Mitochondrial DNA Variation among Anadromous Populations of Cisco (*Coregonus artedii*) as revealed by Restriction Analysis[1]

Louis Bernatchez and Julian J. Dodson

Département de biologie, Université Laval, Sainte-Foy (Québec). G1K 7P4 Canada

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Mitochondrial DNA (mtDNA) restriction analyses were done to test the hypothesis that river populations of anadromous cisco from James-Hudson Bays that exhibit life-history variations are reproductively isolated. MtDNA variation among 141 cisco (*Coregonus artedii*) from eight rivers was studied with eight hexameric, four multihexameric and one multipentameric restriction enzymes which generated a mean of 78 fragments per fish. Pairwise sequence divergence estimates among the 19 mtDNA clones resolved were generally low but highly variable (mean: 0.52%, range: 0.08 to 1.03%). UPGMA and Wagner parsimony analysis revealed two major clonal groups which differed in diversity and geographic distribution; the more diversified (nucleon diversity index; 0.70) and less diversified (0.31) groups were twice as abundant in James and Hudson Bays, respectively. This suggests that cisco of James-Hudson Bays are derived from two glacial refugia and that they recolonized the area by two major postglacial routes. The frequency distribution analysis of all defined clones revealed significant stock discreteness among Hudson Bay populations and a lack of population subdivision in James Bay. The genetic structuring and diversity of anadromous cisco in James-Hudson Bay is discussed in relation to historical biogeographical events and to interactions of various life-history stages with the physical oceanographic environment.

Nous avons utilisé l'analyse de polymorphisme de longueur de fragments de restriction (PLFR) de l'ADN mitochondrial (ADNmt) pour tester l'hypothèse voulant que les populations de ciscos (*Coregonus artedii*) anadromes des baies James et d'Hudson démontrant des différences dans leur cycle vital soient isolées reproductivement. Nous avons étudié la variabilité de l'ADNmt de 141 ciscos provenant de huit rivières avec huit enzymes hexamériques, quatre multihexamériques et un multipentamérique qui ont généré en moyenne 78 fragments par poisson. L'estimé du pourcentage de divergence de séquence entre les 19 clones mitochondriaux identifiés était généralement faible mais très variable (moyenne: 0.52%, étendue: 0.08 à 1.03%). Les analyses de groupement selon l'association moyenne et de parcimonie de Wagner ont révélé deux groupes clonaux qui diffèrent par leur diversité et leur distribution géographique. Le plus diversifié (indice de diversité nucléique; 0.70) et le moins diversifié (0.31) de ces groupes étaient respectivement deux fois plus abondants dans la baie James et la baie d'Hudson. Ceci suggère que les ciscos des baies James et d'Hudson proviennent d'au moins deux refuges glaciaires et qu'ils ont recolonisé cette région par deux routes postglaciaires distinctes. L'analyse des fréquences de distribution de tous les clones identifiés a démontré que les populations de la baie d'Hudson mais non celles de la baie James étaient isolées reproductivement. Nous discutons l'hypothèse voulant que la structure et la diversité génétique des ciscos anadromes des baies James et d'Hudson soient déterminées par l'histoire biogéographique de l'espèce de même que par l'intéraction des différents stades de développement avec l'environnement océanographique physique du milieu.

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Anadromous populations of lake cisco (*Coregonus artedii*) inhabit large river systems of James and Hudson Bays. On the eastern coast, the species is found in variable abundance from the Rupert River to the Povungnituk River (Fig. 1) where it represents a major component of the domestic fisheries and a resource with potential for commercial exploitation by the native people (Morin and Dodson 1986). Knowledge of its population structure is thus becoming of major concern for adequate management and resource partitioning among native communities whose populations are expected to double within the next 25 yr.

Migration is an essential component at all stages of the life cycle of anadromous cisco (Dodson et al. 1986). In James Bay, the spring hatching period coincides with spring thaw, and larvae are flushed from the rivers into the bay. Juveniles and nonreproductive adults reenter the lower reaches of the rivers during the fall where they overwinter. The following spring, fish of all ages aggregate in the estuaries waiting for the retreat of the ice pack on the bay to migrate to feeding grounds. The upstream migration of mature fish begins by late summer and spawning occurs in late fall. Populations of Hudson Bay differ from those of James Bay by the absence of juvenile fish in the spring aggregation in estuaries which suggests that they either overwinter in different rivers or migrate early to the bay under the ice pack (Kemp et al. 1989).

Fish from different rivers exhibit differences in growth, age at maturity, reproductive effort, and mortality rate (Morin et al. 1982). These differences are believed to represent local adjust-

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ments of the life history strategy resulting from trade-offs in energy allocation among growth, maintenance, and reproduction in response to different migration costs associated with the difficulty of upstream migration in each river. (Lambert and Dodson 1990; Kemp 1989). These differences fit the predictions of theoretical models of optimal reproductive strategies and thus fulfil the major criterion of local adaptation of the stock concept. However, the reproductive isolation of these populations remains to be demonstrated.

Morphological and ecological differences have been widely used to discriminate fish stocks (Ihssen et al. 1981). However, the problems in extrapolating phenotypic data to the underlying genotypic information are numerous (Ferris and Berg 1987). In the case of cisco species, the phentoypic variation observed among populations may be largely due to the influence of environmental factors during early development and thus cannot always provide strong evidence for reproductive isolation (Todd and Smith 1980; Todd et al. 1981). Because they are not environmentally influenced, genetic markers are more reliable than other traits for stock identification. Allozyme analysis has been widely used for this purpose and examples of its usefulness are numerous (Allendorf et al. 1986). However, this technique has failed to reveal differences between four species of Great Lakes ciscoes due primarily to a lack of allozyme polymorphism in this group of fish (Todd 1981).

In recent years, the analysis of mitochondrial DNA (mtDNA) polymorphism by restriction endonucleases has often been recognized as approaching the ideal method for quantifying genetic differences among populations (Avise 1987; Ferris and Berg 1987; Wilson et al. 1985). Two features of mtDNA enhance its utility for stock identification; its rapid evolution relative to the nuclear genome and its maternal mode of inheritance (Moritz et al. 1987). Currently used mtDNA analyses appear inadequate for the discrimination of mtDNA genotypes differing solely by the accumulation of mutations over the approximately 18 000 yr that have elapsed since the end of Wisconsinan glaciation (Wilson et al. 1985). However, stocks can be discriminated by the study of distribution frequencies of mtDNA polymorphisms that predate the genetic isolation of populations (Bentzen et al. 1989; Bernatchez and Dodson 1990; Bernatchez et al. 1989). Furthermore, the same mtDNA restriction analysis used for stock discrimination may provide insight into the historical biogeography of the species under study (Avise 1987).

In this paper, we report the results of mtDNA restriction analysis carried out to test the hypothesis that river populations of anadromous cisco from James-Hudson Bays are reproductively isolated. This study also provides a first database of mtDNA fragment size variability which will help orient sampling effort in studies of other populations of this species.

Methods

Sampling

Cisco from eight populations of the eastern coast of James-Hudson Bays were sampled at the mouth of their overwintering rivers during the spring of 1987 and at the end of the summer 1988 (Fig. 1; Table 1). In 1987, livers and developing ovaries were extracted from freshly caught specimens, frozen immediately and kept on dry-ice for several months before mtDNA was extracted. In 1988, mature ovaries were kept on wet ice and mtDNA was extracted within 10 d following capture.

Isolation and Restriction Enzyme Analysis of mtDNA

MtDNA was purified according to Gonzalez-Villa Señor et al. (1986) as modified by Bernatchez et al. (1988). MtDNA was digested separately with eight hexameric, four multihexameric and one multipentameric restriction endonucleases (Table 2). Mitochondrial DNA fragments were electrophoretically separated on 0.8 or 1.2% agarose gels for 16 h at 25 V.

Visualization of Restriction Fragments

When mtDNA was obtained in sufficient quality and quantity, ethidium bromide staining was sufficient to reveal the digested fragments. For many of the frozen samples with low or poor quality yield of mtDNA, DNA was denatured, neutralized, and transferred to nitrocellulose filters by the procedure described by Maniatis et al. (1982). Filters were hybridized with a highly purified radiolabeled total mtDNA probe. ^{32}P-labeled DNA with a specific activity of at least 10^8 cpm/μg was prepared with ^{32}P-dATP and ^{32}P-dCTP. The nick translation reaction contained 2 μL of highly purified mtDNA (40–80 ng$\cdot\mu$L^{-1}), 2.5 μL of 10$\times$ NT buffer (Maniatis et al. 1982), 1 μL of each unlabelled dGTP and dTTP (1 μmol$\cdot\mu$L^{-1}), 5 μL of each labeled ^{32}P-dATP and ^{32}P-dCTP (29.6 TBq = 800 Ci$\cdot$mmol^{-1}), 2.0 μL of EDTA (1 mM) and 1.0 μL of DNase (10 ng$\cdot\mu$L^{-1}). Distilled water was added to a final volume of 25 μL. The reaction was left 10 min at room temperature, then 1 μL of DNA polymerase 1 (10$\cdot\mu$L^{-1}) was added to the mixture and left 45 min at room temperature. The reaction was stopped with 500 mM EDTA to a final concentration of 20 mM and the volume adjusted to 40 μL with 1 $\times$ TE buffer (Maniatis et al. 1982). The nick-translated DNA was then separated from unincorporated nucleotides by column chromatography on Sephadex G-100 medium (Maniatis et al. 1982). The nitrocellulose filters were prehybridized and hybridized individually in a plastic bag following the procedures of Wahl et al. (1979). Filters were autoradiographed using an intensifying screen (Cronix lightning-plus) for 2 to 16 h.

Data Analysis

Size estimates of fragments were made by running simultaneously into the agarose gel digests of phage lambda DNA with *Hind*III and *Eco*RI-*Hind*III double digest. No attempt to score fragments less than 350 base pairs was made. Distinct single endonuclease patterns were identified by a specific letter in order of appearance. Each fish was assigned a multi-letter code which described its composite mtDNA genotype.

Sequence divergence between genotypes was estimated according to Upholt's (1977) fragment method. Sequence divergence and standard deviation were estimated independently for hexameric and multihexameric enzymes (*Ava*II was treated with multihexameric) and both estimates were then pooled following weighting for the number of base pairs sampled by each type of enzyme. The resulting distance matrix was clustered by UPGMA (Sneath and Sokal 1973) using the average linkage algorithm of the SAS statistical package. The diversity of mtDNA lineages was estimated with Nei and Tajima's (1981) nucleon diversity index;

$$h = \frac{1}{n-1}\left[n\left(1 - \sum_{i=1}^{l} x_i^2\right)\right],$$

where x_i is the frequency of the ith type of mtDNA in a population of n specimens and l is the number of mtDNA types.

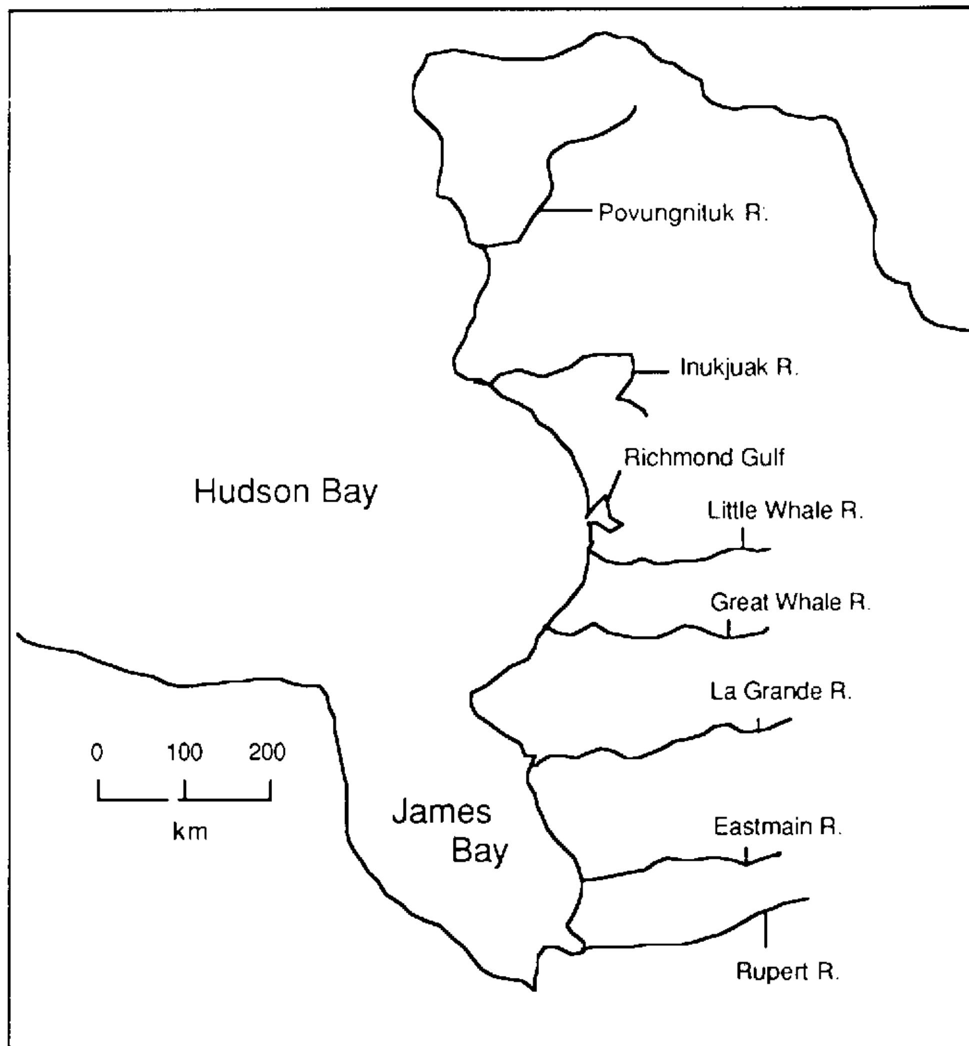

FIG. 1. Location map with sampling sites of anadromous cisco.

TABLE 1. Sample locations, sample sizes, number of clonal lines, and estimated nucleon diversity for anadromous cisco populations sampled in James-Hudson Bays. Nucleon diversity was not estimated for the Great Whale and Inukjuak because of small sample sizes.

Population	Sample size	Genotypes number	Nucleon diversity
James Bay			
Rupert River	22	9	0.83
Eastmain River	29	9	0.85
La Grande River	27	8	0.83
Hudson Bay			
Little Whale River	17	4	0.55
Richmond Gulf	17	2	0.52
Povungnituk River	19	4	0.71
Great Whale River	4	3	—
Inukjuak River	6	4	—

The mtDNA clones were organized into a Wagner network using the Metro algorithm of the Phylip package provided by Joe Felsenstein (Department of Genetics, University of Washington, Seattle, WA 98195).

The geographical heterogeneity in the frequency of mtDNA genotypes among populations was analyzed using the randomized generation of chi-square distribution by a Monte Carlo technique (Roff and Bentzen 1989). This procedure circumvents the problem of low sample size by estimating the probability of obtaining a chi-square value larger than that observed by randomization of the original data sets. By this method, the accuracy of the estimate of Type 1 error depends only upon the number of randomizations of the original data sets. In this case, 1000 randomizations were performed.

We also estimated the likelihood of detecting mitochondrial DNA diversity at the population and regional levels within the confines of our sampling protocol. To do so, we adopted the combinatorial approach of Hebert et al. (1988). The relationship between the number of clones detected as a function of sample size was estimated by an incremental random choice of individuals. This procedure was done at the population level and also at the regional level by pooling all fish from a given region. An increment choice of three fish at the population level and five fish at the regional level was used to estimate the number of clones detected for each incremental step. The procedure was repeated 10 times for each sampling intensity to generate an estimate of random sampling variance. The relationship between the number of clones detected as a function of genome

Table 2. Fragment size estimates (base pairs) for all fragment patterns resulting from the restriction analysis of cisco mtDNA. No attempt to estimate fragments below 350 base pairs was made.

*Ava*I					*Ava*II					*Ban*I	
A	B	C	D	E	A	B	C	D	E	A	B
3 550	3 550	3 740	3 550	3 550	4 800	4 800	4 800	4 800	4 800	3 700	3 700
2 950	2 950	3 550	2 950	2 950	1 675	1 675	1 675	1 675	2 160	2 550	2 550
2 500	2 650	2 500	2 500	2 500	1 450	1 450	1 450	1 450	1 675	2 200	2 200
2 400	2 500	2 400	2 500	2 400	1 300	1 300	1 425	1 425	1 450	2 000	2 000
1 550	1 550	1 550	1 550	1 550	1 250	1 250	1 300	1 300	1 300	1 700	1 575
1 250	1 250	1 250	1 250	1 250	970	970	970	1 075	970	1 150	1 150
1 150	1 150	1 150	1 150	1 150	910	970	910	970	770	880	880
850	850	850	850	790	770	770	770	770	750	800	800
790	790		790	620	750	750	750	750	450	740	740
					450	450	450	450	450	380	380
					450	450	450	450	360		
					360	360	360	360	350		
					350	350	350	350			
16 990	17 140	16 990	17 090	16 760	15 485	15 545	15 660	15 825	15 485	16 100	15 975

*Dra*I		*Hae*II						*Hinc*II				*Pvu*II
A	B	A	B	C	D	E	F	A	B	C	D	
5 600	6 150	4 300	4 300	4 850	4 300	4 300	4 850	6 400	6 400	6 400	6 400	9 500
4 600	4 600	4 300	4 300	4 300	3 900	4 300	4 300	2 900	2 900	2 150	2 900	5 000
3 300	3 300	3 700	3 700	3 700	3 700	3 700	3 700	2 050	2 050	2 050	2 050	1 640
1 625	1 625	1 400	1 400	1 400	1 400	1 400	1 400	1 575	1 750	1 575	1 700	860
690	690	1 100	1 200	1 100	1 100	1 100	1 100	1 450	1 575	1 450	1 450	
630	630	920	1 100	920	920	1 000	1 100	1 125	1 125	1 125	1 125	
550		825	825	825	825	825	825	840	840	840	840	
		550	550		550	550				750		
					400							
16 995	16 995	17 095	17 375	17 095	17 095	17 175	17 275	16 340	16 640	16 340	16 465	17 000

*Bgl*I		*Hind*III			*Sma*I	*Xmn*I			*Bam*HI	*Pst*II	
A	B	A	B	C		A	B	C		A	B
8 100	7 600	4 220	4 220	4 220	9 400	7 600	5 050	7 600	17 000	11 780	11 780
7 600	7 600	3 500	3 800	3 740	7 550	5 050	5 050	5 050		2 700	4 750
1 300	1 300	3 500	3 500	3 500		4 200	4 200	2 850		2 050	470
	500	3 200	3 200	3 200			2 550	1 350		470	
		2 400	2 400	2 400							
17 000	17 000	16 820	17 120	17 060	16 950	16 850	16 850	16 850	17 000	17 000	17 000

sampling intensity was estimated by incremental random choice of restriction enzymes. Results were obtained by an incremental choice of two restriction enzymes at both population and regional levels. The random choice of enzymes was repeated 10 times for each incremental step. As different restriction enzymes produced different numbers of fragments, we attributed the fragment numbers to their respective restriction enzymes which yielded a sum of the fragments for any enzyme combination.

Results

Restriction Sites Variation

The 13 enzymes used generated a total of 105 restriction fragments with a mean of 78 per individual. Considerable polymorphism was observed among the 141 cisco analyzed. All enzymes but three were polymorphic (Table 2) which allowed identification of 19 mitochondrial genotypes (Table 3). The estimated nucleon diversity among all fish analyzed was 0.79. This value is intermediate within the values calculated for other fish species that were studied over a wide geographic range using similar analyses (Table 4). The mean size of mtDNA genome as estimated by averaging the sums of all digestion patterns but *Ava*II (omitted because of the number of undetected small fragments) was 16 945 bp ± 240 (SD). The length estimate for the mtDNA molecule of *C. artedii* falls within the values observed in other fishes studied to date (data compiled in Billington and Hebert 1988; Kornfield and Bogdanowicz 1987; Bentzen et al. 1989). Pairwise sequence divergence estimate between all mtDNA clones was generally low but highly variable (mean: 0.52 ± 0.22 (SD), range: 0.08 to 1.03%) (Table 5). Clustering of the distance matrix by UPGMA revealed two major clonal groups (A, B) separated by a mean sequence divergence of 0.52% (Fig. 2). These composed respectively 50 and 45% of the 141 fish analyzed. The remaining 5% was distributed among five highly divergent clones (group C). The Wagner parsimony analysis generated a total of 31 equally parsimonious networks requiring a minimum

TABLE 3. Description of clonal lines, number of individual fish, and frequency distribution of each clone observed among anadromous cisco from James-Hudson Bays. Composites represent the following order of enzymes; *Ava*I, *Ava*II, *Ban*I, *Bgl*I, *Dra*I, *Hae*II, *Hinc*II, *Hind*III, *Pvu*II, *Sma*I, *Xmn*I, *Pst*I, *Bam*HI.

Clone	Composite	No. of fish	James Bay			Hudson Bay				
			Rupert	Eastmain	LaGrande	Great Whale	Little Whale	Richmond Gulf	Inukjuak	Povungnituk
1	A A A A A A A A A A A A A	53	7	6	8	1	11	10	1	9
2	A A A A A A A A A A A A B	1					1			
3	A A A A A A A B A A A A A	1				1				
4	A A A A A A A C A A A A A	1	1							
5	A A A A A D A A A A A A A	2	1						1	
6	A A A A B A A A A A A A A	4								4
7	A C A B A C A A A A A A A	1	1							
8	A C B B A A A A A A A A A	33	6	8	4		4	7	2	2
9	A C B B A A A A A A B A A	1		1						
10	A C B B A A A A A A C A A	15	1	6	7		1			
11	A C B B A A C A A A A A A	8	3	2	3					
12	A C B B A C A A A A A A A	11	1	3	1				2	4
13	B B B B B B A A A A A A A	1				1				
14	C C B B A C A A A A A A A	1			1					
15	D B A A A E B A A A A A A	1				1				
16	D D B B A C D C A A A A A	2			2					
17	D D B B A F D C A A A A A	2	1	1						
18	D E B B A E B A A A A A A	1		1						
19	E A A A A A A A A A A A A	2		1	1					

TABLE 4. mtDNA nucleon diversity index estimated for various species studied by conventional restriction analysis in a wide geographic range. Maximum possible value is 1.0. Only studies covering a wide geographic distribution of the species and performed with a comparable number of restriction enzymes were selected to reduce sampling biases. However, these values should be considered with caution as different enzymes were used in different studies.

Species	Diversity	Reference
Atlantic menhaden *(Brevoortia tyrannus)*	0.99	Avise et al. 1989
Warmouth sunfish *(Lepomis gulosus)*	0.97	Bermingham and Avise 1986
European whitefish *(Coregonus lavaretus)*	0.95	Bernatchez et al. 1989
Atlantic herring *(Clupea harengus)*	0.91	Kornfield and Bogdanowicz 1987
Pacific herring *(C. harengus pallasi)*	0.91	Schweigert and Withler 1990
Bowfin *(Amia calva)*	0.81	Bermingham and Avise 1986
Cisco *(Coregonus artedii)*	0.79	Present study
American shad *(Alosa sapidissima)*	0.79	Bentzen et al. 1989
Lake trout *(Salvelinus namaycush)*	0.77	Grewe and Hebert 1988
Brown trout *(Salmo trutta)*	0.72	Gyllensten and Wilson 1986
Redear sunfish *(Lepomis microlophus)*	0.68	Bermingham and Avise 1986
Chinook salmon *(Oncorhynchus tshawytscha)*	0.60	Wilson et al. 1987
Walleye *(Stizostedion vitreum)*	0.55	Billington and Hebert 1988
American eel *(Anguilla rostrata)*	0.54	Avise et al. 1986
Hardhead catfish *(Arius felis)*	0.47	Avise et al. 1987b
Lake whitefish *(Coregonus clupeaformis)*	0.31	Bernatchez et al. 1989

of 35 site changes. Figure 3 illustrates the mutational steps required to move from one clone to another. Clone 8 (group B) appeared central in the minimum number of steps required to move to any clonal line. It was never more than eight steps distant from any clone while the maximum number of steps required between two clones was 15 (clones 13 and 17). Clonal group A and B were separated by one *Ban*I and *Bgl*I site gain and two *Ava*II site losses. Clonal lines pooled into group C in UPGMA differed from one another and from group A and B by many site gains or losses. Other trees obtained differed from Fig. 3 mainly by alternative positions and mutational steps related to these clonal lines.

Geographic Variation

A significant difference in the frequency distribution of clonal groups A and B was observed among populations (Table 6). It is apparent that this heterogeneity is due to a genetic split between James and Hudson Bays. Clonal group A was most abundant in populations of Hudson Bay while clonal group B was most abundant in populations of James Bay (Fig. 4). The overall ratio of A/B was 1.94 in Hudson Bay compared with 0.52 in James Bay. James Bay also differed by the presence of group C clones in all populations as opposed to their absence in Hudson Bay. Clonal group B exhibited a much higher nucleon diversity index (0.70) than A (0.31). Thus, the estimated diversity index was higher for any population of James Bay than Hudson Bay (Table 1).

The frequency distribution analysis of all defined clones revealed no evidence of stock discrimination among James Bay populations ($X^2 = 25.49$, df = 6, $P > 0.25$, 506/1000 random-

TABLE 5. Pairwise percent sequence divergence estimates (below diagonal) with corresponding standard deviation (above diagonal) calculated for clonal lines observed among anadromous cisco from James-Hudson Bays. This distance matrix was used to cluster clonal lines by UPGMA.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	0	0.12	0.15	0.15	0.18	0.18	0.2	0.2	0.23	0.17	0.22	0.23	0.28	0.25	0.27	0.27	0.29	0.24	0.18
2	0.13		0.13	0.13	0.18	0.13	0.24	0.24	0.26	0.21	0.26	0.27	0.3	0.29	0.21	0.3	0.33	0.28	0.18
3	0.09	0.23		0.15	0.16	0.24	0.23	0.22	0.24	0.18	0.25	0.26	0.29	0.28	0.2	0.27	0.29	0.28	0.16
4	0.09	0.23	0.09		0.16	0.24	0.23	0.22	0.24	0.18	0.25	0.26	0.29	0.28	0.2	0.24	0.26	0.28	0.16
5	0.13	0.26	0.21	0.21		0.18	0.21	0.23	0.26	0.23	0.24	0.23	0.3	0.25	0.32	0.27	0.3	0.25	0.25
6	0.14	0.28	0.24	0.24	0.26		0.24	0.23	0.25	0.18	0.26	0.27	0.24	0.29	0.22	0.3	0.33	0.29	0.18
7	0.35	0.49	0.44	0.44	0.39	0.5		0.25	0.21	0.17	0.29	0.18	0.26	0.25	0.25	0.17	0.21	0.39	0.23
8	0.34	0.48	0.44	0.44	0.48	0.49	0.27		0.17	0.17	0.14	0.17	0.23	0.25	0.25	0.21	0.21	0.29	0.23
9	0.48	0.63	0.59	0.59	0.61	0.65	0.38	0.13		0.19	0.15	0.17	0.26	0.21	0.29	0.27	0.26	0.22	0.26
10	0.36	0.51	0.47	0.47	0.48	0.53	0.26	0.25	0.39		0.2	0.21	0.29	0.23	0.27	0.25	0.28	0.24	0.23
11	0.43	0.57	0.52	0.52	0.57	0.58	0.36	0.08	0.21	0.34		0.23	0.24	0.29	0.25	0.22	0.21	0.32	0.34
12	0.48	0.63	0.57	0.57	0.53	0.63	0.13	0.13	0.25	0.38	0.22		0.24	0.18	0.26	0.2	0.19	0.34	0.24
13	0.67	0.82	0.77	0.77	0.81	0.54	0.79	0.48	0.61	0.75	0.57	0.63		0.26	0.27	0.29	0.28	0.23	0.3
14	0.64	0.78	0.72	0.72	0.68	0.78	0.27	0.27	0.38	0.53	0.36	0.13	0.79		0.27	0.21	0.21	0.39	0.26
15	0.32	0.45	0.39	0.39	0.46	0.44	0.68	0.67	0.8	0.66	0.78	0.84	0.62	1.02		0.27	0.29	0.21	0.32
16	0.63	0.78	0.63	0.54	0.67	0.78	0.26	0.54	0.66	0.52	0.64	0.39	0.87	0.55	0.67		0.23	0.22	0.28
17	0.87	1.02	0.87	0.79	0.92	1.02	0.49	0.48	0.61	0.76	0.58	0.35	0.71	0.49	0.81	0.22		0.21	0.31
18	0.63	0.77	0.71	0.71	0.78	0.76	0.68	0.36	0.47	0.62	0.45	0.51	0.48	0.68	0.39	0.64	0.48		0.25
19	0.13	0.26	0.21	0.21	0.27	0.26	0.48	0.48	0.61	0.48	0.57	0.63	0.81	0.79	0.46	0.77	1.03	0.78	0

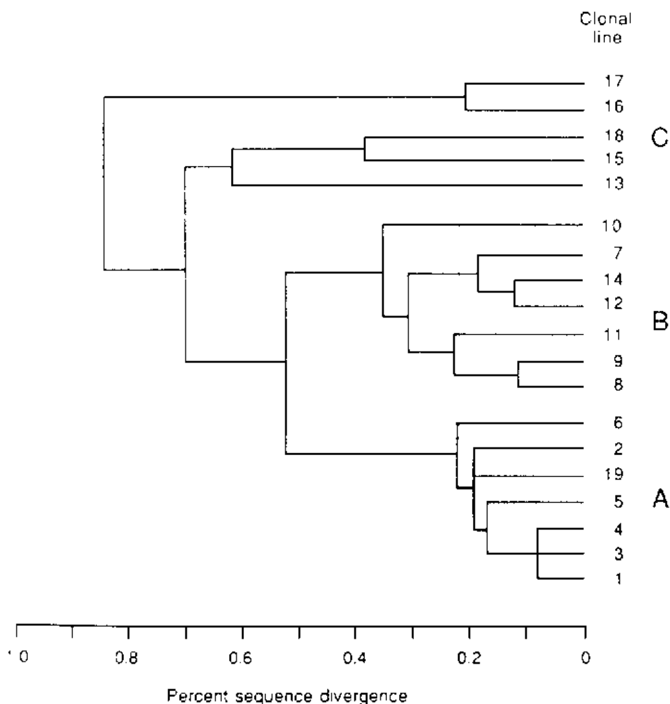

FIG. 2. UPGMA phenogram clustering the distance matrix of sequence divergence between anadromous cisco clonal lines.

ized $\chi^2 > \chi^2$ observed). In Hudson Bay, the Povungnituk River population clearly differed from the homogeneous assemblage of Little Whale River and Richmond Gulf populations ($\chi^2 = 18.24$, df = 5, $P < 0.005$, 0/1000 randomized $\chi^2 > \chi^2$ observed). The Little Whale River - Richmond Gulf assemblage also differed significantly from James Bay ($\chi^2 = 25.62$, df = 14, $P < 0.05$, 11/1000 randomized $\chi^2 > \chi^2$ observed). Although not statistically demonstrated, further structuring in Hudson Bay is suggested by the addition of Great Whale and Inukjuak Rivers. For example, three unique clonal lines were found among four fish sampled in the Great Whale River (Table 3).

Sampling and Detection of mtDNA Diversity

The number of mtDNA clones detected as a function of number of individuals or restriction fragments samples was always higher in James Bay. At the population level, the number of genotypes increased smoothly with the number of fish sampled and approached an asymptotic value of close to nine with a sample of 21 fish in James Bay compared with a value of three with a sample of 15 fish in Hudson Bay (Fig. 5). This suggests that 21 and 15 specimens were sufficient to detect all variability within a given population of James and Hudson Bay, respectively. The same relationship did not tend towards an asymptotic value at the regional level which demonstrates that detection of all mitochondrial variability in both regions would require higher sample sizes (Fig. 5).

The number of genotypes detected at the population level increased with the number of restriction fragments sampled and reached an asymptotic value of close to three with a sample of about 50 fragments in Hudson Bay (Fig. 6). In James Bay, however, a sample of 78 fragments was still insufficient to detect all mtDNA variants. This conclusion also applied at the regional level in both James and Hudson Bay.

Discussion

Mitochondrial DNA Variation and Postglacial Colonization

It has been suggested that contemporary observations of intraspecific genetic structure and diversity have been determined to a large degree by historical geographic factors (Avise et al. 1987a; Bermingham and Avise 1986). The extent to which the Wisconsinan glaciation may have influenced the structure and diversity of cisco populations of James-Hudson Bays is illustrated by a comparison of the nucleon diversity index observed for anadromous populations of cisco with those of lake whitefish (*C. clupeaformis*) and European whitefish (*C. lavaretus*). Lake whitefish from James-Hudson Bays exhibit the lowest nucleon diversity reported in the literature while European whitefish from the Baltic Sea have one of the highest (Table 4), despite similar life cycles and close phylogenetic relationships. We have previously argued that this difference

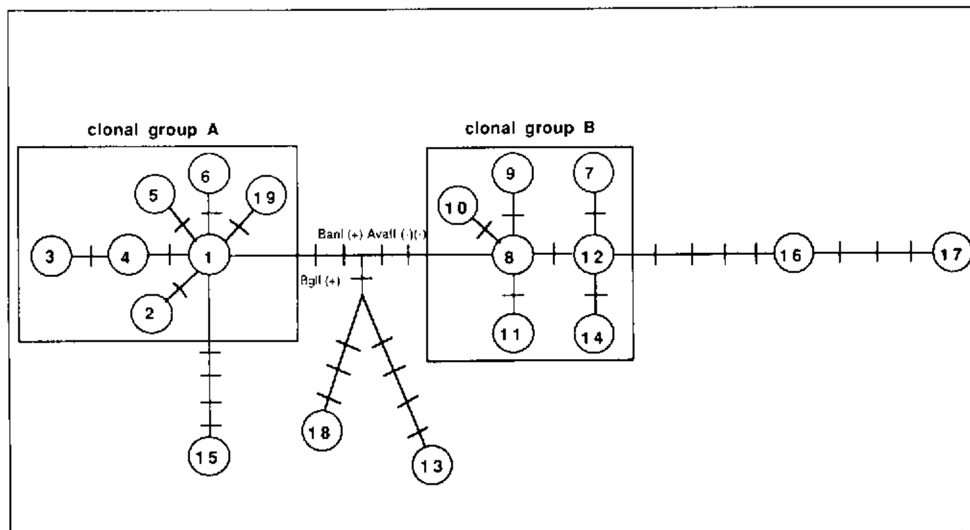

FIG. 3. Parsimony network linking the 19 cisco mtDNA clones revealed by restriction analysis. Clonal groups A and B refer to clusters identified by UPGMA. Site gains (+) or losses (−) that differentiate the two clonal groups are indicated. Sites differences were inferred from differences in fragment patterns among clonal lines.

TABLE 6. Chi-square analysis of the observed frequency distribution of clonal groups A and B among anadromous populations of cisco in James-Hudson Bays. Expected frequencies are given in brackets. $X^2 = 14.35$, df = 5 $P < 0.05$.

	Populations					
	James Bay			Hudson Bay		
Clonal group	Rupert	Eastmain	LaGrande	Little Whale	Richmond Gulf	Povungnituk
A	9(10)	7(12.9)	9(11.9)	12(8.1)	10(8.1)	13(9.1)
B	12(11)	20(14.1)	16(13.1)	5(8.9)	7(8.9)	6(9.9)

may be due to the greater extent of the ice sheet in North America compared with Eurasia during the last glaciation events that led to a more intense bottleneck effect for lake whitefish thus reducing mtDNA variability (Bernatchez et al. 1989). However, cisco from James-Hudson Bays exhibit a nucleon diversity more than twice that of lake whitefish from the same area, and consequently other factors must be considered to explain such differences.

A major genetic difference between the two species in James-Hudson Bays is the existence of two major divergent mtDNA clusters revealed in *C. artedii* as opposed to only one for *C. clupeaformis*. In addition, highly divergent lineages were observed in *C. artedii*. Avise et al. (1984) demonstrated that mtDNA variability observed today is largely influenced by stochastic lineage extinction which in turn is a function of time since population founding, generation time, density regulation, long term effective population size, and population subdivision. The first three parameters are quite similar for these two species such that they are unlikely to have led to dissimilar stochastic lineage extinction. A possible hypothesis is that numerous highly distinct lineages were retained in *C. artedii* because of a much higher abundance of the ancestral population that recolonized James-Hudson Bays. However, while the sorting and subsequent coexistence of highly divergent lineages within a single large population lacking geographic subdivision is theoretically possible, such a situation has not been observed in any species assayed to date (Avise et al. 1988).

An alternative and more plausible hypothesis is that historical population subdivisions in *C. artedii* led to retention of divergent lineages in different refugia. Such population subdivisions provide a buffering effect against lineage extinction (Avise et al. 1984). Hence, although the diversity of lineages within each isolated population was low, the overall diversity within the species could remain high. For example, Billington and Hebert (1988) observed two major mtDNA clonal groups among walleye (*Stizostedion vitreum*) which they associated with different glacial refugia. Nucleon diversity indices calculated independently for each group were low (0.16 and 0.18) but the overall estimate remained relatively high (0.55). In the case of *C. artedii*, it is possible that the two major mtDNA clusters A and B identify ancient populations that evolved in two separate glacial refugia which subsequently recolonized James-Hudson Bays following the retreat of the ice sheet. Fish from only one refugium would have recolonized this area in the case of *C. clupeaformis*. The analogy in overall diversity observed between *C. clupeaformis* (0.31) and cluster A of *C. artedii* (0.31) suggests these two groups of fish may have survived in the same refugium and have undergone similar bottleneck effects during the last glaciation.

Further evidence that clonal groups A and B are derived from two different refugia is provided by their different geographic distributions. Cluster A is twice as abundant as cluster B in populations of Hudson Bay, whereas the reverse is true in James Bay (Fig. 4). Crossman and MacAllister (1985) argued that

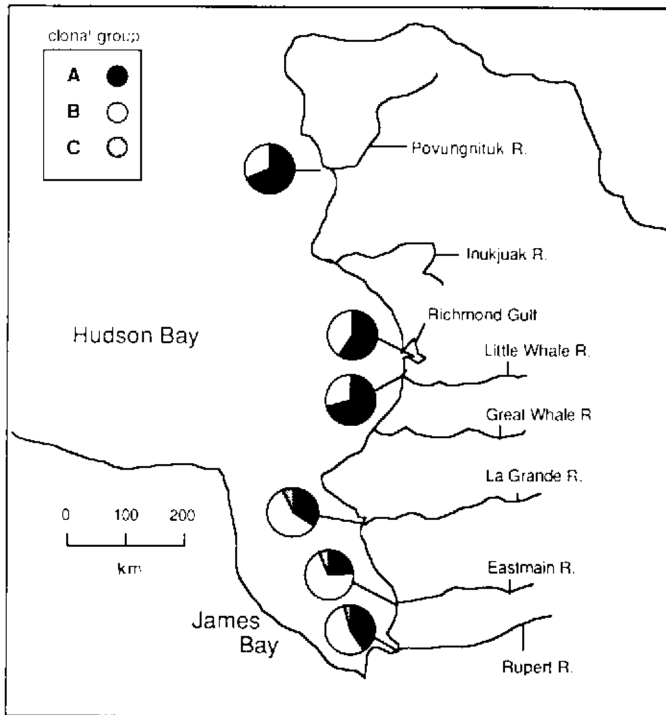

FIG. 4. Distribution of the A (black), B (white), and C (dotted) mtDNA clonal groups identified by UPGMA.

recolonization of the modern Hudson Bay system was possible by two major routes. The earliest connection was via the northwest coast of Hudson Bay followed later by the opening of southern James Bay. Therefore, one group of cisco (clonal group A) may have recolonized the area from the north and the other (clonal group B) from the south. Mixing of the two lineages has occurred since then without completely overriding the historical geographic influences on population subdivision. The genetic evidence for the existence of two major glacial refugia was also noted in lake trout (*Salvelinus namaycush*) and walleye (Grewe and Hebert 1988; Billington and Hebert 1988). Both these studies revealed two major mtDNA clonal groups among fish from the Great Lakes area that the authors associated with recolonization of the area from different refugia. Such concordant gene pool fragmentation among independently evolving species may indicate influences of a shared biogeographic history and suggest the existence of generalized tracks for these species (McDowall 1978).

The presence of five highly divergent clonal lines (cluster C) in James Bay also contributes to the higher nucleon diversity observed for *C. artedii* as opposed to *C. clupeaformis*. However, their origin is problematic as they diverge highly either from clonal group A or B and among themselves. It is possible that they represent introgressed mtDNA of other cisco species, but this hypothesis remains to be tested.

Geographic Variation of mtDNA Lineages

Based on life history variations, it was tacitly accepted that river populations of anadromous cisco in James-Hudson Bays are reproductively isolated. Thus, the premise of this study was that mtDNA restriction analysis should have differentiated each of them. While no unique population specific markers were found, statistical differences in the frequency distribution of mtDNA clones demonstrate that cisco spawning in particular river systems form discrete reproductive units. Three distinct genetic pools were identified; (1) Povungnituk River, (2) Richmond Gulf-Little Whale River, and (3) all James Bay rivers. Further structuring in Hudson Bay is also suggested by the clonal lines distribution in Great Whale and Inukjuak Rivers. Results obtained for Hudson Bay contrast with the absence of population subdivisions revealed in James Bay. Several reasons could explain the apparent low population structuring in James Bay; (1) the stochastic effect of low sample size, (2) genotypes common to all populations are ancestral mtDNAs which remained widespread following the genetic isolation of populations, and (3) the homogeneity of mtDNA lineage distribution reflects ongoing gene flow among river populations. The first two explanations cannot be strictly ruled out. However, it is hardly conceivable that these two phenomena would prevent stock discrimination only among populations of James Bay and not those from Hudson Bay. For instance, sample sizes were even smaller in Hudson Bay and yet allowed stock discrimination.

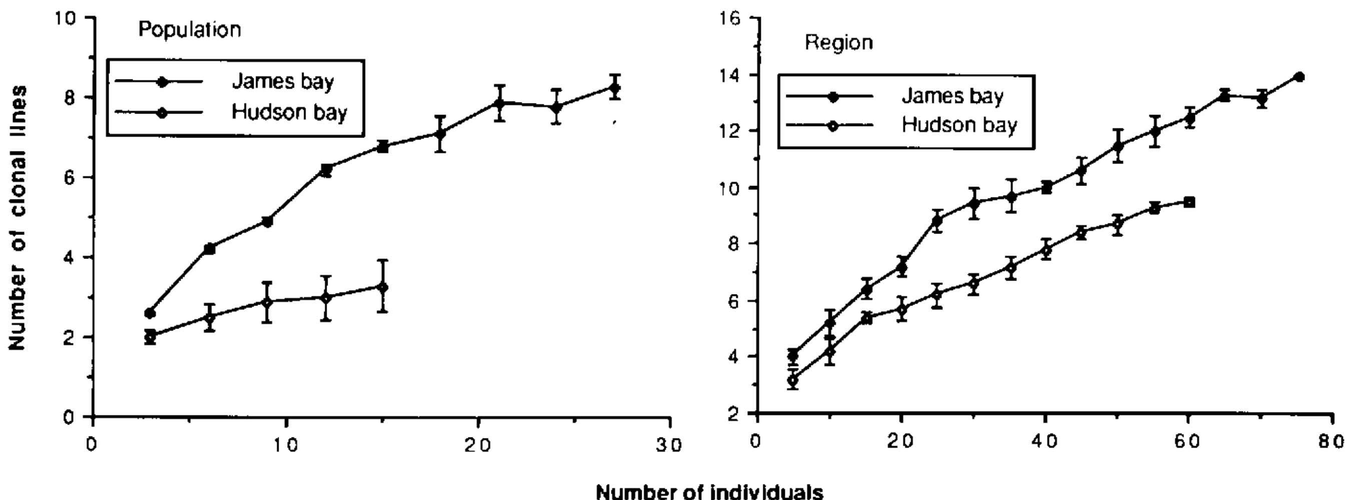

FIG. 5. Effect of fish sample size on the number of mtDNA clonal lines detected within populations and within each the James Bay region and the Hudson Bay region. Results were obtained by an incremental random choice of three fish at the population level and of five fish at the regional level. The procedure was repeated 10 times for each incremental step. Standard error is indicated by vertical bar.

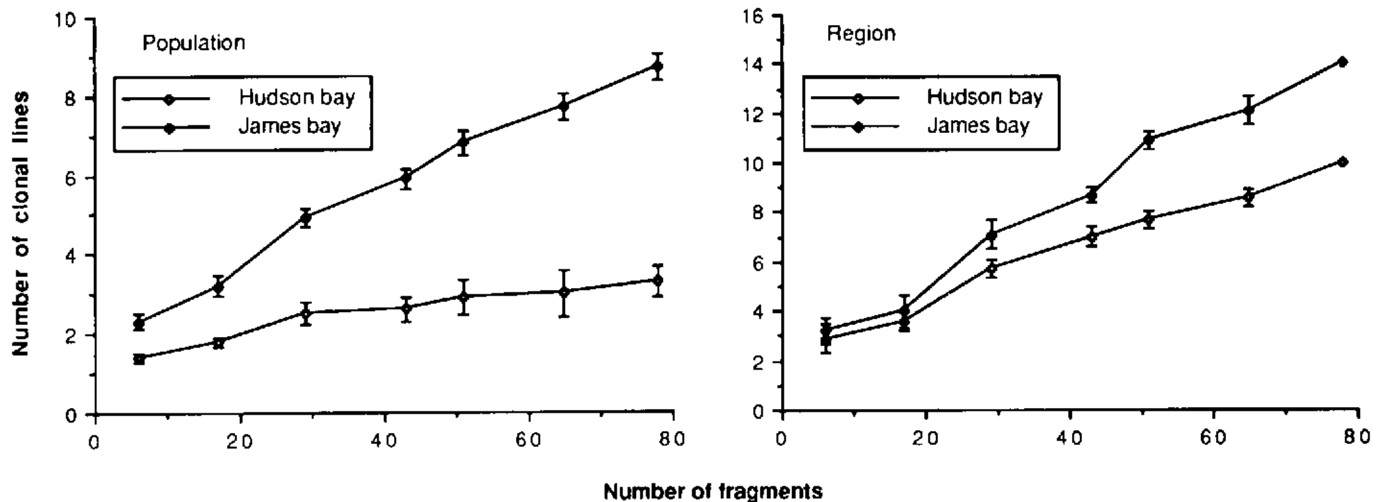

Fig. 6. Effect of the resolution of mtDNA restriction analysis on the number of clonal lines detected within populations and within regions (James Bay and Hudson Bay). Results were obtained by an incremental random choice of two restriction enzymes. Number of enzymes were then converted into the corresponding number of fragments analysed for each incremental step. The procedure was repeated 10 times for each incremental step. Standard error is indicated by vertical bars.

We propose that the absence of population structure in James Bay is the result of gene flow among rivers. There is considerable evidence that the population structure of many fish species is defined during the early life history stages. Two factors are essential for maintaining population integrity; (1) the existence of well defined physical oceanographic or geographic features in which larvae maintain themselves through behavioral and passive mechanisms, and (2) the homing capacity of the adult fish to spawning grounds (Sinclair and Iles 1988). Evidence of homing to natal sites in coregonid fishes is provided by tagging experiments but the degree of precision in this behaviour is not documented. In the case of anadromous cisco from James Bay, tagging experiments did not reveal homing but instead demonstrated straying among rivers (Lambert and Dodson 1982). For instance, four out of six recaptured fish tagged in the Eastmain River were caught in three rivers located between 50 and 170 km from the tagging site. In addition, the study of larval movements suggests that larvae are not retained within each river or estuary. Ochman and Dodson (1982) found that coregonine larvae from the Eastmain River are flushed out of the river and transported passively into the bay soon after hatching. This appears to represent a mechanism to exploit the greater availability of food in the Bay. A major oceanographic feature of James Bay is the existence of a cyclonic gyre during the summer months (Prinsenberg 1986). In this system, cold and highly saline Arctic water masses from Hudson Bay enter along the western shore of James Bay, are heated and diluted as they progress southward, resulting in a cyclonic current of warmer and brackish water (as low as 10 $g \cdot kg^{-1}$) which can reach speeds of up to 19 $cm \cdot s^{-1}$ on the northeastern coast of James Bay. It is likely that cisco larvae flushed into the Bay may be trapped in this gyre. In such a case, all of James Bay would act as a geographic retention zone. This, along with an apparent imprecision of adult homing, is consistent with the hypothesis that cisco from all James Bay rivers represent a unique gene pool. An analogous situation in which fish do not home to specific natal rivers but to a wide geographic area of retention has been documented for the rainbow smelt (*Osmerus mordax*) in the Gulf of St. Lawrence (Fréchet et al. 1983; Ouellet and Dodson 1985).

The same potential for gene flow also exists in Hudson Bay but its realization may be hampered by ecological barriers. A cyclonic pattern of circulation analogous to James Bay also exists in Hudson Bay (Prinsenberg 1986) in which larval cisco could be trapped. However, the waters of the two systems differ in respect to many physico-chemical characteristics. Unlike James Bay, Hudson Bay is more typically oceanic and high salinity (>24 $g \cdot kg^{-1}$) is found all along the coast, except in the plumes of major rivers. The high salinity encountered in Hudson Bay may prevent transport of larval cisco over long distances. Studies of salinity tolerance of larvae of anadromous populations of two species of cisco, *C. albula* and *C. sardinella* revealed a maximum salinity tolerance between 8 and 13 $g \cdot kg^{-1}$ for the two species (Girsa et al. 1980; Jager et al. 1981). If *C. artedii* exhibits a similar salinity tolerance, larval cisco trapped in the gyre of Hudson Bay and transported away from river plumes would not survive. This would limit gene flow along the coast and river populations would be maintained mainly by larvae remaining in the estuary of each river, resulting in population subdivision.

Management Implications

The basic premise of fish stock conservation and sustainable harvesting is that each stock should be managed separately (MacClean and Evans 1981). This study revealed at least two separate reproductive units of *C. artedii* in Hudson Bay and suggests that there may be more structuring. Therefore, it is advisable that each river system of Hudson Bay should be managed individually, except for the homogeneous Little Whale–Richmond Gulf complex. Results for James Bay do not support the stock hypothesis. Nevertheless, caution must be taken in concluding that no stock structure exists as the absence of genetic differences among populations does not allow us to categorically conclude that there is gene flow among rivers. Based on the number of clonal lines detected as a function of restriction enzymes (Fig. 6), it is clear that more variability would have been detected by increasing the number of enzymes used. Although it is theoretically possible that a more comprehensive analysis may reveal population structuring, the absence of pop-

ulation subdivision is corroborated by the apparent absence of larval retention in each river and by the demonstration of straying among rivers in tagging experiments. We thus conclude that it is advisable to consider the eastern coast of James Bay as a management unit.

Mitochondrial DNA restriction analysis allowed us to identify distinct genetic pools among anadromous *C. artedii* of James-Hudson Bay. Thus, our results support the genetic model of life history variations observed among anadromous cisco populations of Hudson Bay and between populations from James and Hudson Bay. On the other hand, the lack of genetic substructuring observed in James Bay suggests that reproductive isolation is not a prerequisite for life history variation.

In conclusion, the genetic structuring of anadromous cisco in James-Hudson Bay appears to be determined by historical biogeographical events and by the interaction of the various life-history stages with the physical oceanographic environment of the area.

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