Genetic and Phenotypic Variation through the Migratory Season Provides Evidence for Multiple Populations of Wild Steelhead in the Dean River, British Columbia

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Abstract.—We provide evidence for previously undetected population structure in a wild run of summer steelhead *Oncorhynchus mykiss* within a river that has considerable recreational importance (Dean River, British Columbia). Data were gathered from an existing catch-and-release fishery and examined for phenotypic and genetic variation through the migratory season. Specifically, we compared fish captured in different periods during the migration: early (July 2–30), middle (July 31–September 5), and late (September 6–30). Age (freshwater and saltwater), sex ratio, and body girth did not differ significantly among these groups for females or males. Body length increased through the migratory season for both sexes, perhaps because late-migrating fish had more time to feed in the ocean. Based on genotypes at 10 microsatellite loci, early and late groups showed highly significant genetic differences ($P < 0.001$). Assignment tests were able to classify individuals back to early or late groups with 84% accuracy (122 of 145 tests). These results suggest the presence of at least two populations that migrate at different times in the Dean River system. The magnitude of the genetic difference was small ($F_{ST} = 0.007$; Nei's unbiased $D = 0.0149$, Reynolds coancestry coefficient = 0.007) but comparable to values for other anadromous Pacific salmon species over similar spatial scales. Moreover, the coarse level of our sampling, and possible overlap in migratory timing among populations, suggests that the observed differentiation underestimates the true differentiation. A deficit of heterozygotes in the late group suggests further population substructure within late-migrating groups of fish. Examining temporal variation through a migratory season proved a useful approach for obtaining preliminary evidence of population structure in migrating salmonids within a small river system.

Steelhead, the anadromous form of rainbow trout *Oncorhynchus mykiss*, support a large and lucrative sport fishery in rivers that harbor robust populations. Unfortunately, steelhead populations have been extirpated or have declined dramatically in abundance in many parts of their native range (Nehlsen et al. 1991; Slaney et al. 1996; Smith and Ward 2000; Ward 2000). At least 23 major populations have disappeared from California, Oregon, Washington, and Idaho (Nehlsen et al. 1991), and 151 populations are of ''special concern'' or at high risk of extinction in British Columbia and the Yukon (Slaney et al. 1996). Current management efforts for steelhead and other anadromous salmonids focus on restoring extirpated or endangered populations and on maintaining the productivity and genetic integrity of healthy populations (CPMNAS 1996). This focus at the level of populations (rather than entire species) is important because (1) healthy local populations provide benefits to humans and other organisms that reside nearby, (2) the maintenance of healthy populations is expected to improve the long-term genetic and demographic health of an entire metapopulation (a group of populations exchanging migrants; Cooper and Mangel 1999; Young 1999), and (3) the U.S. Endangered Species Act mandates protection of ''any distinct population segment of any species of vertebrate fish or wildlife which interbreeds when mature'' (Utter 1981; Waples 1991, 1995).

Protection of local populations requires the identification of groups of fish within which interbreeding is common, but among which interbreeding is rare. Such distinct populations should be identified not only during breeding, but also at other stages of their life history when they might be differentially impacted by human exploitation (e.g., mixed-stock fisheries). Historically, such

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identification relied on the distribution of phenotypic variation within and among populations (Swain and Foote 1999). Analyses of phenotypic variation often reveal large differences among populations, some of which seem to represent adaptations to local environments (Taylor 1991). More recently, the identification of distinct populations has relied primarily on genetic markers that are assumed to be selectively neutral (allozymes, mitochondrial DNA, microsatellites), and thus indicative of gene flow among populations. In reality, the appropriate identification of populations warranting separate management ultimately requires an understanding of both neutral and adaptive genetic variation (Waples 1991, 1995; Utter et al. 1993; Hard 1995).

Studies of neutral genetic variation have enriched our understanding of steelhead population structure, but a lack of congruence among studies from different regions highlights the need for further study. At large spatial scales, steelhead populations probably exchange very few migrants and are essentially independent, both genetically and demographically. For example, considerable genetic differences have been documented among steelhead from (1) the upper Columbia River, coastal rivers in Washington, and coastal rivers in California (Reisenbichler et al. 1992); (2) the Fraser River, Vancouver Island, and the Columbia River (Beacham et al. 1999); (3) different river systems in California (Berg and Gall 1988); (4) different river systems in British Columbia (Heath et al. 2001); and (5) northern British Columbia and Alaska (Taylor 1995).

At smaller spatial scales, however, patterns of genetic variation become more complicated. For coastal rivers, the level of variation among populations is lower than that among regions, and is sometimes not statistically significant. For example, Reisenbichler and Phelps (1989) did not find significant genetic variation among populations from the north coast of Washington; and Beacham et al. (2000) and Heath et al. (2001) found that differences between the Nass River and Skeena River watersheds (British Columbia) were less than among tributaries within those watersheds. Among tributaries within large rivers, some populations appear genetically distinct whereas others do not (Beacham et al. 2000; Heath et al. 2001). At very small spatial scales (within tributaries), several studies have documented weak but significant genetic differentiation for steelhead that migrate at different times (e.g., summer versus winter run, Leider et al. 1984; Nielsen and Fountain 1999; but see Chilcote et al. 1980).

The general picture of population structure in steelhead is thus roughly similar to that in other anadromous Pacific salmonids. There are typically large differences among regions (perhaps related to postglacial colonization from separate refuges; Nielsen 1999; McCusker et al. 2000). Populations from major river systems within regions typically differ from each other, but to a lesser extent, and the degree of differentiation continues to decrease as one compares tributaries within river systems and seasonal races within tributaries. However, unlike other salmonid species that almost invariably show significant genetic differentiation among tributaries within river systems, in steelhead the degree of differentiation is highly dependent on the specific location. This ambiguity and the desirability of making management decisions at the level of distinct populations indicate a need for additional studies of steelhead population structure, particularly within smaller river systems. Unfortunately, such studies are extremely rare and are entirely lacking for most systems, even those with tributaries that could easily harbor separate local populations.

Our study tests for evidence of distinct populations within the Dean River, British Columbia, a small river system supporting a premier sport fishery but lacking any information on population structure. An investigation of population structure would ideally include multiple years of samples from discrete breeding aggregations, as well as sampling of fish throughout the migratory period. This sampling scheme allows identification of distinct breeding populations and their contribution to mixed groups that migrate at different times and locations (e.g., Beacham et al. 1999, 2000). In many cases, however, such sampling may be logistically difficult, particularly when breeding locations are unknown or difficult to access. Under these circumstances, it may be profitable to first collect temporally spaced samples through the migratory period. Examination of such samples may provide preliminary indications that distinct populations are present and hint at the degree to which these populations are separated during migration and exploitation. If evidence for multiple populations is found, more intensive research is justified, and managers may want to implement provisional strategies related to migratory timing. Our study of Dean River steelhead shows the value of this approach in providing preliminary information about population structure.
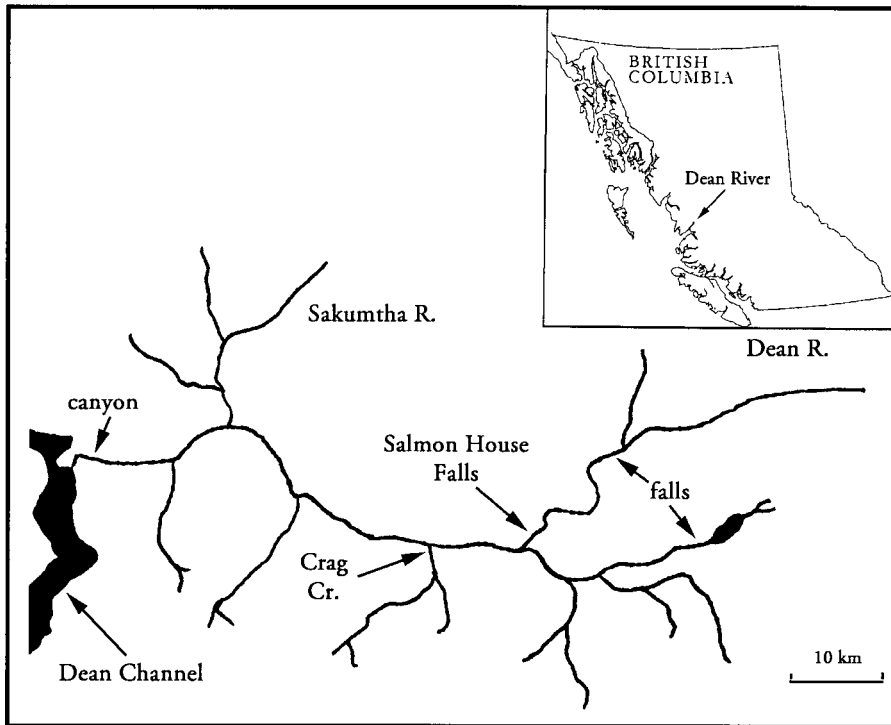
FIGURE 1.—Map of the Dean River study system, with British Columbia in the inset. The highest set of falls shown on the main stem of the Dean River marks the upstream limit of steelhead migration.

The Dean River

The Dean River originates near an elevation of 1,200 m and flows generally westward for approximately 225 km. The river winds through coastal mountains and over several waterfalls, reaching the ocean near the end of Dean Channel, in the central coast of British Columbia (Figure 1). Distinct regions of the Dean River are separated by a range of coastal mountains and are differentially influenced by glacial melt and late-summer runoff from snowmelt. The upper reaches receive little glacial runoff, are relatively stable during the summer months, and have summer water temperatures that can exceed 17.5°C. As the river flows westward, it is joined by several glacial tributaries. One such river, the Sakumtha, is characterized by highly variable flows and turbidity, and summer temperatures typically about 5°C cooler than the adjacent Dean River main stem. Another tributary, the Takia, receives glacial and nonglacial runoff and contains a system of small lakes. All of these areas appear to be used by adult and juvenile steelhead.

The Dean River watershed has several physical barriers that potentially isolate distinct populations and impose restrictions on migration timing. At least two waterfalls, including Salmon House Falls (Figure 1), can be negotiated by steelhead during high water associated with peak snow runoff in the early summer but are impassable during the typically low flows of late summer and fall. Between these times, discharge varies by a factor of four (84–339 m³/s; Hemus 1973). Other waterfalls, however, are not ascended by steelhead under any conditions, and limit spawning to the lower 75 km of the Dean River and its tributaries.

The Dean River supports a catch-and-release steelhead fishery from June through September, with overall catches typically ranging between 3,000 and 5,000 fish (Peard and Leggett 2001). Current regulations restrict fishing to the waters below Crag Creek (approximately 40 km from the river mouth; Figure 1), but the relative inaccessibility of the upper reaches further limits most fishing to the lowest 30 km. All fishing occurs during migration or during the holding period before spawning (none takes place during spawning).

The diverse physical characteristics of the Dean River watershed, the seasonal nature of some barriers to migration, and the variable timing of ar-

rival by steelhead (June through September), all suggest the possibility for genetically distinct and locally adapted populations within the watershed. This possibility, combined with concentrated fishing effort in the lower river, makes the Dean River system excellent for investigating steelhead population structure. We sampled steelhead caught in the sport fishery and examined genetic, phenotypic, and life history data for evidence of variation through the spawning migration.

Methods

Steelhead were captured by anglers using single barbless hooks. Angler effort was concentrated between the lower Dean River canyon (4 km upstream from the ocean) and the mouth of the Sakumtha River (18 km from the ocean; Figure 1), with 80% of the 591 total captures (291 males, 300 females) taking place in this area. The remaining fish were caught between the Sakumtha River and a location approximately 5 km further upstream (12%) and between Crag Creek and a location approximately 5 km downstream (8%).

For each captured fish, we used measuring tapes to determine body (fork) length and body girth (nearest cm), tweezers to remove 3–5 scales, and scissors to clip a tissue sample of approximately 0.5 cm^2 from the lower tip of the caudal fin. Tissue samples were preserved in 95% ethanol, and scales were stored on paper cards. The lower tip of the caudal fin of each captured fish was examined for evidence of a clip mark, which was used to identify individuals that had been captured and sampled previously. When a fish was captured more than once, only data from the first capture were used in our analyses of life history and genetic variation. Measuring and sampling were performed by professional fishing guides; their efforts were coordinated and standardized by M. Hendry, who was present on the river each day of the fishing season.

All scales were scanned visually for quality. For each fish, one or two of the scales most suitable for aging were mounted on gummed cards and impressed in transparent diethyl acetate (0.5-mm thick, cut into 7.6- × 12.7-cm cards, capacity of up to 30 fish per card) with a heated hydraulic press (100°C at a pressure of 34,475 kPa for 3 min; Koo 1955, 1962). Acetate scale impressions were viewed with a microfiche reader (100×) for age determination. Ages were estimated by counting the number of freshwater and saltwater annuli (bands of closely spaced, narrow, broken, interbraided, or resorbed circuli that form once a year),

and any spawning checks. Spawning checks on steelhead scales are formed by resorption of saltwater circuli when a fish returns to spawn in freshwater (Bali 1959). Freshwater ages were not estimated for scales that were even slightly regenerated (formed later in life to replace lost scales), because the first freshwater annulus in steelhead may be located very close to the focus of the scale. Owing to this conservative approach, freshwater age data were obtained for only a subset of the fish with saltwater ages. In our discussion, the freshwater or saltwater age of each fish is the number of winters that it spent in each environment. Thus, a fish that spent two winters in freshwater and two winters in saltwater would be freshwater age 2 and saltwater age 2. A fish that lived through a total of five winters, in freshwater or saltwater, would have a total age of 5 years.

The samples were divided into three groups based on date of capture: early (July 2–30; $N = 110$), middle (July 31 to September 5; $N = 394$), and late (September 6–30; $N = 87$). Dates separating the three groups were chosen to partition the migratory season roughly into thirds, with at least 70 tissue-sampled individuals in each group. The early, middle, and late groups were then compared for differences in sex ratio, age composition, body length, and body girth. Chi-square tests were used to compare differences in sex ratio and to compare differences in age composition in terms of total age at first maturity, freshwater age, and saltwater age, with separate tests for each sex. Body length was compared among the three groups by simple linear regression of length on sample date for all ages pooled and for saltwater age-2 fish only, with separate regressions for each sex. Analysis of covariance (ANCOVA) was used for each sex to standardize girth to a common body length. All statistical analyses were conducted with Statistical Package for the Social Sciences (SPSS, version 7.5).

Our analysis assumes that the date of capture for each fish is correlated with the date it entered the river. We tested this assumption with mark–recapture data (678 steelhead tagged, 108 recaptured) and angler-effort data from 1973 (June 1 to September 15), collected from the same general area as our own samples (Hemus 1973). If fish entering the river distribute themselves randomly through the accessible portions of the watershed, a consistent proportion of the fish entering each week should remain within the sampling area. Under this scenario, an individual captured on a given date should have a probability of being recaptured
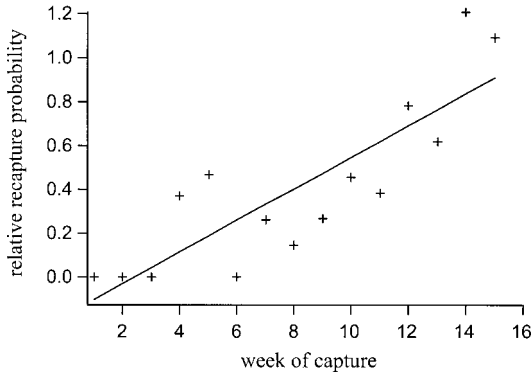
FIGURE 2.—Recapture probability index (actual recapture probability/expected recapture probability; plus signs) for fish tagged in a given week between June 1 (the start of week 1) and September 15 (the end of week 15). Expected recapture probability for fish tagged in week 1 is assigned a value of 1.0. Expected recapture probability for fish tagged in subsequent weeks is scaled by the fraction of angler-days that remain in the season. Actual recapture probability for a fish is the fraction of fish from those tagged in a given week that were later recaptured. If a constant proportion of the fish that enter the river in a given week remains in the sampling area, the ratio of actual recapture probability to theoretical recapture probability would be constant. The observed increase in this ratio indicates that fish entering the river early are likely to move upstream out of the sampling area and those entering late are likely to remain in the sampling area. Since sampling takes place in the section of the river closest to the ocean, this indicates that, in general, date of initial capture is well correlated with the date of river entry.

that is directly proportional to the number of subsequent angler-days in the season. We devised a recapture index that should remain relatively constant through the migratory season for the case of a random distribution: the proportion of fish tagged in a given week that were later recovered divided by the proportion of angler-days after tagging. Our analysis of the tagging data shows that this index does not remain constant: fish tagged late are actually much more likely to be recaptured than fish tagged early (Figure 2). In fact, none of the fish tagged during the first 3 weeks of the migratory season were ever recaptured. This pattern indicates that most of the fish caught in the first half of the season move rapidly through the sampling area. Fish caught later in the season are more likely to have entered the river later and are more likely to remain in the sampling area. Given this result, and evidence that run timing is consistent and heritable (see Discussion), our sampling of fish caught by

anglers in the lower river should yield dates of capture that are correlated with dates of river entry.

We focused our genetic analysis on the early and late groups because these seemed to possess the greatest a priori potential for differentiation. All individuals in the early and late groups from which tissue samples were taken ($N = 71$ early and 74 late) were genotyped using two multiplexed sets of fluorescently labeled microsatellite primer pairs (set A: *Oneμ11, Omy77, Ssa85, Sfo8, Oneμ14,* and *Ots1*; set B: *Oneμ2, Omy325, Ssa14,* and *Oneμ8*). Primer pair sequences, primer concentrations, polymerase chain reaction (PCR) conditions, electrophoresis details, and methods for scoring amplification products followed those described by Wenburg et al. (1996; see also Olsen et al. 1996). Briefly, PCR reactions were carried out in a Perkin-Elmer 9600 thermocycler with a profile consisting of one cycle at 94°C (180 s), 10 cycles at 94°C (60 s) + X°C (30 s) + 72°C (15 s), 14 cycles of 94°C (30 s) + X°C (30 s) + 72°C (15 s), and 1 cycle at 94°C (30 s) + X°C (30 s) + 72°C (300 s), where X represents an annealing temperature of 56°C for set A and 52°C for set B. Reactions were carried out in 10-μL volumes containing 10 mM TrisHCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.8 mM deoxynucleotide triphosphates (dNTPs; 0.2 mM each), 0.4 units *Taq* DNA polymerase, 1 μL crude quick-lysis DNA extract (as described in Olsen et al. 1996), and primer concentrations as given in Table 2 of Wenburg et al. (1996). Polymerase chain reaction products were electrophoresed on a 6% denaturing polyacrylamide gel with a Perkin-Elmer Applied Biosystems, Inc. 373A automated sequencer and scored individually as described by Olsen et al. (1996) and Wenburg et al. (1996).

The program GENEPOP (versions 3.2a and 3.3; see Raymond and Rousset 1995) was used to (1) calculate observed and expected heterozygosity at each locus in each sample group (early and late), (2) test for deviations from Hardy–Weinberg equilibrium at each locus in each sample group, (3) test for linkage disequilibrium between all possible pairs of loci in each sample group, and (4) test for significant genetic differentiation between the early and late groups (genotypic differentiation). The program FSTAT (Goudet 1995, 2000) was used to calculate F_{ST} (proportion of the total genetic variation attributable to differences among sample groups) between the early and late sample groups at each locus and over all loci combined. FSTAT was also used to calculate bootstrapped 95% confidence intervals for overall F_{ST}. The program

TABLE 1.—Distribution of age at first maturity for Dean River steelhead captured in the early (July 2–30), middle (July 31–September 5), and late (September 6–30) periods of the migration. Percentages for each age-group within each row are given in parentheses.

		Age at first maturity (years)				
	N	3	4	5	6	7
Females						
Early	25	0	8 (32.0)	14 (56.0)	3 (12.0)	0
Middle	62	0	16 (25.8)	38 (61.3)	7 (11.3)	1 (1.6)
Late	18	1 (5.6)	9 (50.0)	7 (38.9)	1 (5.6)	0
Total	105	1 (1.0)	33 (31.4)	59 (56.2)	11 (10.5)	1 (1.0)
Males						
Early	22	0	12 (54.5)	8 (36.4)	2 (9.1)	0
Middle	64	1 (1.6)	20 (31.3)	29 (45.3)	14 (21.9)	0
Late	10	0	2 (20.0)	6 (60.0)	2 (20.0)	0
Total	96	1 (1.0)	34 (35.4)	43 (44.8)	18 (18.8)	0
Both sexes	201	2 (1.0)	67 (33.3)	102 (50.7)	29 (14.4)	1 (0.5)

Tools for Population Genetic Analysis (TFPGA; Miller 1997) was used to calculate Nei's (1978) unbiased genetic distance (D) and the Reynolds et al. (1983) coancestry coefficient.

Assignment tests (see review in Hansen et al. 2001) in WHICHRUN (Banks and Eichert 2000) determined how well each individual fish could be classified back to its sample group based on a background of allele frequencies in the two groups. This was done with a jackknife procedure, in which each fish being tested was removed when determining the baseline frequencies for each group. In order to examine interannual variation in allele frequencies, GENEPOP was also used to test for genotypic differentiation and F_{ST} (over all loci) between 4- and 5-year-old fish. For multiple tests, we report results that remain significant after sequential Bonferroni corrections, so as to maintain consistency with previous studies. We note, however, that inferences are best drawn from combined statistical tests (e.g., genotypic differentiation for all loci combined) because the Bonferroni procedure is excessively conservative for the number of comparisons used here (e.g., if genotypic differentiation was $P = 0.006$ at each of the ten loci, the Bonferroni procedure would say that none of the loci was significant).

Results

Of the 591 steelhead we captured between July 2 and September 30, 1996, 55 (9.3%) had been captured and sampled previously. Of fish tagged in the early group (July 2–30; $N = 110$), none of the females and two males (3.6% of males) were later recaptured. Of the fish tagged in the middle group (July 31–September 5; $N = 394$), 21 females (10.6% of females) and 15 males (7.7% of males) were later recaptured. Of the fish tagged in the late group (September 6–30; $N = 87$), 3 females (7.9% of females) and 14 males (28.6% of males) were later recaptured. It thus appears that fish in the lower river early in the migratory season are rapidly replaced by new arrivals and that later fish have a greater tendency to remain in the area of their original capture. Our results and those of Hemus (1973) suggest that early fish rapidly migrate upriver and that date of capture in the lower river is correlated with the date of river entry.

Life History

Excluding recaptures, we analyzed a total of 267 females and 269 males. Sex ratios did not differ between the early (55 females, 53 males), middle (177 females, 181 males), and late (35 females, 35 males) sample groups ($P = 0.987$). The most common ages at first maturity were 4 years (33.3% of all fish), 5 years (50.7%) and 6 years (14.4%; Table 1), and the proportion of individuals in each age-class did not differ among the sample groups for females ($P = 0.349$) or males ($P = 0.239$). The only freshwater ages were 2 (27.4%), 3 (64.7%), and 4 (8.0%; Table 2) years, and the proportion of individuals in each age-class did not differ among the sample groups for females ($P = 0.096$) or males ($P = 0.146$). The near significance of female freshwater age composition could be attributed to more freshwater age-2 females in the late group (55.6%) than in the early (32.0%) or middle (22.6%) groups. The most common saltwater ages were 1 (10.0%), 2 (70.6%), and 3 (18.9%; Table 3) years, and the proportion of individuals in each age-class did not differ among the sample groups for females ($P = 0.249$) or males ($P = 0.628$). Of the fish for which we obtained saltwater ages ($N = 441$), six females (2.9% of females) and six males (3.0% of males) had spawned at least once

TABLE 2.—Freshwater ages (number of winters in freshwater) for Dean River steelhead captured in the early (July 2–30), middle (July 31–September 5), and late (September 6–30) periods of the migration. Percentages for each age group within each row are given in parentheses.

| | N | Years in freshwater | | |
		2	3	4
Females				
Early	25	8 (32.0)	15 (60.0)	2 (8.0)
Middle	62	14 (22.6)	43 (69.4)	5 (8.1)
Late	18	10 (55.6)	8 (44.4)	0
Total	105	32 (30.5)	66 (62.9)	7 (6.7)
Males				
Early	22	7 (31.8)	13 (59.1)	2 (9.1)
Middle	64	14 (21.9)	46 (71.9)	4 (6.3)
Late	10	2 (20.0)	5 (50.0)	3 (30.0)
Total	96	23 (24.0)	64 (66.7)	9 (9.4)
Both sexes	201	55 (27.4)	130 (64.7)	16 (8.0)

TABLE 3.—Saltwater ages (number of winters in the ocean) for Dean River steelhead captured in the early (July 2–30), middle (July 31–September 5), and late (September 6–30) periods of the migration. Percentages for each age-group within each row are given in parentheses.

| | N | Years in saltwater | | | |
		1	2	3	4
Females					
Early	44	1 (2.3)	40 (90.9)	3 (6.8)	0
Middle	133	6 (4.5)	111 (83.5)	16 (12.0)	0
Late	31	3 (9.7)	22 (71.0)	6 (19.4)	0
Total	208	10 (4.8)	173 (83.2)	25 (12.0)	0
Males					
Early	44	6 (13.6)	27 (61.4)	10 (22.7)	1 (2.3)
Middle	124	22 (17.7)	71 (57.3)	31 (25.0)	0
Late	32	3 (9.4)	17 (53.1)	11 (34.4)	1 (3.1)
Total	200	31 (15.5)	115 (57.5)	52 (26.0)	2 (1.0)
Both Sexes	408	41 (10.0)	288 (70.6)	77 (18.9)	2 (0.5)

previously, including two males that had spawned twice previously.

For fish of all ages combined, regression analyses showed that body length increased through the season for females (0.045 cm/d, $r^2 = 0.022$, $P = 0.016$) and males (0.075 cm/d, $r^2 = 0.017$, $P = 0.031$). Grouping fish into early, middle, and late samples (as for age composition) revealed a similar trend (Table 4). When regression analyses were restricted to the most common saltwater age (2 years), body length still increased through the migratory season for females (0.031 cm/d, $r^2 = 0.024$, $P = 0.040$) and males (0.050 cm/d, $r^2 = 0.048$, $P = 0.019$).

Body girth increased with body length ($P < 0.001$ for both males and females) but did not differ among the sample groups ($P = 0.472$ for females; $P = 0.584$ for males), and the interaction between body length and sample group was not significant ($P = 0.385$ for females; $P = 0.489$ for males). Removal of the interaction term from the ANCOVA confirmed that body girth increased with body length (slope = 0.479, $P < 0.001$ for females; slope = 0.535, $P < 0.001$ for males) and showed that body girth standardized to a common body length did not differ appreciably among the sample groups ($P = 0.064$ for females; $P = 0.289$ for males). The near significance for females was due to the fact that early females had slightly smaller girths (37.2 cm) than did middle (38.7 cm) or late females (38.2 cm) after standardizing each group to the mean body length of 75.3 cm (i.e., adjusted means from ANCOVA).

Genetic Variation

In the early sample group, all ten loci were in Hardy–Weinberg equilibrium (HWE), even before Bonferroni corrections for multiple comparisons (Table 5). In the late group, five of the ten loci were out of HWE, and *Ots1* and *One*μ*2* remained out of HWE after Bonferroni corrections (adjusted α = 1 − [1 − 0.05]^{0.1} = 0.0051; Table 5). For all loci combined, the early sample group conformed to HWE ($P = 0.842$) but the late group did not ($P < 0.001$). For each locus, and overall, the departures from HWE appeared to be caused by a heterozygote deficiency (Table 5).

Linkage disequilibrium in the early sample group was significant in three of the 45 pairwise comparisons of loci: *Omy77* versus *Omy325* ($P = 0.002$), *One*μ*14* versus *Ssa14* ($P = 0.023$), and *One*μ*8* versus *Ssa14* ($P = 0.043$). Linkage disequilibrium in the late sample group was also significant in three of 45 pairwise comparisons: *One*μ*14* versus *Omy325* ($P = 0.013$), *Sfo8* versus *Ssa14* ($P = 0.003$), and *Omy325* versus *Ssa14* ($P = 0.028$). None of the significant pairs of loci were the same in the early and late sample groups, none of the pairs remained significant after correction

TABLE 4.—Variation in fork length (cm) of male and female Dean River steelhead captured in the early (July 2–30), middle (July 31–September 5), and late (September 6–30) periods of the migration.

	Early	Middle	Late	Total
Females				
Mean	74.3	75.3	76.5	75.3
SD	4.6	5.6	7.2	5.7
N	55	177	35	267
Males				
Mean	76.5	78.1	80.6	78.1
SD	9.5	10.6	9.4	10.2
N	54	180	35	269

TABLE 5.—Summary of microsatellite variation in Dean River steelhead captured in the early (July 2–30) and late (September 6–30) periods of the migration. The total number of alleles and the allele size range (number of base pairs) observed at each locus for both groups combined is shown. For individual groups, sample size (N), observed heterozygosity (H_o), expected heterozygosity (H_e), and P-values of tests for deviations from Hardy–Weinberg equilibrium are shown. Tests that remain significant after sequential Bonferroni corrections are indicated with an asterisk.

Locus	Alleles	Size	Early				Late			
			N	H_o	H_e	P	N	H_o	H_e	P
Omy77	8	106–138	71	0.77	0.77	0.919	74	0.77	0.77	0.711
Oneμ11	3	144–148	60	0.45	0.43	0.607	54	0.56	0.59	0.473
Oneμ14	8	147–163	69	0.46	0.51	0.487	74	0.50	0.58	0.039
Ots1	10	163–251	71	0.55	0.59	0.275	69	0.43	0.55	0.001*
Sfo8	19	219–307	69	0.81	0.88	0.324	72	0.81	0.87	0.210
Ssa85	11	104–136	71	0.85	0.79	0.567	73	0.73	0.79	0.726
Omy325	17	99–155	71	0.73	0.72	0.316	69	0.70	0.78	0.104
Oneμ2	33	226–328	67	0.90	0.93	0.869	69	0.86	0.91	0.003*
Oneμ8	9	156–180	70	0.50	0.52	0.516	67	0.40	0.50	0.017
Ssa14	9	129–153	60	0.47	0.53	0.522	69	0.54	0.65	0.036

for multiple comparisons (original $\alpha = 1 - [1 - 0.05]^{0.02} = 0.001$), and approximately three of 45 tests would be expected to be significant ($P < 0.05$) by chance alone. We conclude that all 10 loci were assorting independently.

The early and late sample groups were generally similar in allele frequencies (Figure 3), but nevertheless showed evidence of significant genetic differentiation. Values of F_{ST} for individual loci ranged from less than zero for five loci, to 0.032 for *Sfo8* and 0.034 for *Oneμ11* (Table 6). For all loci combined, F_{ST} was 0.007 (95% confidence interval [CI] bootstrapped across loci: −0.001 to 0.017). Tests for genotypic differentiation between the early and late sample groups were significant at four of 10 loci (Table 6), one after Bonferroni corrections (*Sfo8*). For all loci combined, genotypic differentiation was highly significant ($P < 0.001$), even after removing *Sfo8* ($P = 0.001$). Nei's (1978) unbiased genetic distance between the early and late sample groups was 0.0149, and the Reynolds et al. (1983) coancestry coefficient was 0.007. Assignment tests in WHICHRUN (Banks and Eichert 2000) showed that individuals could be classified back to their group of origin with a reasonable degree of precision: 84.2% success (60 of 71 in the early group, 62 of 74 in the late group; Figure 4).

Our data were produced by sampling within a single year, but included fish from several different age-groups (Table 1). By considering genotypes of 4- and 5-year-old fish to be representative of allele frequencies of adults in successive years, we examined our data for evidence of significant differences in allele frequencies between years. There were a total of 50 (early and late groups combined) age 4 ($N = 21$) and age 5 ($N = 29$) fish for which

we also obtained genotypic data. We found no evidence of significant genotypic differentiation at any of the loci we examined or over all loci combined ($P > 0.05$ for each test). Similarly, the overall F_{ST} value between the age-classes was −0.0042. We conclude that allele frequencies were relatively stable between the 2 years.

Discussion

Our Phenotypic Data in the Context of Other Studies

Direct quantitative comparisons between our results and earlier work in the Dean River (George and Leggett 1982), as well as other steelhead populations (e.g., Withler 1966), are difficult to make for two reasons. First, many of the steelhead in previous studies were killed by anglers, who have a tendency to release females and smaller fish (George and Leggett 1982). These biases might influence estimates of sex ratio, body size, and ocean age composition. Second, the previous studies did not consider within-season variation, or if they did (e.g., Withler 1966), no indication is given as to whether the temporal samples reflect actual variation in migration timing. We therefore limit ourselves to a few qualitative comparisons among studies.

Saltwater and freshwater ages in 1996 were similar to those in previous Dean River studies (George and Leggett 1982): most fish were freshwater age 3 (1973–1982, 70.8% to 89.5%; 1996, 66.4%; Table 2) and saltwater age 2 (1973 and 1981; 48.8% to 94.8%; 1996, 70.6%; Table 3). Thus, Dean River steelhead do not appear to have undergone a dramatic shift in age composition, but subtle shifts may have taken place, such as a pos-
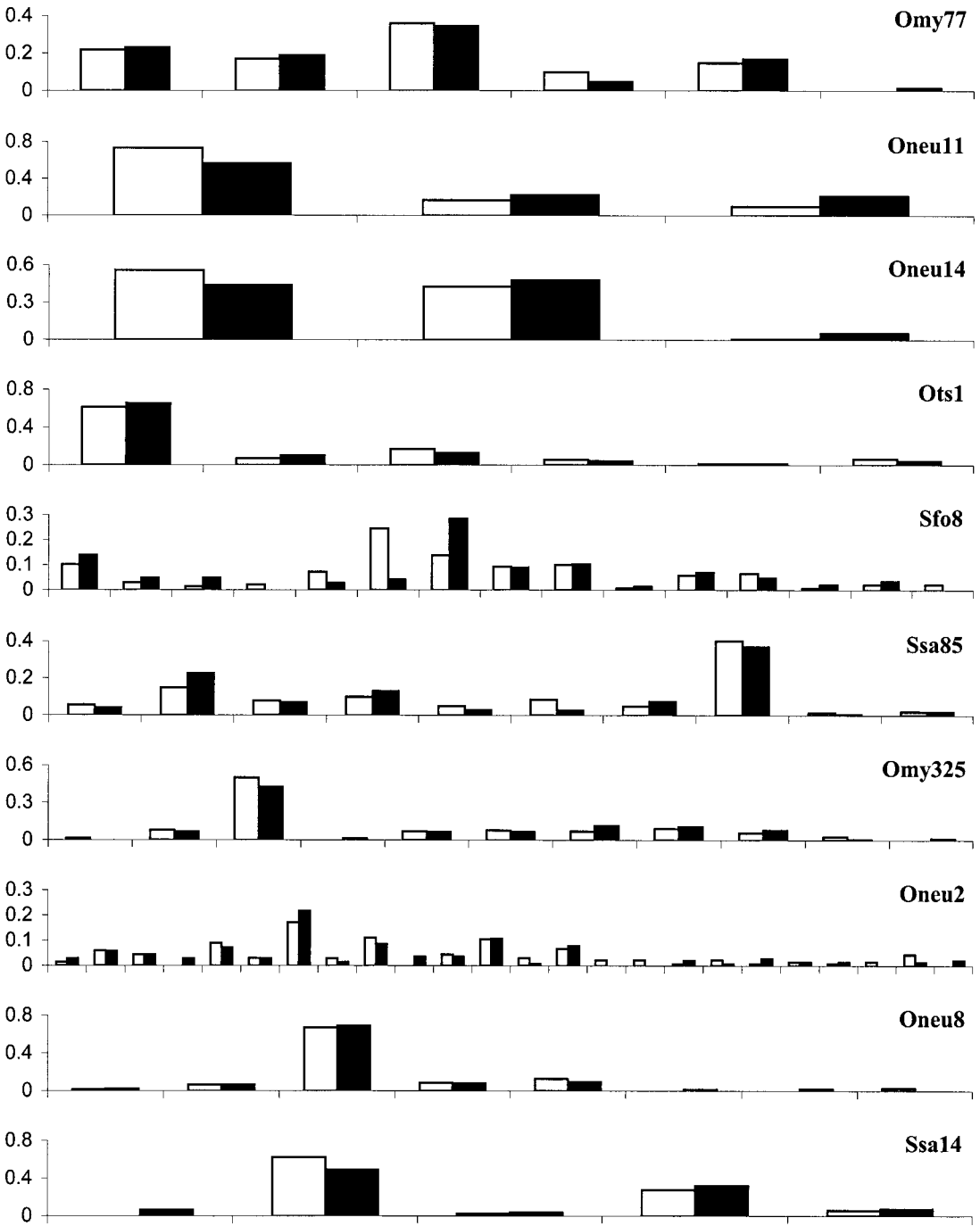
FIGURE 3.—Allelic variation in steelhead in the early (white bars) and late (black bars) sample groups. Different alleles are shown on the *x*-axis and the proportional representation in the sample is shown on the *y*-axis. To simplify presentation, only alleles present at a frequency of at least 0.01 in the early or late group are shown.

Locus	Genotypic differentiation (P-value)	F_{ST}
Omy77	0.378	−0.004
Oneμ11	0.022	0.034
Oneμ14	0.027	0.010
Ots1	0.822	−0.004
Sfo8	<0.001*	0.032
Ssa85	0.433	0.001
Omy325	0.407	−0.001
Oneμ2	0.069	−0.002
Oneμ8	0.176	−0.005
Ssa14	0.016	0.011
All loci combined	<0.001	0.007

FIGURE 4.—Freshwater age of Dean River steelhead in comparison to high seas steelhead samples.

sible reduction in freshwater age. We can roughly compare Dean River age composition to summer steelhead populations from southwestern British Columbia (including Vancouver Island) and northwestern British Columbia (Skeena River system). Steelhead life histories in each of these areas shows considerable variation but, in general, Skeena River steelhead have older freshwater ages, younger saltwater ages, and older total ages than southwestern British Columbia steelhead (Table 7). The Dean River steelhead are intermediate between the two regions in freshwater age, similar to Skeena River populations in saltwater age, and similar to southwestern British Columbia populations in total age (Table 7). These comparisons are tentative, however, because no data are available to test for shifts in age composition in these populations. Nevertheless, the trend toward increasing freshwater age with latitude is robust because it occurs in other Pacific salmon species (Groot and Margolis 1991). The trend probably arises because shorter summers and colder water necessitate a longer period of growth before fish can successfully smolt and migrate to the ocean.

The high-seas mixing of steelhead populations from a broad geographic range provides a means of estimating average freshwater ages at the species level. Of 3,280 steelhead captured in high seas samples between 1955 and 1985 (Burgner et al. 1992), 17.7% spent 1 year in freshwater, 26.7% spent 2 years, 42.0% spent 3 years, 12.7% spent 4 years, and 0.9% spent 5 years. Dean River steelhead differ from this species average in having
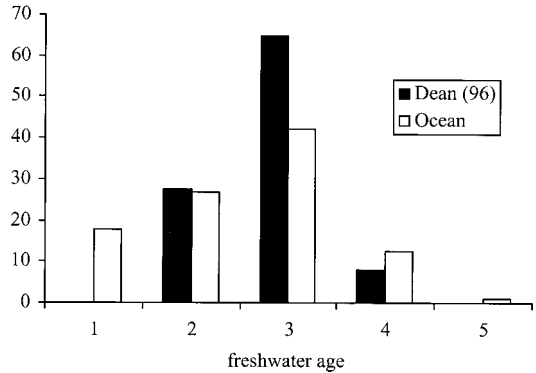
fewer freshwater age-1 fish and more freshwater age-3 fish (Figure 5). The higher percentage of freshwater age-1 fish in the ocean sample is probably attributable to the presence of hatchery-produced steelhead (Bernard and Myers 1996; Burgner et al. 1992), which are absent from the Dean River. On average, Dean River steelhead are older in freshwater age than steelhead captured in the high-seas samples (3.0 versus 2.5 years).

Mean lengths of Dean River steelhead in 1996 (males: 78.3 cm; females: 75.4 cm) were roughly similar to mean lengths recorded in previous years (1973–1981; males: 73.5–85.5 cm, average = 77.5 cm; females: 70.3–76.8 cm, average = 73.9 cm; George and Leggett 1982), suggesting the lack of a major shift in body size. In general, Dean River steelhead tend to be larger than steelhead from southwestern British Columbia and smaller than steelhead from the Skeena River (Table 7). We caution that this comparison is limited and that some populations probably represent exceptions. Other anadromous salmon species fail to show consistent increases in body size with latitude—chinook salmon O. tshawytscha (Roni and Quinn 1995) and sockeye salmon O. nerka (McGurk 2000); so the observed trend for steelhead may depend more on specific local conditions than on latitude.

The proportion of repeat spawners in the Dean River seems to have changed. Between 1973 and 1976, repeat spawners averaged 13.8% of the run, and between 1977 and 1982 they averaged 8.3% of the run (George and Leggett 1982; Evans 1983). In 1996, only 2.9% of males and 3.0% of females were repeat spawners. This percentage is the lowest in 11 years of Dean River data and is lower than in other British Columbia populations for which we obtained data (although percentages for

TABLE 7.—Summary of life history characteristics in Dean River steelhead, with comparisons to other populations of summer steelhead. Data include the percentage of spawners that spawned at least once previously (repeat spawn), the fork length of males (male FL) and females (female FL), and the average freshwater age (FW age), saltwater age (SW age), and total age (total age) of first time spawners.

River	Repeat spawn (%)	Male FL (cm)	Female FL (cm)	FW age (years)	SW age (years)	Total age (years)
Dean (1996)[a]	3.0	78.3	75.4	2.8	2.1	4.8
Dean (1973–1981)[b]	10.5	77.5	73.9	3.0	2.1	5.1
Southwestern British Columbia						
Capilano[c]	6.1	74.2	71.1	2.6	2.3	4.9
Coquihalla[c]	6.3	69.3	66.8	2.8	2.3	5.0
Seymour[c]	4.4	77.0	69.9	2.7	2.3	5.0
Vancouver Island[d]	7.0	64.1	64.3	2.2	2.7	4.9
Skeena River						
Babine[e]	3.6			3.2	2.1	5.3
Kispiox[f]	17.9	89.0	77.9	3.6	2.4	6.0
Morice[f]	6.6			3.8	1.4	5.2
Sustut[g]	5.5	84.5	76.1	3.8	2.2	6.0

[a] Present study.
[b] George and Leggett (1982).
[c] Withler (1966).
[d] Hooton et al. (1987).
[e] Whately and Chudyk (1979).
[f] Various unpublished agency reports summarized in Combs (1991).
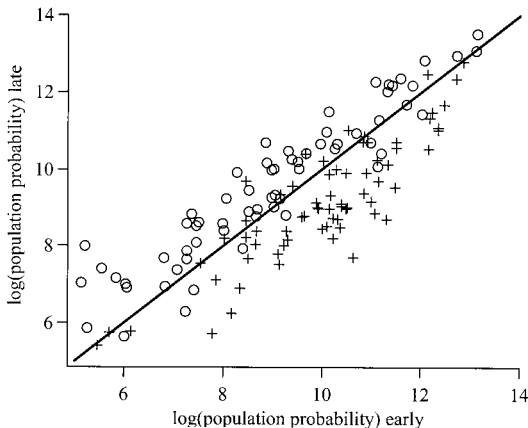[g] Spence et al. (1990) and Saimoto (1994).

FIGURE 5.—Population assignments of individuals using WHICHRUN (Banks and Eichert 2000). The horizontal and vertical axes represent a logged and scaled population probability (as defined in WHICHRUN). The values of the x and y coordinates for each individual represent the probabilities of that fish belonging to the early (x) and late (y) groups. Crosses represent fish from the early group and open circles represent fish from the late group. Fish that lie above or below the line of equal probability have been assigned to the late or early populations, respectively. Individuals that lie on the line have an equal probability of belonging to either population. Axes limits have been adjusted to allow greater resolution, and excluded 11 individuals as a result. Out of 145 of individuals, 122 (84%) were classified correctly to their group of origin (early or late).

Babine River steelhead are almost as low; Table 7). The dramatic decrease in repeat spawners in the Dean River from a fairly high level relative to other populations (Table 7) to the lowest observed level calls for explanation. The simplest explanation would be that we aged steelhead differently from previous studies. This explanation seems unlikely because (1) our result is consistent with a continuation of the decrease observed between the periods 1973–1976 and 1977–1982 and (2) our analysis yielded similar conclusions to previous Dean River data for freshwater, saltwater, and total ages (Table 7).

Several other explanations for the dramatic decline in postspawning survival deserve consideration. Changes in commercial interception of kelts (postspawning adults) may be partly responsible. Unlike immature steelhead that generally range well into the Pacific, kelts are most common in coastal and nearshore waters (Burgner et al. 1992) and may thus be more susceptible to the commercial Pacific salmon fishery, in which a substantial number of Dean River steelhead are bycatch (George and Leggett 1982). Local marine and freshwater conditions, or angler bias in the earlier years toward keeping larger (older) fish, may also contribute to this observation. Further work will be necessary to evaluate the generality of our result and to determine its cause. We en-

TABLE 8.—Summary of F_{ST} values for nuclear markers in steelhead. Spatial scale is the shortest water distance between the nearest and farthest populations in the study; BC = British Columbia, CA = California, WA = Washington, OR = Oregon.

Study	F_{ST} (range among loci)	Geography of sampling	Number of populations	Spatial scale	Genetic markers
Present study	0.007 (0–0.034)	Different times in a single migratory run (Dean River, BC)	2	~20–60 km	10 microsatellites
Nielsen and Fountain (1999)	0.01 (0.00–0.33)	Seasonal races in one river (Middle Fork Eel River, CA)	2	<100 km (?)	16 microsatellites
Reisenbichler and Phelps (1989)	0.015	Rivers into ocean with tributaries (WA)	27	~10–450 km	23 polymorphic allozymes
Reisenbichler et al. (1992)	0.015	Rivers into ocean with tributaries (CA, WA, OR)	19	~20–420 km	8 polymorphic allozymes
Heath et al. (2001)	0.039	Rivers into ocean with tributaries (BC)	10	~80–850 km	6 microsatellites
Beacham et al. (1999)	0.076 (0.063–0.143)	Rivers into ocean with tributaries (BC and WA)	22	~50–1,600 km	8 microsatellites
Beacham et al. (2000)	0.026 (0.008–0.039)	Tributaries to river system (Skeena River, BC)	7	~20–300 km	8 microsatellites
Beacham et al. (2000)	0.024 (0.011–0.033)	Tributaries to river system (Nass River, BC)	10	~20–150 km	8 microsatellites

courage such work because dramatic decreases in postspawning survival could have considerable impacts on population dynamics, effective population sizes (it reduces the overlap between generations), and the evolution of reproductive behavior and energy allocation. For example, decreases in the probability of surviving to a subsequent reproductive episode should favor increased reproductive effort during the current episode, which should then further decrease the probability of postbreeding survival (Stearns 1992; Jonsson et al. 1997).

Genetic Evidence for Distinct Populations

Our genetic results, as well as temporary physical barriers to migration (late fish would have difficulty surmounting waterfalls), suggest that the Dean River contains at least two, and perhaps more, discrete populations that migrate at different times. Samples of steelhead in the lower river from early (July 2–30) versus late (September 6–30) in the season showed highly significant genotypic differentiation (Table 6), and assignment tests correctly matched individuals to these sample groups 84.2% of the time. The observed differentiation was small relative to that commonly seen in other studies of anadromous salmonids, including steelhead (Table 8). However, studies documenting large genetic differences are usually conducted on much larger spatial scales than even the maximum possible separation of spawning sites in the Dean River (approximately 70 km). Studies conducted on a spatial scale similar to ours (i.e., within

moderate-sized watersheds or for nearby streams) typically show low levels of differentiation (e.g., steelhead: $F_{ST} = 0.01$ in Nielsen and Fountain 1999; chinook salmon: $F_{ST} = 0.006$ in Carl and Healey 1984; chum salmon *O. keta*: $F_{ST} = 0.016$ in Tallman and Healey 1994; sockeye salmon: $F_{ST} = 0.006$ in Woody et al. 2000, and $F_{ST} = 0.011$, average value within nine lake systems, in Varnavskaya et al. 1994). Genetic variation in our study is thus consistent with previous work in anadromous salmonids: nearby populations are weakly, but often significantly, differentiated at neutral genetic markers.

The observed level of genetic differentiation was likely an underestimate of the actual differentiation. Sampling took place along a single stretch of river and, because migratory timing of the populations may have overlapped, our two temporal sample groups could include fish from the same breeding population (e.g., Olsen et al. 2000). Our results further suggest that the late sample group may include individuals from at least two populations, as indicated by deviations from Hardy–Weinberg equilibrium (heterozygote deficits, Wahlund effect; Table 5). Heterozygote deficits in the late group (none were seen in the early group) may have been produced by a mixture of early and late fish or by a mixture of fish from more than one late-migrating population. Sampling fish from specific spawning sites would resolve this question and better define migratory timing of the different populations.

Phenotypic Variation through the Season

Variation in life history and morphology can arise owing to spatial and temporal variation in selection, and may thus vary among fish that migrate or spawn at different times (e.g., Hendry et al. 1999; Woody et al. 2000). We did not observe any temporal variation in sex ratio or age composition in our samples. We also failed to find strong evidence of temporal variation in relative girth (girth standardized to a common body length), although the trend was nearly significant for females (early females tended to have smaller girths than middle or late females). Differences in the shape of adult spawners among populations can reflect adaptations to different spawning environments (e.g., Blair et al. 1993; Hamon et al. 2000). In the Dean River, however, it is perhaps more likely that the observed variation in female girth was simply the result of a change in gonad maturity between early and middle or late fish. That is, early migrating fish were less mature and might therefore have smaller girths (e.g., Hamon and Foote 2000).

Body length increased through the migratory season for both females and males. Body length often varies among different salmonid populations within a river or lake system (e.g., Blair et al. 1993), and can even vary through the season within individual populations (although the trend is usually for a decrease in length with time; Hendry et al. 1999). Variation in body length may be adaptive, or simply the result of different environmental conditions experienced by individuals migrating or spawning at different times or places (Swain and Foote 1999). The increase in body length through the season in Dean River steelhead (0.031 cm/d for females and 0.050 cm/d for males; ocean age-2 fish only) is roughly comparable to steelhead summer growth rates in the ocean (0.049 cm/d, calculated from Burgner et al. 1992). Thus, the increase in length through the season in the Dean River could potentially be explained by the greater opportunity for growth in fish that leave the ocean later in the season.

Interpreting Temporal Variation

Numerous studies have documented genetic and phenotypic variation through the season in samples of migrating or spawning anadromous salmonids (e.g., McGregor et al. 1998; Smoker et al. 1998; Brykov et al. 1999; Hendry et al. 1999; Woody et al. 2000). Indeed, the timing of migration, maturation, and spawning all seem highly

heritable (see citations in Hendry et al. 1999). For example, the heritability of return date of offspring from pink salmon spawned on the same date was 0.40 ± 0.20 for females (Smoker et al. 1998). In chinook salmon, the heritability of return timing within two populations was 1.08 ± 0.28 (Quinn et al. 2000). Temporal genetic or phenotypic variation during the migratory period could result from continuous variation within a single population, as is the case for fish on the spawning grounds (Hendry et al. 1999). In this case, variation in neutral genetic markers could result either from genetic drift, coupled with decreasing gene flow between fish that breed at increasingly different times (''isolation-by-time,'' Hendry et al. 1999) or from genetic linkage between neutral genetic markers and quantitative trait loci that influence spawning date (Fishback et al. 2000). Variation in phenotypic traits might then result from temporal clines in selective pressures (''adaptation-by-time,'' Hendry et al. 1999) or from phenotypic plasticity.

Temporal variation through the migratory period might also arise from differential migratory timing of distinct populations. In this case, samples of migrants might be composed of varying proportions of fish from separate populations. The present study provides more support for this scenario than for continuous variation within a single population. A genetic difference between early and late-migrating fish could arise in either situation, but the deficit of heterozygotes in only one group provides evidence that the early group was composed mainly of a single population and the later group included fish from at least two and possibly more populations. Some of the fish contributing to the late group could conceivably be from the same population as the fish captured in the early group. However, our analysis of tagging data suggests that very few early fish remain in the lower river late in the season. We thus have evidence for at least one population with an early migration and one or more populations with later migrations.

Our results provide evidence of genetic variation through the migratory period, but little evidence of noteworthy phenotypic variation. Moreover, the only observed variation in phenotypic traits (body length and girth) could *potentially* be explained by differential growth opportunity and maturity state. Variation arising through such means can be useful for identifying fish from different groups (e.g., Craig 1985) but it provides little evidence of genetically based, adaptive phenotypic variation. Although our results do not re-

fute adaptive differences (we measured only a few traits, and some differences that *might* be adaptive were present), they raise the interesting question of what might promote phenotypic similarity among populations that migrate at different times. One possibility is similar selective regimes. If the migratory or spawning conditions do not vary appreciably among populations, then adaptive differences are not expected. Another possibility is opposing environmental and genetic effects, or ''countergradient'' variation (sensu Conover and Schultz 1995). In the Dean River, for example, early migrating steelhead have less opportunity for growth in the ocean and might therefore be smaller through an environmental effect. However, selection could still favor large size, which would lead to the evolution of higher genetically based growth rates in the early group. If the two groups were reared in common conditions, the early group might actually grow more rapidly (see examples in Conover and Schultz 1995).

Implications

Understanding both genetic and phenotypic variation through a migratory period, as well as discriminating between two possible scenarios for the origin of the variation (continuous variation in a single population versus mixtures of populations migrating at different times), is critical for managing anadromous salmonids. In the case of a single continuous population, temporal genetic variation at selectively neutral markers suggests that fish migrating at different times also breed at different times and that fish breeding at separate times have reduced levels of gene flow. Under these circumstances, differential fishing pressure through the season might alter the timing of migration and spawning. For example, increased fishing pressure early in the season will reduce the fitness of early migrants and spawners and favor an evolutionary shift toward later migration. A shift in timing will likely reduce the productivity of the population as a whole because natural migration and spawning periods are presumably matched to ''optimal'' time windows. Moreover, variation in other traits through the season may be adaptive, reflecting temporal clines in selective pressures. A shift in timing will thus further depress fitness because other phenotypic traits will no longer be well-matched to migratory timing.

When discrete populations migrate during distinct time periods that overlap, management decisions can also have severe consequences for population persistence and productivity. In many larg-

er systems, such as the Fraser and Columbia rivers, biologists have long recognized that different populations migrate during different periods, and that those migration periods facilitate passage to natal spawning sites (e.g., populations facing longer migrations start their migration earlier in the year). The large size of these river systems, and their large numbers of commercially important species, increase the likelihood that populations will migrate during distinct periods and that research will be conducted to determine those periods. Management strategies that recognize differences in timing and spread exploitation among populations in relation to their abundance will then maximize persistence and productivity of the entire system.

In smaller river systems, or on smaller scales in large systems, different seasonal races (e.g., summer and winter steelhead, fall and winter chum salmon) are recognized, and can be managed separately because they show only minimal overlap in migratory timing. In these same systems, however, some discrete populations may migrate at overlapping times. Indeed, research on commercially important or endangered populations within small-to-moderate river systems has sometimes provided a means of discriminating between populations with overlapping timing (e.g., Craig 1985; Olsen et al. 2000). However, such studies are relatively rare and are absent for species of limited commercial importance. Our study of Dean River steelhead shows that a premier sport fishery is probably composed of multiple populations that vary in migratory timing. Many other wild steelhead rivers are similar to the Dean River in run size, river length, and the potential for isolation of spawning populations, but very little is yet known about their population structure and the run timing of separate populations.

A complete study of population structure requires samples from discrete breeding aggregations, as well as samples from different times throughout the migratory period. This is an expensive and time-consuming endeavor that has been conducted in only a few cases for steelhead, typically for much larger systems (e.g., Nass and Skeena rivers, Beacham et al. 2000). We have shown how an existing sport fishery can be used to collect information that provides preliminary inferences regarding the possibility of discrete populations and their migratory timing within a small river system. This information can be used to justify more intensive sampling efforts and to facilitate provisional management strategies that maximize the protection of any discrete popula-

tions that may be present. In the Dean River, for example, managers should recognize that early migrating steelhead may represent a separate population, and should perhaps modify commercial regulations in mixed-stock fisheries to further protect this group from concentrated commercial fishing pressure (bycatch) early in the year (George and Leggett 1982).

Acknowledgments

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