

### Chapter 3

Population structure and mitochondrial DNA diversity of North American white sturgeon  
(*Acipenser transmontanus*): An empirical expansive gene flow model

Paul J. Anders

*Center for Salmonid and Freshwater Species at Risk,  
University of Idaho, Moscow, Idaho, 83844-2260, USA*

Madison S. Powell

*Center for Salmonid and Freshwater Species at Risk,  
University of Idaho, Hagerman Fish Culture Experiment Station,  
3059 F. National Fish Hatchery Road, Hagerman, Idaho, 83332, USA*

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*Abstract* - Due to their evolutionary significance and the dramatic extent of habitat alteration and degradation globally, sturgeons (Acipenseridae) have become the subject of intense conservation efforts. Despite recent phylogenetic reconstruction of Acipenseriformes, past and contemporary gene flow between and among white sturgeon populations (*Acipenser transmontanus*), as well as many aspects of their population structure remain largely unknown. We sequenced a hypervariable 453 bp. segment of the *A. transmontanus* mitochondrial control region to: 1) characterize mitochondrial sequence diversity and divergence, population structure, and phylogenetic assemblages from a major portion of the species' geographic range; 2) quantify gene flow within and among major rivers across this range; and 3) assess whether population structure was best explained by isolation by distance, stepping stone, or by continent-island models of dispersal and gene flow, or alternative models. White sturgeon samples (n=260) were collected from 20 fish at each of 13 locations from six major rivers in western North America (Columbia, Snake, Kootenai, Fraser, Nechako, and Sacramento). Concordant results (AMOVA, Monte Carlo  $X^2$ , NCA, PHYLIP, PAUP, REAP, linear regression) including observed  $F_{ST}$  and  $Nm$  values revealed that expansive gene flow over hundreds to thousands of kilometers best explained observed population structure. Observed population structure likely resulted from a mosaic of range expansions and contractions in conjunction with geographically and temporally variable gene flow over evolutionary time. We present and discuss the "expansive gene flow model" (EGF) to explain observed population structure of white sturgeon in western North America. The EGF model was strongly supported by empirical white sturgeon genetic, dispersal, life history, and reproductive data.

## Introduction

Acipenseriformes (sturgeons and paddlefishes) occupy a unique position in the evolution of fishes (Bemis et al. 1997). Fossil records suggest that Acipenseriformes (sturgeons and paddlefishes) and Acipenseridae (sturgeons) date from the Lower Jurassic (~200 Mya) and upper Cretaceous periods (>100 Mya) respectively, and that both extinct and extant forms appear exclusively Holarctic (Patterson 1982; Gardiner 1984; Bemis et al. 1997; Bemis and Kynard 1997). Worldwide diversity of sturgeons and paddlefishes is currently threatened, as evident by the extirpation of some European and Asian forms, and the concurrent depletion of many North American Acipenserids (Rieman and Beamesderfer 1990; Birstein 1993; Waldman 1995; Wirgin et al. 1997; Birstein et al. 1997a, 1997b; Khodorevskyaya et al. 1997; Kryhtin et al. 1997; Wei et al. 1997; Campton et al. 2000). With few exceptions, population abundance of most *Acipenser* species, including many populations of North American taxa, is currently at historically low levels (Wirgin et al. 1997; Beamesderfer and Farr 1997). Recent phylogenetic reconstructions using morphological, anatomical, and molecular genetic data have contributed to an improved, yet incomplete understanding of the unique evolutionary history of Acipenseriformes (Bemis et al. 1997; Birstein et al. 1997b; Findeis 1997; Birstein and Bemis 1997).

Like many sturgeon species worldwide, white sturgeon (*Acipenser transmontanus*) constitute an economically, ecologically, recreationally, and culturally valuable resource (Anders and Powell 1999). Because of these characteristics, their evolutionary significance, and the dramatic extent to which their habitats have been altered and degraded globally, sturgeons have become the subject of intense conservation efforts (Campton et al. 2000 Birstein et al. 1997a, and references therein, Birstein 1993). White sturgeon and their most closely related sister taxon green sturgeon (*A. medirostris*) are generally sympatric, and endemic to the North American Pacific Coast and large coastal river systems west of the Rocky Mountain continental divide from central California north to the Gulf of Alaska and the Aleutian Islands (Scott and Crossman 1973; Wydowski and Whitney 1979; Moyle and Cech 1996). Individual white sturgeon have been observed as far south as Ensenada Mexico, but did not appear to represent spawning populations (Moyle 1976). In addition to general sympatry with

*A. transmontanus* along the Pacific Coast of North America, *A. medirostris* also occurs in Asian marine waters off Japan, Korea, and Sakhalin (Wydowski and Whitney 1979).

Currently, reference to white sturgeon “populations” is simply a convenient way to describe groups of fish in particular geographic areas. Contemporary gene flow between and among populations or locations has not been well-characterized (Brown et al. 1993). In addition, appropriate biological and ecological data needed to accurately define white sturgeon populations and putative population structure remain inadequate. Miller et al. (1995) reported that population status and recruitment success of *A. transmontanus* varied considerably across the Columbia River Basin. More recently, Parsley et al. (*In Press*) described white sturgeon population sizes and trends as high and generally stable in the lower Columbia River, low to moderate but variable, and relatively stable in the middle reaches, and low to negligible in upper reaches of the Basin, where population trends were either unknown or generally declining.

In 1990, the Canadian government classified white sturgeon as a Species of Special Concern (COSEWIC 2001). Subsequently, the provincial government of British Columbia listed white sturgeon in British Columbia as imperiled including them on the Red List (Harper et al. 1994). White sturgeon populations in the impounded Columbia River reaches in British Columbia were also listed as endangered/critically imperiled, largely due to poor survival of early life stages, or recruitment failure (Hildebrand et al. 1999). The Kootenay/i population, occupying BC, Idaho and historically Montana waters was federally listed as endangered under the US Endangered Species Act in 1994 (USFWS 1994). Conversely, the Sacramento River population has fluctuated in size during the past several decades due to infrequently strong year classes and highly variable natural recruitment, but appeared viable (Schaffter and Kohlhorst 1999).

Effective conservation and management strategies for endemic fish populations require functional knowledge of historical and contemporary population structure of the managed species. Genetic techniques can generate valuable information concerning such structure, and provide a standardized, reproducible means of evaluation to complement ongoing

biological and ecological investigation. Mitochondrial DNA (mtDNA) regions possess genetic markers that can provide information about historical population genetic structure and contemporary patterns of gene flow within and among areas, or populations (Awise 1994). Geographic distribution and relationships among white sturgeon mtDNA haplotypes can provide important insight into current and historical population structure (i.e. phylogeography, Awise et al. 1987) and mechanisms responsible for such structure. Such insight is currently incomplete, but remains crucial for successful white sturgeon conservation and management. White sturgeon stock delineation research using protein allozymes (Setter and Brannon 1992; Bartley et al. 1985) and mtDNA sequencing (Brown et al. 1992) from a relatively limited number of samples and sampling locations suggested differences in white sturgeon allele and haplotype frequencies across geography.

White sturgeon are the largest, longest-lived fishes inhabiting North American freshwater systems (Wydowski and Whitney 1979; Scott and Crossman 1973). Unlike salmonid fishes, white sturgeon do not require specific physiological changes (smoltification) prior to entering saltwater and can freely migrate between freshwater and saltwater environments or remain in estuarine habitat for prolonged periods (DeVore et al. 1999). Empirical data have confirmed their ability to migrate in excess of 2,500 km within, between, and among major river systems of western North America (DeVore et al. 1999; ODFW 1996). However, all sturgeons spawn exclusively in freshwater (Bemis and Kynard 1997).

Extensive development of hydropower dams throughout the Columbia River Basin during the past century has severely fragmented free-flowing, large river habitats (National Research Council 1996) occupied by white sturgeon. Conversely, dams have not fragmented white sturgeon habitats in the mainstem Sacramento and Fraser rivers. One important result of such river fragmentation for white sturgeon is the creation of a series of relatively isolated sub-populations (Secor et al. *In press*, Jager et al. 2001, Jager et al. 2000). This is especially critical for a migratory species like *A. transmontanus*, where fragmentation by dams may artificially impose exclusively downstream migration and subsequent gene flow. Altered seasonal river discharge and thermal regimes resulting from impoundment and dam

operations may also alter migration and limit habitat availability (Cooke et al., *In Press*; Jager et al. *In Press*; Secor et al. *In press*; Auer 1996).

To address these critical uncertainties, comprehensive white sturgeon genetic research at an appropriately large geographic scale with adequate geographic representation is urgently needed to characterize white sturgeon population structure and its underlying formative mechanisms. Such data can then be used to assess effects of fragmentation on *A.*

*transmontanus* population structure, viability, and persistence. Accordingly, the objectives of this study were to: 1) characterize white sturgeon mitochondrial sequence diversity and divergence, population structure, and phylogenetic assemblages from a major portion of the species' geographic range; 2) quantify gene flow within and among major river basins across this range; and 3) determine if white sturgeon population structure is best explained by isolation by distance, stepping stone, or continent-island models of dispersal and gene flow, or by alternative models.

## Methods

### Sample collection

White sturgeon samples (pectoral fin clips and blood samples; n=260) were collected from 20 fish each at a total of 13 study sites from six major rivers in western North America (Columbia, Snake, Kootenai, Fraser, Nechako, and Sacramento, Table 1, Figure 1). Tissue samples were stored in liquid lysis buffer (EDTA 0.5 M, 2 M Tris, % M NaCl, SDS 20%, dH<sub>2</sub>O). Blood samples were frozen on dry ice in the field and stored at -20°C at the lab. Sampling sites were chosen to represent putative white sturgeon genetic diversity and variation across much of the species' range. Sampling sites outside the Columbia River Basin also enabled comparisons of white sturgeon genetic diversity and populations structure between impounded (Columbia, Snake, Kootenai, Nechako) and unimpounded (Fraser, Sacramento) rivers.

## Mitochondrial DNA

Genomic DNA was isolated from individual white sturgeon using standard chloroform-isoamyl alcohol extraction procedures outlined in Hillis et al. (1996). PCR reactions were performed using published primers L185 (5' -CATCTACCATTAATGTTATAC-3') and H740 (5'-ATCAAGGTATGTCGATGACA-3') to amplify a 453 bp section of the white sturgeon mtDNA control region (Brown et al. 1993). PCR reactions (40 µl final volume) contained 1 µl of extracted genomic DNA, 1 µl 100% DMSO, 3.20 µl 100 mM BSA, 4 µl Perkin-Elmer 10X PCR Buffer II, 4 µl 25 mM MgCl<sub>2</sub>, 0.8µl of each primer (10 mM concentration), and 200µm of each dNTP (10 µm and 0.10 µl Perkin-Elmer Amplitaq DNA polymerase (0.25 units/µl). Amplifications were performed using an MJ Research PTC-100 thermal cycler. The thermal cycle program consisted of 4 min at 94° C, followed by 35 cycles of 94° C for 1 min, 50° C for 2 min and 30 sec, and an extension step at 72° C for 2 min 30 sec. A final elongation step at 72° C for 4 min was attached to the final cycle. Amplified products were treated using standard shrimp alkaline phosphatase pre-sequencing purification techniques. Bases were called and aligned using Sequencher™ software (Gene Codes Corp. Ann Arbor MI.). Amplified products were electrophoresed in 2.9% low-melting-point TBE gels and run at 100 V. for 25 minutes. Gels were buffered in 750 ml of TBE with 37.5 µl of 5 % ethidium bromide added to visualize PCR-amplified DNA fragments. PCR products were sequenced using Big Dye Terminator sequencing chemistry and an Applied Biosystems, Inc. Model 377 automated DNA sequencer. Each sample representing a unique mtDNA sequence was re-extracted, re-amplified, and re-sequenced to confirm haplotype designation.

## Data analysis

*Regressions* - Simple linear regressions were performed using Microsoft XL and Statistical Analysis Software (SAS 1991) to assess various aspects of white sturgeon genetic and geographic population structure. Specifically, regression analysis was used to investigate the relationships between: 1) the number of haplotypes, haplotype diversity, and geographic distance from the Pacific Ocean (minimum inland distance, km); 2) mean percent sequence

difference among individuals within a site and minimum inland distance for each site; and 3) estimates of gene flow ( $F_{ST}$ ,  $N_m$ ) and nucleotide divergence and geographic distance between all paired study sites.

*Haplotype frequency tests* - Haplotype frequency distribution comparisons between all paired study sites were performed using Monte-Carlo simulations (10,0000 iterations) of the exact, weighted, Pearson Chi-Square ( $X^2$ ) test (SAS 1991). The following null hypotheses regarding population structure of white sturgeon in western North America were tested ( $P \leq .05$ ) using the above procedure:

H<sub>0</sub>1: White sturgeon mtDNA haplotype frequency distributions from all 13 sampling sites in western North America are not significantly different.

H<sub>0</sub>2: White sturgeon mtDNA haplotype frequency distributions from 6 large Pacific Coast river systems in western North America are not significantly different.

H<sub>0</sub>3: White sturgeon mtDNA haplotype frequency distributions between all paired study sites in western North America are not significantly different.

*Phylogenetic analyses* - A nucleotide substitution matrix of genetic distance ( $d$  values) between all haplotype pairs was generated from mtDNA sequence data using the two-parameter model of Kimura (1980) in the "K" program in REAP (Restriction Enzyme Analysis Package, Version 4.0, McElroy et al. 1991). This matrix, along with haplotype frequency data from all sites, provided input data to estimate haplotype and nucleotide diversity of mtDNA within sites and nucleotide divergence ( $D_A$ ) between all paired sites, using the DA program in REAP (McElroy et al. 1991). The KITCH program in PHYLIP 3.5 (Felsenstein 1989), which assumes independence and equal rates of divergence, was then used to generate UPGMA, neighbor-joining, and parsimony population trees to illustrate evolutionary relationships of white sturgeon. Haplotype trees were generated using PAUP software (Swofford 1998), involving a full heuristic search with 1000 iterations. An analogous 453 bp mtDNA control region sequence from green sturgeon (*A. medirostris*, GenBank accession number ) was used as an outgroup for all phylogenetic analyses.

*AMOVA* - White sturgeon genetic population structure was evaluated using the nested analysis of molecular variance program (AMOVA, Excoffier et al. 1992). AMOVA input involved a Euclidean distance matrix containing absolute genetic distance values for all haplotype pairs. AMOVA hierarchically partitioned white sturgeon mtDNA sequence variation into three strata: variability among geographic regions, among sites, and within sites. Samples were stratified by the 13 sampling sites, and nested within geographic regions. Three, five, and six geographic region designations were tested to identify potential effects of region classification on partitioning of white sturgeon genetic variability (Table 2). Significance of variance components was tested using 10,000 random permutations to generate null distributions for each variance component (Excoffier et al. 1992).

*Haplotype network* - To further illustrate relationships among haplotypes, a minimum spanning network was generated using absolute genetic distance values from all paired haplotypes, calculated by the MINSPNET software program (Excoffier 1993). Major river basins (Columbia, Fraser, Sacramento) from which samples were collected were indicated for all haplotypes in the network to illustrate potential geographic population structure.

*Gene Flow* - Gene flow was estimated using the product  $N_m$  (number of individuals exchanged between populations per generation; Avise 1994).  $N_m$  estimates were derived from Wright's F-statistics (1951), which showed that for neutral alleles in an island model equilibrium expectations are:

$$\text{(Eq. 1)} \quad F_{ST} = 1 / (1 + 4 N_m)$$

Solving equation 1 for  $N_m$  yields:

$$\text{(Eq. 2)} \quad N_m = (1 - F_{ST}) / 4 F_{ST}$$

Phi statistics ( $\theta$ ) generated by the AMOVA analysis were substituted into Equation 2 as  $F_{ST}$  analogues (Weir 1996) to calculate pairwise  $N_m$  values for white sturgeon from all paired study sites. White sturgeon  $F_{ST}$  values were calculated using Equation 1.

*NCA* - Nested clade analysis (*NCA*) provides a methodology to separate genetic population structure from population history (Templeton et al. 1992; Posada et al. 2000). Nested clade analysis was performed to investigate white sturgeon phylogeography using the GEODIS program (Templeton 1993; Templeton et al. 1995; Posada et al. 2000). We performed a two-part *NCA* using GEODIS (Version 2.0, Posada et al. 2000) as reported by Good and Sullivan (2001), which first involved a  $X^2$  contingency test of geographic association of haplotypes. This test calculated a  $X^2$  statistic from a contingency table that was generated by treating haplotype composition of each clade and the geographic locations as categorical values. Unlike Good and Sullivan (2001), we generated and used a matrix of intersite geographic distances rather than using latitude and longitude data because white sturgeon migration and subsequent gene flow are linear rather than multidirectional across terrestrial landscape. Statistical significance was tested by comparing the observed  $X^2$  statistic with the null generated by 10,000 permutations of haplotypes randomly assigned to locations. Secondly, a geographic distance analysis was performed, also using the GEODIS program, which calculated clade distance ( $D_c$ ) and nested clade distance ( $D_n$ ). Finally, the *NCA* tested for geographic association within and among the resulting clades (Templeton et al. 1995; Templeton 1998).

## Results

### White sturgeon mtDNA sequence diversity and distribution

Sequence analysis revealed twenty-six unique mtDNA sequences, subsequently referred to as haplotypes (GenBank accession numbers pending; Table 3). Thirty-three variable sites were observed, including 28 transitions, 2 transversions, and 3 indels (Appendix Table A).

The most common haplotype (H1) was represented by 4-19 of the 20 fish from each of the 13 study sites. Of the remaining 25 haplotypes, 16 were represented by 2-13 fish, and 9 by a

single fish (Figure 2). The two most common haplotypes (H1 and H2) were represented by 165 (64%) of the 260 fish sequenced, and were observed at 13 and 11 study sites respectively. The four most common haplotypes accounted for 198 of the 260 fish sequenced (76%), and were observed at 100%, 85%, 54% and 54% of the 13 sampling sites respectively. The remaining 22 were observed at 1-5 sites.

Common haplotypes were widely distributed among the 13 sampling sites. Two to eleven haplotypes were observed at each of the 13 study sites (Table 3). Total numbers of haplotypes at each study site were negatively correlated with inland distance from the Pacific Ocean in all river systems studied ( $r^2 = 0.67$ ). Eleven haplotypes were observed at the Columbia River estuary (CRE), adjacent to the Pacific Ocean, and centrally located within the species' geographical range. Likewise, the other three study sites with unimpounded access to the Pacific Ocean had a comparatively large number of haplotypes (LFR 9 haplotypes, LCR 8 haplotypes, SAC 7 haplotypes). Conversely, only two haplotypes were observed in each of two distant inland sampling sites (KL 1270 km, KR 1400 km), for a total of three in the Kootenai River/Kootenay Lake system. In this system, located in the Upper Columbia River Basin (Figure 1), eighteen of 20 (90%) Kootenay Lake fish, and 19 of 20 (95%) Kootenai River fish shared H1, the most numerically and geographically predominant haplotype. Two private haplotypes were observed in the Nechako River (H13, n=4; H14, n=5), a Fraser River tributary in British Columbia, approximately 800 km upstream from the Pacific Ocean. All nine rare haplotypes (< 5% prevalence within a site, H5 H16, H12, H18, H19, H22, H23, H25, H26) were observed either near the Pacific Ocean (CRE, LFR, < 80 km inland) or far upriver (HC, CJS, 919-1350 km inland). Three of the nine (H5, H16, H12) were observed only in the Fraser River and were considerably diverged from H1, differing by 1.8-3.1% sequence difference, or 8-14 nucleotide substitutions (Figure 2). The remaining six (H18, H19, H29, H22, H23, H25, H26) were far less divergent, differing from H1 by one-three nucleotide substitutions (Figure 2), or 0.2-0.7% sequence difference.

Haplotype diversity ranged from 0.1 in the post-glacially isolated Kootenai River population to 0.86 in the Columbia River estuary (mean 0.67 for all sites, Table 4). Haplotype diversity was also negatively correlated with inland geographic distance ( $r = -0.65$ ). Mean haplotype

diversity among impounded sites (0.82) was greater than that among unimpounded sites (0.60). However, unimpounded sites were all in close proximity (0-250 km) to the Pacific Ocean, while impounded sites were located from 250-1,400 km inland. Thus, location of sites within river drainages may also have contributed to the observed difference in mean haplotype diversity between impounded and unimpounded sites. White sturgeon from the Nechako and Sacramento rivers exhibited haplotype diversities nearly identical to Columbia and Snake River fish (Table 4).

Nucleotide diversity ranged from 0.04 at KR to 0.72 for fish sampled from CRE, with a mean value of 0.51 for all sites (Table 4). Mean nucleotide diversity from impounded sites (0.39) was considerably lower than mean nucleotide diversity among unimpounded sites (0.79). As with the previous comparison of mean haplotype diversity between impounded and unimpounded sites, site location within river basins (inland distance) presumably contributed to this difference.

Eighteen of the 26 haplotypes (69%) differed from H1 by five or fewer nucleotide substitutions (Figure 2). Pairwise haplotype frequency comparisons revealed 0.2-4.2% sequence difference among all haplotypes (mean 1.57%; 1-19 nucleotide substitutions). Pairwise comparisons between an analogous sequence from the phylogenetic outgroup green sturgeon (*Acipenser medirostris*) and all white sturgeon haplotypes revealed 10.6 -11.7 % sequence difference between the two sturgeon species (47-53 substitutions, mean 50, 11% sequence difference). Consistent with the negative correlation between numbers of haplotypes per site and inland geographic distance, percent sequence difference among haplotypes within study sites was also negatively correlated with inland distance in all river systems studied ( $r^2 = 0.59$ ).

#### Population structure

Monte-Carlo  $X^2$  analysis resulted in rejection of all null hypotheses due to statistically significant differences in haplotype distribution comparisons: 1) among all 13 study sites as a group, 2) between all paired river systems, and 3) between 47 of the 78 paired study sites (59%), 11 at the  $P \leq 0.05$  level, and 36 at the  $P \leq 0.01$  level (Table 5,6,7). All pairwise river

system comparisons were significantly different ( $P < 0.05$ ,  $n=1$ ;  $P \leq 0.01$ ,  $n=14$ , Table 5), with the exception of the Columbia-Snake comparison when excluding the CJS dataset. The CJS site exhibited considerable variability, largely contributing to the statistically significant differences observed between the Snake and Columbia River sample groups when compared by region (Table 5).

Large river system geographic scales were generally required to produce significant differences in haplotype frequency distributions between paired study sites. Chi-square analysis revealed that mean geographic distances required for significant differences in haplotype frequency distributions between paired locations were 1,199 km (at  $P \leq 0.05$ , range 611-1865 km,  $n=10$ ) and 1,510 km (for  $P \leq 0.01$ , range 311-2,655 km,  $n=37$ , Table 6). However, geographic distances within this range did not necessarily result in significant differences. Nine of 15 (60%) of all pairwise haplotype frequency distribution comparisons between Columbia and Snake River sites were not significantly different, and were separated by  $\leq 959$  km (Table 6).

Haplotype frequency distribution comparisons between all paired sites also revealed that distributions at the LFR and NKO sites were significantly different than distributions from all other locations (Table 7). Similarly, haplotype frequency distributions from KR and KL were significantly different than those from all other large river systems sampled in western North America (Table 7). However, KR and KL haplotype frequency distributions were not significantly different ( $P = 0.48$ ; Table 7). The CJS haplotype frequency distribution was significantly different than those from all other locations except LGO and HC, which were the two most geographically proximate sites to CJS, and the only other Snake River sites sampled. Finally, the SAC haplotype frequency distribution was significantly different than those from all other sites, except CRE and LCR, which were the two most geographically proximate sites to the Sacramento River (Table 7).

Phylogenetic analysis

All UPGMA, neighbor-joining, parsimony, and PAUP generated population and haplotype trees consistently lacked meaningful clade structure and geographic or bootstrap support (figures not shown). However, all haplotype dendrograms contained the same 3 bifurcations, consistently giving rise to the same three haplotype pairs (H12-H22, H4-H16, H4-H7). Haplotypes 12 and 22 were strongly supported in haplotype dendrograms (bootstrap support > 97%), while the H4-H16 and H4-H7 pairs exhibited less support (bootstrap support 57-81%). These findings were corroborated by their placement in the haplotype network (Figure 2). Terminal clades in population dendrograms also consistently lacked geographic support (figures not shown).

#### AMOVA

Nested AMOVA analysis estimated that 87-90% of white sturgeon mtDNA sequence variability was partitioned into the smallest geographic scale (within-population), with less than 10% observed among geographic regions (Table 8). Similarly, AMOVA analyses among regions estimated that 88-96% of white sturgeon sequence variability was observed within regions (Table 8). Finally, AMOVA analyses among sites revealed that approximately 89% of sequence variability was observed within study sites, with less than 2% of that variation observed among sites (Table 8). Geographic region classifications had little effect on the partitioning of white sturgeon sequence variation in this study (Table 8).

#### Haplotype network

Consistent with the AMOVA results, superimposition of river basin of origin on all haplotypes in the minimum spanning network (Figure 2) strongly suggested no distinct geographic population structuring of white sturgeon in western North America. In order to infer distinct geographical population structuring of white sturgeon, most sequence variability should be partitioned among regions or among study sites. The opposite was consistently observed in this study. Furthermore, if distinct white sturgeon geographic population structure had existed, the three geographic regions superimposed on the 26 haplotypes in Figure 2 should have been clustered in relatively distinct areas of the network.

## Gene flow

White sturgeon gene flow, indicated as  $F_{ST}$  values, ranged widely (<0.01-0.51, Table 6), but with two exceptions indicated little genetic divergence among white sturgeon from most locations (mean  $F_{ST}$  excluding KR and KL sites: 0.04). Mean  $F_{ST}$  value for all sites, including KR and KL increased to 0.12. White sturgeon  $F_{ST}$  values from Kootenai River and Kootenay Lake sites were consistently an order of magnitude greater than those from paired comparisons from all locations other than the Kootenai system. Mean  $F_{ST}$  values involving Kootenai River and Kootenay Lake sites were 0.33 (range: 0.22-0.51) and 0.28 (range: 0.17-0.41) respectively. When statistically significant haplotype frequency differences occurred between paired locations (n=47, Table 6), corresponding  $F_{ST}$  values consistently reflected moderate to considerable genetic divergence (mean 0.17, range 0.01-0.51).  $F_{ST}$  values were not significantly correlated with geographic distance between all paired study sites ( $r^2 = <0.0001$ , n=78).  $N_m$  estimates ranged from <1-138 migrants per generation (mean 11) between all paired study sites (Table 6).  $Nm$  values also failed to exhibit a discernible correlation with geographic distance ( $r^2=0.01$ , n=78). Similarly, nucleotide divergence values ( $D_A$ ) were not well correlated with geographic distance ( $r^2=0.2$ , n=78).

## NCA

Permutation analysis of the total cladogram (Figure 3) did not reveal statistically significant association between geographic and genetic histories of white sturgeon ( $P=0.21$ ). However, four individual nested clades within the total cladogram (clades 1.4, 1.6, 2.1, 3.1) exhibited significant association with geography, although only two of these clades were significantly associated with geography based on the geographic distance analysis of the GEODIS program (clade 1.6,  $P < 0.01$ ; clade 3.1,  $P=0.047$ ). Furthermore, sampling design was inadequate to discriminate between isolation by distance (short distance migration) and long distance dispersal for clade 1.6. Similarly, the geographical sampling scheme was inadequate to discriminate between fragmentation and isolation-by-distance for clade 3.1, which only included haplotypes from the Fraser (LFR) and Nechako (NKO) rivers, which are

approximately 800 km apart. The latter limitation was due to the fact that samples were unavailable from the 800 km reach of the Fraser River separating LFR from NKO.

## Discussion

*Population structure* - Based on concordant results from this study, white sturgeon population structure was not supported by isolation by distance, stepping stone, or continent-island gene flow and dispersal models. Alternatively, observed gene flow across nearly 2,700 km, encompassing several large Pacific Coast river systems in western North America, appeared responsible for widespread geographic distribution of common haplotypes. This expansive haplotype distribution, along with relatively low  $F_{ST}$  values (<0.01-0.05, mean 0.04, excluding KR and KL sites) and high  $Nm$  values (<1-139, mean 12) supported considerable gene flow throughout a major portion of the species' range. Most notable were the two most common haplotypes (H1 and H2), observed at 13 and 11 of the 13 study sites in western North America. The observations that 8 of 26 haplotypes comprised 81% of the fish sequenced (210 of 260), and that these haplotypes existed in either two or three of the three major Pacific Coast river systems studied, also suggested high gene flow, and possible historical panmixia at continental and smaller geographic scales.

No significant relationship existed between measures of gene flow ( $F_{ST}$ ,  $Nm$ ) and geographic distance between all population pairs ( $r^2 \leq 0.01$ ). This observation was inconsistent with operative mechanisms of isolation by distance, stepping stone, and continent-island gene flow and dispersal models. The lack of significant relation between  $F_{ST}$ ,  $Nm$  values, and intersite geographic distance also indicated gene flow-mediated genetic similarity at these large geographic scales. High gene flow was further revealed by the observation that nearly 70% of all haplotypes (18 of 26), which existed as a mosaic throughout the entire study area, differed by just 1-4 substitutions (0.2-0.8% sequence difference). Finally, as with gene flow estimates, nucleotide divergence and intersite geographic distance were not significantly correlated ( $r^2 = 0.2$ ).

AMOVA analysis was conducted to further evaluate these findings. AMOVA consistently partitioned 87-90% of the observed sequence variation into the smallest geographic scale (within-population), regardless of geographic region designation. In general, pairwise comparisons of haplotype frequency distributions were not significantly different within river systems but did differ among all paired river system comparisons. Monte-Carlo  $\chi^2$  tests revealed that the mean geographic distance associated with significant differences in haplotype frequency distributions between paired locations exceeded 1,000 km; no significant differences occurred between population pairs < 311 km apart. Furthermore, up to 959 km (mean 662 km, n= 31 pairwise comparisons) separated population pairs that lacked significant differences in haplotype frequency distributions. However, sufficient differentiation in haplotype frequency distributions across geography resulted in the rejection of the three null hypotheses, which tested for geographic homogeneity of the distributions.

Phylogenetic analyses were subsequently performed to further investigate population structure. Despite robust geographical and numerical sample representation, haplotype and population trees consistently lacked informative clade structure and geographic or bootstrap support. This outcome was consistent with results of previously published white sturgeon phylogenetic analyses, which suggested that: 1) mtDNA haplotypes were little diverged; 2) topologies of gene trees were not statistically significant; and 3) the species generally lacked any significant geographic substructure (Brown 1991; Brown et al. 1992, 1993). Assignment of major river basins to each haplotype in the network in this study also failed to identify any decipherable geographic population structure. Finally, the higher resolution NCA was performed to further explain observed white sturgeon population structure. Consistent with previous results of this study, the NCA failed to find significant association between genetic and geographic white sturgeon histories with the exception of three consistently clustered haplotype pairs considerably diverged from the most common haplotype (1.7 – 3.1% sequence difference). However, these three haplotype pairs were collectively observed in all three major Pacific Coast river systems studied.

Thus, corroborative results from this study revealed that expansive gene flow over hundreds to thousands of kilometers, rather than isolation by distance, stepping stone, or continent-island models, best explained observed white sturgeon population structure. Observed population structure may likely have resulted from a temporal mosaic of range expansions and contractions in conjunction with temporally and geographically variable gene flow over evolutionary time. Accordingly, we present a new descriptive gene flow and dispersal model, the “expansive gene flow model” (EGF) to explain white sturgeon population structure revealed by this study.

Results of our analysis were well supported by the few genetic analyses of *A. transmontanus* in the literature (Setter and Brannon 1992; Bartley et al. 1985; Brown 1991; Brown et al. 1992, 1993, 1996). Results of two independent protein allozyme studies suggested that white sturgeon heterozygosity varied considerably across geography (Bartley et al. 1985; Setter and Brannon 1992). The mean percentage of 29 loci that were polymorphic was lowest in the Kootenai River population (27.6%), compared with white sturgeon from the Snake (31.0%) and the mid-Columbia (44.8%) rivers, and Lake Roosevelt (55.2%, Setter and Brannon 1992). Kootenai River white sturgeon are believed to be a post-glacially isolated population of ancestral Columbia River stock (Duke et al. 1999; USFWS 1999). Consistent with this notion, no unique alleles were found in Kootenai River fish relative to downstream populations, based on allozyme analysis (Setter and Brannon 1992). Setter and Brannon (1992) suggested that due to lower diversity and genetic distance estimates separating white sturgeon in the Kootenai system from other areas, the Kootenai River population constituted a stock within a species. This population was subsequently listed as endangered under the US Endangered Species Act in 1994 (USFWS 1994).

Based on RFLP analysis of 178 white sturgeon (105 and 73 from the Fraser and Columbia rivers respectively), Brown et al. (1993) reported that nearly 61% of individuals from both rivers shared a common haplotype. As in our study, these authors reported that the most common haplotype was found at all sampling locations. Brown et al. (1992) also reported that a unique haplotype represented by a pooled sibling sample of white sturgeon from the Sacramento River (n=1) clustered closely with haplotypes from Columbia and Fraser river

fish in UPGMA phylograms. This pattern of association of haplotypes from distant locations in different river systems was repeatedly observed in the haplotype network (Figure 2).

The proposed EGF model of white sturgeon population structure was also supported by independent, empirical white sturgeon movement and dispersal data. Because these fish were historically capable of migrating and successfully reproducing throughout major portions of their geographic range within as few as a single to several generations, the widespread distribution of common haplotypes reported appeared somewhat intuitive. DeVore et al. (1999) reported that 471 white sturgeon originally tagged in the unimpounded lower Columbia River were recaptured at 23 separate locations outside the Columbia River, including the Fraser and Sacramento rivers, and 21 additional geographically intermediate locations along the Pacific Coast of Oregon and Washington. Empirical data from white sturgeon recaptured in the unimpounded lower Columbia River but tagged elsewhere were unavailable. However, such dispersal was independently supported by the widespread geographic distribution of haplotypes and gene flow estimates reported in our study. Empirical data to support overwhelmingly or exclusively downstream migration in the lower Columbia River could not be found.

In addition to documented migration in unimpounded areas, recent empirical data also suggested that white sturgeon were not completely isolated within individual Columbia River reservoirs (ODFW 1996). Movement between reservoirs was believed to occur through navigation locks, over spillways, and through fish bypass facilities (Warren and Beckman 1993; DeVore et al. 1999). In the ODFW study (1996), of 661 recaptured white sturgeon tagged in the four furthest downstream Columbia River reservoirs, 591 (89.4%) were recaptured in the reservoir of original tagging, 68 (10.3%) were recaptured in downstream reservoirs adjacent to those of original tagging or in the unimpounded lower Columbia River, and 2 (0.3%) were recaptured in adjacent upstream reservoirs during a seven year period (i.e. < half of a white sturgeon generation). Finally, Warren and Beckman (1993) reported that nearly 3,500 white sturgeon were counted in observation windows in the fish bypass facilities at the three furthest downstream Columbia River dams from March through November,

1986-1991. However, relating these fish count data to gene flow measures is problematic due to uncertainties associated with migration direction (upstream vs. downstream) and potential multiple counting error. Regardless, the EGF population structure model for white sturgeon was independently supported by empirical movement and migration data from impounded and unimpounded portions of the species' range.

Concordance between the aforementioned empirical white sturgeon dispersal data and results of our genetic analysis provided further support for the EGF model. In addition, empirical dispersal data and  $Nm$  estimates generated by this study were supported by published ranges of numbers of migrants per generation theoretically needed to counteract population differentiation due to genetic drift in isolated populations. Wright (1931) showed that under the infinite island model at migration-drift equilibrium, a migration rate of only one per generation tends to maintain (biallelic) polymorphism in subpopulations and substantially limits divergence among subgroups (i.e.  $F_{ST(equilibrium)} = 0.2$ ). White sturgeon  $F_{ST}$  values generated by our study ranged from  $< 0.01 - 0.51$  (mean 0.12) including KR and KL sites. However, with these outliers removed,  $F_{ST}$  values ranged from  $< 0.01$  to 0.05, with a mean of 0.04 indicating, on average, little genetic divergence (Wright 1931) with the exception of these two sites. When pooled with samples from all sites, the KR and KL samples elevated mean  $Nm$  values (11 vs. 14 estimated migrants per generation) and greatly reduced mean  $F_{ST}$  values (0.12 vs. 0.4). This effect was presumably due to the observation that in all pairwise comparisons involving both gene flow estimators and the KR and KL sites, mean intersite geographic distances were very large (KR: 441-2,560 km, mean 1,451; KL: 311-2650 km, mean 1,324 km). Thus, consistent with the proposed EGF model, considerable genetic divergence was seen in white sturgeon, however, only involving paired samples from 100's to 1,000's of kilometers apart (KR and KL vs. all other sites; Table 7). Furthermore, the low number of haplotypes at the KR and KL sites, due to post-glacial refounding effects, and/or subsequent loss of previously rare haplotypes due to drift, also played a role in the low  $Nm$  and high  $F_{ST}$  values in all comparisons involving KR and KL (Table 7).

Since Wright's original work (1931), many authors reported that under an island model one migrant per generation was sufficient for subpopulations to theoretically exhibit (approximate) panmixia (Kimura & Ohta 1971, Lewontin 1974, Spieth 1974). Based on analysis of pre-equilibrium trends in the finite island model, Varvio et al. (1986) reported that loss of variation depended strongly on the size and number of subpopulations, with approximate panmixia occurring between 0.9 and 4.7 migrants per generation. Mills and Allendorf (1996) considered consequences of likely violations of the island model and concluded that in the real world  $\leq 10$  migrants per generation between two populations were necessary to accomplish conservation objectives. Finally, Vucetich and Waite (2000) reported that for many animal populations the combination of a small  $N_e/N$  ratio and fluctuating population size required  $>10$  migrants per generation to counteract effects of drift. Despite the diverse array of cited requirements regarding numbers of migrants per generation needed to counteract effects of genetic drift in isolated populations,  $Nm$  values reported from many paired population comparisons in our analysis ( $<1 - 138$  migrants per generation, mean 11) suggested sufficient migration to retain local genetic variability (in the absence of fragmentation by hydropower dams).

We concur with Brown et al. (1992), who suggested that genetic drift in relatively isolated white sturgeon demes was unlikely to have caused any reduction in the lower Columbia River impoundments because fewer than four consecutive white sturgeon generations had passed since initial impoundment by Bonneville Dam in 1938. We also agree that abrupt separation created by installation of individual hydropower dams, each during relatively few years, may have been responsible for observed segregation of certain haplotypes in areas upstream or downstream from these dams (Brown et al. 1992). Finally, if particular groups of females exhibited synchronous migration patterns distinguished by mtDNA haplotype, contemporary haplotype distributions could have reflected the locations of these maternal cohorts when the dams were completed (Brown et al 1992). However, as our data suggested, if many haplotypes geographically transcended areas fragmented by two adjacent dams, such a pattern could be obscured to varying degrees relative to the magnitude of geographic transcendence.

The EGF model was further supported by empirical white sturgeon life history and reproductive attributes. White sturgeon are characterized by delayed onset of first reproduction. First maturation generally occurs from 10 to 20 years of age for males, and from 15 to 30 for females (Scott and Crossman 1973; Semakula and Larkin 1968; Conte et al. 1988; Ireland et al. *In Press*). This trait, coupled with empirically confirmed migratory and dispersal ability, supported gene flow estimates generated by this study. In addition, individual longevity ( $\leq 82$  years of age, Simpson and Wallace 1982), infrequently exceeding 100 years of age (M. Rosenau, British Columbia Ministry of Environment, Lands, and Parks, Personal communication), may have also contributed to observed migration, dispersal, and gene flow (Brown et al. 1993, 1996).

White sturgeon are iteroparous spawners, which broadcast gametes into the water column where fertilization occurs before the demersal, adhesive embryos settle to the substrate (Paragamian et al 2001, and references therein). In demographically viable white sturgeon populations, iteroparity provides the opportunity for within-year reproduction by numerous generations of fish. Reproductive periodicities vary between sexes; males may reproduce every 2-4 years, while females may reproduce at no less than five year intervals (Conte et al. 1988; Chapman et al. 1996, Anders et al. *In Press*). Simpson and Wallace (1982) reported 4-11 year spawning periodicity for white sturgeon, but made no mention of gender. Little is known regarding reproductive senescence in *A. transmontanus*. However, mature adults are thought to spawn numerous times over a 30-40 year period, and possibly longer (S. Doroshov, University of California, Davis, personal communication). If an individual female initially reproduced as age 25 and successfully spawned in subsequent 5-year intervals until age 65, it theoretically could contribute gametes to subsequent generations up to nine times. Furthermore, communal spawning, along with the above reproductive mechanisms, likely contributed to increased gene flow in white sturgeon relative to that of paired, semelparous fishes (e.g. Salmonidae).

In the context of the EGF model, consistent, significant negative correlations between haplotype diversity measures, percent sequence difference among individuals within sites,

and inland geographic distance revealed diminished gene flow as a function of upstream distance in the Columbia River Basin ( $r^2 = 0.42 - 0.67$ ). Inadequate sample representation precluded assessment of this concept in the Fraser and Sacramento rivers. Unbiased comparisons of gene flow among locations within currently impounded and unimpounded river reaches were not possible due to autocorrelation between locations of sampled unimpounded areas and proximity to the Pacific Ocean (0-250 km). However, Brown et al. (1992) reported that hydropower dams on the Columbia River may have been responsible for reduced haplotype diversity in the Columbia River compared to the Fraser; although these authors reported little divergence among haplotypes from the impounded and unimpounded reaches of the Columbia River, and from the Fraser River.

*Evolutionary history* - Nucleotide substitution rates in mammalian mtDNA have been reported as approximately 2% per million years (Brown et al. 1996; Brown 1985; Wilson 1985). However, substitution rates for the mtDNA control region of *A. transmontanus* were reported to be considerably higher (8% per million years, Brown et al. 1993). We observed 1-19 nucleotide substitutions among control region sequences of *A. transmontanus* constituting 0.2-4.2 % sequence difference. This range was nearly identical to earlier estimates of sequence difference (0.1 – 4.2%) for the same segment of the control region, based on sequencing of 27 white sturgeon samples from the Fraser and Columbia rivers (Brown et al. 1996). Applying the nucleotide substitution rate of 8% per million years (Brown et al. 1993) suggested that diversification observed in this study occurred during approximately the past 50,000-500,000 years. Using the same substitution rate suggested that *A. transmontanus* and *A. medirostris* (observed 11% mean sequence difference) diversified some 1.4 million years ago. The 11% mean interspecific sequence difference observed in our study was nearly four times greater than the 4.14% sequence difference reported between the two species by Brown et al. (1996). However, Brown et al.'s 1996 estimate was based on sequence analysis of 27 *A. transmontanus* specimens (11 and 16 from the Columbia and Fraser rivers respectively), compared to 260 sequences from 13 sites in three major Pacific Coast river systems in this study.

*Comparisons with other Chondrosteian taxa* - Low genetic variability among other North American chondrosteans has also been reported. Low levels of intraspecific variation were reported based on protein allozyme analysis of paddlefish populations (*Polyodon spathula*) (Carlson et al. 1992; Fries and Hutson 1993). Epifanio et al. (1996) performed a more robust genetic analysis of *P. spathula*, using allozyme and mtDNA RFLP techniques to analyze 189 individuals from six geographic regions of the Mississippi River drainage. Results of this study (Epifanio et al. 1996) differed from earlier studies (Carlson et al. 1992; Fries and Hutson 1993) in two ways: 1) the 1996 and 1993 studies revealed the *PGM-1\*108* allele not reported by Carlson et al. (1992); and 2) Epifanio et al. uncovered four alleles at *CK-B\**, while Carlson et al (1992) observed two, and Fries and Hutson (1993) observed none.

Restriction enzyme analysis also revealed low interspecific mtDNA variation in northern populations of several sturgeon species, *A. fulvescens*, *A. medirostris*, and *A. oxyrinchus* (Brown 1991). Bowen and Avise (1990) reported that populations of *A. oxyrinchus* in the southeastern United States had very low mtDNA diversity. Similarly, Guenette et al. (1993) reported relatively low genetic divergence ( $d=0.219-0.75\%$ ), and a total of three haplotypes from an RFLP analysis of mtDNA involving 82 specimens of lake sturgeon (*A. fulvescens*) from the St. Lawrence River and James Bay drainages in Quebec, Canada.

Until recently, little was known about stock structure of Atlantic sturgeon (*A. oxyrinchus oxyrinchus*). However, a latitudinal cline in mtDNA haplotype diversity and differences in RFLP haplotype frequencies were reported among populations from Canada, the Hudson River, and the southeastern United States (Waldman et al. 1996a, 1996b, in Wirgin et al. 2000). Sequencing of the control region (203 bp) from Gulf of Mexico sturgeon (*A. oxyrinchus*) and the subspecies Atlantic sturgeon (*A. o. oxyrinchus*) revealed three fixed nucleotide differences between the two taxa (Ong 1996). In direct contrast to results of our genetic analysis of white sturgeon, another study of Gulf sturgeon mtDNA showed strong genetic structuring of populations on regional and even river-specific scales (Stabile et al. 1996).

More recently, Wirgin et al. (2000) sequenced a 203 bp section of the control region of 322 Atlantic sturgeon (*A. o. oxyrinchus*) from 11 river systems on the east coast of North America from the Saint Lawrence River to southern Georgia to elucidate range-wide population structure. One to 17 haplotypes per population were reported in this study, with haplotype diversity ranging from 0.0-0.90. This haplotype diversity range was nearly identical to that observed for white sturgeon in this study (0.01-0.86) at a similar (continental) geographic scale. Wirgin et al. (2000) also reported that greater genetic diversity was observed in southern populations, likely due to the persistence of these populations through the Pleistocene and to faster mutation rates associated with their shorter generation times at southern latitudes relative to northern populations sampled. Results of our *A. transmontanus* population structure analysis were inconsistent with finer scaled population structure and clinal variation of haplotype diversity reported for *A. oxyrinchus* and *A. o. oxyrinchus* over similarly large geographic expanses (Ong 1996; Stabile et al. 1996; Waldman et al. 1996a, 1996b; Wirgin et al. 2000). However, these interspecific differences were strongly supported by data that contributed to the EGF model for white sturgeon.

Brown et al. (1992) reported that the lower Columbia River represented a Pleistocene glacial refuge that may have supported a population that provided the founders of post-glacially recolonized Fraser River populations. However, unlike reported effects of Pleistocene glaciation on Atlantic sturgeon diversity and significant RFLP haplotype frequency differences (Waldman et al. 1996a, 1996b; Wirgin et al. 2000), post-glacially founded Fraser River populations consistently exhibited greater mtDNA heterozygosity and nucleotide diversity relative to the putative Columbia River source population (Brown et al. 1992). Our white sturgeon sample from the Lower Fraser River (n=20) also exhibited the highest haplotype diversity and second highest nucleotide diversity values of all sites sampled (Table 4). This condition of greater genetic diversity in a founded compared to a source population is generally the reverse of most studies of mtDNA diversity between refugium and subsequently founded animal populations (Brown et al. 1992). These authors proposed an explanation to this conundrum that involved subsequent artificially reduced diversity measures in the Columbia system due to recent impoundment and over-exploitation. The

latitude-based clinal variation exhibited by Atlantic sturgeon (Wirgin et al. 2000) may not apply to white sturgeon, possibly due to potential historical panmixia of *A. transmontanus* across the range of latitudes studied. However, inadequate sample availability from the Sacramento and Fraser rivers prevented us from testing such a hypothesis.

Two North American sturgeon species, *Scaphirhynchus albus* and *S. platyrhynchus*, were initially indistinguishable at 37 protein loci (Phelps and Allendorf 1983). Subsequently, Campton et al. (2000) provided a more robust mtDNA sequence analysis to resolve taxonomic questions regarding three currently recognized species in the southeastern and central United States: Pallid, shovelnose and Alabama sturgeon (*S. albus*, *S. platyrhynchus*, and *S. suttkusi*). Maximum sequence divergence among these three species (2.06%) was less than values usually observed between fish species (Campton et al. 2000), and was approximately half of maximum intraspecific differences observed in *A. transmontanus* control region sequences in our study. The Campton et al. (2000) study provided the first molecular genetic evidence for distinguishing the three *Scaphirhynchus* species. This study, coupled with morphological and biogeographical data, led these authors to conclude that pallid and Alabama sturgeon should be evaluated as distinct species under the ESA.

Most recently, Tranah et al. (2001) reported different patterns of genetic structure among sympatric populations of *S. albus* and *S. platyrhynchus* in the Mississippi and Missouri river drainages based on data from five nuclear DNA microsatellite loci. These results were reported to represent reproductive isolation between sympatric populations of the two species (Tranah et al. 2001). These authors found that shovelnose sturgeon from the upper Missouri and lower Mississippi rivers were genetically indistinguishable, due to their known widespread distribution and inferred gene flow. In this case, shovelnose sturgeon population structure data more closely resembled our data, which supported the white sturgeon EGF model. However, Tranah et al. (2001) also reported that pallid sturgeon from the Atchafalaya River (lower Mississippi River drainage) were genetically differentiated from both upper Missouri River populations sampled. This result may have been due to polyphyletic origin of pallid sturgeon, sympatric speciation, genetic drift, and/or introgressive hybridization in the Atchafalaya River (Campton et al 2000; Tranah et al 2001).

Associated  $F_{ST}$  values resulting from comparisons among the two *Scaphirhynchus* species and confirmed hybrids ranged from 0.01 to 0.25 (mean 0.12, n=21), with greater  $F_{ST}$  values for all *S. albus* X *S. platyrhynchus* comparisons, excluding hybrids (0.12 – 0.22, mean 0.19, n=6)(Tranah et al. 2001). Consistent with results of the mtDNA sequencing analysis of *S. albus* and *S. platyrhynchus* (Campton et al. 2000), the highest interspecific  $F_{ST}$  value based on nuclear microsatellite analysis (0.25, Tranah et al. 2001) was approximately half of the maximum interspecific  $F_{ST}$  value observed in *A. transmontanus* (0.51, Table 6). Interestingly, the mean  $F_{ST}$  values for all comparisons between *S. albus* and *S. platyrhynchus* and confirmed hybrids based on nuclear microsatellite analysis (Tranah et al. 2001) and all intraspecific comparisons of *A. transmontanus* in our study were identical ( $F_{ST} = 0.12$ ). Thus, additional, independent molecular genetic evidence of high gene flow in white sturgeon relative to other North American sturgeon species (*S. albus* and *S. platyrhynchus*, Campton et al. 2000; Tranah et al. 2001) further supported the uniqueness of *A. transmontanus* population structure and its associated EGF gene flow and dispersal model among Chondrosteian fishes.

Finally, population fragmentation poses a serious threat to many sturgeon taxa, including *A. transmontanus* (Secor et al. In Press; Jager 2001, Jager et al. 2000). Hydropower development at the Columbia River Basin scale has extensively fragmented numerous historically free-flowing river reaches (NRC 1996). Potential effects of fragmentation by hydropower dams on white sturgeon population structure, viability, and persistence are of utmost importance because it has long been known that isolated populations lose neutral genetic variability at a rate directly proportional to how small they are (Wright 1931). Because fragmentation can reduce population or deme size, and small population size can negatively affect genetic variation, fragmentation can indirectly reduce genetic variability. One important effect of such river fragmentation on white sturgeon is the artificial creation of a series of relatively isolated sub-populations (Secor et al. In press, Jager 2001, Jager et al. 2000). This is especially critical for a migratory species like *A. transmontanus* because fragmentation by dams may also impose exclusively downstream gene flow. Altered daily and seasonal river discharge and thermal regimes resulting from impoundment and dam

operations may also alter migration, limit habitat availability, and may affect timing, location or success of reproduction (Anders et al. In Press; Cooke et al. In Press; Jager et al. In Press; Secor et al. In press; Holderman et al., submitted; Auer 1996). Thus, prudent future white sturgeon conservation and management programs might consider evaluating various options to restore historic gene flow, based on findings from this analysis.

In conclusion, based on our results of mtDNA sequencing of 260 white sturgeon from 13 sites across three major Pacific Coast river systems in western North America, *A. transmontanus* population structure was best explained by the proposed expansive gene flow model rather than by traditional isolation by distance, stepping stone, or continent-island models. As a result, observed sequence differences within *A. transmontanus* were nearly two-fold greater than those distinguishing three sturgeon species (*S. albus*, *S. platyrhynchus*, and *S. suttkusi*) across similarly large geographic expanses in central and southeastern United States. Concordant results of AMOVA, Monte Carlo  $\chi^2$ , NCA, PHYLIP, PAUP, REAP, linear regression analyses, and moderate to high  $F_{ST}$  and  $Nm$  values revealed that expansive gene flow over hundreds to thousands of kilometers best explained observed white sturgeon population structure. The proposed EGF population structure model was well supported by our genetic data, and by independent, empirical white sturgeon genetic, dispersal, life history, and reproductive data. In the context of the EGF model, consistent, significant negative correlations between haplotype diversity measures, percent sequence difference among individuals within sites, and inland geographic distance revealed diminished gene flow as a function of upstream distance in the Columbia River Basin.

Although we provided new, valuable insight into white sturgeon population structure, the need to further describe *A. transmontanus* population genetic attributes at local, regional, and range-wide geographic scales using biparentally inherited nuclear markers remains critical. Published nuclear microsatellite primers and protocols have only recently become available for such analyses (McQuown et al. 2000; May et al. 1997). Results from such studies can provide urgently needed higher-resolution population genetic data to facilitate informed conservation and management decisions. Such decisions are key to restoring and

maintaining population viability and persistence of white sturgeon, as well as that of other imperiled sturgeons at population, subspecies, and species levels worldwide.

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Table 1. Sampling locations, location codes, and sample information from 260 white sturgeon from western North America.

River	Location	Code	n	Inland distance (km)	Year sampled	Sampling Agency
Columbia	Columbia River estuary	CRE	20	<20	1998	ODFW
	Lower Columbia River	LCR	20	<50	1997	ODFW
	The Dalles Pool	TD	20	308	1997	ODFW
	McNary Pool	MCN	20	470	1995	ODFW
	Lake Roosevelt	LKR	20	960	1998	ODFW, WDFW
Kootenai	Kootenay Lake (BC)	KL	20	1270	1995	IDFG, KTOI, BCMELP
	Kootenai River (US)	KR	20	1400	1997	IDFG, KTOI
Snake	Little Goose Pool	LGO	20	635	1998	WDFW
	Hell's Canyon	HC	20	850	1998	IPC
	CJ Strike Reservoir	CJS	20	1365	1996	IPC
Fraser	Lower Fraser River	LFR	20	<80	1997	RL&L, BCMELP
Nechako	Nechako River	NKO	20	790	1995	RL&L, BCMELP
Sacramento	Sacramento River	SAC	20	<50	1999	CDFG, UC Davis

Table 2. AMOVA analysis geographic region classifications.

Test	Regions	Sampling locations
3-Region AMOVA analysis	Fraser	LFR, NKO
	Columbia	CRE, LCR, TD, MCN, LKR, LGO, HC, CJS, KL, KR
	Sacramento	SAC
5-Region AMOVA analysis	Fraser	LFR, NKO
	Columbia	CRE, LCR, TD, MCN, LKR
	Snake	LGO, HC, CJS
	Kootenai	KL, KR
6-Region AMOVA analysis	Sacramento	SAC
	Fraser	LFR
	Nechako	NKO
	Columbia	CRE, LCR, TD, MCN, LKR
	Snake	LGO, HC, CJS
	Kootenai	KL, KR



Table 4. Within-location white sturgeon haplotype frequency and haplotype and sequence diversity in western North America.

Region	Location code	n	Number of haplotypes	Haplotype diversity <sup>1</sup>	Nucleotide diversity <sup>2</sup>
Columbia	CRE	20	11	0.86	0.72
	LCR	20	8	0.81	0.67
	TD	20	6	0.66	0.65
	MCN	20	7	0.80	0.60
	LKR	20	4	0.63	0.66
Snake	LGO	20	7	0.75	0.55
	HC	20	6	0.72	0.26
	CJS	20	6	0.83	0.21
Kootenay/i	KL	20	2	0.19	<0.01
	KR	20	2	0.01	0.04
Fraser	LFR	20	9	0.85	1.14
	NKO	20	4	0.76	0.16
Sacramento	SAC	20	7	0.78	0.51

1 = Haplotype diversity estimated according to Nei (1987, eqs. 8.5 and 8.12)

2 = Nucleotide diversity estimated Nei and Tajima (1981).

Table 5. Results of Monte-Carlo simulated exact weighted Pearson Chi-Square comparisons of white sturgeon haplotype frequencies by major river system (\* =  $P \leq .05$ , \*\* =  $P \leq .01$ ).

	Columbia	Snake	Kootenai/y	L. Fraser	Nechako
Columbia					
Snake	<0.001** <sup>a</sup>				
Kootenai/y	<0.001**	<0.001**			
L. Fraser	<0.001**	<0.001**	<0.001**		
Nechako	<0.001**	<0.001**	<0.001**	<0.001**	
Sacramento	0.016*	<0.001**	<0.001**	<0.001**	<0.001**

a:  $P=0.17$  excluding CJS dataset from Columbia and Snake River inter-regional analysis.

Table 6. Geographic intersite distance (km), gene flow estimates ( $F_{ST}$ ,  $Nm$ ), and  $\chi^2$  comparison  $P$  values of white sturgeon haplotype frequency distributions at 13 sites in western North America. (\* =  $P \leq .05$ , \*\* =  $P \leq .01$ ).

Location	CRE	LCR	TD	MCN	LKR	LGO	HC	CJS	KL	KR	LFR	NKO
CRE	0											
LCR	<150 19 0.03 0.88	0							Legend  Km $Nm$ $F_{st}$ $\chi^2 P$ value			
TD	308 23 0.01 0.55	158 11 0.02 0.19	0									
MCN	469 41 0.01 0.54	315 25 0.01 0.24	161 3 0.07 0.07	0								
LKR	959 4 0.07 0.06	809 7 0.03 0.09	651 1 0.15 **	490 8 0.03 0.06	0							
LGO	634 15 0.02 0.36	484 80 <0.01 0.34	326 3 0.01 0.13	165 10 0.03 0.84	551 11 0.02 0.53	0						
HC	919 11 0.02 0.15	769 25 0.01 0.19	611 3 0.08 *	450 29 0.01 0.37	836 78 <0.01 0.20	285 6 0.04 0.99	0					
CJS	1365 10 0.02 *	1215 9 0.03 **	1057 3 0.01 *	896 13 0.02 *	1262 3 0.09 **	731 21 0.01 0.17	446 76 <0.01 0.30	0				
KL	1270 <1 0.35 **	1120 <1 0.30 **	962 <1 0.41 **	801 1 0.25 **	311 1 0.17 **	862 1 0.18 *	1147 1 0.20 *	1593 1 0.31 **	0			
KR	1400 <1 0.39 **	1250 <1 0.35 **	1092 <1 0.51 **	931 1 0.30 **	441 1 0.22 **	992 1 0.24 **	1277 1 0.26 **	1732 <1 0.37 **	120 138.64 <0.01 0.48	0		
LFR	500 11 0.02 0.63	750 28 0.01 0.20	808 9 0.01 0.11	969 27 0.01 0.16	1459 5 0.05 0.12	1134 15 0.02 0.27	1419 9 0.03 0.08	1865 7 0.03 *	1770 <1 0.36 **	1900 <1 0.40 **	0	
NKO	1290 5 0.05 *	1440 6 0.04 **	1598 2 0.11 **	1759 5 0.05 **	2249 3 0.07 **	1924 6 0.04 **	2209 6 0.04 **	2655 3 0.07 **	2560 1 0.30 **	2650 <1 .035 **	790 4 0.06 **	0
SAC	900 6 0.04 0.18	1050 8 0.03 0.08	1208 1 0.11 **	1369 10 0.02 *	1859 4 0.06 **	1534 11 0.02 *	1819 5 0.05 **	2265 4 0.06 **	2170 1 0.23 **	2300 1 0.27 **	1400 3 0.07 **	2190 3 0.07 **

Table 7. Results of Monte-Carlo simulated exact weighted Pearson Chi-Square pairwise comparisons of white sturgeon haplotype frequencies by location from 13 study sites in western North America (\*=  $P \leq 0.05$ ; \*\*=  $P \leq 0.01$ ).

	NKO	LFR	CRE	LCR	TD	MCN	LKR	KL	KR	LGO	HC	CJS
NKO												
LFR	**											
CRE	*	*										
LCR	**	**	0.88									
TD	**	**	0.55	0.19								
MCN	**	*	0.54	0.24	0.07							
LKR	**	**	0.06	0.09	**	0.06						
KL	**	**	**	**	**	**	**					
KR	**	**	**	**	**	**	**	**	0.48			
LGO	**	*	0.36	0.34	0.13	0.84	0.53	*	**			
HC	**	*	0.15	0.19	*	0.37	0.20	*	**	1.00		
CJS	**	**	*	**	*	*	**	**	**	0.17	0.30	
SAC	**	**	0.18	0.08	**	*	**	**	**	*	**	**

Table 8. Results of AMOVA analysis of 26 white sturgeon haplotypes from western North America. (See Table 2 for geographic region definitions).

Variance component	3-Region Analysis		5-Region Analysis		6-Region Analysis	
	Variance	%	Variance	%	Variance	%
<u>Nested Analysis</u>						
Variance among regions	-0.002	-0.59	0.039	9.97	0.041	10.61
Variance among study sites within regions	0.042	11.02	0.009	2.48	0.007	1.83
Variance within study sites	0.344	89.57	0.344	87.54	0.344	87.55
<u>Analysis among study sites</u>						
Variance among study sites	0.041	10.75	0.014	10.75	0.041	10.75
Variance within study sites	0.344	89.25	0.344	89.25	0.344	89.25
<u>Analysis among regions</u>						
Variance among regions	0.014	3.65	0.043	10.96	0.045	11.51
Variance within regions	0.377	96.35	0.350	89.04	0.348	88.49

Appendix A. Observed mitochondrial control region haplotypes in white sturgeon (*Acipenser transmontanus*). The most common haplotype, 9, is used as a reference. Dots indicate identity, colon indicates insertion or deletion.

Haplotype	Variable sites																																							
	7	8	10	28	50	52	57	58	73	114	116	117	135	171	179	191	201	215	283	285	286	291	299	300	304	332	356	365	366	378	389	390	401							
9	G	G	G	A	A	C	A	G	C	T	A	T	A	G	A	G	T	C	C	C	G	T	G	A	A	:	T	A	G	A	A	A	A	T						
17	A	.	A	G	.	.	G	.	.	.	G	C	.	A	.	.	.	T	.	T	.	.	.	G	.	T	C	.	A	.	A	:	.							
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