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Final Report: Terms of Endangerment: Use of Modern Genetic Techniques to Measure the Status of Protected Species

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Terms of Endangerment: Use of Modern Genetic Techniques to Measure the Status of Protected Species

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Abstract

Historically, field methods such as mark-recapture analysis were the main approaches used for monitoring wildlife populations. Fieldwork, however, is time consuming, labor intensive, and therefore costly. Genetic monitoring can directly estimate population size, trends, and connectivity from a sample of individuals taken at a single point in time, and over a much smaller space geographically, and therefore can be much quicker and cheaper than traditional field methods. Herein, we test the power of genetic tools to monitor wildlife, focusing on two species that are listed under the U.S. Endangered Species Act (Island Night Lizard, *Xantusia riversiana*, and Island Fox, *Urocyon littoralis*) and one species that is not listed (Pacific Treefrog, *Pseudacris regilla*) on the Channel Islands. The proposed work addresses the Legacy FY2012 Area of Emphasis on species at-risk, species of concern, and declining species and habitats. Specifically, this project matches the RFP objective of funding "innovative projects that serve as pilot or demonstration efforts of new techniques, methodologies, and management practices, including monitoring and predictive modeling." Moreover, our project advances the three Strategic Goals of DoD. PARC (Partners in Amphibian and Reptile Conservation) Draft Strategic Plan of: (1) providing technical expertise to the DoD community in support of effective amphibian and reptile management and stewardship practices; (2) sustaining viable amphibian and reptile populations and habitats through sound management and stewardship on DoD lands; and (3) promoting surveying and monitoring of amphibians and reptiles on DoD lands (Lovich et al. 2014).

Introduction

Using genetic tools to monitor populations is a new, powerful approach for wildlife management (Schwartz et al. 2007, Luikart et al 2010). DoD natural resource managers responsible for conserving wildlife need efficient, cost-effective, and informative methods for monitoring wildlife populations. The eight California Channel Islands (northern islands: Anacapa, Santa Cruz, Santa Rosa, San Miguel; southern islands: Santa Catalina, Santa Barbara, San Nicolas, San Clemente; see Fig. 1) represent a unique challenge for wildlife managers. The Navy has significant installations on San Clemente and San Nicolas Islands, a radar installation on Santa Cruz Island, and owns San Miguel

Island (which is managed by the National Park Service). These islands have been isolated from the mainland for millions of years and thus support a diversity of endemic taxa. In particular, many mammal, reptile, and amphibian species and populations on the Channel Islands are of conservation concern due to a history of habitat degradation caused by livestock and feral grazers, invasive plants, and now climate change. Recent efforts by the Navy have eliminated most non-native species and enabled natural vegetation to recover. However, rugged terrain and the logistical difficulties of conducting island-wide, long-term monitoring of multiple taxa have limited our knowledge of population sizes and trends of native wildlife populations as well as movement (i.e., gene flow) within and among islands and between the islands and the mainland. Effective, integrated monitoring methods are thus essential to ensure that unique Channel Islands populations and species do not decline to precariously low levels, potentially hampering military operations and readiness if they are listed on the endangered species list or remain on it. Thus, genetic monitoring has the potential to revolutionize wildlife management on DoD installations.

Study Objectives

1. Estimate effective population sizes (i.e., a genetic estimate of the number of breeding individuals that pass their genes on to subsequent generations) of: Island Fox (listed on Santa Cruz, San Miguel, Santa Rosa, and Santa Catalina Islands), recently delisted Island Night Lizard (previously listed on San Nicolas, San Clemente, and Santa Barbara Islands), and the Pacific Treefrog (not listed). We are conducting our research on three islands with active Navy Installations: San Clemente (Island Fox, Island Night Lizard); San Nicolas (Island Fox, Island Night Lizard); and Santa Cruz (Island Fox, Pacific Treefrog) (Fig. 1).

2. Test for changes (increases or declines) in population sizes.

3. Estimate genetic connectivity among populations.

4. Test the statistical power of genetic monitoring to detect population increases or declines.

5. Use mark-recapture data to estimate population sizes and then compare the statistical power of genetic monitoring vs. mark-recapture analysis to determine population status.

6. Compare the costs, resulting data, and overall efficiency of field surveys vs. genetic monitoring for assessing population status.

7. Communicate our findings to DoD natural resource managers and work with them to integrate our research findings into management plans.

Background

Tissue sample collection of all target species has been completed (blood from Island Foxes; toe tips and/or liver tissue from Island Night Lizards; and liver tissues from Pacific chorus frogs) from all Navy Installations on the Channel Islands (Fig. 1; Table 1). Total tissues collected and/or analyzed are 200 Island Foxes, 150 Island Night Lizards, and 140 Pacific Chorus Frogs. This was a major accomplishment given the large number of islands and individuals, as well as the number of collaborators that we had to reach out to that had tissues, permits, or otherwise to facilitate such work on 2 of the 3 target endangered species. Second, we have collected next-generation sequence (NGS) data (single-nucleotide polymorphism or "SNP" data) for all three species on the islands on which they occur and from mainland outgroups. This project involved learning the latest in next-generation sequencing technology, skills for application to the species management on DoD lands. Finally, we have analyzed these SNP data to estimate population structure (i.e., differences and similarities among populations), genetic variation within populations, and effective population sizes (N_e). Below, we provide more details about or Methods, Results, and Conclusions to date.

Materials and Methods

Field data collection—Tissue samples were collected from Island Foxes, Island Night Lizards, and Pacific chorus frogs using standard methods for each taxonomic group from respective islands (Fig. 1; Table 1). For Island Foxes, blood samples were collected by fox biologists currently working on San Clemente Island (SCL), San Nicolas Island

(SNI), and Santa Cruz Island (SCI). Blood was stored in EDTA buffer for long-term storage. For Island Night Lizards, toe tips were taken from 1-2 toes per individual on SCL and SNI and stored in 95-100% ethanol. Finally, for Pacific chorus frogs, liver samples were taken from euthanized specimens (that will serve as museum vouchers) on SCI and stored in 95-100% ethanol. All tissues are currently stored in an ultra-cold freezer (-80C) at Colorado State University (CSU).

Genomic data collection—Although we originally proposed to use microsatellite loci (short, tandem repeat DNA) as molecular markers for this project, we decided to take advantage of rapid advances in DNA sequencing technology to increase the accuracy of our estimates of effective population size (N_e) and our power to detect changes in population size. These new "next-generation sequencing (NGS)" technologies are dramatically increasing the number of loci (genetic markers) available for genetic analysis, allowing for "population genomic" analysis. Population genomics is the analysis of hundreds or thousands of SNP loci—rather than 10-20 loci which is typical for traditional microsatellite studies—in order to characterize genetic variation among and within populations. In the context of genetic monitoring, population genomics will allow much more accurate estimates of N_e, changes in genetic variation, and genetic structure. By using genomics, we are fulfilling the goal embodied in our proposal title of using the most advanced technology possible to monitor populations.

We collected genomic data using a recently developed approach called "RAD tag", which stands for restriction-site associated DNA tags (Rowe et al. 2011). This approach involves five main steps: (1) genomic DNA is digested with restriction enzymes; (2) P1 adapters with individual barcodes are ligated to DNA fragments; (3) DNA from different individuals is pooled and sheared to 300-800 bp; and (4) the resulting RAD tag "libraries" (barcoded DNA fragments from multiple individuals) is sequenced at a core facility using an Illumina HiSeq sequencer. In our case, we paid the University of Oregon core facility to sequence our RAD tag libraries because of their competitive prices and superior technical support.

Genomic data analysis—Sequencing is complete for island Foxes, Island Night Lizards, and Island Chorus Frogs. We identified SNPs using the bioinformatics pipeline implemented in the program STACKS (Catchen et al. 2011). We then used two approaches to characterize population structure among populations: (1) pairwise F_{ST} values, a measure of genetic differentiation ranging from zero (no differences among populations) to one (maximum degree of differentiation, in which populations are fixed for different alleles—variants of a gene), calculated in GENEPOP '007 (Rousset 2008); and (2) neighbor-net trees inferred using SPLITSTREE4 (Bryant & Moulton 2004). Next, we estimated within population genetic variation using expected heterozygosity, H_e, in Genotype Viewer (http://www.montana.edu/kalinowski/Software.htm). Finally, we estimated N_e for each population using the linkage disequilibrium method implemented in NeEstimator 2.01 (Do et al. 2014).

Results

Sample collection—In less than a year since beginning the project, we completed sampling of our three focal species on all Navy Installations, as detailed in Table 1.

Genomic results—Genomic data collection and analysis has gone extremely well. Below, we outline our results to date, including discovery of variable SNP markers; analysis of population structure and gene flow (comparisons AMONG populations); analysis of genetic variation WITHIN populations; and $\boldsymbol{N_e}$ estimates.

Numbers of variable SNPs. We found high levels of genomic variation within most populations. After all quality filters, RAD sequencing provided 4858, 1035, and 2591 variable SNP loci for analysis for island foxes, island night lizards, and Pacific chorus frogs, respectively. These high numbers of SNPs provide high statistical power for analysis of population structure, genetic variation, and N_e.

Population structure analysis. Pairwise F_{ST} values were high among most island populations, likely due to a lack of gene flow among islands. For island foxes, pairwise F_{ST} values ranged from 0.46-0.96; for island night lizards, they ranged from 0.23-0.88; and for Pacific chorus frogs, they ranged from 0.23-0.68. The high degree of population structure was also evident in our Neighbor-net trees (Figs. 2-4). In general, geographically proximate populations were genetically more similar to each other.

However, for island night lizards, Santa Barbara Island was more similar genetically to San Clemente Island than San Nicolas Island, even though it is geographically closer to San Nicolas Island.

Genetic variation within populations was high. There were substantial differences among populations in the amount of within population genetic variation, as measured using expected heterozygosity (H_e; Table 1). In particular, for all three species, H_e was highest in mainland outgroup populations compared to island populations.

N_e estimates. Finally, we estimated N_e for all populations using the linkage disequilibrium method implemented in NeEstimator (Table 1). Overall, N_e estimates were smallest for island foxes, largest for Pacific chorus frogs, and intermediate for island night lizards. In fact, for all Pacific chorus frog populations and most island night lizard populations, N_e estimates were indistinguishable from infinite, because of low linkage disequilibrium. These populations likely have N_e values in the hundreds or larger.

Discussion and Conclusions

As hoped, we were able to genotype thousands of SNPs for each species using the new RAD sequencing approach. This allowed us to characterize genetic structure among populations, genetic variation within populations, and estimate N_e.

In all three species, we found high levels of genetic differentiation among populations, as revealed by our F_{ST} estimates and Neighbor-net trees. For all species, individuals grouped by population, indicating little to no gene flow among populations. Moreover, most populations were most similar to other geographically proximate populations. For example, northern populations of island foxes were more similar to each other than they were to southern populations of island foxes (and vice versa). We expected these populations to be genetically divergent from each other based on previous studies with microsatellites (Goldstein et al. 1999; Aguilar et al. 2004).

We also found that in all three species, within-population genetic variation was higher in the mainland (outgroup) species (in the case of island foxes and island night lizards) or all Pacific chorus frogs. This agrees with previous meta-analyses that have found that island populations in general have lower genetic variation and are more prone

to inbreeding depression than closely related mainland populations (Frankham 1998).

Effective population sizes (N_e) were smallest in island foxes and indistinguishable from infinite in all Pacific chorus frog populations and most island night lizard populations. The observation that N_e is small for island foxes, particularly on SNI, is likely due to historic bottlenecks and variance in reproductive success among individuals. N_e is usually significantly smaller than N_c, the "census" or field-based estimate of population size. Many Island Fox populations have gone through severe bottlenecks (temporary reductions in population size) over time, and bottlenecks are expected to drive N_e downwards. Small N_e in turn results in low genetic variation, which can also result in inbreeding depression. Our results do not demonstrate inbreeding depression, but managers should be aware of the possibility of negative inbreeding effects that could reduce fitness (reduce survival or fecundity), especially during environmentally stressful periods (e.g., drought).

Next steps—We have already written and submitted a manuscript for publication based on our island fox population genomics results. Our next steps will be to do the same for our island night lizard and Pacific chorus frog results. We plan to continue using our genomic SNP data to monitor these island species into the future to ensure they continue to flourish and therefore maintain military readiness.

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Table 1. Samples collected and analyzed for genetic monitoring and population genomic analysis. Heterozygosity (H_e) was estimated using Genotype Viewer and effective population size (N_e) was estimated using NeEstimator. We could not estimate N_e for grey foxes because they were sampled from a large area, violating the assumption of panmixia (indicated with NA). "Infinite" means that N_e was too large for NeEstimator to accurately estimate N_e (and likely indicates an N_e in the hundreds or greater).

Species	**Island or site**	**No. individuals analyzed**	H_e	N_e **(95% CI)**
Island fox	Miguel	24	0.06	16.8 (16.3-17.4)
	Rosa	23	0.15	14.2 (14.1-14.3)
	Cruz	24	0.11	28.6 (28.2-29.1)
	Catalina	46	0.25	47.1 (46.8-47.4)
	Clemente	19	0.06	81.0 (72.4-91.8)
	Nicolas	46	0.01	1.2 (1.1-1.2)
Grey fox (outgroup)	Mainland southern CA	18	0.26	NA
Island night lizard	Barbara (north)	25	0.01	Infinite (274.1-infinite)
	Barbara (south)	20	0.01	Infinite
	Clemente (north)	25	0.02	106.3 (64.0-281.6)
	Clemente (south)	15	0.02	Infinite
	Nicolas (NW)	23	0.02	21.5 (18.5-25.6)
	Nicolas (SE)	18	0.02	120.2 (17.6-infinite)
Desert night lizard (outgroup)	Mainland southern CA	24	0.06	Infinite
Pacific chorus frog	Rosa (west)	20	0.05	Infinite
	Rosa (east)	20	0.04	Infinite
	Cruz (west)	20	0.06	Infinite
	Cruz (east)	21	0.06	Infinite
	Catalina (north)	20	0.06	Infinite
	Catalina (south)	19	0.06	Infinite
	Mainland southern CA	20	0.14	Infinite

Fig. 1. California Channel Islands, Navy installations on islands, and distribution of focal species for genetic monitoring project. Blue shading shows Navy installations and property and red outline shows installations (San Clemente, San Nicolas, and Santa Cruz Islands).

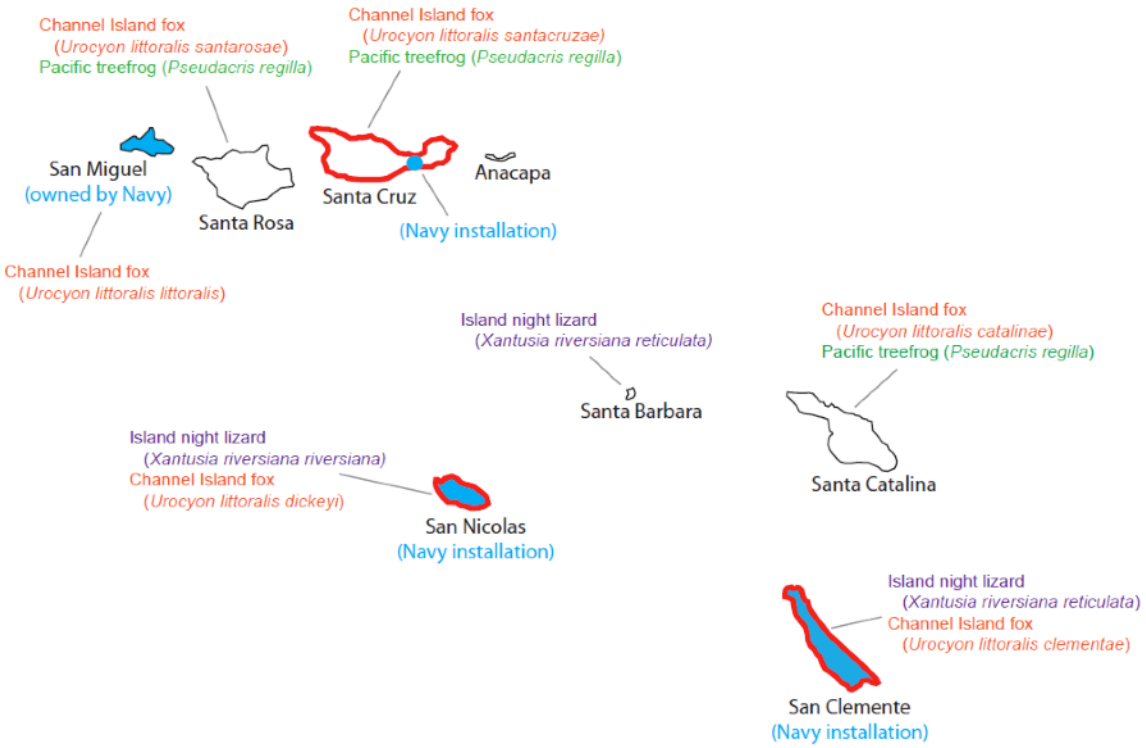

Fig. 2. Neighbor-net tree showing genetic relationships among 6 island fox populations and grey fox outgroup from the mainland. SMI = San Miguel Island; SRI = Santa Rosa Island; SCI = Santa Cruz Island; SCA = Santa Catalina Island; SNI = San Nicolas Island; SCL = San Clemente Island.

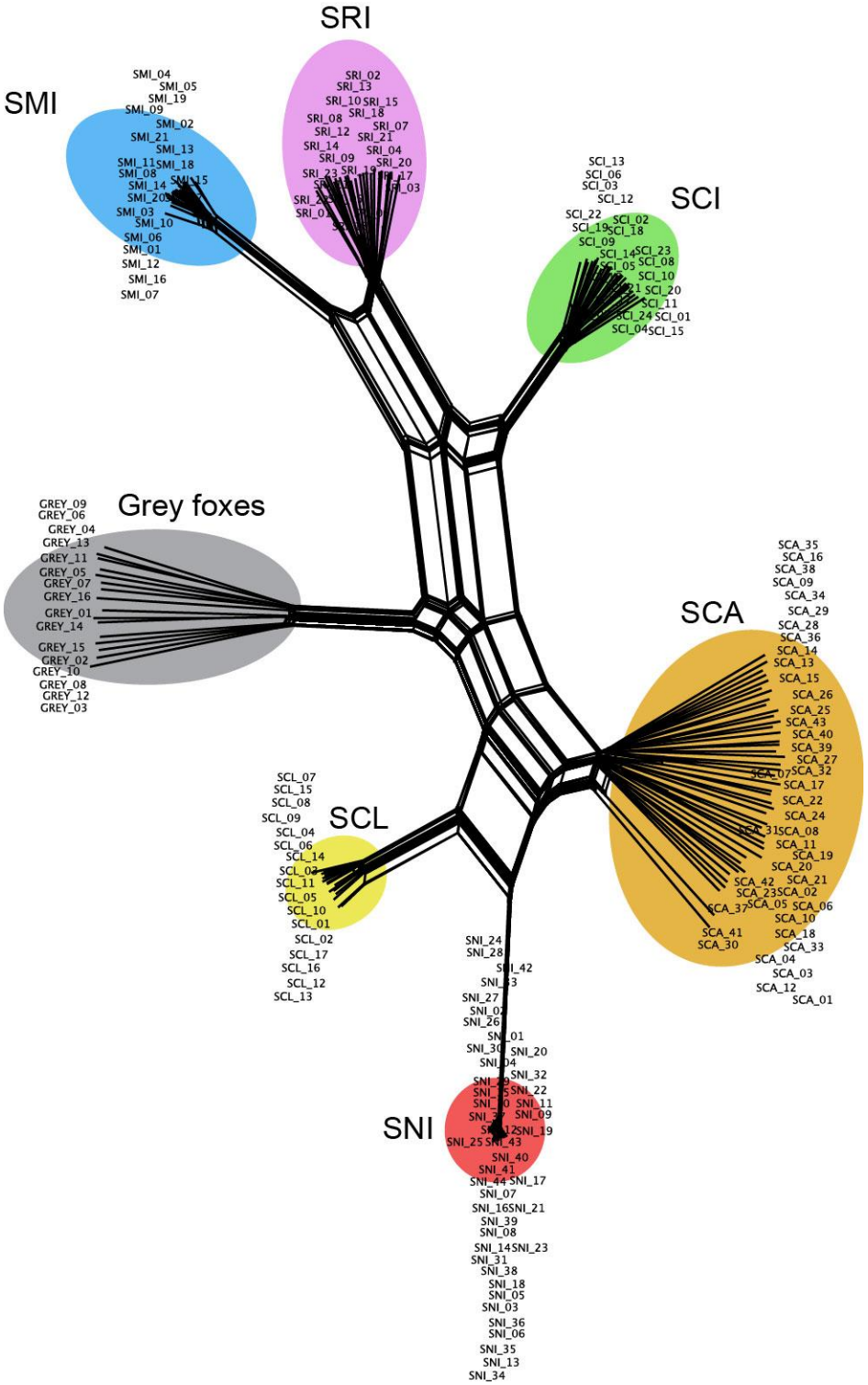

Fig. 3. Neighbor-net tree showing genetic relationships among 3 island night lizard populations and desert night lizard outgroup from the mainland (desert night lizard, *Xantusia vigilis*). Inset shows zoomed in view of 3 island night lizard populations. SBI = Santa Barbara Island; SCL = San Clemente Island; SNI = San Nicolas Island.

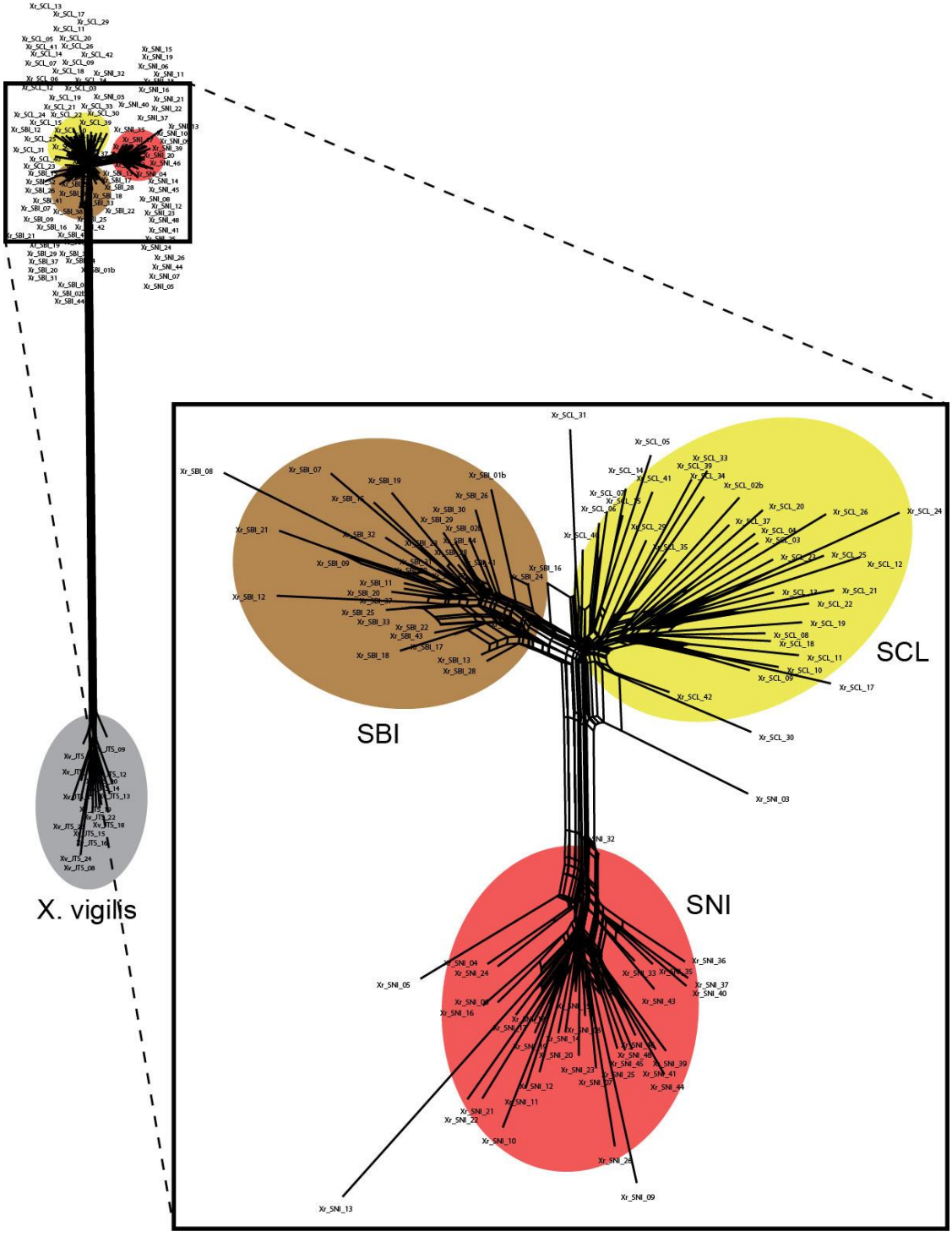

Fig. 4. Neighbor-net tree showing genetic relationships among 3 Pacific chorus frog populations on the Channel Islands and one outgroup population from the mainland. SRI = Santa Rosa Island; SCI = Santa Cruz Island; SCA = Santa Catalina Island.