The evolutionary history of steelhead (Oncorhynchus mykiss) along the US Pacific Coast: Developing a conservation strategy using genetic diversity

J. L. Nielsen

Changes in genetic variation across a species range may indicate patterns of population structure resulting from past ecological and demographic events that are otherwise difficult to infer and thus provide insight into evolutionary development. Genetic data is used, drawn from 11 microsatellite loci amplified from anadromous steelhead (Oncorhynchus mykiss) sampled throughout its range in the eastern Pacific Ocean, to explore population structure at the southern edge in California. Steelhead populations in this region represent less than 10% of their reported historic abundance and survive in very small populations found in fragmented habitats. Genetic data derived from three independent molecular systems (allozymes, mtDNA, and microsatellites) have shown that the southernmost populations are characterized by a relatively high genetic diversity. Two hypothetical models supporting genetic population substructure such as observed were considered: (1) range expansion with founder-flush effects and subsequent population decline; (2) a second Pleistocene radiation from the Gulf of California. Using genetic and climatic data, a second Pleistocene refugium contributing to a southern ecotone seems more feasible. These data support strong conservation measures based on genetic diversity be developed to ensure the survival of this uniquely diverse gene pool.

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Key words: conservation, evolution, genetic diversity, microsatellite DNA, steelhead.

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J. L. Nielsen: USGS/BRD, Alaska Biological Science Center, 1011 East Tudor Rd., Anchorage, AK 99503, USA; tel: +1 907 786 3670; fax: +1 907 786 3636; e-mail: jennifer_nielsen@usgs.gov

Introduction

Conservation and recovery strategies for threatened or endangered aquatic species are usually developed using demographic and/or ecological (habitat based) data. These data typically derive from surveys of a small number of populations over a short period of time. Concordance of results drawn from demographic and ecological data is not guaranteed. Extinction risk models used to analyse population trends at the local and global scales consider three kinds of stochastic change: environmental, demographic, and catastrophic (Mace and Lande, 1991; Boyce, 1992; Caughley, 1994; Taylor, 1995), but neglect potential information derived from genetic effects (Dunham et al., 1999). Environmental and catastrophic change affect all individuals within a population and can have large negative results on genetic population structure. Demographic change, on the other hand, affects each individual differently and can result in multiple dispensations of fitness among individuals within the population.

Population decline, quasi-extinction, and low overall abundance may result from any of the above effects, but genetic structure within and among survivors will differ. A catastrophic loss of most individuals within a population in a given area can lead to recolonization by neighbour populations with similar ecological and reproductive requirements. Colonizers, however, can quickly swamp most of the original gene pool. Expansion of a species range due to environmental shifts in habitat conditions or invasions following catastrophic change can retain significant genetic structure with sufficient gene flow, and theoretically lead to unique patterns of increased genetic diversity due to founder-flush effects.
events (Slatkin, 1996). On the other hand, altered environmental conditions that accompany demographic expansion may shift fitness and survival patterns within a population and can have a variety of effects on the gene pool. Changes in effective population size (the number of individuals actually contributing genes to the next generation), population bottlenecks, and movement (i.e. gene flow) among surviving groups must be considered (Avise, 1994; Allenford and Waples, 1996).

Under all of the above conditions, the degree of genetic variation found among and between existing populations provides information concerning past patterns of demographic and ecological events in the history of each population (Weir, 1996). Patterns of genetic variation have a strong historical component (Slatkin, 1985, 1993) and trends in isolation, gene flow, and evolutionary history derived from these may provide insight into historic population dynamics (Avise, 1994). These changes are especially informative in fragmented populations or those found near the edge of a species range. The suite of events reflected in the gene pool can be used in the analyses of population viability or extinction risk (Lande, 1995; Hedrick et al., 1996; Allenford et al., 1997). Therefore, genetic data can serve as indicators of conservation and recovery requirements that are otherwise difficult to infer.

Genetic diversity can be measured on multiple scales. Evolutionary divergence is a dynamic process with markers acting across many different temporal and spatial patterns (Avise, 1994; Nielsen et al., 1997a). Changes in molecular markers used in population studies are generally thought to be selectively neutral throughout their evolutionary range and have no direct effect on individual fitness (Kimura, 1983). Change is assumed to result from stochastic events involving differences in genetic drift and/or gene flow among the different populations (Nei, 1987). Neutrality, therefore, is the most parsimonious explanation for patterns of genetic variation found in fish population studies.

DNA mutation rates are known to vary extensively among genes and across taxa (Wilson et al., 1977; Nei, 1987; Weber and Wong, 1993). With highly variable mutation rates and no evidence of selection, the three most common molecular systems—allozymes, mitochondrial DNA (mtDNA), and microsatellite repeat loci—can present three different temporal or spatial scales for measuring genetic diversity in any population (Boyce et al., 1996; Nielsen et al., 1997b). Allozyme electrophoresis reflects protein-coding genetic structure and loci in this system are thought to have the slowest mutation rate among the three markers. Allozyme analysis was the only molecular system applied to fish population studies until the early 1980s when the development of DNA technology provided new tools (Utter, 1995).

Mitochondrial DNA (mtDNA), passed from the female of the species to her offspring, has been shown to provide strong biogeographic resolution in many species (Avise, 1994). Maternally inherited, non-recombining mtDNA markers with high evolutionary rates of sequence divergence can show a degree of variation among populations not found in more slowly evolving proteins and enzymes (Brown et al., 1979; Moritz et al., 1987).

Microsatellite DNA represents a class of highly polymorphic, simple sequence tandem repeat loci, usually containing two (di-), three (tri-), or four (tetra-nucleotide) core units. These units repeat at varying lengths in different individuals, groups, or populations. Microsatellites often have more alleles per locus by species than allozymes and are more variable than haplotype diversity depicted by mtDNA. These markers exhibit high levels of variability due to high mutation rates ($10^{-2}$–$10^{-3}$) and the large numbers of unlinked loci available from most animal genomes (e.g. Queller et al., 1994). Additionally, microsatellites can be amplified from non-destructive tissue and changes recorded in the loci are frequently used to determine genetic variation in studies of closely related or endangered species (Ashley and Dow, 1994; May et al., 1997). With molecular changes occurring at different mutation rates in different markers, congruence among these markers would require some degree of consistency in population history and structure across multiple temporal and/or spatial scales (Boyce et al., 1996).

Under the ecological “dispersal” model, populations found at the edge of their species’ range are thought to be made up of colonizers from the centre of the range that have adapted to new environmental conditions through active or passive dispersal across a pre-existing geographic or ecological barrier (Avise, 1994). In the alternative model of “vicariance” a more or less continuous distribution of a species across the landscape has been split by large-scale geologic or hydrologic events such as uplifting, continental break-up, or glaciation. In this study I examine microsatellite genetic diversity for 11 loci among four California anadromous steelhead (Oncorhynchus mykiss) populations and one population from southeast Alaska. Genetic diversity analyses are used to examine the population structure of steelhead at the southern extent of their range, and to gain insight into their past evolutionary history with consideration of two very different genetic dispersal models. Microsatellite diversity measures from several aquatic and terrestrial species from the published literature are compiled for comparison with diversity measures developed from Pacific steelhead.

Material and methods

Individual fish used in this paper were collected for various projects, 1989–1997 (Table 1). Southern steelhead were represented by 30 fish captured in the Santa
Ynez River (36 km north) and Malibu Creek (96 km south) near the Point Conception species boundary in southern California (Figure 2). These fish represented the total observed anadromous contribution (spawning year 1993/1994) for the southern most populations in the Eastern Pacific Ocean. Northern California steelhead (n=27) were collected from eight coastal rivers found south of Cape Mendocino. Middle Fork Eel River fish were collected from winter-run steelhead (summer-run fish also occur in this basin). The Eel River flows into the Pacific Ocean approximately 200 km north of Cape Mendocino and 150 km south of the Oregon border with California. Southeast Alaska steelhead were collected by the National Marine Fisheries Service in the Sashin Creek drainage on the southeast portion of the Baranof Island near Little Port Walter.

Previously published data for California steelhead based on allozymes (Busby et al., 1996) and mtDNA (Nielsen et al., 1994; Nielsen et al., 1998) provide protocols and methodologies for these two molecular systems. New genetic data presented here are based on amplification of 11 microsatellite loci (Omy77, Omy207, Omy325, One2, One8, One11, One14, Ot5, Ssa14, Ssa85, and Ssa289) drawn from other research reports and publications. My laboratory has previously reported data on Omy77, Omy207, and Ssa289 for California steelhead. Our microsatellite DNA extraction and amplification protocols are available in this literature (Nielsen et al., 1997a).

The 11 loci reported here have shown significant polymorphisms in previous studies across a broad range of salmonid species. The Omy-series of microsatellite loci were developed specifically for *O. mykiss* (Morris et al., 1996; M. O’Connell, Dalhousie University, pers. comm.), the One-series was developed for *O. nerka* (Scribner et al., 1996), Ot5-series for *O. tshawytyascha* (M. Banks, University of California, Davis, pers. comm.), and the Ssa-series of primers were developed for Atlantic salmon, *Salmo salar* (McConnell et al., 1995; O’Reilly et al., 1996).

The underlying molecular mechanisms of the mutation process remain unknown for microsatellites leading to controversy in analytical assumptions used to measure genetic diversity and differentiation. For this study, I ran two pairwise genetic distance matrices based on the current most popular mutation models. The first was calculated for microsatellite allelic diversity using methods described in Goldstein et al. (1995) for the 11 microsatellite loci combined. Their \((\delta \mu)^2\) genetic distance measure is equivalent to a general analysis of variance using an average sum of squares of differences in allelic size within each population, and the average squared difference between all possible pairs of populations. Both distance measures represent a scale of variation for allelic frequencies among and between populations and were calculated using the program (MICROSAT 1.4) available from Dr E. Minch, Department of Genetics, Stanford University, USA (http://lotka.stanford.edu/distance.html).

Table 1. Anadromous steelhead populations sampled for microsatellite genetic analyses.

<table>
<thead>
<tr>
<th>Region</th>
<th>Population</th>
<th>Year(s)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>California:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>South of Point Conception</td>
<td>Malibu Creek</td>
<td>1993–1994</td>
<td>14</td>
</tr>
<tr>
<td>North of Point Conception</td>
<td>Santa Ynez River</td>
<td>1993–1994</td>
<td>16</td>
</tr>
<tr>
<td>South of Cape Mendocino</td>
<td>Albion River</td>
<td>1993</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Cottonova Creek</td>
<td>1992</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Garcia River</td>
<td>1992</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Guadal River</td>
<td>1991</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Howard Creek</td>
<td>1992</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Navarro River</td>
<td>1992</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Usal Creek</td>
<td>1991</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Van Duzen River</td>
<td>1993</td>
<td>5</td>
</tr>
<tr>
<td>North of Cape Mendocino</td>
<td>Middle Fork River</td>
<td>1992–1993</td>
<td>11</td>
</tr>
<tr>
<td>Southeast Alaska</td>
<td>Sashin Creek</td>
<td>1996</td>
<td>59</td>
</tr>
</tbody>
</table>

Evolutionary history of *Oncorhynchus mykiss*
assess reproducibility of branching patterns found in the consensus tree.

A measure of the effects of population subdivision based on a variance-ratio test commonly used in population genetic studies (Fst) was calculated for 11 microsatellite loci combined using MICROSAT. MICROSAT was also used to estimate the extent of gene flow (M) in an island model at equilibrium (Slatkin, 1993) among all possible pairs of steelhead populations. Geographic distance between any two populations was calculated as coastal kilometres between the centre of the geographic distribution of each collection. We tested for a pattern of isolation by distance among the five steelhead populations. Geographic distance between any two populations was calculated as coastal kilometres between the centre of the geographic distribution of each collection. We tested for a pattern of isolation by distance among the five steelhead populations by regressing \( \log(M) \) on \( \log(\text{distance}) \) according to methods given in Slatkin (1993). Based on simulation and tests of empirical data, Slatkin (1993) showed that a negative slope resulting from this type of regression analysis would suggest some degree of isolation by distance among equilibrium populations.

Results

Analyses of steelhead genetics used 11 microsatellite loci, 9 of which have published allelic ranges for steelhead populations from Washington state (Wenburg et al., 1996). Allelic size (including the amplified primer) ranged from 87 to 320 base pairs (bp). Allelic size ranges presented here exceeded (high or low) previously published ranges for all 9 loci (Table 2). For all populations combined, the number of alleles per locus averaged 16, and ranged from 4 (Oneì11) to 35 (Oneì2). Average heterozygosity for the 11 loci was 0.68. Average Fst for these loci in the five steelhead populations used in this study was 0.17.

We found 23 unique alleles in the southern steelhead samples from the Santa Ynez River and Malibu Creek. The average frequency of four of these alleles exceeded 0.05 (traditional cut-off for the definition of "rare alleles" in population genetics) in the combined southern steelhead collection. At the Oneì2 locus we found a group of 24 alleles ranging in size from 242 to 320 bp, that were common in both northern California and Alaska steelhead, but were never found in southern stocks. This large size range expansion of alleles for this locus suggests a possible duplication event in steelhead evolutionary history similar to the many duplicated allozyme loci thought to be related to the tetraploid ancestry of all salmonids (Allendorf and Thorgaard, 1984).

Genetic distance analysis based on allelic structure at the 11 loci combined showed the greatest genetic distance between southern (from the Santa Ynez River and Malibu Creek) and southeast Alaska steelhead \([\delta_{\mu}^2=51.17; D=1.18]\). In comparison, genetic distance between steelhead from the Middle Fork Eel River and northern California populations found south of Cape Mendocino was only \([\delta_{\mu}^2=2.68; D=0.094]\). Genetic distance between the Alaska steelhead and those found in California rivers south of Cape Mendocino was \([\delta_{\mu}^2=21.72; D=0.77]\). Neighbour joining analysis of the \([\delta_{\mu}^2]\) genetic distance data supported separation of the southern steelhead populations from all other groups with a bootstrap value of 57% and separation between Santa Ynez River fish and those collected in Malibu Creek at 73% (Figure 1).

Table 2. Allelic size distributions found in 11 microsatellite loci for six anadromous steelhead populations (cf. Table 1; SEAA: southeast Alaska; PS: Puget Sound, Washington state; MFER: Middle Fork Eel River; NCA: northern California south of Cape Mendocino; SYR: Santa Ynez River; MC: Malibu Creek). n: number of fish sampled. Numbers in parentheses represent positive observations of the number of alleles per locus.

<table>
<thead>
<tr>
<th>Locus</th>
<th>n</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omy77</td>
<td>103–137 (9)</td>
<td>117–137 (11)</td>
</tr>
<tr>
<td>Omy207</td>
<td>102–138 (10)</td>
<td>n.a.</td>
</tr>
<tr>
<td>Omy325</td>
<td>95–135 (11)</td>
<td>95–151 (20)</td>
</tr>
<tr>
<td>Oneq2</td>
<td>204–284 (22)</td>
<td>237–350 (23)</td>
</tr>
<tr>
<td>Oneq11</td>
<td>143–149 (4)</td>
<td>144–146 (2)</td>
</tr>
<tr>
<td>Ots1</td>
<td>159–239 (9)</td>
<td>159–251 (10)</td>
</tr>
</tbody>
</table>

*From Wenburg et al., 1996.
proline tRNA end of the control-region in walleye. Variable sections containing tandem repeats found at the mtDNA sequence data. However, the latter were based only on walleye showed significantly more diversity for lotype diversity in walleye (diversity=0.0007; Bernatchez and Dodson, 1994); haplotype diversity ranged from 0.17 to 0.48 and nucleotide diversity=0.014 (Nielsen et al., 1994). Allozyme analyses by the National Marine Fisheries Service of some of the same populations of southern steelhead we used for this study showed unprecedented levels of allelic diversity in southern stocks (Busby et al., 1996).

Control-region mtDNA sequence for southern steelhead in California showed haplotype diversity=0.57 and nucleotide diversity=0.014 (Nielsen et al., 1997; Nielsen et al., 1998). We can compare these values to other published diversity estimates for mtDNA sequence data in fish: nucleotide diversity in California rainbow trout=0.0034 (Bagley, 1997); chinook (O. tshawytscha) haplotype diversity ranged from 0.17 to 0.48 and nucleotide diversity from 0.0019 to 0.0044 (Adams et al., 1994); North American whitefish (Coregonus sp.) nucleotide diversity=0.0007 (Bernatchez and Dodson, 1994); haplotype diversity in walleye (Stizostedion vitreum)=0.36–0.79 (Stepien and Faber, 1998). In these comparisons only walleye showed significantly more diversity for mtDNA sequence data. However, the latter were based on an analysis of larger segments of mtDNA including variable sections containing tandem repeats found at the proline tRNA end of the control-region in walleye.

Our current analyses using 11 microsatellite loci showed unique genetic diversity in southern California steelhead stocks. Recent studies of genetic diversity among diverse taxa suggested that (\(\delta\mu\))² and D are efficient measures of tree branch length estimates and can be used to reconstruct an evolutionary topology for unique genetic diversity found at the edge of this species’ range. One hypothesis following the “dispersal” model cites climatic cycles in ocean conditions lasting 40–200 years which can cause a shift in abundance of anadromous salmon and steelhead among habitats throughout the eastern Pacific Ocean (Pearcy et al., 1990; Peary, 1992). Under this hypothesis, as the cycles repeat themselves there are periodic or episodic opportunities for significant demographic and genetic exchange among populations. As favourable ocean conditions return, salmon and steelhead stocks could recolonize southern habitats with increased survival (Lawson, 1993). But steelhead have shown significant declines coast wide since the 1980s (Busby et al., 1996), not just in southern California.

Cyclical change in ocean conditions could have theoretically populated the southern California coastal streams with fish from diverse lineage’s breeding in more
northern environments. Much of the unique genetic diversity found for allozymes, mtDNA, and microsatellites in southern steelhead has not been found at all in contemporary Pacific steelhead collections from more northern areas, leading to questions about where parental populations for the southern steelhead were located. Congruence among the three independent genetic markers for unique diversity suggests that past events leading to genetic structure in these populations were consistent with population history and structure over a large range of temporal scales. Otherwise, independent molecular systems with very different mutation rates would show unique scales of diversity without any expectation of congruence (Boyce et al., 1996).

Common alleles found in the northern California populations are also found in southern areas supporting a hypothesis of significant gene flow between these areas. Our analyses indicated a limited pattern of isolation by distance with a slope of $-0.11$ in the regression of Slatkin’s $M$ (gene flow) on geographic distance among our study populations. This analysis, however, showed that only 12% of the variation found in the microsatellite data could be explained by isolation by distance. In a similar analysis using 24 allozyme loci in gulls (*Larus glaucescens*) collected along the west coast of North America, isolation by distance explained 37% of the variance (Slatkin, 1993).

Low regression slope and $r^2$ values found for comparisons using southern steelhead could result from two factors. Either there is significant geneflow among these populations and very little actual isolation by distance, or one or more population is not in genetic equilibrium.

Figure 2. Mitochondrial DNA haplotype frequency distributions in three bio-regions of California and from southeast Alaska. Two transition zones or species boundaries are shown, one near Point Conception, and the subarctic boundary near Cape Blanco in southern Oregon. MYS5 and MYS8 were only found in California populations; MYS12 and MYS21 represent the third and fourth most common haplotypes in southeast Alaska populations. Numbers of individuals sampled exceed 80 per region (for details see Nielsen et al., 1994 and Nielsen, 1996).
Table 3. A comparison of molecular diversity measures calculated using microsatellites. Slatkin (1993) isolation by distance measure calculated from $F_{st}$ is given as $M$. Genetic distance values based on $(\delta\mu)^2$ were derived from Goldstein et al. (1995). Nei’s distance (1972) is given as $D$. Maximum distance values are those recorded in source population comparisons (see individual papers for exact population distributions). Hz=heterozygosity.

<table>
<thead>
<tr>
<th>Population</th>
<th>n</th>
<th>#loci</th>
<th>Average Hz</th>
<th>Mean $F_{st}$</th>
<th>M</th>
<th>Max. $(\delta\mu)^2$</th>
<th>Max. D</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between southern California and Alaska steelhead</td>
<td>89</td>
<td>11</td>
<td>0.66</td>
<td>0.029</td>
<td>8.37</td>
<td>51.17</td>
<td>1.182</td>
<td>This paper</td>
</tr>
<tr>
<td>Between Alaska and northern California steelhead</td>
<td>86</td>
<td>11</td>
<td>0.71</td>
<td>0.025</td>
<td>7.56</td>
<td>21.71</td>
<td>0.772</td>
<td>This paper</td>
</tr>
<tr>
<td>Between southern and northern California steelhead</td>
<td>57</td>
<td>11</td>
<td>0.72</td>
<td>0.064</td>
<td>10.17</td>
<td>12.52</td>
<td>0.249</td>
<td>This paper</td>
</tr>
<tr>
<td>Between Mendocino coast and M. F. Eel River steelhead</td>
<td>38</td>
<td>11</td>
<td>0.72</td>
<td>0.035</td>
<td>6.69</td>
<td>2.68</td>
<td>0.094</td>
<td>This paper</td>
</tr>
<tr>
<td>Among 11 populations Sacramento R. rainbow trout</td>
<td>732</td>
<td>12</td>
<td>0.68</td>
<td>0.018</td>
<td>13.64</td>
<td>8.36</td>
<td>0.182</td>
<td>Nielsen unpub. data</td>
</tr>
<tr>
<td>Among 3 populations Alaska rainbow trout/steelhead</td>
<td>59</td>
<td>10</td>
<td>0.46</td>
<td>0.087</td>
<td>2.62</td>
<td>13.22</td>
<td>0.289</td>
<td>Nielsen unpub. data</td>
</tr>
<tr>
<td>Among 3 populations chinook salmon Yukon River</td>
<td>83</td>
<td>16</td>
<td>0.27</td>
<td>0.053</td>
<td>4.47</td>
<td>0.33</td>
<td>0.084</td>
<td>Scribner et al., 1996</td>
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<tr>
<td>Among 7 Atlantic salmon populations in Quebec</td>
<td>183</td>
<td>5</td>
<td>0.83</td>
<td>0.075</td>
<td>3.08</td>
<td>n.a.</td>
<td>0.897</td>
<td>Fountain et al., 1997</td>
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<tr>
<td>Between Canadian and north Ireland Atlantic salmon</td>
<td>120</td>
<td>3</td>
<td>0.53</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>0.900</td>
<td>McConnell et al., 1995</td>
</tr>
<tr>
<td>Among 5 populations North Atlantic cod</td>
<td>101</td>
<td>6</td>
<td>0.90</td>
<td>0.014</td>
<td>17.61</td>
<td>50.60</td>
<td>n.a.</td>
<td>Bentzen et al., 1996</td>
</tr>
<tr>
<td>Mammals</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among 8 international human populations</td>
<td>318</td>
<td>8</td>
<td>0.63</td>
<td>0.107</td>
<td>2.09</td>
<td>n.a.</td>
<td>0.346</td>
<td>Deka et al., 1995</td>
</tr>
<tr>
<td>Between Navajo Native Americans and Finnish Europeans</td>
<td>402</td>
<td>33</td>
<td>0.67</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>0.399</td>
<td>Urbanek et al., 1966</td>
</tr>
<tr>
<td>Among 14 indigenous human populations</td>
<td>140</td>
<td>25</td>
<td>0.57</td>
<td>0.163</td>
<td>1.29</td>
<td>25*</td>
<td>0.548</td>
<td>Bowcock et al., 1994</td>
</tr>
<tr>
<td>Comparing humans and chimpanzees</td>
<td>186</td>
<td>8</td>
<td>0.61</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>1.901</td>
<td>Deka et al., 1995</td>
</tr>
<tr>
<td>Among 1 wild and 3 domestic bighorn sheep populations</td>
<td>250</td>
<td>8</td>
<td>0.77</td>
<td>0.155</td>
<td>1.36</td>
<td>39*</td>
<td>2.797</td>
<td>Forbes et al., 1995</td>
</tr>
<tr>
<td>Among bighorn sheep in 15 southwest populations</td>
<td>252</td>
<td>4</td>
<td>0.56</td>
<td>0.272</td>
<td>0.67</td>
<td>n.a.</td>
<td>1.283</td>
<td>Boyce et al., 1996</td>
</tr>
</tbody>
</table>

*Distance values taken from estimates made in Takezaki and Nei (1996).
A second example given by Slatkin (1993) concerned pocket gophers (*Thomomys bottae*) in central California where he concluded that the time scale of approach of $F_{st}$, and hence $M$, to equilibrium (where isolation by distance becomes apparent) is under the influence of local effective population size. A recent immigration event (past 50–2000 years) forced him to reject a pattern of isolation by distance. A similar conclusion was reached for these populations using genetic distance (Lessa, 1990).

A population is in genetic equilibrium when there are more or less constant allele rations in a gene pool through successive generations over a significant period of time. The number of rare alleles found to be unique to the very small southern steelhead populations argue against equilibrium. Analysis of northern California and Alaska steelhead without southern California stocks produced slope ($\sim 0.28$) and $r^2$ (0.34) values similar to those reported as significant in Slatkin (1993). These results suggest the northern California populations may fit the assumptions of equilibrium and do show significant isolation by distance, but southern stocks do not.

Founder-flush effects represent one theoretical condition under which extensive genetic diversity can evolve rapidly from a single, panmictic source, although the theory remains controversial (Templeton, 1980; Slatkin, 1996; Charlesworth, 1997). Founder-flush models assumed that populations that are founded by a small number of individuals, and then grow rapidly, are characterized by a distinct genetic evolution. If the founding population represents a random sample from the parent population, the model predicts that during the period of rapid growth, genetic drift is much weaker than would normally be expected, particularly for low-frequency alleles. Therefore, the probability of loss of a neutral lineage is low and the probability of fixation of advantageous alleles is much higher than in populations that remain a constant size (Slatkin, 1996).

For founder-flush effects to have contributed to the observed genetic diversity we would need to find support for range expansion into the southern habitats, a period of rapid growth, and a period of habitat reduction (ocean and fresh water) leading to a significant and rapid population decline. Range expansion and habitat decline seem to be feasible circumstances in the recent evolutionary history of southern California steelhead. While ecological conditions in this region during the Pleistocene are not well documented, the extreme temperatures and arid environment found in today’s freshwater habitats argue strongly against rapid population growth over recent geologic history.

Putting aside the founder-flush hypothesis due to lack of supporting evidence, we are left with one alternative to explain the unique genetic diversity observed. This second hypothesis follows the “vicariance” model of genetic variation outlined in Avise (1994). Behnke (1992) suggested a Gulf of California Pleistocene refugium for Pacific salmonids. Perhaps some of the genetic diversity in southern steelhead represents lineage effects from populations that evolved from a Gulf of California refugium, rather than reflecting particular processes in a marginal population with common ancestry from a Beringia refugium. If this is true, then current populations would be located close to the center of their Pleistocene divergence and not at the southern edge. A Gulf of California refugium has been documented for many marine and nearshore organisms (Lynn and Simpson, 1987; Thomas and Strub, 1990; Bernard et al., 1991).

This hypothesis suggests that southern California may play the role of an evolutionary ecotone (after Smith et al., 1997) between two Pleistocene source populations. Interfacing divergent conditions found in ecotones play a significant role in generating genetic diversity (Mace et al., 1996). Small isolated populations found in such regions may exhibit greater interpopulation diversity because individuals are more subject to drift and/or directional selection (Smith, 1993; Risser, 1995).

Physical ocean conditions support the ecotone hypothesis. The California Transition Zone (equivalent to the southern species boundary in Figure 2) represents a region of steep thermal gradients situated adjacent to Point Conception, California (34°N). It has been shown that boundaries of distributions of individual species will cluster in regions of pronounced environmental change (Bernard et al., 1991). This may also be true for populations of steelhead that carry variable biogeographic histories, having evolved from two divergent Pleistocene refugia (Nielsen et al., 1994).

If the theory of two Pleistocene refugia and an ecotone in southern California where the two evolutionary histories meet and combine is true, it has important implications for management, because differing processes or pathways of evolution need to be considered in conservation programmes (Smith et al., 1993). The National Marine Fisheries Service has recently determined steelhead in southern California as an Evolutionarily Significant Unit (ESU) of *O. mykiss* and listed it as an endangered “species” under the US Endangered Species Act. Although some portion of their genetic diversity has survived, extinction of this unique gene pool is highly likely unless conditions in freshwater habitats improve (Busby et al., 1996). Recolonization and/or restoration of these areas by Beringia derived fish (i.e. northern stocks) would not adequately reflect their existing genetic diversity. Unique evolutionary histories found in any population provide a mandate for careful and considered recovery activities (Nielsen, 1995; Tissier et al., 1995; Allendorf and Waples, 1996). This is clearly the case for the southern steelhead in California.
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