Phylogeography and postglacial dispersal of lake trout (*Salvelinus namaycush*) in North America

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Abstract: We used restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA) diversity to assess the complex postglacial history of lake trout (*Salvelinus namaycush*) and test existing dispersal hypotheses. A pilot survey with 30 restriction enzymes was carried out on lake trout from 16 geographically representative populations to determine phylogenetically informative characters. Subsequent screening of 1416 lake trout from 93 populations across the species' range with nine variable restriction enzymes showed that lake trout from at least five glacial refugia contributed to extant populations. Three major mtDNA lineages were observed, with sufficient differences to suggest their divergence during the mid-Pleistocene. Geographic and genetic differences within two lineages suggested further vicariant divergence caused by Wisconsinan glacial advances. In contrast with more southern freshwater species, no correlation was observed between the geographic proximity of glacial refugia and relatedness of mtDNA lineages. Current distributions of refugial lineages are readily explained by consideration of timing and connections of proglacial lakes. These lakes facilitated large-scale dispersal from multiple refugia, particularly enabling long-distance dispersal from the Mississippian and northwestern refugia. Proglacial lakes also enabled extensive secondary contact among refugial groups, resulting in high levels of intrapopulation mtDNA diversity within their former boundaries.

Résumé : Nous avons analysé le polymorphisme de taille de fragments de restriction (RFLP) de l'ADN mitochondrial (ADNmt) pour caractériser l'histoire post-glaciaire complexe du touladi (*Salvelinus namaycush*) et vérifier les hypothèses actuellement avancées au sujet de la dispersion de cette espèce. Nous avons effectué une étude pilote au moyen de 30 enzymes de restriction sur le touladi de 16 populations géographiquement représentatives afin de choisir les caractères apportant de l'information d'intérêt phylogénétique. Après cette étape, nous avons tracé le profil de 1 416 touladis, provenant de 93 populations réparties dans l'ensemble de l'aire de distribution de l'espèce, au moyen de neuf enzymes de restriction variables; cette analyse a révélé que les touladis d'au moins cinq refuges glaciaires ont contribué à la formation des populations actuelles. Nous avons mis en évidence trois grandes lignées d'ADNmt; elles diffèrent suffisamment les unes des autres pour qu'on puisse envisager qu'elles ont divergé au pléistocène moyen. À en juger d'après les différences géographiques et génétiques observées entre deux des lignées étudiées, les avancées glaciaires wisconsiniennes auraient causé une nouvelle divergence vicariante. Contrairement à ce qui s'observe chez les espèces d'eau douce vivant plus au sud, aucune corrélation n'a été mise en évidence entre la proximité géographique des refuges glaciaires et la parenté des lignées d'ADNmt. La distribution actuelle des lignées issues de refuges s'explique facilement par le facteur chronologique ainsi que par le fait que les lacs proglaciaires étaient reliés. Ces lacs ont favorisé une dispersion dans un rayon étendu depuis des refuges multiples, et, plus particulièrement, la dispersion à grande distance des éléments des refuges mississippien et du nord-ouest. Ils ont aussi permis d'importants contacts secondaires entre les groupes des refuges, ce qui explique la grande diversité que présente l'ADNmt à l'intérieur d'une même population dans le territoire qu'occupaient autrefois les lacs proglaciaires. [Traduit par la Rédaction]

Introduction

The Pleistocene glaciations had unprecedented impacts on the ecology and genetic structure of North American species. The genetic and ecological upheavals caused by repeated glacial advances and retreats were especially pronounced among freshwater species, due to their restrictive dispersal requirements (Pielou 1991). Shifting ice fronts frequently altered habitat conditions drastically through the formation and failure of ice dams, drainage shifts, cascading overflows, and sudden emptying or flooding of ice-margin lakes (Dyke and Prest 1987; Pielou 1991). As a result of these large-scale disruptions, species diversities in formerly glaciated areas are far below those observed in neighbouring nonglaciated regions (McAllister et al. 1986). Conversely, the vast proglacial lakes formed from glacial meltwater provided tremendous dispersal opportunities for many aquatic species. The use of these drainage connections as avenues of dispersal has resulted in these taxa having much larger ranges than most species in nonglaciated regions (McAllister et al. 1986).

It is ironic that most studies examining the phylogeographic impacts of Pleistocene glaciations have so far been conducted on species that occur well south of glaciated areas (e.g.,

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Bermingham and Avise 1986; Avise 1992). Within regions formerly covered by the ice sheets, broad-scale phylogeographic studies have been conducted on only a few fish species (e.g., Ward et al. 1989; Bernatchez and Dodson 1991; Billington et al. 1992). Nevertheless, the limited information available suggests that species from glaciated and nonglaciated regions exhibit both quantitative and qualitative differences in genetic structure, with species from glaciated regions having reduced levels of intraspecific divergence and diversity (Bernatchez et al. 1989; Billington and Hebert 1991).

The lake trout (*Salvelinus namaycush*) is an ideal species for studying glacial impacts on phylogeographic structure, as its history and biology are intimately linked to the Pleistocene glaciations. The species' range corresponds closely to glacial limits, and lake trout are largely restricted to deepwater habitats created by glacial scouring (Martin and Olver 1980). The genetic structure of lake trout would have been strongly influenced by Pleistocene events, as repeated glacial cycles would have caused severe reductions in habitat availability. Surviving populations were displaced and isolated into separate refugia for long periods (>50 000 years), providing opportunities for their genetic divergence. Conversely, proglacial lakes present during glacial retreat would have facilitated dispersal and contact among refugial groups. This complex zoogeographic history has made resolution of the species' history difficult and has resulted in numerous, frequently conflicting hypotheses regarding the number and locations of glacial refugia utilized by lake trout (summarized in Crossman and McAllister 1986; Wilson and Hebert 1996). Despite this, lake trout have been used as a model system to explain postglacial dispersal of other aquatic species (Crossman and McAllister 1986; Pielou 1991). Until recently, the general consensus was that lake trout from a Mississippian refuge colonized much of Canada whereas lake trout from Beringian and Atlantic refugia made only local contributions (Black 1983*a*, 1983*b*; Crossman and McAllister 1986). This conclusion was indirectly supported by genetic analyses of lake whitefish (*Coregonus clupeaformis*) that showed phylogeographic patterns concordant with this proposal (Bernatchez and Dodson 1991; Bodaly et al. 1992).

Previous genetic studies of lake trout in eastern Canada have detected several lineages that show considerable geographic structure, which has been attributed to recolonization of this region by fish from several glacial refugia (Grewe and Hebert 1988; Ihssen et al. 1988; Wilson and Hebert 1996). This study extends previous work by using mitochondrial DNA (mtDNA) to examine the impacts of Pleistocene and postglacial events on the genetic structure of lake trout from across the full species range and compares the phylogeographic data obtained with those from other freshwater fishes.

Methods

A total of 1416 lake trout were sampled/collected from 93 populations across the species' range (Table 1), including 30 eastern populations examined previously (Wilson and Hebert 1996). Sample sizes ranged from 3 to 34, with a mean of 15.2 fish per population (Table 1). Where possible, fresh tissues were taken and extracted as described in Wilson and Hebert (1996). For most populations, total DNA (nuclear DNA plus mtDNA) was extracted with an SDS – proteinase K protocol (Bardakci and Skibinski 1994), using 50–100 mg of frozen or ethanol-preserved muscle, liver, or adipose fin tissue per sample.

DNA samples were digested with restriction enzymes and run on 0.8–1.2% agarose gels alongside molecular size standards. The DNA was then denatured, neutralized, and transferred to a nylon membrane (Amersham Hybond-N) by capillary blotting. Membranes were baked at 80°C for 2 h and probed with purified lake trout mtDNA labelled with a chemiluminescent chemical (digoxigenin (DIG), Boehringer Mannheim). Membrane hybridization and visualization were carried out as described by Höltke et al. (1992).

To assess the species' phylogenetic structure, mtDNA from 64 fish from 16 geographically distant populations was screened for variation with 30 restriction enzymes (Table 2). In addition, a 1100 base pair portion of the mtDNA molecule containing the D-loop was amplified using primers and amplification protocol from Bernatchez and Danzmann (1993) and screened for variation with six restriction enzymes that recognize tetranucleotide sequences. Restriction fragment homologies were confirmed using double digests with reference enzymes. Recognition sites were inferred from fragment patterns, as most mutational shifts could be explained by single-site gains or losses. The inferred restriction site data were used to construct a mutational network among haplotypes with the maximum likelihood program RESTML from PHYLIP 3.5c (Felsenstein 1993). The inferred site matrix was also subjected to bootstrapping and majority rule consensus, using the SEQBOOT, MIX, and CONSENSE modules of PHYLIP. Nucleotide divergences among haplotypes were estimated using the formulae of Nei and Tajima (1981) in the REAP computer package (McElroy et al. 1992) and clustered using the neighbour-joining method of Saitou and Nei (1987) offered by the NEIGHBOR program in PHYLIP. Divergence times among mtDNA lineages were estimated using a molecular clock estimate for salmonid mtDNA of 1% per million years (Smith 1992), after correcting for diversity within lineages (Wilson et al. 1985).

The remaining fish were screened with nine restriction enzymes that detected variation (*Ase*I, *Ava*I, *Bam*HI, *Eco*O109I, *Eco*RV, *Hin*dIII, *Sca*I, *Stu*I, and *Sty*I) to determine their phylogeographic origins. Haplotype (nucleon) and nucleotide (π) diversities of populations were calculated using REAP. For nucleotide diversity values calculated from this reduced set of enzymes, it was assumed that enzymes that did not detect more than one haplotype in the initial population screening (Table 3) were invariant in all other populations. Although this assumption may be incorrect, it prevented overinflation of estimated values. Geographic relationships and statistical significance of phylogeographic structure were assessed using the AMOVA program of Excoffier et al. (1992), which partitions variance into within- and among-population components, based on both genetic diversity of populations and relatedness of haplotypes within populations.

Results

Intraspecific phylogeny

Of the 30 restriction enzymes used to screen fish from the 16 test populations (Tables 2 and 3), four enzymes (*Bgl*II, *Cla*I, *Kpn*I, and *Mlu*I) failed to cut lake trout mtDNA. The remaining 26 enzymes recognized an average of 121 cut sites (702 bases) per fish, or 4.2% of the mtDNA genome. Thirteen of these enzymes failed to detect variation among the fish screened and recognized 48 cut sites. Fragment data for these enzymes are presented elsewhere (Wilson 1995).

Thirteen enzymes detected variation (Table 2), revealing 14 haplotypes among fish from the test populations (Table 3). Seven additional haplotypes were detected during the larger geographic survey and were characterized for fragment patterns with all variable enzymes (Table 3). Of the six four-base restriction enzymes used to digest the polymerase chain

Table 1. Collection sites for lake trout across its native range showing population numbers, geographic location, sample sizes (N), number of haplotypes present (N_h), and nucleon (h) and nucleotide (π) diversities.

No.	Lake	Location	Lat.	Long.	N	N_h	h	π
1	Trouser Lake	Labrador	56°32′	61°46′	14	1	0	0
2	**Chamcook Lake**	New Brunswick	45°09′	67°06′	15	1	0	0
3	Togue Pond	Maine	46°56′	68°53′	11	1	0	0
4	**Moosehead Lake**	Maine	45°40′	69°38′	15	2	0.133	0.009
5	**Long Pond**	Maine	45°58′	70°09′	5	1	0	0
6	Lake Winnepesaukee	New Hampshire	43°36′	71°22′	12	1	0	0
7	Raquette Lake	New York	43°48′	74°36′	15	1	0	0
8	Seneca Lake	New York	42°45′	76°55′	7	2	0.571	0.192
9	Lac Letemplier	Quebec	49°27′	68°47′	20	1	0	0
10	Lac Albanel	Quebec	51°05′	73°00′	12	4	0.455	0.203
11	Lac Mistassini	Quebec	50°30′	73°30′	23	4	0.625	0.246
12	**Lac Beland**	Quebec	48°51′	73°19′	12	1	0	0
13	**Lac Normand**	Quebec	46°29′	73°14′	24	1	0	0
14	Lac Archambault	Quebec	46°19′	74°15′	11	1	0	0
15	Lac des 31 Milles	Quebec	46°12′	75°49′	23	2	0.474	0.156
16	Lac Polonais	Quebec	47°00′	75°22′	7	1	0	0
17	**Lake Opeongo**	Ontario	45°42′	78°23′	30	2	0.129	0.043
18	Clear Lake	Ontario	45°15′	78°32′	30	1	0	0
19	**Tim Lake**	Ontario	45°45′	79°02′	15	1	0	0
20	Elliot Lake	Ontario	46°23′	82°42′	11	2	0.509	0.171
21	**Lake Superior**	Ontario	47°30′	87°00′	21	7	0.784	0.416
22	**Lake Nipigon**	Ontario	49°50′	88°30′	30	9	0.825	0.387
23	Little Sparkling Lake	Ontario	49°50′	90°13′	12	1	0	0
24	Confederation Lake	Ontario	51°05′	92°44′	10	2	0.200	0.013
25	**Daniels Lake**	Ontario	49°55′	93°50′	14	2	0.143	0.102
26	**Scattergood Lake**	Ontario	49°18′	92°43′	11	3	0.691	0.203
27	Lake Mameigweiss	Ontario	49°34′	91°49′	17	2	0.221	0.057
28	Hawley Lake	Ontario	54°30′	84°39′	9	1	0	0
29	Trout Lake	Wisconsin	46°02′	89°49′	17	1	0	0
30	God's Lake	Manitoba	54°40′	94°15′	15	5	0.695	0.323
31	Dymond Lake	Manitoba	58°48′	94°32′	8	4	0.821	0.386
32	Croll Lake	Manitoba	59°34′	98°22′	15	3	0.562	0.324
33	Clearwater Lake	Manitoba	54°05′	101°05′	29	4	0.603	0.267
34	Lake Athapapuskow	Manitoba	54°33′	101°40′	30	6	0.703	0.338
35	Reindeer Lake	Saskatchewan	57°15′	102°15′	20	5	0.784	0.379
36	Little Bear Lake	Saskatchewan	54°20′	104°35′	3	2	0.667	0.341
37	Lac la Ronge	Saskatchewan	55°04′	105°19′	15	5	0.762	0.343
38	Nemieben Lake	Saskatchewan	55°20′	105°20′	19	5	0.790	0.295
39	**Kingsmere Lake**	Saskatchewan	54°06′	106°27′	17	2	0.118	0.031
40	Lac la Plonge	Saskatchewan	55°14′	107°34′	31	4	0.759	0.335
41	Pierce Lake	Saskatchewan	54°30′	109°42′	10	3	0.689	0.382
42	Milliken Lake	Saskatchewan	59°27′	108°45′	3	1	0	0
43	Lake Athabasca	Saskatchewan	59°15′	109°15′	10	3	0.711	0.401
44	Cornwall Lake	Alberta	59°36′	110°35′	9	2	0.500	0.130
45	Peerless Lake	Alberta	56°37′	114°40′	19	2	0.351	0.227
46	Swan Lake	Alberta	52°07′	115°10′	20	1	0	0
47	Rock Lake	Alberta	53°27′	118°15′	20	1	0	0
48	Waterton Lake	Alberta	49°03′	113°54′	13	2	0.513	0.329
49	Cosley Lake	Montana	49°29′	114°00′	18	1	0	0
50	**Twin Lake**	Montana	46°00′	113°00′	5	1	0	0
51	Moose Lake	British Columbia	53°00′	119°00′	32	1	0	0
52	Francois Lake	British Columbia	54°03′	125°45′	34	1	0	0
53	Burnt Rose Lake	British Columbia	59°00′	128°00′	8	1	0	0
54	Graveyard Lake	British Columbia	59°00′	127°00′	5	1	0	0
55	**Muncho Lake**	British Columbia	58°59′	125°47′	20	1	0	0
56	Summit Lake	British Columbia	58°40′	124°20′	12	1	0	0
57	Nueltin Lake	Northwest Territories	60°30′	99°30′	11	3	0.655	0.352

Table 1 (*concluded*).

No.	Lake	Location	Lat.	Long.	N	N_h	h	π
58	Hawk Lake	Northwest Territories	63°38′	90°40′	8	1	0	0
59	Lailor Lake	Northwest Territories	69°17′	82°50′	27	1	0	0
60	Grinnell Lake	Northwest Territories	69°34′	83°55′	15	1	0	0
61	Sarcpa Lake	Northwest Territories	68°32′	83°15′	29	1	0	0
62	Hall Lake	Northwest Territories	68°40′	82°30′	23	1	0	0
63	Pelly Bay	Northwest Territories	68°33′	89°47′	11	1	0	0
64	Spence Bay	Northwest Territories	69°34′	93°30′	8	1	0	0
65	Unnamed lake	Northwest Territories	69°09′	104°43′	5	1	0	0
66	Unnamed lake	Northwest Territories	69°07′	105°07′	16	1	0	0
67	Great Slave Lake	Northwest Territories	61°23′	115°38′	27	4	0.658	0.277
68	Great Bear Lake	Northwest Territories	66°00′	119°00′	5	2	0.400	0.205
69	Fish Lake	Northwest Territories	71°50′	124°33′	14	1	0	0
70	Raddi Lake	Northwest Territories	71°41′	123°43′	14	1	0	0
71	Itkrilek Lake	Northwest Territories	69°33′	132°05′	12	1	0	0
72	Tassiruak Lake	Northwest Territories	69°37′	132°12′	12	1	0	0
73	Sitidgi Lake	Northwest Territories	68°32′	132°40′	25	1	0	0
74	Unnamed lake	Northwest Territories	69°17′	133°52′	6	1	0	0
75	Peter Lake	Northwest Territories	68°46′	134°08′	11	1	0	0
76	Noell Lake	Northwest Territories	68°32′	133°34′	21	1	0	0
77	Frances Lake	Yukon Territory	61°23′	129°35′	12	1	0	0
78	Ethel Lake	Yukon Territory	63°22′	136°05′	14	1	0	0
79	Teslin Lake	Yukon Territory	60°15′	132°58′	17	1	0	0
80	Quiet Lake	Yukon Territory	61°05′	133°05′	10	2	0.467	0.239
81	Marsh Lake	Yukon Territory	60°25′	134°18′	15	2	0.476	0.244
82	Tagish Lake	Yukon Territory	60°10′	134°20′	13	3	0.410	0.154
83	Lake Laberge	Yukon Territory	61°11′	135°12′	14	1	0	0
84	Fish Lake	Yukon Territory	60°37′	135°14′	11	1	0	0
85	Braeburn Lake	Yukon Territory	61°27′	135°48′	11	1	0	0
86	Kusawa Lake	Yukon Territory	60°20′	136°22′	15	2	0.133	0.009
87	Dezadeash Lake	Yukon Territory	60°28′	136°59′	11	2	0.509	0.260
88	Aishihik Lake	Yukon Territory	61°26′	137°15′	13	1	0	0
89	**Kluane Lake**	Yukon Territory	61°15′	138°45′	13	1	0	0
90	**Toolik Lake**	Alaska	68°38′	149°36′	20	1	0	0
91	Island Lake	Alaska	68°31′	149°32′	14	1	0	0
92	Galbraith Lake	Alaska	68°28′	149°25′	8	1	0	0
93	Ugashik Lake	Alaska	56°48′	156°52′	12	1	0	0

Note: The 16 lakes shown in bold type were used in the initial screening for geographically informative restriction enzymes.

reaction (PCR) amplified D-loop region, only *Dde*I and *Sty*I detected more than one haplotype (Table 2). The *Sty*I polymorphism in the PCR-amplified fragment coincided with the polymorphism detected by digestion of the entire mtDNA molecule, localizing this variable site. *Dde*I fragment patterns were not included in Table 4, as only fish with "B" patterns for *Ava*I and *Bam*HI were routinely screened with this enzyme; random sampling and digestion of mtDNA from fish from other lineages revealed only the *Dde*I "A" pattern.

The mutational network constructed from the inferred restriction sites (Fig. 1) revealed three major groups distinguishable by two or more restriction site differences that corresponded to lineages observed in earlier studies (Grewe and Hebert 1988; Wilson and Hebert 1996). Haplotypes 1–4 and 15–19 corresponded to group A of Grewe and Hebert (1988) and differed from all other haplotypes by at least three mutational steps, excepting haplotype 4, which differed from haplotype 13 by only two steps. Haplotypes 5–7 corresponded to group B of Grewe and Hebert (1988) and were distinguishable from all other haplotypes by unique *Dde*I and *Sty*I fragment patterns (Fig. 1). Haplotypes 8–12, 14, and 20 differed from all other haplotypes by a minimum of seven mutational steps (Fig. 1) and belong to Grewe and Hebert's (1988) C lineage. Haplotypes 13 and 21 were very similar to group B haplotypes except for their *Dde*I and *Sty*I fragment patterns. These two haplotypes were assigned their own group (D), as they differed from groups A, B, and C by at least two restriction site characters. Bootstrap analysis supported two clades with 100% certainty, group C versus A, B, and D (Fig. 2). Reduced bootstrap separation of group A from groups B and D (78%) was caused by the near-intermediacy of haplotype 4, which occurred as one individual in Lake Superior, Ontario. Groups A, B, and D were nonetheless considered valid, as each group could be distinguished by two or more restriction site characters. Divisions within group C lacked significant structure, due to unresolved *Ase*I and *Ava*I homoplasies.

Estimated pairwise nucleotide divergences (±1 SE) among haplotypes ranged from 0.07 to 1.03%, with a mean value of 0.54%. Groups B and D differed by 0.24% (±0.02) (Fig. 2) and showed limited divergence from group A haplotypes (0.42%

Table 2. Haplotypes for restriction enzymes that detected variation in lake trout mtDNA and their resulting fragment patterns showing fragment sizes in kilobases.

***Ase*I**	
A	3.02 2.93 2.75 2.19 2.00 1.75 1.75 0.57
B	3.02 2.75 2.19 2.15 2.00 1.75 1.75 0.80 0.57
C	2.93 2.75 2.19 2.10 2.00 1.75 1.75 1.49
D	4.40 3.02 2.93 2.19 2.00 1.75 0.57
***Ava*I**	
A	5.49 2.68 2.22 2.12 1.68 1.36 1.10 0.57
B	5.49 2.68 2.22 1.68 1.52 1.36 1.10 0.60 0.57
C	5.49 2.68 1.84 1.68 1.52 1.36 1.10 0.60 0.57 0.37
D	5.49 2.68 1.84 1.68 1.36 1.10 0.90 0.62 0.60 0.57 0.37
***Bam*HI**	
A	16.80
B	9.48 7.32
C	9.48 5.00 2.32
*Bsp*HI	
A	10.10 3.41 3.24
B	10.10 6.65
*Bst*EII	
A	9.46 7.17
B	9.46 6.55 0.68
***Eco*O109I**	
A	4.39 3.98 2.75 1.17 0.73 0.67 0.64 0.40 0.30
B	4.39 3.15 2.75 1.17 0.84 0.73 0.67 0.64 0.40 0.30
C	4.71 4.39 2.75 1.17 0.67 0.64 0.40 0.30
D	4.39 4.20 2.75 1.17 0.67 0.64 0.40
***Eco*RV**	
A	14.25 1.55 0.96
B	15.80 0.96
C	14.25 2.52
***Hin*dIII**	
A	9.10 2.24 1.96 1.76 1.48 0.26
B	9.10 3.44 2.24 1.76 0.26
C	9.10 2.24 1.96 1.76 1.74
*Nco*I	
A	7.46 6.83 1.83 1.19
B	8.29 7.46 1.83
***Sca*I**	
A	10.20 6.57
B	6.57 5.35 4.84
C	11.95 4.84
D	16.80
*Sph*I	
A	12.97 3.86
B	9.85 3.86 3.12
***Stu*I**	
A	5.10 2.36 1.68 1.55 1.22 1.04 0.54 0.49 0.45 0.31
B	5.10 2.36 1.68 1.55 1.35 1.22 0.54 0.49 0.45
C	5.10 2.36 1.68 1.55 1.35 0.82 0.54 0.49 0.45 0.40
***Sty*I**	
A	2.20 1.30 1.05 1.00 0.85 0.80 0.74 0.70 0.56 0.52 0.48 0.36 0.25
B	2.20 1.05 1.00 0.85 0.83 0.80 0.74 0.70 0.56 0.52 0.48 0.46 0.36 0.25
D-loop	
***Dde*I**	
A	0.62 0.57
B	0.62 0.27 0.20
***Sty*I**	
A	0.78 0.28
B	1.10

Note: Enzymes used for geographic survey of lake trout populations are indicated in bold type.

Table 3. Composite haplotypes for restriction enzyme polymorphisms detected among lake trout populations showing locations of individuals with each haplotype among 16 test populations, and additional haplotypes detected during the geographic survey.

Haplotype	Composite fragment pattern														Location
Test populations															
1	A	A	A	A	A	A	A	A	A	A	A	A	A	A	17, 21, 22, 25, 26, 39
2	A	A	A	A	A	A	A	B	A	A	A	A	A	A	19
3	A	A	A	A	B	A	A	A	A	A	A	A	A	A	22
4	A	A	B	A	A	A	A	A	A	A	A	A	A	A	21
5	A	B	B	A	A	A	A	A	A	A	B	A	B	B	2, 4, 5, 12, 13, 17, 21, 22
6	B	B	B	A	A	A	A	A	A	A	B	A	B	B	22
7	A	B	C	A	A	A	A	A	A	A	B	A	B	B	4
8	B	C	C	B	A	B	B	A	A	B	B	B	A	A	21, 22, 25, 26
9	A	C	C	B	A	B	B	A	A	B	B	B	A	A	21, 22, 90
10	B	C	C	B	A	B	B	A	B	B	B	B	A	A	21
11	B	D	C	B	A	B	B	A	A	B	B	B	A	A	22
12	A	D	C	B	A	B	B	A	A	B	B	B	A	A	21, 22
13	A	B	B	A	A	A	A	A	A	A	B	B	A	A	21, 22, 26, 39, 55, 89
14	A	C	C	B	A	B	B	A	A	C	B	A	A	A	21, 22, 50
Additional haplotypes															
15	A	A	A	A	A	A	A	A	A	D	A	A	A	A	33
16	A	A	A	A	A	C	A	A	A	A	A	A	A	A	24
17	C	A	A	A	A	A	A	A	A	A	A	A	A	A	11
18	D	A	A	A	A	A	A	A	A	A	A	A	A	A	30
19	A	A	A	A	A	A	C	A	A	A	A	A	A	A	37, 38
20	A	C	C	B	A	B	B	A	A	B	B	C	A	A	34
21	A	B	B	A	A	D	A	A	A	A	B	B	A	A	82, 86

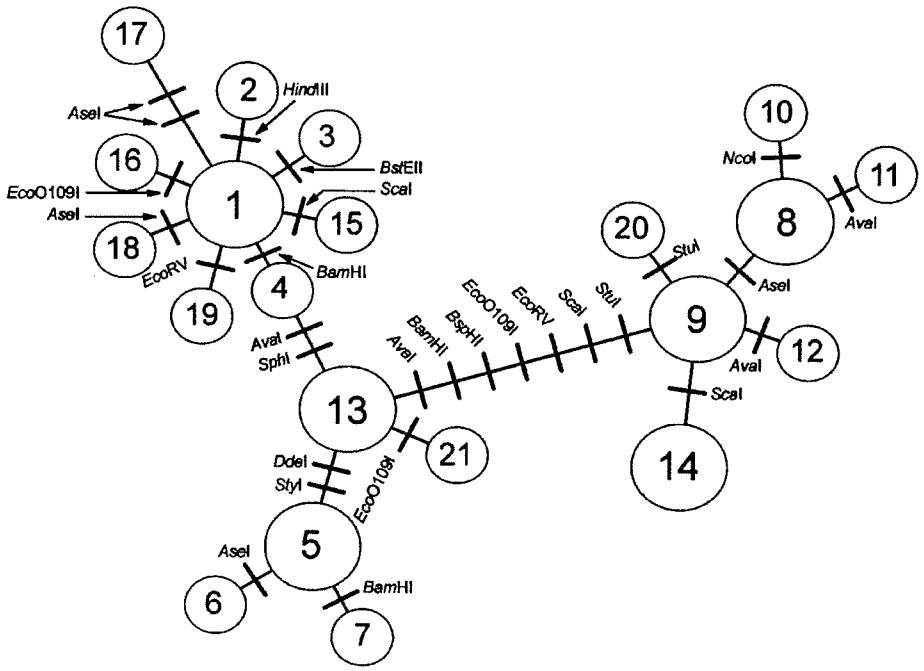
Note: Enzymes from Table 2 are listed in order (*Ase*I, *Ava*I, *Bam*HI, *Bsp*HI, *Bst*EII, *Eco*O109I, *Eco*RV, *Hin*dIII, *Nco*I, *Sca*I, *Sph*I, *Stu*I, and *Sty*I), as well as *Dde*I digestion of the amplified D-loop. Names for locations are given in Table 1.

Table 4. Haplotypes detected during the geographic survey with nine informative restriction enzymes (*Ase*I, *Ava*I, *Bam*HI, *Eco*O109I, *Eco*RV, *Hin*dIII, *Sca*I, *Stu*I, and *Sty*I) showing abundances and locations for uncommon haplotypes.

Haplotype	Composite fragment pattern									*N*	Location
A1	A	A	A	A	A	A	A	A	A	270	(31)
A2	A	A	A	A	A	B	A	A	A	15	19
A3	A	A	A	C	A	A	A	A	A	5	24, 40
A4	C	A	A	A	A	A	A	A	A	2	11
A5	D	A	A	A	A	A	A	A	A	1	30
A6	A	A	A	A	C	A	A	A	A	9	35, 37, 38
A7	A	A	B	A	A	A	A	A	A	1	21
A8	A	A	A	A	A	A	D	A	A	1	33
B1	A	B	B	A	A	A	A	A	B	205	(20)
B2	B	B	B	A	A	A	A	A	B	1	22
B3	A	B	C	A	A	A	A	A	B	1	4
C2	A	C	C	B	B	A	B	B	A	411	(40)
C1	B	C	C	B	B	A	B	B	A	51	(13)
C3	A	C	C	B	B	A	C	A	A	39	(8)
C4	A	C	C	B	B	A	B	C	A	1	34
C5	B	D	C	B	B	A	B	B	A	1	22
C6	A	D	C	B	B	A	B	B	A	7	21, 22, 34
D1	A	B	B	A	A	A	A	B	A	391	(44)
D2	A	B	B	D	A	A	A	B	A	28	2, 86

Note: Numbers of populations containing common haplotypes are given in parentheses. Locations of other haplotypes indicate populations listed in Table 1.

Fig. 1. Parsimony network showing mutational relationships among lake trout haplotypes listed in Table 3. Larger ellipses indicate abundant haplotypes (>30 individuals).



(±0.02)). Group C was the most divergent, differing from group A by 0.88% (±0.01) and from groups B and D by 0.83% (±0.02). Mean divergence within each lineage was equal to or less than 0.15% (Fig. 2).

Geographic structure

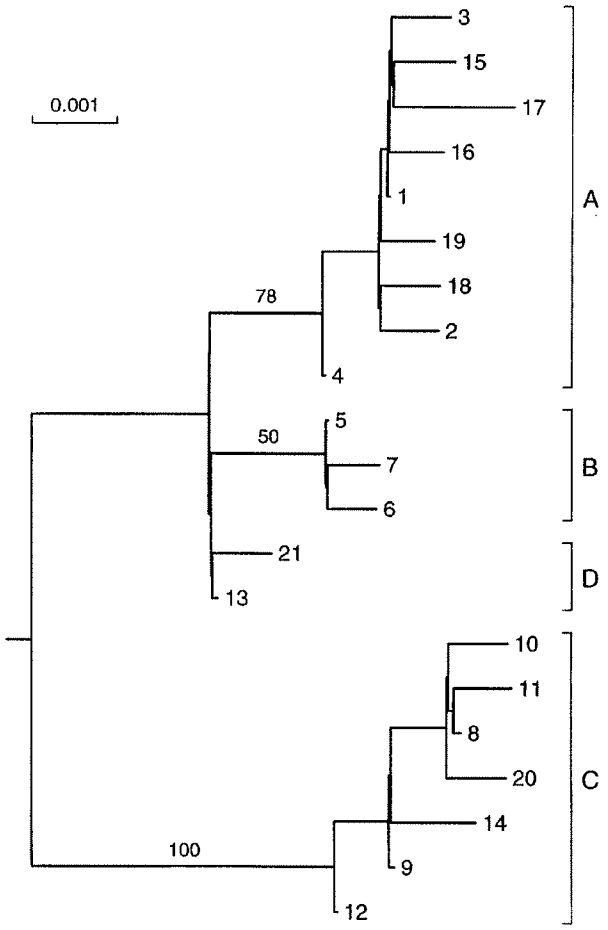
The geographic survey of the remaining populations with nine informative enzymes revealed that groups A, B, C, and D had differential geographic distributions with considerable overlap (Fig. 3). There was little correspondence between geographic distance and genetic divergence, with closely related lineages showing largely allopatric distributions. Group B fish occurred from central Ontario to the Atlantic coast and dominated populations in Quebec, New Brunswick, and New England. By contrast, lake trout with group D haplotypes dominated southern Alaska and Yukon populations and occurred in populations throughout central Canada, extending as far east as western Ontario. Groups B and D were both present in Lake Nipigon and Lake Superior, which formed the western limit for lake trout belonging to group B (Fig. 3). The origin of group D fish in Waterton Lake, Alberta, is uncertain, as this lake has been stocked with fish from Swan Lake (D. Donald, Environment Canada, Regina, Sask., personal communication). Group A fish occurred throughout central Canada, from Quebec to Alberta, and into the southern Northwest Territories (Fig. 3). Group C was widespread throughout northern Alaska and arctic Canada, as well as inland throughout central Canada (Fig. 3), with disjunct occurrences in Montana, southern British Columbia, Alberta, and Lakes Albanel and Mistassini in western Quebec.

The majority of rare haplotypes differed from common haplotypes by single mutations (Fig. 1; Tables 3 and 4) and typically occurred as single individuals in large lakes such as Nipigon and Superior, although several haplotypes (A2, A3, A6, and C6) were either common within single sites or occurred in several populations (Table 4). The three most common group C haplotypes (C1, C2, and C3) showed strong geographic patterning, although all three haplotypes cooccurred in central Canada (Fig. 4). Lake trout with haplotype C1 occurred primarily in Ontario, Quebec, and Manitoba, with Great Slave Lake as the northwest limit to their observed distribution, whereas haplotype C2 dominated populations in arctic and western Canada (Fig. 4). Haplotype C3 fish were largely limited to lakes in Montana and southwest Alberta (Waterton Lake), but also occurred sporadically in Saskatchewan, Manitoba, and western Ontario (Fig. 4).

Contact among lineages was extensive, particularly among groups A, C, and D (Fig. 3). All three of these groups cooccurred throughout central Canada, extending northwest into Lake Athabasca and Great Slave Lake. Groups C and D showed the most extensive codistributions, with additional sympatry in Great Bear Lake, the Mackenzie Delta, and the southern Yukon Territory. Groups B and D showed virtually no overlap, co-occurring only as individuals in Lakes Albanel, Nipigon, and Superior (Fig. 3).

Variation of both nucleon and nucleotide diversity within populations was largely influenced by the number of major lineages present. High levels of diversity occurred almost exclusively within areas formerly covered by major proglacial lakes (Fig. 5). The majority of populations within former lake margins contained haplotypes from more than one mtDNA lineage, with the greatest regional diversity occurring in populations within or near areas formerly covered by Lakes Algonquin, Agassiz, and McConnell (Fig. 5). By contrast, virtually all populations beyond the former proglacial lake margins contained only single haplotypes. This relationship held true even

Fig. 2. Neighbour-joining dendrogram of estimated nucleotide divergences among lake trout haplotypes within geographically representative populations showing divergences among phylogenetic groups described in the text. Numbers above branches represent percent bootstrap support based on 10 000 replications. Scale bar indicates nucleotide divergence of 0.001 (0.1%).



for apparently anomalous populations in the Yukon Territory (Fig. 5), where populations with diversities greater than zero occurred within the former boundaries of glacial Lake Champagne (Lindsey et al. 1981).

The significance of genetic heterogeneity among populations and regions was assessed with the AMOVA program (Excoffier et al. 1992), using two predictive models of regional partitioning to test phylogeographic structure. Populations were partitioned on the basis of modern drainage basins (Arctic, Hudson Bay, Atlantic, Gulf of Mexico, and Pacific drainages) and by postulated refugia (Atlantic, Mississippian, Missourian, and two Beringian refugia) based on earlier multiple-refugia hypotheses (Lindsey 1964; Khan and Qadri 1971; Black 1983*a*, 1983*b*). Both models indicated significant geographic structuring ($p < 0.001$) within and among populations and regions. The refuge model was more effective than the drainage model in partitioning variance components such that variance among regions was maximized ($V_A = 1.42$ (54%) versus 0.71 (29%)) and variance within regions was minimized ($V_B = 0.49$ (19%) versus 1.03 (42%)), indicating that the refuge model best explained geographic patterns of genetic variation. This suggests that the observed genetic structure reflects historical rather than present-day events and that current conditions do not favour gene flow among populations. Much of the difference between the two models resulted from the occurrence of several genetic lineages within the Atlantic and Hudson Bay drainages whereas refugial delineations suggested by Khan and Qadri (1971) and Black (1983*a*, 1983*b*) were largely dominated by single clades (Fig. 3). Both models had high levels of variation in central Canada (Hudson Bay drainage – Mississippian refuge), reflecting the extensive admixture of multiple genetic groups. This highlights the extensive dispersal of lake trout from refugia via proglacial lakes (Fig. 6) and provides an explanation for the many conflicting interpretations of lake trout postglacial dispersal among previous studies.

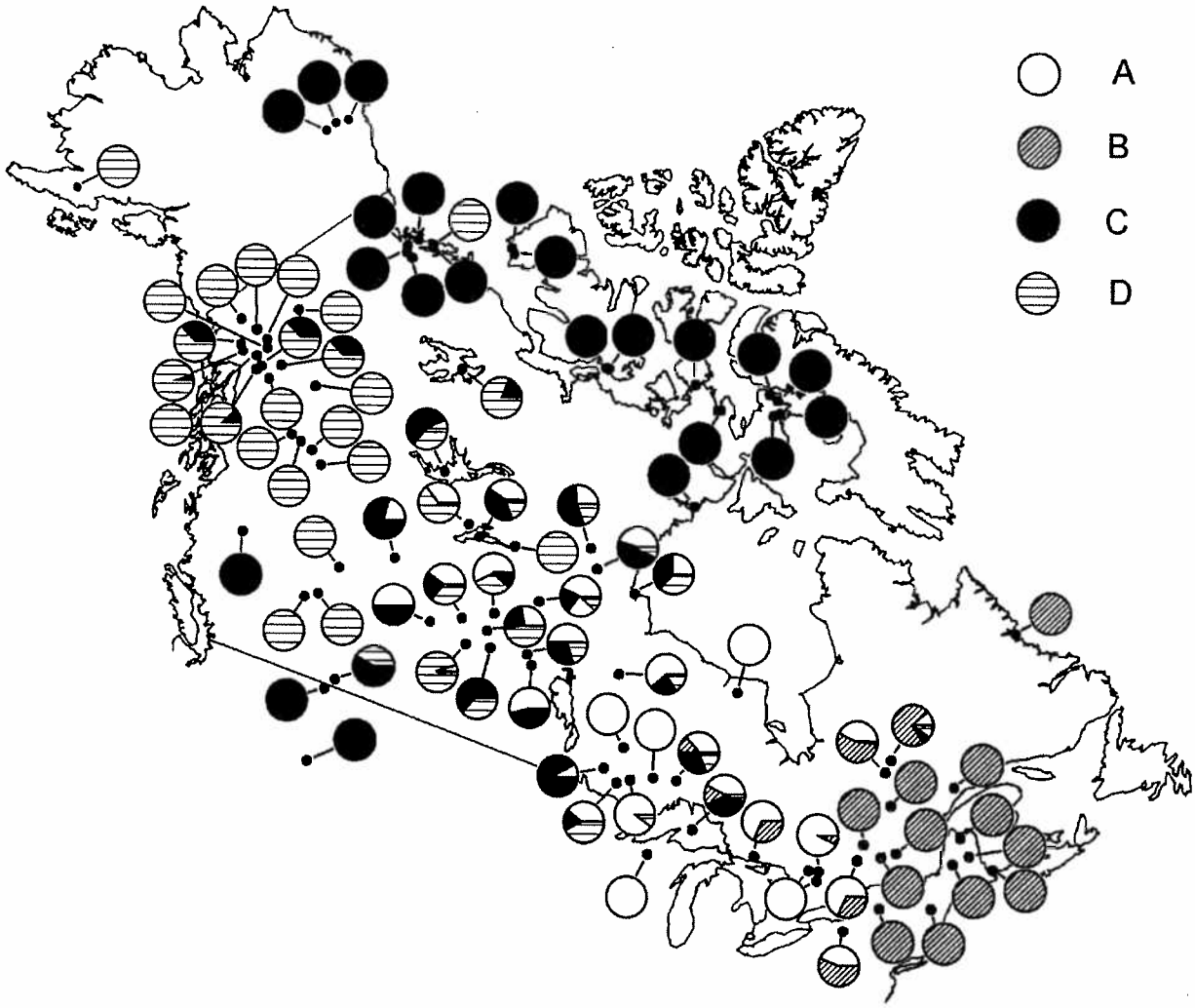
Discussion

Intraspecific divergence

Levels of diversity within and among lake trout mtDNA lineages (<1%) are consistent with data from other northern freshwater species (Bernatchez and Dodson 1991; Billington and Hebert 1991; Wilson et al. 1996) and are probably due to repeated glacial disturbances causing reduced mtDNA diversity through habitat loss, displacement, and persistence in suboptimal habitats during glacial events (Avise et al. 1984). The limited divergence among lineages and low diversity within each group suggest their origin during the Pleistocene. Based on a mtDNA divergence rate of 1% per million years (Smith 1992), group C diverged from other lake trout lineages about 450 000 – 700 000 years ago. This may be an underestimate, however, as the calculation assumes a highly diverse ancestral population (Wilson et al. 1985). In addition, slower estimates for salmonid mtDNA divergence such as 0.5–0.9% per million years (Martin and Palumbi 1993) suggest longer divergence times. Using the lower bound of Martin and Palumbi's estimate, divergence among lake trout lineages could potentially have occurred as early as the beginning of the Pleistocene epoch, about 1.6 million years ago. Although divergence times were not estimated for groups A, B, and D, their levels of genetic divergence suggest that these lineages all diverged well before the Wisconsinan glaciation.

The extent of genetic divergence among the lineages showed no clear correlation with geographic distance. Mitochondrial lineages B and D were closely related, but occupied opposite ends of the continent. Similarly, the three most common group C haplotypes differed by only single cut sites, but had largely disjunct distributions. These groups probably originated from mid-Pleistocene populations that were subdivided by glacial advances, with subsequent divergence in separate refugia through either mutation or lineage extinction. Given the close relationship of the species' range to Pleistocene glacial limits, this cycle of displacement, divergence, and recolonization has probably occurred repeatedly and been a key factor in the formation and differentiation of the major lake trout lineages.

Fig. 3. Distributions of mtDNA lineages A, B, C, and D among sampled lake trout populations. Populations and sample sizes are listed in Table 1.



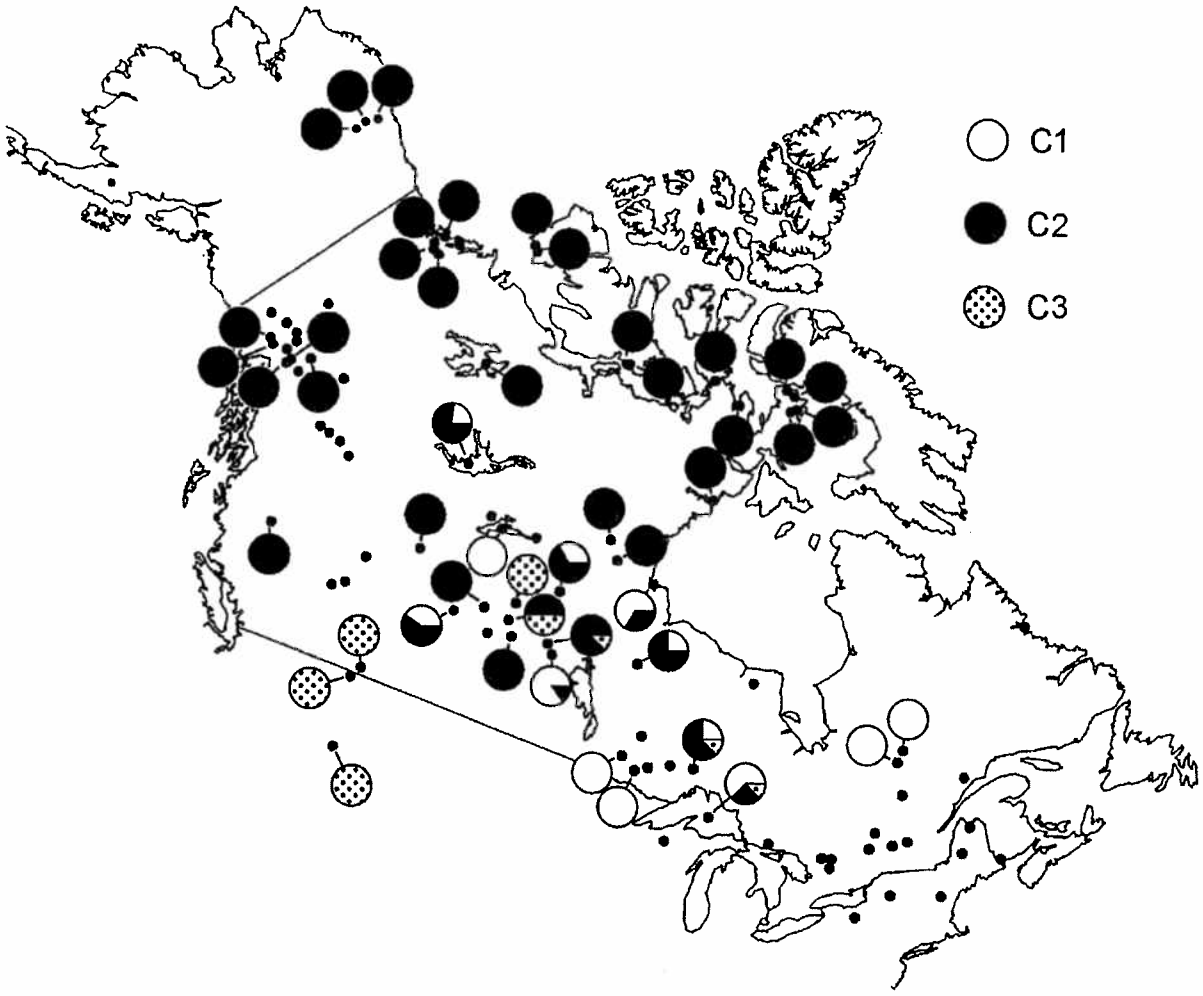
Refugial origins and postglacial dispersal

The geographic distributions of the major lake trout mtDNA lineages strongly suggest their persistence in and dispersal from separate refugia, supporting previous multiple-refugia hypotheses (Lindsey 1964; Khan and Qadri 1971). Groups A, B, and D appear to have each dispersed from single refugia (Fig. 6). Group A fish appear to have dispersed from a Mississippian refuge and group B from an Atlantic source (Wilson and Hebert 1996). These major refugia have been documented as sources for an array of species with widely varying distributions and ecology (Crossman and McAllister 1986), including lake trout (Lindsey 1964; Khan and Qadri 1971; Black 1983*a*, 1983*b*).

Group D lake trout apparently dispersed from a southern Beringian refuge (Fig. 3). Although much of Alaska was ice-free and could have served as a refuge, the absence of lake trout from the lower Yukon River in Alaska argues against this (Lindsey 1964). The Nahanni Valley in the western Northwest Territories is a likely refugial location, as it formed the terminus of a valley between the Cordilleran and Laurentide ice sheets and contained periglacial lakes throughout the Wisconsinan glaciation (Dyke and Prest 1987). The probable use of a Nahanni refuge is supported by group D's virtual absence from the Yukon and Alaska north slope, as well as its abundance in southern Yukon populations (Fig. 3). A Nahanni refuge has previously been proposed for lake whitefish based on allozyme data (Foote et al. 1992), and the same refuge may also have been important for other taxa (Lindsey and McPhail 1986).

Geographic patterning within group C was more complex and suggests vicariant disruption by glacial advances, with the three most common haplotypes dispersing from separate refugia (Fig. 4). The distribution of haplotype C1 (Fig. 4) can best be explained by its dispersal from a southern (Mississippian or Missourian) refuge, as its distribution is strongly concordant with group A fish from the Mississippian refuge (Fig. 3). It is therefore probable that group C fish in Quebec (Figs. 3 and 4) came from a southern rather than a Beringian

Fig. 4. Distributions of the common group C haplotypes (C1, C2, and C3). Dots indicate sites where group C haplotypes are absent. Pies indicate relative and not absolute proportions of these haplotypes; presence–abundances of other haplotypes are not shown.

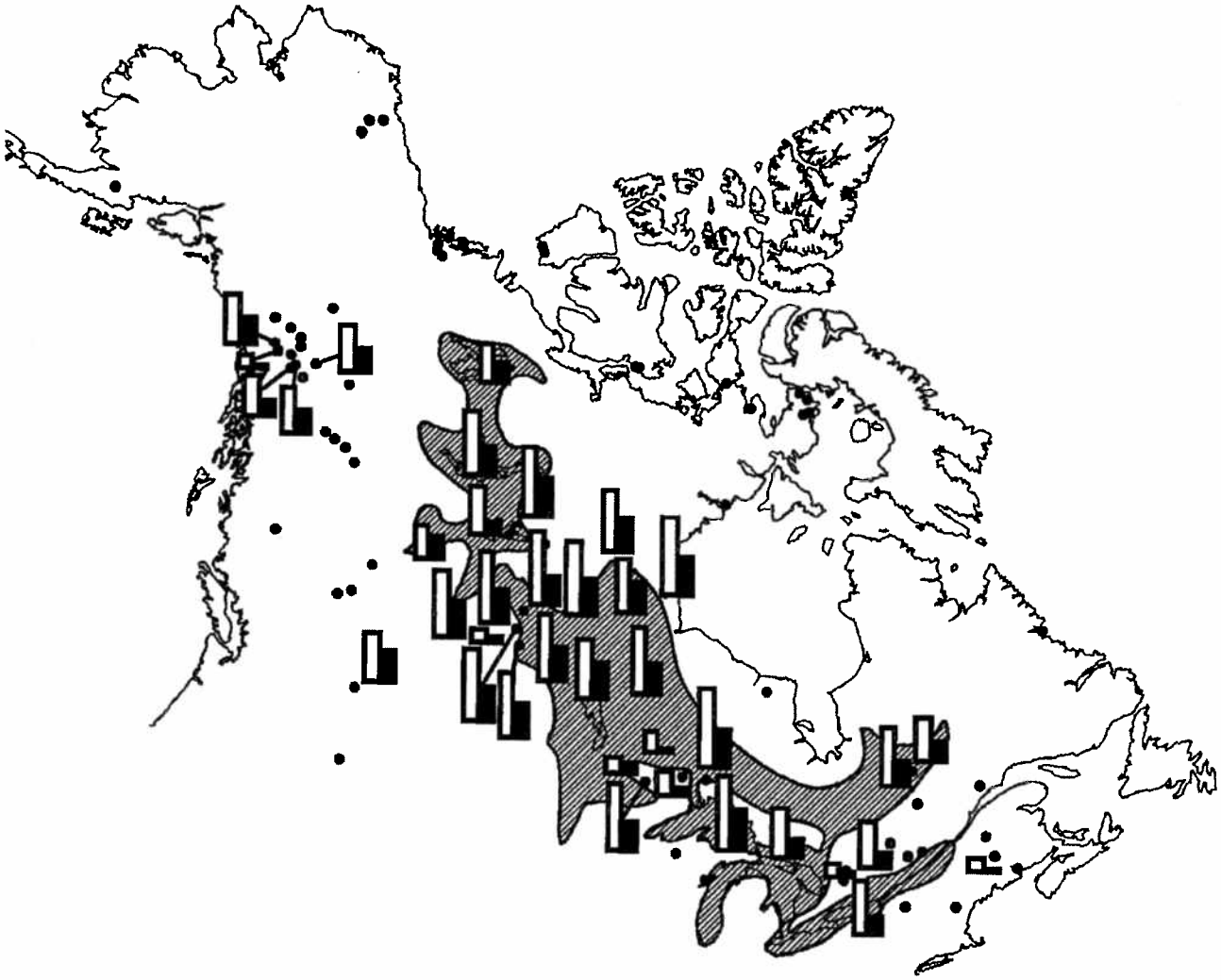


source, as previously suggested by Wilson and Hebert (1996). By contrast, haplotype C2 and derivative fish appear to have persisted in a Beringian refuge separate from that occupied by group D, as the two lineages show largely separate arctic distributions (Fig. 3).

The localized distribution of haplotype C3 in western Montana and southwestern Alberta suggests its persistence in a nearby refuge, as suggested by Lindsey (1964). This lineage does not appear to have utilized a Missourian refuge, as emigrants from this system would have had early access to Lake Agassiz with subsequent widespread dispersal. Crossman and McAllister (1986) differentiated between a Missourian refuge and potential refugia in western Montana and Alberta, based on the presence of several endemic and cold-water fish and invertebrate taxa. Additional evidence for a cold-water refuge in this region comes from fossil remains of Arctic grayling (*Thymallus arcticus*) from January Cave in southwestern Alberta, dated at 23 000 – 31 000 years ago (Burns 1991).

The geographic distribution of mitochondrial diversity suggests that lake trout dispersal was primarily dependent on the network of proglacial lakes that spanned much of Canada, although headwater exchange and coastal dispersal were also important (Figs. 5 and 6). Dispersal of group B lake trout from their Atlantic refuge throughout eastern Canada and New England occurred primarily via regional access through glacial Lakes Vermont and Ojibway-Barlow and by following retreating ice margins (Wilson and Hebert 1996). Colonization of Ontario by group B occurred via a connection between glacial Lakes Iroquois and Vermont, and thence into the Laurentian Great Lakes. The distribution of Mississippian (group A) lake trout in eastern North America approximates that of the proglacial lakes in the Great Lakes basin such as glacial Lakes Algonquin and Iroquois (Dyke and Prest 1987). Similarly, Mississippian lake trout had repeated intermittent access to Lake Agassiz 12 800 to 9000 years ago (Dyke and Prest 1987) and colonized western Canada via Lakes Agassiz and McConnell, probably reaching Lake McConnell through a direct connection during the Emerson phase of Lake Agassiz 9900–9500 years ago (Smith and Fisher 1993). Although Mississippian lake trout would have been able to disperse eastward from Lake McConnell 9000–8000 years age as ice margins retreated (Black 1983*a*; Dyke and Prest 1987), the absence of

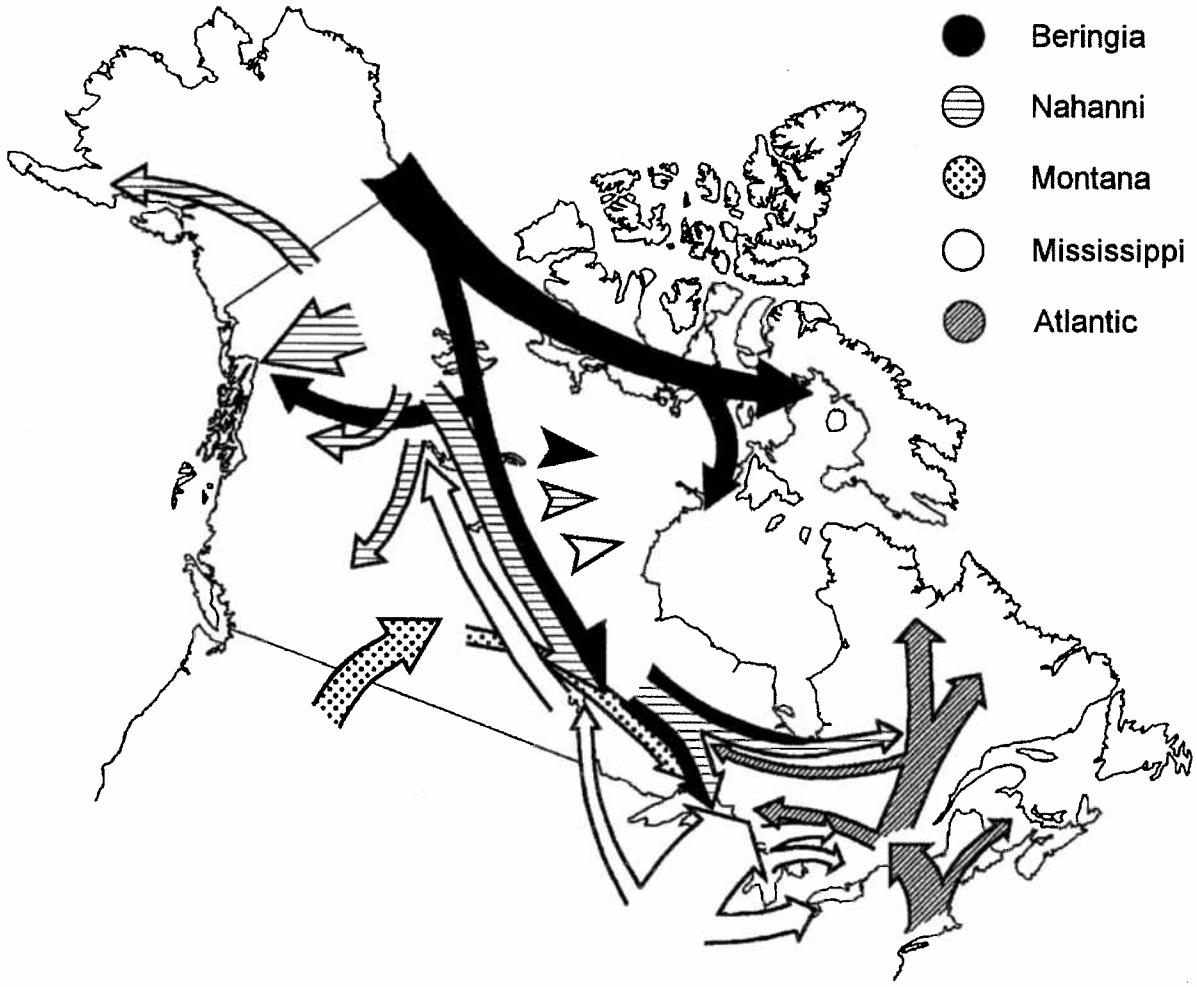
Fig. 5. Geographic distribution of diversity within lake trout populations with respect to former maximal extents of major proglacial lakes (Ojibway-Barlow, Algonquin, Iroquois, Agassiz, Peace, and McConnell) indicated by shaded areas. White and black bars represent values of nucleon and nucleotide diversities from Table 1, respectively. Dots represent populations with single haplotypes only.



group A haplotypes in regions northwest of Great Slave Lake (Fig. 3) suggests that Mississippian lake trout were not the primary colonists of the Mackenzie Delta, in contrast with earlier expectations (Black 1983*a*; Crossman and McAllister 1986).

Glacial retreat and ensuing dispersal opportunities began much earlier in western Canada than in the east. Dispersal of lake trout from the two northwestern refugia probably began soon after the glacial maximum 18 000 years ago (Dyke and Prest 1987), as ice retreat was rapid in the northwest and much of the Yukon and Alaska was deglaciated by 14 000 years ago. The resulting meltwater would have facilitated lake trout dispersal both inland via proglacial lakes and along the Arctic coast by reducing salinity to levels tolerated by lake trout (Martin and Olver 1980). Beringian lake trout dispersing along the Arctic coast would have followed glacial margins closely, gradually colonizing eastward until the demise of the Laurentide ice sheet about 6000 years ago (Dyke and Prest 1987). Similar dispersal patterns have been observed for other Beringian emigrants such as Arctic char (*Salvelinus alpinus*) (Wilson et al. 1996). Evidence from other populations indicates that Beringian group C lake trout dispersed along the coast at least as far as the Melville Peninsula (Wilson and Hebert 1993). Coastal movements may have also facilitated inland dispersal, as dispersal from a Beringian refuge by many species is thought to have been prevented by a barrier waterfall on the lower Mackenzie River that lasted from 11 500 to 6100 years age (Lindsey and McPhail 1986). Based on the distributions of groups C and D in eastern Canada (Figs. 3 and 4), it is certain that Beringian- and Nahanni-refuge lake trout were able to colonize glacial Lake McConnell by 9500 years ago, with subsequent dispersal into Lake Agassiz (Smith and Fisher 1993). Movement of Beringian group C fish into southern Yukon lakes probably resulted from connections between the Liard River and glacial Lake Champagne about 10 000 years ago, as this proglacial lake covered four of the

Fig. 6. Hypothesized refugial origins and dispersal for extant lake trout populations based on distributions of mitochondrial haplotypes shown in Figs. 3 and 4 and geological data of proglacial lake locations and connections (Dyke and Prest 1987).



five lake basins where group C fish were detected (Lindsey et al. 1981). Dispersal through the southern Yukon Territory and northern British Columbia was similarly governed by a complex series of ice movements and drainage transfers (Bodaly and Lindsey 1977; Lindsey and McPhail 1986). Dispersal of group D lake trout from the Nahanni refuge into southern Alaska probably occurred via drainage reversals in the Peel River system during early stages of deglaciation (Bodaly and Lindsey 1977). The eastward dispersal of group D lake trout mirrored that of Beringian group C emigrants (Fig. 6). Both groups reached the Great Lakes region (Figs. 3, 4, and 6), and the presence of group D lake trout in Lake Albanel (Fig. 3) indicates that some of these fish were able to colonize Quebec via Lake Ojibway-Barlow.

Dispersal of lake trout from the Montana refuge appears to have been quite limited (Figs. 4 and 6). Suboptimal dispersal conditions were caused by ice stagnation and low volumes of meltwater as well as a shortage of suitable habitats (Christiansen 1979; Dyke and Prest 1987). Proglacial lakes and drainages were localized and laden with glacial till and were subject to catastrophic outburst floods which frequently drained the lakes completely (Christiansen 1979). Lake trout are poorly suited to such conditions (Martin and Olver 1980), although the presence of haplotype C3 in Saskatchewan, Manitoba, and western Ontario indicates that some fish were able to disperse into Lake Agassiz, probably via glacial Lake Saskatchewan, which existed from 14 000 to 11 000 years ago and was connected to Lake Agassiz 11 500 – 11 000 years ago (Christiansen 1979).

Secondary contact among refugial groups

The striking variation in mtDNA diversity among lake trout populations is largely due to the number of refugial lineages present. The close relationship between the geographic distribution of diverse populations and former proglacial lakes (Fig. 5) strongly suggests secondary contact among refugial groups within these lakes as the cause for intrapopulation diversity. By contrast, populations of lake trout beyond proglacial lake limits were probably founded by relatively few individuals from single refugial sources.

This study makes it clear that secondary contact among groups was extensive throughout central Canada. The

co-occurrence of all of the refugial groups in Lakes Superior and Nipigon (Figs. 3 and 4) underscores the tremendous dispersal facilitated by postglacial drainage events (Fig. 6) and highlights the importance of the major proglacial lakes in facilitating transcontinental dispersal. The dispersal opportunities provided by these meltwater lakes were unparalleled in nonglaciated habitats (Fig. 6).

The correspondence between proglacial lake connections and haplotype distributions suggests that colonization order significantly influenced modern abundances of lake trout refugial groups. As the probabilities for persistence of mtDNA haplotypes within populations are proportional to their initial abundances (Birky et al. 1983), the rapid expansion of population sizes by early colonists in newly formed habitats would place later arrivals at a numerical disadvantage. The predominance of the Beringian and Nahanni lake trout lineages in northwestern Canada would therefore have been assured by their early dispersal into glacial Lakes Peace and McConnell. However, late arrival times could potentially be offset by large number of colonists. For example, despite the likelihood that Mississippian lake trout were present in Lake Agassiz prior to its connection with Lake McConnell, the large numbers of Beringian and Nahanni refuge fish in Lakes Peace and McConnell would have ensured their current abundances in lakes within the former Lake Agassiz basin. By contrast, the low numbers of lake trout with haplotype C3 in central Canada indicate that fish from the Montana refuge had little impact on the genetic composition of proglacial lake populations, due to either low numbers of colonists or their late arrival. At the nuclear level, however, genetic contributions of each group could diffuse through proglacial populations via random mating events (Birky et al. 1983; Billington and Hebert 1991).

Comparative patterns of intraspecific phylogeography

Comparison of intraspecific nuclear and mitochondrial data shows that impacts of drainage events during glacial–interglacial transitions on phylogeographic patterns varied considerably among species. For example, allozyme and mtDNA studies of lake trout from Ontario and Manitoba yielded opposing zoogeographic interpretations. Allozyme data suggested two relatively homogeneous major geographic groups that originated from Mississippian and Atlantic refugia (Ihssen et al. 1988) whereas the mtDNA data show the sympatric occurrence of fish from four out of the five refugial groups in this region. The extensive secondary contact among refugial groups resulted in the homogenization of nuclear markers among previously isolated groups, indicating the geographic extent of these contact zones, rather than distributions of refugial groups themselves as proposed by Ihssen et al. (1988). Combined allozyme–mtDNA analysis of walleye (*Stizostedion vitreum*) populations similarly showed that mtDNA was more effective than allozymes in elucidating phylogeographic patterns, due to reduced signal from nuclear characters as a result of secondary contact among refugial groups (Ward et al. 1989). By contrast, separate allozyme and mtDNA phylogeographic analyses of lake whitefish (Bernatchez and Dodson 1991; Bodaly et al. 1992; Foote et al. 1992) produced nearly identical interpretations of postglacial dispersal, with widespread distribution of Mississippian fish and only localized contributions from other refugia, with little or no intergradation of geographic races.

The extent and timing of proglacial lakes as well as connections and conditions among them produced qualitative differences in dispersal opportunities and resulting phylogeographic structure among the species comprising regional faunas (Ward et al. 1989; Bernatchez and Dodson 1991; Wilson and Hebert 1996). In contrast with phylogeographic concordance among aquatic species from nonglaciated areas (Bermingham and Avise 1986; Avise 1992), phylogeographic patterns among northern species show marked differences that can readily be interpreted in light of postglacial history and species-specific ecological characteristics. It is becoming clear that many northern fish species possess multiple mtDNA lineages that represent ancestral populations from separate glacial refugia. With the low levels of intraspecific diversity and divergence often observed (Billington and Hebert 1991), it is somewhat remarkable that genetic signatures of multiple refugia can be detected. The modal patterning and discontinuous geographic distribution of intraspecific divergence in many of these species highlight the tremendous demographic and evolutionary impacts that Pleistocene glaciations have had on the genetic structure of northern species.

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References

Avise, J.C. 1992. Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. Oikos, **63**: 62–76.

Avise, J.C., Neigel, J.E., and Arnold, J. 1984. Demographic influences on mitochondrial DNA lineage survivorship in animal populations. J. Mol. Evol. **20**: 99–105.

Bardakci, F., and Skibinski, D.O.F. 1994. Application of the RAPD technique in tilapia fish: species and subspecies identification. Heredity, **73**: 117–123

Bermingham, E., and Avise, J.C. 1986. Molecular zoogeography of freshwater fishes in the southeastern United States. Genetics, **113**: 939–965.

Bernatchez, L., and Danzmann, R.G. 1993. Congruence in control region sequence and restriction site variation in mitochondrial DNA of brook charr (*Salvelinus fontinalis* Mitchill). Mol. Biol. Evol. **10**: 1002–1014.

Bernatchez, L., and Dodson, J.J. 1991. Phylogeographic structure in mitochondrial DNA of the lake whitefish (*Coregonus clupeaformis*) and its relation to Pleistocene glaciations. Evolution, **45**: 1016–1035.

Bernatchez, L., Dodson, J.J., and Boivin, S. 1989. Population bottlenecks: influence on mitochondrial DNA diversity and its effect in coregonine stock discrimination. J. Fish Biol. **35**(Suppl. A): 233–244.

Billington, N., and Hebert, P.D.N. 1991. Mitochondrial DNA diversity in fishes and its implications for introductions. Can. J. Fish. Aquat. Sci. **48**(Suppl. 1): 80–94.

Billington, N., Barrette, R.J., and Hebert, P.D.N. 1992. Management implications of mitochondrial DNA variation in walleye stocks. N. Am. J. Fish. Manage. **12**: 276–284.

Birky, C.W., Jr., Maruyama, T., and Fuerst, P. 1983. An approach to population and evolutionary genetic theory for genes in mitochondrial and chloroplasts, and some results. Genetics, **103**: 513–527.

Black, G.A. 1983*a*. Origin, distribution, and postglacial dispersal of a swimbladder nematode, *Cystidicola stigmatura*. Can. J. Fish. Aquat. Sci. **40**: 1244–1253.

Black, G.A. 1983*b*. *Cystidicola farionis* (Nematoda) as an indicator of lake trout (*Salvelinus namaycush*) of Bering ancestry. Can. J. Fish. Aquat. Sci. **40**: 2034–2040.

Bodaly, R.A., and Lindsey, C.C. 1977. Pleistocene watershed exchanges and the fish fauna of the Peel River basin, Yukon Territory. J. Fish. Res. Board Can. **34**: 388–395.

Bodaly, R.A., Clayton, J.W., Lindsey, C.C., and Vuorinen, J. 1992. Evolution of lake whitefish (*Coregonus clupeaformis*) in North America during the Pleistocene: genetic differences between sympatric populations. Can. J. Fish. Aquat. Sci. **49**: 769–779.

Burns, J.A. 1991. Mid-Wisconsinan vertebrates and their environment from January Cave, Alberta, Canada. Quat. Res. **35**: 130–143.

Christiansen, E.A. 1979. The Wisconsinan deglaciation of southern Saskatchewan and adjacent areas. Can. J. Earth Sci. **16**: 913–938.

Crossman, E.J., and McAllister, D.E. 1986. Zoogeography of freshwater fishes of the Hudson Bay drainage, Ungava Bay and the Arctic Archipelago. *In* The zoogeography of North American freshwater fishes. *Edited by* C.H. Hocutt and E.O. Wiley. John Wiley & Sons, New York. pp. 53–104.

Dyke, A.S., and Prest, V.K. 1987. Late Wisconsinan and Holocene history of the Laurentide Ice Sheet. Geogr. Phys. Quat. **41**: 237–263.

Excoffier, L., Smouse, P.E., and Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics, **131**: 479–491.

Felsenstein, J. 1993. PHYLIP (Phylogeny Inference Package) version 3.5c. Department of Genetics, University of Washington, Seattle, Wash.

Foote, C.J., Clayton, J.W., Lindsey, C.C., and Bodaly, R.A. 1992. Evolution of lake whitefish (*Coregonus clupeaformis*) in North America during the Pleistocene: evidence for a Nahanni glacial refuge race in the Northern Cordillera region. Can. J. Fish. Aquat. Sci. **49**: 760–768.

Grewe, P.M., and Hebert, P.D.N. 1988. Mitochondrial DNA diversity among broodstocks of the lake trout, *Salvelinus namaycush*. Can. J. Fish. Aquat. Sci. **45**: 2114–2122.

Höltke, H.J., Sagner, G., Kessler, C., and Schmitz, G. 1992. Sensitive chemiluminescent detection of digoxigenin-labeled nucleic acids: a fast and simple protocol and its applications. BioTechniques, **12**: 104–113.

Ihssen, P.E., Casselman, J.M., Martin, G.W., and Phillips, R.B. 1988. Biochemical genetic differentiation of lake trout (*Salvelinus namaycush*) stocks of the Great Lakes region. Can. J. Fish. Aquat. Sci. **45**: 1018–1029.

Khan, N.Y., and Qadri, S.U. 1971. Intraspecific variations and postglacial distribution of lake char (*Salvelinus namaycush*). J. Fish. Res. Board Can. **28**: 465–476.

Lindsey, C.C. 1964. Problems in zoogeography of the lake trout *Salvelinus namaycush*. J. Fish. Res. Board Can. **21**: 977–994.

Lindsey, C.C., and McPhail, J.D. 1986. Zoogeography of fishes of the Yukon and Mackenzie basins. *In* The zoogeography of North American freshwater fishes. *Edited by* C.H. Hocutt and E.O. Wiley. John Wiley & Sons, New York. pp. 639–674.

Lindsey, C.C., Patalas, K., Bodaly, R.A., and Archibald, C.P. 1981. Glaciation and the physical, chemical and biological limnology of Yukon lakes. Can. Tech. Rep. Fish. Aquat. Sci. No. 966.

Martin, A.P., and Palumbi, S.R. 1993. Body size, metabolic rate, generation time, and the molecular clock. Proc. Natl. Acad. Sci. U.S.A. **90**: 4087–4091.

Martin, N.V., and Olver, C.H. 1980. The lake charr, *Salvelinus namaycush*. *In* Charrs: salmonid fishes of the genus *Salvelinus*. *Edited by* E.K. Balon. Dr. W. Junk Publishers, The Hague, The Netherlands. pp. 205–277.

McAllister, D.E., Platania, S.P., Schueler, F.W., Baldwin, M.E., and Lee, D.S. 1986. Ichthyofaunal patterns on a geographic grid. *In* The zoogeography of North American freshwater fishes. *Edited by* C.H. Hocutt and E.O. Wiley. John Wiley & Sons, New York. pp. 17–51.

McElroy, D.M., Moran, P., Bermingham, E., and Kornfield, I. 1992. REAP: an integrated environment for the manipulation and phylogenetic analysis of restriction data. J. Hered. **83**: 157–158.

Nei, M., and Tajima, F. 1981. DNA polymorphism detectable by restriction endonucleases. Genetics, **97**: 145–163.

Pielou, E.C. 1991. After the Ice Age: the return of life to glaciated North America. University of Chicago Press, Chicago, Ill.

Saitou, N., and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**: 406–425.

Smith, D.G., and Fisher, T.G. 1993. Glacial Lake Agassiz: the northwestern outlet and paleoflood. Geology (Boulder), **21**: 9–12.

Smith, G.R. 1992. Introgression in fishes: significance for paleontology, cladistics, and evolutionary rates. Syst. Biol. **41**: 41–57.

Ward, R.D., Billington, N., and Hebert, P.D.N. 1989. Comparison of

allozyme and mitochondrial DNA variation in populations of walleye, *Stizostedion vitreum*. Can. J. Fish. Aquat. Sci. **46**: 2074–2084.

Wilson, A.C., Cann, R.L., Carr, S.M., George, M., Gyllensten, U.B., Helm-Bychowski, K.M., Higuchi, R.G., Palumbi, S.R., Prager, E.M., Sage, R.D., and Stoneking, M. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. Biol. J. Linn. Soc. **26**: 375–400.

Wilson, C.C. 1995. Phylogeography and postglacial dispersal of two North American *Salvelinus* species. Ph.D. thesis, University of Guelph, Guelph, Ont.

Wilson, C.C., and Hebert, P.D.N. 1993. Natural hybridization and introgression between lake trout (*Salvelinus namaycush*) and Arctic charr (*S. alpinus*) in the Canadian arctic. Can. J. Fish. Aquat. Sci. **50**: 2652–2658.

Wilson, C.C., and Hebert, P.D.N. 1996. Phylogeographic origins of lake trout (*Salvelinus namaycush*) in eastern North America. Can. J. Fish. Aquat. Sci. **53**: 2764–2775.

Wilson, C.C., Hebert, P.D.N., Reist, J.D., and Dempson, J.B. 1996. Phylogeography and postglacial dispersal of Arctic charr *Salvelinus alpinus* in North America. Mol. Ecol. **5**: 187–198.