Western Kentucky University
TopSCHOLAR®

Honors College Capstone Experience/Thesis Projects

Honors College at WKU

6-28-2017

Analysis of Population Structure in a California Newt (Taricha torosa) Metapopulation

Jessica Vincent
Western Kentucky University, jessica.vincent863@topper.wku.edu

Follow this and additional works at: http://digitalcommons.wku.edu/stu_hon_theses

Part of the Animal Experimentation and Research Commons, Genetics Commons, Population Biology Commons, and the Terrestrial and Aquatic Ecology Commons

Recommended Citation
Vincent, Jessica, "Analysis of Population Structure in a California Newt (Taricha torosa) Metapopulation" (2017). *Honors College Capstone Experience/Thesis Projects.* Paper 710.
http://digitalcommons.wku.edu/stu_hon_theses/710

This Thesis is brought to you for free and open access by TopSCHOLAR®. It has been accepted for inclusion in Honors College Capstone Experience/Thesis Projects by an authorized administrator of TopSCHOLAR®. For more information, please contact topscholar@wku.edu.

ANALYSIS OF POPULATION STRUCTURE IN A CALIFORNIA NEWT (*TARICHA TOROSA*) METAPOPULATION

A Capstone Experience/Thesis Project

Presented in Partial Fulfillment of the Requirements for

The Degree of Bachelor of Science in Biology with

Honors College Graduate Distinction at Western Kentucky University

By

Jessica Jo Vincent

*****

Western Kentucky University

2017

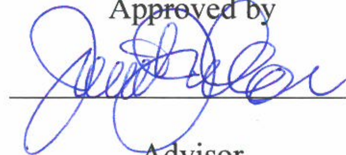
CE/T Committee:

Professor Jarrett Johnson

Professor Ajay Srivastava

Professor Christopher Keller

Approved by



Advisor

Department of Biology

Copyright by

Jessica Jo Vincent

2017

ABSTRACT

As anthropogenic influences take an ever-increasing toll on the environment, understanding how environmental change affects species is paramount. Concern regarding decline in amphibian populations has spurred research examining the effects of habitat change on the dynamics of populations at landscape levels. One important goal is to understand how gene flow among populations is affected by changes in habitat. Biologists need to consider the relationship between gene flow and habitat alterations so that movements among individual breeding ponds can be maintained over time, reducing risk of local extinction events. This study focuses on patterns of gene flow among thirteen populations of California newt (*Taricha torosa*). We examined genetic structure using five microsatellite markers. We found little evidence of genetic structure on the studied landscape despite extensive variation at our microsatellite loci. Support for models of population structure was weak, and estimates of gene flow among population pairs were high, suggesting that interpond movements on this landscape are extensive. These findings are contrary to our expectations and deviate from other work on these landscapes for other species. Further analyses are required to determine the scale at which we would observe a pattern of genetic structure in this species, but ultimately, these data will be informative for conservation efforts directed at the California Newt.

Key words: Amphibian conservation, Landscape genetics, Population structure

Dedicated to my Friends and Family

ACKNOWLEDGEMENTS

I would like to thank my CE/T advisor, Dr. Jarrett Johnson for constant help and encouragement throughout this process, as well as all my fellow in his lab who were always readily available to assist or commiserate, whatever the case; Especially, I would like to thank Dana Leigh for her contribution to the data set.

This project would not have been possible without the Biology Department and the Biotechnology Center who never begrudged their time and resources; The Faculty-Undergraduate Student Engagement program, which funded the project; and the Honors College, whose staff was always there for guidance and support.

Finally, I would like to thank my Mother and Father, my brothers, and my close friends for never failing to remind me that I can accomplish whatever I put my mind to.

VITA

July 21,1995………………………………………………… Born – Lexington, Kentucky

2013………………………….. Anderson County High School, Lawrenceburg, Kentucky

2015…………………………….... Laboratory Technician, Western Kentucky University

2015…………………………………. Faculty-Undergraduate Student Engagement Grant

2016……………………………….…… Kentucky Academy of the Sciences Presentation

Fields of Study

Major: Biology

Major: Psychological Science

Minor: Neuroscience

TABLE OF CONTENTS

Abstract....iii

Dedication....iv

Acknowledgements....v

Vita....vi

List of Tables....viii

List of Figures....ix

Chapters:

1. Introduction....1
2. Methods and Materials 5
3. Results....11
4. Discussion....14

References....19

Appendix of Tables....23

Appendix of Figures....27

Appendix of Solutions....33

LIST OF TABLES

Table 1: Index of Populations..23

Table 2: Aspects of Microsatellite Loci...24

Table 3: P- values for Hardy Weinberg Exact Tests with Locus......................................25

Table 4: Table of F_{st} Values...26

LIST OF FIGURES

Figure 1: A) County map of California showing range of *Taricha torosa;* B) Adult California Newt, *Taricha torosa;* C) Larval California Newt, *Taricha torosa*.................27

Figure 2: Bar plots from STRUCTURE depicting assignment of individuals from the eight populations into four putative populations 28

Figure 3: Delta K-values from Structure denoting likelihood of various genetic subpopulations..29

Figure 4: Mean log-likelihood of data under sequential models of the number of populations 30

Figure 5 Map of pond locations 31

Figure 6: Scatter plot depicting relationship between geographic and genetic distance for pairwise data between populations 32

CHAPTER 1

INTRODUCTION

Population declines culminating in extinction are a major threat to biodiversity, and recently the rates of declines have been increasing (Ricciardi and Rasmussen, 1999). Further, population declines are proceeding at a rate that is greater in some taxa than in others (Stuart et al., 2004). Specifically, amphibian populations have been more dramatically affected by this apparent global decline in biodiversity when compared to other vertebrate taxa such as birds or mammals (Baillie et al., 2004; Halliday, 2008; Houlahan et al., 2000). Nearly one-third of amphibian populations were shown to be experiencing population decline in 2004, with that number only expected to increase (Carey et al., 2001; Mendelson et al.,2006).

Although many factors, such as pollution, disease, and invasive species play a role in the decline of amphibian populations, scientists consistently find that the largest factor is habitat loss and fragmentation due to anthropogenic activities (Cushman, 2006; McKinney, 2008; Salice, 2012). Human land-use, such as destruction of forests for agricultural or urban purposes, creates problems for the over-land movements of forest-associated amphibians and limits the connections that should exist between adjacent populations. Monitoring the relationship between amphibian populations and their

landscape is crucial to providing conservation efforts to combat population decline. (Storfer et al., 2007)

Fortunately, many amphibian populations are easy to delineate compared to populations of other organisms because of their dependency on discrete aquatic breeding sites (Johnson et al. 2007). Conceptually, we can think of natural landscapes as supporting multiple semi-independent populations that exchange dispersers and exist as a "meta-population", or "population of populations" (Hanski, 1998). Pond-breeding amphibians fit the metapopulation paradigm well, with small breeding subpopulations centered on aquatic breeding habitat distributed across heterogeneous landscapes. High quality subpopulations create a surplus of individuals, which then disperse to other, potentially less sustainable subpopulations within the whole. Under this model, the more successful subpopulations are referred to as sources, while their lower quality counterparts are known as sinks. The immigration and emigration dynamics of metapopulations decrease the likelihood of regional extinction events because any subpopulation that goes extinct locally will reliably be recolonized with dispersers from other extant subpopulations. Understanding interactions among subpopulations within metapopulations is a crucial early step in developing plans to moderate the effects of anthropogenic habitat changes and promote the health of amphibian species.

An important dynamic of an amphibian metapopulation is gene flow, or the movement of organisms among individual breeding ponds over the course of many

generations. Understanding this movement enables us to identify sources and sinks of the metapopulation and determine which populations, if any, are isolated.

The field of landscape genetics provides a conceptual framework for understanding the connectivity among breeding ponds within a metapopulation. Landscape genetics combines genetic, ecological, and geographical inferences in a combined statistical framework to determine the relationship between landscape features and the movement of individuals to understand patterns of gene flow and the genetic structure of populations (Holderegger and Wagner, 2006). Landscape genetic studies provide empirical data that describe the dynamics of metapopulations and play a major role in driving modern conservation approaches, and therefore, are closely aligned with the initiatives of conservation biologists (Manel et al., 2003; Storfer et al., 2007).

With more and more amphibians facing placement on the endangered species list, it is critically important that ecologists and conservation biologists understand the patterns of connectivity and genetic structure of metapopulations as well how anthropogenic environmental changes disrupt dispersal and gene flow, further jeopardizing biodiversity. The objective of this study is to gain a better understanding of the pattern of dispersal and gene flow among subpopulations of the California Newt (*Taricha torosa*) on two largely intact contiguous landscapes in central California. This study uses variable, neutral genetic markers to reveal genetic structure within the metapopulation that exists on these landscapes. Information generated from this project can be applied to conservation efforts aimed at preserving natural interactions among

subpopulations within the metapopulation, and decrease the extinction risk of the population and the species as a whole.

As with many previous studies, we expect to find a general relationship between geographic distance and the genetic differentiation among pairs of subpopulations, called “isolation by distance.” Such a relationship results from diminishing rates of gene flow between pond pairs that are separated geographically. We also expect to reveal a relationship between landscape features, such as roads, on our estimates of gene flow between sites. This expectation is based both on published landscape genetic literature (*e.g.*, Manel et al., 2003; Storfer et al., 2007) and previous unpublished work (Leigh and Johnson, unpublished). Landscape features, both natural and anthropogenic, can affect rates of gene flow among populations, resulting in population genetic structure across a landscape; however, it is important to recognize that the degree to which the study landscape is heterogeneous will affect the intensity of genetic structure observed.

CHAPTER 2

METHODS AND MATERIALS

Study Species

The California Newt, *Taricha torosa* inhabits coastal regions of California and the southern portion of the Sierra Nevada (Fig. 1A). Their habitat consists of woodlands and chaparral along with small breeding ponds and creeks. During hot summer months, California Newts remain terrestrial, preferring to stay in moist environments. Newts become aquatic during breeding seasons and migrate over land to breeding ponds (Nafis, 2016).

The coloration of adult newts varies between a rough yellowish to brown on the dorsal side and light yellow to orange on the ventral side (Fig 1B). Larval California newts are lightly colored with narrow dark bands along their back (Fig. 1C). Adult California Newts produce tetrodotoxin, but eggs and larvae are not poisonous, leaving them vulnerable to predation, especially from introduced invasive species such as mosquitofish and crayfish (Gamradt and Kats, 1996).

Although conservation biologists are concerned about California newt populations they are currently only considered a California Species of Special Concern (SSC). However, some subpopulations in San Diego County have become extinct due to human activities (Thompson et al., 2016).

Study Sites

Samples were collected from thirteen breeding ponds located in adjacent protected regions in Santa Clara County near San Jose, California in 2007. The Blue Oak Ranch Reserve is part of the California Natural Reserve System and is operated by University of California, Berkeley. Because of its educational uses, the reserve does contain manmade research facilities and classrooms for those working on projects in the area. The Blue Oak Ranch encompasses 1,319 ha of land situated at an elevation of 454-870 m. (University of California Regents, 2017)

At 4,403 ha, Joseph Grant County Park is the largest park and recreational area in Santa Clara County. This area on the western slope of Mt Hamilton receives approximately 600 mm of precipitation per year. As a government run park, Grant County does have 51 miles of hiking and mountain biking trails and facilities. These areas are characterized by a mix of forests of a variety of Oak trees and grasslands with permanent and seasonal streams and ponds throughout. Blue Oak Ranch Reserve and Grant County Park are home to approximately 660 different species of plants and vertebrate animals. (County of Santa Clara, CA, 2017)

Molecular Markers

Microsatellite DNA markers are commonly used to discern genetic variation for landscape genetics studies because they are highly variable due to elevated mutation rates versus other genetic marker types (Chistiakov et al., 2006). Further, because

microsatellites are found in non-coding DNA, mutations are not selected against and accumulate in populations. Using highly variable microsatellite loci as a target, landscape geneticists are able to identify changes in connectivity over short periods of evolutionary time, even between generations (Schlötterer, 2000; Toonen, 2006). Microsatellite markers have flanking regions that are typically conserved among multiple alleles for the same gene allowing for these high variation alleles to be amplified by PCR. Comparing the frequencies of these alleles among subpopulations within the metapopulation gives ecologists accurately quantified rates of organism dispersal from sources and sinks as well as the overall connectivity of the population.

Data Collection

Samples were collected as part of a different project aimed at detecting the prevalence of chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis*, at the two reserves (Padgett-Flohr, 2008). Between sampling in 2007 and the onset of the current study, whole larvae were kept in pond specific vials of 95% ethanol and stored at -20C. Approximately 0.10 mg of tissue was removed from the tail of each larval individual and the tissue was digested in a guanidine thiocyanate cell lysis and Proteinase K (20mg/mL) solution. These tissue samples were incubated in a heat bath at 55C for 3-6 hours or until no visible tissue was left. The tubes were vortexed and treated with 0.6 uL RNAse (10mg/mL) and placed in a second heat bath at 37C for 30-45 minutes. Afterwards, samples were cooled to room temperature and a protein precipitation solution was added.

Tubes were placed in a microcentrifuge for 5 minutes at 1,300 rpm. The DNA-containing supernatant was moved to a new microcentrifuge tube and 300 uL of 100% isopropyl alcohol was added to precipitate the DNA. The tube was inverted several times to mix the solution before the centrifugation step was repeated. The supernatant was emptied and 300 uL of 70% ethanol was added to wash the pelleted DNA and centrifugation was repeated. After thorough rinsing, the DNA was left to air-dry over-night. The now dried and purified genomic DNA was resuspended in 100 uL of 10mM tris-CL buffer, pH 8.0, and concentrations were quantified using a NanoDrop© NC-1000 Spectrophotometer and stored at -20C.

Microsatellite primers designed for a closely related species, *Taricha granulosa*, were optimized for PCR in *Taricha torosa.* After extensive annealing temperature and magnesium concentration optimization, 5 microsatellite loci were found to be compatible with the target *T. torosa* DNA, see Table 1 (Jones et al., 2001). A selection of 12 samples from each breeding pond, excluding Dean's Valley, which only had 7 samples total, were selected for PCR analysis using 5uL of GoTaq Master Mix (2X), 0.2 uL of the forward primer and 0.2 uL of the reverse primer for each microsatellite locus, 2.0 uL of DNA template (diluted to 25mg/mL), and 2.6 uL of nuclease free water on an Eppendorf Mastercycler.

PCR products were electrophoresed on 1% agarose gels (0.5g agarose, 50mL of 1X TBE buffer, 2 uL of ethidium bromide). Images of the success or failure of PCR were captured using an Alpha Innotech multi image light cabinet. Successful PCRs were

repeated using forward microsatellite primers fluorescently labeled with PET (red), 6-FAM (blue), or NED (yellow). All five PCR products were combined along with the DNA Ladder LIZ600 (orange) and analyzed in multiplexed assays using capillary electrophoresis in an ABI 3130 Sanger Sequencer using GeneScan. The chromatograms were scored using ABI GeneMapper software.

Data Analysis

Scored allelic data was exported from GeneMapper and input to Genepop (Raymond et al., 1995) for calculation of allelic diversity, differentiation among populations (F_{ST}), and an analysis of molecular variance. F_{ST} measures a reduction in heterozygosity between a pair of subpopulations compared to the total population. The genetic markers used for analysis of gene flow within the metapopulation are expected to be neutral; that is, they should not be subject to selection or closely associated to any genes that are under the influence of evolutionary forces, the neutrality of the genetic markers can be estimated using Hardy-Weinberg Exact Tests implemented in Genepop.

Scored allelic data were also imported in to STRUCTURE (Pritchard et al., 2000) to genetically determine the level of subdivision in the total sample. In STRUCTURE, 10 replicate tests for all integer values of K, or the number of separate genetic populations within the metapopulation, between K=1 and K=10 were tested with 1,000,000 MCMC iterations after a 100,000-iteration burn-in. This allowed patterns of genetic structure to be evaluated without geographical bias. Data from STRUCTURE was used in

conjunction with the program HARVESTER (Earl and vonHoldt, 2012) to estimate the most likely K-value for the study landscape based on the method of Evanno (2005).

CHAPTER 3

RESULTS

Hardy Weinberg Equilibrium and Fst

We analyzed genetic data for 96 larval newts from eight breeding ponds (Table 1) using the five microsatellite loci (Table 2) that were optimized for *Taricha torosa*. Table 3 shows data for Hardy-Weinberg Equilibrium (HWE) exact tests by both locus and population. Minor idiosyncratic deviances from HWE expectations were shown, but overall HWE equilibrium was supported. Do to the lack of a consistent bias against HWE across populations or loci and limited nature of the data, no populations or loci were excluded from data analysis.

Data for differentiation between ponds, F_{st}, is shown in Table 4. F_{st} values can vary between 1.0 and 0.0 and represent the reduction in heterozygosity observed as a result of population subdivision. When the total population (T) is divided into subpopulations (S), the value of F_{st} is close to 1.0 if differentiation among subpopulations is high and close to 0.0 if differentiation is low; therefore, measures of F_{st} can be used to infer gene flow between pairs of subpopulations, which reduces differentiation due to genetic drift and results in low F_{st} values. For nearly all comparisons, estimates of heterozygosity across putative subpopulations were not shown to be significantly

different from the observed heterozygosity for the whole population. The only notable exception is for Mudd pond, which may suffer from some sampling bias.

Genetic Assignment Tests

Structure analysis showed slight qualitative support for dividing the data into four separate genetic populations within the metapopulation (i.e., K=4), see Fig.2). The deltaK values, which are used to objectively determine the best supported value of K, have a several peaks (Fig. 3).; however, visual inspection of the mean likelihood values for alternative values for K produced in HARVESTER disputes this result. The log-likelihood values for different values of K decrease for values of K greater than 1 (Fig. 4). These lines of evidence support the existence of a single well-connected panmictic population, rather than the metapopulation being broken into independent genetic subpopulations. The lack of support for population subdivision from the HARVESTER analysis supports the hypothesis of frequent gene flow among sampling sites in this system, which was indicated by low F_{st} values above shown in Table 4.

Isolation by Distance

Populations tend to be more genetically differentiated as geographic distance increases, creating a positive correlation between genetic and geographic distances. High connectivity between discrete breeding ponds within this metapopulation should diminish the expected effect of isolation by distance. The relationship between geographic distance

and the pairwise F_{st} values among our sampling sites do not show a strong relationship (Fig 6).

CHAPTER 4

DISCUSSION

The purpose of this study was to quantify the gene flow among breeding ponds within a metapopulation of an anthropogenically altered landscape. Having high levels of connectivity between populations promotes the longevity of the population and prevents local extinction events. Multiple studies of this type have been completed with various species over multiple years in the same sampling sites (Leigh and Johnson, unpublished). Comparison of these sets of data can illuminate the overall health of the amphibian populations in the area and the effect that human activity is having on their survival.

Microsatellite loci are located within the introns of the DNA and therefore are expected to be under low selective force. Due to this, these loci should be acting within HWE. Deviations from HWE would suggest that our loci are either under the influence of evolutionary forces such as drift or natural selection or closely linked (*i.e.,* nearby on the chromosome) to genes that are. For our data, significant deviances from HWE were exhibited only in locus Tgr14 (P=0.0479, Table 3); however, these deviations were not consistent throughout the data set. The average population P-value for this locus was depressed by outlier values from the 'Yerba Buena' and 'Mudd Pond' populations, which had p-values for deviation from HWE at this locus of 0.0155 and 0.0152, respectively.

Mudd Pond demonstrated significant deviation from HWE across multiple loci with an overall P-value of 0.0018 (Table 3). A single sample site could exhibit consistent deviation from HWE across all loci due to sampling error. For example, our sample of individuals could be providing a biased estimate of the true allele frequencies in the population due to the sampling of closely related individuals (*e.g.*, full-siblings.) Since our samples were collected as free-swimming larvae, we expect that we are obtaining an unbiased sample when we drag a seine or net through the pond. However, if the related larvae form "schools" perhaps we did not obtain a random draw of allelic diversity.

Differentiation between ponds, as depicted by F_{st}, was very low across all sampling sites. Low F_{st} values reflect a high level of gene flow among ponds within the metapopulation. F_{st} values closer to 1.0 significantly different from zero would indicate a pairing of ponds that do not have sufficient gene flow. Whether the observed value for F_{st} indicates sufficient gene flow is occurring between pond pairs between is demonstrated by a P-value <0.05 in Table 4. Mudd pond again demonstrated significant P-values for F_{st} when paired with all other ponds in the data set. This suggests an alternative scenario regarding Mudd pond than the one described above. If we now assume that Mudd is isolated from the rest of the ponds, we would expect genetic drift to be playing a large role in altering allele frequencies from one generation to the next. If the effective population size of the pond is also low, the effects of drift could be great, and therefore drive allele frequencies out of HWE.

Significant differentiation *(i.e.,* high F_{ST}) was also seen between Hotel Pond and North Pond as well as Hotel Pond and Washburn Pond. This would not be unexpected considering the geographic distance between these ponds (Fig. 5). However, a strong relationship between F_{st} and distance was not demonstrated by the entire metapopulation. When F_{st} is plotted in relation to distance, high connectivity between breeding ponds is supported. Since an increase in geographic distance is expected to correlate to an increase in genetic differentiation, the absence of a significant slope for the line of best fit for these data suggests that overall, even ponds that are distant from one another have a high level of gene flow (Fig. 6). Landscape features, whether natural or anthropogenic, are not having a significant effect on gene flow.

When data were analyzed using STRUCTURE, there was no reliable indication of any subdivision of breeding ponds within the metapopulation. We chose to include a K=4 model (Fig. 4) based on the results from data analyzed for *Taricha torosa* for data collected from this same landscape in 2006 (Leigh and Johnson, unpublished) which did suggest the K=4 population structure (Fig. 3) However, the K=4 model was not supported by further analysis using HARVESTER. Instead, the HARVESTER data strongly support the conclusion of a highly interconnected panmictic population comprised of multiple breeding areas on this landscape (Fig. 4).

The expected result of this analysis was that the metapopulation would demonstrate at least weak genetic structure because there are some anthropogenic landscape changes, such as roads, even though the landscape is protected.; and because,

even without strong physical barriers to movement of individuals between breeding ponds, gene flow should be naturally restricted by distance with ponds that are further from one another showing higher levels of differentiation. Instead,our data supports the null hypothesis that the metapopulation consists of one highly connected group of breeding ponds. However, this is perhaps incongruent with the results of the analysis of the same landscape for 2006, (Leigh and Johnson, unpublished). Data collected in 2006, one year prior to the current study much more strongly suggested four genetic subpopulations within the metapopulation (Fig 4.). However, the structure detected in the 2006 data was not indicative of a strong pattern of isolation among subpopulations.

Conclusions and Future Directions

Having a higher level of gene flow within the metapopulation is an encouraging sign for the continued health of the metapopulation. Individuals are able to disperse among breeding ponds, increasing levels of heterozygosity, maintaining genetic diversity, and enhancing the likelihood of the population's survival. The minimal disturbances present on this landscape do not appear to be substantially reducing gene flow among breeding sites; therefore, the levels of gene flow currently occurring will likely ameliorate the differentiation of subpopulations owing to genetic drift, and the genetic diversity of this metapopulation will not dramatically decline.

Data for both *Taricha torosa* and other species over a large span of time have been and will be analyzed over this landscape. Data from these populations will be

compiled and compared to each other as well as geographic information to get an idea of the corridors of gene flow on the landscape and how possible anthropogenic activities could influence the movement of organisms. These data can be used by land management and conservation teams to further promote the preservation of amphibians on this landscape and to help develop strategies to stem the decline of amphibians globally.

REFERENCES

Baillie, J., Hilton-Taylor, C., Stuart, S.N., Commission, I.S.S., (2004). 2004 IUCN red list of threatened species: a global species assessment. IUCN.

Carey, C., Heyer, W., Wilkinson, J., Alford, R., Arntzen, J., Halliday, T., Rand, A. (2001). Amphibian declines and environmental change: Use of remote-sensing data to identify environmental correlates. *Conservation Biology, 15*(4), 903-913.

Chistiakov, D. A., Hellemans, B., & Volckaert, F. A. (2006). Microsatellites and their genomic distribution, evolution, function and applications: A review with special reference to fish genetics. *Aquaculture*, *255*, 1-29.

County of Santa Clara, CA. (2017). Joseph D. Grant County Park. Retrieved 2017, from https://www.sccgov.org/sites/parks/parkfinder/Pages/JosephDGrant.aspx

Cushman, S.A. (2006). Effects of habitat loss and fragmentation on amphibians: A review and prospectus. *Biological Conservation*, *128*, 231–240.

Croshaw, D. A., & Glenn, T. C. (2003). Seven polymorphic microsatellite DNA loci from the red-spotted newt (*Notophthalmus viridescens*). *Molecular Ecology Notes*, *3*, 514-516.

Earl, D.A. & vonHoldt, B.M. (2012). Structure Harvester: A website and program for visualizing structure output and implementing the Evanno method. *Conservation Genetics Resources, 4*: 359.

Evanno, G., Regnaut, S. and Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, *14*(8), 2611-2620.

Gamradt, S., & Kats, L. (1996). Effect of introduced crayfish and mosquitofish on California newts. *Conservation Biology*, *10*(4), 1155-1162.

Halliday, T. R. (2008). Why amphibians are important. *International Zoo Yearbook, 42*(1)*,* 7-14.

Hanski, I. (1998). Metapopulation dynamics. *Nature*, *396*, 41.

Holderegger, R., & Wagner, H. H. (2006). A brief guide to landscape genetics. *Landscape Ecology, 21*(6), 793-796.

Houlahan, J. E., Findlay, C. S., Schmidt, B. R., Meyer, A. H., & Kuzmin, S. L. (2000). Quantitative evidence for global amphibian population declines. *Nature*, *400*, 752-755.

Johnson, J.R., Knouft, J.H., Semlitsch, R.D. (2007). Sex and seasonal differences in the spatial terrestrial distribution of gray treefrog (*Hyla versicolor*) populations. *Biological Conservation*. *140*, 250–258.

Jones, A. G., Blouin, M. S., & Arnold, S. J. (2001). Genetic variation in two populations of the rough-skinned newt (*Taricha granulosa*) assessed using novel tetranucleotide microsatellite loci. *Molecular Ecology Notes*, *1*, 293-296.

Manel, S., Schwartz, M.K., Luikart, G., Taberlet, P. (2003). Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology & Evolution*, *18*, 189–197.

McKinney, M.L. (2008) Effects of urbanization on species richness: A review of plants and animals. *Urban Ecosystems*, *11*, 161–176.

Mendelson, J., Lips, K., Gagliardo, R., Rabb, G., Collins, J., Diffendorfer, J., Brodie, E. (2006). Confronting amphibian declines and extinctions. *Science*, *313*(5783), 48-48.

Nafis, G. (2000-2016). California Newt-*Taricha torosa*. Retrieved 2017, from http://www.californiaherps.com/info/citationinfo.html

Padgett-Flohr, G.E. (2008). Pathogenicity of *Batrachochytium dendrobatidis* in two threatened California amphibians: *Rana draytonii* and *Ambystoma californiense. Herpetological Conservation and Biology, 3*(2), 182-191.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics, 155*(2), 945-959.

Raymond, M. (1995). GENEPOP (v1.2.): Population genetics software for exact tests and ecumenicism. *Journal of Heredity, 86*, 248-249.

Ricciardi, A., & Rasmussen, J. B. (1999). Extinction rates of North American freshwater fauna. *Conservation Biology*, *13*(5), 1220-1222.

Salice, C. (2012). Multiple stressors and amphibians: Contributions of adverse health effects and altered hydroperiod to population decline and extinction. *Journal of Herpetology*, *46*(4), 675-681.

Schlötterer, C. (2000). Evolutionary dynamics of microsatellite DNA. *Chromosoma*, *109*, 365–371.

Storfer, A., Murphy, M.A., Evans, J.S., Goldberg, C.S., Robinson, S., Spear, S.F., Dezzani, R., Delmelle, E., Vierling, L., and Waits, L.P. (2007). Putting the 'landscape' in landscape genetics. *Heredity, 98*, 129-142.

Stuart, S. N., Chanson, J. S., Cox, N. A., Young, B. E., Rodrigues, A. S., Fischman, D. L., & Waller, R. W. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science*, *306*(5702), 1783-1786.

Thomson, R. C., Wright, A. N., & Shaffer, H. B. (2016). California amphibian and reptile species of special concern. Berkeley: University of California Press.

Toonen, R. (2006). Microsatellites for ecologists: A practical guide to using and evaluating microsatellite markers. *Ecology Letters*, *9*, 615-629.

UC Regents, (2017). Blue Oak Ranch Reserve Facts. Retrieved 2017, from http://blueoakranchreserve.net/about/facts-at-a-glance.html

APPENDIX OF TABLES

Num	Name
1	North
2	Pig
3	Yerba Buena
4	Hotel
5	Eagle
6	Dan
7	Mudd
8	Washburn

Table 1: Index of populations.

Name	Sequence Motif	Annealing Temperature (C)	Size	# of alleles
Tgr 01	$(TATC)^{13}$	56	~228	12
Tgr 10	$(TAGA)^{14}$	55	~183	16
Tgr 13	$(TAGA)^{7}$	56	~140	3
Tgr 14	$(GATA)^{31}$	54	~279	26
Nvi 19	$(ACAT)^{9}$	48	~177	8

Table 2: Key aspects of the five microsatellite loci optimized for *Taricha torosa* including the sequence of repeats conserved between different alleles, the temperature used for the annealing step of PCR, the approximate fragment size, and the number of alleles found at each locus in the sampled population.

Pop	Tgr 01	Tgr 10	Tgr 13	Tgr 14	Nvi 19	Total
1	1.0000	1.0000	0.5628	0.5445	N/A	0.9677
2	0.7711	1.0000	0.3217	1.0000	1.0000	0.9860
3	0.2339	N/A	0.8310	**0.0155**	1.0000	0.1692
4	0.2917	N/A	1.0000	0.1224	1.0000	0.5732
5	0.8561	1.0000	0.6164	0.5164	1.0000	0.9893
6	0.1161	1.0000	0.8356	0.5222	1.0000	0.8182
7	0.0442	N/A	0.1020	**0.0152**	0.0645	**0.0018**
8	0.0771	N/A	0.7831	0.4251	0.1485	0.5525
Total	0.1475	1.0000	0.8576	**0.0479**	0.8116	

Table 3: P- values for Hardy Weinberg Exact Tests with Locus on the X-axis and Population on the Y-axis. Significant P-values, bolded, denote data sets that are inconsistent with HWE expectations.

Pop	1	2	3	4	5	6	7	8
1	--	0.340	0.519	0.001	0.306	0.812	**0.008**	0.075
2	0.029	--	0.299	0.203	0.905	0.964	**0.010**	0.122
3	0.013	0.003	--	0.472	0.203	0.550	**0.043**	**0.025**
4	0.098	0.015	-0.006	--	0.091	0.258	**<0.001**	**0.004**
5	0.018	-0.015	-0.001	0.021	--	0.827	**<0.001**	0.075
6	0.003	-0.014	-0.011	0.063	0.066	--	**<0.001**	0.253
7	0.085	0.065	0.066	0.127	0.063	0.066	--	**0.002**
8	0.041	0.018	0.015	0.319	0.016	0.002	0.081	--

Table 4: Fst data for comparisons of all 8 populations with Fst values below the diagonal and P-values above. Significant P-values, bold, denote populations pairs that are more highly differentiated than expected.

APPENDIX OF FIGURES

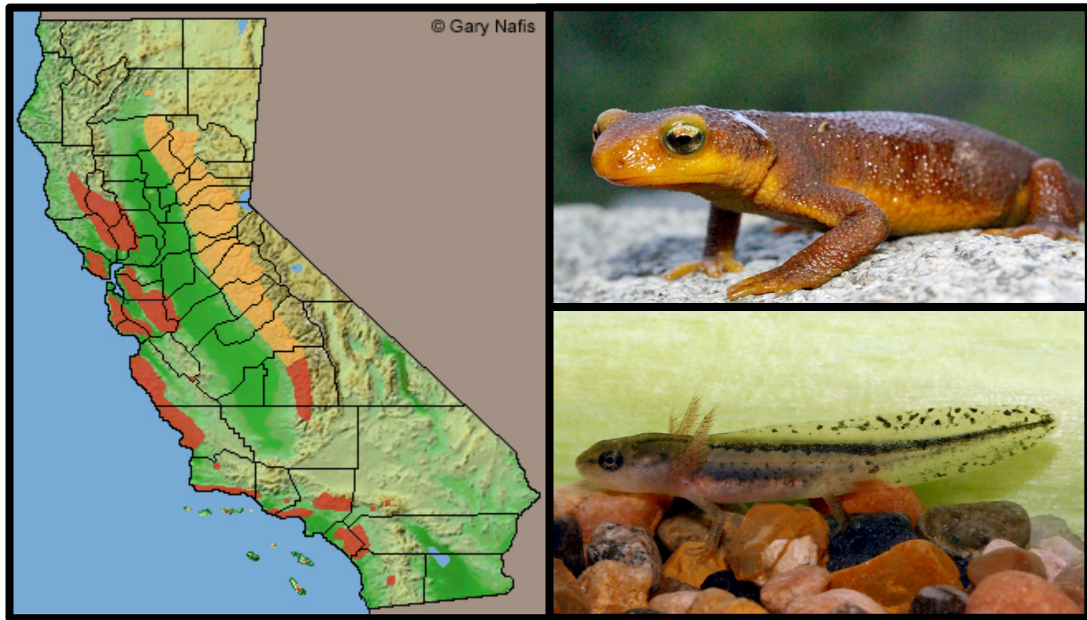


Fig. 1A: County map of California showing range of *Taricha torosa* (in red); 1B: Adult California Newt, *Taricha torosa* (Photo by Nathan Ray); 1C: Larval California Newt, *Taricha torosa* (Photo by Gary Nafis).

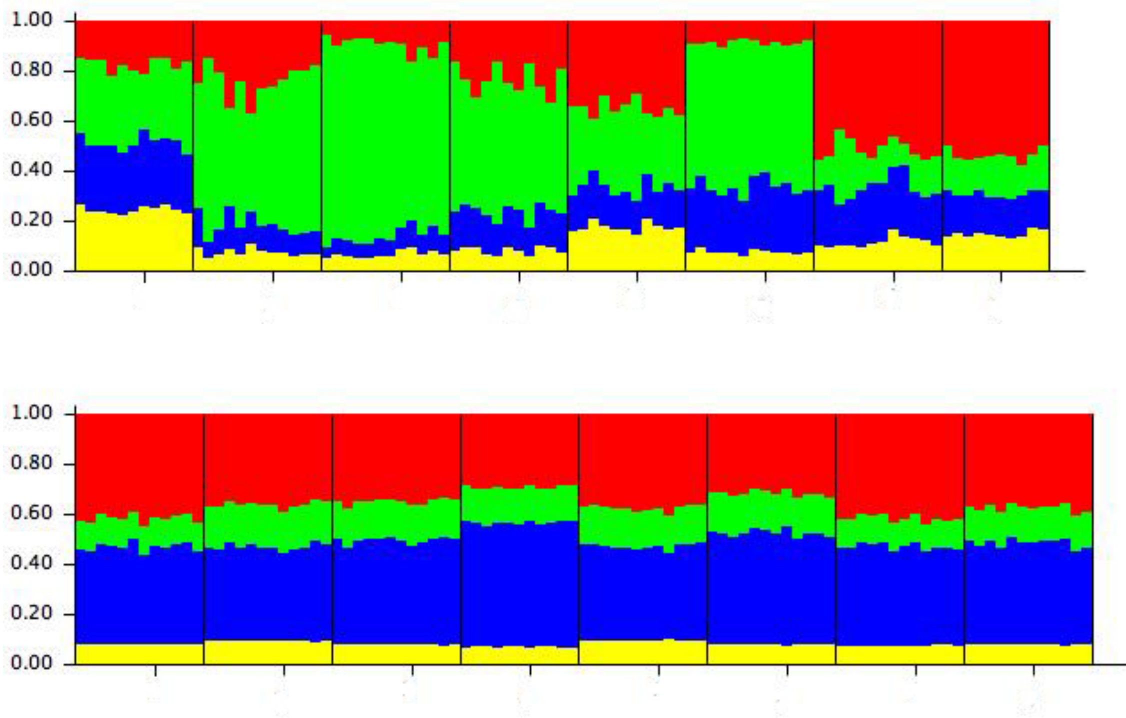


Fig. 2: Bar plots from STRUCTURE depicting assignment of individuals from the eight populations into four putative populations from 2006, top, and 2007, bottom.

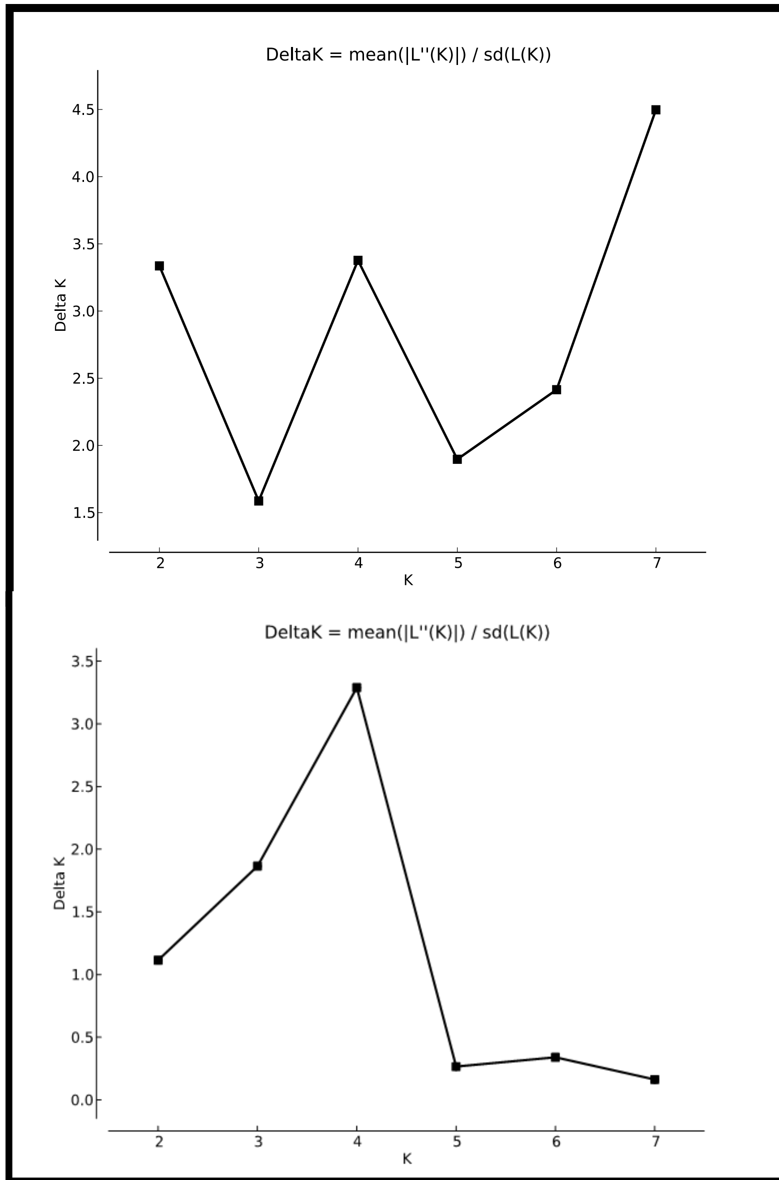


Fig 3: Delta K-values from Structure denoting likelihood of various genetic subpopulations from 2006 (top) and 2007 (Bottom).

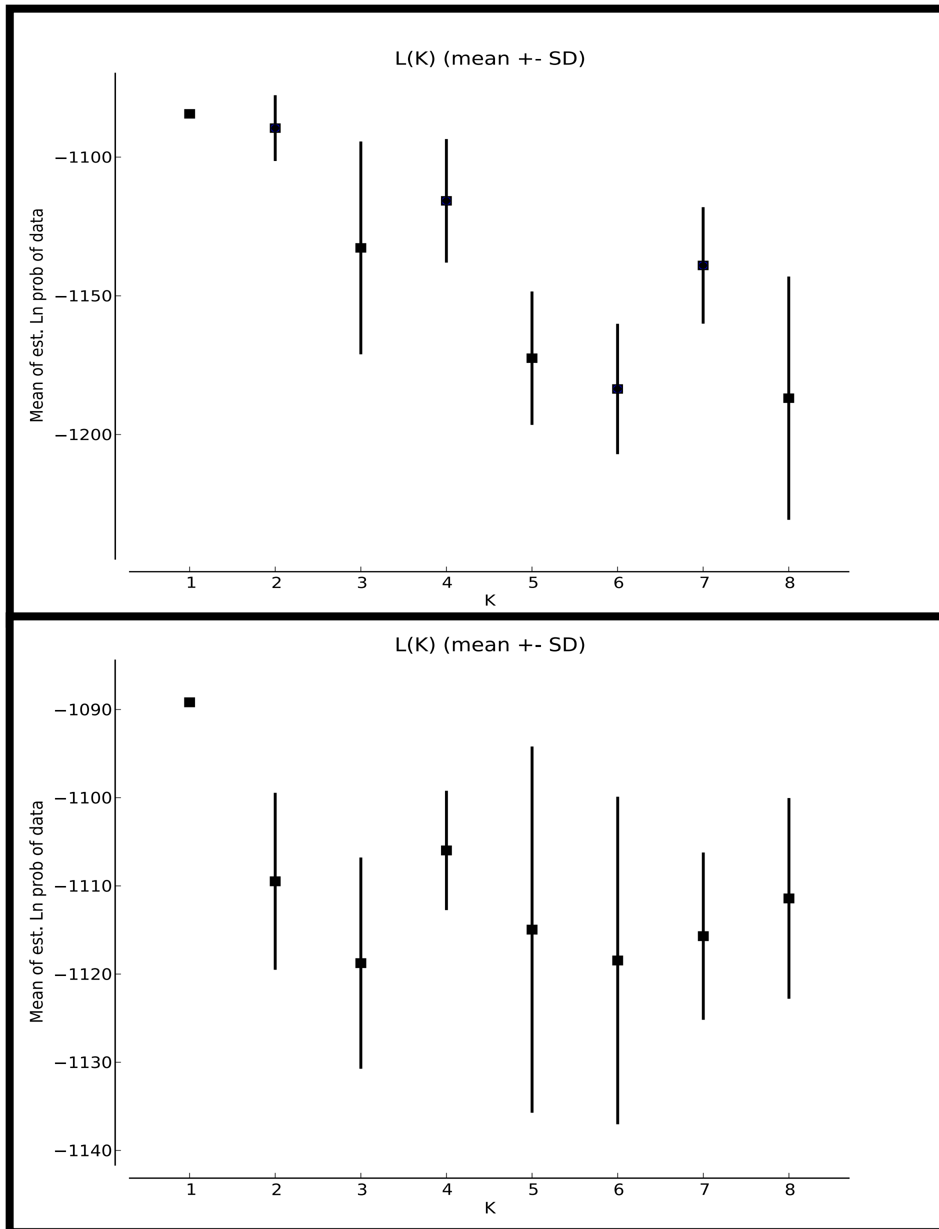


Fig. 4: Mean log-likelihood of data under sequential models of the number of populations for 2006 (top) and 2007 (bottoms).

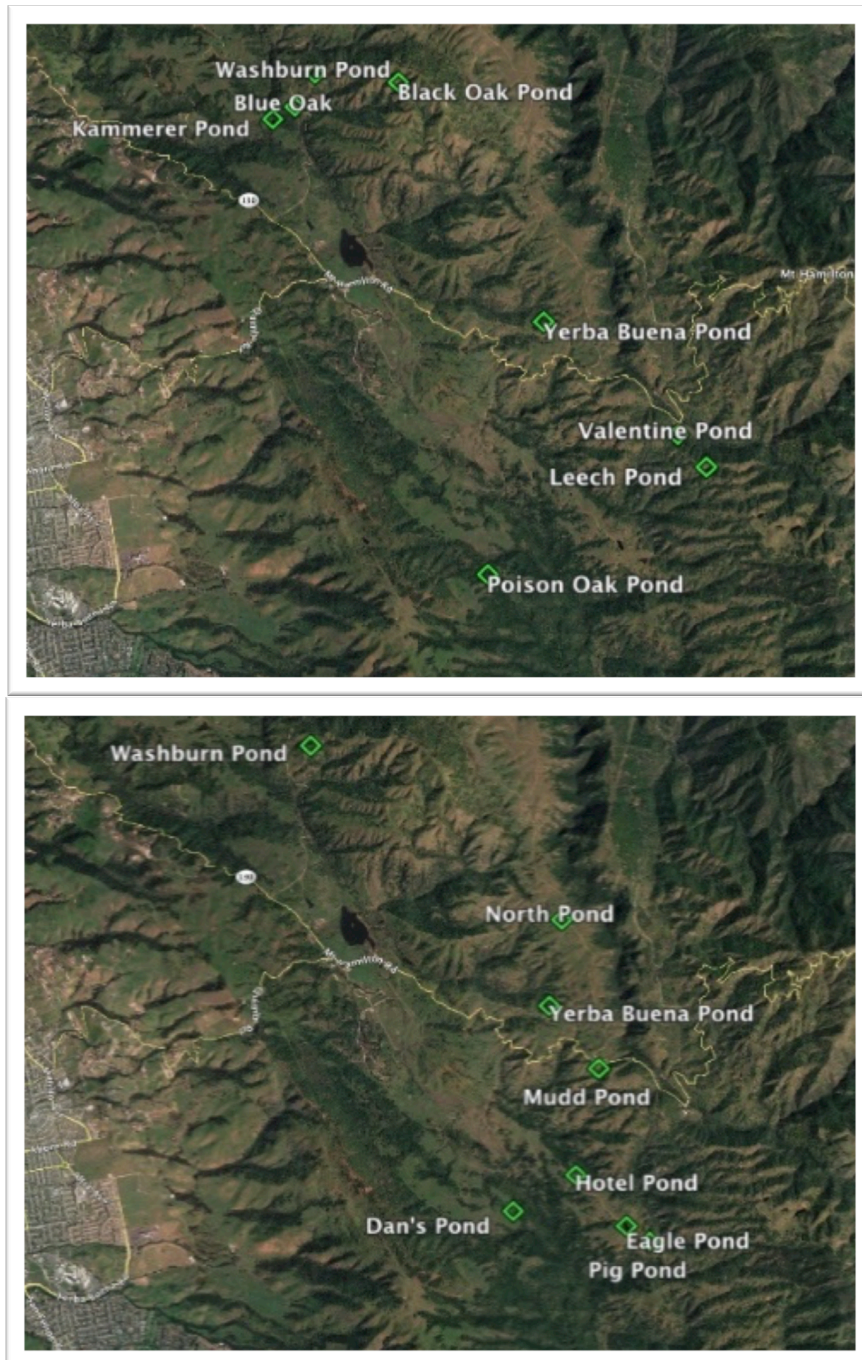
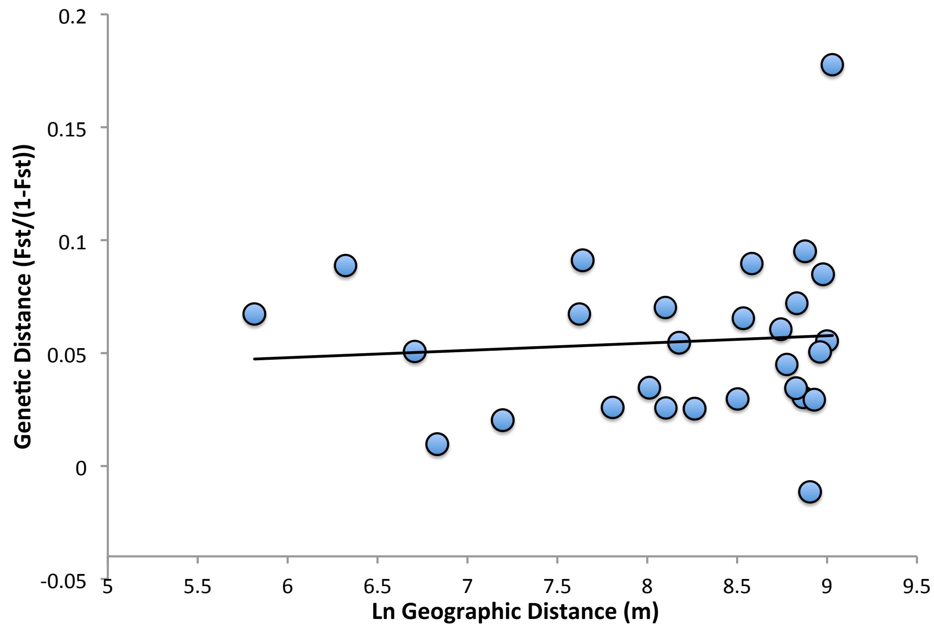


Fig 5: Map of pond locations for 2006 (top) and 2007 (bottom).



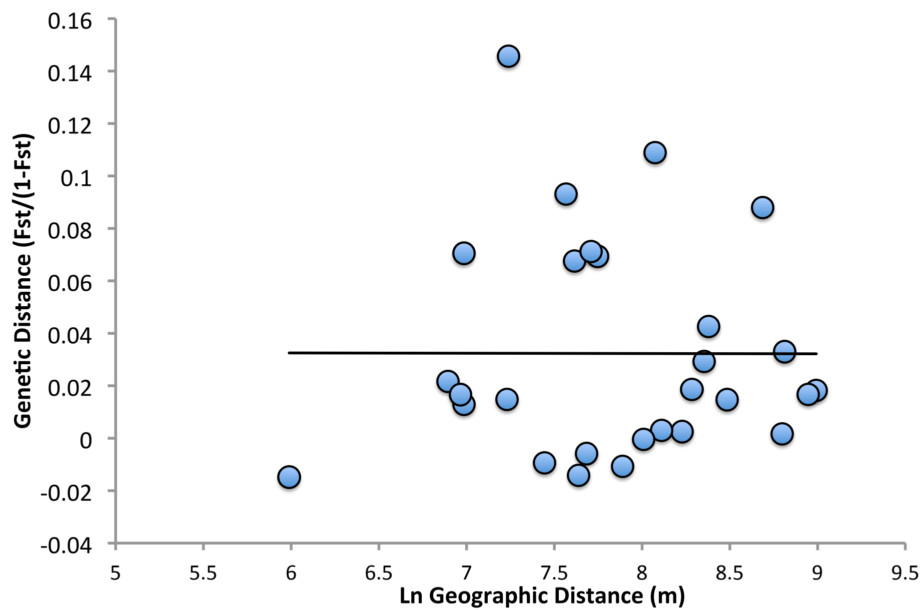


Fig. 6: Scatter plot depicting relationship between geographic and genetic distance for pairwise data between populations for 2006 (top) and 2007 (bottom).

APPENDIX OF SOLUTIONS

CELL LYSIS BUFFER

20 mL	5M stock NaCl
100 mL	1M stock Tris-Cl pH 8.0
100 mL	0.5M stock EDTA
25 mL	20% stock SDS
755mL	dd H_2O
1 L	

0.22 Mm filtered*

PROTEIN PRECIPITATION SOLUTION

473.04 g	118.26 MW Guanidine Thiocyanate
100 mL	1M stock Tris-Cl pH 7.5
900 mL	Deionized H_2O
1 L	

0.22 Mm filtered*