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ORIGINAL PAPER

Introgression between Lahontan and endangered Owens tui chubs, and apparent discovery of a new tui chub in the Owens Valley, California

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Abstract Owens tui chubs (*Siphateles bicolor snyderi* [Miller]) have become extirpated throughout most of their range by introgression with introduced Lahontan tui chubs. The remaining non-introgressed Owens tui chub populations persist in a small number of fragmented habitats. These survivors are listed as ‘‘endangered’’ under both the state and federal endangered species acts. This study employs six microsatellite DNA loci to assess degrees of genetic difference within and among populations of Owens and Lahontan tui chubs and their putative hybrids. It reveals four distinct groups of tui chubs: Owens, Lahontan, hybrid Owens × Lahontan, and Cabin Bar. Patterns of microsatellite DNA variation confirm that tui chubs in the Owens River and its tributaries, and Mono Lake tributaries comprise a hybrid swarm. The overall degree of introgression between Lahontan and Owens tui chubs is approximately 40%, while the remaining 60% derives from Owens tui chub's original genetic composition. Unexpectedly, Owens tui chubs are more genetically similar to Lahontan tui chubs than to Cabin Bar tui chubs, although they share the same river basin with the latter. Cabin Bar tui chubs possess a 3 bp deletion in the flanking region of microsatellite locus Gbi-G79. We give the Cabin Bar tui chub a common name ‘‘toikona tui chub’’ to distinguish it from the Owens tui chub, and recommend independent protection and recovery efforts. Management practices should prevent any future genetic mixing of non-introgressed Owens, toikona, or Lahontan tui chubs with each other, or with the hybrid swarm. Habitats of Owens and toikona tui chubs should remain isolated from the Owens River. Toikona tui chubs should be established in multiple sites.

Keywords Microsatellite DNA · Tui chub · Introgression · Conservation · Toikona · Owens Valley

Introduction

North American fish species are becoming extinct at an accelerating pace as a result of nonnative species introductions and habitat alteration (Moyle and Williams 1990; Minckley and Douglas 1991). One factor in these extinctions is introgressive hybridization (introgression) of previously separated species or subspecies brought into contact through human activities (Allendorf 1988). Native fish conservation in the face of threats of introgression requires assessing genetic mixing, identifying and maintaining genetically distinct taxa, and assuring viable populations for the future (Allendorf and Leary 1988; Haig 1998).

The Owens tui chub (*Siphateles bicolor snyderi* [Miller]) is a subspecies endemic to the Owens River basin (Moyle 2002; Leunda et al. 2005) in Mono and Inyo Counties of eastern California, described by Miller (1973) using meristic and osteological criteria. It

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was common in a variety of aquatic habitats of the basin at the beginning of the 20th century (Snyder 1917; Miller 1973). Since then, their survival has been jeopardized by invasions of alien species and degradation and loss of habitat. By 1974, the number of Owens tui chubs had diminished so precipitously that the state of California added it to its endangered species list. In 1985, this fish became listed as "endangered" under the federal Endangered Species Act (U.S. Fish and Wildlife Service 1998).

The most insidious threat facing Owens tui chubs comes from Lahontan tui chubs (*Siphateles bicolor obesa*) that were presumably introduced as bait fish and spread throughout the Owens River basin since 1960s (Miller 1973). These two forms of tui chub readily interbreed. Most populations of Owens tui chubs, such as the population in Crowley Reservoir, are believed to have been lost through introgression (Miller 1973). Adding to the loss of populations by introgression, predaceous non-native fishes, mainly brown trout (*Salmo trutta*) and largemouth bass (*Micropterus salmoides*) have excluded Owens tui chubs from much of their native range. Extensive development of water resources further diminished the viability of Owens tui chub habitats and helped spread Lahontan tui chubs throughout the Owens River basin.

The persistence of non-introgressed populations of Owens tui chubs can be credited in part to chance physical or hydrologic isolation from the introduced chubs, and in part to active management, including transplantation. Relictual Owens tui chubs are found in AB Spring and CD Spring which provide the water supply to Hot Creek State Fish Hatchery. Prior to their appreciation as an endemic species (Miller 1973), eradication attempts reduced these populations to extreme low levels on multiple occasions. A second native population occurs in the uppermost reach of the Owens River Gorge (Upper Owens Gorge), subsisting in dam seepage and protected from immigration below by an eleven-kilometer reach of unsuitable habitat populated by brown trout (Jenkins 1990, unpublished data). The effort required to capture individuals from this population implies a low density or small population size.

A demographically robust population of presumed non-introgressed tui chubs occurs at an impoundment of Little Hot Creek originally created for waterfowl. Transplants were made from CD Spring and Upper Owens Gorge to the currently defunct Owens Valley Native Fishes Sanctuary in Fish Slough. Progeny of these transfers were successfully established in the Little Hot Creek reservoir.

Non-introgressed Owens tui chubs were also identified in a surprising finding in the newly re-watered Lower Owens River Gorge (Fransz 1995, unpublished data). The origin of these fish is unclear, because this reach of the river was wholly diverted for hydroelectric generation between 1953 and 1992. Twenty-two chubs from this population were rescued to found a new population at the University of California's White Mountain Research Station (WMRS) near Bishop in 1997. State fishery managers were prevented by the landowner from obtaining a larger representative sample of the source population before it crashed in the face of a burgeoning brown trout population.

In 1987 a presumed new relictual population of Owens tui chubs (Miller 1997, unpublished letter to California Department of Fish and Game), was discovered inhabiting irrigation ditches and a spring at Cabin Bar Ranch on the shore of dry Owens Lake. Exhaustive efforts rescued a total of 24 individuals that were placed in an artificial pond on the ranch property in 1989. The remainder of the population is believed to have been extirpated by illegally introduced largemouth bass (*Micropterus salmoides*) and bluegill sunfish (*Lepomis macrochirus*). The rescued fish produced abundant offspring, from which 52 juveniles were transferred to an artificial pond at Mule Spring in 1990. All extant fish of this group descend from this transplant because the source population was lost when the property owner failed to maintain the pond.

Microsatellite DNA loci, characterized by a core sequence consisting of a number of identical repeated units of 2–6 base pair (bp), are extraordinarily variable even within populations that have low levels of allozyme and mitochondrial DNA variation (Tautz 1989; Weber and May 1989). Microsatellite variation has been applied extensively in assessing genetic relationships among various subpopulations (Forbes et al. 1995), detecting the degree of introgression between closely related species and subspecies (Roy et al. 1994), and estimating dispersal and migration (Allen et al. 1995). Using six microsatellite loci, this study (i) assessed the degree of genetic diversity within and among extant populations of Owens tui chub; (ii) compared genetic variation among Owens and geographically proximate Lahontan tui chub populations to reassess the subspecific status of Owens tui chub; and (iii) determined the extent of introgression by Lahontan tui chubs into Owens populations.

Materials and methods

Sample collection and DNA extraction

A total of 360 individual tui chubs were analyzed. We took 152 samples from nine Owens River basin locales presumed to hold non-introgressed populations, and 124 from another eight locales representing every known occurrence of the widespread putative hybrids (see Table 1 and map in Fig. 1). Collections for presumed non-introgressed tui chubs were made under federal recovery permits issued by U. S. Fish and Wildlife Service. In addition, 84 samples from eight disjunct Lahontan tui chub populations, including the Lahontan creek-form (*S. b. obesa*) and Lahontan lake-form (*S. b. pectinifer*), were assessed as reference populations to ensure that population-specific genotypes are not confused with subspecies-specific genotypes. Fish were sampled using electrofishing, minnow traps, gill nets, and seines. A subset of tui chubs were sacrificed to permit parallel osteological and meristic studies conducted by S. Parmenter and other researchers. Tissue samples were taken from the tips of each pelvic fin. One piece (10–20 mm^2) was air-dried and stored in a paper envelope, while another was preserved in DMSO buffer. Genomic DNA was extracted from fin clips using the Sigma Tissue Kit (Sigma), and stored at –70°C until needed.

Table 1 Presumed status, population location, code, sample size, and year of collection of tui chub samples

Presumed taxon	Population location	Longitude and latitude	Code	Sample size	Year of collection
S. b. snyderi	Cabin Bar Ranch; Inyo Co., California	36°18′53″ N,118°01′25″ W	CB	16	2002
S. b. snyderi	Mule Spring; Inyo Co., California	37°06′25″ N, 118°12′10″ W	MS	14	1997 and 2002
S. b. snyderi	Lower Owens Gorge; Inyo Co., California	37°26′24″ N, 118°33′20″ W	OG-L	11	1997
S. b. snyderi	White Mountain Research Station; Inyo Co., California	37°21′39″ N, 118°19′45″ W	WMRS	15	2002
S. b. snyderi	Sotcher Lake; Madera Co., California	37°37′27″ N, 119°04′23″ W	SOT	14	2002
S. b. snyderi	AB Spring, Hot Creek Hatchery; Mono Co., California	37°38′19″ N, 118°51′50″ W	ABS	15	2002
S. b. snyderi	CD Spring, Hot Creek Hatchery; Mono Co., California	37°38′18″ N, 118°51′37″ W	CDS	15	2002
S. b. snyderi	Little Hot Creek; Mono, Co., California	37°41′21″ N, 118°50′19″ W	LHC	35	1997 and 2002
S. b. snyderi	Upper Owens Gorge; Mono Co., California	37°35′06″ N, 118°39′31″ W	OG-U	17	2002
Hybrid	Hot Creek; Mono Co., California	37°40′33″ N, 118°48′31″ W	HC	20	1997
Hybrid	Mammoth Creek; Mono Co., California	37°38′36″ N, 118°51′13″ W	MC	15	2001
Hybrid	Twin Lakes-Mammoth; Mono Co., California	37°37′05″ N, 119°00′33″ W	TLM	15	2001
Hybrid	Upper Gorge Tailbay; Mono C., California	37°32′47″ N, 118°35′24″ W	OG-TB	15	2001
Hybrid	A1 Drain; Inyo Co., California	37°21′44″ N, 118°25′14″ W	A1D	13	2001
Hybrid	C2 Ditch; Inyo Co., California	37°26′02″ N, 118°34′07″ W	C2D	15	2001
Hybrid	McNally Canal; Inyo Co., California	37°24′44″ N, 118°20′20″ W	MNC	16	2001
Hybrid	June Lake; Mono Co., California	37°46′57″ N, 119°04′35″ W	JL	15	2001
S. b. obesa	East Walker River; Mono Co., California	38°19′38″ N, 119°12′51″ W	EWR	22	1997 and 2001
S. b. obesa	Twin Lakes-Bridgeport; Mono Co., California	38°10′18″ N, 119°19′56″ W	TLB	15	2001
S. b. obesa	Walker Lake; Mineral Co., Nevada	38°41′54″ N, 118°42′59″ W	W	11	1997 and 2001
S. b. obesa	Lake Tahoe; El Dorado Co., California	38°56′20″ N, 120°00′10″ W	TAH	15	2001
S. b. obesa	Toulon Drain; Pershing Co., Nevada	40°05′01″ N, 118°34′42″ W	TD	5	1997
S. b. pectinifer	Pyramid Lake; Washoe Co., Nevada	40°02′00″ N, 119°34′02″ W	PLP	5	1997
S. b. obesa	Pyramid Lake; Washoe Co., Nevada	40°02′00″ N, 119°34′02″ W	PLO	5	1997
S. b. obesa	Independence Lake; Nevada Co., California	39°26′34″ N, 120°18′35″ W	L	6	1997

The 1997 collection was authorized by federal permit # PRT-829201, and collection for the presumed non-introgressed Owens tui chub taxa in 2002 was made under federal permit # TE049668–0

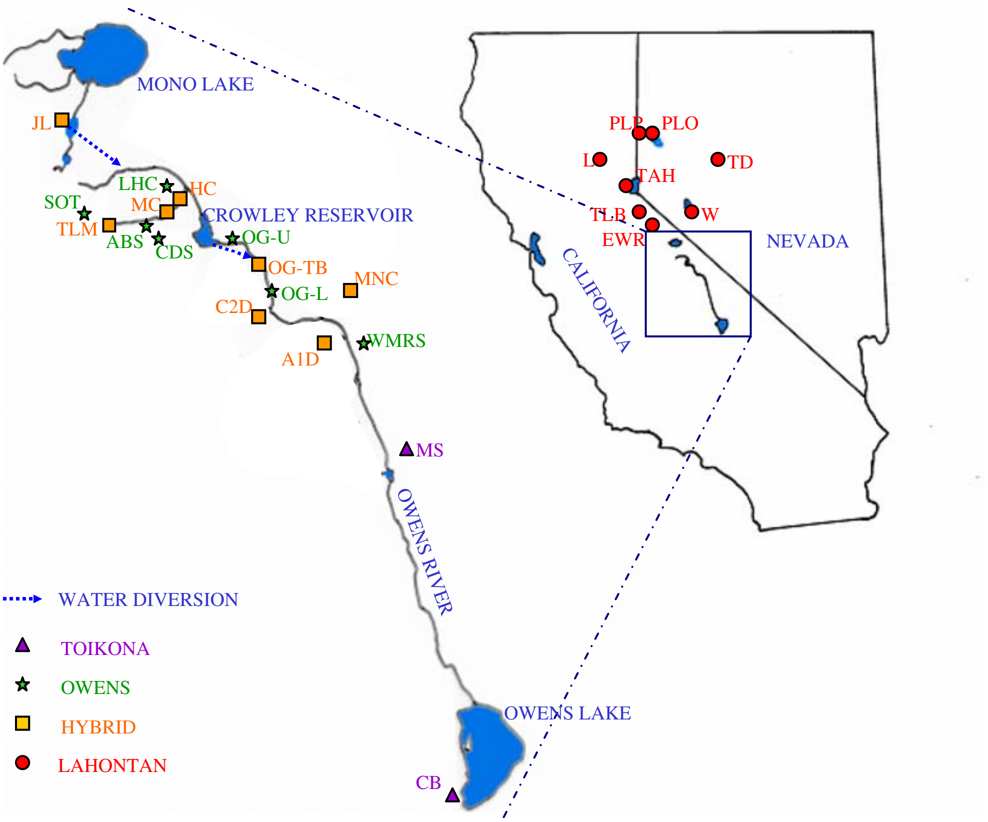

Fig. 1 Map of sample locations (see Table 1 for population codes)

PCR amplification and microsatellite DNA screening

Six microsatellite loci (Gbi-G3, Gbi-G10, Gbi-G13, Gbi-G38, Gbi-G79, and Gbi-G87) developed in Lahontan tui chubs by Meredith and May (2002) were utilized in this study. Microsatellite DNA was amplified via the polymerase chain reaction (PCR). Each 10 µl PCR contained: 20 mM Tris–HCl, pH 8.4, 1.5 mM $MgCl_2$ (3.0 mM for Gbi-G13 and Gbi-G38) 0.2 mM dNTPs, 0.5 µM primers, and 0.4 units Taq DNA polymerase (Promega). Reaction mixtures were amplified using the following conditions: 96°C for 2 min, then followed by 35 cycles (30 cycles for Gbi-G38 and 40 cycles for Gbi-G79 and Gbi-G87) of 95°C for 40 s, 52°C for 1 min (50°C for Gbi-G13 and 60°C for Gbi-G38), and 72°C for 1 min, and ended with an extension of 72°C for 10 min. The PCR-generated products of microsatellite DNA were electrophoresed on a 5.5% denaturing polyacrylamide gel, and visualized on a BaseStation 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).

DNA sequencing

We sequenced Gbi-G79, in which alleles of irregular sizes were discovered during microsatellite screening. These irregular alleles appear either 1 bp or 3 bp smaller than would be expected in regular alleles composed of tetra-nucleotide repeats. To understand this cryptic irregularity of Gbi-G79 alleles, 11 representative homozygote samples of Owens and Lahontan tui chubs were selected for PCR-direct sequencing. The PCR for sequencing remained the same as that for genotyping, except that the forward primers were unlabeled. Sequencing reactions were performed in the DNA Sequencing Facility (Division of Biological Sciences, The University of California, Davis) using Applied Biosystems BigDye® Terminators v3.0 Cycle Sequencing Kit. Each sequence was generated by both forward and reverse primers.

Population genetic analyses

FSTAT 2.9.3 was used to compute allele frequency and allele number for each locus and each population (Goudet 1995). ARLEQUIN 3.0 was performed to estimate observed and expected heterozygosities, and to test deviations of Hardy–Weinberg equilibrium (Excoffier et al. 2005). A factorial correspondence analysis (FCA) was conducted to project the genetic relationships of populations of multiple dimensions on the basis of the matrix of allele counts per individual with GENETIX 4.04 program (Belkhir et al. 2003). FCA offers the advantage of simultaneous expression of the genetic differences contributed by each allele, and it has proven well-suited for introgression tests (Lu et al. 2001; Roques et al. 2001). Pairwise *F*-statistics (F_{ST}; Weir and Cockerham 1984) were computed using GENETIX 4.04 (Belkhir et al. 2003) to determine the degree of population divergence and subdivision.

We examined spatial patterns of divergence among relictual Owens tui chub populations (including Cabin Bar). Using maps and air photos, we estimated stream distances from the uppermost populations (ABS and CDS) to all other populations along the Owens River. The Lower Owens Gorge (OG-L) and Upper Owens Gorge (OG-U) populations lie intermediate between AB and CD Springs and Cabin Bar Ranch (see Fig. 1). A test of the relationship between pairwise F_{ST} and geographical distance along stream was performed using isolation-by-distance (IBD) web service (Jensen et al. 2005).

The degree of introgression equivalent to the proportion of admixture was determined using LEADMIX 1.0 (Wang 2003). Miller (1973) concluded that the introduced Lahontan tui chubs originated from the Walker basin. In this aspect of the study, East Walker River (EWR), Twin Lakes-Bridgeport (TLB), and Walker Lake (W) were selected to represent Walker basin tui chubs. The Walker basin tui chub samples were pooled as the first parental sample, and all non-introgressed Owens tui chubs were used as the second parental sample. All individuals of introgressed Owens tui chub populations were pooled for the estimation of overall degree of introgression. The degree of introgression was independently determined for each separate hybrid population. The estimable genetic drift was assumed to be 0.0001. LEADMIX also gave the estimates of degree of introgression from two moment estimators, one by Roberts and Hiorns (1965) and the other developed by Long (1991) and later elaborated by Chakraborty et al. (1992). The 95% confidence intervals (CIs) were obtained from 1000 bootstrapping samples over all six microsatellite DNA loci.

Results

Microsatellite DNA variation

Individual genotypes were either single-banded or symmetrically double-banded, suggesting that these loci are disomic. A range of 11 (Gbi-G13) to 27 (Gbi-G3) alleles was scored per locus. Alleles at five loci, Gbi-G3, Gbi-G10, Gbi-G13, Gbi-G38, and Gbi-G87 varied in sizes composed of repeats of 4 bp, but Gbi-G79 is exceptional in that it encompasses alleles which cannot be factored by 4 bp. Appendix 1 shows allele frequency and allele number (N_A), observed (H_O) and expected heterozygosity (H_E), and *P*-value for Hardy–Weinberg equilibrium (P_{HW}) tests for each locus and each population (Guo and Thompson 1992). Fifteen out of 144 HW tests indicated statistical significance ($P \leq 0.01$), 10 of which were observed in Gbi-G13. The extraordinary number of Hardy–Weinberg deviations at a single locus is likely a consequence of null alleles.

Sequence indels for Gbi-G79

Sequence data indicate that the actual length of the microsatellite, Gbi-G79 is 157–197 bp with the exclusion of both primers (see Table 2; GenBank accession numbers DQ471889–471899). The number of repeat motifs, $(TCTA)_n$ varied from 4 to 14. Two nucleotide indels were found in the flanking region of the locus between the forward primer and repeat motif. The first is a deletion of cytosine (C) at the 14th nucleotide position from the forward primer, denoted as Sb-D. Microsatellite alleles of Gbi-G79 with Sb-D are characterized by odd sizes (Table 2) and are primarily found in Lahontan tui chubs; a single odd-sized allele of 237 was detected in Little Hot Creek at a frequency of 0.02 (Appendix 1). A 3 bp deletion of ATT, termed " Tk-D," was found in the microsatellite allele 231. This variant was found at Cabin Bar (0.80), and Mule Spring (0.71), but not in any other Owens tui chub populations.

Population divergence and subdivision

Factorial correspondence analysis depicts the 25 tui chub populations in four discrete groups: Owens (OG-L, WMRS, SOT, ABS, CDS, LHC, and OG-U), Lahontan (EWR, TLB, W, TAH, TD, PLP, PLO, and L), hybrid Owens × Lahontan (HC, MC, TLM, OG-TB, A1D, C2D, MNC, and JL) and Cabin Bar (CB and MS) (see Fig. 2). The first two axes account for 36.5% of the total variation among populations. Adding a third dimension would only increase the percent of

Table 2 Sequence deletions found in the microsatellite locus, Gbi-G79

Presumed taxon	Sample code	Homozygote	GenBank accession number	Microsatellite repeat	Deletion
S. b. snyderi	OG-U	242/242	DQ471889	14	–
S. b. snyderi	ABS	234/234	DQ471890	12	–
S. b. snyderi	LHC	234/234	DQ471891	12	–
S. b. snyderi	WMRS	218/218	DQ471892	8	–
S. b. snyderi	WMRS	202/202	DQ471893	4	–
S. b. snyderi	CB	231/231	DQ471894	12	Tk-D
S. b. snyderi	MS	231/231	DQ471895	12	Tk-D
S. b. obesa	W	237/237	DQ471896	13	Sb-D
S. b. obesa	W	225/225	DQ471897	10	Sb-D
S. b. obesa	PLO	213/213	DQ471898	7	Sb-D
S. b. obesa	EWR	209/209	DQ471899	6	Sb-D

Sb-D refers to deletion of a single cytosine (C) at the 14th nucleotide position from the forward primer; Tk-D denotes an ATT deletion at a position 35–37 bp from the forward primer

variation explained to 44.7% while not changing the constitution or relationships among the groups. FC 1 extracts Cabin Bar tui chubs, representing that they are the most significantly different group. Hybrid populations are located intermediately between Owens and Lahontan tui chubs along FC 2; the FCA also shows that Lahontan tui chubs in the Walker basin are more closely related to *S. b. obesa* (PLO) than to *S. b. pectinifer* (PLP) from Pyramid Lake.

The analysis of pairwise F_{ST} reveals significant degrees of population divergence and subdivision among these groupings (Table 3), similar to the FCA results. F_{ST} values averaged over population pairs between Cabin Bar and Owens, hybrid, and Lahontan tui chubs are 0.39, 0.35 and 0.42, respectively; markedly greater than corresponding comparisons between Owens, hybrid, and Lahontan tui chubs. Even though Cabin Bar tui chubs share the same river basin with the Owens chubs, they are more different from Owens chubs than are Lahontan tui chub populations. Likewise, the F_{ST} between Owens and Lahontan tui chubs (0.18) is greater than the F_{ST} pair either between hybrid and Owens (0.11) or hybrid and Lahontan tui chubs (0.09). In addition, compared to between-group F_{ST} values, within-group values are relatively small (0.03–0.10).

The stream distance from Hot Creek Springs (ABS and CDS) to OG-U is 34 kilometers (km), to OG-L is 61 km, and to CB is 224 km, and that between ABS and CDS is approximately 1 km. The IBD analysis illustrates that pairwise F_{ST} between the Owens populations is positively correlated to stream distance (Fig. 3; $Z = 357.85$, $R^2 = 0.887$, one-sided $P \leq 0.0161$

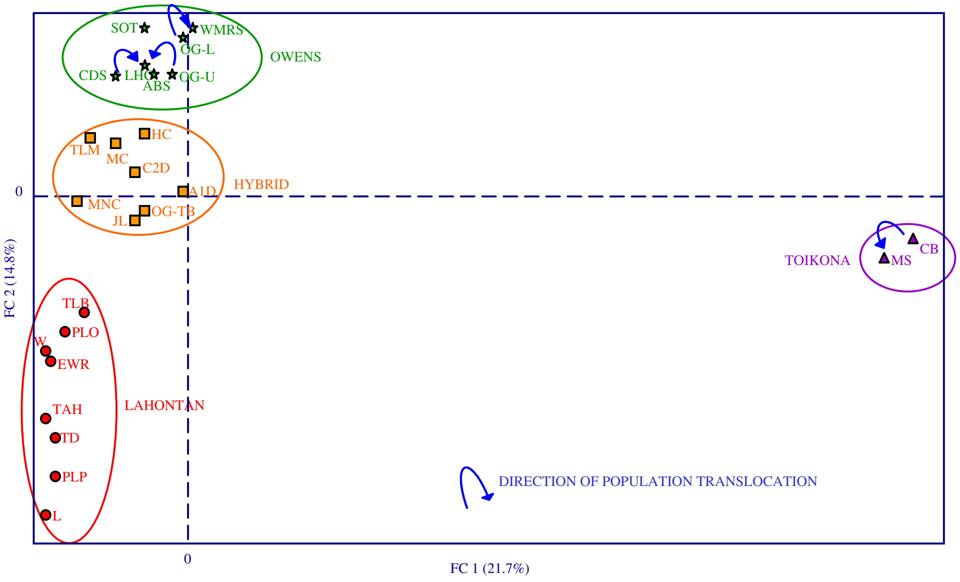

Fig. 2 Factorial correspondence analysis of toikona, Owens, hybrid and Lahontan tui chubs. The projection of populations on the surface is defined by the first two factorial axes of FCA

Table 3 Estimates of pairwise F_{ST} values between tui chub populations and tests of statistical significance at 10,000 permutations are displayed above diagonal (P-values ≤0.01 indicated by "†" are considered significant); F_{ST} values between pairs of groups averaged over populations are displayed below diagonal

	TOIK-ONA		OWENS							HYBRID								LAHONTAN							
	CB	MS	OG-L	WMRS	SOT	ABS	CDS	LHC	OG-U	HC	MC	TLM	OG-TB	A1D	C2D	MNC	JL	EWR	TLB	W	TAH	TD	PLP	PLO	L
CB		0.03	0.46 †	0.46 †	0.45 †	0.40 †	0.44 †	0.36 †	0.36 †	0.36 †	0.37 †	0.47 †	0.36 †	0.35 †	0.39 †	0.43 †	0.37 †	0.42 †	0.38 †	0.42 †	0.41 †	0.50 †	0.48 †	0.46 †	0.53 †
MS	0.03		0.40 †	0.41 †	0.38 †	0.33 †	0.37 †	0.30 †	0.30 †	0.29 †	0.31 †	0.41 †	0.29 †	0.29 †	0.31 †	0.37 †	0.30 †	0.36 †	0.32 †	0.36 †	0.36 †	0.42 †	0.40 †	0.38 †	0.46 †
OG-L				0.02	0.13 †	0.09 †	0.20 †	0.14 †	0.09 †	0.10	0.11 †	0.23 †	0.14 †	0.12 †	0.15 †	0.22 †	0.16 †	0.26 †	0.19 †	0.23 †	0.21 †	0.27 †	0.24 †	0.17 †	0.30 †
WMRS					0.13 †	0.13 †	0.20 †	0.15 †	0.12 †	0.13 †	0.13 †	0.27 †	0.17 †	0.17 †	0.15 †	0.23 †	0.19 †	0.28 †	0.22 †	0.26 †	0.24 †	0.30 †	0.27 †	0.22 †	0.34 †
SOT						0.06	0.09	0.09 †	0.10 †	0.09 †	0.07 †	0.17 †	0.13 †	0.14 †	0.11 †	0.14 †	0.13 †	0.21 †	0.14 †	0.17 †	0.19 †	0.21 †	0.17 †	0.14 †	0.25 †
ABS							0.07	0.06 †	0.05	0.03	0.02	0.09	0.06	0.06	0.07 †	0.10 †	0.06	0.16 †	0.09 †	0.13 †	0.14 †	0.17 †	0.14 †	0.09	0.21 †
CDS	0.39				0.10			0.03	0.13 †	0.08 †	0.05	0.07 †	0.08 †	0.13 †	0.10 †	0.06	0.10 †	0.14 †	0.12 †	0.10 †	0.14 †	0.17 †	0.13 †	0.09	0.22 †
LHC									0.09 †	0.06 †	0.05	0.12 †	0.06 †	0.10 †	0.08 †	0.11 †	0.07 †	0.16 †	0.12 †	0.12 †	0.14 †	0.17 †	0.14 †	0.11 †	0.21 †
OG-U										0.04	0.05	0.15 †	0.08 †	0.05	0.07 †	0.13 †	0.09 †	0.20 †	0.11 †	0.17 †	0.17 †	0.18 †	0.18 †	0.12 †	0.22 †
HC											0.01	0.09 †	0.03	0.02	0.04	0.08 †	0.04	0.12 †	0.07 †	0.09 †	0.11 †	0.13	0.11 †	0.05	0.16 †
MC												0.07 †	0.01	0.04	0.03	0.05	0.03	0.07 †	0.04	0.04 †	0.07 †	0.09 †	0.06	0.03	0.12 †
TLM													0.07 †	0.12 †	0.10	0.09 †	0.11 †	0.14 †	0.12 †	0.11 †	0.12 †	0.14	0.13 †	0.07	0.20 †
OG-TB														0.05	0.05	0.07 †	0.03	0.06 †	0.06 †	0.04	0.07 †	0.08	0.06	0.03	0.12 †
A1D	0.35				0.11							0.06			0.08 †	0.11 †	0.07	0.14 †	0.08 †	0.11 †	0.14 †	0.11 †	0.11 †	0.08	0.17 †
C2D																0.06	0.04	0.11 †	0.07	0.09 †	0.10 †	0.11 †	0.09	0.08	0.17 †
MNC																	0.08 †	0.08 †	0.09 †	0.06	0.10 †	0.11 †	0.10	0.08	0.17 †
JL																		0.06 †	0.07	0.04	0.08 †	0.09	0.07	0.05	0.10 †
EWR																			0.08 †	0.00	0.07 †	0.08	0.06	0.07	0.12 †
TLB																				0.06	0.08 †	0.07	0.03	0.06	0.14 †
W																					0.04	0.05	0.02	0.02	0.08
TAH																						0.07	0.04	0.03	0.06
TD	0.42				0.18							0.09								0.06			0.05	0.05	0.11
PLP																								0.02	0.11
PLO																									0.07
L																									

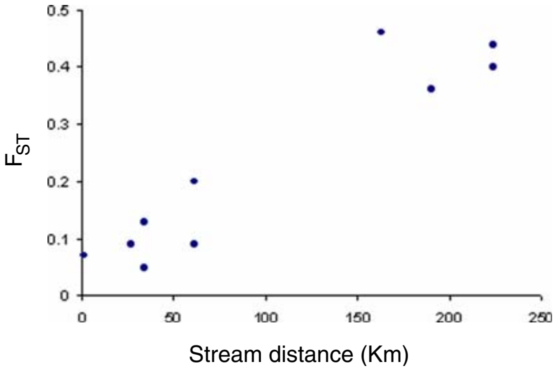

Fig. 3 The correlation of pairwise F_{ST} values and geographical (stream) distances of relictual natural Owens tui chub populations: ABS, CDS, OG-U, OG-L, and CB ($y = 0.001898x + 0.03563$; $R^2=0.887$)

from 10,000 randomizations). Two discrete clusters appear on Fig. 3, one associated with Cabin Bar tui chubs, and the other with Owens tui chubs from the Gorge and Hot Creek Springs.

Genetic variation in Cabin Bar tui chubs

Despite reasonable sample sizes, we found only 18 total alleles in Cabin Bar tui chubs. This is considerably fewer than the allele numbers present in Owens (64), hybrid (102), and Lahontan tui chubs (119) (Appendix 1). In addition to the fewest alleles, Cabin Bar tui chubs also possess the least heterozygosity of the sampled populations. The expected heterozygosity (H_E) of Cabin Bar tui chubs is 0.34 averaged over all the microsatellite loci; much lower than those of Owens (0.71), hybrid (0.83), and Lahontan tui chubs (0.88). Notably, six of the 18 alleles (1/3) observed in Cabin Bar tui chubs are not found in non-introgressed Owens tui chub populations. These six private alleles are distributed across four microsatellite loci, and four of the alleles have high frequencies ranging from 0.52 to 1.00 (see Appendix 1): alleles 293 at Gbi-G10 (0.52), 272 at Gbi-G38 (0.85), 231 at Gbi-G79 (0.76), and 172 at Gbi-G87 (1.00). No evidence was found of hybrid contact between Lahontan and Cabin Bar tui chubs.

Introgression between Lahontan and Owens tui chubs

We looked into distribution patterns of private alleles across tui chub populations of Owens (non-introgressed), hybrid, and Lahontan (Walker basin) origin to determine degrees of introgression. Confirmation of the hybrid populations is evidenced by the alleles only found in the Walker basin and hybrid populations, or the Owens and hybrid populations (Appendix 1). The numbers of alleles in Owens, hybrid, and Walker basin tui chubs are 64, 102, and 92, respectively. A total of fifty alleles were scored across Owens, hybrid and Walker basin tui chubs; 42 alleles from Walker basin tui chub are absent in Owens tui chubs, while 32 of these (76%) are present in hybrid populations. In contrast, all 14 private alleles from Owens populations are present in the hybrids. Six orphan alleles found at low frequencies in the hybrids were not detected in either parental sample.

We compared degrees of introgression of Lahontan tui chubs into hybrid populations by three different estimators, RH (Roberts and Hiorns 1965), LC (Long 1991; Chakraborty et al. 1992), and W (Wang 2003) in Table 4. The overall proportions of introgression estimated using these methods are 0.43, 0.37, and 0.42, respectively. The similarity of the means and 95% confidence intervals of three estimations shows close

Table 4 Estimated degree of introgression by Lahontan tui chubs from the Walker basin into hybrid populations

Hybrid population	RH	LC	W	N_S
HC	0.26 (0.15–0.33)	0.46 (0.31–0.64)	0.24 (0.12–0.37)	20
MC	0.40 (0.26–0.50)	0.45 (0.34–0.54)	0.29 (0.18–0.43)	15
TLM	0.39 (0.20–0.54)	0.90 (0.64–1)	0.08 (0–0.35)	15
OG-TB	0.53 (0.39–0.71)	0.57 (0.43–0.71)	0.56 (0.39–0.73)	15
A1D	0.27 (0.10–0.61)	0.36 (0.22–0.54)	0.57 (0.37–0.82)	13
C2D	0.39 (0.21–0.69)	0.46 (0.35–0.66)	0.44 (0.27–0.62)	15
MNC	0.60 (0.41–0.73)	0.73 (0.68–0.76)	0.56 (0.36–0.76)	16
JL	0.57 (0.35–0.82)	0.67 (0.51–0.85)	0.58 (0.41–0.78)	15
Overall	0.43 (0.34–0.51)	0.37 (0.25–0.52)	0.42 (0.30–0.52)	124

Shaded rows indicate populations with consistently high proportions of introgression estimated by the models of Roberts and Hiorns 1965 (RH), Long (1991) and Chakraborty et al. (1992) (LC); and Wang (2003) (W). The 95% confidence intervals (CIs) obtained from 1000 bootstrapping samples over the six microsatellite loci are in parenthesis. N_S represents the sample size of each population in the study agreement among the methods. However, estimated introgression into individual hybrid populations is not uniform. The three estimators do not show strong agreement in these paired population tests, likely because of the smaller sample sizes.

Discussion

Introgression and hybrid swarm between Lahontan and Owens tui chubs

The presence of native relictual Owens tui chubs was confirmed at AB Spring, CD Spring, Upper Owens Gorge, and Cabin Bar Ranch. Non-introgressed Owens populations of transplanted or unknown origin were found in isolated habitats at Little Hot Creek, Sotcher Lake, Lower Owens Gorge, and WMRS. Except for these fragmented habitats, the Lahontan × Owens hybrid swarm has replaced the original Owens populations in the remainder of the Owens River watershed.

Our microsatellite DNA analysis supports Miller's (1973) scenario of introgression by Lahontan tui chub genes from the Walker basin into Owens populations in three ways. First, the FCA projects Lahontan and Owens tui chubs and their hybrids into separate groupings, and places all the hybrid populations intermediately between the two presumed donor populations (Fig. 2). Second, all Owens tui chub alleles were incorporated into the hybrid swarm, while the invading Walker basin tui chubs successfully contributed only a subset (76%) of their private alleles. The six orphan alleles found in hybrid populations may represent endemic Owens basin alleles which have been lost in the smaller refugial populations through genetic drift, although the possibility that we insufficiently sampled the parental stocks cannot be excluded. Finally, estimated levels of introgression qualitatively agree with the FCA result, and quantitatively agree with the distribution of private alleles. The overall degree of introgression ranges from 0.37 (LC), 0.42 (W), to 0.43 (RH). Though introgression by Walker basin tui chubs into individual hybrid populations varies widely, all three estimators consistently indicate high degrees of introgression in tui chub populations of June Lake (0.57–0.67), Upper Gorge Tailbay (0.53–0.57), and McNally Canal (0.56–0.73).

The odd-sized alleles of Gbi-G79 are rarely found in non-introgressed Owens tui chub populations, absent in Cabin Bar and Mule Spring, yet common in Lahontan tui chubs. The sole exception to this pattern is a single 237 bp allele found at Little Hot Creek. This population is presumed to consist of non-introgressed Owens tui chubs, originally transplanted from Hot Creek Springs and Upper Owens Gorge. If this single allele results from introgression, the event may have occurred in one of the ancestral populations. Compared with the relictual sites, the Little Hot Creek site is more distant from sources of potential introgression. Alternatively, a slight incidence of Sb-Ds may naturally occur in Owens tui chubs. More sequencing would be needed to better understand the evolutionary history of this indel.

Natural introgression can play an important role in the diversification of species (Arnold 1992; Dowling and DeMarais 1993; Dowling and Secor 1997), or could be detrimental if either parental population is better adapted to the local environment (Echelle 1991; Haig 1998). Nonetheless, anthropogenic introgression is considered a serious challenge to the conservation of endangered and threatened populations (Allendorf and Leary 1988; Echelle 1991; Allendorf et al. 2001), because it can quickly degrade locally adapted gene pools. Introgression by Lahontan tui chub genes has diminished genetic integrity throughout Owens basin populations except in four relictual populations (ABS, CDS, OG-U, and CB). Identification of hybrids and genetic characteristics of non-introgressed Owens tui chubs by this study is vital to preserving and restoring potentially locally adapted Owens populations.

Miller (1973) used meristics and morphometrics to demonstrate the widespread introgression by Lahontan tui chubs in the Owens River and interconnected waterways. Samples from Hot Creek, Mammoth Creek, Twin Lakes Mammoth, Upper Gorge Tailbay, A1 Drain, C2 Ditch, and McNally Canal encompass the putative hybrid swarm. Our microsatellite data sustain Miller's findings. The Upper Gorge Tailbay site is a small pond through which passes the entire outflow of Crowley Reservoir, where hybrid tui chubs were initially discovered by Miller (1973). The water arrives via a hydroelectric penstock and turbine whose design permits the passage of live fish, creating a conduit for hybrids or eggs to move from Crowley Reservoir through the tailbay and into the Owens River Gorge below this point. The tailbay has been sporadically dewatered, and presumably recolonized via the penstock. We speculate that upstream movement from the tailbay into federally designated "Critical Habitat" is prevented by a streamflow measurement weir, above which a dense brown trout population renders many kilometers of stream unsuitable for tui chubs (Jenkins 1990, unpublished data). This study confirms that tui chubs in the tailbay are introgressed, and shows the Upper Owens Gorge population has escaped this fate. Downstream tui chub populations (McNally Canal, C2 Ditch, and A1 Drain) are introgressed, very likely through downstream movement of fish from Crowley Reservoir.

Extralimital populations of tui chubs are known from the Mono Lake basin, upper Mammoth Lakes basin, and Sotcher Lake (Madera County, California). Sotcher Lake and June Lake lie outside of the Owens tui chub's native watershed. The origins of these populations are unknown, but are reputed to result from either the use of tui chubs as live bait for sportfishing in June Lake, or in the case of Sotcher Lake, incidental to the stocking of trout. June Lake was chosen to represent the Rush Creek watershed, consisting of four interconnected lakes containing robust introduced populations of tui chubs in the Mono Lake basin. Water from this drainage is exported to the Owens River above Crowley Reservoir through the Mono Craters Tunnel, a trans-basin water diversion. Tui chubs in June Lake genetically resemble the downstream hybrids. It seems likely that introgressed tui chubs from the Owens River or Crowley Reservoir were introduced into both the Rush Creek and Mammoth Lakes watersheds, although it is also plausible that independent introductions of each parental stock may have occurred.

The highest levels of introgression were found in June Lake, Upper Gorge Tailbay (a proxy for Crowley Reservoir population) and McNally Canal. These localities are downstream of potential introduction sites on the mainstem Owens River or Rush Creek. Lesser degrees of introgression were observed in sites where upstream dispersal would be required to account for gene flow: A1 Drain, C2 Ditch, Mammoth Creek, and Hot Creek. Waterfalls physically prevent upstream gene flow into Twin Lakes-Mammoth. Mammoth Creek and Hot Creek potentially receive downstream gene flow from hybrids in Twin Lakes-Mammoth, as well as from the relictual populations in AB Spring and CD Spring.

The tui chub in Sotcher Lake is recognized as non-introgressed *S. b. snyderi* by this study. Sotcher Lake is geographically proximate to the Owens River basin, but drains to the San Joaquin River on the distal slope of the Sierra Nevada Mountains. Field surveys of California Department of Fish and Game on file in the Bishop Office indicate the Sotcher Lake introduction occurred after 1951 but before 1955. The Hot Creek State Fish Hatchery stocked trout in Sotcher Lake during this period, and tui chubs from the springs may have accidentally arrived with the trout. Although similar trout introductions occur elsewhere including the Lahontan basin, we find no evidence of gene flow from Owens into native Lahontan tui chub populations.

Divergence and taxonomic status of Owens and toikona tui chubs

The Owens tui chub is morphologically most similar to the Lahontan tui chub in the East Walker River (Miller 1973). Similarly, our results suggest that Lahontan Creek chubs from both Pyramid Lake and East Walker River are the populations most similar to *S. b. snyderi*. The formal description of Owens tui chub as a subspecies (Miller 1973) is based on subtle anatomical differences such as the scale shape and number of radii. These characters are of questionable significance (Moyle 2002), and no single character is diagnostic. Historically, these two subspecies presumably were isolated when tectonic and climate changes shifted the drainage of Pleistocene Lake Russell (modern Mono Lake) from the Lahontan basin to the Owens River (Hubbs and Miller 1948), 1.3 Ma before present (Reheis et al. 2002). Genetic divergence between Owens and Lahontan tui chubs was suggested by a previous genetic analysis of allozymes and amplified fragment length polymorphisms (AFLPs) (May, Rodzen, and Agresti 1997, unpublished report to the United States Department of the Navy). The microsatellite study reported here confirms the subspecific status of Owens tui chubs by demonstrating evolutionary distinctness at the population level.

Divergent microsatellite allele frequencies between samples from Cabin Bar Ranch (including Mule Spring) and the non-introgressed Owens tui chub populations suggest the existence of two distinct evolutionary entities within the Owens basin. Except for the Cabin Bar population, the remaining relictual Owens tui chub populations are all found in or upstream of the Owens River Gorge; these and their descendants are here recognized as "Owens tui chub" (*S. b. snyderi*). The pairwise F_{ST} values between Cabin Bar and Owens tui chub populations are much greater than those between the recognized subspecies *S. b. obesa* and *S. b. snyderi*, signifying that Cabin Bar tui chubs might be a distinct unrecognized taxon. We give the tui chub originating from Cabin Bar Ranch a common name "toikona tui chub" to distinguish it from the Owens. Toikona is a name for tui chubs used by Paiute people in the Owens Valley, California. It is a shortened form of "*toikonanishu*," which means "standing in the cattails." Recent mitochondrial DNA data do not indicate difference between Owens and toikona (Belfiore et al., unpublished data). Formal taxonomic description of toikona tui chubs requires more complete examination of meristic and osteological characters. This analysis has not yet been possible because, in their present restricted and biologically unproductive habitats, these fish do not attain sufficient body size at maturity for the indicative characters to fully develop (Miranda and Escala 2000).

Despite geographic proximity, and notwithstanding the demonstrated tendency of tui chubs to freely interbreed (Hubbs and Miller 1943; Miller 1973; Leunda et al. 2005), our results demonstrate substantial reproductive isolation between the two tui chub groups in the Owens River basin. The low genetic variation found in toikona tui chubs is likely a consequence of a severe bottleneck, inbreeding, and recent isolation. Nonetheless, the relative abundance and high frequencies of private alleles, including Tk-D in the 231 bp allele of Gbi-G79, suggest that this fish is distinctive.

Our findings suggest that the divergence between toikona and Owens tui chubs may reflect differentiation over a long period, or local adaptation. Owens and Lahontan tui chubs presumably diverged over the past 1.3 Ma (Reheis et al. 2002), implying separation of the more distinctive Owens and toikona tui chubs may be even older. Cabin Bar Ranch lies on the shore of Owens Lake, a pluvial lake that became functionally dewatered by upstream diversions in the 20th century. The springs and ditches where this fish was discovered lie more than 45 m below the outlet elevation through which Owens Lake discharged. At the Owens Lake highstand, standing water submerged the Cabin Bar site and much of the Owens Valley north approximately to Independence, California. As recently as the late 19th century, tui chubs were observed in Owens Lake (Gilbert 1893). U. S. Fish and Wildlife Service (1998) reviewed early collections of tui chubs in and near the Owens River and concluded "Owens tui chubs were common and occupied all valley-floor wetlands." It is challenging to envision the protracted persistence of a distinctive tui chub in a hypothetical refugium along the steep western shore of pluvial Owens Lake throughout the Holocene. If these fish evolved allopatrically, it follows that either the fish were recently introduced, or that geographic distance or migration barriers separated the Owens and toikona forms. To the north of Bishop, California, the Owens River cascades through a gorge some 30 km in length in which it drops 730 m in elevation. The Gorge is a recognized biogeographic barrier that prevented the colonization of headwater areas by Owens pupfish (*Cyprinodon radiosus*) (Miller and Pister 1971). All populations classified as Owens tui chub by this study originate in or upstream of the Gorge, and only the toikona and hybrid forms are found to the south. One interpretation of these observations is that toikona tui chubs represent an original form, which was widespread in the Owens Valley downstream of the Gorge. Ultimately, explanation of the distinctiveness of toikona tui chubs calls for meristic and osteological studies, for which few suitable specimens currently exist.

While the correlation between pairwise F_{ST} and stream distance suggests that isolation-by-distance (Wright 1943) could explain some of the differences between upstream (Owens) and downstream (toikona) populations, the pattern is probably more complex. Although Fig. 3 shows a linear increase in F_{ST} with distance, the cluster of points with large spatial separation and high F_{ST} derives entirely from comparisons involving toikona tui chubs. This pattern is consistent with divergence due to spatial distance, but could alternatively be an artifact—if truly isolated samples were improperly combined in a single analysis. Fragmentation of the original tui chub population leaves data gaps which confound the identification of preexisting geographic patterns of variation. The hydraulics of the 760,000-year-old (Sarna-Wojcicki et al. 2000) Owens Gorge would have impeded upstream gene flow into the upper part of the Owens River watershed. However, it is probable that unidirectional dispersal occurred in the downstream direction thorough larval drift. To the extent that isolation-by-distance may explain the observed differences between Owens and toikona tui chubs, we suggest that the prehistoric populations below the Gorge would have to be quite large in order to maintain their distinctiveness in the face of continual immigration.

Conservation and management of Owens and toikona tui chubs

Owens and toikona tui chubs may represent important independent lines of evolution. We recommend both tui chubs receive independent protection and recovery efforts, in order to avoid irreversible alteration of possibly unique evolutionary lineages. Future management practices should strive to protect, enhance, and expand habitats of both Owens and toikona tui chubs. If toikona tui chubs can be established in more productive habitats, we believe it would be appropriate to sacrifice a sufficient number of specimens to conduct meristic and osteological studies.

AB and CD Springs at Hot Creek Hatchery became isolated in 1931 with the construction of dams (Needham 1936), Upper Owens Gorge became cut off by Long Valley Dam in 1941 (Kahrl 1982); and Cabin Bar was presumably isolated when Owens Lake dried up around 1924. Our microsatellite results reveal spatial patterns of population divergence not only between Owens and toikona tui chubs, but also within Owens. The smaller differences found within Owens tui chub populations probably result from habitat fragmentation and drift in the historical period. Because the existing degree of variation in remnant populations seems reasonable for their viability, augmentation of genetic diversity by moving individuals among the relictual populations of Owens tui chubs is not indicated.

Hybrid tui chubs are so abundant and widespread throughout the Owens River basin that eradication is unrealistic. Existing management strategies of containment should be continued and strengthened to prevent any future mixing of non-introgressed Owens, toikona, or Lahontan tui chubs with each other, or with the hybrid swarm. Habitats of Owens and toikona tui chubs should remain isolated from potential gene flow from the Owens River.

Genetic studies have received increasing attention in the conservation and management of endangered and threatened species (O'Brien 1994; Amos and Balmford 2001; Frankham et al. 2002). Small and isolated populations have a heightened risk of extinction as a result of inbreeding depression and loss of genetic diversity (Franklin 1980; Chesser 1983; Hedrick 1983; Meffe 1986), as well as stochastic events. The remnant toikona tui chubs descend from a total of 24 founders rescued from the Cabin Bar Ranch in 1989, and their extant populations are confined to two diminutive artificial ponds. We speculate that the low genetic variation in this fish may be a consequence of founder effects. Establishing new populations and increasing effective population size in existing ones can avert further losses of genetic diversity due to inbreeding or genetic drift (Minckley 1995). To this extent, additional toikona tui chub populations need to be established in the Owens Valley. Because we know so little about toikona tui chubs, additional understanding of their origin, genetics and ecophysiology would better inform efforts to save these unique fish. Indeed, recovery efforts come with many costs; recreational fisheries, fish hatchery operations, and the development of water supplies in the town of Mammoth Lakes have all been constrained by tui chub protection (U. S. Fish and Wildlife Service 1998). This study provides the genetic background to guide recovery strategies, monitor long-term genetic change, and establish additional populations of rare tui chubs.

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Appendix 1 Summary of allelic frequency for each microsatellite locus and each population. H_O and H_E are observed and expected heterozygosities, respectively; P_{HW} represents the *P*-value of Hardy–Weinberg equilibrium test (100,000 steps in Markov Chain, and 10,000 Dememorization Steps); N_A and N_S represent the number of alleles and samples, respectively

LOCUS	ALLELE	TOIKONA		OWENS							HYBRID								LAHONTAN							
	Size (bp)	CB	MS	OG-L	WMRS	SOT	ABS	CDS	LHC	OG-U	HC	MC	TLM	OG-TB	A1D	C2D	MNC	JL	EWR	TLB	W	TAH	TD	PLP	PLO	L
Gbi-G3	228	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.03	–	–	0.06	–	–	0.10	–	–
	232	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.06$^{©}$	0.07	–	–	–	–
	236	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.07	–	–	–	–
	240	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.03	0.20	0.10	0.20	0.17
	244	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.10	–	–	–
	248	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.33	–	0.20	–	0.17
	252	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05$^{©}$	0.04$^{©}$	–	0.10	–	–	0.10	0.08
	256	–	–	–	–	–	–	–	–	–	–	–	–	0.10	0.04	–	–	–	–	0.04	–	0.07	–	0.10	–	–
	260	–	–	–	–	–	–	–	–	–	–	0.09	–	–	–	–	–	0.07	0.05	0.42	–	0.03	0.20	0.10	–	0.08
	264	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.03	–	0.23	0.06	–	–	0.10	0.10	–
	268	–	–	–	–	–	0.03	0.13	0.12	–	–	–	0.15	–	–	–	0.27	0.10	0.14	–	0.22	0.03	0.10	–	–	–
	272	–	–	0.45	0.29	0.07	0.40	0.13	0.07	0.12	0.23	0.18	0.31	0.23	0.19	0.07	0.08	0.10	0.07	–	0.06	0.03	–	–	0.30	–
	276	–	–	–	–	–	–	–	–	–	0.08	0.09	–	0.03	0.04	0.25	0.04	0.03	0.11	0.04	0.06	–	–	0.10	–	–
	280	–	–	–	–	–	–	–	–	–	–	–	–	–	0.12	0.04	0.08	–	0.27	–	0.28	0.03	0.10	–	0.10	0.08
	284	0.27	0.39	–	–	–	–	–	–	0.06	–	–	–	0.03	0.08	–	–	0.07	0.02	–	–	–	–	–	–	0.17
	288	0.04	0.23	0.10	0.29	0.32	0.23	0.27	0.40	0.21	0.23	0.14	0.04	0.10	–	0.32	0.08	0.27	–	0.08	0.06	0.07	–	–	–	–
	292	–	–	–	–	–	–	–	0.02	0.09	0.03	–	0.35	0.03	0.12	–	–	–	0.02	0.12	0.06	0.03	0.30	–	–	–
	296	–	–	–	–	0.18	0.03	–	0.03	0.03	0.13	–	0.08	0.10	0.08	0.11	0.04	0.07	0.11	–	0.06	–	–	0.10	0.10	–
	300	–	–	–	–	0.21	0.10	0.27	0.12	0.18	0.10	0.27	0.04	0.13	0.04	0.14	0.35	0.07	0.14	–	0.06	–	–	–	–	–
	304	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.25
	308	0.54	0.19	0.20	0.32	–	–	–	–	–	0.03	–	–	0.03	0.08	–	–	0.10	0.02	0.04	–	–	–	–	–	–
	312	0.15	0.19	–	–	–	0.17	0.03	0.15	0.09	0.05	0.09	–	0.07	–	0.04	–	–	–	–	–	0.07	–	0.10	–	–
	316	–	–	–	–	–	–	–	0.02	–	0.03	–	–	–	–	–	–	–	–	–	–	0.03	–	–	–	–
	320	–	–	–	–	–	–	0.13	0.07	0.06	0.03	0.14	–	0.07	–	–	–	–	–	–	–	–	–	–	–	–
	324	–	–	–	–	–	–	–	–	–	–	–	0.04§	–	0.15§	–	0.08§	0.03§	–	–	–	–	–	–	0.10	–
	328	–	–	0.25	0.07	0.11	0.03	0.03	0.02	0.03	0.08	–	–	0.03	0.08	–	–	–	–	–	–	–	–	–	–	–
	332	–	–	–	0.04	0.11	–	–	–	0.15	0.03	–	–	0.03	–	0.04	–	0.03	–	–	–	–	–	–	–	–
	H_O	0.69	0.77	0.80	0.79	0.79	0.67	0.93	0.74	0.94	0.85	0.55	0.54	0.80	0.92	0.79	0.77	0.93	0.77	0.54	1.00	0.80	0.60	1.00	0.80	1.00
	H_E	0.64	0.75	0.76	0.79	0.84	0.77	0.85	0.79	0.89	0.88	0.88	0.82	0.92	0.93	0.82	0.81	0.92	0.88	0.81	0.90	0.90	0.91	0.98	0.93	0.91
	P_{HW}	0.27	1.00	0.53	0.86	0.06	0.30	1.00	0.25	0.13	0.60	0.04	0.24	0.02	0.09	0.41	0.55	0.57	0.36	0.02	1.00	0.08	0.16	1.00	0.53	1.00
	N_A	4	4	4	5	6	7	7	10	10	12	7	7	13	11	8	8	13	11	8	11	14	6	9	7	7
	N_S	13	13	10	14	14	15	15	34	17	20	11	13	15	13	14	13	15	22	13	9	15	5	5	5	6
Gbi–G10	237	0.38	0.36	0.36	0.23	0.21	0.50	0.37	0.41	0.50	0.58	0.37	0.32	0.27	0.73	0.13	0.19	0.25	–	0.30	–	–	–	–	–	–
	253	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05$^{©}$	0.03	–	–	–	–
	257	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.10	–	0.10
	261	–	–	0.23	0.17	–	–	–	–	0.09	0.13	0.03	–	0.03	–	0.07	0.13	0.21	0.05	0.03	0.14	0.23	–	–	0.20	0.40
	265	–	–	–	–	–	–	0.07	0.06	–	0.03	0.17	0.25	0.10	0.04	0.10	–	0.17	0.11	0.03	0.09	0.23	–	0.10	–	–
	269	–	–	–	–	–	–	–	–	–	0.05	0.03	–	–	–	–	0.03	–	0.05	0.07	–	0.27	0.20	0.10	–	0.30
	273	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	0.06	–	0.11	–	0.09	0.07	0.10	0.10	0.20	0.10
	277	–	0.04	–	–	–	–	–	–	0.06	–	0.03	0.14	0.03	0.04	0.43	0.22	0.17	0.16	0.17	0.05	–	0.10	–	–	0.10
	281	–	–	–	–	–	–	0.23	0.29	–	–	–	–	0.10	–	–	–	–	0.09	0.07	0.23	–	0.10	0.10	0.10	–

Appendix 1 continued

LOCUS	ALLELE	TOIKONA		OWENS							HYBRID								LAHONTAN							
	Size (bp)	CB	MS	OG-L	WMRS	SOT	ABS	CDS	LHC	OG-U	HC	MC	TLM	OG-TB	A1D	C2D	MNC	JL	EWR	TLB	W	TAH	TD	PLP	PLO	L
	285	–	–	–	–	–	–	–	–	–	–	–	–	0.03	–	–	0.22	–	0.02	–	0.14	–	0.20	–	0.10	–
	289	–	0.14®	–	–	–	–	–	–	–	–	–	–	–	–	0.03	–	–	0.11	–	–	0.07	–	0.10	0.10	–
	293	0.56®	0.46®	–	–	–	–	–	–	–	–	0.03	–	0.07	–	0.07	0.03	–	0.05	–	0.09	0.03	0.10	0.10	0.10	–
	297	0.03	–	–	–	0.25	0.17	–	–	0.15	0.08	0.13	0.25	0.20	0.15	0.10	0.06	–	0.05	0.23	0.05	0.03	–	0.20	0.10	–
	301	0.03	–	–	–	–	0.03	0.03	0.01	–	0.03	–	–	0.10	–	0.07	–	–	0.02	0.03	–	–	–	–	–	–
	305	–	–	–	–	–	–	–	–	–	–	–	–	0.03	–	–	0.03	0.08	0.05	–	0.05	–	–	–	–	–
	309	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.07©	0.03©	–	–	–	0.10	–	–
	313	–	–	–	–	–	0.03	–	–	–	0.05	–	–	–	–	–	–	0.08	0.05	–	0.05	–	–	–	0.10	–
	317	–	–	–	–	0.04	0.03	–	–	0.03	–	–	–	–	–	–	0.03	0.04	0.02	0.03	–	0.03	0.20	–	–	–
	321	–	–	0.36	0.57	0.50	0.20	0.30	0.23	0.18	0.05	0.20	0.04	0.03	–	–	–	–	–	–	–	–	–	–	–	–
	325	–	–	0.05	0.03	–	0.03	–	–	–	0.03	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	H_O	0.81	0.71	0.36	0.67	0.64	0.67	0.60	0.66	0.65	0.50	0.80	0.71	1.00	0.46	0.80	1.00	0.50	0.82	0.80	0.36	0.80	1.00	0.80	1.00	1.00
	H_E	0.56	0.66	0.76	0.62	0.66	0.70	0.74	0.70	0.71	0.68	0.80	0.79	0.88	0.51	0.79	0.87	0.90	0.93	0.84	0.96	0.85	0.93	1.00	0.96	0.80
	P_{HW}	0.04	0.93	0.03	1.00	0.50	0.74	0.34	0.29	0.21	0.07	0.57	0.64	0.85	0.51	0.49	0.33	0.00*	0.12	0.55	0.00*	0.05	1.00	0.12	1.00	1.00
	N_A	4	4	4	4	4	7	5	5	6	9	8	5	11	5	8	10	7	15	10	11	9	7	9	8	5
	N_S	16	14	11	15	14	15	15	35	17	20	15	14	15	13	15	16	12	22	15	11	15	5	5	5	5
Gbi–G13	210	–	–	–	–	0.25	–	–	–	–	–	–	0.08§	–	–	–	–	–	–	–	–	–	–	–	–	–
	214	–	–	–	–	–	0.10	0.11	0.04	–	0.13	0.25	–	0.12	0.10	0.19	0.25	0.21	0.53	0.37	0.41	0.37	0.20	0.30	–	0.20
	218	1.00	1.00	1.00	1.00	0.56	0.57	0.21	0.54	0.91	0.50	0.33	0.04	0.35	0.55	0.54	0.13	0.43	–	0.27	0.05	0.03	0.10	–	0.20	0.10
	222	–	–	–	–	0.06	0.20	0.61	0.30	0.09	0.05	0.13	0.67	0.12	–	0.12	0.28	0.07	0.08	–	0.14	0.27	–	0.10	0.30	–
	226	–	–	–	–	–	0.07	–	–	–	0.29	0.21	0.21	0.15	0.10	–	–	0.11	0.18	0.07	0.18	0.17	–	–	0.30	0.40
	230	–	–	–	–	–	0.07	–	–	–	0.03	–	–	0.15	0.05	0.08	0.06	0.18	0.23	0.17	0.14	0.07	0.20	0.20	–	0.10
	234	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–	–	–	–	0.10	0.05	0.03	–	–	–	–
	238	–	–	–	–	–	–	–	–	–	–	–	–	–	0.10	–	–	–	–	0.03	0.05	–	0.40	0.10	–	0.20
	242	–	–	–	–	–	–	–	–	–	–	–	–	–	0.10§	–	0.28§	–	–	–	–	0.03	0.10	0.10	0.10	–
	246	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.03	–	–	0.10	–
	250	–	–	–	–	0.13	–	0.07	0.13	–	–	0.08	–	0.08	–	0.08	–	–	–	–	–	–	–	0.20	–	–
	H_O	–	–	–	–	0.25	0.20	0.21	0.33	0.06	0.32	0.42	0.17	0.62	0.10	0.23	0.50	0.21	0.55	0.67	0.91	0.73	0.80	1.00	0.80	1.00
	H_E	–	–	–	–	0.73	0.69	0.65	0.65	0.17	0.70	0.82	0.58	0.86	0.71	0.74	0.80	0.80	0.66	0.80	0.79	0.82	0.82	0.89	0.89	0.82
	P_{HW}	–	–	–	–	0.00*	0.00*	0.00*	0.01*	0.09	0.00*	0.00*	0.00*	0.15	0.00*	0.00*	0.02	0.00*	0.43	0.48	0.76	0.56	0.33	1.00	0.35	1.00
	N_A	1	1	1	1	4	5	4	4	2	5	5	4	7	6	5	5	5	4	6	7	8	5	6	5	5
	N_S	16	10	9	13	8	15	14	27	17	19	12	12	13	10	13	16	14	20	15	11	15	5	5	5	5
Gbi–G38	240	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.03	–	–	–	–
	248	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.10	–	–
	256	–	–	–	–	–	–	–	–	–	0.03	–	–	0.03	–	–	–	–	0.07	0.03	–	–	–	–	–	–
	260	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05©	0.03	–	0.10	0.10	–
	264	–	–	–	–	–	–	–	–	–	–	0.07	–	0.03	–	–	0.03	–	0.09	0.07	0.09	0.03	0.10	0.10	–	–
	268	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.03§	–	–	–	–	–	–	–	–
	272	0.88®	0.81®	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.10©	–	–	–	–	–	–
	280	–	–	–	–	–	–	0.04	0.04	–	–	0.03	–	0.03	–	–	0.06	–	–	0.07	–	–	–	–	–	–
	284	–	–	0.18	0.07	0.04	0.17	0.04	0.03	0.06	0.13	0.13	0.10	–	0.12	0.04	–	0.13	0.05	0.03	0.14	–	–	0.10	0.10	–

Appendix 1 continued

LOCUS	ALLELE	TOIKONA		OWENS							HYBRID								LAHONTAN							
	Size (bp)	CB	MS	OG-L	WMRS	SOT	ABS	CDS	LHC	OG-U	HC	MC	TLM	OG-TB	A1D	C2D	MNC	JL	EWR	TLB	W	TAH	TD	PLP	PLO	L
	288	–	–	–	–	–	–	–	–	–	–	–	–	–	0.08	0.04	0.03	–	0.09	–	0.05	0.03	–	–	0.10	0.08
	292	–	–	0.23	0.03	0.14	0.37	0.11	0.06	0.18	0.08	0.07	0.23	0.07	0.15	0.11	0.19	0.13	0.07	0.13	0.05	0.13	–	0.10	–	0.25
	296	–	–	–	0.13	–	–	–	–	0.18	0.03	0.03	0.07	0.07	0.12	0.11	0.06	0.03	0.05	0.10	–	0.10	–	–	–	–
	300	–	–	–	–	–	0.03	–	–	–	–	–	–	0.07	0.04	0.04	–	–	0.16	0.03	0.05	0.07	–	–	–	–
	304	–	–	0.46	0.47	0.32	0.10	0.21	0.21	–	0.25	0.07	0.17	0.10	–	0.14	0.03	0.07	–	–	–	0.17	–	0.20	0.20	0.08
	308	0.03	0.04	–	0.07	–	0.03	–	–	0.12	0.13	0.03	0.17	0.07	0.15	0.25	0.13	0.03	0.05	0.17	0.05	0.17	0.50	–	–	–
	312	0.06®	0.15®	–	–	–	–	–	–	–	0.05	0.03	–	0.17	–	0.04	–	0.20	0.05	–	0.09	0.03	–	0.10	–	–
	316	0.03	–	–	–	–	0.03	0.04	0.09	0.12	0.10	0.13	0.23	0.17	0.19	–	0.03	0.20	0.09	–	0.14	0.07	0.30	–	0.20	0.50
	320	–	–	–	–	0.04	0.07	0.14	0.09	0.03	–	0.13	–	–	–	–	0.19	0.03	0.02	–	0.09	–	–	–	0.10	–
	324	–	–	–	–	0.21	0.03	0.14	0.06	0.18	0.08	0.10	–	–	–	0.04	0.06	–	–	0.07	0.05	–	–	–	–	–
	328	–	–	–	–	–	0.07	–	0.02	–	–	–	–	–	0.04	–	–	–	0.02	0.07	–	0.03	–	–	–	–
	332	–	–	–	–	0.04	–	–	0.02	0.15	0.03	0.03	–	0.03	0.08	–	–	–	0.02	0.13	–	–	–	–	0.20	0.08
	336	–	–	0.14	0.23	0.21	0.10	0.29	0.40	–	0.03	0.13	0.03	–	–	0.11	0.13	0.03	–	–	–	0.07	–	–	–	–
	340	–	–	–	–	–	–	–	–	–	–	–	–	0.03	0.04	0.04	–	0.03	0.02	–	–	0.03	–	0.20	–	–
	344	–	–	–	–	–	–	–	–	–	0.10	–	–	0.13	–	–	–	–	0.07	–	0.05	–	–	–	–	–
	348	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.07	0.06	0.07	0.09	–	0.09	–	0.10	–	–	–
	356	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	–	–	–	–
	H_O	0.13	0.31	0.91	0.80	0.79	0.80	0.93	0.82	0.94	1.00	0.93	0.80	1.00	1.00	0.86	0.88	0.80	0.91	1.00	0.91	0.93	0.80	0.60	0.80	0.67
	H_E	0.29	0.40	0.72	0.73	0.81	0.83	0.85	0.79	0.88	0.89	0.93	0.85	0.92	0.91	0.90	0.92	0.91	0.94	0.93	0.96	0.93	0.71	1.00	0.96	0.76
	P_{HW}	0.07	0.16	0.64	0.01*	0.83	0.80	0.93	0.98	0.98	0.52	0.61	0.63	0.95	0.19	0.62	0.38	0.37	0.63	0.65	0.54	0.64	1.00	0.01*	0.39	1.00
	N_A	4	3	4	6	7	10	8	10	8	12	13	7	13	10	12	12	12	16	12	14	14	4	8	7	5
	N_S	16	13	11	15	14	15	14	34	17	20	15	15	15	13	14	16	15	22	15	11	15	5	5	5	6
Gbi–G79	202	–	–	0.55	0.57	0.31	0.27	0.07	0.14	0.09	0.08	0.17	0.07	0.12	0.27	0.07	0.03	0.07	–	–	–	–	–	–	–	–
	209	–	–	–	–	–	–	–	–	–	0.03	0.17	–	0.04	–	–	–	–	0.28	0.13	0.10	–	–	–	–	–
	210	–	–	0.23	0.03	0.19	0.03	0.03	–	0.12	0.03	0.08	0.04	–	0.15	–	–	0.10	–	–	–	–	–	–	–	–
	213	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–	–	–	0.07	–	–	0.13	–	0.20	–
	214	–	–	0.05	0.03	0.15	0.37	0.07	0.17	0.15	0.22	0.13	0.21	–	–	0.25	0.07	0.33	–	0.03	–	–	–	–	–	–
	217	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.14	–	–	–	0.07	–	0.07	–	–	–	–
	218	–	–	0.18	0.30	0.12	0.07	0.20	0.09	0.32	0.25	0.17	0.18	0.12	0.19	0.29	0.53	–	0.05	0.10	0.05	–	–	–	–	–
	221	–	–	–	–	–	–	–	–	–	0.03	–	–	–	–	0.04	–	0.07	0.03	–	–	0.14	0.13	–	0.30	0.17
	222	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.23©	0.10©	–	–	0.40	–	–
	225	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	0.03	–	0.10	–	0.15	0.14	–	–	0.10	0.08
	226	–	–	–	–	–	0.03	0.03	0.02	–	0.03	0.08	–	0.08	–	–	–	–	0.15	0.17	0.05	0.11	0.25	–	0.10	–
	229	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05©	0.21	0.13	–	–	0.67
	230	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.11	–	0.20	–	–
	231	0.80®	0.71®	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–	–	–
	233	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–	–	–
	234	0.03	0.13	–	0.07	–	0.20	0.40	0.36	0.09	0.19	0.13	0.50	0.39	0.15	0.21	0.30	0.23	0.28	0.10	0.10	–	0.13	0.10	0.10	–
	237	–	–	–	–	–	–	–	0.02	–	–	–	–	0.04	–	–	–	0.03	0.05	–	0.20	–	–	–	–	–
	238	–	–	–	–	–	–	–	–	0.06	0.03	0.04	–	0.19	–	–	–	0.03	0.05	0.07	0.05	–	–	–	0.10	–
	241	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.10	–	–
	242	0.17	0.17	–	–	0.23	0.03	0.17	0.13	0.15	0.06	–	–	–	0.15	–	–	0.10	0.03	–	–	–	0.13	–	–	0.08

Appendix 1 continued

LOCUS	ALLELE	TOIKONA		OWENS							HYBRID								LAHONTAN							
	Size (bp)	CB	MS	OG-L	WMRS	SOT	ABS	CDS	LHC	OG-U	HC	MC	TLM	OG-TB	A1D	C2D	MNC	JL	EWR	TLB	W	TAH	TD	PLP	PLO	L
	245	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.03$^{\copyright}$	0.10$^{\copyright}$	0.07	–	0.10	–	–
	250	–	–	–	–	–	–	0.03	0.08	0.03	0.06	0.04	–	0.04	–	–	0.03	–	–	–	0.05	0.04	0.13	–	0.10	–
	258	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–	–	–
	266	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.10	–	–
	270	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.03§	–	–	–	–	–	–	–	–
	H_O	0.40	0.50	0.55	0.60	0.62	0.87	0.80	0.81	0.88	1.00	0.92	0.64	0.69	0.77	0.93	0.53	0.73	0.85	1.00	0.80	0.93	1.00	1.00	0.80	0.50
	H_E	0.34	0.47	0.72	0.62	0.81	0.77	0.79	0.81	0.84	0.86	0.91	0.69	0.84	0.86	0.81	0.67	0.83	0.84	0.89	0.96	0.91	0.96	0.84	0.93	0.67
	P_{HW}	1.00	1.00	0.68	0.93	0.31	0.07	0.89	0.49	0.74	0.71	0.94	0.45	0.05	0.58	0.96	0.30	0.55	0.71	0.28	0.28	0.82	1.00	0.79	0.51	0.53
	N_A	3	3	4	5	5	7	8	8	8	11	9	5	8	7	6	6	9	9	10	11	11	7	6	7	4
	N_S	15	12	11	15	13	15	15	32	17	18	12	14	13	13	14	15	15	20	15	10	14	4	5	5	6
Gbi–G87	164	–	–	–	–	–	–	–	–	–	0.08	0.04	–	0.10	–	0.04	–	–	–	–	0.09	–	–	–	–	–
	168	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.30	0.10	–	–
	172	1.00$^{\circledR}$	1.00$^{\circledR}$	–	–	–	–	–	–	–	–	–	–	0.07§	0.15§	–	–	–	–	–	–	–	–	–	–	–
	176	–	–	–	–	–	0.07	0.17	0.18	–	–	0.07	–	–	–	–	0.03	–	–	0.04	–	–	–	0.10	–	–
	180	–	–	–	–	–	0.07	0.13	0.23	–	0.08	0.14	–	0.30	0.04	0.08	0.22	0.50	0.50	0.04	0.36	0.23	0.10	0.10	–	0.33
	184	–	–	–	–	–	0.07	0.03	–	0.06	0.08	–	0.11	–	0.12	0.08	0.03	0.03	0.08	–	–	–	0.40	0.10	0.10	–
	188	–	–	0.11	0.27	0.71	0.43	0.47	0.16	0.12	0.19	0.18	0.29	–	0.04	0.19	0.38	0.10	0.11	0.29	0.18	–	–	–	0.10	–
	192	–	–	–	–	–	–	0.13	0.11	0.03	0.03	0.04	–	–	–	0.04	–	0.13	0.11	0.14	0.05	0.03	–	0.10	0.10	0.17
	196	–	–	–	–	–	–	–	–	–	–	–	–	–	0.19	–	–	–	–	0.14	0.05	–	–	0.40	0.10	0.08
	200	–	–	0.06	–	0.04	–	–	–	0.12	–	0.04	0.07	0.07	–	–	0.03	–	–	–	0.05	0.03	–	–	0.10	0.42
	204	–	–	0.39	0.47	0.04	0.13	–	–	0.24	0.06	0.18	0.07	0.07	0.04	0.04	0.03	0.03	–	0.07	–	0.23	0.10	0.10	0.10	–
	208	–	–	0.28	0.03	0.21	0.13	0.03	0.27	0.27	0.08	0.11	0.18	0.20	0.08	0.12	0.13	–	0.03	0.11	–	0.20	0.10	–	0.10	–
	212	–	–	0.17	0.23	–	0.07	0.03	0.05	0.09	0.22	0.18	0.14	0.10	0.12	0.31	0.13	–	–	0.07	0.05	0.03	–	–	0.10	–
	216	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–	0.03	0.05	–	–	0.13	–	–	0.10	–
	220	–	–	–	–	–	0.03	–	–	0.09	0.06	0.04	0.14	0.07	–	0.08	0.03	0.07	–	–	–	0.03	–	–	–	–
	224	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.10	–	0.04	0.05	–	–	–	–	–
	228	–	–	–	–	–	–	–	–	–	0.11	–	–	0.03	0.15	–	–	–	0.08	–	0.09	0.03	–	–	–	–
	232	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.03	–	–	–	–
	236	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04§	–	–	–	–	–	–	–	–	–	0.10	–
	240	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05$^{\copyright}$	0.07$^{\copyright}$	–	–	–	–	–	–
	244	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–	–	–	0.05	–	–	–	–	–
	H_O	–	–	0.89	0.53	0.57	0.67	0.80	0.58	0.88	0.72	0.79	0.79	0.87	1.00	0.69	0.75	0.53	0.63	0.50	0.64	0.87	0.80	0.80	1.00	0.67
	H_E	–	–	0.77	0.72	0.46	0.79	0.74	0.82	0.86	0.91	0.90	0.86	0.86	0.91	0.87	0.83	0.77	0.76	0.90	0.86	0.87	0.80	0.87	1.00	0.85
	P_{HW}	–	–	0.15	0.47	0.73	0.35	0.87	0.04	0.67	0.16	0.16	0.17	0.42	1.00	0.09	0.48	0.13	0.24	0.00*	0.05	0.95	1.00	0.60	1.00	0.63
	N_A	1	1	5	4	4	8	7	6	8	10	10	7	9	11	10	9	8	8	10	10	10	5	7	10	4
	N_S	16	11	9	15	14	15	15	31	17	18	14	14	15	13	13	16	15	19	14	11	15	5	5	5	6
	$H_E(\Sigma N_A)$	0.34(18)		0.71(64)							0.83(102)								0.88(119)							

Note, private alleles present in toikona, but absent in Owens tui chubs are indicated by $^{\circledR}$; orphan alleles found in hybrid, but neither in Owens nor Lahontan Walker basin tui chubs by §; private alleles of Lahontan Walker basin tui chubs absent in hybrid and Owens tui chub populations by $^{\copyright}$; absent alleles and non–existing heterozygosities are displayed by ‘‘– ‘‘. Asterisks indicate P-values ≤ 0.01 for tests of deviations from Hardy–Weinberg expectations (P_{HW})

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