Genetics and management of endangered Mohave tui chub, *Siphateles bicolor mohavensis*

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ABSTRACT

Using 12 microsatellite DNA loci, we characterized genetic structure of four populations of Mohave tui chub, and examined the taxonomic status of the introduced cyprinid fish common in the Mojave River today. We found only unhybridized Mohave tui chubs in the four populations, and unhybridized arroyo chubs in the river. Contrary to our expectation, the source population at MC Spring has significantly fewer alleles and lower heterozygosity than populations historically derived from it. Our findings suggest that genetic drift due to a small effective population size in MC Spring has reduced genetic diversity in the five decades since the original transplants were made. A bottleneck of 10 individuals during the founding of the Camp Cady population is reflected in significantly lower genetic diversity and divergence of that population from all others. Two additional populations possess significantly higher levels of diversity, Lake Tuendae and China Lake. We recommend instituting artificial gene flow to rebuild genetic diversity in MC Spring and Camp Cady, and to better conserve allelic diversity in the species as a whole. New populations established in the future should be derived from Lake Tuendae and China Lake.
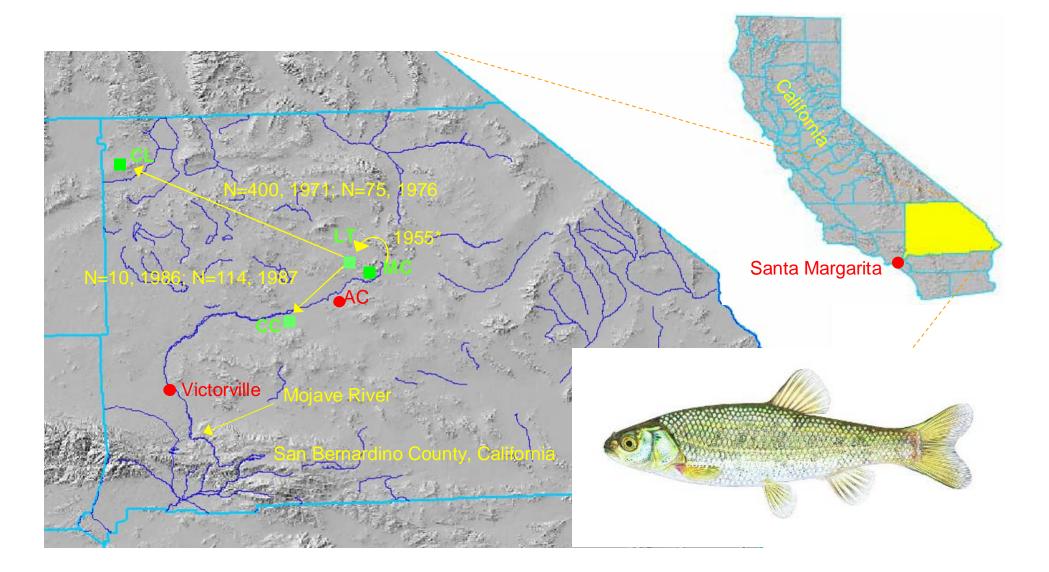
Fig. 1: Locality and transplant history of *S. b. mohavensis*

BACKGROUND

1. Mohave tui chub, *S. b. mohavensis* is the only fish native to the Mojave River basin, California.

2. Tui chubs were displaced by introduced arroyo chubs (*Gila orcutti*) throughout most of their range in 1930s (Hubbs and Miller 1943).

3. Extant Mohave tui chubs persisted in one relictual population, Mohave Chub Spring, from which three transplanted populations were derived: Lake Tuendae, China Lake, and Camp Cady.

OBJECTIVES

1. Assess historical hybridization between Mohave tui chub and arroyo chub in the Mojave River.

2. Analyze the population structure and genetic variation within and among Mohave tui chub populations.

3. Evaluate contemporary populations for evidence of recent genetic bottleneck.

4. Recommend appropriate stocks and genetic strategies for reestablishment of Mohave tui chub.

5. Suggest methods for long-term maintenance of genetic integrity in the recovery populations.

MATERIALS AND METHODS

Fifty individual samples were collected in 2005 from each of five locations: Camp Cady (CC), China Lake (CL), Lake Tuendae (LT), MC Spring (MC), and Afton Canyon (AC), San Bernardino County, California (see map in Fig. 1, and sites in Fig. 2). Fish were captured with minnow traps. Noninvasive genetic specimens were sampled and all fish were released at the point of capture. Pelvic fin clips (10-20 mm^2) were air-dried, placed in paper envelopes. Whole genomic DNA was extracted from tissue samples using the Promega 96-well Tissue Kit.

Camp Cady   **China Lake**   **Lake Tuendae**   **MC Spring**   **Afton Canyon**

Fig. 2. Sample collection Sites in Mojave River basin, California

We used 12 selected microsatellite loci developed by Meredith and May (2002), and Baerwald and May (2004). The forward primer of each primer pair was labeled with a fluorescent phosphoramidite. The PCR-generated microsatellite DNA products were visualized on a Base-Station gel imaging system (BioRad). Composite genotypes for individual fish were compiled by scoring co-dominant alleles at each microsatellite locus using Cartographer 1.2.6 software (BioRad). Genetic structure analyses were performed with programs of STRUCTURE (Pritchard et al. 2000) and Arlequin (Excoffier et al. 2005).

RESULTS

Taxonomic Status of the River Population

Out of 12 microsatellite DNA loci screened, we found two diagnostic alleles at two loci which distinguish Mohave tui chubs from arroyo chubs and presumed introgressed fish from Afton Canyon in the Mojave River. The first allele was 162 at locus CypG41, which was fixed in Mohave tui chub (see Fig. 3). The second was 165 at CypG47, which is fixed in arroyo chub but has two alternate alleles, 169 and 173, in Mohave tui chub.

Table 1. Total number of alleles ($\sum N_A$) and private alleles ($\sum N_P$), M-ratio (M), and expected heterozygosity ($H_E\hat{}$).

	Camp Cady	China Lake	Lake Tuendae	MC Spring
$\sum N_A (\sum N_P)$	41 (0)	62 (6)	57 (4)	34 (1)
$M\hat{} \pm$ C.I.	0.61 ± 0.12	0.70 ± 0.13	0.64 ± 0.15	0.65 ± 0.15
$H_E\hat{}$	0.54	0.61	0.59	0.41

Table 2. Pairwise F_{ST} (above diagonal) and percentage of private allele (below diagonal) (* indicates statistical significance).

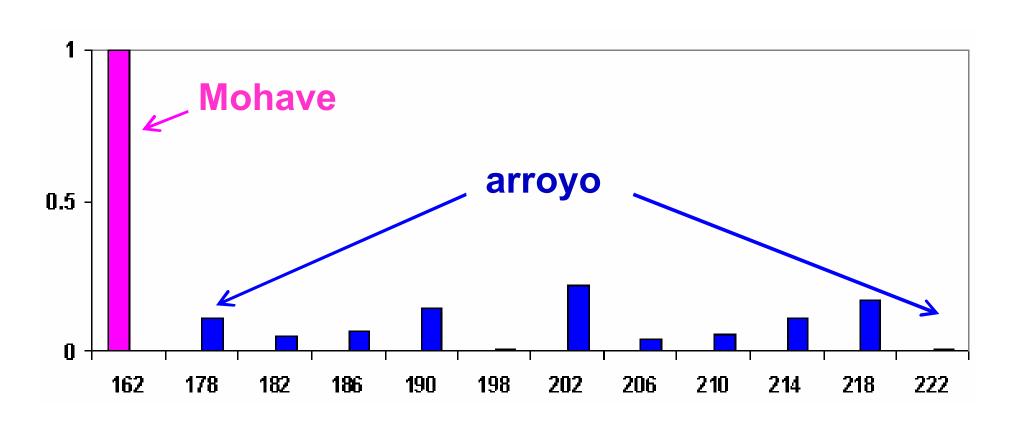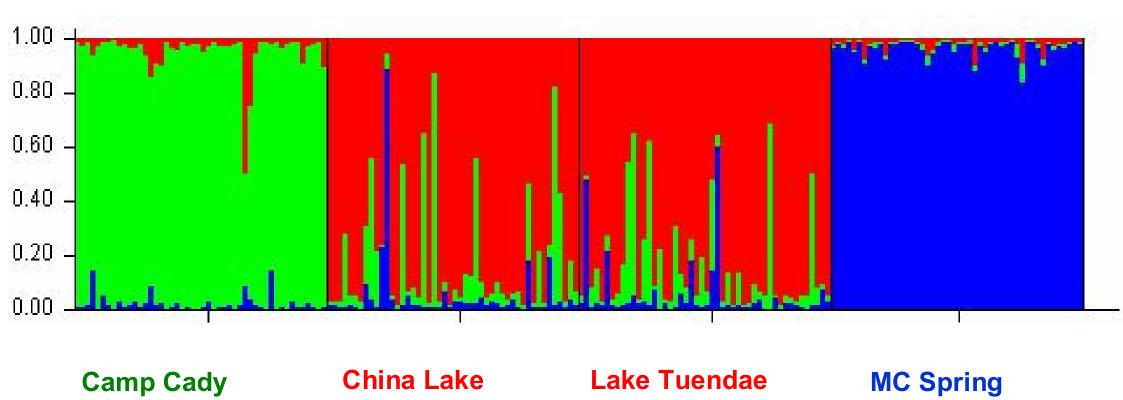| Population	Camp Cady	China Lake	Lake Tuendae	MC Spring
Camp Cady		0.07*	0.10*	0.24*
China Lake	0.34		0.02*	0.16*
Lake Tuendae	0.31	0.20		0.17*
MC Spring	0.40	0.48	0.48	

Fig. 3: Diagnostic allele of Mohave tui chub, 162 at locus CypG41

Genetic Structure of the Mohave Tui Chub Populations

Mohave tui chubs had comparatively low degrees of genetic diversity, reflected by both allele number and heterozygosity. Among the four populations, MC Spring exhibited the lowest variation at 11 polymorphic microsatellite loci (see Table 1). All pairwise F_{ST} values were statistically significant (Table 2; p-value ≤ 0.01 at 10,000 permutations). Among-population divergence was also supported by AMOVA results, in which the total variation among-population was 13% (d. f. = 3; p-value ≤ 0.0001), and within-population variation was 87% (d. f. = 380).

The log-likelihood probabilities for the modes of K=1~6 in STRUCTURE were maximized for K=3. The Bayesian bar plot of inferred probabilities of individual classification is used to visualize the data for K=3 (Fig. 4). The MC Spring population formed the first cluster independently; the majority of Camp Cady tui chubs went to the second cluster; most of China Lake and Lake Tuendae individuals comprise a third cluster, while the remaining individuals were more widely distributed.

Camp Cady   **China Lake**   **Lake Tuendae**   **MC Spring**

Fig. 4. Bayesian Bar Plot of Inferred Populations

CONCLUSIONS

1. The chub population in the Mojave River is non-introgressed *Gila orcutti*

2. All four protected populations are non-introgressed *Siphateles bicolor mohavensis*

3. MC Spring and Camp Cady have diverged from the larger populations through loss of genetic variation

4. Camp Cady narrow bottleneck ➔ single founder effect (n=10)

5. MC Spring wider bottleneck ➔ chronic low effective population size

MANAGEMENT IMPLICATIONS

1. Increase number of populations (Recovery Plan calls for six)

2. Exchange individuals among populations

3. Use China Lake & Lake Tuendae when founding new populations

4. Lack of introgression suggests recovery activities in Mojave River may be feasible, but should be the subject of experimentation

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