

Influence of Pleistocene Glaciations and Human Intervention upon Mitochondrial DNA Diversity in White Sturgeon (*Acipenser transmontanus*) Populations

James R. Brown,¹ Andrew T. Beckenbach, and Michael J. Smith

Institute of Molecular Biology and Biochemistry, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C. V5A 1S6, Canada

Brown, J. R., A. T. Beckenbach, and M. J. Smith. 1992. Influence of Pleistocene glaciations and human intervention upon mitochondrial DNA diversity in white sturgeon (*Acipenser transmontanus*) populations. *Can. J. Fish. Aquat. Sci.* 49: 358–367.

The influence of recent genetic bottlenecks on two Northwest Pacific populations of white sturgeon (*Acipenser transmontanus*) was studied using restriction enzyme site differences in mitochondrial DNA (mtDNA). A total of 10 mtDNA genotypes were detected in 178 sturgeon from the Columbia and Fraser rivers. Nearly 61% of individuals from both rivers had a common genotype. However, one genotype was found only in the Fraser River. Geographical substructuring was not evident in the phylogenetic analysis. The Columbia River, a Pleistocene refugium, is suggested as the source of founders for the Fraser River following the last glacial recession, about 10 000–12 000 yr ago. MtDNA genotypic frequencies were significantly different between the rivers. Estimates of mtDNA (h) and nucleotide (π) diversity were consistently higher for the recently colonized Fraser River compared with the Columbia River refugium. This condition is the reverse of all known studies of mtDNA diversity between refugium and colonized animal populations. Recent overexploitation and habitat destruction are suggested as a severe genetic bottleneck that has reduced mtDNA diversity in Columbia River sturgeon.

Les auteurs ont examiné l'influence de récents points d'étranglement génétiques sur deux populations d'esturgeon blanc (*Acipenser transmontanus*) du Pacifique nord-ouest en établissant les différences du site d'hydrolyse de l'ADN mitochondrial (ADNmt) par des endonucléases de restriction. Ils ont ainsi relevé 10 génotypes de l'ADNmt chez 178 esturgeons des fleuves Columbia et Fraser. Presque 61 % des individus de ces deux cours d'eau partageaient le même génotype. Par contre, un seul génotype a été identifié dans le fleuve Fraser. Une sous-structure géographique n'a pas été révélée par une analyse phylogénétique. Selon les auteurs, le fleuve Columbia, un refuge du Pléistocène, serait la source des colonisateurs du fleuve Fraser qui s'y auraient dispersés après la dernière récession glaciaire, il y a de cela environ 10 000 à 12 000 années. Les fréquences génotypiques de l'ADNmt étaient nettement différentes entre les rivières. Les estimations de la diversité de l'ADNmt (h) et des nucléotides (π) étaient régulièrement plus élevées dans le cas du fleuve Fraser récemment colonisé par rapport au refuge du Columbia. Cette situation va à l'encontre de toutes les études de la diversité de l'ADNmt entre des populations d'un refuge et d'un site colonisé. Selon les auteurs, la récente surexploitation et la destruction de l'habitat sont à la source d'un grave point d'étranglement génétique qui a réduit la diversité de l'ADNmt chez l'esturgeon blanc du fleuve Columbia.

Received April 10, 1991
Accepted August 26, 1991
(JA990)

Reçu le 10 avril 1991
Accepté le 26 août 1991

Mitochondrial DNA (mtDNA) has been widely used as a genetic marker in population studies because of its higher mutation rate relative to nuclear DNA, generally maternal inheritance, and lack of sexual recombination (reviewed in Brown 1985). Several North American fish species have been subjected to mtDNA analysis including walleye (*Stizostedion vitreum*; Ward et al. 1989), American shad (*Alosa sapidissima*; Bentzen et al. 1989), lake whitefish (*Coregonus clupeaformis*; Bernatchez et al. 1989), and chinook salmon (*Oncorhynchus tshawytscha*; Wilson et al. 1987).

In the above studies, the northern distributions of intraspecific mtDNA genotypes suggested possible dispersal patterns of founder groups from one or several southern refugia. Southern river drainages, located beyond the maximum excursions

of Pleistocene ice sheets, have had prolonged periods of ecological stability. In contrast, northern freshwater habitats have been completely deglaciated for only the last 10 000–12 000 yr (Smith 1981). Postglacial dispersal from southern refugia was probably a severe genetic bottleneck, which has typically lead to a reduction in mtDNA diversity among northern populations of widely distributed species. In this study, we describe mtDNA variation between two populations of white sturgeon (*Acipenser transmontanus*) from refugium and formerly glaciated river systems.

Sturgeon and their closest living relatives, paddlefish, are members of the Infraclass Chondrostei, a pre-Jurassic diversion from the teleost lineage (Lauder and Liem 1983; Gardiner 1984). Knowledge of the life history and population genetics of sturgeon species, particularly *A. transmontanus*, is sparse. The white sturgeon is the largest freshwater fish in North America, and individuals may exceed 600 kg in weight and 6 m in length (Scott and Crossman 1973). Reproductive maturity is

¹Author to whom correspondence should be addressed. Present address: Department of Biochemistry, Sir Charles Tupper Medical Building, Dalhousie University, Halifax, N.S. B3H 4H7, Canada.

attained between ages 11 and 34 yr and specimens older than 70 yr have been recorded (Semakula and Larkin 1968).

Acipenser transmontanus is anadromous and readily moves between freshwater and marine coastal habitats. Conventional and radiotagging studies have yielded little information regarding either the length of time sturgeon spend at sea, their fidelity to river of birth, or migration patterns (Miller 1972a, 1972b; Haynes and Gray 1981; Haynes et al. 1978; Dixon 1986), although individuals may travel distances of several hundred kilometres in marine coastal waters (Galbreath 1985). Because sturgeon are capable of repeated spawnings, the potential exists for significant gene flow among sturgeon populations with access to the Pacific Ocean.

The Fraser and Columbia rivers have the largest *A. transmontanus* populations in the Pacific Northwest (McPhail and Lindsay 1986). Both rivers empty into the Pacific Ocean, with the mouth of the Fraser River located about 500 km north of that of the Columbia River. The southern two thirds of the Columbia River remained ice-free during the Pleistocene glaciations (McPhail and Lindsay 1986). In comparison, the entire Fraser River was completely covered by the Cordilleran Ice Sheet as recently as 10 000–12 000 yr ago (Clague and Luternauer 1983). Based on the paleogeography of the region, it is probable that Columbia River sturgeon migrating northward through marine coastal waters colonized the Fraser River after the last glacial retreat. Therefore, this founder effect may have acted as a significant genetic bottleneck and caused notably lower mtDNA diversity in the Fraser River sturgeon population relative to the Columbia River population.

Human activity may have imposed a second genetic bottleneck of unknown magnitude. Nearly all North American sturgeon populations were extensively overfished from the late 1800's to the early 1900's (reviewed in Binkowski and Doroshov 1985). Later construction of large hydroelectric dams on the Columbia River in the 1930's and 1950's may have accentuated this bottleneck effect through alteration of migration routes and destruction of spawning habitat (Galbreath 1985).

In this study, mtDNA variation was determined in *A. transmontanus* populations of the Fraser and Columbia rivers over a 3-yr period (i) to investigate within-river substructuring of populations, (ii) to provide estimates of existing genetic diversity in light of recent bottleneck events, and (iii) to determine the extent of past and contemporary gene flow among populations.

Materials and Methods

Sample Collection and mtDNA Isolation

mtDNA was extracted from fresh sturgeon liver tissue removed in the field and transported on ice to the laboratory. mtDNA's were prepared from 105 sturgeon caught in the Fraser River, British Columbia, and 73 sturgeon from the Columbia River, Washington State. Most sturgeon were between 1 and 2 m fork length, with estimated ages ranging from 9 to 30 yr (D. Lane, Malaspina College, Nanaimo, B.C., unpubl. data).

State of Washington Department of Fisheries personnel collected liver samples from commercially caught sturgeon in the late summers of 1987 and 1988 and the spring of 1990. Sturgeon were caught near the mouth of the lower Columbia River ($n = 19$) and in the upper Columbia River ($n = 54$) above Bonneville Dam, located about 230 km from the river

mouth. This dam is the last impoundment of the Columbia River before it flows into the Pacific Ocean and has effectively isolated upper river sturgeon populations since the mid-1930's. Upper Columbia River sturgeon were caught from three separate reservoirs (in order upstream from Bonneville Dam): Bonneville Pool ($n = 25$), The Dalles Pool ($n = 24$), and John Day Pool ($n = 5$). The latter two pools were created by dams built in the 1950's.

Commercial sturgeon fishing is prohibited on the Fraser River, so most of the liver samples came from sturgeon caught by the seasonal (May–November) sport fishery. A few sturgeon were caught either incidentally in salmon gill nets or in a separate tagging study (D. Lane, pers. comm.). Sample sites ranged from the mouth of the Fraser River to the town of Lillooet, nearly 320 km upstream. The largest sample was taken in 1987 ($n = 66$), with smaller samples in 1988 ($n = 19$) and 1989 ($n = 20$). It is not known whether the lower sample sizes in latter years reflect changes in the sturgeon population or a reduction in angler effort.

A single mtDNA sample was also prepared from liver tissue, pooled from 20 sibling juvenile sturgeon (2.5 mo old) from the Sacramento River, California.

mtDNA was extracted from liver tissue within 6–72 h of collection. mtDNA was isolated using a modified protocol of Lansman et al. (1981) that omitted the sucrose gradient step but included an increase in CsCl – ethidium bromide centrifugation time to 60–72 h. Typical mtDNA yields were 300–500 ng/g homogenized tissue⁻¹.

mtDNA Analysis

Comparisons were based on digests with 12 restriction enzymes with specificities for six-nucleotide (*ApaI*, *BclI*, *BglII*, *EcoRI*, *HindIII*, *PvuII*, and *XbaI*), multiple six-nucleotide (*AccI*, *AvaI*, *HaeII*, and *HincII*), and multiple five-nucleotide (*AvaII*) recognition sequences. The mtDNA genome of one individual was digested with paired combinations of several enzymes (*AvaI*, *BclI*, *EcoRI*, *HindIII*, *KpnI*, *PvuII*, *SacI*, and *XbaI*) to map the relative locations of specific restriction sites. Enzyme digest conditions were as specified by the vendors (BRL and Pharmacia). DNA fragments were visualized under ultraviolet light after electrophoresis in ethidium bromide stained agarose gels.

The observed intraspecific differences in DNA fragment patterns for each enzyme were due to relatively few restriction site changes. Therefore, it was possible to base divergence estimates on the presence or absence of restriction sites located on specific DNA fragments. Differences in fragment length caused by the loss or gain of restriction sites were distinguished from length polymorphisms found in the control or D-loop region of *A. transmontanus* mtDNA (Buroker et al. 1990). Southern blot analysis and direct sequencing identified DNA fragments containing the control region in specific restriction enzyme digests (Brown 1991). In the phylogenetic analysis, mtDNA genotypes were defined by the loss or gain of restriction enzyme sites. Phylogenetic analysis based on mtDNA length variation is inappropriate because the mechanism and rate of mutations for the addition or deletion of repeat sequences in the control region are considerably different from those of nucleotide substitutions and may not be entirely neutral (Rand and Harrison 1989; Buroker et al. 1990; Brown 1991).

Nucleotide substitutions per site (δ) between pairs of mtDNA genotypes were estimated using the maximum likelihood

method of Nei and Tajima (1983). A UPGMA tree of percent sequence divergence (d) was constructed with internal nodes plotted as $1/2d$ (Nei 1987, p. 293–296). Standard errors of internal nodes were calculated using the method of Nei et al. (1985).

The presence or absence of restriction sites was also analyzed using the Wagner parsimony method. The bootstrapped mixed parsimony algorithm (BOOT) of the PHYLIP 3.3 program package was used with 100 replicates (Felsenstein 1990). The estimate of haplotypic or nucleon diversity (h) of Nei and Tajima (1981), used here to evaluate the diversity of mtDNA lineages within a population, was calculated as

$$h = n \left(1 - \sum_{i=1}^r x_i^2 \right) / (n - 1)$$

where x_i is the frequency of the i th type of mtDNA in a population of n specimens and r is the number of mtDNA types. Although this index is usually applied to the heterozygosity at nuclear loci, it is an appropriate measure for the diversity of maternal lineages (Nei 1987). Its application here permits comparisons with published mtDNA diversity estimates for other species.

A second estimate of nucleotide diversity (π) was calculated as

$$\pi = \sum_{ij} x_i x_j \pi_{ij}$$

where x is the frequency of the i th and j th type of mtDNA in a population and π_{ij} is the percent sequence difference between the two types (Nei and Li 1979). Therefore, this index of nucleotide diversity considers the frequency of occurrence of mtDNA types in a population as well as the divergence among mtDNA lineages.

Sensitivity of the sampling regime to detect mtDNA genotypes was evaluated using the combinatorial analysis of Hebert et al. (1988) as applied by Bernatchez et al. (1989). The relationship between sample size and the number of mtDNA genotypes observed in the Fraser and Columbia rivers was determined from the cumulative number of mtDNA genotypes detected in a random choice of 60 individuals. Increments of three individuals ($n = 3, 6, 9, 12, \dots, 60$) were selected at random, without replacement, using the random case selection algorithm in the data management program (PID) of the BMDP statistical software package (Dixon 1983). The mean number of genotypes detected in 10 randomizations was plotted against sample size.

Results

Restriction Map of Sturgeon mtDNA

The size of *A. transmontanus* mtDNA varies between 16.1 and 16.7 kb depending on the number of tandemly repeated 82 nucleotide sequences in the D-loop region (Buroker et al. 1990). Figure 1 shows several restriction enzyme sites, the D-loop region, and genes for apocytochrome *b*, tRNA^{Thr}, and tRNA^{Pro} (Gilbert et al. 1988; Brown et al. 1989; Buroker et al. 1990). The order and transcriptional polarity of these genes are identical to that of other vertebrates.

Intraspecific Phylogeny

The 12 restriction enzymes detected between 52 and 56 sites per *A. transmontanus* mtDNA genome. Because most of the

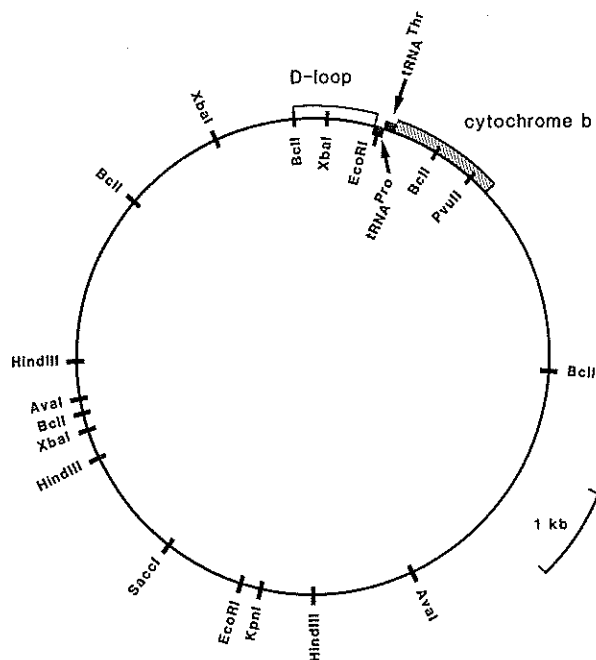


FIG. 1. Restriction enzyme map of the *A. transmontanus* mtDNA genome showing the locations of known coding sequence.

enzymes cleaved at six-nucleotide recognition sites, between 285 and 323 nucleotides or 1.8–2.0% of the genome was sampled. DNA fragment lengths for each enzyme are presented in Appendix A. Nearly 50% of individuals were heteroplasmic (i.e. had multiple copies of different mtDNA types within an individual) for length variation. This length variation was localized to the control region using a cloned 0.8-kb fragment of the entire control region from *A. transmontanus* mtDNA to probe Southern blots (Buroker et al. 1990; Brown 1991).

Five of the 12 restriction enzymes used in the phylogenetic analysis (*AccI*, *AvaI*, *AvaII*, *HincII*, and *BclI*) revealed intra-specific restriction site polymorphisms in *A. transmontanus*. The loss or gain of single restriction sites was observed for the enzymes *AccI*, *AvaII*, and *HincII* (Appendix A). There were two predominant *AvaI* fragment patterns, with a third pattern occurring in a single individual. Three individuals had a parallel loss and gain of two different *BclI* sites relative to the most frequent *BclI* fragment pattern.

In total, 11 mtDNA genotypes or clonal lines (based on the loss or gain of restriction sites) were detected in *A. transmontanus*. Of the 179 individuals examined, 109 (61%) shared a single mtDNA genotype, At2 (Fig. 2). The next three most common mtDNA genotypes (At1, At3, and At4) occurred in 13, 21, and 22 individuals, respectively. The seven remaining genotypes occurred in one to seven individuals.

Sequence divergence between genotypes ranged between 0.17 and 1.07% (Table 1). UPGMA analysis revealed three major genotypic clusters (Fig. 2). Genotypes At1, At3, At4, At5, and At6 formed one cluster. The branch ordering of four of these genotypes is arbitrary because At1, At4, and At5 are equally diverged from At6. The second major cluster consists of At2, At7, and At8. The third cluster, as defined by two *BclI* restriction site differences, included three rare genotypes (At9, At10, and At11). Low estimates of intraspecific sequence divergence resulted in overlapping standard errors about the internal nodes. Therefore, the topology of the UPGMA tree is not significant.

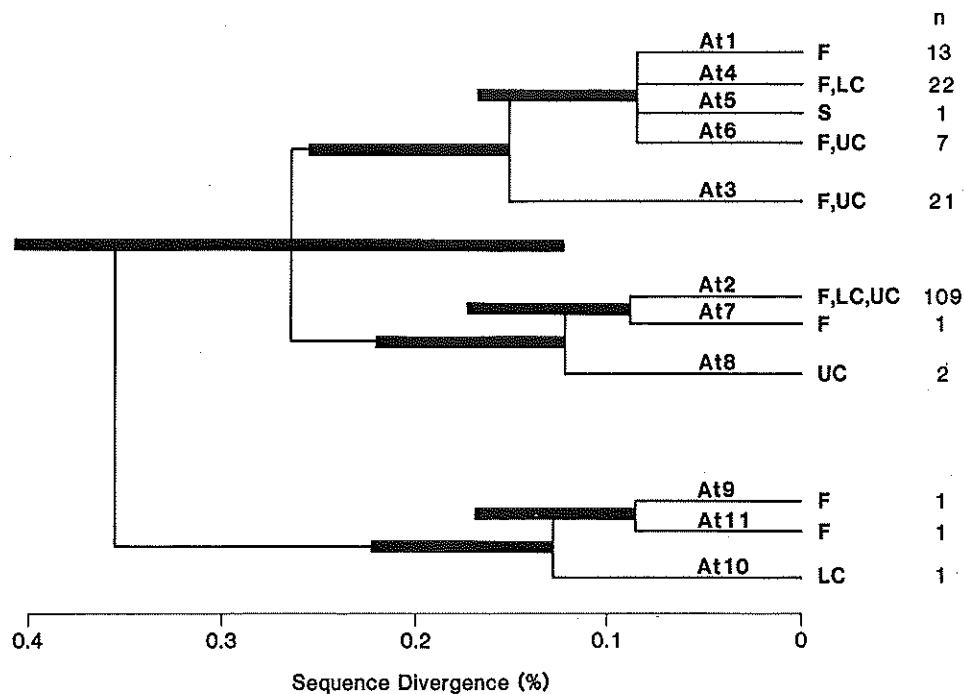


FIG. 2. UPGMA phylogeny of *A. transmontanus* genotypes (At1-11). Branching points are plotted as $1/2d$. Bars represent 1 SE on either side of the branching point. Number of individuals (n) and occurrence in the Fraser (F), Lower Columbia (LC), Upper Columbia (UC), and Sacramento (S) rivers are shown.

TABLE 1. Percent pairwise sequence divergence estimates (lower diagonal) and standard errors (upper diagonal) for mtDNA lineages of *A. transmontanus* (At1-11).

	At1	At2	At3	At4	At5	At6	At7	At8	At9	At10	At11
At1	—	0.355	0.171	0.242	0.242	0.168	0.397	0.308	0.297	0.302	0.349
At2	0.708	—	0.308	0.246	0.246	0.302	0.174	0.177	0.397	0.404	0.355
At3	0.173	0.533	—	0.171	0.302	0.242	0.355	0.251	0.349	0.247	0.302
At4	0.344	0.350	0.173	—	0.242	0.168	0.302	0.308	0.297	0.302	0.242
At5	0.344	0.350	0.524	0.344	—	0.168	0.302	0.308	0.297	0.397	0.297
At6	0.170	0.523	0.344	0.170	0.170	—	0.349	0.355	0.238	0.349	0.297
At7	0.881	0.176	0.708	0.523	0.523	0.695	—	0.251	0.435	0.443	0.397
At8	0.533	0.179	0.357	0.533	0.533	0.708	0.357	—	0.443	0.362	0.404
At9	0.514	0.880	0.695	0.514	0.514	0.338	1.051	1.072	—	0.242	0.168
At10	0.523	0.897	0.351	0.523	0.886	0.695	1.072	0.721	0.344	—	0.171
At11	0.692	0.707	0.523	0.344	0.695	0.514	0.880	0.897	0.170	0.173	—

A closely related species, *A. medirostris*, was used as an outgroup taxon in the Wagner parsimony analysis. Presence or absence data on a total of 64 restriction sites were entered into the analysis (Appendix A). In 100 bootstrap replicates, only one intraspecific branching point occurred more than 50 times (Fig. 3). However, the topology of the parsimony tree was generally congruent with that of the UPGMA tree, with similar clusterings of genotypes At2, At7, and At8 and genotypes At9, At10, and At11.

Distinctive geographical groupings of Fraser and Columbia River genotypes were not evident in either phylogenetic analysis. The pooled sibling sample from the Sacramento River was a separate genotype (At5) that clustered closely with genotypes found in the Fraser and Columbia rivers. In the nomenclature used in Table 2, the composite genotype of this California sample was ABABA.

Geographical Occurrence of Genotypes

The preceding phylogenetic analysis showed that topologies of the two genotypic trees were not statistically significant.

Genotypes were characterized on the basis of the presence or absence of restriction sites which indicates actual nucleotide sequence changes between the mtDNA genomes of individuals. Therefore, analysis of genotypic frequencies is justified even though the inferred ancestral relationships between genotypes are not statistically well supported.

The geographical range of the most frequent genotype (At2) extended throughout the sampled range in the Columbia and Fraser rivers (Fig. 4 and 5; Table 2). However, the observed frequency of all genotypes was significantly different between river systems (log-likelihood ratio chi-square or $G = 36.84$, $df = 5$, $p < 0.0001$). Genotypic frequencies were also distinctive between the upper and lower Columbia River ($G = 21.99$, $df = 5$, $p < 0.001$).

Genotype At2 was found throughout the Columbia River. However, less frequent genotypes were specific to areas above (At3, At6, and At8) or below (At4 and At10) Bonneville Dam (Fig. 4). Two of the rare genotypes (At8 and At10) were unique to the Columbia River.

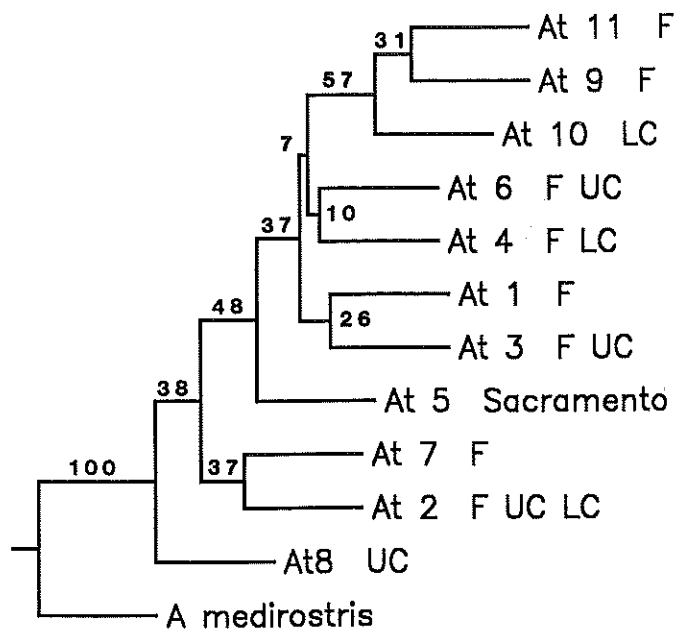


FIG. 3. Wagner parsimony phylogeny of *A. transmontanus* genotypes. The number of times each branching point occurred in 100 bootstrap replications is shown. Letter codes for geographical location of genotypes follow Fig. 2.

In the Fraser River, the most common genotypes (At2, At3, and At4) were distributed throughout the middle and lower sections of the river (Fig. 5). In the nine individuals sampled upstream of Hell's Gate Rapids (280–320 km from the river mouth), only genotypes At2 ($n = 8$) and At4 ($n = 1$) were detected. The Hell's Gate Rapids, located 200 km upstream, have completely isolated upper Fraser River sturgeon populations since 1912. In comparison, eight separate genotypes were detected downstream of the rapids.

Only one genotype (At1) had a narrow distribution. This unique genotype was found in 13 individuals caught in a 45-km-long section of the Fraser River, 90 km from the river mouth (Fig. 5). This genotype was represented in roughly the same proportions in samples collected over a 3-yr period.

mtDNA Diversity

Both estimators of mtDNA diversity showed that mtDNA diversity in the Fraser River ($h = 0.70$, $\pi = 0.157$) is nearly

twice that in the Columbia River ($h = 0.36$, $\pi = 0.076$) (Table 3). In the Columbia River, greater mtDNA diversity was found below ($h = 0.49$, $\pi = 0.103$) than above ($h = 0.30$, $\pi = 0.067$) Bonneville Dam. MtDNA diversity in the upper Columbia River was considerably lower in the Bonneville Pool in comparison with The Dalles and John Day pools. Lower mtDNA diversity ($h = 0.22$) was also estimated for isolated sturgeon populations above Hell's Gate Rapids (Fig. 5) compared with the lower Fraser River ($h = 0.72$).

Diversity estimates for the Fraser River derived from annual subsamples in 1987 ($n = 66$), 1988 ($n = 19$), and 1989 ($n = 20$) were consistently higher than estimates for the Columbia River ($n = 73$), suggesting that low diversity estimates for the Columbia River population were not artifacts of sample size.

Sample Size and Detection of Genotypes

In the combinatorial analysis, the number of genotypes detected rose with sample size until an asymptote was reached for samples larger than 36–39 individuals (Fig. 6). Fewer genotypes were detected in the Columbia River (four to five genotypes) compared with the Fraser River (five to six genotypes).

Discussion

Intraspecific Phylogeny

The phylogenetic analysis indicates that *A. transmontanus* mtDNA genotypes are little diverged and generally lack any significant geographical substructure. Low confidence intervals around intraspecific branching points can be attributed to either a deficiency in the number of nucleotides sampled or historical events that have greatly reduced mtDNA diversity in this species.

An examination of the recognition sites of the polymorphic enzymes, *AccI* (GT[AG]AC), *AvaI* (GPyCGPuG), *AvaII* (GG[AT]CC), and *HincII* (GTPyPuAC), reveals that all have multiple recognition sequences. Of the fixed hexameric enzymes used (*ApaI*, *BclI*, *BglII*, *EcoRI*, *HindIII*, *PvuII*, and *XbaI*), only *BclI* was polymorphic, with three individuals displaying a variant fragment pattern. In other studies of intraspecific mtDNA variation, including some of the fish population studies cited here, fixed hexameric enzymes were polymorphic. DNA sequence comparisons of a hypervariable

TABLE 2. MtDNA genotypic frequencies of *A. transmontanus* for entire samples from the Fraser and Columbia rivers as well as for various subsamples of the Columbia River. The letter code in parentheses denotes polymorphic DNA fragment patterns for the enzymes *BclI*, *HincII*, *AvaI*, *AvaII*, and *AccI*.

Genotype	Entire rivers		Columbia		Upper Columbia pools		
	Fraser	Columbia	Lower	Upper	Bonneville	The Dalles	John Day
<i>n</i>	105	73	19	54	25	24	5
At1 (AAABB)	0.12	0.0	0.0	0.0	0.0	0.0	0.0
At2 (ABBAA)	0.49	0.80	0.68	0.83	0.92	0.75	0.80
At3 (AABBB)	0.18	0.03	0.0	0.04	0.04	0.04	0.0
At4 (ABBBB)	0.16	0.07	0.26	0.0	0.0	0.0	0.0
At6 (ABABB)	0.02	0.07	0.0	0.09	0.04	0.17	0.0
At7 (ABCAA)	0.01	0.0	0.0	0.0	0.0	0.0	0.0
At8 (AABAA)	0.0	0.03	0.0	0.04	0.0	0.04	0.20
At9 (BBABB)	0.01	0.0	0.0	0.0	0.0	0.0	0.0
At10 (BABBB)	0.0	0.01	0.05	0.0	0.0	0.0	0.0
At11 (BBBBB)	0.01	0.0	0.0	0.0	0.0	0.0	0.0

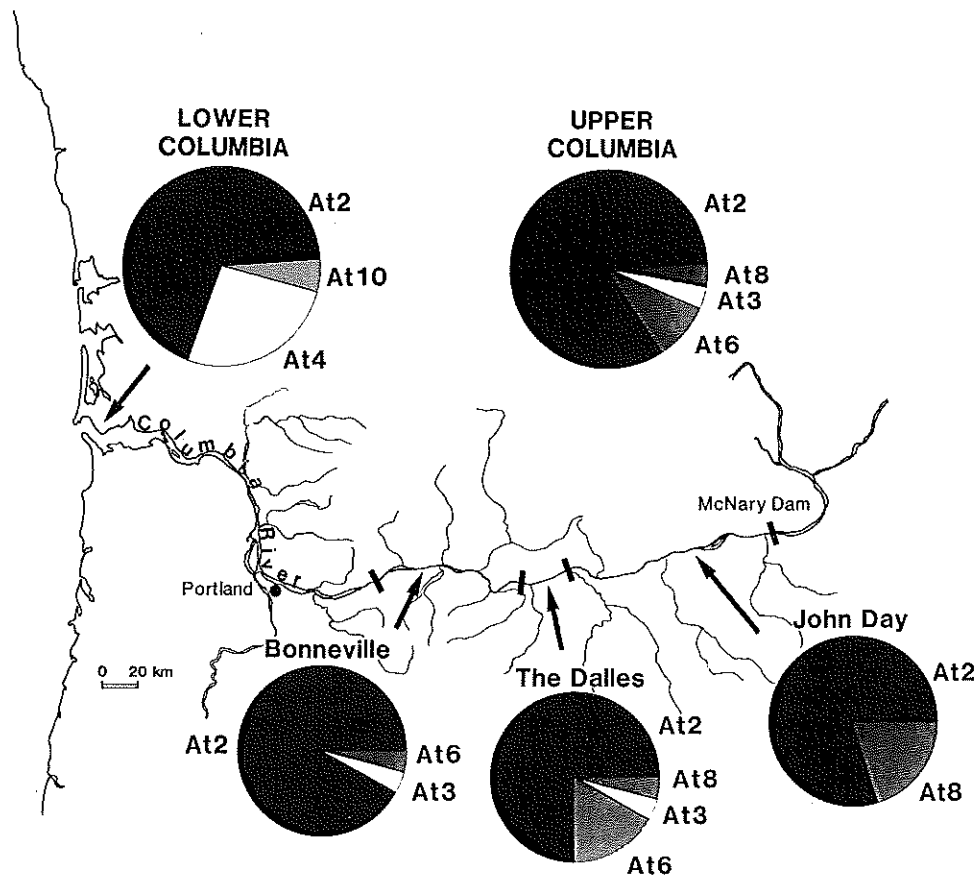


FIG. 4. MtDNA genotypic frequencies in the Columbia River. Upper pie charts show frequencies in the lower ($n = 19$) and upper ($n = 54$) Columbia River as delineated by the Bonneville Dam. Bottom pie charts subdivide genotypic frequencies of the upper Columbia sample into reservoirs: Bonneville Pool ($n = 25$), The Dalles Pool ($n = 24$), and John Day Pool ($n = 5$). Solid bars across the river show the location of each dam.

segment in the control region of *A. transmontanus* mtDNA generally concur with the low intraspecific divergence estimates obtained from restriction enzyme data (Brown 1991). The low levels of mtDNA sequence variation detected in *A. transmontanus* are thus probably reflective of the lack of sequence differences among individuals rather than inadequate sampling of nucleotides or populations (see below).

Low genetic variability has been noted for other North American chondrosteans. Little intraspecific variation was observed in a protein electrophoretic study of paddlefish (*Polyodon spathula*) populations (Carlson et al. 1982). Two other species of sturgeon, *Scaphirhynchus albus* and *S. platyrhynchus*, were indistinguishable at 37 protein loci (Phelps and Allendorf 1983). Restriction enzyme analysis has revealed low intraspecific mtDNA variation in northern populations of the sturgeon species, *A. fulvescens*, *A. medirostris*, and *A. oxyrhynchus* (Brown 1991). Populations of *A. oxyrhynchus* in the southeastern United States also show very low mtDNA diversity (Bowen and Avise 1990).

Postglacial dispersal and possibly more contemporary migrations would minimize geographical substructuring of *A. transmontanus* mtDNA genotypes, while recent, severe genetic bottleneck effects would reduce mtDNA diversity. Bidirectional gene flow between the Fraser and Columbia rivers could be occurring, although the fidelity of migratory *A. transmontanus* to natal rivers is unknown. Sturgeon tagged in the Columbia River have been recaptured in marine waters

off the coast of northern Washington State (Galbreath 1985). A low number of migratory female spawners per generation could prevent significant divergence of mtDNA genotypes between these river systems (Slatkin 1987).

The most geographically segregated sturgeon mtDNA genotype was At1. In 3-yr of study, all individuals with this genotype were caught in a 45-km section of the lower Fraser River. This genotype ($n = 13$) comprised about 12% of the Fraser River sample. The restricted range of At1 and higher divergence ($>1.0\%$) between certain conspecific genotypes (Table 1) suggest the existence of moderate isolation processes. Discussion regarding reproductive barriers is highly speculative given our limited understanding of sturgeon behavior. Potential microgeographic isolation of spawning females could occur in two large lakes draining into the Fraser River. Sturgeon may also be segregated into nonsynchronous spawning cohorts separated by reproductive cycles of several years (Roussow 1957; Semakula and Larkin 1968; Doroshov 1985).

mtDNA Diversity

Previous studies have consistently shown that intraspecific mtDNA diversity (h) is lower in recently founded populations relative to populations in areas of long-term ecological stability (Table 3). As an example, North American lake whitefish populations in recently ($<18\ 000$ yr ago) deglaciated rivers had much lower diversity ($h = 0.26 \pm 0.25$) than Eurasian pop-

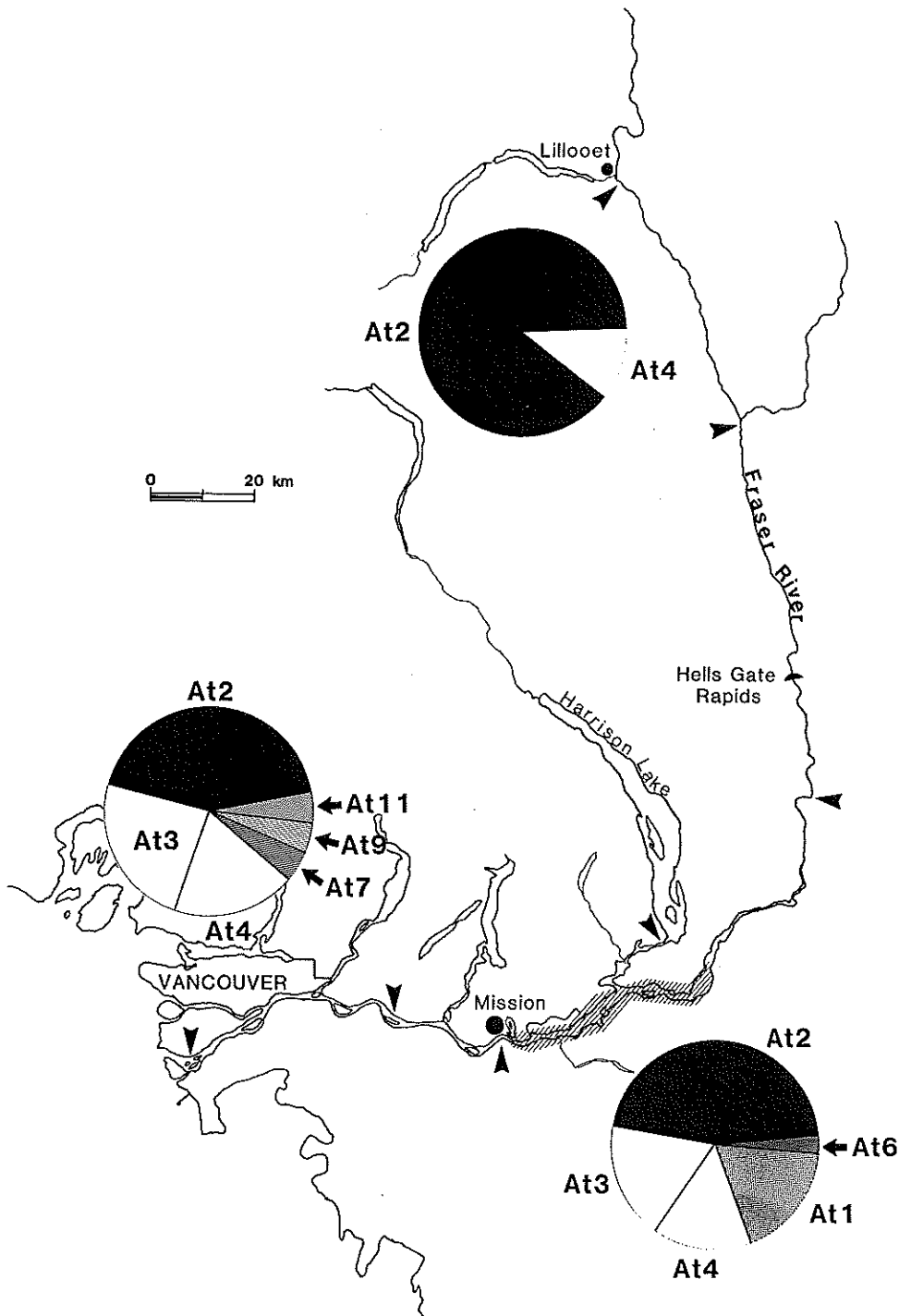


FIG. 5. MtDNA genotypic frequencies in the Fraser River. Arrows indicate the edges of sample areas for the lower ($n = 22$), middle ($n = 74$), and upper ($n = 9$) river. Shaded area upstream of Mission City covers the range of genotype At1.

ulations ($h = 0.90 \pm 0.06$) of a closely related species, *C. lavaretus* (Bernatchez et al. 1989). Genetic bottleneck effects were probably less severe for the Eurasian species because over its contemporary range, glaciation was incomplete and several local refugiums probably existed. Ashley and Wills (1987) concluded that, in the deer mouse (*Peromyscus maniculatus*), a few recent colonization events were responsible for lower mtDNA heterogeneity of island populations ($h = 0.0-0.44$) compared with mainland groups ($h = 0.67-1.0$).

The *A. transmontanus* population that colonized the Fraser River ($h = 0.70$) had considerably higher mtDNA diversity than the putative founder population of the Columbia River ($h = 0.36$), contrary to expectation. Further, the upper Columbia River population ($h = 0.30$) had reduced diversity relative to the lower Columbia River population ($h = 0.49$). Log-likelihood ratio chi-square tests confirmed genotypic frequencies were significantly different in all comparisons between the Fraser River, the lower Columbia River, and the upper Columbia River.

TABLE 3. Estimates of mtDNA diversity (h) and nucleotide diversity (π) using the methods of Nei and Tajima (1981) and Nei and Li (1979), respectively.

Location	n	h	π
Fraser and Columbia rivers	178	0.65	0.135
Fraser River	105	0.70	0.157
1987	66	0.75	0.151
1988	19	0.53	0.134
1989	20	0.51	0.142
Columbia River	73	0.36	0.076
Lower	19	0.49	0.103
Upper	54	0.30	0.067
Bonneville	25	0.16	0.039
The Dalles	24	0.42	0.096
John Day	5	0.40	0.029

It is unlikely that additional samples would have increased the detection of novel mtDNA genotypes in either river system (given the enzymes used in this study). The combinatorial analysis estimated that a minimum sample size of 33–39 individuals is sufficient to fully detect mtDNA diversity in both river systems (Fig. 6). Samples for the Columbia ($n = 73$) and Fraser ($n = 105$) rivers were considerably larger than this estimate. The consistently fewer number of genotypes detected over a range of sample sizes in the Columbia River, relative to the Fraser River, is further evidence of lower genetic diversity in the Columbia River.

The mtDNA diversity of both river samples combined ($h = 0.65$) is near the lower estimates for natural populations of Atlantic salmon (*Salmo salar*) ($h = 0.60$) and brown trout (*Salmo trutta*) ($h = 0.70$ – 1.0 ; Gyllensten and Wilson 1987). In these same salmonids, mtDNA diversity levels determined for hatchery populations ($h = 0.0$ – 0.02) founded by small maternal groups approached the diversity estimate of the entire upper Columbia River sturgeon population ($h = 0.30$) and, in particular, the Bonneville reservoir sample ($h = 0.17$).

There are two possible explanations for higher mtDNA diversity in sturgeon populations of the recolonized Fraser River relative to

the Columbia River refugium. First, migrants from another southern population may have significantly added to the Fraser River mtDNA gene pool. However, with the exception of the Sacramento River, other Pacific Coast rivers do not support substantial sturgeon populations. The single individual from the Sacramento River examined had a unique, but closely related genotype, not found in either of the more northerly rivers.

The second, and more plausible, explanation is that although the Columbia River was the source of present mtDNA lineages in the Fraser River, postglacial events have lowered mtDNA diversity in the Columbia River population. Recent overexploitation and habitat destruction could have greatly reduced genetic diversity in sturgeon. Semakula and Larkin (1968) documented a precipitous decline in the commercial catch of sturgeon on the Fraser River from over 2 500 000 kg in 1897 to below 44 000 kg by 1916. Columbia River stocks suffered a parallel decline over the same time period (Galbreath 1985).

Habitat changes have occurred in both river systems but have been more extensive in the Columbia River basin. Hydroelectric dams built on the Columbia River in the 1930's and 1950's have probably caused major alterations in sturgeon migration patterns and spawning areas. The abrupt appearance of these barriers over a period of only a few years may have been responsible for the observed segregation of certain genotypes in areas either upstream or downstream of the Bonneville Dam.

In 1912, an accidental rock slide blocked the Fraser River at Hell's Gate, about 200 km from the mouth. mtDNA diversity was reduced in the sample collected above Hell's Gate ($h = 0.22$) relative to the lower Fraser River ($h = 0.72$). This intrariver difference in diversity estimates is similar to that observed in the Columbia River between sturgeon populations located above and below the Bonneville Dam.

Similar patterns of lower genetic diversity in recently isolated, freshwater populations have been observed in other fish species. A freshwater *S. trutta* population trapped above a waterfall had lower heterozygosity at protein loci compared with an anadromous

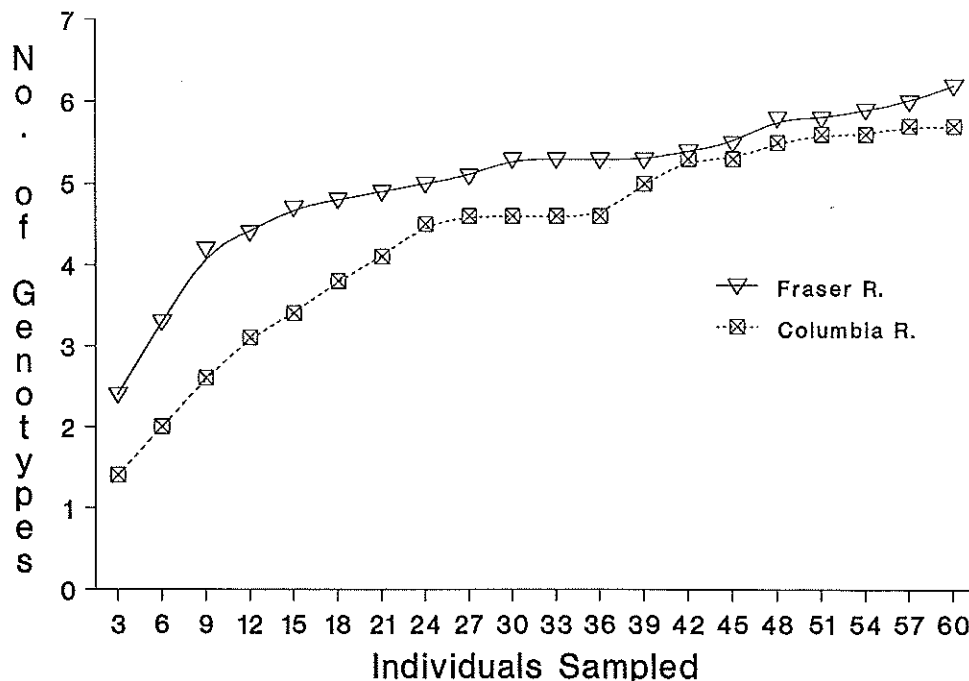


FIG. 6. Sample size and detection of *A. transmontanus* genotypes in the Fraser and Columbia rivers. Each data point represents the mean of 10 random subsamples.

population found in the same river basin (Hindar et al. 1991). Genetic differentiation between landlocked and anadromous forms was significantly greater than that between sympatric *S. trutta* populations with different life histories traits. Australian populations of *Galaxias ruttaceus* landlocked in lakes had lower mtDNA diversity than stream populations with access to estuaries and the sea (Ovenden and White 1990). The recent formation of these Australian lakes (3000–7000 yr ago) was suggested as a severe but transient bottleneck which reduced mtDNA diversity.

There is no apparent divergence between mtDNA genotypes found above Bonneville Dam and those occurring in the lower Columbia River and the Fraser River. The probability of detecting nucleotide changes so soon after the occurrence of a genetic bottleneck is extremely low (Wilson et al. 1985). However, a steep reduction in mtDNA or genetic diversity has occurred in the landlocked sturgeon population.

Genetic drift in isolated demes is unlikely to have caused a reduction in mtDNA diversity in upper Columbia River sturgeon populations because fewer than four consecutive generations have passed since the closure of the Bonneville Dam. Lower mtDNA diversity in the upper Columbia River is most probably the result of selective pressures for individuals which can reproduce in the landlocked, freshwater habitat. This is not to suggest that selection is acting at the mtDNA level, but that mtDNA genotypes are neutral markers associated with surviving maternal lineages.

Human pressures upon populations can be highly selective and result in a net reduction in genetic diversity (Nelson and Soule 1987). Dams on the Columbia River may have reduced sturgeon spawning success by denying lower river sturgeon access to upstream spawning grounds. Upstream sturgeon populations are similarly excluded from estuaries that have greater food productivity. Also the dams have greatly altered natural current velocities that may be critical to sturgeon spawning success (Doroshov 1985; Brannon et al. 1985).

If particular groups of females with synchronous migration patterns are distinguishable by mtDNA genotype, the present-day distribution of mtDNA genotypes could be indicative of the location of these maternal cohorts when the dams were closed. The presence of two genotypes (At3 and At6) in both the Fraser and upper Columbia rivers, and their absence from the lower Columbia River, proves that upper Columbia sturgeon once migrated throughout the basin and into marine coastal waters.

Any reduction in genetic diversity in sturgeon populations should be of special concern in the conservation of this species given its long generation time and advanced age of reproductive maturity. The endangered or threatened status of two thirds of the North American sturgeon indicates that this ancient group has not fared well in recent times (Williams et al. 1989).

Previous evolutionary studies of intraspecific mtDNA variation have been mainly concerned with the phylogeographical relationship of mtDNA genotypes as a reflection of the influence of natural geographical events on population structure. We suggest that human intervention in animal populations can also produce detectable shifts in mtDNA genotypic frequencies over a relatively short time.

Acknowledgements

We thank L. Beckman, U.S. Fish and Wildlife Service; G. Edmundson and D. Lane, Malaspina College; V. Swiatkiewicz, British Columbia Ministry of Environment; and C. Tracy, Washington Department of Fisheries; and the many sport fishermen for providing sturgeon livers. We also thank L. Bernatchez and S. Palumbi for their comments on the manuscript. This research was supported by Simon Fraser University Special Research Project and Natural Sci-

ences and Engineering Research Council of Canada grants to A.T.B. and M.J.S. A British Columbia Science Council Graduate Research and Engineering and Technology Award provided funding to J.R.B.

References

- ASHLEY, M., AND C. WILLS. 1987. Analysis of mitochondrial DNA polymorphisms among Channel Island deer mice. *Evolution* 41: 854–863.
- BENTZEN, P., G. G. BROWN, AND W. C. LEGGETT. 1989. Mitochondrial DNA polymorphism, population structure, and life history variation in American shad (*Alosa sapidissima*). *Can. J. Fish. Aquat. Sci.* 46: 1446–1454.
- BERNATCHEZ, L., J. J. DODSON, AND S. BOIVIN. 1989. Population bottlenecks: influence on mitochondrial DNA diversity and its effect in coregonine stock discrimination. *J. Fish. Biol.* 233–244.
- BINKOWSKI, F. P., AND S. I. DOROSHOV [ED.]. 1985. North American sturgeons: biology and aquaculture potential. Dr. W. Junk, Dordrecht, The Netherlands. 163 p.
- BOWEN, B. W., AND J. C. AVISE. 1990. Genetic structure of Atlantic and Gulf of Mexico populations of sea bass, menhaden, and sturgeon: influence of zoogeographic factors and life history patterns. *Mar. Biol.* 107: 371–381.
- BRANNON, E., S. BREWER, A. SETTER, M. MILLER, F. UTTER, AND W. HERSHBERGER. 1985. Columbia River white sturgeon (*Acipenser transmontanus*) early life history and genetics study. Bonneville Power Administration, Division of Fish and Wildlife, Portland, OR.
- BROWN, J. R. 1991. Molecular evolution and population genetics of sturgeon (genus *Acipenser*) based on mitochondrial DNA analysis. Ph.D. dissertation, Simon Fraser University, Burnaby, B.C. 191 p.
- BROWN, J. R., T. L. GILBERT, D. J. KOWBEL, P. J. O'HARA, N. E. BUKOKER, A. T. BECKENBACH, AND M. J. SMITH. 1989. Nucleotide sequence of the apocytochrome B gene in white sturgeon mitochondrial DNA. *Nucleic Acids Res.* 17: 4389.
- BROWN, W. M. 1985. Evolution of animal mitochondrial DNA, p. 62–88. *In* M. Nei and R. K. Koehn [ed.] *Evolution of genes and proteins*. Sinauer, Sunderland, MA.
- BUKOKER, N. E., J. R. BROWN, T. A. GILBERT, P. J. O'HARA, A. T. BECKENBACH, W. K. THOMAS, AND M. J. SMITH. 1990. Length heteroplasmy of sturgeon mitochondrial DNA: an illegitimate elongation model. *Genetics* 124: 157–163.
- CARLSON, D. M., M. K. KETTLER, S. E. FISHER, AND G. S. WHITT. 1982. Low genetic variability in paddlefish populations. *Copeia* 3: 721–725.
- CLAGUE, J. J., AND J. L. LUTERNAUER. 1983. Late quaternary geology of southwestern British Columbia. Field Trip 6. Geological Association of Canada, Victoria Section, Victoria, B.C.
- DIXON, B. N. 1986. Age, growth and migration of white sturgeon in the Nechako and upper Fraser rivers of British Columbia. B.C. Minist. Environ. Fish. Tech. Circ. 70.
- DIXON, W. J. 1983. BMDP statistical software. University of California Press, Berkeley, CA.
- DOROSHOV, S. I. 1985. Biology and culture of sturgeon *Acipenseriformes*, p. 251–274. *In* J. F. Muir and R. J. Roberts [ed.] *Recent advances in aquaculture*. Croom Helm, London.
- FELSENSTEIN, J. 1990. PHYLIP manual 3.3. University Herbarium, University of California, Berkeley, CA.
- GALBREATH, J. L. 1985. Status, life history and management of Columbia River white sturgeon, *Acipenser transmontanus*, p. 119–125. *In* F. P. Binkowski and S. I. Doroshov [ed.] *North American sturgeons: biology and aquaculture potential*. Dr. W. Junk, Dordrecht, The Netherlands.
- GARDINER, B. G. 1984. Sturgeon as living fossils, p. 148–152. *In* N. Eldredge and S. M. Stanley [ed.] *Living fossils*. Springer Verlag, New York, NY.
- GILBERT, T. L., J. R. BROWN, P. J. O'HARA, N. E. BUKOKER, A. T. BECKENBACH, AND M. J. SMITH. 1988. Sequence of tRNA^{Thr} and tRNA^{Pro} from white sturgeon (*Acipenser transmontanus*) mitochondria. *Nucleic Acids Res.* 16: 11825.
- GYLLENSTEN, U., AND A. C. WILSON. 1987. Mitochondrial DNA of salmonids: inter- and intraspecific variability detected with restriction enzymes, p. 301–317. *In* N. Ryman and F. Utter [ed.] *Population genetics and fishery management*. University of Washington Press, Seattle, WA.
- HAYNES, J. M., AND R. H. GRAY. 1981. Diel and seasonal movements of white sturgeon, *Acipenser transmontanus*, in the mid-Columbia River. *Fish. Bull.* 79: 367–370.
- HAYNES, J. M., R. H. GRAY, AND J. C. MONTGOMERY. 1978. Seasonal movements of white sturgeon (*Acipenser transmontanus*) in the mid-Columbia River. *Trans. Am. Fish. Soc.* 107: 275–280.
- HEBERT, P. D. N., R. D. WARD, AND L. J. WEIDER. 1988. Clonal-diversity patterns and breeding-system variation in *Daphnia pulex*, an asexual-sexual complex. *Evolution* 42: 147–159.

- HINDAR, K., B. JONSSON, N. RYMAN, AND G. STAHL. 1991. Genetic relationships among landlocked, resident, and anadromous brown trout, *Salmo trutta* L. *Heredity* 66: 83-91.
- LANSMAN, R. A., R. O. SHADE, J. F. SHAPIRA, AND J. C. AVISE. 1981. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations: III. Techniques and potential applications. *J. Mol. Evol.* 17: 214-226.
- LAUDER, G. V., AND K. F. LIEM. 1983. The evolution and interrelationships of the Actinopterygian fishes. *Bull. Mus. Comp. Zool.* 150: 95-197.
- MCPHAIL, J. D., AND C. C. LINDSAY. 1986. Zoogeography of the freshwater fishes of Cascadia (The Columbia system and rivers north of the Stikine), p. 615-638. *In* C. H. Hocutt and E. O. Wiley [ed.] *The zoogeography of North American freshwater fishes*. John Wiley and Sons, New York, NY.
- MILLER, L. W. 1972a. White sturgeon population characteristics in the Sacramento - San Joaquin estuary as measured by tagging. *Calif. Fish Game* 58: 94-101.
- 1972b. Migrations of sturgeon tagged in the Sacramento - San Joaquin estuary. *Calif. Fish Game* 58: 102-106.
- NEI, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York, NY. 512 p.
- NEI, M., AND W-H. LI. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. USA* 76: 5269-5273.
- NEI, M., J. C. STEPHENS, AND N. SAITOU. 1985. Methods for computing the standard errors of branching points in an evolutionary tree and their application in molecular data from humans and apes. *Mol. Biol. Evol.* 2: 66-85.
- NEI, M., AND F. TAJIMA. 1981. DNA polymorphisms detectable by restriction endonucleases. *Genetics* 97: 145-163.
1983. Maximum likelihood estimation of the number of nucleotide substitutions from restriction sites data. *Genetics* 105: 207-217.
- NELSON, K., AND M. SOULÉ. 1987. Genetical conservation of exploited fishes, p. 345-368. *In* N. Ryman and F. Utter [ed.] *Population genetics and fishery management*. University of Washington Press, Seattle, WA.
- OVENDEN, J. R., AND R. W. G. WHITE. 1990. Mitochondrial and allozyme genetics of incipient speciation in a landlocked population of *Galaxias truttaceus* (Pisces: Galaxiidae). *Genetics* 124: 701-716.
- PHELPS, S. R., AND F. W. ALLENDORF. 1983. Genetic identity of pallid and shovelnose sturgeon (*Scaphirhynchus albus* and *S. platyrhynchus*). *Copeia* 3: 696-700.
- RAND, D. M., AND R. G. HARRISON. 1989. Molecular population genetics of mtDNA size variation in crickets. *Genetics* 121: 551-569.
- ROUSSOW, G. 1957. Some considerations concerning sturgeon spawning periodicity. *J. Fish. Res. Board Can.* 14: 553-572.
- SCOTT, W. B., AND E. J. CROSSMAN. 1973. *Freshwater fishes of Canada*. Bull. Fish. Res. Board Can. 184.
- SEMAKULA, S. N., AND P. A. LARKIN. 1968. Age, growth, food and yield of the white sturgeon (*Acipenser transmontanus*) of the Fraser River, British Columbia. *J. Fish. Res. Board Can.* 25: 2589-2602.
- SLATKIN, M. 1987. Gene flow and the geographic structure of natural populations. *Science (Wash., DC)* 236: 787-792.
- SMITH, G. R. 1981. Late cenozoic freshwater fishes of North America. *Annu. Rev. Ecol. Syst.* 12: 163-193.
- WARD, R. D., N. BILLINGTON, AND P. D. N. HEBERT. 1989. Comparison of allozyme and mitochondrial DNA variation in populations of walleye, *Stizostedion vitreum*. *Can. J. Fish. Aquat. Sci.* 46: 2074-2084.
- WILLIAMS, J. E., J. E. JOHNSON, D. A. HENDRICKSON, S. CONTRERAS-BALDERAS, J. D. WILLIAMS, M. NAVARRO-MENDOZA, D. E. MCALLISTER, AND J. E. DEACON. 1989. Fishes of North America endangered, threatened or of special concern: 1989. *Fisheries* 14: 2-20.
- WILSON, A. C., R. L. CANN, S. M. CARR, M. GEORGE, U. B. GYLLENSTEN, K. M. HELM-BYCHOWSKI, R. G. HIGUCHI, S. R. PALUMBI, E. M. PRAGER, R. D. SAGE, AND M. STONEKING. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. *Biol. J. Linn. Soc.* 26: 375-400.
- WILSON, G. M., W. K. THOMAS, AND A. T. BECKENBACH. 1987. Intra- and interspecific mitochondrial DNA sequence divergence in *Salmo*: rainbow, steelhead, and cutthroat trout. *Can. J. Zool.* 63: 2088-2094.

Appendix A

TABLE A.1. DNA fragment lengths (in base pairs) for the restriction enzymes used in this study. Species are *A. transmontanus* (A.t.) and *A. medirostris* (A.m.). Intraspecific polymorphic DNA fragment patterns are denoted by the letter codes (A, B, or C) used in Table 2. Variable length fragments known to span the control region are indicated by an asterisk.

<i>Apa</i> I		<i>Eco</i> RI		<i>Hind</i> III		<i>Bgl</i> II		<i>Pvu</i> II		<i>Xba</i> I	
A.T.	A.m.	A.t.	A.m.	A.t.	A.m.	A.t.	A.m.	A.t.	A.m.	A.t.	A.m.
7200	7200	8400	8600	12 000	12 000	16 500	16 500	16 500	10 000	11 000	8200
5400	5400	8200	7600	3 000	3 000				6 400	4 700	4700
											2800
4300	4300			1 100	1 100					1 100	1100

<i>Acc</i> I			<i>Hae</i> II		<i>Ava</i> II			<i>Bcl</i> I		
A.t.-A	A.t.-B	A.m.	A.t.	A.m.	A.t.-A	A.t.-B	A.m.	A.t.-A	A.t.-B	A.m.
4600*	4600*	4600*	15 000	15 000	6200	4000	8300*	8000	8000	4800
3600	2800	2500	1 800*	1 800*	2700*	2700*	1700	2500	2500	4800*
1900	1900	1900			2100	2200	1600	2300	2300	2500
1750	1750	1750			1400	2100	1100	1800*	1370	2300
1550	1550	1550			1000	1400	1000	750	900*	750
1300	1300	1300			760	1000	760	680	820	680
1100	1100	1100			620	760	620			
560	800	1100			560	620	560			
500	560	560			520	560	520			
	500	500			400	520	400			
					340	400	340			
						340				

<i>Ava</i> I				<i>Hinc</i> II			
A.t.-A	A.t.-B	A.t.-C	A.m.	A.t.-A	A.t.-B	A.m.	
6600	11 000	11 000	6600	3700	5000*	5000*	
5400	5 400	3 400	5400	2700	3700	3700	
4600		2 000	4600	2500	2700	2700	
				2500*	1400	1400	
				1400	1400	1100	
				1400	1100	940	
				1100	580	580	
				580	450	480	
				450		450	