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RESEARCH ARTICLE

Conservation genetics of a desert fish species: the Lahontan tui chub (*Siphateles bicolor* ssp.)

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Abstract Analysis of the genetic diversity and structure of declining populations is critical as species and populations are increasingly fragmented globally. In the Great Basin Desert in particular, climate change, habitat alteration, and fragmentation threaten aquatic habitats and their endemic species. Tui chubs, including the Lahontan tui chub and Dixie Valley tui chub, (*Siphateles bicolor* ssp.) are native to the Walker, Carson, Truckee and Humboldt River drainages in the Great Basin Desert. Two populations, Walker Lake and Dixie Valley, are under threat from habitat alteration, increased salinity, small population sizes, and nonnative species. We used nine microsatellite markers to investigate the population genetic structure and diversity of these and nine other tui chub populations to provide information to managers for the conservation of both Walker Lake and Dixie Valley tui chubs. Genetic population structure reflects both historical and contemporary factors, such as connection with Pleistocene Lake Lahontan in addition to more recent habitat fragmentation. Dixie Valley was the most highly differentiated population (pairwise $F_{ST} = 0.098-0.217$, $p < 0.001$), showed evidence of a past bottleneck, and had the lowest observed heterozygosity ($H_o = 0.607$). Walker Lake was not substantially differentiated from other Lahontan tui chub populations, including those located in different watersheds (pairwise $F_{ST} = 0.031-0.103$, $p < 0.001$), and had the highest overall observed heterozygosity ($H_o = 0.833$). We recommend that managers continue to manage and monitor Dixie Valley as a distinct Management Unit, while continuing to maximize habitat size and quality to preserve overall genetic diversity, evolutionary potential, and ecological processes.

Keywords Microsatellite · Desert fishes · Tui chub · Great Basin · Walker Lake · Dixie Valley

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Introduction

The field of conservation genetics operates on the premise that the genetic diversity of a population has consequences for its health and viability (Frankham 2002). Small populations in particular are at greater risk of extinction due to demographic (e.g. stochastic or catastrophic events Lande 1993) and genetic [inbreeding, increased genetic load, and reduced evolutionary potential, (Frankham 2002)] reasons, the combination of which can lead to what is known as an "extinction vortex". Therefore managers are increasingly looking to genetic studies to quantify and monitor the genetic diversity of threatened populations as part of comprehensive management plans. Such genetic studies have the added benefit of helping managers prioritize conservation actions when resources are limited and populations are fragmented, such as those of aquatic species in arid regions.

The Great Basin desert, located primarily in Nevada, exhibits extreme aquatic endemism due to climatic and geological processes—Pleistocene lakes in the area receded around 10,000 ya allowing aquatic populations to speciate in isolation (Hubbs and Miller 1948; Hershler 1998; Smith et al. 2002). Many of these endemic species are vulnerable as a result of habitat alteration, water extraction, introduced species, population fragmentation, and climate change (Sada and Vineyard 2002). Sada and

Vineyard (2002) reviewed the status of aquatic taxa in 2002 and found sixteen to be extinct, 78 declining, and only 28 to be somewhat stable, yet still facing threats. Efforts to preserve this threatened biodiversity may necessitate management actions that benefit from conservation genetics research (e.g. Frankham et al. 2002). Such actions can include, but are not limited to, founding refuge populations, habitat restoration, reintroductions and translocations, designating Evolutionarily Significant (Moritz 1994) or Management Units (Avise 2000), and genetic monitoring (e.g. Frankham et al. 2002).

The Lahontan tui chub (*Siphateles bicolor obesa*, *S. b. pectinifer*), and the closely related Dixie Valley tui chub (*S. b.* ssp.) are two Great Basin subspecies. Lahontan and Dixie Valley tui chubs are in the minnow family (Cyprinidae) and serve as a major prey item for native species such as migratory birds and the threatened native Lahontan cutthroat trout (Coffin and Cowan 1995). Tui chubs are typically abundant when found; archeological evidence suggests they were used as a food source for Native Americans (Raymond and Soble 1990). Like many desert fishes, tui chubs are adapted to harsh and variable environments due to their longevity (up to 35 years) and high fecundity. Tui chubs can mature at 2–3 years (Kucera 1978) and produce as many as ~70,000 eggs per year (Kimsey 1954). This life history enables them to survive unfavorable conditions and rapidly repopulate, much like the cui ui (*Chasmistes cujus*; Scoppetone et al. 1986) and native fishes in the Colorado River (e.g. Garrigan et al. 2002).

Two tui chub populations in particular, Walker Lake and Dixie Valley, are currently managed and monitored by Nevada Department of Wildlife (NDOW). Lahontan tui chubs in Walker Lake, one of the largest in both habitat area and historical census size, are threatened by upstream water extraction, which has dramatically increased salinity in the lake (Russell 1885; Stockwell 1994; Lopes and Allender 2009). Between 1882 and 2010, mostly as a result of upstream water diversions, the lake level dropped more than 150 ft and total dissolved salts have increased to 19,200 mg/L (Lopes and Allender 2009; NDOW 2011). This increase in salinity has extirpated all other fish species in the lake, leaving Lahontan tui chubs as the last self-sustaining fish species in the lake (NDOW 2011). The Dixie Valley tui chub, on the other hand, is endemic to Dixie Valley and restricted to a few small geothermal and cold springs on the Fallon Naval Air Station. Dixie Valley tui chubs are considered by some to be an un-described subspecies (Moyle 1995). Previous allozyme research (May 1999) and phylogenetic analysis using Cytochrome B (Harris 2000) found them to be genetically differentiated from Lahontan tui chubs, although geographically proximate locations such as Topaz Lake and Little Soda Lake were not included in either analysis. Dixie Valley tui chub are listed as a category 2 species by the USFWS (USFWS 1985), and managed as a species of concern by NDOW. Threats include introduced species, habitat alteration, small population size, and current and proposed geothermal power plant operations that may alter spring hydrology (Kris Urqhart, NDOW, personal communication).

For conservation of both Dixie Valley and Walker Lake tui chub populations, it is important to understand levels of differentiation between these and other populations in the Lahontan basin. Many of these populations are fragmented, recipients of conspecific translocations, or of unknown origin. In the event of further declines or increased threats, refuge populations of Walker Lake or Dixie Valley tui chubs can be founded as a source of backup genetic material if future augmentation or reintroductions are deemed necessary. Alternatively, if Walker Lake or Dixie Valley is extirpated, managers may introduce fish from genetically similar populations for reintroduction or restoration. By estimating population structure, estimating effective population size (N_e), and assessing overall genetic diversity, managers can identify suitable populations for reintroductions and use the information for future genetic monitoring (Schwartz et al. 2007).

The objectives of this genetic study are to (1) use microsatellite data to assess population genetic structure of Lahontan and Dixie Valley tui chubs; and (2) assess genetic diversity, test for genetic bottlenecks, and estimate effective population size N_e of eleven Lahontan and Dixie Valley tui chub populations, and (3) discuss the conservation implications of our findings.

Methods

Sample collection

Between 10 and 50 individual Lahontan and Dixie Valley tui chub samples were collected from each of eleven locations: Topaz Lake, NV (TPZ), Spooner Lake, NV (SPN), Little Soda Lake, NV (LSL), Stillwater National Wildlife Refuge, NV (STW), Tahoe Keys, CA (TKS), Pyramid Lake, NV (PYR), East Fork Walker River, CA (EWR), South Fork Reservoir, NV (Humboldt River; SFH), Twin Lakes, CA (TWN), Walker Lake, NV (WLK), and Casey Pond, Dixie Valley, NV (DXV; Table 1; Fig. 1). These locations are in or are adjacent to the Walker, Carson, Humboldt, or Truckee River drainages. Each sample consists of a single 1 mm^2 pelvic fin clip placed in a coin envelope and dried for storage. Whole genomic DNA was extracted using the Promega Wizard SV 96 Genomic DNA Purification System (Promega Corporation, Madison, WI).

Table 1 List of sample locations, location code, number of samples (N) analyzed, known stocking history, and year collected for all samples analyzed

Location	Code	N	Stocking history	Year
Topaz Lake, NV	TPZ	49	Formerly Alkali Lake; connected to the West Fork Walker River through a diversion ditch in 1922	2006
Little Soda Lake, NV	LSL	50	Originally stocked in 1931; source is unknown (Kim Tisdale, personal communication)	2006
Spooner Lake, NV	SPN	48	No recorded stocking	2008
Tahoe Keys, CA	TKS	10	No recorded stocking	2007
Pyramid Lake, NV	PYR	33	No recorded stocking	2008
Twin Lakes, CA	TWN	15	No recorded stocking	2008
Walker Lake, NV	WLK	50	No recorded stocking	2007
Stillwater National Wildlife Refuge, NV	STW	32	Fisheries inventory records indicate fish were present and common; later stocked with fish from Sleep Mine Wetlands and Walker Lake	2007
Dixie Valley (Casey Pond), NV	DXV	17	No recorded stocking	1998
East Fork Walker River, CA	EWR	20	No recorded stocking	1997
South Fork Reservoir, NV	SFH	24	No recorded stocking	2007

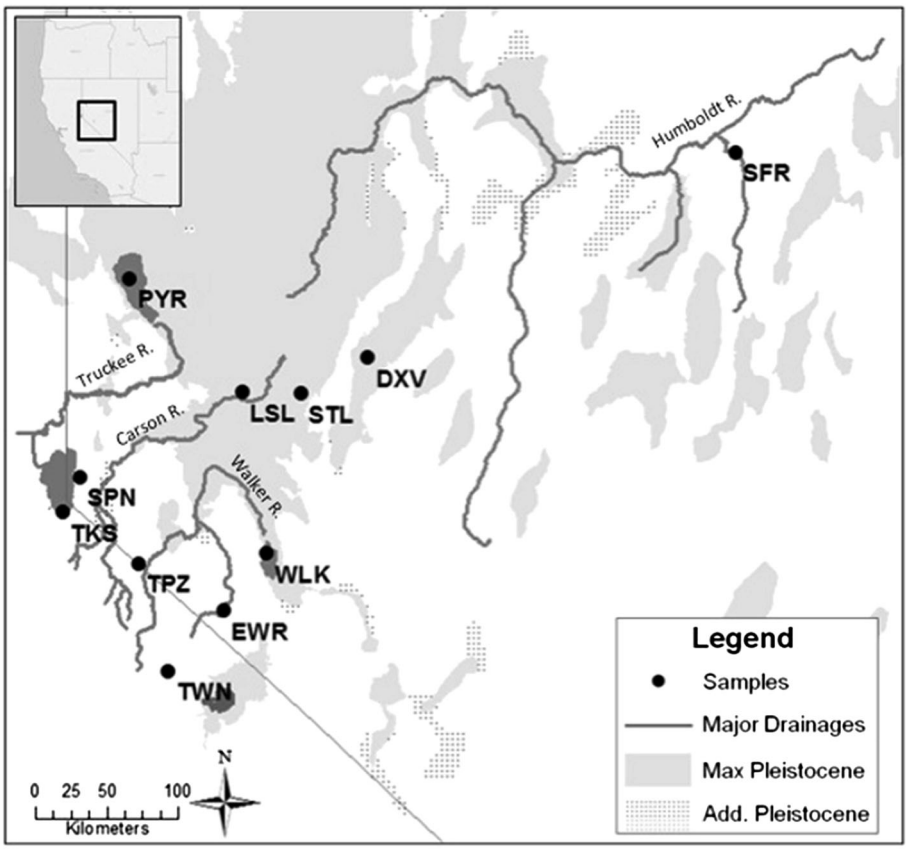

Fig. 1 Map of sample locations where Lahontan and Dixie Valley tui chubs were collected. Location codes are listed in Table 1. *Gray shading* indicates maximum area of Lake Lahontan during the late Pleistocene (Max Pleistocene). *Stippled shading* indicates other potential Pleistocene connections (Add. Pleistocene). Pleistocene Lake Lahontan layer taken from USGS (http://pubs.usgs.gov/mf/1999/mf-2323/)

Microsatellite genotyping

Samples were genotyped at five microsatellite loci from [Meredith and May (2002); Gbi-G13, Gbi-G38, Gbi-G39, Gbi-G79 and Gbi-G87)] and four from [Baerwald and May (2004); Cyp-G3, Cyp-G41, Cyp-G47, and Cyp-G48]. PCR reactions were conducted under conditions from (Chen 2013) and electrophoresed on an ABI 3730XL capillary electrophoresis instrument (Applied Biosystems, Carlsbad, California) after a 1:5 dilution with water. Peaks were

scored using GeneMapper software (Applied Biosystems). We used the software *MICRO-CHECKER* 2.2.3 (Van Oosterhout et al. 2004) to detect and correct any unusual values in the data set and to look for significant homozygote excess that might indicate the presence of null alleles.

Population structure

Pairwise F_{ST} values, a measure of the proportion of genetic diversity due to allele frequency differences among populations, were calculated with the software package *Arlequin* version 3.5 (Excoffier and Lischer 2010), using the option of exact tests of population differentiation. Significance of each pairwise F_{ST} value was calculated in *Arlequin* using 5,000 bootstrap permutations. We used SPAGeDi v1.4 (Hardy and Vekemans 2002) to calculate R_{ST} and its significance, and to test for significant difference between pairwise F_{ST} and R_{ST} values using 5,000 iterations of the allelic identity permutation test (Hardy et al. 2003). R_{ST} is a measure of global variance in allele size, rather than allele identity, as in F_{ST}. When $R_{ST} = F_{ST}$, one can assume that genetic differentiation is largely caused by genetic drift, rather than stepwise mutations (Hardy et al. 2003). We applied a sequential Bonferroni correction ($\alpha = 0.05$) to comparisons between F_{ST} and R_{ST} to correct for multiple tests.

To determine the optimal number of genetic clusters (K) and to assign individuals to specific genetic clusters, we used *STRUCTURE* 2.3.3 (Pritchard et al. 2000). We performed three independent runs of $K = 1$–11, each with a burn-in period of 100,000 and 1,000,000 MCMC repetitions. We assumed no prior information, admixture, and correlated allele frequencies (Falush et al. 2003). We used STRUCTURE HARVESTER (Earl and vonHoldt 2012) to implement the DeltaK method (Evanno et al. 2005) to determine the optimal K. The three *STRUCTURE* outputs for each K (total N outputs = 33) were compiled with the software *CLUMPP* (Jakobsson and Rosenberg 2007) using the Greedy K algorithm (described in Jakobsson and Rosenberg 2007). *CLUMPP* aligns multiple replicate analyses of the same data set and creates an infile for the software *DISTRUCT* (Rosenberg 2004). *DISTRUCT* was used to create a graphical representation of the mean *STRUCTURE* outputs for a chosen K. We used *GENETIX* (Belkhir et al. 2003) to perform a factorial correspondence analysis (FCA) to visually depict the genetic relationships between individuals and populations and confirm STRUCTURE results.

Genetic diversity

The scoring of private alleles, calculations of allelic frequencies, observed heterozygosity (H_o), expected heterozygosity (H_e) were calculated in *GDA* (Lewis and Zaykin 2001). Deviations from Hardy–Weinberg equilibrium (HWE) and detection of linkage disequilibrium (LD) were calculated using Genepop ver. 4.0 (Raymond and Rousset 1995). A sequential Bonferonni correction ($\alpha = 0.05$) was applied to determine the significance of multiple tests in LD and HWE calculations (Rice 1989). We used the software program HP-Rare (Kalinowski 2005b) to calculate allelic richness (A_r), a measure of genetic diversity, and private allelic richness (A_P), a measure of genetic distinctiveness using rarefaction to correct for sample size (Kalinowski 2004). F_{IS} values and their significance ($\alpha = 0.05$) were calculated using the software program SPAGeDi v1.4 (Hardy and Vekemans 2002) with 5,000 permutations.

Population bottlenecks and effective population size

We used two methods to estimate genetic bottlenecks. First, to detect the probability of a more recent population bottleneck (between 0.8 and 4.0 N_e generations ago) we used the software program Bottleneck 1.2.02 (Piry et al. 1999), which uses simulations to determine if the population of interest has an excess of heterozygosity greater than expected at mutation-drift equilibrium, an indication of a genetic bottleneck (Cornuet and Luikart 1996). For this test we conducted the Wilcoxan sign-rank test over 5,000 iterations for two mutation models: the stepwise mutation model (SMM) and the two-phase model (TPM; Di Rienzo et al. 1994). TPM parameters were 12 % variance in non-stepwise mutations, 95 % stepwise and 5 % non-stepwise mutations (Estoup and Cornuet 1999; Spencer et al. 2000). The most likely mutation model is likely somewhere between the two models (Di Rienzo et al. 1994). The second bottleneck test is the M-ratio test, which calculates the ratio "M" of number of alleles (k)/allele size range (r). Populations where M is smaller than expected are likely to have undergone a severe genetic bottleneck (Garza and Williamson 2001). M was calculated in M_p_val (http://swfsc.noaa.gov/textblock.aspx?Division=FED&id=3298), using the following parameters: proportion of one-step mutations (p_s) = 0.9, average size of non-one-step mutations ($delta_g$) = 3.5, and $\ominus = 10$. The $\ominus$ parameter is calculated with the equation $\ominus = 4Ne\mu$, where μ is $= 5.0 \times 10^{-4}$ mutations/locus/generation (Estoup and Angers 1998). To determine the sensitivity of M to changes in $\ominus$, we calculated M varying $\ominus$ from 0.1 to 20, corresponding with N_e values of 50–10,000. M was compared to the critical M (M_c) calculated in Mcrit (http://swfsc.noaa.gov/textblock.aspx?Division=FED&id=3298), where 95 % of 10,000 simulations have $M > M_c$. M_c parameters were $p_s = 0.9$, $delta_g = 3.5$, and $\ominus = 10$. As with calculating

M, we varied $\ominus$ from 0.1 to 20 to determine sensitivity of M_c to changes in $\ominus$.

To estimate the N_e of individual sample locations, we used the corrected linkage disequilibrium method (Waples and Do 2008) implemented in NeEstimator (Do et al. 2014), using a $P_{crit} = 0.02$ for populations where $N > 25$ samples and $P_{crit} = 0.03$ where $N \leq 25$ individuals. The assumptions of this method include random mating, isolation, selective neutrality of the markers used, no genetic structure within the population, and discrete generations. Do et al. (2014) performed simulations and found that the linkage disequilibrium method performed better than both the molecular coancestry method (Zhdanova and Pudovkin 2008) and the heterozygote excess method (Nomura 2008).

Results

One test in *MICRO-CHECKER* detected possible null alleles ($p < 0.01$) at Gbi-G87 in Twin Lakes. Out of 432 tests for HWE, 12 were significant after a sequential Bonferroni correction ($p < 0.05$). There is no consistent pattern across populations or loci for significant Hardy–Weinberg disequilibrium, so no loci were dropped. For LD in individual populations, after a sequential Bonferroni correction, there were 26 significant tests out of 550, but no significant LD tests across all loci and all populations. See Appendix I for allele frequencies in each sampling location.

Population structure

Computed pairwise F_{ST} values ranged from 0.024 to 0.217 (Table 2). All F_{ST} values were statistically significant ($p < 0.001$). The lowest pairwise F_{ST} value was between Pyramid Lake and Tahoe Keys, which were once connected by the now dammed Truckee River. The highest pairwise F_{ST} values were between Dixie Valley and other populations, and the lowest were between Walker Lake or Pyramid Lake and all other populations (Table 2). Pairwise R_{ST} values ranged from −0.025 to 0.175. No R_{ST} values were significantly greater than F_{ST} values after a Bonferroni correction.

The optimal K-value for the *STRUCTURE* analysis based on the Evanno et al. (2005) method is $K = 3$, with additional substructure at $K = 8$ (see Fig. 2 for plot of DeltaK). When $K = 3$, the main cluster includes Topaz Lake, Tahoe Keys, Pyramid Lake, Twin Lakes, Walker Lake, Stillwater, East Walker River, and South Fork Humboldt. The second cluster includes Little Soda Lake and Dixie valley, and Spooner Lake forms its own cluster (Fig. 3). When $K = 8$, additional substructure is evident, with seven locations forming distinct clusters (Topaz Lake, Little Soda Lake, Spooner Lake, Stillwater, Dixie Valley, East Walker River, and South Fork Humboldt). The eighth genetic cluster is comprised of Tahoe Keys, Pyramid Lake, Twin Lakes and Walker Lake (Fig. 4). The FCA analysis supports the STRUCTURE analysis where $K = 3$; Walker Lake, Tahoe Keys, Twin Lakes, Pyramid Lake, Topaz Lake, Stillwater National Wildlife Refuge, East Walker River and South Fork Reservoir form an overlapping

Table 2 Pairwise F_{ST} below the diagonal, and R_{ST} values above the diagonal, calculated with nine microsatellite loci. F_{ST} values in bold are significant ($p < 0.001$). R_{ST} values in bold are significant after a sequential Bonferroni correction ($p < 0.05$). R_{ST} values in italics are significantly greater than F_{ST} values before a Bonferroni correction. No R_{ST} values were significantly greater than F_{ST} values after a Bonferroni correction

Location	TPZ	LSL	SPN	TKS	PYR	TWN	WLK	STW	DXV	EWR	SFH
TPZ	–	**0.042**	**0.072**	0.045	***0.084***	**0.060**	***0.085***	**0.081**	**0.115**	0.022	**0.050**
LSL	**0.080**	–	**0.170**	0.119	**0.057**	**0.115**	**0.053**	**0.090**	**0.175**	**0.051**	**0.149**
SPN	**0.082**	**0.141**	–	−0.025	***0.164***	**0.071**	***0.135***	**0.094**	**0.151**	**0.069**	**0.095**
TKS	**0.151**	**0.193**	**0.063**	–	***0.140***	0.032	***0.103***	0.079	**0.154**	0.009	0.080
PYR	**0.116**	**0.177**	**0.070**	**0.024**	–	**0.084**	0.010	**0.041**	**0.090**	**0.054**	***0.134***
TWN	**0.070**	**0.133**	**0.079**	**0.075**	**0.062**	–	**0.062**	**0.089**	**0.160**	-0.011	**0.082**
WLK	**0.031**	**0.079**	**0.054**	**0.059**	**0.044**	**0.040**	–	**0.033**	**0.090**	**0.053**	***0.146***
STW	**0.065**	**0.116**	**0.067**	**0.095**	**0.081**	**0.088**	**0.038**	–	**0.070**	**0.074**	***0.167***
DXV	**0.098**	**0.120**	**0.149**	**0.217**	**0.202**	**0.174**	**0.103**	**0.164**	–	**0.146**	**0.166**
EWR	**0.051**	**0.099**	**0.122**	**0.144**	**0.126**	**0.038**	**0.042**	**0.093**	**0.157**	–	**0.049**
SFH	**0.056**	**0.138**	**0.059**	**0.083**	**0.058**	**0.071**	**0.038**	**0.048**	**0.151**	**0.095**	–

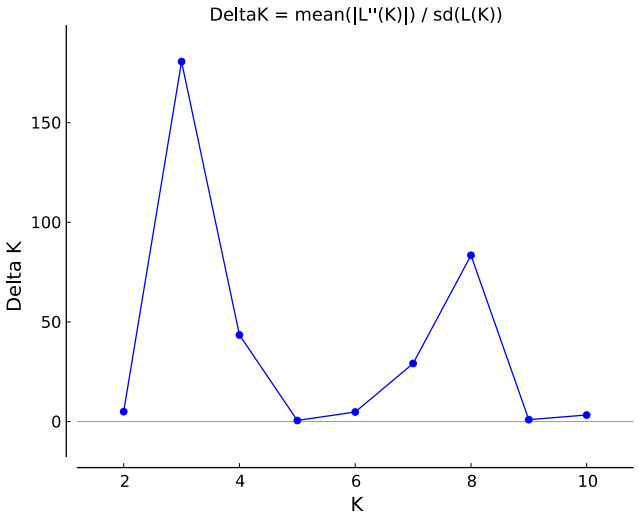

Fig. 2 Plot of DeltaK (Evanno et al. 2005) produced in STRUCTURE HARVESTER (Earl and vonHoldt 2012) showing optimal STRUCTURE K = 3, with additional substructure at K = 8

cluster. Spooner Lake forms a distinct cluster, and Little Soda Lake and Dixie Valley form an overlapping cluster (Fig. 5).

STRUCTURE results, the FCA analysis, and low to moderate pairwise F_{ST} values indicate that there is a single main overlapping cluster (hereafter Walker-Pyramid cluster) the core of which consists of Tahoe Keys, Pyramid Lake, Walker Lake, and Twin Lakes. East Walker River, Topaz, South Fork Humboldt, and Stillwater NWR are differentiated from this core when substructure is examined, but group with it when K = 3.

Population genetic diversity

The average number of alleles per locus (N_A) ranged from 5.44 to 17.11 (Table 3). H_o values ranged from 0.607 to 0.833, and H_e values ranged from 0.623 to 0.831 (Table 3). Walker Lake had the most private alleles (N = 16), while several populations had only one private allele. A_r and A_P were calculated with a minimum number of genes N = 10, the smallest sample size included in the analysis; A_r varied from 3.80 to 6.22. Walker Lake had the highest private allelic richness ($A_P = 0.730$), and Twin Lakes had the lowest ($A_P = 0.160$) (Table 3). F_{IS} values ranged from −0.011 to 0.124, three of which were significant ($p < 0.05$).

Population bottlenecks and effective population size

Only Spooner Lake showed evidence of a population bottleneck using the H_k test (TPM, p = 0.082). The *M*-ratio test also showed evidence of a more severe bottleneck in Spooner Lake, Dixie Valley, and Tahoe Keys (Table 4). N_e values ranged from 35.9 to ∞, and had wide confidence intervals, often with ∞ as an upper limit (Table 3).

Discussion

In this study we analyzed the population genetic structure and diversity of 11 tui chub populations to measure the genetic distinctiveness of two populations of concern: Walker Lake and Dixie Valley. This analysis provides genetic data in the event of future monitoring, and can be used to recommend source populations for reintroduction in the event of extirpation of the Walker Lake or Dixie Valley populations of tui chubs.

Genetic structure

Both contemporary and historical factors, as well as relative habitat size and isolation have contributed to the population genetic structure of Lahontan-Dixie tui chubs today. All of the populations analyzed here were once connected by Lake Lahontan. At its last highstand 14,500–13,000 ya, Lake Lahontan covered much of Northwestern Nevada and collected waters from the Truckee, Carson, Walker, Humboldt, Susan and Quinn rivers (Benson 1991). At this time, the fish populations in these watersheds were connected, allowing gene flow. When Lake Lahontan receded, gene flow was restricted to connected inundated areas. Though Lake Lahontan has receded since ~13,000 ya, flood events may have

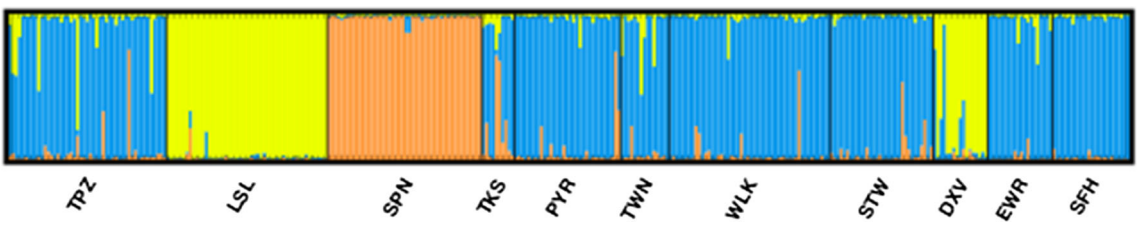

Fig. 3 *STRUCTURE* output with $K = 3$ showing population substructure among 11 sample locations. *Blue cluster* consists of Topaz Lake, Tahoe Keys, Pyramid Lake, Twin Lakes, Walker Lake, Stillwater, East Walker River and South Fork Humboldt. *Yellow cluster* is Little Soda Lake and Dixie valley, and the orange cluster is Spooner Lake. (Color figure online)

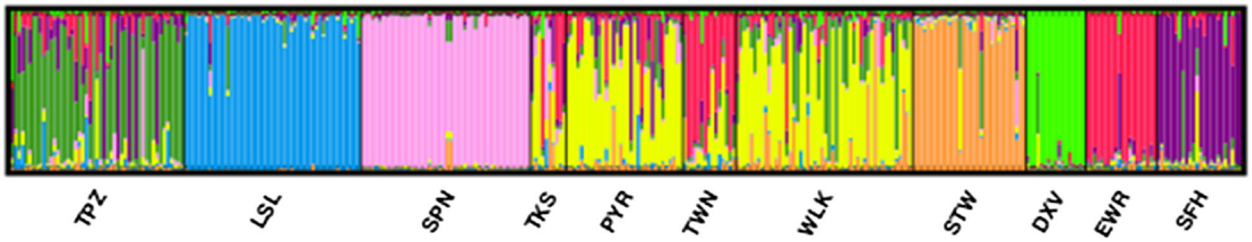

Fig. 4 *STRUCTURE* output with $K = 8$ showing population substructure among 11 sample locations. Topaz Lake, Little Soda Lake, Spooner Lake, Stillwater, Dixie Valley, East Walker River, and South Fork Humboldt form independent clusters, while Tahoe Keys, Pyramid Lake, Twin Lakes and Walker Lake form a cluster

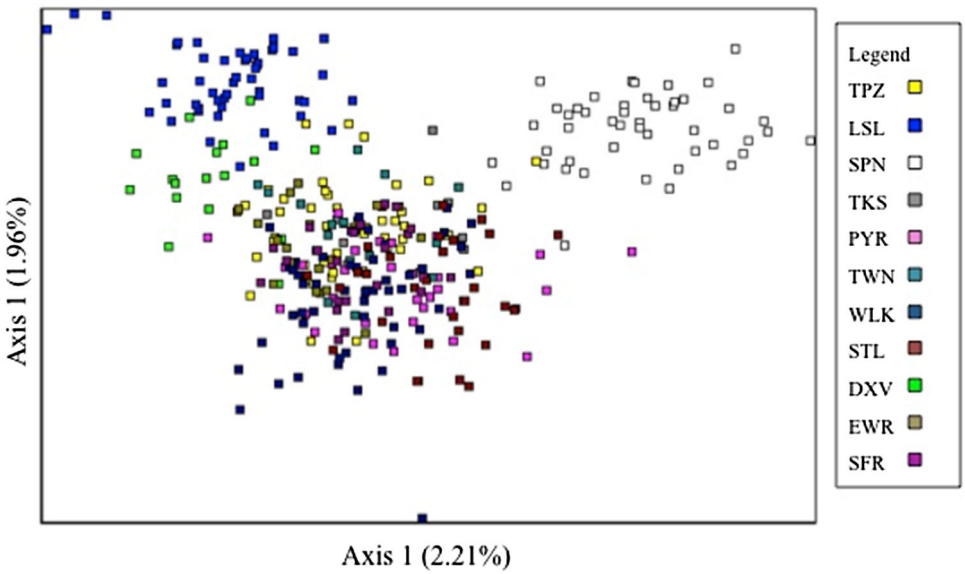

Fig. 5 Graphical representation of the FCA analysis with 11 sampled locations

Table 3 Sample location, expected (H_e) and observed (H_o) heterozygosity, inbreeding coefficient (F_{IS}), average number of alleles across loci (N_A), and number of private alleles (N_P), Allelic richness (A_r) and private allelic richness (A_P), and effective population size (N_{eLD}) with 95 % confidence intervals (CI) after jackknifing over loci. F_{IS} values in bold are significant

Location	N	H_e	H_o	F_{IS}	N_A	A_r	N_P	A_P	N_{eLD} (CI)
TPZ	49	0.787	0.796	−0.011	13.89	5.86	9	0.41	3,013.3 (365.8–∞)
LSL	50	0.670	0.622	**0.072**	7.33	4.24	2	0.25	239.8 (102.1–∞)
SPN	48	0.772	0.758	0.019	8.78	4.78	5	0.55	160.2−(63.5–∞)
TKS	10	0.760	0.715	0.061	6.78	5.37	1	0.42	∞ (8.6–∞)*
PYR	33	0.815	0.716	**0.124**	13.33	6.10	4	0.47	344.8 (51.9–∞)
TWN	15	0.774	0.698	**0.102**	8.44	5.15	1	0.16	∞ (∞–∞)[a]
WLK	50	0.831	0.833	−0.002	17.11	6.22	16	0.73	∞ (414.1–∞)
STW	32	0.796	0.804	−0.010	10.44	5.38	3	0.45	133.1 (61.9–∞)
DXV	17	0.623	0.607	0.027	5.44	3.80	2	0.31	26.7 (13.0–128.1)[a]
EWR	20	0.725	0.678	0.067	8.33	4.92	1	0.23	122.6 (39.8–∞)[a]
SFH	24	0.781	0.778	0.003	9.78	5.23	3	0.31	158.9 (63.8–∞)[a]
Mean		0.758	0.723	0.041	9.97	5.19	4.27	0.39	–

[a] Indicates populations where $N \leq 25$ and $P_{crit} = 0.03$. For all other populations $P_{crit} = 0.02$

Table 4 Results from two bottleneck tests

Location	H_k model significance	*M*	M_c
TPZ	NS	0.915	0.698
LSL	NS	0.743	0.697
SSPN	TPM, p = 0.082	0.671*	0.697
TKS	NS	0.654	0.590
PYR	NS	0.930	0.676
TWN	NS	0.711	0.621
WLK	NS	0.930	0.697
STW	NS	0.828	0.676
DXV	NS	0.557*	0.633
EWR	NS	0.785	0.646
SFH	NS	0.714	0.658

Table includes location, heterozygote excess model showing significance (and *p* value reported if $p < 0.10$) estimated in the software Bottleneck 1.2.02 (Piry et al. 1999), *M* ratio results calculated with the parameters: $\ominus = 10$, proportion of non-stepwise mutations, $p_s = 0.10$, and average step of non-stepwise mutation of 3.5 are reported

* Indicates that *M* is significantly less than M_c (calculated with the same parameters as *M*, $p < 0.05$)

reconnected populations more recently, allowing gene flow and reducing differentiation. This has been observed in the golden perch (*Macquaria ambigua*) in Australia (Faulks et al. 2010). As indicated by significantly greater F_{ST} values versus R_{ST} values, genetic drift rather than mutation may be the main cause of existing population differentiation (Hardy et al. 2003). It should be noted that Kalinowski (2005a) suggests that if the true F_{ST} of a population is greater than 0.05, fewer than 20 individuals may be used, though when the F_{ST} is very low (0.01), fewer than 20 may not be not sufficient for accurate estimation (Table 1).

Surprisingly, even when substructure is examined, Pyramid Lake and Walker Lake cluster together despite being separated by the Carson River drainage. Both Walker and Pyramid Lakes have the lowest pairwise F_{ST} values between them and other populations, and both have among the greatest observed heterozygosity levels. One explanation for these results is the large sizes of both Pyramid ($\sim$49,000 ha) and Walker Lake ($\sim$13,000 ha) that likely supported very large population sizes over time since the Lakes were connected. Pyramid Lake and Walker Lake are the main remnants of Pleistocene Lake Lahontan, and it is possible their large population sizes resulted in less genetic drift. This would have allowed retention of a greater proportion of historical neutral genetic diversity from Lake Lahontan, resulting in both higher genetic diversity and lower differentiation between Pyramid and Walker Lakes today.

Dixie Valley is the most differentiated population of those sampled based on pairwise F_{ST} values and structure analysis. This finding, in combination with previous genetic research (May 1999; Harris 2000) and the unique and isolated habitat in Dixie Valley (Garside and Schilling 1979), supports continued efforts to conserve Dixie Valley as a Management Unit. Little Soda Lake and Spooner Lake are also genetically distinct. Both Dixie Valley and Little Soda Lake are small locations (<200 m across). Dixie Valley is thought to be a natural population, while Little Soda Lake was stocked from an unknown source in 1931 (Kim Tisdale, NDOW, personal communication). Spooner Lake, created in 1927, is a relatively small impoundment (0.4 ha) thought to be a natural (unstocked) population. Though tui chubs are currently abundant in Spooner Lake and Little Soda Lake (Kim Tisdale, NDOW, personal communication), isolation, founder effect, genetic drift, and/or bottlenecks may have led to differentiation of this population.

Genetic diversity

The mean heterozygosity value of sampled tui chub populations is higher than the mean of most freshwater fishes (0.54, DeWoody and Avis 2000), though sample sizes from some locations were lower than recommended. Hale et al. (2012) recommends at least 25–30 individuals to accurately estimate allele frequencies using microsatellites, and for five of the populations, 25–30 individuals were not available (Table 1). Only Spooner Lake and Dixie Valley presented evidence of genetic bottlenecks, though it is possible that the sample sizes of some populations or the markers used do not provide enough power to detect bottlenecks (Peery et al. 2012). The significant positive F_{IS} values in Little Soda Lake, Twin Lakes, and Pyramid Lake suggest increased inbreeding, though the sample size in Twin Lakes was too small to accurately calculate a F_{IS} value. Our N_e estimates with infinite confidence intervals are due to small sample sizes relative to true N_e (Waples and Do 2010). Many of these populations, such as Walker and Pyramid Lakes, are thought to have census sizes in the hundreds of thousands (Kris Urqhart, NDOW, personal communication). A sample of only 30–50 individuals from such locations may not be enough to get a precise estimate, even if true N_e is as low as $\sim$1,000 (Waples and Do 2010). Given the overall wide confidence intervals and small sample sizes, we recommend N_e be used in the context of additional genetic analysis and monitoring when considering management actions.

Both Walker and Pyramid Lakes are expected to have higher genetic diversity relative to other populations due to their larger population sizes and terminal locations. Large populations experience less genetic drift than small populations. In addition, terminal or downstream locations like Pyramid and Walker Lakes will accrue genetic diversity

from their upstream populations, a pattern observed in *Gambusia holbrooki* (Hernandez-Martich and Smith 1997). Life history also plays a role in the strength of genetic drift. Populations of longer-lived organisms often contain multiple generations that act as a reservoir of diversity, making it more difficult to detect increasing genetic drift associated with declining populations (e.g. ornate box turtles, Kuo and Janzen 2004). Similar patterns have been observed in other inland fish species of concern, such as the copper redhorse (*Moxostoma hubbsi*) which has an estimated census size of 500 and low recruitment, yet has both high genetic diversity and N_e (Lippe et al. 2006). Another example is the endangered razorback sucker (*Xyrauchen taxanus*), a species that has declined steeply over the last few decades with little evidence of successful recruitment. Most surviving razorback suckers are large, old adults, and the population has maintained high genetic variation (Dowling et al. 1996a, b; Garrigan et al. 2002; Dowling et al. 2005). Garrigan et al. (2002) suggests that this is due to the long generation time and the historically large and geographically wide range of the razorback sucker. As recently as the mid-20th century, there was probably a large population of razorback suckers in the Lower Colorado River (Hedrick 2004). Therefore, any decline in census number has not yet significantly reduced the genetic diversity of the remaining population, though if declines continue genetic diversity will be lost. Similarly, if the tui chub population in Walker Lake continues to experience low recruitment, it will ultimately lose genetic variation.

Conservation implications

Our analysis has different implications for the conservation of tui chub populations in Walker Lake and Dixie Valley. First, Dixie Valley is genetically differentiated from other tui chub populations, and has lowest genetic diversity, lowest estimated N_e, and evidence of a genetic bottleneck. Small, isolated populations such as Dixie Valley are at increased risk of inbreeding depression and extinction by stochastic events (e.g. Gilpin and Soulé 1986; Caughley 1994; Frankham et al. 2002). Given these findings and known threats, we recommend continuing to manage Dixie Valley tui chub as a separate Management Unit (Avise 2000). We also recommend genetic monitoring, and maximizing habitat area and quality through control of emergent vegetation, managing grazing, and control of introduced species. If further declines of Dixie Valley tui chub occur, managers may consider founding one or more refuge populations of Dixie Valley tui chub. If founding refuge population(s) is deemed necessary, managers should follow recommendations of population size requirements that retain and sustain both short and long term evolutionary potential of both the founding and the refuge population (i.e., 50/500 rule; for discussion and clarification of the "rule" see Jamieson and Allendorf (2012), but also consider Lynch and Lande (1998), and Franklin and Frankham (1998)).

Walker Lake on the other hand, is not substantially differentiated from other sampled Lahontan tui chub populations, including those that are in other watersheds. However Walker Lake is a valuable population, with the greatest overall genetic diversity and an important ecological role as the last self-sustaining native fish in the Lake. The recovery of the threatened Lahontan cutthroat trout is dependent upon a healthy self-sustaining Walker Lake tui chub population. By preserving overall genetic diversity managers also preserve evolutionary potential, allowing populations to continue to adapt to changing environments, fill ecological roles, and conserve evolutionary processes.

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Appendix

See Table 5.

Table 5 Loci used in this study with allele sizes and frequency of each allele in each location, and over all locations, weighted for sample size

Allele size	TPZ	LSL	SPN	TKS	PYR	TWN	WLK	STW	DXV	EWR	SFH	All (weighted)
Gbi-G13												
206	–	–	–	–	0.037	–	0.010	–	–	–	–	0.005
210	0.102	0.270	0.125	0.375	0.278	0.633	0.229	0.065	0.156	0.550	0.188	0.225
214	0.082	–	–	0.063	0.111	0.067	0.042	0.129	–	–	0.167	0.055
218	0.082	0.210	0.167	0.125	0.148	–	0.094	0.145	0.125	0.050	0.063	0.122
222	0.092	0.150	0.271	0.313	0.111	0.033	0.271	0.468	–	0.200	0.208	0.201

Table 5 continued

Allele size	TPZ	LSL	SPN	TKS	PYR	TWN	WLK	STW	DXV	EWR	SFH	All (weighted)
226	0.204	0.210	0.115	–	0.130	0.233	0.135	0.081	–	0.175	0.063	0.140
230	0.041	–	–	–	0.037	–	0.073	–	0.125	–	0.125	0.034
234	0.092	–	–	–	0.111	0.033	0.063	0.048	–	–	0.042	0.040
238	0.194	–	0.323	–	0.019	–	0.021	0.065	0.125	0.025	0.146	0.103
242	0.092	–	–	–	–	–	0.021	–	–	–	–	0.016
246	0.010	0.120	–	0.063	0.019	–	0.031	–	–	–	–	0.027
250	0.010	0.020	–	–	–	–	0.010	–	0.469	–	–	0.028
254	–	0.020	–	0.063	–	–	–	–	–	–	–	0.005
N	49	50	48	8	27	15	48	31	16	20	24	336
Gbi-G38												
244	–	0.020	–	–	–	–	–	–	–	–	–	0.003
248	–	0.130	–	–	0.018	–	–	–	–	–	–	0.021
252	–	–	–	–	0.036	–	0.010	–	–	0.075	–	0.009
256	–	–	0.052	–	0.018	–	0.031	0.125	–	–	–	0.025
260	0.052	–	–	0.056	0.018	0.100	0.102	–	0.059	0.050	–	0.035
262	0.010	–	–	–	0.018	–	–	–	–	–	–	0.003
264	0.073	0.020	0.031	–	0.018	–	0.010	–	–	–	0.021	0.022
268	0.083	–	0.094	–	0.054	0.033	0.041	0.016	–	–	–	0.038
272	–	–	0.042	–	0.018	–	0.020	0.094	–	–	–	0.019
276	0.042	0.030	–	–	–	–	0.010	0.016	–	–	–	0.013
280	0.031	–	0.010	0.056	0.018	–	0.020	–	0.147	0.075	–	0.024
284	0.073	–	–	0.056	0.036	–	0.031	–	–	0.100	–	0.025
288	0.010	0.320	0.021	0.167	0.125	0.100	0.051	0.359	–	0.050	0.167	0.127
292	0.063	–	0.156	0.167	0.089	0.200	0.092	0.094	–	0.100	0.104	0.087
296	0.073	0.230	0.031	0.111	0.107	0.033	0.112	0.047	0.088	0.175	0.042	0.100
300	0.063	0.160	0.042	0.167	0.036	0.033	0.133	–	0.559	–	0.021	0.096
304	0.104	0.030	0.156	0.111	0.125	0.133	0.112	0.094	–	0.025	0.063	0.091
308	0.125	–	0.083	–	0.018	–	0.020	–	–	0.025	0.292	0.056
312	0.052	–	0.094	0.056	0.036	–	0.031	0.063	–	0.125	0.146	0.053
316	0.042	–	–	–	0.036	–	0.010	0.047	–	0.025	0.021	0.018
322	0.021	–	0.052	0.056	0.107	0.033	0.041	–	–	–	–	0.028
326	0.042	0.060	0.094	–	0.054	0.133	0.020	0.016	–	0.025	–	0.044
330	–	–	–	–	0.018	0.133	0.020	0.016	0.147	0.025	–	0.021
334	0.021	–	–	–	–	–	0.031	–	–	–	–	0.007
338	0.010	–	–	–	–	–	0.010	–	–	–	0.021	0.004
342	0.010	–	0.010	–	–	–	0.010	0.016	–	0.050	0.104	0.016
346	–	–	0.031	–	–	0.067	–	–	–	0.075	–	0.012
350	–	–	–	–	–	–	0.020	–	–	–	–	0.003
354	–	–	–	–	–	–	0.010	–	–	–	–	0.002
N	48	50	48	9	28	15	49	32	17	20	24	340
Gbi-G39												
186	–	–	–	–	–	–	0.041	–	–	–	–	0.006
190	–	–	–	–	–	–	0.010	–	–	–	–	0.002
194	–	–	–	–	0.053	–	0.041	0.109	–	–	–	0.020
198	–	–	–	–	0.079	0.033	0.133	0.031	–	–	–	0.030
202	0.031	–	–	–	0.026	–	0.092	0.016	–	–	0.021	0.023
206	–	–	–	0.083	–	0.067	0.041	0.016	–	0.175	–	0.023
210	0.031	0.120	0.032	–	0.079	0.033	–	–	0.318	–	0.063	0.050

Table 5 continued

Allele size	TPZ	LSL	SPN	TKS	PYR	TWN	WLK	STW	DXV	EWR	SFH	All (weighted)
214	0.174	0.420	–	0.167	0.184	–	0.061	–	–	0.100	0.125	0.130
218	0.092	0.170	0.170	–	0.158	0.100	0.051	0.047	–	0.150	0.042	0.104
222	0.174	0.060	0.053	0.083	0.026	0.133	0.031	0.016	0.091	–	–	0.062
226	0.041	0.070	–	–	–	0.167	0.051	0.031	–	–	0.021	0.037
230	0.041	0.010	0.064	–	0.079	0.233	0.051	0.141	0.091	0.100	0.042	0.067
234	0.112	0.070	0.043	0.083	0.026	0.067	0.051	0.078	0.409	0.175	0.104	0.089
238	0.092	0.040	0.053	0.083	0.079	–	0.051	0.047	–	–	0.167	0.059
242	0.071	0.040	–	–	0.105	0.033	0.051	0.172	–	0.225	0.313	0.087
246	0.071	–	0.011	0.167	–	0.100	0.082	0.078	–	0.050	0.104	0.051
250	0.071	–	–	–	0.026	–	0.082	0.109	–	0.025	–	0.037
254	–	–	0.213	–	0.053	–	0.031	0.109	–	–	–	0.050
258	–	–	0.106	–	–	–	0.010	–	–	–	–	0.017
262	–	–	0.181	0.083	–	–	0.041	–	–	–	–	0.034
270	–	–	0.064	0.083	0.026	–	–	–	–	–	–	0.012
274	–	–	0.011	–	–	–	–	–	–	–	–	0.002
278	–	–	–	0.083	–	0.033	–	–	–	–	–	0.003
282	–	–	–	0.083	–	–	–	–	0.046	–	–	0.003
286	–	–	–	–	–	–	–	–	0.046	–	–	0.002
N	49	50	47	6	19	15	49	32	11	20	24	332
Gbi-G79												
226	–	–	–	–	0.061	–	–	–	–	–	–	0.006
228	0.010	0.040	0.096	0.100	–	0.167	0.010	0.078	–	0.200	–	0.051
230	–	–	–	–	–	–	0.010	–	–	–	–	0.002
232	0.163	0.100	0.234	–	0.015	0.200	0.063	–	–	–	0.044	0.092
234	–	–	–	–	–	–	–	0.016	–	–	–	0.002
236	0.112	–	0.064	–	0.015	0.133	0.042	0.156	0.059	0.100	–	0.061
240	0.082	–	–	0.100	0.227	–	0.125	0.031	0.088	0.075	0.044	0.068
244	0.061	0.120	0.181	0.500	0.091	0.300	0.135	0.016	0.147	0.275	0.065	0.135
248	0.102	0.100	0.287	0.250	0.106	0.033	0.146	0.188	0.206	–	0.435	0.164
252	0.163	0.030	0.117	–	0.061	0.067	0.104	0.203	0.177	0.175	0.152	0.115
256	0.174	0.060	–	–	0.121	–	0.083	0.109	–	0.150	0.196	0.089
260	0.071	0.550	–	0.050	0.091	0.067	0.073	0.078	0.294	0.025	–	0.137
264	0.051	–	–	–	0.046	0.033	0.135	0.063	–	–	0.044	0.041
268	0.010	–	–	–	–	–	0.010	0.031	0.029	–	–	0.007
272	–	–	–	–	0.061	–	0.031	–	–	–	0.022	0.012
276	–	–	0.021	–	0.015	–	0.010	–	–	–	–	0.006
280	–	–	–	–	0.015	–	0.010	0.031	–	–	–	0.006
284	–	–	–	–	0.030	–	–	–	–	–	–	0.003
290	–	–	–	–	–	–	0.010	–	–	–	–	0.002
292	–	–	–	–	0.030	–	–	–	–	–	–	0.003
298	–	–	–	–	0.015	–	–	–	–	–	–	0.002
N	49	50	47	10	33	15	48	32	17	20	23	
Gbi-G87												
181	0.133	–	–	–	–	–	–	–	–	0.050	0.042	0.026
185	0.041	0.110	–	–	–	–	–	–	–	–	0.021	0.024
193	0.102	0.090	0.177	–	0.022	–	–	0.067	–	–	0.104	0.070
197	0.133	0.060	0.094	0.300	0.044	0.033	0.010	0.033	0.067	0.450	0.417	0.117
201	0.071	–	0.156	0.100	0.044	–	0.010	0.017	0.067	0.075	0.021	0.050

Table 5 continued

Allele size	TPZ	LSL	SPN	TKS	PYR	TWN	WLK	STW	DXV	EWR	SFH	All (weighted)
205	0.020	–	–	–	0.022	0.300	0.031	–	0.400	0.025	0.188	0.056
207	–	–	–	–	0.022	–	–	0.050	–	–	–	0.006
209	0.031	0.080	–	–	0.044	–	0.031	0.033	–	0.100	0.063	0.038
213	0.041	0.220	–	0.200	0.109	0.300	0.051	0.017	–	–	0.021	0.075
215	–	–	–	–	0.022	–	0.071	–	–	–	–	0.012
217	0.020	–	0.010	–	0.044	–	0.061	–	–	–	–	0.017
219	–	–	–	–	0.065	0.033	0.020	0.183	–	–	–	0.026
221	0.041	–	0.292	0.200	0.087	0.067	0.061	0.050	–	–	–	0.075
223	–	–	–	–	–	–	0.031	–	–	–	–	0.005
225	0.204	0.320	0.156	0.100	0.087	0.067	0.041	0.083	–	0.050	–	0.130
227	–	–	–	–	–	–	0.010	–	–	–	–	0.002
229	0.041	–	0.031	–	0.022	0.100	0.143	0.117	–	–	–	0.049
233	0.031	–	–	0.100	0.130	–	0.082	0.083	0.067	–	0.042	0.041
237	0.051	–	–	–	0.065	0.033	0.092	0.017	–	0.025	–	0.031
241	0.031	0.090	–	–	0.130	0.033	0.051	0.067	–	–	0.083	0.049
245	–	0.010	–	–	0.022	–	0.071	0.050	0.367	0.075	–	0.040
249	–	–	–	–	–	–	0.061	0.033	–	0.025	–	0.014
253	–	–	–	–	0.022	–	0.051	0.083	–	–	–	0.017
257	–	–	0.010	–	–	–	–	–	–	0.125	–	0.009
261	–	–	0.063	–	–	0.033	0.010	0.017	–	–	–	0.014
265	0.010	0.020	0.010	–	–	–	–	–	–	–	–	0.006
281	–	–	–	–	–	–	–	–	0.033	–	–	0.002
289	–	–	–	–	–	–	0.010	–	–	–	–	0.002
N	49	50	48	5	23	15	49	30	15	20	24	328
Cyp-G3												
205	–	–	–	–	–	0.067	–	–	–	0.050	–	0.006
213	–	–	–	–	–	–	–	–	–	0.025	–	0.002
217	0.063	–	–	–	–	–	0.010	–	–	0.075	0.125	0.023
221	0.177	0.380	0.365	0.500	0.172	0.067	0.255	0.109	0.706	0.075	0.063	0.252
223	–	0.010	–	–	–	–	–	–	–	–	–	0.002
225	0.042	–	–	–	0.094	–	0.010	0.063	0.029	–	0.063	0.028
227	–	–	–	–	–	–	–	0.172	–	–	–	0.016
229	0.031	0.050	0.135	–	0.094	0.033	0.051	0.078	–	0.050	0.104	0.066
233	0.073	0.030	0.021	–	0.016	0.033	0.031	0.047	0.147	–	0.042	0.039
237	0.094	–	0.052	–	0.031	0.067	0.112	0.234	–	0.025	–	0.066
239	–	–	–	–	–	–	0.010	–	–	–	0.042	0.004
241	0.063	–	–	–	0.156	0.167	0.051	–	–	0.050	0.063	0.045
243	0.010	–	–	–	–	–	–	–	–	–	–	0.002
245	0.052	–	0.031	0.125	0.031	0.133	0.020	0.125	–	0.075	0.063	0.047
247	0.021	–	–	–	–	–	–	–	–	–	–	0.003
249	0.021	0.030	–	–	0.047	0.033	0.051	–	–	–	0.146	0.031
251	0.010	–	–	0.063	–	–	0.020	–	–	–	0.021	0.007
253	0.010	0.020	–	–	0.078	0.200	0.010	–	0.029	0.125	0.021	0.032
255	0.021	–	–	–	0.031	–	0.031	0.078	–	0.025	0.021	0.020
257	–	–	0.010	–	0.063	0.067	0.020	–	–	–	–	0.013
259	0.010	–	–	0.063	0.016	0.033	0.010	–	–	0.075	–	0.012
261	0.010	0.030	0.021	0.063	0.047	–	0.010	–	–	–	0.083	0.022
263	0.135	–	–	0.063	0.016	0.067	0.041	0.016	–	0.075	–	0.036

Table 5 continued

Allele size	TPZ	LSL	SPN	TKS	PYR	TWN	WLK	STW	DXV	EWR	SFH	All (weighted)
265	0.031	–	–	–	0.031	–	0.041	–	0.029	0.025	0.021	0.018
267	0.021	–	–	–	–	–	–	–	–	–	–	0.003
269	0.021	–	–	–	0.031	–	0.041	0.016	–	–	0.021	0.015
271	0.010	0.060	–	–	0.016	0.033	0.010	–	–	0.225	–	0.028
273	–	–	–	–	–	–	0.041	0.016	0.059	–	0.021	0.012
277	0.010	0.290	0.354	–	0.016	–	0.020	0.016	–	–	0.021	0.101
281	0.010	0.100	–	–	–	–	0.010	0.031	–	–	–	0.020
283	–	–	–	–	–	–	–	–	–	–	0.042	0.003
285	–	–	–	–	–	–	0.020	–	–	–	–	0.003
289	–	–	–	–	–	–	–	–	–	–	0.021	0.002
291	–	–	–	–	–	–	0.020	–	–	–	–	0.003
293	0.010	–	–	–	–	–	0.020	–	–	0.025	–	0.006
297	–	–	0.010	–	0.016	–	0.010	–	–	–	–	0.004
309	0.010	–	–	–	–	–	–	–	–	–	–	0.002
325	0.010	–	–	–	–	–	–	–	–	–	–	0.002
327	0.010	–	–	–	–	–	–	–	–	–	–	0.002
333	–	–	–	–	–	–	0.010	–	–	–	–	0.002
337	–	–	–	–	–	–	0.010	–	–	–	–	0.002
339	0.010	–	–	–	–	–	–	–	–	–	–	0.002
371	–	–	–	0.125	–	–	–	–	–	–	–	0.003
N	48	50	48	8	32	15	49	32	17	20	24	343
Cyp-G41												
167	0.929	0.940	0.490	0.100	0.129	0.583	0.604	0.654	0.971	0.850	0.568	0.661
171	0.071	0.060	0.510	0.900	0.823	0.417	0.375	0.346	0.029	0.150	0.409	0.330
175	–	–	–	–	0.048	–	0.021	–	–	–	0.023	0.009
N	49	50	48	10	31	12	48	26	17	20	22	333
Cyp-G47												
170	–	–	0.010	–	–	–	0.020	–	–	–	–	0.004
174	0.010	–	–	–	–	–	0.030	–	–	–	–	0.006
178	0.520	0.710	0.292	0.350	0.359	0.393	0.570	0.391	0.441	0.700	0.318	0.480
182	0.367	0.290	0.510	0.400	0.484	0.500	0.350	0.406	0.559	0.300	0.614	0.416
186	0.061	–	0.188	0.250	0.156	0.036	0.030	0.203	–	–	–	0.081
190	0.020	–	–	–	–	–	–	–	–	–	–	0.003
194	0.020	–	–	–	–	0.071	–	–	–	–	0.068	0.010
N	49	50	48	10	32	14	50	32	17	20	22	344
Cyp-G48												
118	–	–	–	–	–	–	0.022	–	–	–	–	0.003
122	–	–	–	–	0.063	–	0.056	0.016	0.177	0.125	0.021	0.032
126	–	–	–	–	0.016	–	0.044	–	0.029	–	–	0.009
130	–	–	–	–	–	0.033	–	–	–	–	–	0.002
134	0.020	–	–	–	0.156	–	0.033	0.031	–	–	–	0.025
138	–	–	–	–	–	–	0.011	0.125	–	–	0.104	0.021
142	–	–	0.010	–	0.016	–	0.033	–	–	–	–	0.007
146	0.010	0.100	–	–	–	–	0.044	0.203	–	0.050	0.021	0.045
150	0.133	0.060	0.021	–	0.047	0.067	0.044	0.016	0.118	–	0.167	0.063
154	0.041	0.140	–	–	0.063	0.067	0.033	–	–	0.050	0.125	0.051
158	0.163	0.100	–	0.100	0.063	0.067	0.044	–	–	–	0.063	0.060
160	–	–	0.125	–	–	–	–	–	–	–	–	0.018

Table 5 continued

Allele size	TPZ	LSL	SPN	TKS	PYR	TWN	WLK	STW	DXV	EWR	SFH	All (weighted)
162	0.071	–	0.177	–	0.063	0.067	0.033	–	0.088	–	–	0.053
164	0.020	–	0.167	–	–	–	–	–	–	–	–	0.026
166	0.041	0.100	0.031	0.200	0.141	0.033	0.056	0.016	0.235	0.050	0.083	0.075
170	0.061	0.010	0.010	0.100	0.141	0.167	0.144	–	–	0.200	0.042	0.069
172	–	–	–	–	–	–	–	–	–	–	0.021	0.002
174	0.031	0.230	–	0.100	0.047	0.033	0.067	0.094	0.029	0.050	0.063	0.073
176	–	–	–	–	–	–	0.011	–	–	–	–	0.002
178	0.092	0.020	0.146	0.100	0.047	0.233	0.044	0.172	0.029	0.125	0.042	0.088
180	–	–	0.042	–	–	–	–	–	–	–	–	0.006
182	0.061	–	–	0.050	0.031	–	0.022	0.047	0.118	0.025	0.063	0.032
184	–	–	0.031	–	–	–	–	–	–	–	–	0.004
186	0.092	0.010	–	0.050	0.063	0.100	0.067	–	–	0.250	–	0.050
188	0.010	–	–	–	–	–	–	–	–	–	–	0.002
190	0.092	0.120	0.104	0.100	0.031	0.033	0.067	0.156	0.029	0.050	0.063	0.085
194	0.031	0.110	–	0.050	0.016	0.100	0.033	0.063	–	0.025	0.063	0.044
198	0.010	–	–	0.050	–	–	–	–	0.147	–	–	0.010
202	–	–	–	0.050	–	–	–	–	–	–	0.021	0.003
206	0.010	–	–	0.050	–	–	0.044	–	–	–	0.042	0.012
210	–	–	0.010	–	–	–	0.022	–	–	–	–	0.004
214	–	–	0.063	–	–	–	0.011	0.016	–	–	–	0.012
218	–	–	0.063	–	–	–	–	–	–	–	–	0.009
222	–	–	–	–	–	–	–	0.047	–	–	–	0.004
234	0.010	–	–	–	–	–	–	–	–	–	–	0.002
262	–	–	–	–	–	–	0.011	–	–	–	–	0.002
N	49	50	48	10	32	15	45	32	17	20	24	342

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