

## Genetic Population Structure and History of Chinook Salmon of the Upper Columbia River

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**Abstract.**—Chinook salmon *Oncorhynchus tshawytscha* that return to the upper Columbia River (upstream from the confluence of the Yakima River) are considered from the perspectives of allelic variation at 32 polymorphic loci, historical activities within this region, and ancestral affinities to downstream populations. Collections of summer–fall-run fish are distinguished from spring-run fish by an eightfold greater genetic distance between groups than exists within either group. Each group was related to but remained distinct from adjacent downstream groups within different major ancestral units, previously identified throughout the Columbia River. Summer–fall-run fish are most closely related to fall-run fish of the mid-Columbia and Snake rivers, and spring-run fish to the spring–summer-run fish of the Snake River. In both groups, the present geographic distributions and genetic population structures within the upper Columbia River reflect translocations, confinements, and cultural activities between 1939 and 1943 under the Grand Coulee Fish Maintenance Project, and subsequent introductions and fish culture. The considerable genetic homogeneity within the summer–fall-run group appears to have been maintained through past and present interbreedings and strayings over a single continuous run. Some degree of genetic distinction persists between cultured and wild spring-run fish; the cultured fish are genetically indistinguishable from their ancestral source of the downstream Carson Hatchery, derived during the 1950s from fish returning to the upper Columbia and Snake rivers. The entire summer–fall-run group and the wild component of the spring-run group qualify for consideration as different evolutionarily significant units. Suggestions to conserve the genetic variation within these groups focus on measures that restrict excessive gene flow and permit maintenance and development of local adaptations.

The Columbia River is the largest river entering the Pacific Ocean from North America, draining 670,810 km<sup>2</sup> of the northwestern United States and southwestern Canada (Figure 1). Historically, this drainage supported the world's greatest runs of chinook salmon *Oncorhynchus tshawytscha*. The present distribution of returning fish in spring, summer, and fall modes contrasts with a continuum of returns and a summer mode recorded in the nineteenth century (Thompson 1951). This altered distribution and an overall numerical decline has been attributed to the combined effects of overharvest and habitat degradation (Mullan 1987; Nehlsen et al. 1991). The currently depleted number of summer-run fish has stimulated petitions for their protection under the U.S. Endangered Species Act (ESA; 16 U.S.C. §§ 1531 to 1544; Rohlf 1993). An adequate understanding of the ancestral relationships among geographically and temporally isolated chinook salmon populations, particularly within the

drainages of the upper Columbia River, is a necessary component of response to these petitions (Waples 1991).

We examine relationships among chinook salmon populations of the upper Columbia River. Biochemical genetic data from 16 summer-run, fall-run, and spring-run collections identify two distinct groupings consistent with those indicated from previous studies in Figure 2. We relate these observations to historical fishery management in the region and discuss the relationships of these groups to other populations, their relevance as evolutionarily significant units (ESUs), and appropriate management strategies.

### Background

#### *Biochemical Genetic Studies*

Biochemical genetic studies involving chinook salmon populations of the Columbia River have

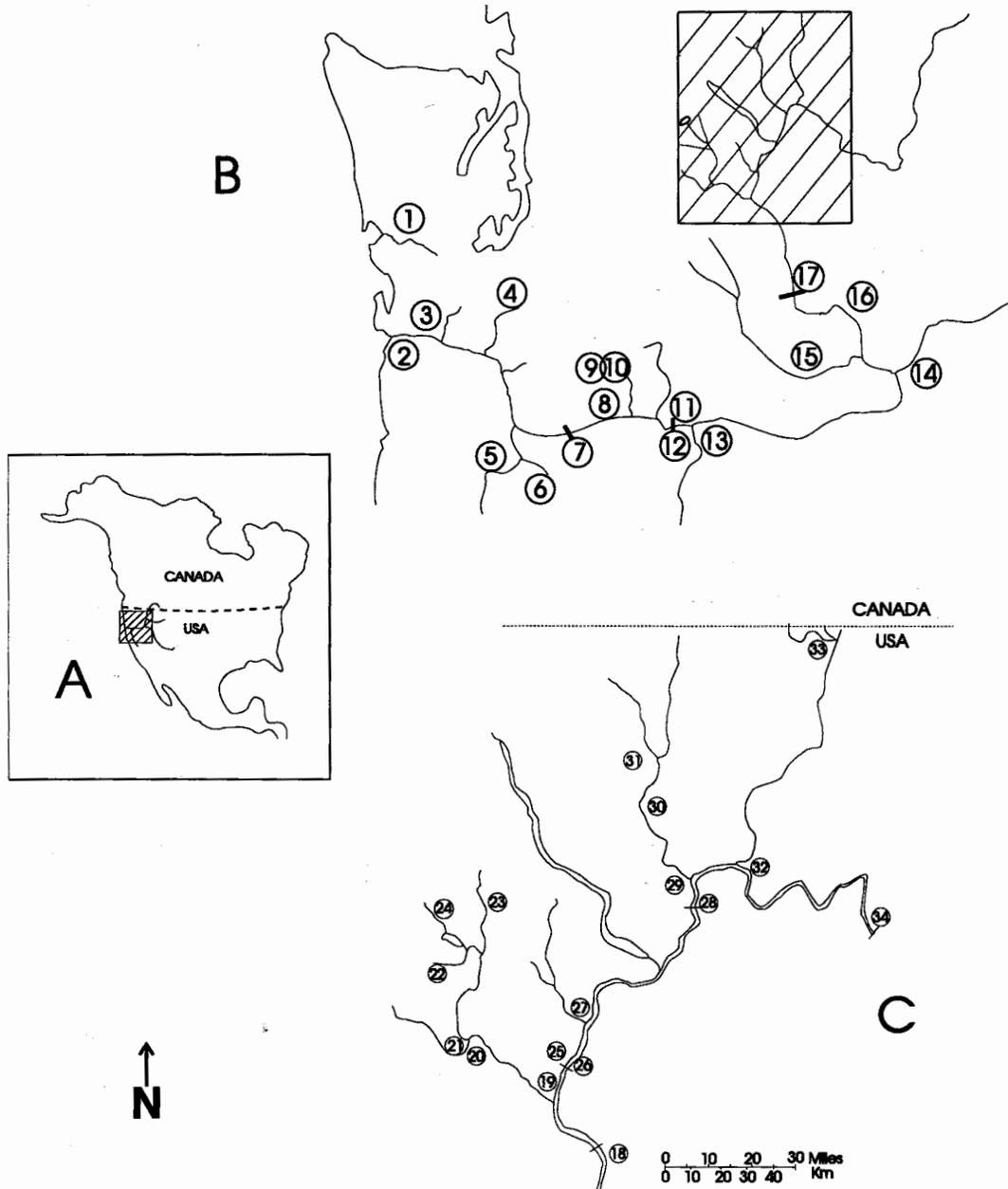


FIGURE 1.—(A) The Columbia River drainage relative to North America. (B) Enlargement of cross-hatched area of (A). The drainage is arbitrarily subdivided into the lower Columbia River below the Dalles Dam, the mid-Columbia River between the Dalles Dam and the confluence of the Yakima River, the Snake River, and the upper Columbia River upstream from the Yakima River. Numbers of specific locations (excluding cross-hatched area) indicate (1) Chehalis River, (2) Columbia River mouth, (3) Elokomin River, (4) Cowlitz River, (5) Willamette River, (6) Eagle Creek, (7) Bonneville Dam, (8) Spring Creek, (9) Carson Hatchery, (10) Wind River, (11) Little White Salmon River, (12) The Dalles Dam, (13) Deschutes River, (14) mouth of the Snake River, (15) Yakima River, (16) Hanford Reach, (17) Priest Rapids Hatchery. (C) Enlargement of cross-hatched area of (B). Numbers of locations indicate (18) Rock Island Dam, (19) Wenatchee River, (20) Leavenworth Hatchery, (21) Icicle Creek, (22) Nason Creek, (23) Chiwawa River, (24) White River, (25) Rocky Reach Dam, (26) Eastbank Hatchery, (27) Entiat River, (28) Wells Dam, (29) Methow River, (30) Carlton Pond, (31) Winthrop Hatchery, (32) Okanogan River, (33) Similkameen River, and (34) Grand Coulee Dam.

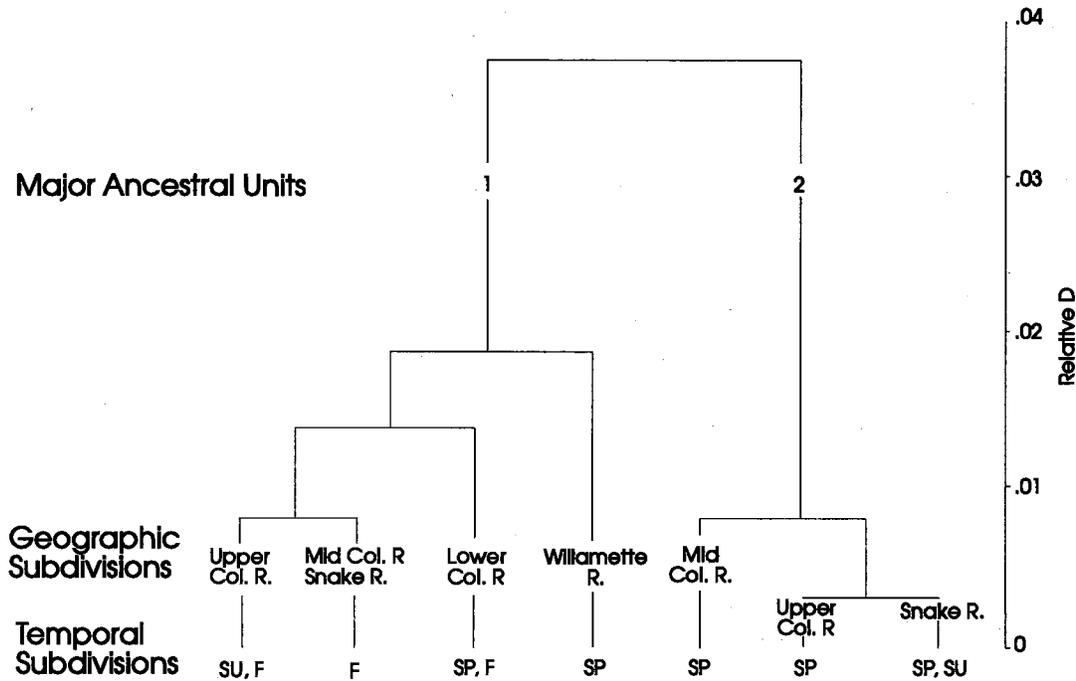


FIGURE 2.—An unweighted pair-group method using arithmetic averages dendrogram indicating the hierarchical subdivision of chinook salmon populations of the Columbia River. Derived from Matthews and Waples (1991: Figure 5) and based on pairwise measurements of genetic distance ( $D$ ; Nei 1972) at 21 polymorphic loci. Multiple temporal subdivisions (SP = spring-run, SU = summer-run, and F = fall-run fish) included within some geographic subdivisions indicate the absence of distinguishing allele frequency patterns based on run timing. (Columbia = Col., River = R.)

revealed a complex population structure (Utter et al. 1989, 1992; Bartley et al. 1992; Waples et al. 1993) both between and within two major ancestral units (Figures 1 and 2). Subgroups of populations within unit 1 extend into major tributaries from the lower Columbia River through the Snake River and upper Columbia River, whereas the natural distribution of unit 2 subgroups is restricted to areas upstream from the lower Columbia River.

Relationships among temporally distinct runs vary with location. Dates for distinguishing spring and summer runs and summer and fall runs at Priest Rapids Dam are 23 June and 1 September (Public Utility Districts upstream from Hanford, Washington) or 17 June and 17 August (U.S. Army Corps of Engineers). In unit 1, spring- and fall-run fish of the lower Columbia River and summer- and fall-run fish of the upper Columbia River do not appear genetically differentiated (Utter et al. 1989). Except where otherwise noted, the convention of Mullan (1987) is followed here in reference to the latter subgroup as summer-fall-run fish. Within unit 2, genetic studies, coupled with juvenile life-history

and habitat data, of spring- and summer-run fish of the Snake River have concluded that these combined runs constitute a common evolutionary grouping (Matthews and Waples 1991).

#### Historical Modifications

*The Grand Coulee Fish Maintenance Project.*—Fishery management associated with the blockage to upstream migration by Grand Coulee Dam must be reviewed when considering the present genetic structure of chinook salmon populations of the upper Columbia River. Grand Coulee Dam permanently blocked access of anadromous salmonids to over 1,609 km of upstream spawning and rearing habitat in 1939. In compensation for this loss, the Grand Coulee Fish Maintenance Project (GCFMP) intercepted upstream migratory salmonids at Rock Island Dam, near Wenatchee (Figure 1), from 1939 through 1943 for relocation in tributaries downstream from Grand Coulee Dam. The details outlined in Table 1 indicate a 5-year period when almost all adult spring-run and summer-fall-run

TABLE 1.—A chronology of events affecting chinook salmon between Rock Island and Grand Coulee dams under the Grand Coulee Fish Maintenance Project (GCFMP) (from Fish and Hanavan 1948; Mullan 1987). Year given represents brood year.

- 1938—Normal spawning occurs upstream from Rock Island Dam, including areas upstream from Grand Coulee site. Juvenile summer-fall fish go to sea in 1939, and some possibly in 1940<sup>a</sup>. Spring-run progeny of the 1938 brood enter sea in 1940.
- 1939—All upstream migrants are trapped at Rock Island Dam, 1939–1943. Releases include mixed spring-run to racked area in Nason Creek (Wenatchee River tributary just downstream from Lake Wenatchee) and summer-fall fish to racked areas in the Entiat River and in the Wenatchee River between Lake Wenatchee and Tumwater. The same pattern of adult treatment continues through 1943, except for ending Entiat releases after 1940 brood.
- 1940—Juveniles of mixed-stock summer-fall chinook salmon and of spring-run chinook salmon are introduced to Methow River, Entiat River, and Icicle Creek (Wenatchee River tributary near Leavenworth, Washington). Fish culture begins in late August at Leavenworth Hatchery with spring-run and summer-fall-run parents.<sup>b,c</sup>
- 1941—Some mixed-origin juveniles of summer-fall chinook salmon are released to Entiat River.
- 1942—Mixed-stock juveniles of summer-fall chinook salmon are released to Methow River, Icicle Creek, and Entiat River. Release of spring-run juveniles of mixed origin is made to Methow River. Some mixed adults, presumably 3-year-old jacks from 1939 brood spawning, are released past Rock Island Dam.
- 1943—Releases of mixed-stock juveniles of summer-fall chinook salmon are made to Icicle Creek and Entiat River. Progeny of mixed spring-run fish are released in Icicle Creek and in Wenatchee, Methow, and Entiat rivers. About 15% of a limited release of jacks upstream from Rock Island Dam return to the Methow River.
- 1944—No trapping at Rock Island Dam, hence adults have access to tributaries and main Columbia River in the upper Columbia region. Returns include progeny of both mixed-stock juvenile releases and of natural spawners. Juveniles from mixed Rock Island summer-fall fish are released in Entiat River. Progeny of mixed spring-run fish are released in Methow River. "Mixed" here, and for subsequent brood years, means adults that returned to GCFMP hatcheries but that originated from adults trapped at Rock Island Dam.
- 1945—Returns include a few progeny of natural spawners in racked areas and hatchery-produced fish. Hatchery-reared spring-run fish are released to Methow River. Hatchery summer-fall chinook salmon are delivered to Entiat River.
- 1946—Returns are as for 1945 brood. Juveniles of mixed summer-fall chinook salmon are released in Icicle Creek, Methow River, and Entiat River. Mixed juvenile spring-run fish are released to Methow River.
- 1947—Returns are as for 1945 and 1946 broods. Mixed spring-run chinook salmon from Leavenworth Hatchery egg take are released in Icicle Creek, and those from Winthrop egg take are released in Methow River. Summer-fall juveniles from Entiat volunteers to trap are delivered to Leavenworth, with liberation point unknown but likely to be Icicle Creek. Remaining Entiat progeny probably are delivered to Entiat River.

<sup>a</sup>Role of stream-annulus summer-fall chinook salmon in pre-dam era is unknown. Present age distributions include some stream-annulus adults, but this may result from effects of hydropower system on seaward migration.

<sup>b</sup>Records do not separate progeny numbers in spring-run and summer-fall-run groups. We assume that maturation timing would prevent extensive mixing of the two groups at spawning time.

<sup>c</sup>Fish and Hanavan (1948) did not distinguish the fall-run component, calling all late-run chinook salmon "summer chinook." Chapman et al. (Don Chapman Consultants, Boise, Idaho, 1994) termed all late-run chinook salmon "summer-fall chinook."

fish, regardless of original destination, were respectively either confined to restricted areas for natural reproduction or used for cultural activities. These interceptions, translocations, and within-group admixtures permanently transfigured the populations of anadromous salmonids above Rock Island Dam and provided a foundation for the present population structures.

*Cultural activities since the Grand Coulee Fish Maintenance Project.*—Additional cultural activities that persist through the present have complicated modifications of population structures imposed through the GCFMP. The most obvious manipulations involve introductions of fish from regions downstream from the upper Columbia River. A review of such introductions (Table 2) indicates a

continual influx from diverse geographic and ancestral origins.

The most persistent and extensive of these introductions involve spring-run fish from the Carson Hatchery on the Wind River (see Figure 1). This population was derived from spring-run fish that were destined for the Snake and the upper Columbia rivers and were intercepted at Bonneville Dam starting in 1955 (Ricker 1972). The spring-run fish of the adjacent Little White Salmon River Hatchery were largely derived from Carson fish (Howell et al. 1985).

Spring-run production of both the Leavenworth and Entiat hatcheries depended on eggs from downstream broodfish of mixed origin through the 1970s and into the 1981 brood year. These exoge-

TABLE 2.—Releases of chinook salmon in the upper Columbia River drainage from sources downstream from Rock Island Dam (compiled from Peven 1992); the latest spring-run releases were from the 1982 brood year. See Figure 1 for locations. Abbreviations are creek (Ck.), hatchery (H.), river (R.), subyearling (SY), and yearling (Y).

Origin of released fish	Run of released fish	Release		
		Location	Numbers	Fish size
<b>1960–1970</b>				
Spring Ck. H.	Summer	Entiat R.	990,800	SY
	Fall	Icicle Ck.	2,922,000	SY, Y
Eagle Ck. (Willamette R.)	Spring	Icicle Ck.	251,000	Y
	Fall	Columbia R.	659,000	SY
	Spring	Icicle Ck.	86,000	Y
<b>1971–1980</b>				
Carson H.	Spring	Icicle Ck.	6,978,000	Y
	Spring	Entiat R.	1,677,000	Y
	Spring	Columbia R.	1,183,000	Y
Little White Salmon H.	Spring	Icicle Ck.	1,127,000	Y
	Spring	Entiat R.	1,161,000	Y
Cowlitz H.	Spring	Icicle Ck.	989,000	Y
	Spring	Entiat R.	436,000	Y
Simpson H. (Chehalis R.)	Fall	Columbia R.	715,000	SY, Y
<b>1981–1990</b>				
Carson H.	Spring	Icicle Ck.	155,000	Y
	Spring	Entiat R.	436,000	Y
	Spring	Columbia R.	762,000	Y
Little White Salmon H.	Spring	Entiat R.	622,000	Y
Elokomin H.	Fall	Columbia R.	296,000	Y
Bonneville H.	Fall	Columbia R.	226,000	Y
Snake R. × Priest Rapids H.	Fall	Columbia R.	1,136,000	SY, Y
Priest Rapids H.	Fall	Columbia R.	657,000	Y

nous infusions included intervals of five consecutive years in both hatcheries when all releases were of predominantly Carson Hatchery origins. This dependence on external sources ultimately gave way to full hatchery production from fish returning to the respective hatcheries in 1982 (Mullan 1987; Peven 1992).

The summer and fall hatchery programs of the upper Columbia River have been supported primarily through indigenous populations of this region, centering on the Wells Dam Hatchery for areas above Rocky Reach Dam and the Eastbank Hatchery for the Wenatchee River. The background of broodstocks has been reviewed by D. Chapman et al. (Don Chapman Consultants, Boise, Idaho, 1994). Returns to these hatcheries for a given year-class, in addition to fish released from the hatchery, included naturally produced upriver fish and substantial numbers of strays from downriver (Rocky Reach, Wenatchee, and Priest Rapids) hatchery releases of summer-run and fall-run fish. Since 1991, Priest Rapids Hatchery has supplied fall-run fish to the Rocky Reach Hatchery, and the Wells Hatchery has been the source of summer-run fish released in the Methow and Similkameen rivers. The more limited Bonneville Hatchery fall-run

chinook salmon releases (Figure 1; Table 2) represent fish of mixed upstream ancestry analogous to the spring-run fish of the Carson Hatchery. This stock was created during the 1980s for enhanced downstream production of “upriver bright” fall chinook salmon destined for the upper Columbia River (Smouse et al. 1990).

#### Materials and Methods

Sixteen collections, made between 1989 and 1992 under conditions detailed in Marshall and Young (1994), represented populations of adult or juvenile fish from 10 localities (Figure 1; Table 3). Attempts were made to sample equal numbers of male and female adults throughout the spawning period and over the contiguous spawning range of a given population. Juvenile progeny from separate brood years were sampled from several hatchery programs. Tissues dissected from adult fish in the field and placed directly on dry ice included approximately 1 cm<sup>3</sup> each of heart, liver, cheek muscle, and eye tissue; intact smolt-size juvenile fish were placed on dry ice when collected. All samples were transferred to a -80°C freezer for subsequent storage prior to electrophoresis.

TABLE 3.—Sampling locations for spring-run and summer-fall-run chinook salmon of the upper Columbia River. Collection year for juvenile samples reared at Eastbank Hatchery (†) or rearing ponds at Carlton (Methow River) or Similkameen (††) is year of parental spawning by adults collected at indicated locations. Designations for time of return are based on passage at Priest Rapids Dam: before 25 June, spring run (SP); between 25 June and 13 August, summer run (SU); and after 13 August, fall run (F). Maturity is either adult (A) or juvenile (J).

Collection number and name	Time of return	Collection years	Maturity	Sample size
1 Wenatchee River	SU	1989–1992	A	409
2 Wells Hatchery	SU	1991–1992	A	202
3 Wells Trap	SU	1991–1992	A	180
4 Wenatchee River†	SU	1992	J	86
5 Wells (Carlton)††	SU	1992	J	90
6 Wells (Similkameen)††	SU	1992	J	75
7 Similkameen River	SU	1991–1992	A	81
8 Hanford Reach	F	1990	A	99
9 Priest Rapids	F	1990–1991	A	200
10 Winthrop Hatchery	SP	1992	A	100
11 Leavenworth Hatchery	SP	1991	A	100
12 White River	SP	1989, 1991, 1992	A	113
13 Nason Creek	SP	1989–1992	A	71
14 Chiwawa River	SP	1989, 1991, 1992	A	133
15 Chiwawa River†	SP	1992	J	86
16 Chiwawa River†	SP	1991	J	100

Methods of tissue extraction, electrophoresis, and histochemical staining followed Aebersold et al. (1987). The loci and alleles screened (Table 4) and the laboratory protocol used are described in detail in Marshall and Young (1994). Phenotypes from all gels were independently double scored, and many were screened in two or more tissues and on two different buffers to ensure accuracy of the data and to resolve all known alleles. Genetic nomenclature followed the American Fisheries Society guidelines established by Shaklee et al. (1990). Allelic data for isolocus pairs (*sAAT-1,2\**, *sMDH-A1,2\**, and *sMDH-B1,2\**) were used in comparative analyses under the assumption that all of the variation occurred at one locus.

Genetic data were analyzed with the BIOSYS-1 computer program<sup>1</sup> for pairwise genetic distances (*D*; Nei 1972), and dendrograms were constructed through the unweighted pair-group method (Sneath and Sokal 1973). A *G*-test (log-likelihood ration test; Zar 1974) examined heterogeneity in pairwise comparisons of allele frequencies among samples; the *G* statistic approximates the chi-square distribution based on the same degrees of freedom and critical values. Samples were combined into pooled collections over two or more years for some locations when sample sizes in individual years were less

than 50 and also when *G*-tests between or among years for larger samples from the same location were nonsignificant. Chi-square tests for departures of genotypes from the expected binomial distribution (Hardy–Weinberg equilibrium) were made on all disomic loci when frequencies of the common allele were less than 0.95.

## Results and Discussion

### Genetic Analyses

Genetic variability was detected within 17 classes of enzymatic proteins at 32 presumed single loci or isolocus pairs among the 16 collections of upper Columbia River chinook salmon examined in this study (Table 4). The allelic frequencies of the polymorphic loci (Appendix) provided the basis for genetic comparisons among these collections.

Thirteen out of 227 tests for deviations from Hardy–Weinberg equilibrium were significant at the 0.05 level. No pattern was discernable among the loci for which significant deviations were observed. Because this frequency of deviations would be expected by chance (sampling error) among 227 tests, conditions resulting in Hardy–Weinberg proportions (e.g., random mating and absence of strong selection) were presumed to underlie these collections.

*Between-group distinctions.*—The amounts and distributions of genetic variation varied considerably among loci (Appendix). The ranges of allele frequencies among the 17 most variable loci (Ta-

<sup>1</sup>Swofford, D. L., and R. B. Selander. 1989. BIOSYS-1: a computer program for the analysis of allelic variation in population genetics and biochemical systematics. Release 1.7, Illinois Natural History Survey, Champaign.

TABLE 4.—List of enzyme names and international numbers (IUBNC 1984) of variable enzymes, as well as loci designations, tissue distributions, and relative mobilities of variant allelic forms of the polymorphic loci. Tissue types are eye (E), heart (H), liver (L), and cheek muscle (M).

Enzyme name	Enzyme number	Locus	Relative mobilities of variants	Tissue distribution
Aspartate aminotransferase	2.6.1.1	<i>sAAT-1,2*</i>	85, 105	M, H
		<i>sAAT-3*</i>	90	E
		<i>sAAT-4*</i>	130, 63	L
		<i>mAAT-1*</i>	-77, -104	M, H
Adenosine deaminase	3.5.4.4	<i>ADA-1*</i>	83	M, E, H
		<i>ADA-2*</i>	105	M, E, H
Aconitate hydratase	4.2.1.3	<i>sAH*</i>	86, 112, 108	L
		<i>mAH-4*</i>	119	M, H
Dipeptidase	3.4.--	<i>PEPA*</i>	90, 81	M, E, H, L
Glucose-6-phosphate isomerase	5.3.1.9	<i>GPI-B2*</i>	60	M
		<i>GPI-A*</i>	105	M, E, H
		<i>GR*</i>	85	M, E, H, L
Glutathione reductase	1.6.4.2	<i>HAGH*</i>	143, 131	M, H, L
Hydroxyacylglutathione hydrolase	3.1.2.6	<i>mIDHP-2*</i>	154, 50	M, E
Isocitrate dehydrogenase	1.1.1.42	<i>sIDHP-1*</i>	74, 142, 94, 126	M, H, E, L
		<i>sIDHP-2*</i>	127, 83	H, E, L
		<i>LDH-B2*</i>	112, 71	E, L
L-Lactate dehydrogenase	1.1.1.27	<i>LDH-C*</i>	90, 84	E
Malate dehydrogenase	1.1.1.37	<i>sMDH-A1,2*</i>	27, 160	M, H, E
		<i>sMDH-B1,2*</i>	121, 70, 83, 126	M, H, L
		<i>mMDH-2*</i>	200	M, H
		<i>sMEP-1*</i>	92	M, H
Malic enzyme (NADP <sup>+</sup> )	1.1.1.40	<i>MPI*</i>	109, 95	M, H, E
Mannose-6-phosphate isomerase	5.3.1.8	<i>PEPD-2*</i>	107	M
Proline dipeptidase	3.4.13.9	<i>PEP-LT*</i>	110	M, L
Leucyl-tyrosine dipeptidase	3.4.--	<i>PGDH*</i>	90	M, E
Phosphogluconate dehydrogenase	1.1.1.44	<i>PGK-2*</i>	90, 74	M, E
Phosphoglycerate kinase	2.7.2.3	<i>PGM-2*</i>	136	M
Phosphoglucosmutase	5.4.2.2	<i>sSOD-1*</i>	-260, 580, -175	L
Superoxide dismutase	1.15.1.1	<i>mSOD*</i>	142	H
		<i>PEPB-1*</i>	130, -350	M, E
Tripeptide aminopeptidase	3.4.--	<i>TPI-2.2*</i>	104	M, E
Triose-phosphate isomerase	5.3.1.1			

ble 5) reflect the actual divergence among collections. Typically, the greatest differences occurred between the summer-fall-run and spring-run groups, and no overlap of allele frequency occurred between groups for those nine loci with ranges exceeding 0.20. Particularly notable in this regard were *sMEP-1\** and *PGK-2\**, with respective overall ranges of 0.805 and 0.618.

The clear distinction between these two groups was apparent in other analyses. Significance levels of *G*-tests exceeded 0.001 in all comparisons, and between-group genetic distances ranged between 0.030 and 0.049 (Table 6). The dendrogram of pairwise genetic distances (Figure 3) separates the summer-fall-run and spring-run fish at an average between-group *D* value of 0.04, an eightfold greater distance than the largest pairwise *D* value observed among within-group separations.

Average relative heterozygosity values (based on only polymorphic loci; Appendix) did not overlap among collections representing different groups; differences between summer-fall-run (mean 0.101) and spring-run (mean 0.086) fish were highly signif-

icant ( $P < 0.001$  based on Mann-Whitney *U*-test). These differences comport with previous comparisons of heterozygosities among chinook salmon populations of the Columbia River (Utter et al. 1989; Winans 1989; Waples et al. 1991a). Heterozygosity values were consistently lower for either spring-run fish of the upper Columbia River or spring-summer-run fish of the Snake River when compared with other groups.

These major groupings fell within appropriate subdivisions of the different major ancestral groups inferred from previous studies in Figure 2. The summer-fall-run group of this study merged with the same subdivision indicated in unit 1, having affinities to fall-run fish of the mid-Columbia and Snake rivers, but were distinguished by differing allelic frequencies at several loci including *sAH\**, *sSOD-1\**, and *PEPB-1\** (Utter et al. 1989). Similarly, the spring-run group of this study coincides with the same subdivision of unit 2 in Figure 2, being distinguished from the closely related spring-summer-run subgrouping of the Snake River by



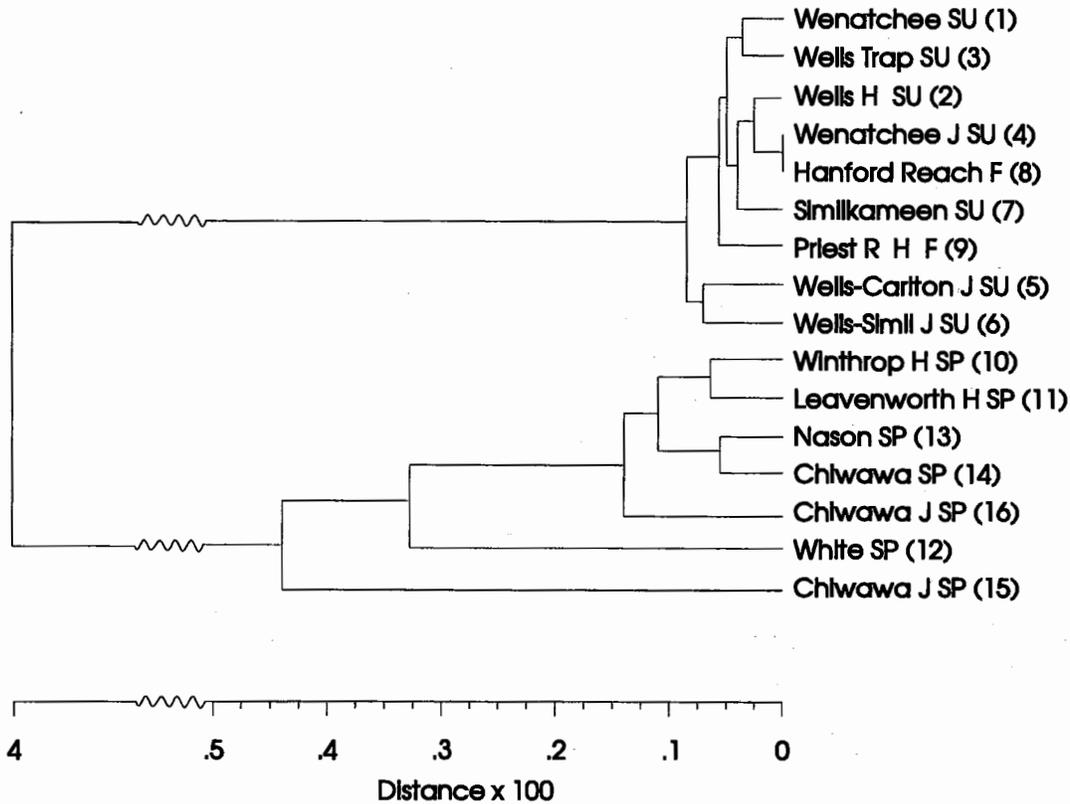


FIGURE 3.—An unweighted pair-group method using arithmetic averages dendrogram of genetic relationships among chinook salmon populations of the upper Columbia River based on pairwise genetic distance values ( $D$ ; Nei 1972) for 36 polymorphic loci. Abbreviations given are fall (F), spring (SP), summer (SU), juveniles (J), hatchery (H), and river (R), and numbers in parentheses represent collection numbers (see Table 3 for sampling details).

males in particular would result in a reduced effective population size and a resultant opportunity for excessive genetic drift (e.g., Allendorf and Ryman 1987; Gall 1987).

At Wells Dam, sufficiently large numbers of adults were available for spawners to reduce the likelihood of genetic drift as a factor in the overall higher levels of significance of  $G$ -tests between Wells-Similkameen juveniles (6) and other summer-fall-run collections. These fish and those reared at Carlton ponds (collection 5) represented subsamples of the same year-class of Wells Hatchery juveniles. Thus, other factors, including differential subsampling of juveniles moved to the respective sites or differential mortalities at the sites, appear to be more likely explanations (Marshall and Young 1994). The presence of unique alleles at  $sAH^*$  in four individuals and at  $sIDHP^*$  and  $sSOD^*$  in single individuals supports the former possibility.

The most notable distinction involving adult col-

lections was the divergence within the spring-run group of the White River (12). Allele frequencies lie beyond the range of other adult spring-run samples at 11 of the 36 loci, although no unique alleles were found (Appendix). These differences indicate that this population should be considered distinct from the other sampled spring-run populations.

Some less obvious differences occurred among the adult samples. The clustering of the Winthrop (10) and Leavenworth (11) hatchery collections, and of the adjacent Nason Creek (13) and Chiwawa River (14) wild samples (Figure 3) supports the possibility of some persisting genetic isolation between these hatchery and wild spring-run populations.

Significant  $G$ -tests occurred between Hanford Reach (8) and all other summer-fall-run collections except Priest Rapids Hatchery (9). These differences were small and insufficient to affect measurements of genetic distance at the reported levels.

However, at loci such as *AAT-4\**, *sIDPH-2\**, *sMDH-A1,2\**, *sMDH-B1,2\**, and *TPI-2.2\** (Appendix), slight outlying of allele frequencies for Hanford Reach from other collections within this group suggests the possibility of some persisting degree of isolation of these wild fall-run fish from upstream populations.

#### *Synthesis from Genetic and Historical Information*

The major points from the separate considerations of genetic and historical information presented separately in the preceding sections include

1. spring-run populations of the upper Columbia River are genetically distinct from summer-fall-run fish;
2. each group has genetic affinities with different major ancestral groups within the Columbia River;
3. the basis for all current distributions above Rock Island Dam lies in relocations over five consecutive years under the GCFMP;
4. releases of cultured fish under the GCFMP included crosses between summer- and fall-run fish and, possibly, between late-spawning spring-run chinook salmon and early-spawning summer-run chinook salmon;
5. extensive releases subsequent to the GCFMP have included origins from gene pools outside the upper Columbia River;
6. cultured summer-fall-run fish include parentage of broad temporal diversity within this group; and
7. there are no allele frequency differences from Priest Rapids Dam upstream that suggest genetic isolation of summer-run and fall-run populations.

Further conclusions can be derived from joint considerations of this information.

*Interactions of distinct ancestral groups.*—The historical information has identified numerous opportunities for breakdown of natural genetic structure through interbreedings among fish of distinct ancestral origins or establishment of exogenous gene pools. Effects of this nature were not apparent from the most diverged groups (Figure 2). There were no detectable residual effects from any possible interbreedings between late-maturing spring-run and early-maturing summer-run fish due to cultural activities under the GCFMP. No intermediate groups were evident to suggest persistence of a hybridized spring-run  $\times$  summer-fall-run ancestry. Inspection of the allelic data of the somewhat distinct White

River spring-run population indicates a divergence from other upper Columbia River spring-run groups and not introgression from the summer-fall-run group.

Introductions from hatcheries on the Chehalis, Elokomin, and Cowlitz rivers and Eagle and Spring creeks (Figure 1) represent lineages distinct from either of the upper Columbia resident groups (Figure 2; Utter et al. 1989). These releases of purely exogenous fish (Table 2) appear to have left no detectable descendants.

*Interactions with populations of mixed upriver ancestry.*—The lower river populations derived from mixed upriver ancestry tell a different story. The presently self-sustaining spring-run populations of upper Columbia River hatcheries resulted from continued infusions of Carson and Carson-derived fish (Table 2) until returning adults were sufficient for an autonomous broodstock. The spring-run fish now returning to these hatcheries were genetically very similar to one another (Figure 3) and to Carson fish (Waples et al. 1991b). However, data of this study did not reflect a major contribution of fish of Carson ancestry, based on the distinction between these hatcheries and wild adult spring-run collections from Nason Creek, the Chiwawa River, and particularly the White River (Figure 3).

The less extensive introductions of fall-run hatchery fish of mixed ancestry (Table 2) presently preclude estimating possible genetic influences due to alleles common to upper Columbia River summer-fall-run and mixed ancestral groups. Sample sizes substantially larger than those reported here would be needed to detect shifts of allelic frequencies because of the close relationship to native fish of contributing exogenous gene pools and because of the high proportion of upper Columbia River ancestry (90%) estimated in the Bonneville fall-run population (Smouse et al. 1990).

However, the unique occurrence of the *sAH\*112* allele in multiple individuals of one collection of juvenile offspring from Wells Hatchery parents (collection 6) could be a reflection of exogenous genes from introductions or strays. This allele occurs at frequencies up to 0.03 in related fall-run populations of the mid-Columbia River (Smouse et al. 1990) and at slightly lower frequencies in Snake River and Yakima basin fall-run chinook salmon populations (A. Marshall, Washington Department of Fisheries and Wildlife, unpublished data). Its presence in these juvenile fish released in the Similkameen River raises the possibility of an exogenous component within this group and warrants close monitoring for persistence of this allele upon

the return of these fish to their point of rearing and release.

#### *Evolutionarily Significant Units*

The focus of these proceedings on defining unique units in population conservation warrants a discussion of the status of the populations under consideration in this paper as evolutionarily significant units (ESUs). The ESA as amended in 1978 (16 U.S.C. § 1532[16]) mandates protection of "distinct population segments" of vertebrates as well as of recognized species and subspecies. The concept of the ESU provides a logical and biologically sound framework for defining such intraspecific segments (Waples 1991). To be considered as an ESU, populations (1) must be substantially reproductively isolated from other conspecific population units and (2) must represent an important component in the evolutionary legacy of the species. Four basic questions should be considered in defining an ESU.

1. Is the population genetically distinct from other conspecific populations?
2. Does the population occupy unique habitat?
3. Does the population show evidence of unique adaptation to its environment?
4. If the population became extinct, would this event represent a significant loss to the ecological or genetic diversity of the species?

Waples (1991) further suggested that ESUs should correspond to more comprehensive units unless there is clear evidence that evolutionarily important differences exist between smaller population segments. Being based on distinctions from other intraspecific groups, the ESU primary value is to provide a sound biological basis for proscribing admixtures beyond their boundaries, and definition of an ESU by no means implies a single panmictic unit. These questions and criteria guide considerations of chinook salmon populations of the upper Columbia River as possible ESUs.

*Spring-run populations.*—The clear genetic isolation of spring-run and summer-fall-run fish of the upper Columbia River (Figures 2, 3) qualifies them for separate consideration as ESUs. The mixed and partially exogenous ancestry within the hatchery component presumably precludes this segment of spring-run fish from ESU status (Hard et al. 1992). However, the differences between hatchery and adult wild collections of this study indicate that the latter group, and similarly distinct wild fish of this region, qualify for consideration as components of a common ESU. The geographic isolation and above-

noted genetic distinction from Snake River spring-summer-run fish (presently designated an ESU; Matthews and Waples 1991) would restrict the ESU to the upper Columbia River. The manipulations under the GCFMP limit any evolutionary divergence to the past 50 years and thus probably preclude subdivision in spite of the apparent distinction of the White River population.

The ESU status of spring-run populations of the Yakima River is unclear because of their exclusion from both the present study and investigations focused on spring-summer-run fish of the Snake River (e.g., Matthews and Waples 1991). Clusterings based on accumulated genetic information and providing the basis for Figure 2 (Waples et al. 1991b) suggest affinities of different populations within this drainage to either mid-Columbia River or upper Columbia River groups. Clarification of this issue awaits collection of more detailed information from within the Yakima River basin.

*Summer-fall-run populations.*—Different circumstances surround the ESU status of the summer-fall group of the upper Columbia River. Based on both genetic and historical data, the ancestry of existing populations appears to be predominantly—perhaps entirely—within the upper Columbia River drainage. The questions of interest converge on the possibility of genetic divergence within this drainage. The available information all points toward sufficient past and present admixture among temporal segments that would work against maintaining or establishing either temporal or geographic diversity.

The common elements within this group resulted in all summer-fall-run fish upstream from McNary Dam (50 km downstream from the confluence of the Columbia and Snake rivers, Figure 1), exclusive of the Snake River, recently being considered a single ESU (Waknitz et al. 1995). The inclusion of Yakima River populations within this ESU identifies a common need within this drainage for more detailed studies of fall-run fish as well as for spring-run fish noted above. Cumulatively, the summer-fall-run populations sampled in this study represent an important segment of the species' evolutionary legacy. They are reproductively isolated from, though related to, fall-run populations of the Snake River and the mid-Columbia River. In addition, upper Columbia River summer-fall-run and Snake River fall-run populations are distinguished by differing oceanic distributions, juvenile and adult sizes, and environmental features (summarized in Waples et al. 1991b).

### Conclusions and Recommendations

The data and discussion to this point provide new information about distribution and relationships among chinook salmon populations of the upper Columbia River that is of potential value for the management of these fish. These perspectives pertain to different levels of genetic variation.

At greater levels of genetic distinction, the apparent failures of introductions from, or interbreedings with, more diverged subgroups contrast with successful introductions from more closely related (e.g., Carson) fish. These failures cause us to question the wisdom of further introductions or interbreedings at this level in the upper Columbia River; past efforts were apparently unsuccessful, and further introductions would continue to threaten the breakdown of existing adapted groups that may be displaced or interbred through initial numerical superiority of the exogenous fish, coupled with the likelihood of subsequent failure of hybrids or exogenous fish (Hindar et al. 1991; Waples 1995).

Introductions and interbreedings involving more closely related subgroups have been more successful and therefore pose a potentially greater threat to the stability of indigenous populations of the upper Columbia River. Two groups of closely related populations within which some genetic distinction exists are (1) the spring-summer-run fish of the Snake River, the mixed spring-run hatchery fish (e.g., Carson) and the spring-run fish of the upper Columbia River; and (2) the fall-run fish of the Snake River and mid-Columbia River, the hatchery populations (Bonneville) derived from mixed upriver fall-run fish, and the summer-fall-run fish of the upper Columbia River (Figure 2). Introductions and crosses among populations within either of these groups appear to be more amenable to producing reproductively viable progeny than introductions and crosses between these and other more distantly related groups. This increased potential viability makes it easier for transplants or strays to become established or to merge, potentially eroding adaptive distinctions between groups that may have arisen in both freshwater and marine habitats (e.g., Waples et al. 1991b). Thus, careful monitoring is necessary for detecting intrusions of closely related exogenous fish and for taking appropriate remedial actions.

The most problematic level of genetic variation is that for which differential adaptations have occurred among breeding groups which remain indistinguishable by biochemical or molecular genetic markers. Persistence or evolution of adaptive dis-

tinctions in the absence of conspicuous genetic differentiation is well documented (e.g., Gharrett and Smoker 1993). Such differences are possible because of the more rapid evolutionary time scale for genetic divergence of strongly adaptive characters (e.g., run timing) in contrast with more neutral characters such as the multiple polymorphic protein-coding loci used in this study (see Utter et al. 1993). Thus, sufficient flow or retarded drift of marker genes within these indistinguishable groupings may mask adaptive differences.

The persisting genetic affinity of the Leavenworth and Carson hatchery populations provides a possible example of this type of divergence. An initial dependence on Carson (and Carson-derived Little White Salmon Hatchery) eggs gradually subsided over three generations (Table 2). Numbers of returning releases from Leavenworth gradually increased from 1970 to the point where returning fish constituted the entire broodstock after 1983. Admittedly, these indirect data alone are not strong evidence for differential adaptation, and definitive data (e.g., based on reciprocal egg lots reared and released at both hatcheries) are needed. Better alternative explanations are presently lacking.

Indeed, most data supporting restricted movements of exogenous populations are indirect (e.g., Hindar et al. 1991; Campton 1995). With a few notable exceptions (e.g., Reisenbichler and McIntyre 1977; Chilcote et al. 1986; Campton et al. 1991), appropriate experiments have not been implemented to address theoretical arguments favoring restricted movements of exogenous populations. As such experimental data accumulate, local populations remain the best starting point for any enhancement activities (Hindar et al. 1991; Waples et al. 1991b).

Divergence of a single-source seeding of chinook salmon in New Zealand into diverse habitats and life history patterns (Quinn and Unwin 1993) attests to the evolutionary flexibility of chinook salmon. Similar population divergence has apparently occurred within the upper Columbia River since the disrupting and homogenizing effects of the GCFMP a half century ago. The White River spring-run fish appear to have diverged genetically and perhaps adaptively. Adaptive differences of hatchery stocks have been suggested in the absence of detectable genetic divergence from the source populations. These divergences have developed through continued breedings of adult fish returning to the locations and habitats of their parents.

However, continual infusions of individuals over wide geographic and temporal ranges, even within a

genetically homogeneous group determined by essentially neutral markers, work against establishing both wild and hatchery adaptations—and thus promote inefficiency. For chinook salmon populations of the upper Columbia River then, the most effective strategy appears to be to reduce culturally induced straying and to permit existing populations to develop and adapt within local temporal, ecological, and geographic ranges.

The biological basis for this strategy has been apparent for a long time (e.g., Ricker 1972). The strategy makes sense from the perspectives of both conservation and production and should be implemented.

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## Appendix: Allelic Frequencies

Allelic frequencies for 35 loci in 16 collections of chinook salmon from the upper Columbia River. Average heterozygosities (HET) are given for each collection. See Table 3 and Figure 1 for names and locations of numbered collections and Table 4 for relative allele mobilities.

Locus, allele, and sample size	Collection															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
<i>sAAT-1,2*</i>																
<i>N</i>	(409)	(404)	(180)	(86)	(180)	(75)	(81)	(99)	(200)	(100)	(100)	(113)	(71)	(133)	(86)	(100)
*100	0.999	0.998	1.000	0.994	0.997	1.000	1.000	0.995	1.000	1.000	1.000	1.000	0.993	0.994	0.988	0.975
*85	0.001	0.001	0.000	0.006	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.007	0.006	0.012	0.025
*105	0.001	0.001	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>sAAT-3*</i>																
<i>N</i>	(405)	(190)	(170)	(86)	(90)	(75)	(76)	(89)	(199)	(98)	(97)	(112)	(68)	(130)	(86)	(100)
*100	0.999	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.997	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*90	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>sAAT-4*</i>																
<i>N</i>	(377)	(180)	(139)	(84)	(89)	(74)	(80)	(97)	(164)	(77)	(79)	(105)	(61)	(107)	(76)	(83)
*100	0.999	1.000	0.996	1.000	1.000	1.000	1.000	0.979	0.988	0.955	0.975	0.986	0.959	0.944	1.000	0.994
*130	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*63	0.001	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.012	0.045	0.025	0.014	0.041	0.056	0.000	0.006
<i>mAAT-1*</i>																
<i>N</i>	(409)	(202)	(180)	(85)	(90)	(75)	(81)	(99)	(200)	(100)	(100)	(113)	(71)	(132)	(86)	(100)
*100	0.980	0.990	0.992	0.994	0.989	0.987	0.969	0.995	0.988	0.990	0.985	0.996	1.000	0.985	1.000	0.980
*77	0.001	0.002	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*104	0.018	0.007	0.008	0.006	0.011	0.000	0.031	0.005	0.013	0.010	0.015	0.004	0.000	0.015	0.000	0.020
<i>ADA-1*</i>																
<i>N</i>	(409)	(201)	(180)	(86)	(90)	(75)	(81)	(99)	(200)	(100)	(100)	(113)	(70)	(130)	(86)	(98)
*100	0.991	0.990	1.000	0.983	1.000	1.000	0.994	0.990	0.995	0.975	0.950	0.929	0.929	0.946	0.949	0.939
*83	0.009	0.010	0.000	0.017	0.000	0.000	0.006	0.010	0.005	0.025	0.050	0.071	0.071	0.054	0.051	0.061
<i>ADA-2*</i>																
<i>N</i>	(409)	(202)	(180)	(86)	(90)	(75)	(81)	(99)	(200)	(100)	(100)	(113)	(71)	(133)	(86)	(100)
*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.995
*105	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005
<i>sAH*</i>																
<i>N</i>	(407)	(202)	(168)	(86)	(90)	(75)	(81)	(99)	(200)	(100)	(100)	(112)	(68)	(129)	(86)	(100)
*100	0.769	0.780	0.786	0.744	0.889	0.820	0.741	0.808	0.805	0.000	1.000	0.991	1.000	0.988	1.000	1.000
*86	0.231	0.220	0.214	0.256	0.111	0.153	0.259	0.192	0.195	0.000	0.000	0.004	0.000	0.012	0.000	0.000
*112	0.000	0.000	0.000	0.000	0.000	0.027	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*108	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000
<i>mAH-4*</i>																
<i>N</i>	(409)	(201)	(169)	(86)	(90)	(75)	(81)	(99)	(200)	(100)	(100)	(113)	(69)	(129)	(84)	(100)
*100	0.911	0.873	0.908	0.895	0.894	0.820	0.846	0.919	0.895	0.985	0.975	1.000	1.000	1.000	0.905	1.000
*119	0.089	0.127	0.092	0.105	0.106	0.180	0.154	0.081	0.105	0.015	0.025	0.000	0.000	0.000	0.095	0.000
<i>PEPA*</i>																
<i>N</i>	(409)	(202)	(180)	(86)	(90)	(75)	(81)	(98)	(200)	(100)	(100)	(113)	(70)	(133)	(86)	(100)
*100	0.972	0.983	0.981	0.983	0.989	0.960	0.975	0.990	0.967	1.000	0.990	0.947	0.993	0.985	1.000	0.955
*90	0.027	0.017	0.019	0.017	0.011	0.040	0.019	0.010	0.030	0.000	0.005	0.035	0.007	0.004	0.000	0.000
*81	0.001	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.003	0.000	0.005	0.018	0.000	0.011	0.000	0.045
<i>GPI-B2*</i>																
<i>N</i>	(408)	(202)	(180)	(83)	(87)	(74)	(81)	(99)	(200)	(100)	(100)	(112)	(69)	(132)	(85)	(95)
*100	0.945	0.948	0.928	0.970	0.948	0.959	0.932	0.929	0.952	1.000	0.995	0.969	0.993	0.966	0.882	0.926
*60	0.055	0.052	0.072	0.030	0.052	0.041	0.068	0.071	0.047	0.000	0.005	0.031	0.007	0.034	0.118	0.074
<i>GPI-A*</i>																
<i>N</i>	(409)	(202)	(180)	(86)	(90)	(75)	(81)	(98)	(200)	(100)	(100)	(113)	(66)	(132)	(86)	(100)
*100	0.998	0.990	0.997	1.000	1.000	0.987	0.988	0.995	0.993	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*105	0.002	0.010	0.003	0.000	0.000	0.013	0.012	0.005	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>GR</i>																
<i>N</i>	(409)	(202)	(180)	(86)	(90)	(75)	(81)	(99)	(200)	(100)	(100)	(113)	(71)	(133)	(86)	(100)
*100	0.976	0.970	0.953	0.953	0.983	0.973	0.975	0.975	0.977	0.995	1.000	0.996	0.993	0.989	1.000	0.975
*85	0.024	0.030	0.025	0.007	0.017	0.027	0.025	0.025	0.023	0.005	0.000	0.004	0.007	0.011	0.000	0.025
<i>HAGH*</i>																
<i>N</i>	(409)	(202)	(180)	(86)	(90)	(75)	(81)	(99)	(200)	(100)	(100)	(113)	(71)	(133)	(86)	(100)
*100	0.998	0.998	0.994	1.000	0.989	1.000	1.000	1.000	0.993	0.900	0.910	0.858	0.887	0.914	1.000	0.870
*143	0.002	0.002	0.006	0.000	0.011	0.000	0.000	0.000	0.007	0.100	0.090	0.142	0.113	0.075	0.000	0.130
*131	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000



## Appendix.—Continued.

Locus, allele, and sample size	Collection															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
<i>PGK-2*</i>																
<i>N</i>	(409)	(202)	(180)	(86)	(90)	(75)	(81)	(99)	(199)	(99)	(100)	(113)	(71)	(133)	(86)	(100)
<i>*100</i>	0.560	0.587	0.581	0.602	0.589	0.600	0.605	0.591	0.608	0.106	0.180	0.119	0.127	0.117	0.080	0.080
<i>*90</i>	0.439	0.411	0.414	0.398	0.411	0.400	0.395	0.404	0.392	0.894	0.820	0.881	0.873	0.883	0.920	0.920
<i>*74</i>	0.001	0.002	0.006	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>PGM-2*</i>																
<i>N</i>	(409)	(202)	(180)	(86)	(90)	(75)	(81)	(97)	(200)	(100)	(100)	(113)	(71)	(133)	(86)	(100)
<i>*100</i>	1.000	0.998	1.000	1.000	1.000	1.000	0.994	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
<i>*136</i>	0.000	0.002	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>sSOD-1*</i>																
<i>N</i>	(409)	(202)	(180)	(86)	(90)	(75)	(81)	(99)	(200)	(100)	(100)	(113)	(71)	(133)	(86)	(100)
<i>*100</i>	0.482	0.485	0.511	0.506	0.550	0.540	0.494	0.535	0.507	0.755	0.755	0.872	0.782	0.737	0.574	0.820
<i>*260</i>	0.517	0.512	0.489	0.494	0.450	0.453	0.506	0.465	0.493	0.245	0.245	0.128	0.218	0.263	0.426	0.180
<i>*580</i>	0.001	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>*175</i>	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>mSOD*</i>																
<i>N</i>	(409)	(202)	(179)	(86)	(90)	(75)	(81)	(98)	(200)	(100)	(100)	(112)	(71)	(133)	(86)	(100)
<i>*100</i>	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.985	0.990	1.000	0.993	0.996	1.000	1.000
<i>*142</i>	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.010	0.000	0.007	0.004	0.000	0.000
<i>PEPB*</i>																
<i>N</i>	(409)	(202)	(180)	(86)	(90)	(75)	(81)	(99)	(199)	(100)	(100)	(113)	(71)	(133)	(86)	(100)
<i>*100</i>	0.729	0.696	0.717	0.739	0.706	0.727	0.691	0.747	0.741	0.840	0.840	0.779	0.803	0.812	0.847	0.845
<i>*130</i>	0.260	0.300	0.269	0.261	0.289	0.247	0.309	0.237	0.241	0.110	0.085	0.080	0.085	0.090	0.051	0.045
<i>*350</i>	0.011	0.005	0.014	0.000	0.006	0.027	0.000	0.015	0.018	0.050	0.075	0.142	0.113	0.098	0.097	0.110
<i>TPI-2.2*</i>																
<i>N</i>	(409)	(202)	(180)	(86)	(90)	(75)	(81)	(99)	(200)	(100)	(100)	(113)	(71)	(133)	(86)	(100)
<i>*100</i>	0.998	1.000	0.997	1.000	1.000	1.000	0.994	0.985	0.993	0.915	0.905	0.841	0.965	0.955	0.960	0.975
<i>*104</i>	0.002	0.000	0.003	0.000	0.000	0.000	0.006	0.015	0.007	0.085	0.095	0.159	0.035	0.045	0.040	0.025
HET	0.116	0.114	0.111	0.112	0.1907	0.109	0.124	0.108	0.112	0.081	0.085	0.093	0.079	0.086	0.076	0.081