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Distribution of mitochondrial DNA variation in lake sturgeon (*Acipenser fulvescens*) from the Moose River basin, Ontario, Canada

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We analysed mitochondrial DNA (mtDNA) variation of lake sturgeon (*Acipenser fulvescens*) from the Moose River basin. Our objective was to address various proximate and ultimate factors which may influence the distribution of lake sturgeon mtDNA haplotype lineages in this watershed. The lake sturgeon sampled were characterized by only two mtDNA haplotypes based on a restriction fragment length polymorphism analysis with 40 restriction endonucleases and direct sequencing of 275 nucleotides in the mtDNA control region. We detected no heterogeneity in the mtDNA haplotype frequencies of lake sturgeon captured from different sites within rivers including those separated by major hydroelectric installations. However, lake sturgeon from one tributary had significantly different haplotype frequencies than those from other tributaries suggesting that they composed a discrete genetic stock. These results suggest that gene flow among most sites is significant and is an important factor affecting the distribution of mtDNA variation in this species. The genetic structuring and diversity are discussed in relation to lake sturgeon management and conservation.

Key words: *Acipenser*; conservation; mtDNA; population structure.

I. INTRODUCTION

Alteration of natural habitats by human activities can lead to declines in abundance and eventual loss of fish stocks (Williams *et al.*, 1989). Fishes with different life cycles and patterns of population structure may be affected by habitat disturbance in different ways. For instance, species normally experiencing gene flow through migration may become fragmented into isolated populations, thus experiencing a loss of genetic variability through inbreeding and genetic drift. Conversely, species characterized by limited migration and/or homing behaviour may have specialized genetic and ecological adaptations to local habitats (STOCS, 1981; Taylor, 1991). Localized habitat alteration can lead to the elimination of discrete or unique stocks which are genetically adapted to a specific environment. The net result is a loss in biodiversity and the adaptive potential of the species. Such considerations illustrate how knowledge of population genetic structure can be used to predict the potential impacts of habitat disturbance and develop sound management policies.

The lake sturgeon, *Acipenser fulvescens* (Rafinesque), is widely distributed in North Eastern America (Houston, 1987) and may be particularly vulnerable to

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future developments because it inhabits large-river systems typically exploited for hydroelectricity. The lake sturgeon may also be susceptible to habitat fragmentation because of its ability to migrate long distances (Olver, 1987). Dam construction, point-source effluents, and commercial overharvesting have all contributed to the decline of lake sturgeon populations (Houston, 1987; Olver, 1987).

Despite the economic value of lake sturgeon and its status as a threatened species (Williams *et al.*, 1989), knowledge of its genetic variation and population structure is limited. Guenette *et al.* (1993) analysed mitochondrial DNA (mtDNA) variation in lake sturgeon from the St Lawrence River, Ottawa River, and Waswanipi River, Quebec, Canada, and found low levels of genetic polymorphism. There was no evidence of population subdivision within the St Lawrence corridor but some differentiation between the St Lawrence and the Waswanipi River. The analysis of Guenette *et al.* (1993) was limited to the screening of seven restriction enzymes only. Thus, one could argue that the low polymorphism revealed in that study was related to an inadequate level of resolution. Since the resolution of restriction fragment length polymorphism (RFLP) analysis is directly proportional to the number of restriction sites surveyed, the use of a larger number of restriction enzymes should increase the probability of detecting sufficient genetic variation to address questions related to population structure.

The recent development of the polymerase chain reaction (PCR) (Saiki *et al.*, 1985) allows one to obtain sequence information on specific sections of the genome in population surveys (Vigilant *et al.*, 1989; Thomas *et al.*, 1990; Bernatchez *et al.*, 1992). A recent study by Brown *et al.* (1993) revealed that the most variable region of the mitochondrial genome of white sturgeon (*Acipenser transmontanus*, Richardson) was located in the non-coding, control region (D-loop). Sequence analysis of the control region for that species revealed four to five times more variation than a RFLP analysis performed over the entire mitochondrial genome. Sequence analysis of the lake sturgeon control region could further increase the level of genetic variation detected.

We studied mtDNA variation in lake sturgeon from the Moose River basin, northern Ontario by surveying variation over the entire mtDNA molecule by RFLP analysis with 40 restriction enzymes, and a sequence analysis of a segment of the control region. Our objective was to investigate within and between-tributary substructuring of lake sturgeon populations in this drainage. We address proximate and ultimate factors which may influence the distribution of lake sturgeon mtDNA haplotype lineages in this watershed.

II. MATERIALS AND METHODS

SAMPLE COLLECTION AND mtDNA PURIFICATION

Lake sturgeon were sampled from 1990 to 1992 at eight sites in the Moose River basin, James Bay (Table I). Immediately after fish death, samples of liver (1–4 g) were removed, placed in cold sterile TEKS buffer (50 mM Tris, 10 mM EDTA, 0·20 M KCl, 0·25 M sucrose, pH to 7·5 with NaOH) on wet ice, transferred to the laboratory and refrigerated until processed.

TABLE I. Summary of lake sturgeon analysed from the Moose River basin and absolute frequency distribution of mtDNA haplotypes among samples

Sampling location	Year	*n*	Haplotype	
			1	2
Carmichael Falls, Groundhog River	1990	8	4	4
	1992	11	4	7
Cypress Falls, Mattagami River	1990	20	10	10
	1992	22	7	15
Kipling Dam, Mattagami River	1990	9	4	5
Lower Abitibi River	1992	24	10	14
Missinaibi River	1990	2	0	2
North French River	1992	21	2	19
Otter Rapids, Abitibi River	1990	5	3	2
Renison, Moose River	1990	7	5	2

Depending on the quality of samples, mtDNA was purified using the rapid extraction method of Chapman & Powers (1984) or following procedures of caesium chloride gradient centrifugation (Dowling *et al.*, 1990).

mtDNA RESTRICTION ENZYME ANALYSIS

Forty five- and six-base cutter restriction endonucleases were used to search for polymorphism over the entire mtDNA molecule (Table II). Restriction fragments were separated on 0·8 and 1·0% agarose gels for 18 h at 22 V. When DNA was obtained in sufficient quantity and quality, ethidium bromide staining was sufficient to detect mtDNA fragments. For samples with either low DNA concentrations or genomic DNA contamination, DNA was transferred to nylon membranes (Amersham, Hybond-N) by vacuum. Membranes were hybridized with an ultrapure lake sturgeon mtDNA probe. The probe was labelled non-radioactively with the nucleotide analog digoxigenin-11-UTP by random priming (Feinberg & Vogelstein, 1983). The DNA hybrids were detected by chemiluminescence (Höltke *et al.*, 1992) followed by exposure of the membranes to X-ray film for 2 to 16 h.

mtDNA AMPLIFICATION AND SEQUENCE ANALYSIS

Partially purified mtDNA produced from the rapid protocol of Chapman & Powers (1984) was used as target templates in PCR amplification. We first used symmetrical PCR (equal concentration of both primers) to produce sequencing template but found it unreliable for lake sturgeon. However, asymmetrical PCR (40 : 1 primer ratio), which generates single-stranded templates for sequencing, produced reliable results. PCR amplifications were performed in 50-µl reaction volumes containing two units of *Thermus aquaticus* DNA polymerase, 5 µl of 10X reaction buffer, and each dNTP at 250 µM. One microlitre of the DNA preparation was added to the PCR mix. We used one primer (HN20 at 1 pmol, $^{5'}$GTG TTA TGC TTT AGT TAA GC$^{3'}$) designed by Bernatchez *et al.* (1992) for a study of *Salmo trutta* (L.). This heavy-strand primer is located within the phenylalanine tRNA gene. The light-strand primer used (tpro-2 at 40 pmol $^{5'}$ACC CTT AAC TCC CAA AGC$^{3'}$) is located in the proline tRNA gene and was designed for sturgeon (Brown, 1991). These primers amplified the entire control region. DNA was amplified in a programmable thermal cycler (Perkin Elmer) using the following profile. The initial denaturation period was 1 min at 95° C followed by 35 cycles (1 min at 92° C for denaturation, 1 min at 45° C for annealing, 1·5 min at 72° C for extension). The amplified mtDNA was purified and cleaned using a commercially available kit (Gene Clean) according to the manufacturer's specifications (Bio101) to be made ready for direct sequencing.

TABLE II. Fragment patterns and corresponding estimates of fragment sizes for 40 restriction enzymes resulting from restriction analysis of lake sturgeon mtDNA, polymorphism was observed for only two enzymes, *Ava* II and *Hinc* II

Enzyme	Pattern	Lengths (kb)*	Total
Asn I	A	6·45, 4·29, 1·79, 1·42, 1·26, 1·09, 0·40, 0·15, 0·13	16·98
Acc I	A	5·40, 2·58, 2·52, 2·45, 1·40, 1·20, 0·69, 0·52, 0·28	17·04
Apa I	A	5·26, 4·14, 3·72, 1·69, 1·40, 0·57	16·78
Ava I	A	5·91, 4·10, 1·42	11·43
Ava II	A	3·45, 2·85, 1·92, 1·44, 1·39, 1·11, 0·95, 0·60, 0·57, 0·52, 0·41, 0·38, 0·32, 0·19	16·10
	B	3·45, 2·21, 1·92, 1·44, 1·39, 1·11, 0·95, 0·60†, 0·57, 0·52, 0·41, 0·38, 0·32, 0·19	16·06
Bam HI	A	11·68, 4·44, 1·59	17·71
Ban I	A	6·78, 5·11, 4·14, 1·13	17·16
Ban II	A	1·97, 1·62, 1·57, 1·42, 1·31, 1·27, 1·18	10·34
Bcl I	A	5·66, 5·05, 2·05, 1·46	14·22
Bgl II	A	12·11, 4·38	16·49
Bst XI	A	9·11, 4·22, 3·06, 1·75	18·14
Cla I	A	Single or no cut site	
Csp 45I	A	7·36, 6·72, 3·35	17·43
Dra I	A	7·03, 5·85, 4·01	16·89
Dra II	A	4·38, 3·47, 1·60, 1·52, 1·36, 1·17, 0·69, 0·66, 0·63, 0·27, 0·12	15·87
Eco RI	A	7·38, 5·12, 4·14, 1·08	17·72
Eco RV	A	Single or no cut site	
Hinc II	A	5·25, 4·92, 2·91, 2·05, 1·53	16·66
	B	5·25, 3·09, 2·91, 2·05, 1·76, 1·53	16·59
Hind III	A	13·63, 2·15, 1·25, 0·90, 0·56	18·49
Hinf II	A	2·01, 1·30, 1·12, 1·08, 1·04, 0·68, 0·14	7·37
Hpa I	A	12·73, 3·16, 2·89	18·78
Mam I	A	9·33, 4·59, 3·71	17·93
Nae I	A	Single or no cut site	
Nhe I	A	6·44, 4·48, 2·47, 2·13	15·52
Nco I	A	11·99, 2·35, 1·86	16·20
Nsi I	A	Single or no cut site	
PmAc I	A	Single or no cut site	
Pst I	A	9·59, 5·51, 2·29, 0·49	17·88
Pvu I	A	9·54, 5·34, 1·74	16·62
Rsr II	A	Single or no cut site	
Sal I	A	11·02, 6·11, 2·16	17·13
Sca I	A	12·47 2·76 1·17	16·40
Sna BI	A	8·97, 3·63, 3·40, 1·51	17·51
Spe I	A	7·36, 2·88, 1·86, 1·37, 0·79	14·49
Ssp I	A	5·28, 2·39, 1·98, 1·45, 1·21, 1·11, 1·09, 0·76, 0·69, 0·46, 0·40	17·22
Sst II	A	11·31, 4·66, 1·59	17·56
Stu I	A	2·83, 2·58, 2·13, 1·95, 1·50, 1·45, 1·39, 0·65, 0·58, 0·52	18·41
Sty I	A	2·21, 2·15, 1·83, 1·73, 1·43	9·35
Xba I	A	12·10, 4·51, 1·48	18·09
Xho I	A	Single or no cut site	

*The restriction patterns from where an enzyme cuts once (circular molecule has been linearized but is still a single piece) or not at all (closed circular molecule) are indistinguishable on electrophoretic gels because of similar migration distance.

†Two fragments comigrate.

The cleaned PCR products were directly sequenced using a commercially available sequencing kit which uses T7 DNA Polymerase (Sequenase Version 2 DNA Sequencing Kit, U.S. Biochemical) and the HN20 primer. The sequencing protocols followed those provided with the kit (based on the method of Sanger *et al.*, 1977) except that the termination reaction was performed at 50° C for 5 min. These conditions produced the heavy strand sequence which was then, by convention, translated into the light strand sequence for presentation.

DESIGNATION OF mtDNA HAPLOTYPES

Restriction fragment patterns obtained for each enzyme were assigned a letter in order of discovery. Each letter represented a different mtDNA pattern for a given enzyme. Only patterns produced by variation in the recognition sites of the enzymes and not those originating from variation in the length of the mtDNA molecule were considered (see below). Similarly, each different control region sequence was represented by a different letter. The mtDNA haplotype of each fish was then determined by the combination or set of letters over all enzymes and the control region sequence.

DATA ANALYSIS

The statistical significance of differences in haplotype frequencies among fish from a pair of sampling locations was tested using the randomized generation of the χ^2 distribution by the Monte Carlo procedure (Roff & Bentzen, 1989). Two populations were considered to have significantly different haplotype frequencies if higher χ^2 values were generated 5% or less of the time in 1000 randomized simulations. This analysis is very conservative and was designed for use with the finite sample sizes typical of most molecular analyses. Log likelihood ratio χ^2 or G-tests were used to test if lake sturgeon from different rivers had significantly different haplotype counts; sampling locations within rivers were combined. The pooling of sampling locations within rivers yielded adequate sample sizes for such tests.

The combinatorial analysis of Hebert *et al.* (1988) as applied by Bernatchez *et al.* (1989) was used to test the sensitivity of the RFLP analysis to detect mtDNA diversity in Moose River lake sturgeon. This analysis determined the relationship between the number of restriction enzymes sampled and the number of mtDNA haplotypes detected. The procedure began by choosing (randomly) two restriction enzymes from those used and determining the resulting number of haplotypes which would have been detected (based on the variation observed). The random choice of enzymes was repeated in increments of two enzymes (2, 4, 6 etc.) until the total number of enzymes used was reached. The mean number of haplotypes detected in 10 randomizations per increment of two enzymes was plotted against number of restriction enzymes. This analysis only included the 33 enzymes which had multiple recognition sites in lake sturgeon mtDNA (Table II).

III. RESULTS

RESTRICTION SITE VARIATION

The average size of the sturgeon mitochondrial genome was estimated at 16·6 kb and therefore comparable to the 16·9 kb reported by Guenette *et al.* (1993). We observed five discrete mtDNA genome length variants among the lake sturgeon studied.

Thirty-eight restriction enzymes produced identical fragment patterns in all fish examined (Table II). The fragment patterns generated by only two enzymes (*Ava* II and *Hinc* II) differed among individuals. These polymorphisms corresponded to those described previously by Guenette *et al.* (1993) and discriminated only two mtDNA haplotypes identical to genotypes 1 and 2 of those

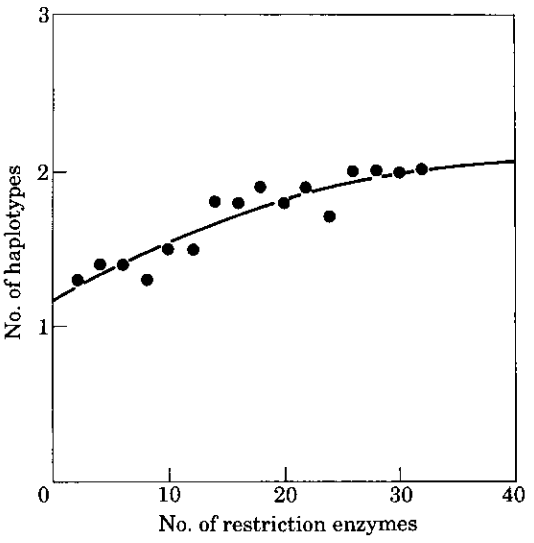

FIG. 1. Number of mtDNA haplotypes detected as a function of randomized incremental choice of restriction enzymes among lake sturgeon from the Moose River basin. Each data point represents the mean of 10 random subsamples of enzymes (see text for explanation).

authors. Despite the use of a greater number of restriction enzymes, we were unsuccessful in detecting additional restriction site polymorphisms. Thus, the number of haplotypes detected in the combinatorial analysis rose with the number of restriction enzymes until an asymptote value of two was reached (Fig. 1).

SEQUENCE VARIATION

A minimum of 275 bp of the 3′ end of the control region was sequenced for 60 individuals (Fig. 2). This sequence characterized 30 of the 60 lake sturgeon. Thirty other fish showed another sequence type differing by a single C–T transition at nucleotide 54. All fish with a particular control region sequence shared the same restriction haplotype. The sequence analysis did not increase the genetic resolution as no additional haplotypes were detected. The sequence data were not considered further for the population analysis.

DISTRIBUTION OF mtDNA HAPLOTYPES AND POPULATION STRUCTURE

We found no evidence of population differentiation among lake sturgeon captured from different sites within the same tributary of the Moose River. The number of lake sturgeon with different haplotypes did not differ significantly among Cypress Falls, Kipling Dam, and Carmichael Falls (1990 and 1992 samples pooled) within the Mattagami/Groundhog system (all adjusted probability values from the Monte Carlo simulations $>0{\cdot}05$). Similarly, lake sturgeon from Otter Rapids did not have significantly different haplotype frequencies from those captured in the lower Abitibi River (Table I).

Lake sturgeon captured from different tributaries of the Moose River had significantly different haplotype frequencies (sampling locations within rivers pooled). North French River lake sturgeon had a significantly higher frequency of haplotype 2 than those from the Mattagami/Groundhog ($G=8{\cdot}56$; 1 d.f.;

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                                CSB 1
TAATAGTGAA TGACTTAATG ACATATCCTG AATATCACAC ATAGTCTGTA    50
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          *
CCATGTACAT GTAGTGAGCG TTTACCGAGG CCTAAGTCTT ACCCCCACAT   100

                                                  CSB 2
AGTAATCAAA TGCCACAAAC GTTTGTTATC GACAAACCCC CTACCCCCTT   150
                                  ----------

                                        CSB 3
TACGCCAGAC AAGCCTTATA TTTCTTGTCA AACCCCAAAA GCAGGACTGA   200
                                  ----------

CTTGTCATCA ACGTACATCC GATTACCCCA ACATGCCTTA GCTGTACAAA   250

TATTTATTCA CTATATTTTC ATGTA                             275
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FIG. 2. Light strand sequence of a 275 bp segment located at the 3′ end of the lake sturgeon control region, haplotype 2. The asterisk indicates nucleotide 54 for which haplotypes 1 and 2 differ by a thymine–cytosine transition. The underlined sequences are the locations of consensus sequence blocks found in control regions of other vertebrates.

P=0·003) and Abitibi Rivers (G=7·98; 1 d.f.; P=0·005) while Mattagami/Groundhog lake sturgeon did not differ significantly from Abitibi River fish (G=0·09; 1 d.f.; P=0·755).

IV. DISCUSSION

LEVELS OF mtDNA VARIATION IN LAKE STURGEON

A salient result of this study was the extremely reduced level of mtDNA nucleotide sequence polymorphism detected in lake sturgeon. The combinatorial analysis suggests that the lack of mtDNA variation is not related to insufficient analytical resolution and may represent the biological reality for the species. These results are congruent with the more general observations that genetic variability is low in most North American chondrosteans studied to date (Carlson *et al.*, 1982; Phelps & Allendorf, 1983; Bowen & Avise, 1990; Brown, 1991; Brown *et al.*, 1992*b*). Reduced mtDNA genetic variation has also been reported in several north temperate fishes for which the distribution range was reduced during the Pleistocene glaciation events (Bentzen *et al.*, 1989; Bernatchez & Dodson, 1991; Danzmann *et al.*, 1991). Both phylogenetic and historical factors could be responsible for the low genetic variability observed in lake sturgeon.

The variation in the total size of the lake sturgeon mtDNA molecule we observed is similar to that reported in previous studies (Brown, 1991; Guenette *et al.*, 1993). Buroker *et al.* (1990) and Brown *et al.* (1992*a*) attributed mtDNA size variation in white sturgeon to be the result of a discrete number of tandem repeats of an 82 bp sequence which are present in one to six copies per individual

fish. Because the inheritance of size variation has not been established, its utility as a genetic marker is suspect until studies on transmission genetics are conducted. We chose, therefore, not to use these data within the present context.

Variation of the mitochondrial D-loop was studied because previous reports had suggested that sequence analysis of this region revealed higher genetic diversity than that detected by RFLP analysis performed over the entire molecule (Vigilant *et al.*, 1989; Brown *et al.*, 1993; Bernatchez & Danzmann, 1993). Brown *et al.* (1993) reported for white sturgeon that the variation in a segment of the control region was four to five times that detected in RFLP analysis. In brook charr (*Salvelinus fontinalis*, Mitchill), Bernatchez & Danzmann (1993) observed twice as much variation in a segment of the control region as that revealed by RFLP analysis. Our results contrast with those observations and suggest that the segment of the control region which we analysed in lake sturgeon is not highly variable, and has limited usefulness for population studies.

GEOGRAPHIC DISTRIBUTION OF mtDNA HAPLOTYPES AND POPULATION STRUCTURE

The statistical analysis of haplotype frequency distributions revealed low levels of genetic differentiation among lake sturgeon captured from different sites within the same watershed. Only lake sturgeon from the North French River showed any genetic differentiation from the other samples, suggesting that they composed a discrete genetic stock. The lack of significant heterogeneity in mtDNA haplotype frequencies among lake sturgeon captured from different sites in the Mattagami, Groundhog, and Abitibi Rivers does not suggest the existence of discrete genetic stocks within this portion of the watershed. This pattern of population structure is consistent with knowledge of lake sturgeon biology (Binkowski & Doroshov, 1985) as well as recent radiotelemetry work (R. S. McKinley, Ontario Hydro Research, unpubl. results) in the Moose River basin which indicated strongly that this species migrates long distance. The life history pattern of lake sturgeon is conducive to extensive gene flow, given that a low number of migratory female spawners per generation is sufficient to prevent significant divergence of mtDNA variation between sites (Allendorf & Phelps, 1981; Chakraborty & Leimer, 1987).

Compared with the limited population differentiation observed within the Moose River basin, a distinct spatial partitioning is observed between James Bay and the St Lawrence River watersheds (Guenette *et al.*, 1993). Guenette *et al.* (1993) observed that most fish (66 out of 70) captured from the St Lawrence corridor were haplotype 1. This pattern of genetic subdivision may reflect historical differences in postglacial recolonization of both watersheds (Crossman & McAllister, 1986). We are currently expanding the phylogeographic analysis to test the hypothesis that lake sturgeon belonging to distinct glacial races have used distinct routes of colonization to reinvade their contemporary range of distribution.

MANAGEMENT AND CONSERVATION OF LAKE STURGEON

A general goal in conservation biology is to conserve as much of the intraspecific genetic diversity as possible, as well as preserving its structural

integrity (Meffe, 1987). Anthropogenic activities can have deleterious effects on both aspects. This is well illustrated by the recent work of Brown *et al.* (1992*b*) with white sturgeon in the Fraser and Columbia Rivers. Fragmented populations in the upper reaches of the Fraser and Columbia Rivers had lower mtDNA diversity than those collected downstream. Furthermore, the overall mtDNA diversity of white sturgeon in the Columbia River has been reduced perhaps through overexploitation.

Our data suggest that gene flow among sampling locations of lake sturgeon from the same river basin is substantial. Habitat alterations such as dam construction or other artificial barriers to migration would have an impact upon the genetic integrity of the species in such systems. One potential outcome is the fragmentation into isolated populations, and loss of genetic variability through evolutionary processes such as random genetic drift and inbreeding. These observations emphasize the importance of considering knowledge of critical life history parameters such as population structure to ensure the long-term survival and evolution of sensitive species such as the lake sturgeon.

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