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Genetic divergence of sympatric resident and anadromous forms of *Oncorhynchus mykiss* in the Walla Walla River, U.S.A.

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Genetic structure (six microsatellites) and gene flow were examined among anadromous (steelhead; $n = 326$), resident (rainbow trout; $n = 52$), and mixed ($n = 407$) *Oncorhynchus mykiss* life-history forms collected from the major drainages the mainstem Walla Walla River and the Touchet River within the Walla Walla River sub-basin, Washington, U.S.A. Genetic structure was detected between the two major drainages. Exact tests, F_{ST}, multi-locus assignment tests and a neighbour-joining dendrogram revealed genetic divergence between sympatric reference populations of adult steelhead and resident rainbow trout in the mainstem Walla Walla River, but not in the Touchet River. Tests of Hardy-Weinberg equilibrium indicated anadromous and resident *O. mykiss* reference populations were in equilibrium, but many of the mixed life-history collections were out of equilibrium. Populations out of equilibrium in the mainstem Walla Walla River appeared to be admixtures as confirmed by multi-locus assignment tests. This is evidence of genetic divergence probably resulting from assortative mating between life-history forms or out-of-basin stocking practices.

Key words: anadromous steelhead; microsatellite; *Oncorhynchus mykiss*; resident rainbow trout; sympatric.

INTRODUCTION

Several species of salmonids exhibit variable life-history strategies (henceforth referred to as 'life-history forms') including rainbow trout *Oncorhynchus mykiss* (Walbaum) (Neave, 1944), cutthroat trout *Oncorhynchus clarki* (Richardson) (Zimmerman *et al.*, 1997), sockeye salmon *Oncorhynchus nerka* (Walbaum) (Wood, 1995), brown trout *Salmo trutta* L. (Skaala & Naevdal, 1989), Arctic charr *Salvelinus alpinus* (L.) (Nordeng, 1983) and Atlantic salmon *Salmo salar* L. (Berg, 1948). Life-history forms range from individuals that remain in fresh

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water throughout their life (resident), those that make migrations from fresh water to estuaries and back (estuarine), and those that make migrations from fresh water to sea and back (anadromous). In many cases, fishes displaying different life-history forms are sympatric in distribution, but the extent of gene flow between life-history forms ranges from high [*Lepomis macrochirus* Rafinesque (Ehlinger & Wilson, 1988) and *S. trutta* (Pettersson *et al.*, 2001)] to relatively low [*O. clarki* (Wilson *et al.*, 1985), *O. nerka* (Foote *et al.*, 1989), *S. salar* (Verspoor & Cole, 1989) and *O. mykiss* (Zimmerman & Reeves, 2000)]. Reproductive isolation between life-history forms has been demonstrated *via* temporal separation (Leider *et al.*, 1984; Burger *et al.*, 2000), spatial separation (Kurenkov, 1978), or both (Zimmerman & Reeves, 2000). Assortative mating between life-history forms has also resulted in reduced gene flow or reproductive isolation between anadromous sockeye salmon and resident kokanee (*O. nerka*; Foote & Larkin, 1988). Reproductive isolation of sympatric life-history forms is not unique to salmonids, as evidenced by research of three-spined stickleback *Gasterosteus aculeatus* L. (Snyder & Dingle, 1990), rainbow smelt *Osmerus mordax* (Mitchill) (Taylor & Bentzen, 1993) and whitefish *Coregonus clupeaformis* (Mitchill) (Bernatchez *et al.*, 1996).

Detecting genetic divergence between resident and anadromous forms is complicated by issues inherent to sympatric life-history forms. Confirmed cases of anadromous salmonid progeny remaining as stream residents have been detected where no resident populations typically occur (residualism; Regan, 1938; Prouzet, 1981). Also, mating involving alternate forms such as anadromous salmonid females and resident males has been observed through 'sneaking' behaviour (McCart, 1970). Furthermore, migratory life histories are not necessarily genetically determined (Skaala & Naevdal, 1989; Hindar *et al.*, 1991) as non-anadromous and anadromous forms can give rise to one another (Johnson, 1980; Nordeng, 1983; Osinov, 1984; Foote *et al.*, 1989). These issues suggest that gene flow should be sufficient to distribute genetic variation between life-history forms. The type of selection experienced by anadromous forms in the ocean, however, is probably quite different from that experienced in freshwater environments (Ricker, 1940) possibly rendering hybrids less fit in either environment, therefore maintaining genetic divergence between life-history forms. For each of these life-history forms to be maintained over evolutionary time, there must be fitness advantages to each strategy.

Oncorhynchus mykiss are native to western North America with both resident and anadromous life-history forms found throughout their range (Behnke, 1992, 2002). Anadromous forms, referred to as steelhead, have highly variable anadromous life histories among onchorhynchid fishes including duration of freshwater rearing (1 to 3 years), and a proportion of steelhead exhibit an iteroparous reproductive strategy. Resident *O. mykiss*, commonly referred to as rainbow trout, spend their entire life in fresh water with potential dispersal to nearby freshwater lakes and streams. Originally, the two life-history forms of *O. mykiss* were classified as two distinct species based on morphology and behaviour, but have been reclassified as a single species (Behnke, 1992). Moreover, *O. mykiss* populations are now further divided into sub-species based primarily upon morphological evidence (Behnke, 1992, 2002). Diagnostic characters to definitively separate sub-species have not been elucidated thus far (Behnke, 2002). Confounding this issue, *O. mykiss* sub-species exhibit resident and anadromous life-history patterns. Additional complexity in *O. mykiss* has been observed on small spatial and temporal scales with respect to population genetic structure (Beacham *et al.*, 1999) and run timing (Hendry *et al.*, 2002).

In the Walla Walla River in south-eastern Washington, U.S.A., sympatric anadromous and resident *O. mykiss* life-history forms have been observed, but the level of genetic divergence and gene flow among these fish remains unknown. Historically, the Walla Walla River sub-basin supported healthy stocks of anadromous steelhead and resident rainbow trout. The Walla Walla River sub-basin is divided into two main drainages (Touchet River and mainstem Walla Walla River) and both have been highly impacted by anthropogenic factors in the past century (Confederated Tribes of the Umatilla Indian Reservation, pers. comm.). These include: river channels have been straightened to reduce flooding and erosion; water levels fluctuate dramatically due to irrigation; dams, diversions and weirs have been placed on several of the rivers in the sub-basin; introductions of hatchery reared rainbow trout (from 1945 to 1993) and steelhead (from 1983 to present) have occurred (Washington Department of Fish and Wildlife, unpubl. data; Oregon Department of Fish and Wildlife, unpubl. data).

In this study, six highly polymorphic microsatellite loci were analysed to determine genetic geographic structure, and asses the extent of divergence between sympatric life-history forms within the Walla Walla sub-basin. Using the level of genetic divergence between anadromous and resident sample collections, an attempt was made to identify individuals of anadromous or resident origin from collections of mixed life-history forms. Finally, the origins of alternate life histories and their impact on geographic distribution of molecular genetic variation within the species were examined.

METHODS

SAMPLE COLLECTIONS

Fin clip samples of *O. mykiss* were collected throughout various tributaries of the Walla Walla River sub-basin, Umatilla River, and Snake River in the Columbia River system from 1998 to 2002 (Table I and Fig. 1). Sample collections consisted of three categories: adult steelhead (ANAD), adult resident rainbow trout (RES) and mixed (MIX) *O. mykiss* collections of undetermined life-history forms of juvenile and adult resident rainbow trout and juvenile anadromous steelhead (Table I). MIX collections were sampled by beach seine from four sites each from the two major tributaries of the Walla Walla River, the Touchet River (T) and mainstem Walla Walla River (WW) for a total of eight MIX samples. Age (determined from scales) and total length (L_T) was recorded for the MIX collections. Individuals collected in beach seines that possessed characteristics exclusive of anadromous *O. mykiss* were moved into distinct resident rainbow trout collections. That is, *O. mykiss* individuals ≥ 3 years of age and ≤ 30 cm L_T were considered adult resident rainbow trout (age of steelhead smolt migration is <3 years, and size of returning adult Walla Walla River steelhead is much larger than 30 cm; pers. obs.). Anadromous samples were collected from morphologically distinct adult steelhead returning to spawn in natal regions. Temporal samples >2 years were taken for both steelhead collections and beach seine collections to represent temporal genetic diversity from the Touchet River and mainstem Walla Walla River. Fin clips were obtained from 841 fish and immediately placed in 95% ethanol until examined in the laboratory. For ease of reference, sample collections are henceforth referred to by

TABLE I. Sample collection information: life-history form, year collected and adjustments to sample size. Sample collection names abbreviated by drainage (T, Touchet River; WW, mainstem Walla Walla River; UM, Umatilla River; SN, Snake River) followed by life-history form (ANAD, anadromous; RES, resident; MIX, mixed life histories)

Sample location	Life history form*	Year collected	Original sample size	Full siblings removed**	Moved to resident rainbow group***	Final sample size
N.F. Touchet (T1-MIX)	Mixed	1999 & 2000	117	18	3	95
S.F. Touchet (T2-MIX)	Mixed	2000	86	9	19	58
N.F. Coppei Creek (T3-MIX)	Mixed	2000	38	1	6	31
S.F. Coppei Creek (T4-MIX)	Mixed	2000	34	5	3	26
Walla Walla mainstem (WW1-MIX)	Mixed	1999	32	1	0	31
Mill Creek (WW2-MIX)	Mixed	1999 & 2000	73	9	5	59
N.F. Walla Walla (WW3-MIX)	Mixed	2000	44	5	16	23
S.F. Walla Walla (WW4-MIX)	Mixed	1999	92	8	0	84
Walla Walla mainstem (WW-ANAD)	Adult steelhead	1998 & 1999	118	0	NA	118
Touchet River (T-ANAD)	Adult steelhead	1999 & 2000	59	0	NA	60
Umatilla River (UM-ANAD)	Adult steelhead	2002	94	0	NA	94
Snake River (SN-ANAD)	Adult steelhead	2000	54	0	NA	54
Touchet River resident (T-RES)	Resident rainbow	1999 & 2000	NA	0	NA	31
Walla Walla mainstem resident (WW-RES)	Resident rainbow	1999 & 2000	NA	0	NA	21
			841	56	–	785

*, 'Mixed' life-history type refers to collections of *O. mykiss* of unknown life history and are presumed to be a mixture of resident and anadromous individuals.

**To avoid over-representation of any single family, all but one individual (randomly chosen) in a family determined to be full siblings (Kinship *v.* 1.31; Queller & Goodnight, 1989) were removed from analysis.

***O. *mykiss* individuals taken in beach seines of age ≥ 3 years with $L_T > 30$ cm were considered to be adult resident rainbow trout, and moved from the mixed group into resident groups.

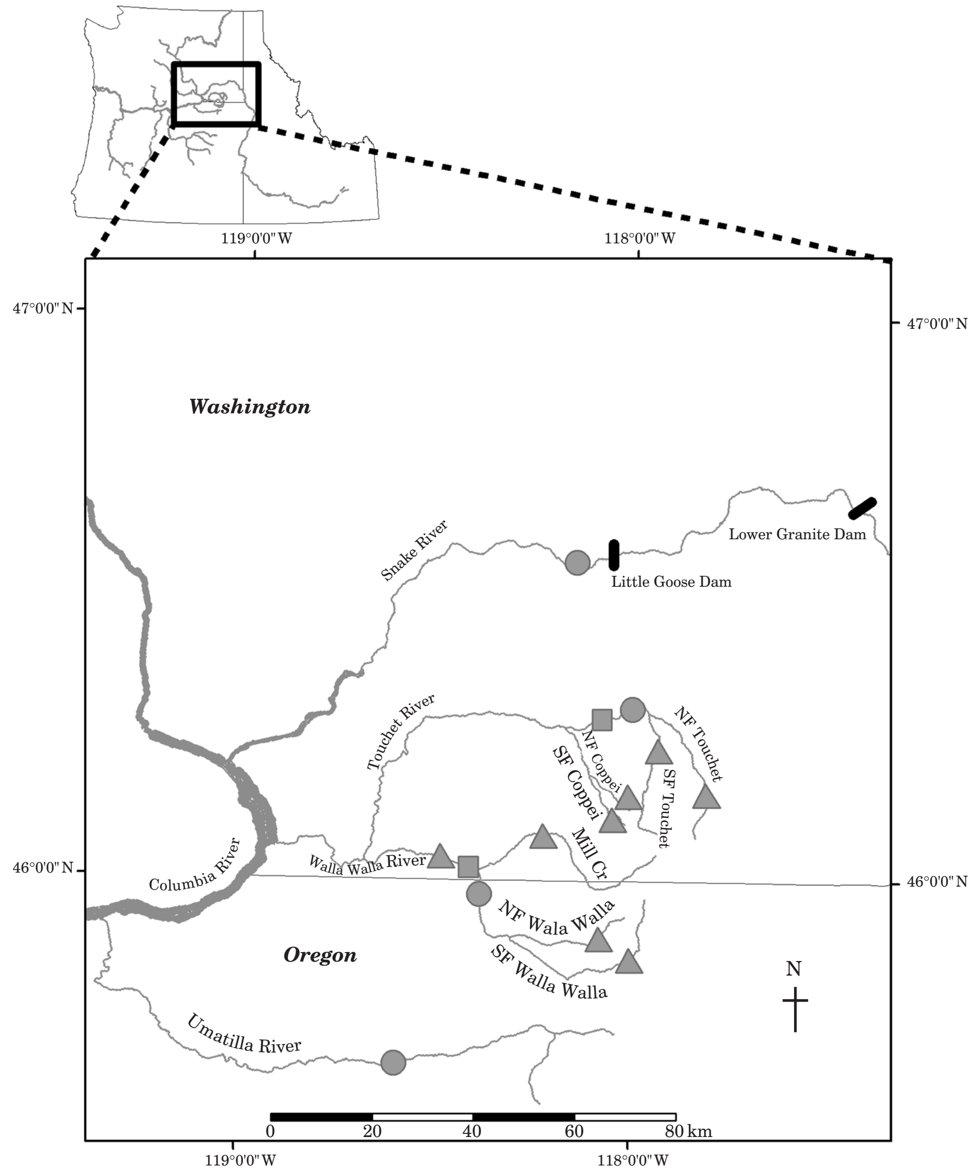

FIG. 1. Map of the general study area of Pacific Northwest, U.S.A. and a detailed map of the sampling locations from the Walla Walla, Umatilla and Snake Rivers, (●, anadromous; ■, resident and ▲, mixed *Oncorhynchus mykiss*).

abbreviated names starting with the tributary followed by the life-history type (*e.g.* T-ANAD = Touchet River, anadromous life history).

LABORATORY ANALYSIS

Fin clips were digested and DNA extracted using the standard manufacture's protocols from Qiagen® DNeasy™ in conjunction with a Qiagen® 3000 robot. Genomic DNA was quantified and arrayed into 96 well plates for high throughput genotyping.

The polymerase chain reaction (PCR) was used to amplify six microsatellite loci designed from *O. mykiss* (*OMM1007*, *OMM1019*, *OMM1020*, *OMM1036*, *OMM1046* and *OMM1050*; GenBank accession numbers respectively AF346669, AF346678, AF346679, AF346686, AF346693, AF346694). Two loci were dinucleotide repeats (*OMM1019*, *OMM1020*), one was a trinucleotide repeat (*OMM1007*) and three were tetranucleotide repeats (*OMM1036*, *OMM1046*, *OMM1050*). PCR amplifications were performed using the AmpliTaq Reagent System (Applied Biosystems®) in an MJ Research® PTC-100 thermal cycler following the manufacturer's protocols, with *c.* 25 ng template genomic DNA in 15 µl total volume. Typical cycling conditions included an initial denaturation of 5 min at 96° C, followed by 30 cycles of 30 s at 94° C, 30 s at 50–62° C and 30 s at 72° C. Final extension was carried out for 10 min at 72° C. Annealing temperature was adjusted to optimize PCR conditions (*OMM1007* = 58° C, *OMM1019* = 58° C, *OMM1020* = 58° C, *OMM1036* = 60° C, *OMM1046* = 60° C and *OMM1050* = 60° C). Forward primers were fluorescently labelled (Applied Biosystems®), and PCR products were genotyped using the manufacture's protocols with an Applied Biosystems® model 3100 genetic analyser.

STATISTICAL ANALYSIS

Since a major portion of the mixed collections were juvenile fish, tests for sibling relationships were performed with the programme Kinship v.1.31 (Queller & Goodnight, 1989). Pair-wise tests between individual fish determine the likelihood of full and half sibling relationships. To avoid over-representation of any single family, all but one individual (randomly chosen) in a family determined to be full siblings were removed from analysis.

To estimate the level of within-population genetic diversity, observed heterozygosity (H_O), expected heterozygosity (H_E; Nei, 1987) and allelic richness (average alleles per locus corrected for sample size) were calculated for all microsatellite loci. Significant differences in heterozygosity among loci were evaluated between sample populations using the Wilcoxon signed ranks test, implemented in SyStat (SPSS Inc.). Power analysis (STATISTICA v. 6.0; StatSoft Inc.) was used to examine reduced sample sizes among aggregate juvenile samples and determine whether an arbitrary difference of 5% in π (as H_E) could be detected given the genetic variance associated with each microsatellite locus used.

Genetic variance was calculated from allele frequencies (F_{ST}; Weir & Cockerham, 1984) using GENEPOP v. 3.3 (Raymond & Rousset, 1995) to estimate pair-wise genetic divergence among collections from the Walla Walla sub-basin. Exact-significance testing methods were used to evaluate conformance to Hardy-Weinberg, linkage equilibria and differences in allele frequency distributions (temporally, geographically and between life-history forms). Unbiased estimators of exact significance probabilities were obtained using the Markov-Chain algorithm described in Guo & Thompson (1993), as implemented in GENEPOP v. 3.3 (Raymond & Rousset, 1995), using 500 000 steps. MicroChecker v. 2.2.1 (www.microchecker.hull.ac.uk) was used to test for null alleles at each of the six loci. Corrections were made against type I error in all exact tests and MicroChecker null allele tests using the sequential Bonferroni method (Rice, 1989).

Assignment tests calculate the probability that an individual's multi-locus genotype derives from alternative groups (species or populations) and assigns membership to the most likely group (Paetkau *et al.*, 1995). Assignment tests were performed using the Bayesian method in GeneClass (Cornuet *et al.*, 1999) in two steps. First, resident and anadromous collections from the Walla Walla River were self classified as reference data. Second, unknown individuals from the mixed life-history sample collections were assigned to either the resident or anadromous group.

In order to infer the degree of relatedness between sample collections, pair-wise genetic distances (Cavalli-Sforza & Edwards, 1967) were calculated between all populations using GENDIST in PHYLIP v. 3.5 (Felsenstein, 1993). Genetic chord distances were then used to construct a neighbour-joining (NJ) tree of sample populations with NEIGHBOR (PHYLIP v. 3.5). Bootstrap replicates were attained using SEQBOOT and CONSENSE in PHYLIP v.3.5 with 1000 iterations.

RESULTS

As a result of scale analysis and length measurements, 52 fish taken in the beach seine samples were determined to be resident rainbow trout by age >3 years and $L_T < 30$ cm. Beach seine collections with the highest percentage of confirmed resident rainbow trout were WW3-MIX (N.F. Walla Walla River; 41%) and T2-MIX (S.F. Touchet River; 25%). After sample populations were evaluated for kinship, all full siblings except one per family were removed from the study resulting in a reduction of total samples from 841 to 785 fish (Table I). Exact tests indicated no significant differences between years within temporal samples from the mainstem Walla Walla River and Touchet River, and therefore temporal samples were pooled at each site.

The six microsatellite loci employed in the analysis ranged from 12 to 38 alleles observed, totalling 147 alleles (Appendix). Allelic richness was 14·5 for anadromous collections, 13·7 for the resident rainbow population and 13·8 for the collections of mixed life-history forms. Observed and expected heterozygosity for each individual collection are shown in the Appendix with medium to high levels of heterozygosity. Average observed heterozygosity for the three collection types was 0·78 for anadromous, 0·70 for resident rainbow trout and 0·67 for mixed collections. Average heterozygosity (H_E) was 0·80 for anadromous and 0·78 for resident rainbow trout, and 0·79 for mixed collections. All of these indicators revealed higher genetic variation in steelhead than resident rainbow trout. Power analysis indicated a sufficient number of samples in the smallest analysis to reliably detect a 5% difference in heterozygosity based upon variance associated with microsatellite loci combined ($P \geq 0{\cdot}827$; $\alpha = 0{\cdot}05$).

Average pair-wise F_{ST} between Touchet River and the mainstem Walla Walla River collections was 0·023 ($P = 0{\cdot}00001$) indicating genetic structure among the two major drainages of the Walla Walla sub-basin. Results of pair-wise F_{ST} calculations between reference collections of steelhead and resident rainbow trout revealed only five of 20 tests were non-significant between analysed collections after sequential Bonferroni correction, but still the largest F_{ST} values were relatively small with values ranging 0·001 to 0·018 (Table II). The largest average F_{ST} detected was between Touchet River steelhead and Walla Walla River resident rainbow trout (0·018; Table II).

TABLE II. Pair-wise F_{ST} values over all loci for reference collections (Weir & Cockerham, 1984). Population abbreviations follow those in Table I

	WW-ANAD	T-ANAD	UM-ANAD	SN-ANAD	T-RES	WW-RES
WW-ANAD						
T-ANAD	0·010					
UM-ANAD	0·006	0·008				
SN-ANAD	0·013	0·009	0·004*			
T-RES	0·003*	0·004*	0·006*	0·009		
WW-RES	0·009	0·018	0·012	0·012	0·001*	

*, values not statistically significant with Bonferroni correction $P = 0{\cdot}05$ divided by $15, = 0{\cdot}0033$.

Tests of Hardy-Weinberg equilibrium (HWE) indicated all loci in anadromous and resident reference populations were in equilibrium, while six of the eight mixed collections were out of equilibrium at one to four loci due to heterozygote deficiencies (Appendix). The two most common loci out of HWE for populations were *OMM1050* and *OMM1020* (each locus out of equilibrium in three collections). While heterozygote deficiencies in these loci could have been the result of amplification problems (*e.g.* null alleles or allele drop out), the fact that these loci were in HWE in all reference populations indicates that the deficiencies were most likely due to effects of non-random mating in the mixed collections rather than amplification problems. Further, tests for null alleles using MicroChecker were not significant at any of the six loci.

Multi-locus assignments of resident and anadromous individuals from the Walla Walla sub-basin resulted in 69·1% success of resident and anadromous individuals assigning to their *a priori* life-history classification. When assignments were examined separately for each of the Walla Walla and Touchet river systems, however, resident and anadromous individuals from the mainstem Walla Walla River were assigned successfully in 91·4%, while those of the Touchet River were assigned successfully in only 67·0%. Using the site specific assignment results from the Touchet River and mainstem Walla Walla River as reference data, unknown individuals from life-history mixture collections taken from each location were assigned to either resident or anadromous groups (Table III). Mixtures from the mainstem Walla Walla River contained a slightly larger proportion of anadromous *O. mykiss* than the Touchet River (56·3 and 50·5% respectively), but reference data for the Touchet River were not robust to provide highly confident assignments. Assignment of mixture samples examined by individual tributary had a range of 67·9% anadromous in WW4-MIX

TABLE III. Assignment test results of Touchet River (T) and mainstem Walla Walla River (WW) collections. RES, resident; ANAD, anadromous; MIX, mixture of resident and anadromous. Population abbreviations follow those in Table I

	Assignment		
a priori group	Resident	Anadromous	Success (%)
T-RES	17	14	54·8
T-ANAD	16	44	73·3
T1-MIX	38	57	N/A
T2-MIX	31	27	N/A
T3-MIX	17	14	N/A
T4-MIX	18	8	N/A
WW-RES	19	2	90·5
WW-ANAD	10	108	91·5
WW1-MIX	17	14	N/A
WW2-MIX	30	29	N/A
WW3-MIX	12	11	N/A
WW4-MIX	27	57	N/A

N/A, not applicable.

(South Fork Walla Walla River) to 30·8% anadromous in T4-MIX (South Fork Coppei Creek; Table III).

The neighbour-joining tree of the sample collections as shown in Fig. 2 indicates geographic genetic differences among drainages within the Walla Walla sub-basin (Walla Walla River collections cluster more closely to each other than to any Touchet River collection and *vice versa*). The T-ANAD and WW-ANAD steelhead collections are more similar to resident and mixed collections within their own drainage than to any collection in the other drainage. Further, all anadromous steelhead collections except T-ANAD cluster together.

DISCUSSION

In this study, slight genetic divergence was detected between sympatric life-history forms of *O. mykiss*. Anadromous and resident forms of *O. mykiss*, however, are more genetically similar within both the Touchet and Walla Walla Rivers than between the two drainages. This corresponds with the general pattern of geographic genetic structure evident in the collections as all samples from tributaries to the mainstem Walla Walla River cluster more closely to each other, than to any Touchet River collection in the NJ tree (Fig. 2). Other sympatric salmonid life-history forms have been shown to be genetically interrelated (Ryman, 1983; Osinov, 1984; Wilson *et al.*, 1985) and more genetically similar within a location than between regions (Stahl, 1987; Foote *et al.*, 1989)

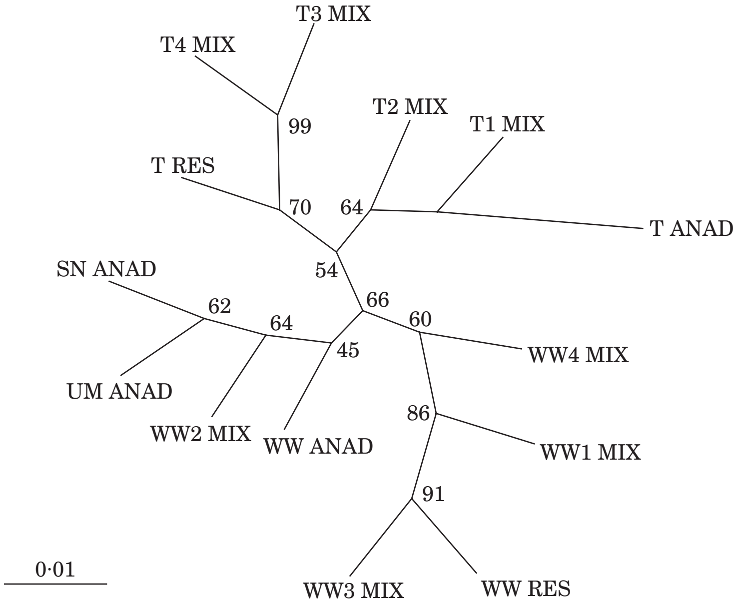

FIG. 2. Neighbour-joining dendrogram (Cavalli-Sforza & Edwards, 1967) displaying the relationship of sample collections. Collections abbreviated by drainage (T, Touchet River; WW, mainstem Walla Walla River) followed by life-history form (ANAD, anadromous; RES, resident; MIX, unknown mixture of resident and anadromous forms of *O. mykiss*., *e.g.* T ANAD = Touchet River anadromous collection). Bootstrap values are present at each node as generated from 1000 iterations.

indicating life-history forms are not derived from separate genetic lineages or from the result of multiple colonizations of allopatric populations.

Genetic differences in life-history forms may represent ecological speciation (Snyder & Dingle, 1990; Taylor & Bentzen, 1993; Bernatchez *et al.*, 1996), or may be associated with anthropogenic factors (Heath *et al.*, 2001). While life-history forms were more genetically similar within each of the drainages of the Walla Walla sub-basin than between drainages, life-history forms were more distinct from one another in the mainstem Walla Walla River than the Touchet River. Results from exact tests, F_{ST}, assignment tests and a NJ tree indicate alternate life-history forms in the Touchet River are not significantly different, while those in the mainstem Walla Walla River are genetically distinct. Resident fish that are genetically divergent from steelhead in the mainstem Walla Walla River may be mating assortatively by size or spawning time (Zimmerman & Reeves, 2000). Alternatively, recent stocking of Snake River origin steelhead in the mainstem Walla Walla River (WDFW; unpubl. data) may account for genetic differentiation among life-history forms in the mainstem Walla Walla River. Evidence for this situation is present in the NJ tree as the clade containing the mainstem Walla Walla River collections is bifurcated with one cluster containing collections primarily of Snake River origin, including the WW-ANAD collection. While this evidence is circumstantial and needs to be further corroborated and quantified by other techniques (*e.g.* parentage analysis), transfers of fish have been shown to influence the genetic structure of receiving populations (Heath *et al.*, 2001). Since genetic differences between life-history forms in the mainstem Walla Walla River may be due to recent hatchery introductions, cumulative occurrences of 'residualism' and sneaking may greatly increase gene flow between life-history forms through time.

While assignment tests confirmed admixtures of genetically distinct life-history forms in heterozygote-deficient, mixed collections from the mainstem Walla Walla River (Wahlund effect), the contributing cause of heterozygote deficiency in the Touchet River mixed collections cannot be unequivocally determined. Life-history forms in the Touchet River were not genetically distinct and therefore would probably not account for heterozygote deficiencies in the mixed collections even in the event of resident and anadromous admixture. Heterozygote deficiencies in the Touchet River mixed collections may be due to circumstances other than a mixture of life-history forms such as hatchery introgression (Currens *et al.*, 1997) resident dispersal (Fausch & Young, 1995), inbreeding (Castric *et al.*, 2002) or family biased juvenile sampling error (Beacham *et al.*, 1999).

Environmental conditions appear to influence juvenile development into life-history types (Lee & Power, 1976), and juvenile development can vary temporally, based on conditions (Metcalf *et al.*, 1989). The final determination of individual life-history form is probably composed of complex interactions between environment and genetics. Furthermore, while reproductive isolation between life-history types in *O. mykiss* commonly involves discussion of only two life-history forms (resident and anadromous), a third life-history form (estuarine) can exist in undisturbed environments such as the Kamchatka Peninsula of Russia (Savvaitova *et al.*, 1997). Relatively undisturbed environments may yield clues to the evolutionary processes in species containing multiple life forms and are prime targets for future research regarding anadromous fish distribution and evolution.

Samples were collected by biologists from multiple agencies including T. Bailey, G. Rowan, P. Kissner, G. Shippentower, D. Thompson, J. Whiteaker and G. Mendel. T. Bailey, G. Mendel and G. Lensegrav provided historical stocking information. R. Henry provided Fig. 1.

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APPENDIX. Summary statistics for all *O. mykiss* collections at six microsatellite loci. N, sample size; A, alleles; AR, allelic richness; H_E, expected heterozygosity; H_O, observed heterozygosity; HWE, Hardy-Weinberg equilibrium FIS value; PA, private alleles. Collection abbreviations as in Table I

Sample collection	Locus	*OMM1007*	*OMM1050*	*OMM1036*	*OMM1020*	*OMM1046*	*OMM1019*	Mean all loci
T1-MIX								
	N	94	93	93	95	93	94	93·7
	A	9	24	27	7	14	11	15·3
	AR	5·6	13·3	15·3	4·8	10·9	8·4	9·7
	H_E	0·591	0·899	0·911	0·469	0·861	0·793	0·754
	H_O	0·564	0·839	0·763	0·505	0·742	0·798	0·702
	HWE	0·047	0·067	0·163	−0·076	0·139	−0·007	0·070
	PA	1	2	1	0	0	0	0·7
T2-MIX								
	N	58	57	46	56	47	55	53·2
	A	6	17	18	8	12	10	11·8
	AR	4·9	11·7	13·3	5·1	9·7	8·9	9·0
	H_E	0·628	0·904	0·902	0·307	0·879	0·840	0·743
	H_O	0·621	0·825	0·674	0·304	0·447	0·709	0·596
	HWE	0·012	0·087	0·254*	0·014	0·494*	0·157	0·199
	PA	0	0	0	0	0	0	0·0
T3-MIX								
	N	31	31	31	31	30	30	30·7
	A	7	15	14	8	9	8	10·2

	AR	6·1	11·9	11·0	7·7	8·4	7·3	8·7
	H_E	0·765	0·887	0·865	0·781	0·860	0·670	0·804
	H_O	0·742	0·742	0·710	0·806	0·733	0·600	0·722
	HWE	0·030	0·166	0·182	−0·033	0·148	0·108	0·104
	PA	0	0	0	0	0	0	0·0
T4-MIX								
	N	22	26	24	25	26	18	23·5
	A	6	11	13	7	9	7	8·8
	AR	6·0	10·2	11·8	6·8	8·3	7·0	8·4
	H_E	0·755	0·885	0·896	0·820	0·831	0·817	0·834
	H_O	0·636	0·654	0·750	0·640	0·538	0·944	0·694
	HWE	0·158	0·263*	0·165	0·223	0·355	−0·161	0·170
	PA	0	0	0	0	0	0	0·0
WW1-MIX								
	N	29	31	31	30	31	31	30·5
	A	7	19	20	6	16	11	13·2
	AR	6·2	14·7	16·4	5·1	13·9	9·5	11·0
	H_E	0·721	0·910	0·939	0·380	0·929	0·848	0·788
	H_O	0·690	0·935	0·935	0·167	0·935	0·774	0·739
	HWE	0·042	−0·028	0·002	0·567*	−0·006	0·089	0·062
	PA	0	0	0	0	2	0	0·3
WW2-MIX								
	N	55	56	58	56	55	58	56·3
	A	9	25	23	8	15	13	15·5
	AR	7·3	17·5	16·5	6·5	11·4	8·5	11·3
	H_E	0·776	0·948	0·938	0·491	0·896	0·828	0·813
	H_O	0·836	0·607	0·776	0·429	0·855	0·776	0·713
	HWE	−0·079	0·361*	0·174	0·130	0·047	0·064	0·124
	PA	0	0	1	0	1	0	0·3

Appendix. Continued

Sample collection	Locus	*OMM1007*	*OMM1050*	*OMM1036*	*OMM1020*	*OMM1046*	*OMM1019*	Mean all loci
WW3-MIX								
	N	22	21	23	23	21	21	21·8
	A	5	14	18	6	13	8	10·7
	AR	4·6	13·1	16·6	5·7	12·3	7·8	10·0
	H_E	0·686	0·876	0·948	0·417	0·895	0·852	0·779
	H_O	0·682	0·857	0·913	0·261	0·905	0·762	0·730
	HWE	0·005	0·024	0·035	0·380*	–0·013	0·110	0·064
	PA	0	0	0	0	0	0	0·0
WW4-MIX								
	N	82	82	76	82	82	82	81·0
	A	9	21	20	7	15	13	14·2
	AR	6·2	13·8	14·1	4·9	10·6	9·5	9·9
	H_E	0·722	0·921	0·921	0·318	0·884	0·834	0·767
	H_O	0·683	0·573	0·618	0·171	0·768	0·805	0·603
	HWE	0·054	0·379*	0·330*	0·465*	0·131	0·035	0·214
	PA	0	0	0	0	1	1	0·3
WW-ANAD								
	N	105	113	116	99	110	118	110·2
	A	10	30	32	10	15	11	18·0
	AR	6·6	15·0	17·8	5·6	11·1	7·9	10·7
	H_E	0·737	0·916	0·946	0·457	0·890	0·799	0·791
	H_O	0·781	0·920	0·922	0·444	0·864	0·763	0·782
	HWE	–0·059	–0·005	0·025	0·027	0·029	0·046	0·011
	PA	1	1	2	0	0	0	0·7

T-ANAD								
	N	54	59	47	52	53	60	54·2
	A	7	19	21	8	13	11	13·2
	AR	5·9	13·2	15·3	5·1	10·0	8·3	9·6
	H_E	0·669	0·907	0·921	0·537	0·870	0·803	0·784
	H_O	0·574	0·881	0·894	0·577	0·925	0·783	0·772
	HWE	0·142	0·028	0·031	−0·076	−0·063	0·026	0·016
	PA	0	0	0	0	1	1	0·3
T-RES								
	N	31	31	28	30	24	26	28·3
	A	7	12	16	8	11	9	10·5
	AR	6·4	14·2	16·0	6·1	11·4	9·0	10·5
	H_E	0·639	0·877	0·932	0·480	0·896	0·846	0·778
	H_O	0·677	0·742	0·714	0·400	0·542	0·846	0·654
	HWE	−0·061	0·158	0·238	0·166	0·400	−0·002	0·163
	PA	0	0	0	0	1	0	0·2
WW-RES								
	N	18	21	20	21	19	20	19·8
	A	8	19	20	5	13	10	12·5
	AR	6·3	15·2	16·4	6·2	11·5	9·0	10·8
	H_E	0·750	0·943	0·945	0·376	0·900	0·840	0·792
	H_O	0·778	0·905	0·900	0·238	0·895	0·850	0·761
	HWE	−0·037	0·042	0·050	0·373	0·006	−0·011	0·041
	PA	0	0	0	0	1	0	0·2
UM-ANAD								
	N	90	80	91	90	82	83	86·0
	A	10	27	27	9	16	13	17·0
	AR	6·3	16·1	16·6	6·1	10·6	8·2	10·7
	H_E	0·690	0·938	0·941	0·548	0·882	0·799	0·799

APPENDIX. Continued

Sample collection	Locus	*OMM1007*	*OMM1050*	*OMM1036*	*OMM1020*	*OMM1046*	*OMM1019*	Mean all loci
	H_O	0·689	0·900	0·890	0·611	0·866	0·771	0·788
	HWE	0·002	0·040	0·054	–0·116	0·019	0·035	0·015
	PA	0	0	1	0	0	1	0·3
SN-ANAD								
	N	51	50	51	54	47	52	50·8
	A	6	26	27	10	16	10	15·8
	AR	5·8	16·9	17·7	7·2	12·3	8·2	11·3
	H_E	0·712	0·932	0·939	0·550	0·909	0·840	0·814
	H_O	0·608	0·860	0·882	0·463	0·915	0·808	0·756
	HWE	0·147	0·079	0·061	0·161	–0·008	0·038	0·071
	PA	0	0	1	1	1	0	0·5
Mean all populations								
	N	53·0	53·6	52·5	53·1	51·4	53·4	52·9
	A	7·6	19·9	21·1	7·6	13·4	10·4	13·3
	AR	6·0	14·1	15·4	5·9	10·9	8·4	10·1
	H_E	0·703	0·910	0·924	0·495	0·884	0·815	0·789
	H_O	0·683	0·803	0·810	0·430	0·781	0·785	0·715
	HWE	0·029	0·060	0·098	0·072	0·091	0·038	0·095
	PA	0·1	0·2	0·4	0·1	0·6	0·2	0·3

*, statistically significant *P* value after sequential Bonferroni correction.