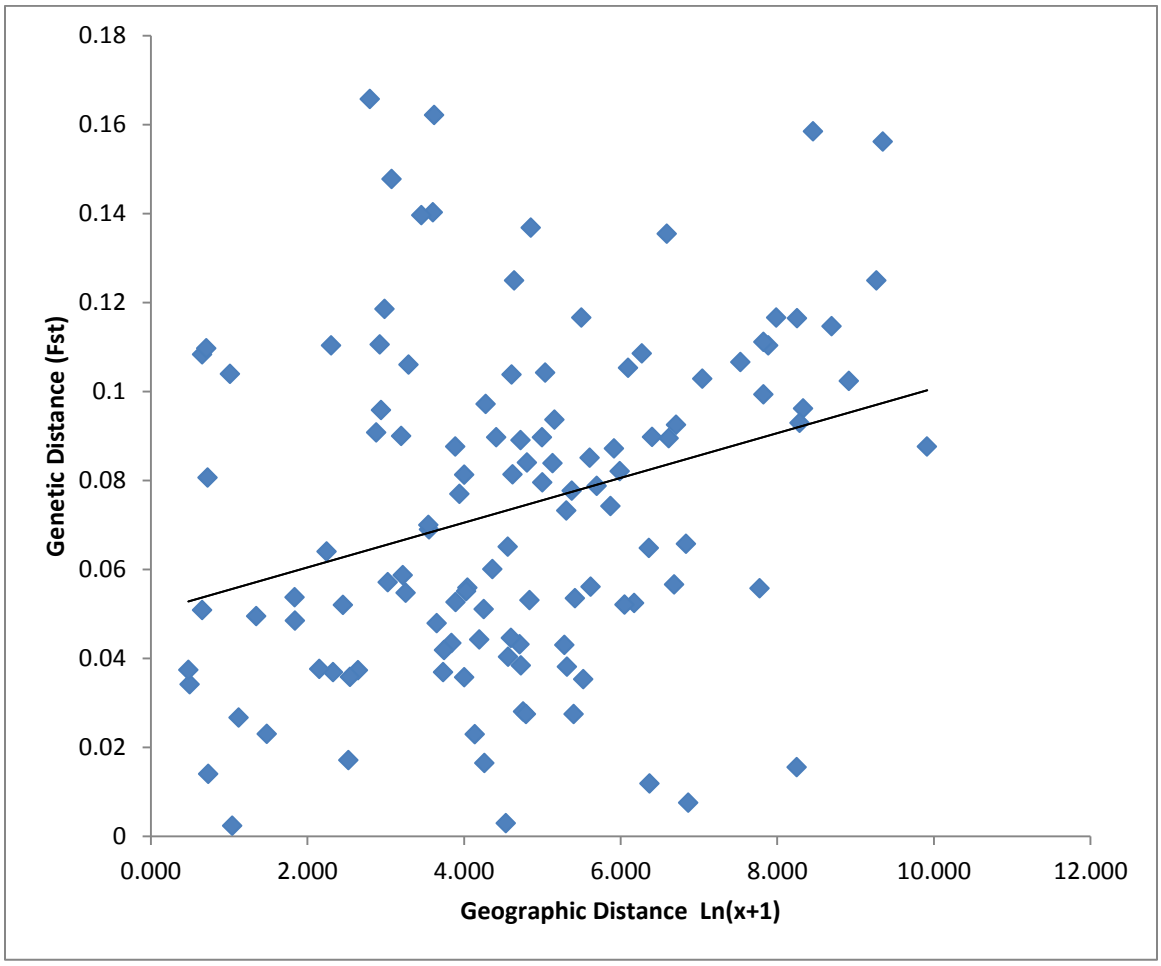
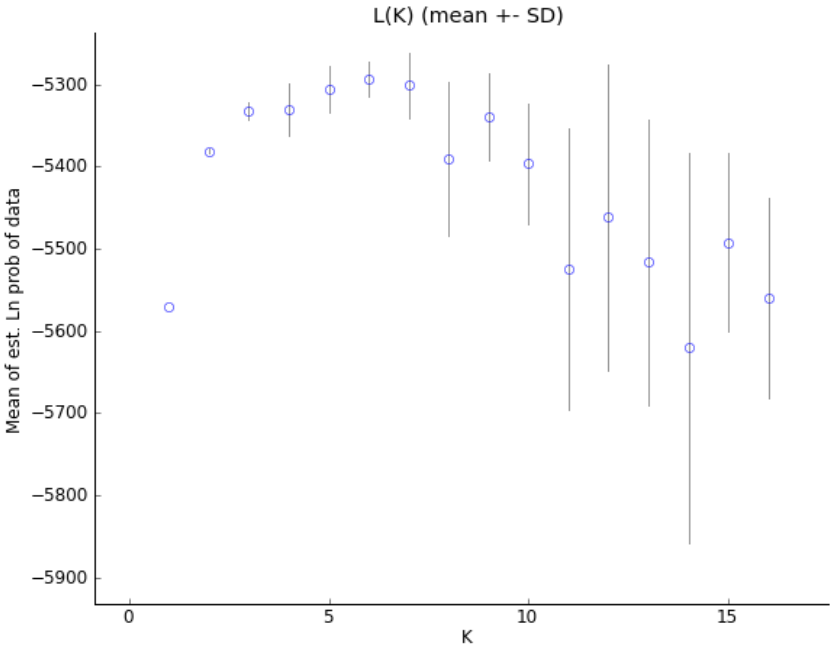


Figure 5. STRUCTURE HARVESTER output. **A)** Mean log-likelihood values (±SD) and **B)** DeltaK for five replicate runs of 1,000,000 iterations at each K value 1-16.



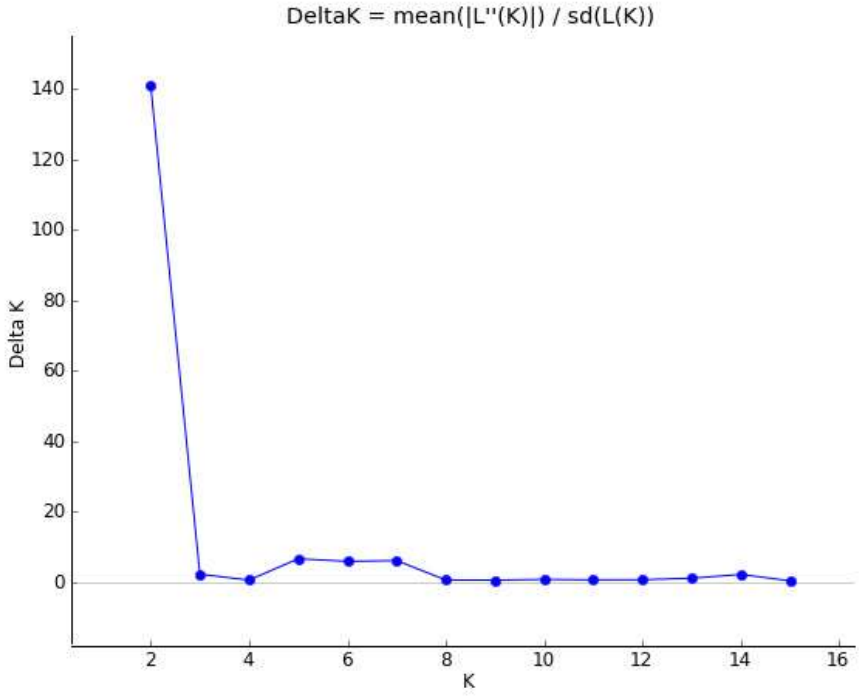


Figure 6. STRUCTURE histogram outputs depicting the assignment probabilities (Y-axis) for a genetic cluster. Each color represents a cluster and each pixel column represents an individual. Sampling sites are delineated along the X-axis by vertical lines.

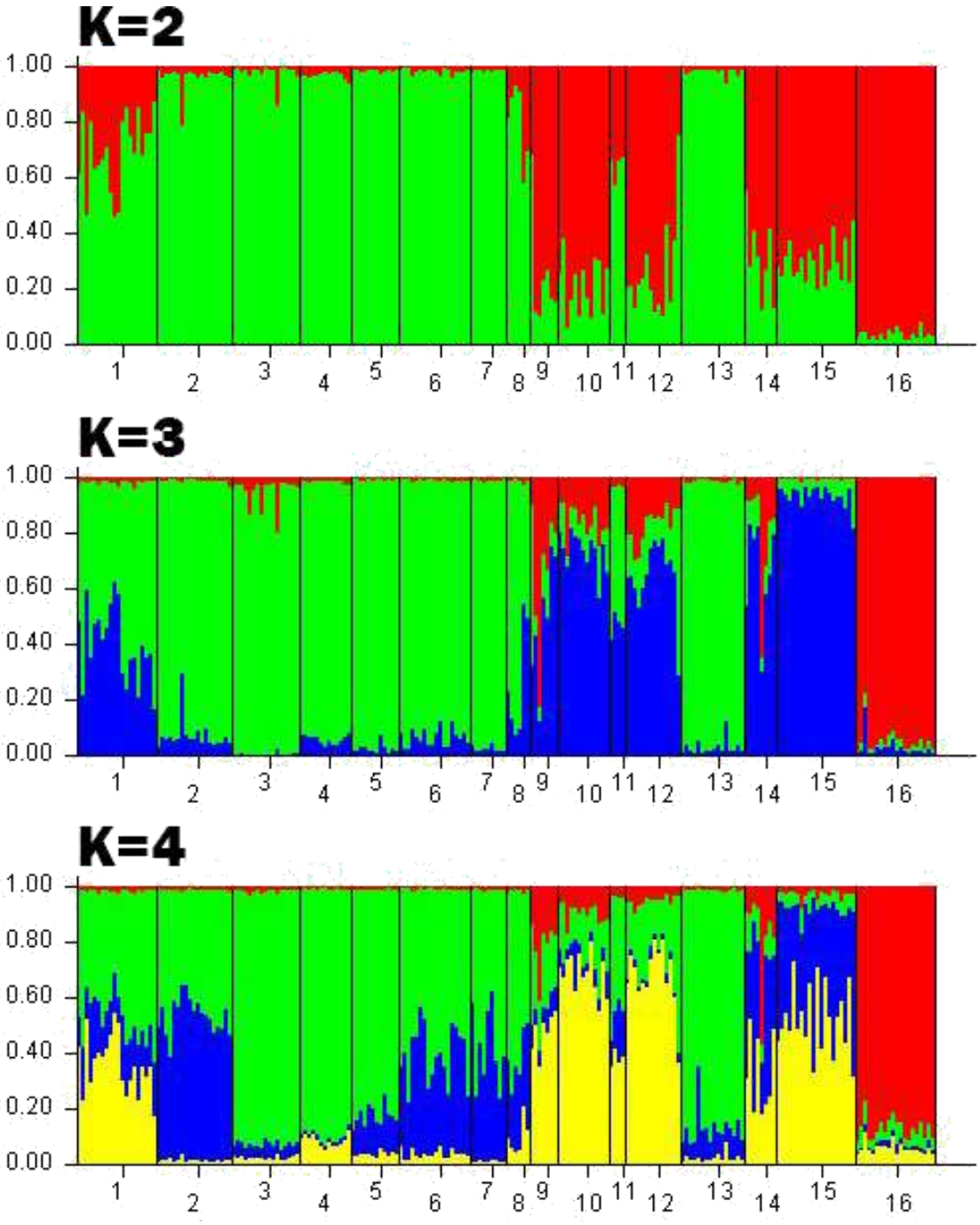


Figure 7. Cluster assignment probabilities depicted as pie charts for each sampling site and superimposed on a map of the Los Vaqueros Watershed. Reservoir included as point of reference. The Alameda County line is visible near the southern-most sampling sites.