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Population bottlenecks: influence on mitochondrial DNA diversity and its effect in coregonine stock discrimination

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This study was designed to test the hypothesis that anadromous populations of North American whitefish, *Coregonus clupeaformis* (Mitchill), believed to have suffered more intense population bottlenecks during past glaciation events, should reveal less mitochondrial DNA (mtDNA) variability and population-genetic structuring than anadromous populations of European whitefish, *C. lavaretus* (L.). *C. clupeaformis* exhibited extremely low levels of diversity and population structuring, in terms of number and frequency of clonal lines as well as sequence divergence estimates, as compared with populations of *C. lavaretus*. These results support the hypothesis that the severity of population bottlenecks related to Pleistocene glaciation events may be largely responsible for the level of mtDNA variability observed. This in turn influences the sampling strategy required to maximize the usefulness of mtDNA analysis in stock discrimination.

Key words: stock discrimination; mtDNA; *Coregonus*; glaciation; bottlenecks.

I. INTRODUCTION

In recent years, mitochondrial DNA (mtDNA) restriction analysis has been used to study population structure of many fish species (review: Avise, 1987). Most studies invoke historical biogeographic events as determinant factors acting on the level of genetic variability and structuring revealed by mtDNA restriction analysis. Pleistocene glaciation appears to have had an important role in shaping the genetic structure of fish populations. Glaciation is believed to have decreased genetic diversity through population bottleneck effects and to have confined populations of northern temperate fishes in separate glacial refugia where they evolved independently for many thousands of years (McAllister *et al.*, 1986). The magnitude and pattern of population genetic structure of these species thus result from a complex interplay between past biogeographic history, the potential for gene flow and the realization of this potential in the face of existing barriers (Avise *et al.*, 1987).

One possible way to study the effect of glaciation on genetic structure is to compare closely-related fish populations that are very similar with respect to their abundance, life history and potential for gene flow but that are believed to have endured differential effects of glaciation. Anadromous whitefish populations of James–Hudson Bay (*Coregonus clupeaformis* (Mitchill) complex) and the Baltic Sea (*C. lavaretus* (L.) complex) provide a unique opportunity to test this hypothesis. These two nomenclatural species are closely related and possibly represent continental variants of the same species (D. Bodaly, pers. comm.). Both forms have similar habitats and population characteristics such as age at maturity, maximal age, fecundity and abundance (Morin *et al.*, 1982; Lehtonen, 1986). Tagging studies have revealed potential for large-scale movements in both forms (Lehtonen, 1986; J. J. Dodson, unpubl. data). While both James–Hudson Bay and the Baltic Sea were covered by ice sheets during the last glaciation, the extent of the glacial

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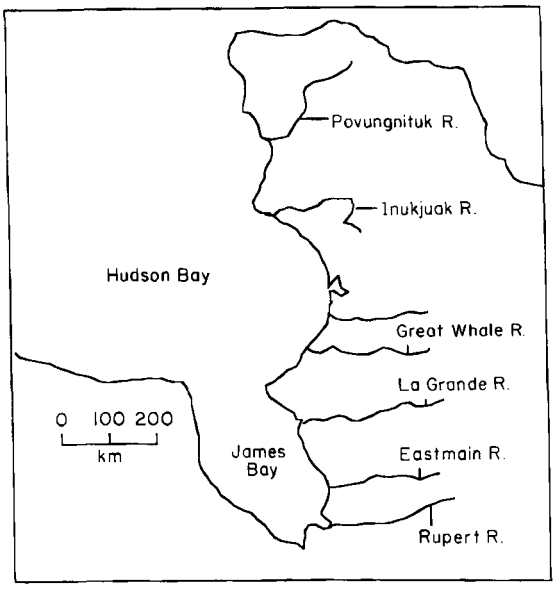

FIG. 1. Location of James–Hudson Bay sampling sites for populations of *Coregonus clupeaformis*.

cover was very different in North America and Eurasia (Denton & Hughes 1981). Therefore, *C. clupeaformis* was probably confined to no more than three or four glacial refugia in North America (D. Bodaly *et al.*, pers. comm.; L. Bernatchez & J. J. Dodson, unpubl. data) while important parts of the present-day distribution range of *C. lavaretus* remained uncovered by glaciers.

This report tests the hypothesis that mtDNA restriction analysis of anadromous *C. clupeaformis* populations should reveal less mtDNA variability and population structuring than *C. lavaretus*. The usefulness of mtDNA analysis to study stock structure is discussed as a function of detectable mtDNA polymorphisms.

II. MATERIALS AND METHODS

SAMPLING

Six populations of anadromous *C. clupeaformis* from James–Hudson Bay were collected from their overwintering rivers during the spring of 1987 (Fig. 1). Seven populations of *C. lavaretus* from the Bothnian Gulf were sampled on their spawning grounds during the autumns of 1987 and 1988 (Fig. 2). Livers and small developing ovaries of fish from James–Hudson Bay were frozen on dry ice soon after capture and kept several months at $-80°$ C before mtDNA was extracted. Mature ovaries of fish from the Bothnian Gulf were shipped on wet ice and mtDNA was extracted within 10 days of capture.

ISOLATION AND RESTRICTION ENZYME ANALYSIS OF mtDNA

Mitochondrial DNA was purified according to Gonzalez-Villa Señor *et al.* (1986) as modified by Bernatchez *et al.* (1988). Aliquots of mtDNA were digested separately with eight hexameric, four multihexameric and one multipentameric restriction endonucleases (enzymes: Table II). Mitochondrial DNA fragments were electrophoretically separated on 0·8% agarose gels.

VISUALIZATION OF RESTRICTION FRAGMENTS

Two methods of visualization were used, depending on the success of the extraction. When mtDNA was obtained in sufficient quality and quantity, ethidium bromide staining

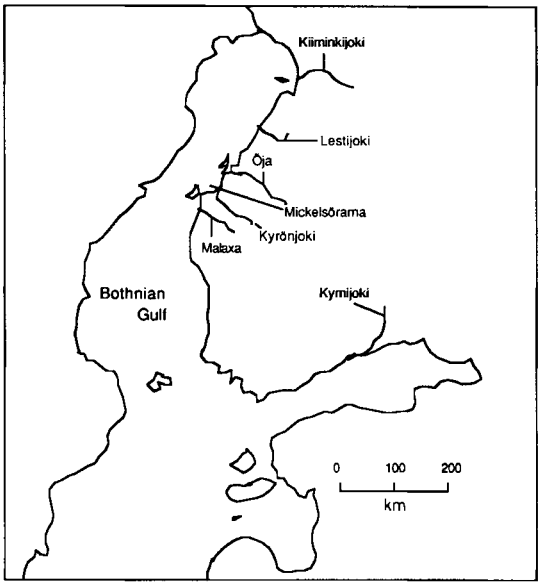

FIG. 2. Location of Bothnian Gulf sampling sites for populations of *Coregonus lavaretus*.

was sufficient to reveal the digested fragments. Otherwise, as for many frozen samples, total DNA was denatured, neutralized and transferred to nitrocellulose filters (procedure: Maniatis *et al.*, 1982). Filters were then hybridized with a highly purified radiolabelled total mtDNA probe following the procedures of Wahl *et al.* (1979). Filters were autoradiographed using an intensifying screen (Cronix lightning-plus) for 2–16 h.

DATA ANALYSIS

Mitochondrial DNA fragments on gel constituted the raw data for further analysis. Endonuclease patterns produced by each restriction enzyme were identified by a specific letter in order of appearance. Thus, each fish was assigned a composite letter code which described its observed mtDNA genotype.

Sequence divergence between genotypes was estimated according to Upholt's (1977) method. The resulting distance matrices were then clustered by UPGMA, using the average linkage algorithm of the SAS statistical package. The diversity of mtDNA lineages with each population was estimated with Nei & Tajima's (1981) nucleon diversity index which takes into account both the number and frequency of mtDNA genotypes.

The likelihood of detecting mtDNA diversity was estimated within the confines of the sampling protocol by adopting 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. The procedure was repeated 10 times for each sampling intensity and applied at the population and regional levels. The relationship between the number of clones detected as a function of genome sampling intensity was estimated by incremental random choice of restriction enzymes. Because restriction enzymes produced different numbers of fragments, the fragment numbers were attributed to their respective restriction enzymes, yielding a sum of the fragments for any enzyme combination. This procedure was applied only at the regional level because of differences in population sample size.

Geographical heterogeneity in the frequency of mtDNA genotypes between populations was analysed following the randomized bootstrap generation of χ^2 distribution elaborated by Roff & Bentzen (1989). Briefly, this procedure circumvents the problem of small sample size by estimating the probability of obtaining a χ^2 value as large as, or larger than, that observed by randomization of the original data sets using a bootstrap approach. In this case, 1000 randomizations were performed.

TABLE I. Mitochondrial DNA variation parameters observed among *Coregonus clupeaformis* of James–Hudson Bay and *C. lavaretus* of the Bothnian Gulf

	C. clupeaformis	*C. lavaretus*
Number of fish	112	79
Number of enzymes	13	13
Informative enzymes	6	11
Mean sites/individual	77	75
Total restriction sites	89	142
Total genotypes	10	35
Mean genotypes/population	2·5±1·2	7·7±4·4
Mean % divergence	0·19±0·05	0·32±0·33

III. RESULTS

RESTRICTION SITE VARIATION

Contrasting differences in restriction site variation were observed between whitefish from James–Hudson Bay and the Bothnian Gulf (Table I). Only six of 13 enzymes used were informative in generating composite genotypes among 112 whitefish from James–Hudson Bay, whereas 11 enzymes were informative among 79 individuals from the Bothnian Gulf. These enzymes sampled a comparable mean number of restriction sites per individual in both cases, but the total number of sites found was higher for *C. lavaretus*. The total number of composite genotypes was greater among whitefish from the Bothnian Gulf (Tables I–III) as was the mean number of composite genotypes per population. Estimates of sequence divergence among all genotypes observed in each region were lower and less variable in James–Hudson Bay.

Striking differences in mtDNA diversity were found between *C. clupeaformis* and *C. lavaretus* (Fig. 3). Only 10 clonal lines were identified in *C. clupeaformis* and the average estimated percent sequence divergence between them was low in all cases. There was no evidence of substructuring among observed mtDNA genotypes. In *C. lavaretus*, greater complexity in genotype diversity was observed. Averaged percent sequence divergence estimates between the 35 observed composite genotypes were highly variable and varied from 0·04 to 1·0%. Sequence divergence between clonal line 18 and others (1·7%) was not considered, as it represents a case of introgressed mtDNA genome of the cisco, *Coregonus albula*, into *C. lavaretus* (L. Bernatchez & J. J. Dodson, upubl. data). Omitting clonal lines 19 and 22, which represented two rare variants, the remaining 32 genotypes (96% of individuals sampled) were grouped in three clusters separated by averaged sequence divergence estimates of 0·62% (cluster A from clusters B and C) and 0·53% (cluster B from cluster C). All populations from the Bothnian Gulf exhibited greater diversity than that of any population from James–Hudson Bay (Table IV).

GEOGRAPHIC VARIATION IN COMPOSITE GENOTYPE FREQUENCY

James–Hudson Bay and the Bothnian Gulf illustrate two extremes of geographic variation in the frequencies of composite genotypes (Tables II, III). In James–

TABLE II. Composite clonal genotype (rows of letters), number of individual fish, and distribution frequency of each clone observed in James–Hudson Bay *Coregonus clupeaformis*. Clonal line 6 is heteroplasmic; fragment patterns were not analysed for all enzymes. Restriction enzymes: 1, AVAI; 2, AVAII; 3, BANI; 4, BGLI; 5, DRAI; 6, HAEII; 7, HINCII; 8, HINDIII; 9, PVUII; 10, SMAI; 11, XMNI; 12, PSTI; 13, BAMHI

Clonal line	Restriction enzyme													Number of fish	Distribution frequency					
	1	2	3	4	5	6	7	8	9	10	11	12	13		Rupert	Eastmain	La Grande	Great Whale	Inukjuak	Povungnituk
1	A	A	A	A	A	A	A	A	A	A	A	A	A	88	14	29	14	9	11	11
2	A	B	A	A	A	A	A	A	A	A	A	A	A	1					1	
3	A	A	A	A	A	A	A	D	A	A	A	A	A	1						
4	A	A	A	A	A	A	B	A	A	A	A	A	A	10						10
5	A	A	A	A	A	A	C	A	A	A	A	A	A	3						3
6	B	C	—	B	B	—	D	B	B	A	B	—	—	1						1
7	A	A	A	A	A	A	A	C	A	A	A	A	A	1			1			
8	A	D	A	A	A	A	A	A	A	A	A	A	A	5		1	4			
9	A	A	A	A	A	A	A	E	A	A	A	A	A	1			1			
10	A	A	B	A	A	A	A	A	A	A	A	A	A	1	1					

TABLE III. Composite clonal genotype (rows of letters), number of individual fish, and distribution frequency of each clone observed in Bothnian Gulf *Coregonus lavaretus*. Restriction enzymes: as in Table II

Clonal line	Restriction enzyme													Number of fish	Distribution frequency						
	1	2	3	4	5	6	7	8	9	10	11	12	13		Kymi-joki	Malaxa	Kyrön-joki	Mickel-sörarna	Öja	Lesti-joki	Kiiminki-joki
1	A	E	A	A	A	A	A	A	A	A	A	A	A	4				1		2	1
2	B	B	A	A	A	B	B	A	A	A	A	A	A	2							2
3	B	A	A	A	A	C	C	A	A	A	A	A	A	10				1	4	2	3
4	B	A	B	A	A	D	B	A	A	A	B	A	A	1							1
5	A	E	A	A	A	A	D	A	A	A	A	A	A	9		1	2	2		2	2
6	C	A	A	A	A	C	E	A	A	A	A	A	A	1							1
7	B	B	A	A	A	C	B	A	A	A	A	A	A	3				1		1	1
8	A	A	A	A	A	A	F	A	A	A	A	A	A	1							1
9	B	A	B	A	A	C	B	A	A	A	A	A	A	1							1
10	A	A	E	A	A	A	F	A	A	A	A	A	A	11	2	2		2		3	2
11	D	A	A	A	A	C	E	B	A	A	A	A	A	1				1			
12	B	A	D	A	A	C	C	A	A	A	D	A	A	1				1			
13	A	A	C	A	A	A	D	A	A	A	A	A	A	1				1			
14	B	B	D	A	A	C	C	A	A	A	A	A	A	4				4			
15	B	B	A	A	A	C	C	A	A	A	A	A	A	1				1			
16	B	A	A	A	A	C	B	A	A	A	A	A	A	3				2	1		
17	A	E	B	A	A	A	F	A	A	A	B	A	A	6			4	1	1		
18	K	H	F	B	A	G	B	A	A	C	A	A	A	1				1			
19	L	A	G	A	A	C	C	A	A	A	A	A	A	1				1			
20	A	I	B	A	A	A	F	A	A	A	B	A	A	1				1			
21	B	A	A	A	A	C	C	B	A	A	A	A	A	1				1			
22	B	B	D	A	A	B	B	A	A	A	A	A	A	1					1		
23	B	A	A	A	A	C	J	A	A	A	A	A	A	2			1		1		
24	A	E	A	A	A	A	D	B	A	A	A	A	A	1					1		
25	F	E	D	A	A	A	D	A	A	A	A	A	A	1					1		
26	B	A	B	A	A	D	B	A	A	B	B	A	A	1					1		
27	G	E	A	A	A	A	F	A	A	A	A	A	A	1					1		
28	B	G	A	A	B	C	B	A	A	A	A	A	A	1			1				
29	C	A	A	A	A	C	C	A	A	A	A	A	A	1			1				
30	B	A	B	A	A	C	B	A	A	A	B	A	A	1					1		
31	A	E	A	A	A	A	F	A	A	A	A	A	B	1		1					
32	A	A	A	A	A	C	B	A	A	A	A	A	A	1	1						
33	A	E	A	A	A	A	M	A	A	A	A	A	A	1	1						
34	A	E	B	A	A	A	F	A	A	A	B	A	C	1	1						
35	A	E	B	A	A	A	F	A	A	E	B	A	A	1	1						

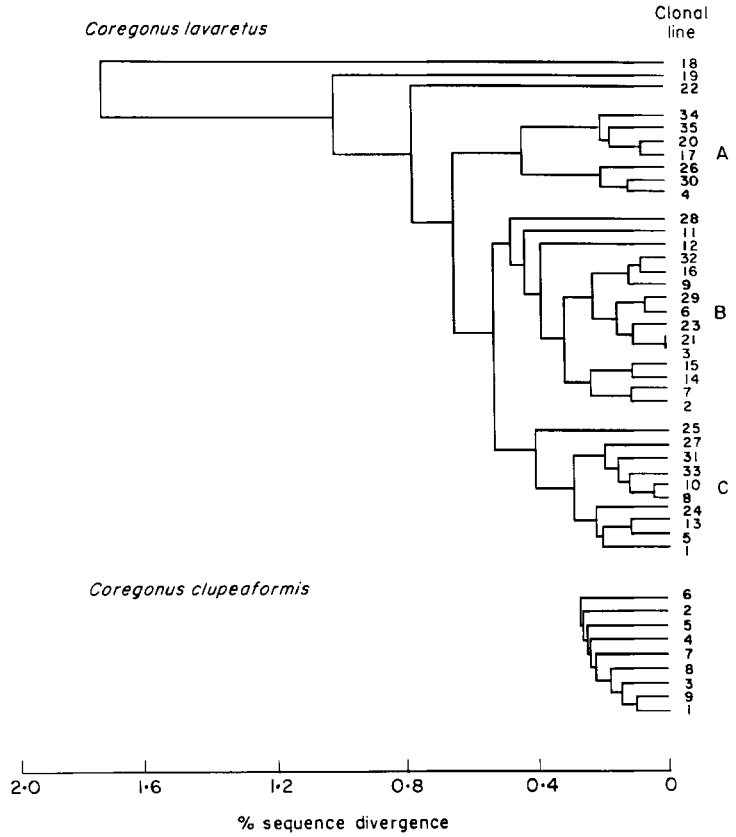

FIG. 3. UPGMA dendrogram clustering the distance matrices of sequence divergence estimates calculated for *Coregonus lavaretus* and *C. clupeaformis*.

Hudson Bay, clonal line 1 made up 79% of all fish observed and was distributed among all rivers. Nevertheless, χ^2 analysis using the bootstrap method revealed heterogeneity in frequency distribution of clonal lines due to the River Povungnituk population ($\chi^2 = 95{\cdot}05$, d.f. $= 45$, $P < 0{\cdot}001$, probability of randomized $\chi^2 >$ observed $= 0/1000$). This was the only population with clonal lines 4 and 5. No population structuring was observed in the other five populations. In the Bothnian Gulf, no statistical difference was observed in the frequency distribution of composite genotypes ($\chi^2 = 233{\cdot}06$, d.f. $= 204$, $P > 0{\cdot}05$, probability of randomized $\chi^2 >$ observed $= 72/1000$). The four most abundant genotypes (clonal lines 3, 5, 10, 17; Table II) were not specifically associated with given populations and were distributed among several rivers. However, each population was characterized by the presence of several rare genotypes not found in other populations. No significant difference was detected in the geographic distribution of the three major genetic clusters identified by UPGMA ($\chi^2 = 17{\cdot}9$, d.f. $= 12$, $P > 0{\cdot}25$).

SAMPLING AND DETECTION OF mtDNA DIVERSITY

For *C. clupeaformis*, the number of genotypes detected as a function of population sample size increased smoothly and approached an asymptotic value of close to 3 at a sample size of 18 individuals (Fig. 4). This suggests that 18 specimens were

TABLE IV. Sample location, sample size, and nucleon diversity estimates of anadromous whitefish populations of the Bothnian Gulf (*C. lavaretus*) and James–Hudson Bay (*C. clupeaformis*)

Population	Spawning	Number of fish	Nucleon diversity
Coregonus lavaretus			
Kymijoki	River	6	0·93
Malaxa	Estuary	4	0·83
Kyrönjoki	Estuary	9	0·81
Mickelsörarna	Sea	22	0·96
Öja	Estuary	12	0·91
Lestijoki	River	11	0·89
Kiiminkijoki	River	15	0·94
			0·90±0·06
Coregonus clupeaformis			
Rupert R.	River	15	0·13
Eastmain R.	River	31	0·13
La Grande R.	River	20	0·49
Great Whale R.	River	9	0
Inukjuak R.	River	12	0·17
Povungnituk R.	River	25	0·66
			0·26±0·25

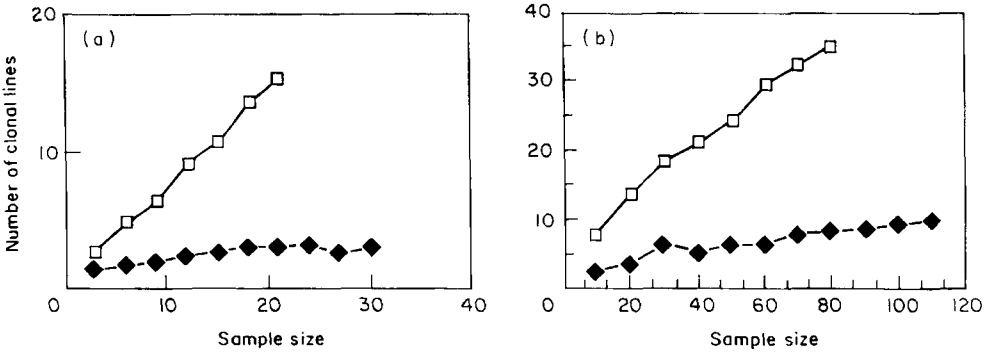

FIG. 4. Effect of fish sample size on the number of mtDNA clonal lines detected (a) within populations (*Coregonus clupeaformis* and *C. lavaretus*) and (b) within regions (James–Hudson Bay and Bothnian Gulf). Results were obtained by an incremental random choice of three fish at the population level and of 10 fish at the regional level. The procedure was repeated 10 times for each incremental step. □, Bothnian Gulf; ◆, James–Hudson Bay.

sufficient to detect all variability within a given population of James–Hudson Bay. However, the same relationship did not reach an asymptotic value at the regional level. Therefore, detection of all mtDNA variability in this region would require more than 110 specimens. At the population level and regionally, the number of genotypes of *C. lavaretus* increased very sharply and did not tend towards an asymptotic value (Fig. 4). In this case, sample sizes of up to 21 individuals within

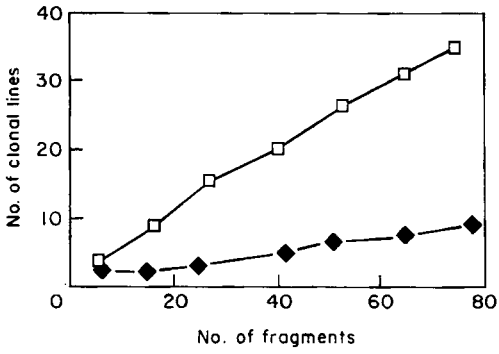

FIG. 5. Effect of level of resolution of mtDNA analysis on the number of *Coregonus clupeaformis* and *C. lavaretus* clonal lines detected within regions (James–Hudson Bay and Bothnian Gulf). Results were obtained by an incremental random choice of two restriction enzymes. Number of enzymes was then converted into the corresponding number of fragments analysed for each incremental step. The procedure was repeated 10 times for each incremental step. □, Bothnian Gulf; ◆, James–Hudson Bay.

populations and 78 individuals from the Bothnian Gulf were inadequate to detect all mtDNA variants.

The number of genotypes detected at the regional level increased with the number of restriction fragments sampled but did not approach an asymptotic value, either for *C. clupeaformis* or for *C. lavaretus* (Fig. 5). Evidently many more fragments were needed to detect variants in James–Hudson Bay than in the Bothnian Gulf.

IV. DISCUSSION

Populations of *C. clupeaformis* from James–Hudson Bay and *C. lavaretus* from the Bothnian Gulf exhibit many differences of mtDNA variation in terms of number and frequency of clonal lines detected, sequence divergence estimates and mtDNA population structuring. In both systems, however, mtDNA data did not allow stock discrimination except in the case of the Povungnituk River population, which showed a highly significant difference from other populations of James–Hudson Bay in frequency distribution of clonal lines.

MITOCHONDRIAL DNA VARIATION

C. clupeaformis exhibited one of the lowest values of mtDNA diversity reported in the literature, with nearly 80% of all fish analysed belonging to the same clonal line. In contrast, *C. lavaretus* showed the other extreme of diversity, with nearly one clonal line detected for every two fish analysed. This may result from differences in mtDNA mutation rate or differences in ancestral demographic conditions of both species. Mitochondrial DNA mutation rate has not been quantified for fish but an estimate of 2% nucleotide substitution per million years is reported for both mammals and birds (Moritz *et al.*, 1987; Shields & Wilson, 1987). Even though the nucleotide substitution rate of fish might differ from that of other vertebrates, it seems unlikely that it would be very different between such closely related species as *C. clupeaformis* and *C. lavaretus*.

Differences in ancestral demographic conditions are more plausible. Avise *et al.* (1984) have demonstrated by means of probability models that mtDNA variability of a population is a function of the stochastic extinction of mtDNA lineages through time. The degree of extinction depends on four main factors: (1) time since population founding; (2) generation time of the population; (3) level of population density regulation; (4) number of founding mtDNA lineages in the parent population. The first three factors are comparable for both species. For example, time since population founding is similar as it followed in both regions the retreat of Wisconsin–Weichselian ice-sheets that started some 18 000 years ago (Denton & Hughes, 1981). Therefore, difference in the number of founding mtDNA lineages in parent populations is a more plausible explanation for differences in mtDNA variability observed between the two regions.

The extremely low diversity in *C. clupeaformis* populations of James–Hudson Bay can be explained by important population bottlenecks associated with the expansion of ice masses during Pleistocene glaciation events. Around 18 000 years ago, most of the North American continent north of the 40th parallel was ice-covered. The Arctic and boreal climatic zones, characteristic of the present-day distribution of *C. clupeaformis*, were telescoped within a relatively narrow band south of the ice-sheet (Flint, 1971; Fulton & Prest, 1987), limiting refugia for cold-adapted species such as whitefish. This inevitably led to population reduction of cold-adapted fish species (McAllister *et al.*, 1986) which may have resulted in an important loss of mtDNA genetic variability of *C. clupeaformis*. Whitefish populations also survived in the unglaciated parts of Alaska and Yukon (Franzin & Clayton, 1977) but did not recolonize eastern North America (D. Bodaly *et al.*, pers. comm.; L. Bernatchez & J. J. Dodson, unpubl. data). The existence of only one major clonal line in most of James–Hudson Bay suggests that this area was recolonized by populations that survived in only one refugium, probably the Mississippian. However, the population-specific clonal lines identified in the Povungnituk River may be associated with a recolonization of northern Hudson Bay by an ancestral population that survived in an Atlantic refugium (L. Bernatchez & J. J. Dodson, unpubl. data).

In contrast to the obvious reduction of potential habitat for *C. clupeaformis* during the Wisconsin glaciation, vast areas of the present-day distribution range of the *C. lavaretus* complex remained unglaciated, especially in Siberia. These areas were associated with an Arctic environment, thus representing potential habitats for whitefish survival. Furthermore, Arctic and boreal climatic zones were more extensive than they are today, expanding over most of the European continent south to the Mediterranean coast (Flint, 1971). Huge freshwater lakes (present-day Black and Caspian Seas) may have provided vast refugia for cold-adapted fishes. Thus, potential population bottlenecks for *C. lavaretus* might not have been as severe, resulting in a preservation of mtDNA variability. The clustering of the clonal lines detected in the Bothnian Gulf into three major groups suggests that this area was recolonized by whitefish that survived in at least three different geographic refugia. Assuming a nucleotide substitution rate of 2% per million years, our results suggest that these three groups last shared a common ancestor 250 000 years ago. This predates Wisconsin–Weichselian glaciation, which began about 100 000 years ago (Fulton & Prest, 1987), and suggests that geographic isolation of these three groups occurred in earlier Pleistocene glacial events.

GENETIC VARIABILITY AND USEFULNESS OF mtDNA ANALYSIS IN STOCK DISCRIMINATION

The present study did not permit stock discrimination in James–Hudson Bay or the Bothnian Gulf, for reasons related to the mtDNA diversity existing in each system. It is unlikely that stocks orginating after the withdrawal of the Wisconsin–Weichselian glaciers may be discriminated by population-specific mtDNA markers. Indeed, currently-used mtDNA analyses (Wilson *et al.*, 1985) appear insufficient to permit discrimination of mtDNA genotypes differing solely by the accumulation of mutations over a period of 18 000 years. Stocks can most probably be discriminated by differential distribution frequencies of mtDNA lineages that predate the genetic isolation of populations. In James–Hudson Bay, discrimination of *C. clupeaformis* stocks was hampered by a lack of mtDNA variants detected and would have been improved only by increasing the number of restriction enzymes. On the other hand, an adequate definition of the stock structure of *C. lavaretus* would require larger fish samples because so many mtDNA genotypes were observed. Hence, the level of variability influences sampling strategy required to maximize the usefulness of mtDNA analysis in stock discrimination.

This study illustrates the importance of considering the historical biogeography of a species in order to understand its population genetic structure. It also illustrates that mtDNA analysis represents a powerful tool to study past and present demographic phenomena but that its usefulness in stock discrimination is maximized if the sampling strategy is adjusted according to a preliminary evaluation of mtDNA variability among populations of interest. This problem partly explains the failure to discriminate stocks in many mtDNA studies of temperate fish species conducted to date.

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