Fanoe Pond Tiger Salamander Genotyping

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Introduction

In early May, 2006, Melissa Denena contacted H. Bradley Shaffer about conducting field sampling and genetic analysis on tiger salamanders from a series of ponds (hereafter referred to as the Fanoe ponds) in the Salinas valley. We agreed to conduct the sampling and laboratory analysis. Our goal was to sample for up to 25 larvae per pond, for five ponds, and to score them for up to 10 genes, and to determine for each gene whether the individual salamander was native *Ambystoma californiense* (CTS) or non-native *A, tigrinum mavortium*. We also agreed to write up a brief report, summarizing our findings across genes and ponds. This document constitutes that final report.

Methods

Tail tips (N=131) were collected from *Ambystoma* larvae at four ponds (Fanoe1-4; Figure 1) 2-3 km NE from Gonzalez, CA on 29 May 2006. No larvae were detected in Fanoe pond 5, even after extensive sampling with a 15 foot long seine. We note, however, that pond 5 is both large and deep, and it may contain animals that we were not able to sample from the deepest part of the pond. Tissue was immediately preserved in 95% ethanol and assigned HBS tissue catalogue #'s (107290-107320, 107350-107449). For each pond, we extracted DNA from 21 individuals for genotyping analyses using standard extraction techniques (Palumbi 1996). We analyzed 21 animals per pond, rather than the full sample of ~32, to retain some samples as backup in case the first round of work was unsuccessful and needed to be repeated.

Individual tissue samples were genotyped for one mitochondrial single nucleotide polymorphism (SNP) locus (Dloop) and up to 7 nuclear SNP loci (FoxG1b, Slc4a4, Dlx3, Contig325, HoxD8, Gnat2, and Gnat1; Voss et al. 2001). For each of these loci, our previous work has identified diagnostic differences between *A. tigrinum* and *A. californiense* (Fitzpatrick and Shaffer 2004). In our previous work, we utilized restriction-fragment-length polymorphism (RFLP) analyses to determine each individual genotype for each animal. However, in the current study, genotyping was performed using the Victor^3 plate reader (Perkin-Elmer) to perform fluorescence polarization (FP) analysis to score each individual's genotype at each locus. FP is a standard technique for the analysis of SNP loci (Xiao and Kwok 2003) and is more efficient and reliable than RFLP analyses. At each SNP locus each individual was scored as 'aa' if it was homozygous for native alleles, 'gg' if it was homozygous for introduced alleles, or 'ga' if heterozygous, with one copy each of a native and introduced allele. These data were summarized, for each gene at each pond, as the total frequencies of each genotype, which provides the basic results of the study. We also summarized the Hybrid Index score for each pond, which simply tallies the proportion of alleles, pooled across individuals and genes, that are native for each pond, using the formula HI=(total # of native alleles

['a'])÷(total # of alleles). The HI score is one way of summarizing the overall level of nativeness/invadedness of a sample of animals from a pond.

Results & Discussion

Raw data are presented in Appendix 1 and genotypic frequency data are presented in Table 1. For the purposes of providing a quantitative assessment of the "nativeness" of each pond, Table 2 contains Hybrid Index (HI) scores for each pond. Higher HI values indicate a greater proportion of native alleles.

The primary conclusion from our data are that all of the animals in all ponds contain primarily non-native gene copies. However, our data also indicate that all ponds contain at least low frequencies of native alleles at some nuclear loci (range=4-7 loci). Interestingly, we detected no native alleles for the single mitochondrial locus. The mitochondrial DNA is a very separate part of an animals overall DNA composition, and our previous work has shown that it sometimes shows a somewhat different pattern than the majority of the nuclear genome (Fitzpatrick and Shaffer, in press). Given this previous work, and the somewhat different pattern seen in the mitochondrial DNA compared to the nuclear DNA, we summarize the data with and without the mtDNA. The nuclear data present a more balanced overall picture of the genetic composition of the populations.

When considering the combined frequencies for all ponds, Table 1 shows that the majority of loci are largely homozygous for introduced alleles. Only HoxD8 displays increased frequencies of heterozygous and homozygous native genotypes. HoxD8 has previously been found to be associated with habitat-dependent heterozygote excess in other study sites in the Salinas Valley (Fitzpatrick and Shaffer 2004), and the pattern found in the Fanoe ponds is consistent with HoxD8 results in other ponds. It is interesting that the same pattern holds, even in the highly impacted ponds in the agricultural landscape of the Fanoe site.

Pond-specific differences among the four ponds do exist, even though all ponds consist of predominantly non-native genes (Tables 1 & 2). Ponds 2 and 4 have the highest HI scores, with about 11-12% native genes, compared to ponds 1 and 3 with 6.5-8% native genes; a similar pattern is present in the higher frequency of heterozygous individuals in ponds 2 and 4, and their lower frequencies of pure non-native (gg) homozygotes. Pond 2 also deviates from the other ponds by the lack of homozygous introduced individuals for the HoxD8 locus. However, even with these differences in the frequency of native alleles among ponds, the raw data indicate that no genotyped individual can be described as putatively "pure" native based on the 8 loci we investigated.

We conclude that the genotypes of salamanders present at Fanoe Ponds 1-4 are comprised of primarily introduced alleles, and that extensive invasion by introduced tiger salamanders and subsequent hybridization has occurred. However, Ponds 2 and 4 each had a somewhat elevated frequency of remnant native California tiger salamander genotypes, and they may have greater biological value than ponds 1 and 3. In addition,

ponds 2 and 4 were also the most "natural" of the ponds on the site—pond 4 was in the process of drying down completely when we visited (as is normally the case for natural vernal pools in the region), and pond 2 had the most extensive open ground with rodent burrows surrounding it.

Literature Cited

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Table 1. Observed genotypic frequencies of 8 SNP loci for Fanoe ponds 1-4. 'Combined' values represent the genotypic frequency for all individual pooled among ponds. 'Average (All)' values represent the average genotypic frequency for all 8 loci. 'Average (Nuclear)' values represent the average of only the 7 nuclear loci.

'gg' freq	FoxG1b	Slc4a4	Dlx3	Contig325	HoxD8	Dloop	Gnat2	Gnat1	Average (All)	Average (Nuclear)
Pond1	1.00	1.00	0.95	1.00	0.38	1.00	0.95	0.85	0.89	0.88
Pond2	1.00	1.00	1.00	0.94	0.00	1.00	1.00	0.65	0.82	0.80
Pond3	1.00	1.00	0.95	1.00	0.21	1.00	1.00	1.00	0.90	0.88
Pond4	0.85	0.83	0.81	1.00	0.38	1.00	0.81	0.75	0.80	0.78
Combined	0.96	0.96	0.93	0.99	0.25	1.00	0.94	0.81	0.85	0.83

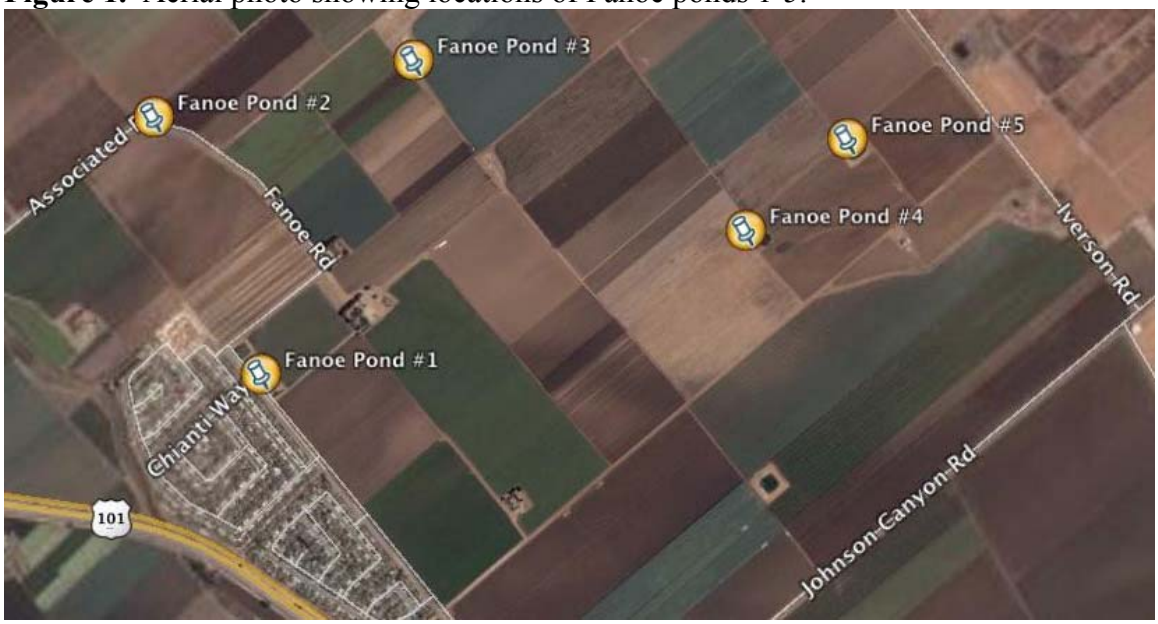
'ga' freq	FoxG1b	Slc4a4	Dlx3	Contig325	HoxD8	Dloop	Gnat2	Gnat1	Average (All)	Average (Nuclear)
Pond1	0.00	0.00	0.05	0.00	0.33	N/A	0.05	0.05	N/A	0.07
Pond2	0.00	0.00	0.00	0.00	0.80	N/A	0.00	0.20	N/A	0.14
Pond3	0.00	0.00	0.05	0.00	0.58	N/A	0.00	0.00	N/A	0.09
Pond4	0.15	0.17	0.14	0.00	0.38	N/A	0.19	0.25	N/A	0.18
Combined	0.04	0.04	0.06	0.00	0.52	N/A	0.06	0.13	N/A	0.12

'aa' freq	FoxG1b	Slc4a4	Dlx3	Contig325	HoxD8	Dloop	Gnat2	Gnat1	Average (All)	Average (Nuclear)
Pond1	0.00	0.00	0.00	0.00	0.29	0.00	0.00	0.10	0.05	0.06
Pond2	0.00	0.00	0.00	0.06	0.20	0.00	0.00	0.15	0.05	0.06
Pond3	0.00	0.00	0.00	0.00	0.21	0.00	0.00	0.00	0.03	0.03
Pond4	0.00	0.00	0.05	0.00	0.24	0.00	0.00	0.00	0.04	0.04
Combined	0.00	0.00	0.01	0.01	0.23	0.00	0.00	0.06	0.04	0.05

Table 2. Hybrid index (HI) score for each Fanoe pond. HI=(total # of native alleles ['a'])÷(total # of alleles).

	HI Scores
Pond1	0.0798
Pond2	0.1132
Pond3	0.0649
Pond4	0.1166

Figure 1. Aerial photo showing locations of Fanoe ponds 1-5.



Appendix 1: Raw genotype data for each individual genotyped at 8 SNP loci. 'gg' represents homozygous introduced, 'aa' represents homozygous native, and 'ga' represents heterozygous genotypes. 'Neg.' refers to absent data.

HBS#	FoxG1b	Slc4a4	Dlx3	Contig325	HoxD8R	Dloop	Gnat2R	Gnat1N	Pond
107350	gg	gg	gg	gg	gg	gg	gg	gg	1
107357	gg	gg	gg	gg	gg	gg	gg	gg	1
107364	gg	gg	gg	gg	gg	gg	gg	aa	1
107351	gg	gg	gg	gg	gg	gg	gg	gg	1
107358	gg	gg	gg	gg	gg	gg	gg	aa	1
107365	gg	gg	gg	gg	gg	gg	gg	Neg.	1
107352	gg	gg	gg	gg	aa	gg	gg	gg	1
107359	gg	gg	ga	gg	ga	gg	gg	gg	1
107366	gg	gg	gg	Neg.	ga	gg	gg	gg	1
107353	gg	gg	gg	gg	ga	gg	gg	ga	1
107360	gg	gg	gg	gg	gg	gg	gg	gg	1
107367	gg	gg	gg	gg	ga	gg	gg	gg	1
107354	gg	gg	gg	gg	aa	gg	gg	gg	1
107361	gg	gg	gg	Neg.	ga	gg	gg	gg	1
107368	gg	gg	gg	gg	aa	gg	gg	gg	1
107355	gg	gg	gg	gg	aa	gg	gg	gg	1
107362	Neg.	gg	gg	gg	aa	gg	gg	gg	1
107369	gg	gg	gg	gg	gg	gg	gg	gg	1
107356	gg	gg	gg	gg	ga	gg	ga	gg	1
107363	gg	gg	Neg.	gg	ga	gg	gg	gg	1
107370	gg	gg	gg	gg	aa	gg	gg	gg	1
107290	gg	gg	gg	gg	aa	gg	gg	aa	2
107297	gg	gg	gg	gg	aa	gg	gg	aa	2
107304	gg	gg	gg	gg	ga	gg	gg	gg	2
107291	gg	gg	gg	gg	ga	gg	gg	ga	2
107298	gg	gg	gg	gg	ga	gg	gg	gg	2
107305	gg	gg	gg	gg	ga	gg	gg	gg	2
107292	gg	gg	gg	Neg.	ga	gg	gg	gg	2
107299	gg	gg	gg	aa	ga	gg	gg	gg	2
107306	gg	gg	gg	gg	ga	gg	gg	ga	2
107293	gg	gg	gg	gg	ga	gg	gg	gg	2
107300	gg	gg	gg	Neg.	ga	gg	gg	gg	2
107307	Neg.	Neg.	Neg.	Neg.	Neg.	gg	gg	gg	2
107294	gg	gg	gg	gg	ga	gg	gg	aa	2
107301	gg	gg	gg	gg	ga	gg	gg	gg	2
107308	gg	gg	gg	gg	ga	gg	gg	gg	2
107295	gg	gg	gg	gg	ga	gg	gg	ga	2
107302	gg	gg	gg	Neg.	ga	gg	gg	gg	2
107309	gg	gg	gg	gg	aa	gg	gg	gg	2

107296	gg	gg	gg	gg	ga	gg	gg	ga	2
107303	gg	gg	gg	gg	ga	gg	gg	gg	2
107310	gg	gg	gg	gg	aa	gg	gg	Neg.	2
107379	gg	gg	gg	gg	ga	gg	gg	gg	3
107386	gg	gg	gg	gg	ga	gg	gg	gg	3
107393	gg	Neg.	gg	gg	ga	gg	gg	gg	3
107380	gg	gg	gg	gg	aa	gg	gg	gg	3
107387	gg	gg	gg	gg	aa	gg	gg	gg	3
107394	gg	Neg.	gg	Neg.	Neg.	gg	gg	gg	3
107381	gg	gg	gg	gg	aa	gg	gg	gg	3
107388	gg	gg	gg	gg	ga	gg	gg	gg	3
107395	gg	gg	ga	gg	gg	gg	gg	gg	3
107382	gg	gg	gg	gg	ga	gg	gg	gg	3
107389	gg	gg	gg	gg	ga	gg	gg	Neg.	3
107396	gg	gg	gg	Neg.	Neg.	gg	gg	gg	3
107383	gg	gg	gg	Neg.	gg	gg	gg	gg	3
107390	gg	gg	gg	gg	gg	gg	gg	gg	3
107397	gg	gg	gg	gg	ga	gg	gg	Neg.	3
107384	gg	gg	gg	gg	ga	Neg.	gg	gg	3
107391	gg	gg	gg	gg	aa	Neg.	gg	gg	3
107398	gg	Neg.	gg	Neg.	gg	Neg.	gg	gg	3
107385	gg	gg	gg	gg	ga	gg	gg	gg	3
107392	gg	gg	gg	gg	ga	gg	gg	gg	3
107399	gg	gg	gg	gg	ga	gg	gg	gg	3
107406	gg	gg	gg	gg	gg	gg	gg	gg	4
107413	gg	Neg.	gg	gg	gg	gg	gg	Neg.	4
107420	gg	Neg.	gg	gg	gg	gg	gg	gg	4
107407	gg	ga	gg	gg	gg	gg	gg	gg	4
107414	gg	gg	gg	gg	aa	gg	ga	gg	4
107421	gg	gg	ga	gg	gg	gg	gg	ga	4
107408	gg	gg	gg	gg	ga	gg	gg	ga	4
107415	Neg.	gg	ga	gg	aa	gg	gg	ga	4
107422	gg	gg	gg	gg	ga	gg	gg	gg	4
107409	gg	gg	gg	gg	ga	gg	ga	gg	4
107416	gg	gg	gg	gg	ga	gg	gg	gg	4
107423	ga	gg	gg	gg	ga	gg	gg	gg	4
107410	gg	gg	gg	gg	gg	gg	ga	gg	4
107417	ga	gg	gg	gg	gg	gg	gg	ga	4
107424	gg	gg	gg	gg	ga	gg	gg	gg	4
107411	gg	gg	aa	gg	gg	gg	gg	gg	4
107418	gg	gg	gg	gg	aa	gg	gg	gg	4
107425	ga	Neg.	gg	gg	aa	gg	ga	gg	4
107412	gg	ga	ga	gg	aa	gg	gg	ga	4

107419	gg	ga	gg	gg	ga	gg	gg	gg	4
107426	gg	gg	gg	gg	ga	gg	gg	gg	4