A major sextet of mitochondrial DNA phylogenetic assemblages extant in eastern North American brook trout (*Salvelinus fontinalis*): distribution and postglacial dispersal patterns

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Abstract: Mitochondrial DNA (mtDNA) restriction fragment length polymorphisms (RFLPs) of 2422 brook trout (*Salvelinus fontinalis*) from 60 units (major drainages, small stream catchments, and isolated lakes) representing 155 populations in eastern North America were examined to test hypotheses regarding postglacial dispersal and recolonization. An analysis of molecular variance (AMOVA) indicated that 38.8% of the variation was partitioned among the units, while approximately 60% was distributed among populations (ϕ_{ST} = 59.3) compared with 40.7% within populations. This distribution of variation suggests a large degree of heterogeneity in population founding events and phylogeographic structuring in this species. Comparisons of mtDNA diversity between fish from putative refugial and recolonization zones for this species indicate that more than one refugial region contributed to northern recolonization. Haplotypic diversities in recolonized regions are greatest in south-central populations (i.e., southern Great Lakes region), while only one haplotype (haplotype 1) predominates in northern, western, and eastern postglacial zones. Large phylogenetic differences were found between northern and southern populations. Populations outside the zone of glaciation were the most genetically heterogeneous and were represented by fish from all six (A–F) of the major evolutionary clades identified. Only fish from the A, B, and C clades were found in glaciated regions, with C lineage fish restricted to south-central glaciation zones. Fish from the C clade are putatively the most ancestral lineage within the species based upon composite shared RFLPs with lake trout (*Salvelinus namaycush*) and Arctic char (*Salvelinus alpinus*).

Résumé : L'étude du polymorphisme de la longueur des fragments d'ADN mitochondrial obtenus au moyen d'enzymes de restriction (RFLP) chez 2422 Ombles de fontaine (*Salvelinus fontinalis*) provenant de 60 habitats aquatiques (bassins hydrographiques principaux, petits bassins hydrographiques et lacs isolés) et appartenant à 155 populations de l'est nord-américain a été entreprise pour vérifier les hypothèses sur la recolonisation et la dispersion post-glaciaires. Une analyse de la variance moléculaire (AMOVA) a démontré que 38,8% de la variation était partagée entre les habitats, alors qu'environ 60% de la variation était partagée entre les populations (ϕ_{st} = 59,3), comparativement à 40,7% répartie au sein des populations. La répartition de la variation indique une grande hétérogénéité des événements qui ont donné lieu aux populations et à la structuration phylogéographique de cette espèce. La comparaison de la diversité de l'ADNmt entre les poissons des refuges et des zones de recolonisation soupçonnés indique que plus d'une zone de refuge a contribué à la recolonisation dans la région nordique. La diversité des haplotypes dans les régions recolonisées est plus grande dans les populations sud-centrales (i.e., la région sud des Grands Lacs) alors qu'un seul haplotype (haplotype 1) prédomine dans les zones post-glaciaires du nord, de l'ouest et de l'est. D'importantes différences phylogénétiques ont été trouvées entre les populations du nord et du sud. Les populations hors des zones glaciées se sont avérées les plus hétérogènes génétiquement et elles sont représentées par des poissons des six (A–F) clades évolutifs principaux reconnus. Seulement des poissons des clades A, B et C ont été trouvés dans les régions glaciées et les poissons de la lignée C sont restreints aux zones glaciées du centre sud. Le clade C représente

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probablement la lignée la plus ancienne de l'espèce d'après l'ensemble des polymorphismes RFLP qu'elle partage avec le Touladi (*Salvelinus namaycush*) et l'Omble chevalier (*Salvelinus alpinus*).

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Introduction

Glacial ice ages have had profound influences upon the evolutionary lineages of species inhabiting regions subjected to such events. Geographic regions once occupied by glaciers are generally characterized as having low faunal diversities (Pielou 1991). Freshwater fish faunas are not only relatively more depauperate in north-temperate zones, but northern populations of fish also have relatively less genetic variability (e.g., mitochondrial DNA (mtDNA) variation) compared with conspecific populations surveyed from unglaciated regions (see references given in Billington and Hebert 1991). Mitochondrial DNA analysis of population structure has been a useful method to ascertain postglacial dispersal routes and phylogeographic structuring in many freshwater fish species (Avise et al. 1987; Bernatchez and Dodson 1991; Wilson et al. 1996; Murdoch and Hebert 1997; Bernatchez and Wilson 1998).

Brook trout (*Salvelinus fontinalis*) are endemic to eastern North America (Scott and Crossman 1973) and have been postulated to have recolonized northern parts of their range from an Atlantic uplands (Appalachian region) refugial zone (Radforth 1944). Radforth (1944) argued that the species' requirement for cool clear water would have precluded its establishment in a southwestern Mississippian refugium. This scenario has been supported by some investigators (e.g., Mandrak and Crossman 1992) but refuted by others (Bailey and Smith 1981) who suggest that northern brook trout populations also arose from a Mississippian source.

We compared the mtDNA diversity in brook trout from the putative Atlantic refugial region with that in conspecifics throughout the species' native range as a test of the single versus multiple refugial origins hypotheses. If the phylogenetic origins of mtDNA haplotypes are deep (= preceding the last glacial ice age), then the distribution of the various haplotypes may be used to unequivocally infer their refugial origins. If all fish arose from a single refugium, then the process of random lineage extinction (Avise et al. 1984; Neigel et al. 1991; Neigel and Avise 1993) should result in a mosaic pattern of haplotypic distributions (i.e., all common refugial haplotypes present throughout the range but not necessarily present in any one population). Since vagility in brook trout is low (Power 1980), such a mosaic pattern should be evident throughout their recolonized range. Conversely, geographic differences within glaciated zones in the occurrence of evolutionarily divergent haplotypes would indicate that fish from multiple refugia are involved in the recolonization process. A mosaic pattern of dispersal would, however, also be expected from multiple refugial origins, owing to low vagility, with the caveat that certain haplotypes would have a relatively widespread intraregion distribution but would not be found throughout the region of recolonization.

Current phylogeographic patterning of mtDNA diversity can be used to infer past demographic events throughout the species range. The process of cladogenesis and lineage extinction may result in a geographic array of haplotypes sharing relative affinities to one another dependent upon past gene flow and isolation. Thus, two regions may be divergent or similar in haplotypic composition. When two regions are divergent in composition, the haplotypes present in each region may be closely related (indicative of a "shallow" phylogenetic origin) or distantly related (= "deep" phylogenetic origin).

Deep phylogeographic groupings would possess fish with mtDNA haplotypes from ancient phylogenetic clades not found in other regions (category I of Avise et al. 1987), while the presence of deep phylogenetic clades scattered throughout the recolonization zone would indicate relatively recent admixture of formerly separate phylogenetic groupings prior to recolonization (category II of Avise et al. 1987). Shallow phylogeographic groupings would be chararactized by the presence of closely related haplotypes with the distribution of the haplotypes reflecting possible differences in refugial origins. A strong phylogeographic distribution of closely related haplotypes (category III of Avise et al. 1987) could indicate their origins from separate refugial centres, while the random distribution of such haplotypes throughout the recolonization zone (category IV of Avise et al. 1987) would likely point to a single refugial source. The presence of a predominant haplotype (usually plesiomorphic) within a clade throughout the zone of recolonization with the presence of closely related (but derived) haplotypes restricted to various regions (category V of Avise et al. 1987) would indicate intermediate past gene flow with the restricted distribution of certain haplotypes reflecting their recent origin. The refugial origins of recently arisen haplotypes are often difficult to infer (e.g., Arctic char (*Salvelinus alpinus*); Danzmann et al. 1991*a*; Wilson et al. 1996). We compared the phylogenetic differences among the major mtDNA lineages in North American brook trout to ascertain estimates of their evolutionary origins and to test the phylogenetic differentiation of phylogeographic groupings (i.e., deep versus shallow).

Materials and methods

Source of the fish

A total of 2422 fish from 175 populations of brook trout were sampled in North America for mtDNA restriction fragment length polymorphism (RFLP) variation (Table 1, Fig. 1). Sample sizes per population ranged from 1 to 44, with most population samplings consisting of from 8 to 20 fish. A population is defined as a group of individuals inhabiting a microgeographically distinct region (lake, stream, or river) with no barriers (i.e., waterfalls, rapids, unsuitable habitat, etc.) present that would impede interbreeding among individuals.

Details of mtDNA variation in brook trout from the Canadian Maritimes is presented in Ferguson et al. (1991), Jones (1995), and Jones et al. (1996). Similar information for Algonquin Park, Ontario, and Big Creek, Ontario, populations is given in Danzmann and Ihssen (1995) and Danzmann et al. (1991*b*), respectively. In Table 1, where reference is made to the analysis of several popula-

Table 1. Sampling locations for 175 populations of brook trout collected in North America.

Population(s)[a]	Number and abbreviation[b]	Latitude, longitude	Reference(s)
Newfoundland			
Avalon Peninsula — Cripple Cove	1. AVA-CC	See reference	Ferguson et al. 1991
Avalon Peninsula — Drook Cove	2. AVA-DR	See reference	Ferguson et al. 1991
Avalon Peninsula — Freshwater	3. AVA-FW	See reference	Ferguson et al. 1991
Avalon Peninsula — Watern Cove	4. AVA-WC	See reference	Ferguson et al. 1991
Humber River	5. HUM	48°58′, 57°54′	
West Salmon River	6. WSR	48°12′, 56°06′	
Western Arm Brook	7. WAB	51°11′, 56°46′	
Nova Scotia			
Mersey River (3)	8. MER	See references	Jones 1995; Jones et al. 1996
Allain River	9. ALL	44°45′, 65°30′	Jones 1995; Jones et al. 1996
Cape Breton			
Warren Brook	10. CBW	46°43′, 60°22′	Jones 1995; Jones et al. 1996
Fortress Brook region (3)	11. CBF	See references	Jones 1995; Jones et al. 1996
Margaree River	12. MAR	46°25′, 61°10′	
Prince Edward Island			
West Branch Morrell River	13. MOR	46°18′, 62°43′	Jones 1995; Jones et al. 1996
New Brunswick			
Tabusintac River (2)	14. TAB	See references	Jones 1995; Jones et al. 1996
Miramichi River (6)	15. MIR	See references	Jones 1995; Jones et al. 1996
Major Kollock Brook	16. MKB	46°48′, 64°56′	Jones 1995; Jones et al. 1996
Fundy National Park (13)	17. FNP	See references	Jones 1995; Jones et al. 1996
Gaspé Bay Peninsula			
Lac Dube	18. DUB	48°13′, 66°51′	
Lac Grand Étang	19. LGE	49°08′, 64°44′	
St. Lawrence River drainage			
Lac Vertnez	20. VER	46°35′, 73°16′	
Lac Banville	21. BAN	47°37′, 71°16′	
Lac Rimouski	22. RIM	48°01′, 68°12′	
Ungava Bay drainage			
Koksoak River	23. KOK	58°07′, 68°29′	
Barry Lake	24. BRY	54°47′, 66°43′	
Hudson Bay drainage			
Great Whale River	25. GWR	55°16′, 77°45′	
Sutton River	26. STN	55°15′, 83°45′	
Brant River	27. BRA	55°12′, 82°54′	
Hawley Lake (Sutton)	28. HAW	54°30′, 84°39′	
Bennett Creek (Mettagami–Moose)	29. BEN	49°57′, 82°16′	
Mowbray Creek (Opasatika–Missinaibi–Moose)	30. MOW	50°02′, 82°13′	
Shekak River—A (Albany)	31. SHK-A	49°17′, 84°29′	
Otasawian River (Albany)	32. OTA	49°47′, 84°51′	
Shekak River—B (Albany)	33. SHK-B	49°46′, 84°25′	
Lake Superior/Michigan drainage			
Forge Creek	34. FRG	49°49′, 87°47′	
Cleaver Lake	35. CLE	48°58′, 87°23′	
Dead Otter Lake	36. DOL	47°39′, 84°48′	
Frost Lake	37. FRS	47°52′, 84°31′	
LePage Creek	38. LeP	45°51′, 88°10′	
Northern Mississippi drainage			
Lawrence Creek	39. LAW	43°54′, 89°34′	
Soper Creek	40. SOP	44°05′, 90°51′	
Lake Huron drainage			
Little White River (Mississagi)	41. LWR	46°41′, 84°54′	
Cedar Creek (Mississagi)	42. CED	46°49′, 82°53′	
Lower Beatty Saugeen (Saugeen)	43. LBS	44°04′, 80°48′	

Table 1 (*continued*).

Population(s)[a]	Number and abbreviation[b]	Latitude, longitude	Reference(s)
Upper Beatty Saugeen — A (Saugeen)	44. UBS-A	44°05′, 80°45′	
Upper Beatty Saugeen — B (Saugeen)	45. UBS-B	44°05′, 80°43′	
Snake Creek (Saugeen)	46. SNK	44°12′, 81°15′	
Traverston Creek (Saugeen)	47. TRA	44°15′, 80°44′	
Barhead Creek (Saugeen)	48. BAR	44°17′, 80°38′	
Rocky Saugeen (Saugeen)	49. R.SAU		
Upper Beaver River (Beaver)	50. UBR	44°19′, 80°26′	
Boyne Creek (Beaver)	51. BOY	44°17′, 80°32′	
Pine River (Nottawasaga)	52. PIN	44°10′, 80°12′	
Sheldon Creek (Nottawasaga)	53. SHL	44°06′, 80°07′	
Noisy River (Nottawasaga)	54. NOI	44°17′, 80°13′	
Upper Nottawasaga River	55. UNR	44°00′, 80°06′	
Erber Creek (Ninemile)	56. ERB	43°56′, 81°32′	
Lucknow Creek (Ninemile)	57. LUC	43°57′, 81°32′	
Bluevale Creek (Maitland)	58. BLV	43°51′, 81°15′	
Blyth Brook (Maitland)	59. BLH	43°44′, 81°21′	
Salem Creek (Maitland)	60. SLM	43°55′, 81°08′	
Wroxeter Creek (Maitland)	61. WRO	43°51′, 81°08′	
Oxtongue River (5)	62. OXT	See reference	Danzmann et al. 1995
Ottawa River drainage			
Crooked Creek	63. CRO	47°57′, 80°05′	
Amable du Fond River (6)	64. AdF	See reference	Danzmann et al. 1995
Petawawa River (28)	65. PET	See reference	Danzmann et al. 1995
Bonnechere River (4)	66. BON	See reference	Danzmann et al. 1995
Madawaska (11)	67. MAD	See reference	Danzmann et al. 1995
York (4)	68. YOR	See reference	Danzmann et al. 1995
Lake Ontario drainage			
Marlbank Creek	69. MRL	44°22′, 77°06′	
Upper Credit River — site A	70. UCR-A	43°50′, 80°02′	
Upper Credit River — site B	71. UCR-B	43°44′, 79°57′	
Black Creek (Credit)	72. BLK	43°39′, 79°57′	
Shelter Valley Brook	73. STR	44°02′, 77°59′	
Lake Erie drainage			
Big Creek (2)	74. BIG	See reference	Danzmann et al. 1991*b*
Kent Creek (Lynn)	75. KNT	42°51′, 80°18′	
Patterson Creek (Lynn)	76. PAT	42°50′, 80°20′	
Spring Brook (Chagrin)	77. SPR	41°31′, 81°20′	
Woodie Brook (Chagrin)	78. WDE	41°30′, 81°18′	
Maine			
Beaver Tail Pond	79. BTP	46°54′, 68°57′	
Trafton Pond	80. TRF	46°33′, 68°38′	
Edray hatchery stock	81. EDR	Source population unknown	
Maryland (Chesapeake Bay drainage)			
Winch Run (Principio)	82. WIN	39°37′, 76°04′	
Panther Branch (Gunpowder)	83. PAN	39°36′, 76°39′	
Kellogg Branch (Susquehanna)	84. KEL	39°39′, 76°26′	
Timber Run (Patapsco)	85. TIM	39°27′, 76°51′	
Tuscarora Creek (Potomac)	86. TUR	39°29′, 77°29′	
Fishing Creek (Potomac)	87. FIS	39°32′, 77°29′	
Maryland (Ohio River drainage)			
Bear Creek (Youghiogheny)	88. BEA	39°39′, 79°16′	
Mill Creek (Youghiogheny)	89. MIL	39°43′, 79°18′	

Table 1 (*concluded*).

Population(s)[a]	Number and abbreviation[b]	Latitude, longitude	Reference(s)
West Virginia (Ohio River drainage)			
Shavers Run (Monongahela)	90. SHA	38°43′, 79°59′	
Crooked Fork (Elk–Kanawha)	91. CRF	38°20′, 80°08′	
Sutton Run (Greenbriar–Kanawha)	92. SUT	38°24′, 79°45′	
Walderman Run (Greenbriar–Kanawha)	93. WAL	38°35′, 79°40′	
West Virginia (Chesapeake Bay drainage)			
Tetercamp Run (Potomac)	94. TET	38°37′, 79°36′	
Horsecamp Run (Potomac)	95. HOR	38°52′, 79°28′	
Virginia (Chesapeake Bay drainage)			
Spy Run (South–Maury–James)	96. SPY	37°55′, 79°18′	
Dry Run (isolated)	97. DRY	38°35′, 79°14′	
Tennessee (Ohio River drainage)			
Indian Camp Creek (Tennessee)	98. IND	35°40′, 83°17′	

[a]Populations are given with the major river drainage in which the population occurs listed in parentheses following the population name. Drainage abbreviations used in Fig. 3 and Table 3 are as follows: AVA, Avalon Peninsula; GBP, Gaspé Bay Peninsula; SLR, St. Lawrence River; UNG, Ungava Bay; HBD, Hudson Bay; NMD, northern Mississippi River; MISS, Mississagi River; SAUG, Saugeen River; BEAV, Beaver River; NOTT, Nottawasaga River; NINM, Ninemile River; MAIT, Maitland River; CRE, Credit River; LYNN, Lynn Creek; CHAG, Chagrin River; MAI, Maine (excluding the hatchery stock); POT, Potomac River; YOUG, Youghiogheny River; KANA, Kanawha River. If a drainage is represented by a single population, then the drainage is represented by the abbreviation listed in the table. Certain drainages have a number in parentheses following the drainage designation. This number represents the number of populations sampled within the drainage or region. The reader is referred to the reference(s) listed for further details on these populations.

[b]The number listed beside the population is used to represent the population in Fig. 1.

tions from a drainage (indicated by the number in parentheses following the drainage designation), the reader is referred to the above-mentioned original references for details on the mtDNA haplotypic composition in these populations. In the Ferguson et al. (1991) study, fish from the Avalon Peninsula, Newfoundland, were not analyzed for variation at the enzyme *Dra*II. New RFLP patterns were subsequently detected in these fish using *Dra*II, which redesignates some mtDNA haplotypes present in these populations. Also, in the study by Jones et al. (1996), RFLP variation was detected in east coast populations using the enzymes *Alu*I, *Asp*700, *Ava*I, *Bgl*II, and *Cla*I. As these enzymes were not routinely examined in the brook trout populations we sampled, the designation of haplotypes 35, 36, 42, 45, 46, and 48 (Jones 1995; Jones et al. 1996) was omitted in the present study. All these haplotypes differ by one or two mutational steps from haplotype 1 and were therefore designated as haplotype 1 for the data analysis in this study. Details on the methods of mtDNA preparation have previously been described (Danzmann and Ihssen 1995).

Statistical analyses

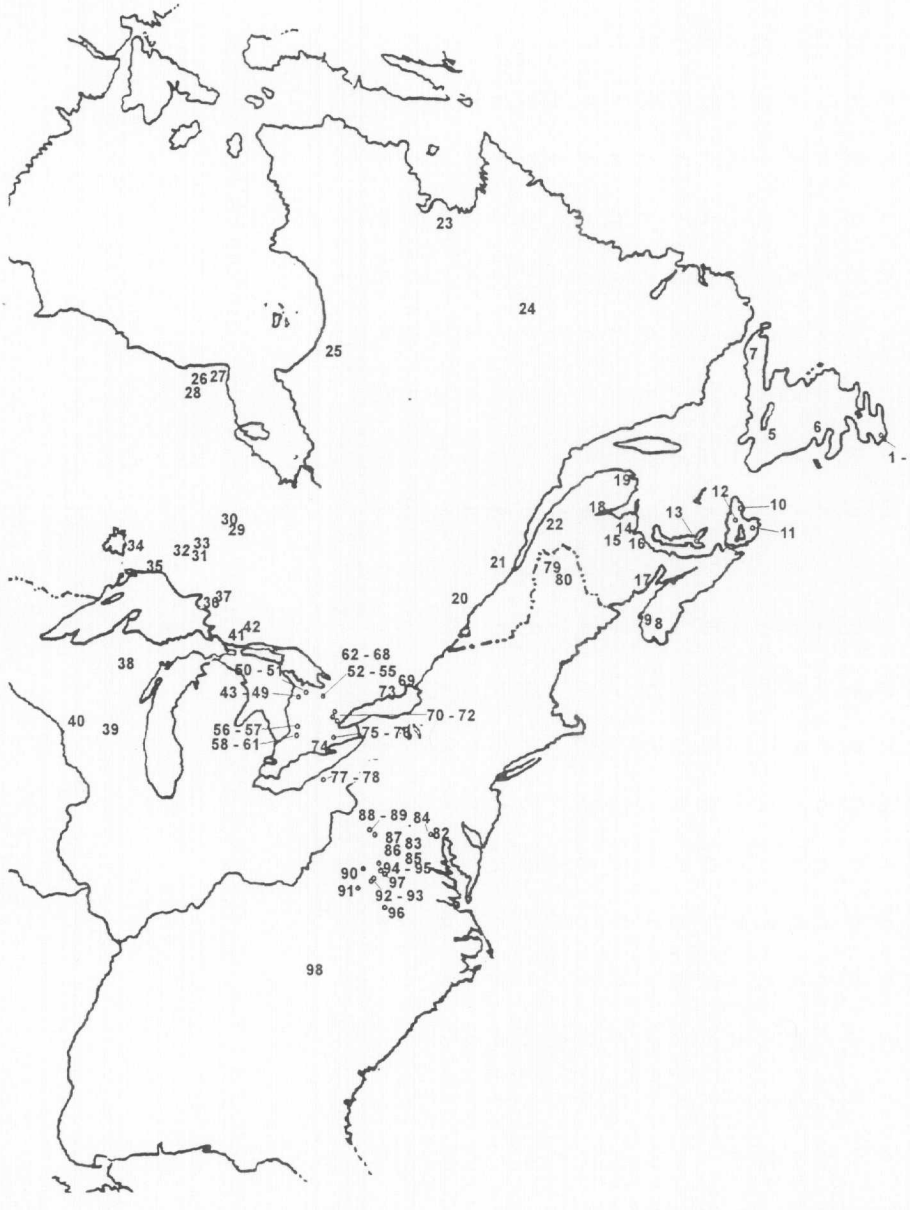
Analysis of molecular variation (AMOVA) among populations within drainages followed Excoffier et al. (1992) using ARLEQUIN (version 1.0). For this analysis, genetic distances among haplotypes were calculated using the presence/absence matrix of restriction sites detected among haplotypes. Of the 175 populations sampled, only 155 were used in the analysis, partitioned into 60 units (drainages, small stream catchments, or single lakes). Some populations were omitted because of sample size (i.e., $N < 6$), and three populations from the Fundy National Park region were omitted because they were obtained as single samples from a drainage and nearby drainages were more extensively sampled in this small geographic region.

Evolutionary relationships among haplotypes were assessed using a neighbour-joining (NJ) tree (Saitou and Nei 1987) and parsimony analysis. Distances (D) for the NJ tree were generated according to the distance algorithm given in Danzmann et al. (1991*a*) using the program MTDIS (Danzmann 1998). The algorithm assumes homology between the pair of sequences being compared and estimates percent sequence divergence by counting the site gains that differ between both sequences assuming that they are generated by 1 base pair changes. Distances generated by MTDIS were used to construct an NJ tree using MEGA (Kumar et al. 1993). The program RESTML in PHYLIP 3.5 (Felsenstein 1989) was used to test for non-negativity and significance of the internal branch lengths within the NJ tree. Bootstrap confidence estimates on each of the major clade branch points were obtained using SEQBOOT, NEIGHBOR, and CONSENSE in PHYLIP 3.5 using the distances calculated with MTDIS for 100 randomized restriction site matrices.

The data set was also subjected to a Dollo parsimony analysis using the program DOLLOP in PHYLIP 3.5. Ancestral RFLP sites were used to root the tree if it was possible to ascertain these sites. RFLP sites that were present in either Arctic char (Danzmann et al. 1991*a*; Wilson 1995; Wilson et al. 1996) or lake trout (*Salvelinus namaycush*) (Wilson 1995; Wilson and Hebert 1996) were considered to be ancestral (Appendix I).[3] The composite Arctic char and lake trout restriction sites gave polarity to the intraspecific phylogeny of haplotypes with brook trout. Dollo parsimony was considered the most appropriate method of analysis, as it conserves site gains and considers site losses to be evolutionarily more likely. For this analysis, the input order of the taxa was mixed until the first 500 trees requiring the minimum number of steps to generate the tree were obtained. One hundred trees were obtained from each input randomization of the data set. The majority rule consensus

[3]Details of the new RFLP variation in brook trout and other information referred to as appendices can be obtained by contacting the senior author or by anonymous ftp to site 131.104.50.2 using the password "danzmann." Then use the command GET to retrieve the file bcna.zip and pkunzip the file.

Fig. 1. Map of collection sites for 175 populations of brook trout (indicated as either separate populations or populations within drainages) in North America. Numbers correspond to the collection sites detailed in Table 1. The source population for the Edray hatchery stock (population 81) is unknown.



tree from these 500 trees was constructed using CONSENSE in PHYLIP 3.5. In addition to the Dollo tree, a Wagner minimum spanning network was generated that lists all the site gains and losses for enzymes producing polymorphic restriction sites (Appendix II, see footnote 3). It was possible to generate several equally parsimonious trees because of sites that could be readily exchanged owing to homoplasy. These sites are indicated with a star on the Wagner tree. The site gains and losses depicted were routed from haplotype 22 (i.e., the haplotype with the closest affinity to Arctic char and lake trout).

Results

Haplotype diversity and phylogenetic groupings

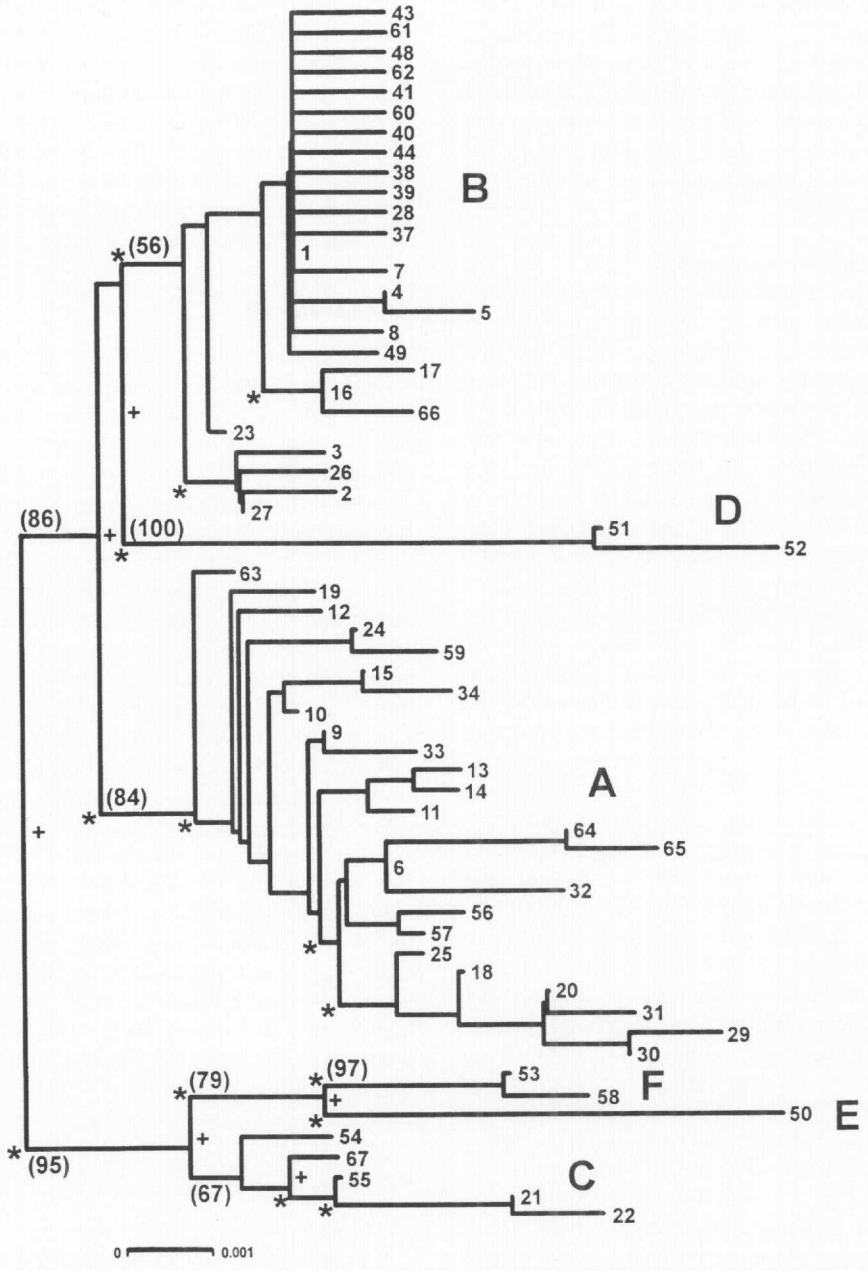
Sixty-one haplotypes were detected among the brook trout populations using 34 hexameric or multihexameric restriction enzymes that produced RFLPs. Forty-three haplotypes have previously been described (Danzmann et al. 1991*b*; Bernatchez and Danzmann 1993; Danzmann and Ihssen 1995; Jones et al. 1996), while 18 are new (Table 2; see Appendix III, see footnote 3). No RFLP variation but 43 restriction sites (in parentheses) were detected with 13 restriction enzymes as follows: *Bcl*I (four), *Bst*EII (four), *Csp*45I (two), *Eco*RI (two), *Hae*II (four), *Nae*I (three), *Pma*CI (one), *Sau*I (three), *Sno*I (one), *Spe*I (four), *Stu*I (nine), *Tth*111I (two), and *Xba*I (four). These enzymes were not screened on all fish but were examined in at least two fish from all new populations. Thus, even though some polymorphisms may have gone undetected with this suite of enzymes, we retained these restriction sites in the analysis to obtain more accurate sequence divergence estimates. The enzymes *Asp*700, *Ava*I, *Bgl*II, and *Cla*I have detected RFLPs

Table 2. Mitochondrial DNA RFLPs identified in brook trout and their haplotypic designations.

Enzyme	Haplotype 21	22	37	38	39	40	41	43	44	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67
67																													
*Acc*I	A	D	A	A	E	A	A	A	A	A	A	F	A	A	B	G	A	A	A	B	A	A	A	I	A	G	H	A	A
*Asn*I	C	C	A	A	A	E	F	A	A	A	A	A	A	B	A	—	—	A	A	B	A	A	A	A	A	A	A	A	C
*Bam*HI	B	B	A	A	A	A	A	A	A	A	A	B	C	C	B	B	B	A	A	B	A	A	A	A	A	A	A	A	B
*Ban*I	A	A	A	A	A	A	A	A	A	A	A	A	F	F	A	A	A	G	H	A	D	A	A	A	A	D	D	A	A
*Ban*II	B	B	A	A	A	A	A	A	A	A	A	C	B	D	B	B	B	B	B	B	E	A	A	A	B	B	B	E	B
*Bst*XI	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C	A	A
*Dra*I	B	B	A	A	A	A	A	A	A	A	A	B	A	A	B	B	B	A	A	B	A	A	A	A	A	A	A	A	B
*Dra*II	A	A	B	C	A	A	A	D	E	A	A	F	A	A	A	A	A	A	A	A	G	H	A	A	A	A	A	A	A
*Eco*RV	A	A	A	A	A	A	A	A	A	A	A	B	A	A	—	—	—	—	—	—	—	A	A	—	A	A	A	—	—
*Hinc*II	D	D	A	A	A	A	A	A	A	B	C	A	C	C	A	A	D	A	A	A	A	A	A	A	A	A	A	A	A
*Hind*III	A	A	A	A	A	A	A	A	A	A	A	A	A	A	D	—	—	A	A	D	A	A	A	A	A	A	A	A	A
*Nco*I	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	A	B	A	A	A	A	B	B	A	A
*Nde*I	B	B	A	A	A	A	A	A	A	A	A	B	B	B	—	—	—	B	B	—	B	A	A	A	B	B	B	A	B
*Nhe*I	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	A	B	A	A	A	B	B	B	A	A
*Nsi*I	A	A	A	A	A	A	A	A	A	A	A	A	A	A	—	—	—	A	A	—	A	A	A	A	A	A	A	—	A
*Pst*I	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	A	B	A	A	A	B	B	B	B	B
*Pvu*II	B	B	A	A	A	A	A	A	A	A	A	B	A	A	B	B	B	A	A	B	A	A	A	A	A	A	A	A	B
*Sca*I	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	—	A	A	A	—	A
*Sna*BI	A	A	A	A	A	A	A	A	A	A	A	B	A	A	B	A	—	A	A	B	A	A	A	—	A	A	A	—	—
*Sph*I	A	A	A	A	A	A	A	A	A	A	A	A	A	A	—	—	—	A	A	—	A	A	A	—	A	A	A	—	—
*Ssp*I	A	A	—	—	—	—	—	—	—	—	—	A	B	B	A	A	—	A	A	A	A	A	A	—	A	A	A	—	—

Note: Haplotypes 21 and 22 are given as the reference C assemblage haplotypes for routing site gains and losses in Appendix II, haplotypes 37–41, 43, 44, 48, and 49 are the common haplotypes surveyed in the present study and Jones et al. (1996), and haplotypes 50–67 are newly described in this study.

Fig. 2. Neighbor-joining tree of interhaplotypic mtDNA distances (*D*) calculated with MTDIS. Distances are additive according to the scale shown. Branch lengths that are supported as being significantly different from zero with RESTML are shown with an asterisk at their base. The phylogenetic groupings that are depicted in descendant taxa at nodes showing a plus sign are supported in 100% of the Dollo parsimony trees produced. Bootstrapped confidence estimates for the branch points of the major clades are indicated in parentheses.



in some east coast fish (Jones et al. 1996) but were omitted from this study because they were not analyzed in all the fish. Twenty-one of the enzymes used generated 52 polymorphic restriction sites (Appendix I) with 90 invariant sites. Thus, a maximum of 185 restriction sites (accounting for semi-isoschizomer sites) were examined representing approximately 6.7% of mitochondrial genome.

The NJ tree (Fig. 2) shows the placement of haplotypes into six clades designated A–E and this is supported by the intrabranch standard error tests from RESTML and the bootstrap confidence levels. Clades A–C were previously identified in a combined DNA sequence and RFLP analysis of northern brook trout populations (Bernatchez and Danzmann 1993). Support for the inclusion of all haplotypes depicted within either the B or C assemblages is weak, as indicated by the bootstrap confidence levels. The equivocal placement of B assemblage haplotypes was previously reported by Bernatchez and Danzmann (1993), and thus, it may be warranted to recognize subassemblage groupings within each clade corresponding to haplotypes 2, 3, 26, and 27 (= B2 clade) and haplotypes 54, 55, and 67 (= C2 clade).

The tree generated by RESTML in PHYLIP and the NJ tree generated from the *D* values given by MTDIS had identical topologies except for the interchange of some terminal

haplotypes in the A assemblage. The branching orders of several assemblage A taxa were not supported by RESTML and their relative affinities remain unresolved with the exception of haplotypes 18, 20, 25, 29, 30, and 31, which constitute a distinct grouping within clade A.

The rooted Dollo parsimony tree requiring 67 steps (not shown) indicated that the C clade haplotypes (haplotypes 21 and 22) are the most ancestral. There is support for the retention of the haplotypes indicated as belonging to clades D, E, and F and for the separation of A and B clade haplotypes as shown by the plus sign at branching nodes in Fig. 2. The branching orders depicted at these nodes were supported in 100% of the trees produced.

Geographical distribution of haplotypes

Most of the North American brook trout sampled belong to the A, B, and, to a lesser extent, C assemblages (Table 3). A assemblage and B assemblage fish appear to be the predominant types that have recolonized northerly latitudes (groups 1 and 3; Fig. 3). Fish from the northern part of the range extending from Newfoundland, the southern Maritimes, Maine, and westward to Hudson Bay are very closely related and predominantly possess B assemblage haplotypes. In fact, the vast majority of these fish have haplotype 1 (group 1; Fig. 3). The majority of the populations sampled from the Great Lakes region possess both A and B assemblage haplotypes (group 3, Fig. 3). Some of the populations in the Chesapeake Bay drainage (Potomac River, Dry Run, Kellogg Branch) also belong to this group. C assemblage fish appear to be in highest frequency in the Lake Erie and lower Lake Huron watersheds (group 4; Fig. 3).

Distinct genetic groupings are present in the Ohio River drainage (groups 2, 5, 6, and 7, Fig. 3), having representative fish from the D, E, and F phylogenetic assemblages. D assemblage haplotypes were found only in the two Youghiogheny River (tributary of the Ohio River) populations sampled (group 2; Fig. 3). A single haplotype (haplotype 50 = assemblage E) was found in the Indian Camp Run (tributary of the Tennessee River) population, which thus forms a distinct grouping. Two additional groupings from the Ohio drainage are composed of fish from Shavers Run, which possess C2 assemblage haplotypes, and the three Kanawha River populations, which possess primarily F assemblage and some A assemblage haplotypes. Interestingly, none of the populations sampled from the Ohio River drainage possessed any B assemblage haplotypes.

Populations were also grouped into 16 major geographic regions that represent major present-day drainage basins derived from postglacial drainage zones (see Table 4). Thus, these regions represent the remnants of glacial lakes, glacial drainages, and geographically isolated regions not impacted by major spillways that are likely to have moulded their present-day faunas by acting as corridors of dispersal and recolonization. Assignment of the phylogenetic clades to these regions was made to assess the relative diversity of the major brook trout phylogenetic lineages in each of these habitat regions (Fig. 4).

Partitioning of genetic variability

The AMOVA analysis comparing population variation within drainages indicates that a large proportion of the genetic variation occurs among populations (mean ϕ_{ST} = 0.593) rather than within (Table 5). Heterogeneity among drainages is also quite high (mean ϕ_{CT} = 0.388), but this value may be influenced by the relative partitioning of populations within drainages. Most groupings consisted of between 2 and 3 populations with some drainage groupings exceeding 10 populations. When the analysis was recalculated taking random subsamples of populations from larger drainage groupings so that population numbers were not greater than four, average ϕ_{CT} estimates (0.426) and average ϕ_{ST} estimates (0.633) were increased (data not shown), indicating that estimates of interpopulation and interdrainage variation based upon unequal samplings of populations from the various drainages may be conservative.

Discussion

The patterning of mtDNA diversity present in North American brook trout indicates that fish recolonizing formerly glaciated regions outside southern Ontario were primarily derived from the B assemblage (Fig. 4). Indeed, a single haplotype (haplotype 1) predominates throughout much of these northern regions. Fish with haplotype 1 or haplotypes one or two mutational steps removed from haplotype 1 compose 98% of the fish sampled in the Canadian Maritime Provinces including the lower St. Lawrence River and 100% of the fish sampled from the Ungava and Hudson Bay drainages and northern Maine. Fish in the southern Ontario region are derived from a mixture of A, B, and C assemblage lineages, while fish from the glacial Lake Iroquois (Lake Ontario and Ottawa River region) were derived from both A and B assemblage sources.

An examination of the mtDNA phylogenetic assemblage diversity in fish derived from the putative Appalachian uplands refugial zone (Radforth 1944) (groups 13 and 15; Fig. 4) indicates that these fish were unlikely to have colonized the entire northern glacial zone. Only haplotypes from the A and B assemblages were sampled in these fish. Since fish from certain populations in the southern Great Lakes region possessed a relatively high frequency of C assemblage haplotypes, it is most likely that these fish were derived from ancestors that had a Mississippian refugium, thus supporting Bailey and Smith's (1981) hypothesis for multiple refugial origins for this species.

Refugial origins

Mississippian

C assemblage fish in recolonized regions appear to have been derived solely from a Mississippian refugium, since C assemblage fish were not detected in the Atlantic refugial populations or the Lake Ontario drainage basin. The restricted dispersal of haplotypes 18 and 20 (A assemblage) to the southern Great Lakes region is also a strong phylogeographic signal that these fish were likely derived from a Mississippian refugium. Haplotypes 18 and 20 were not observed east of this region.

One possible origin for northern C assemblage fish is the Ohio River basin. Fish from the Ohio River may have recolonized northern habitats following stream capture events (e.g., Monongahela/Allegheny River drainage into glacial LakeMaumee) (Hocutt et al. 1986). However, all the

Table 3. Distribution of mtDNA haplotypes among North American brook trout populations.

Population	Assemblage A	B	C	D	E	F	References
Newfoundland							
1. AVA—CC		**1** (15)					Ferguson et al. 1991
2. AVA—DR		**1** (9), **61** (6)					Ferguson et al. 1991
3. AVA—FW		**1** (12), **60** (3)					Ferguson et al. 1991
4. AVA—WC	**6** (7)	**1** (8)					Ferguson et al. 1991
5. HUM		**1** (8), **43** (2)					
6. WSR	**6** (5)						
7. WAB		**1** (10)					
Nova Scotia							
8. MER [3]		**1** (33)					Jones 1995; Jones et al. 1996
9. ALL		**1** (7), **41** (1)					
10. CBW		**1** (15)					
11. CBF [3]		**1** (26), **44** (3)					Jones 1995; Jones et al. 1996
12. MAR		**1** (10)					
Prince Edward Island							
13. MOR		**1** (19)					
New Brunswick							
14. TAB [2]		**1** (49), **40** (3), **43** (3)					Jones 1995; Jones et al. 1996
15. MIR [6]		**1** (87), **37** (4)					Jones 1995; Jones et al. 1996
16. MKB		**1** (14), **39** (1)					
17. FNP [13]	**6** (1)	**1** (130), **16** (1), **38** (3), **49** (5)					Jones 1995; Jones et al. 1996
Gaspé Bay Peninsula							
18. DUB		**1** (15)					
19. LGE		**1** (20)					
St. Lawrence River							
20. VER		**1** (24)					
21. BAN		**1** (20)					
22. RIM		**1** (14)					
Ungava Bay drainage							
23. KOK		**1** (12)					
24. BRY		**1** (12)					
Hudson Bay drainage							
25. GWR		**1** (12)					
26. STN		**1** (30)					
27. BRA		**1** (7), **48** (2)					
28. HAW		**1** (1)					
29. BEN		**1** (3), **62** (1)					
30. MOW		**28** (1)					
31. SHK—A		**1** (10)					
32. OTA		**1** (2)					
33. SHK—B		**1** (7)					

Table 3 (*continued*).

Population	Assemblage A	B	C	D	E	F	References
Lake Superior/Michigan drainage							
34. FRG		**1** (13)					
35. CLE	**6** (8), **12** (3)						
36. DOL		**1** (7)	**67** (1)				
37. FRS		**1** (9)					
38. LeP		**1** (6), **2** (4)					
Northern Mississippi drainage							
39. LAW		**1** (5), **2** (5)					
40. SOP		**1** (2), **2** (7), **7** (1)					
Lake Huron drainage							
Mississagi							
41. LWR		**1** (7), **2** (1), **7** (1)	**67** (1)				
42. CED		**1** (2), **2** (2), **28** (7)					
Saugeen River							
43. LBS	**6** (1), **18** (1), **20** (4), **24** (1)	**1** (10), **7** (2), **27** (1)					
44. UBS—A	**6** (1), **20** (2), **32** (1)	**1** (5), **2** (1), **23** (1), **27** (3)	**21** (8), **22** (3)				
45. UBS—B	**6** (1)	**1** (10)					
46. SNK	**6** (15)	**1** (2), **7** (10), **26** (1)					
47. TRA	**6** (1), **20** (5)	**1** (1), **2** (5), **3** (1), **27** (11)					
48. BAR	**6** (2), **20** (2)	**1** (1), **2** (3), **3** (1), **7** (1)					
49. R.SAU	**20** (4)	**1** (1), **3** (2), **27** (2)					
Beaver River							
50. UBR	**6** (1)	**1** (3), **2** (3), **7** (1)	**21** (4)				
51. BOY	**6** (2), **18** (1), **20** (9), **25** (1)	**1** (2), **2** (8), **27** (1)					
Nottawasaga River							
52. PIN	**6** (2), **20** (7), **30** (1)	**1** (6), **2** (3), **27** (1)					
53. SHL	**20** (6), **29** (1), **30** (4), **31** (1)	**1** (2), **2** (1)					
54. NOI	**20** (9)	**1** (5), **2** (2)					
55. UNR	**20** (3)	**1** (6), **2** (2)					
Ninemile River							
56. ERB	**6** (3)	**1** (2)	**21** (5), **22** (1)				
57. LUC	**6** (1)	**1** (1), **27** (2)	**22** (8)				
Maitland River							
58. BLV		**1** (3)	**21** (13)				
59. BLH	**6** (2)	**1** (1), **7** (9), **27** (4)	**21** (1)				
60. SLM		**1** (2), **3** (3)	**21** (4)				
61. WRO		**1** (1), **27** (2)	**21** (11)				
62. OXT [5]	**6** (36), **9** (2), **10** (1), **34** (2)	**1** (15), **2** (10), **7** (10), **16** (3)					Danzmann et al. 1995
Ottawa River drainage							
63. CRO		**1** (7)					

Table 3 (*continued*).

Population	Assemblage A	B	C	D	E	F	References
64. AdF [6]		**1** (77)					Danzmann et al. 1995
65. PET [28]	**6** (103), **9** (2), **10** (1), **12** (2) **15** (5), **19** (1)	**1** (350), **2** (4), **4** (2), **7** (14) **16** (1), **17** (1)					Danzmann et al. 1995
66. BON [4]	**6** (2), **12** (1)	**1** (59), **7** (1)					Danzmann et al. 1995
67. MAD [11]	**6** (14), **9** (7), **10** (2), **11** (1) **13** (1), **14** (7), **33** (1)	**1** (77), **2** (27), **3** (1), **7** (1) **8** (1), **23** (1)					Danzmann et al. 1995
68. YOR [4]	**6** (36)	**1** (11), **2** (22)					Danzmann et al. 1995
Lake Ontario drainage							
69. MRL		**1** (13), **2** (7)					
Credit River							
70. UCR—A		**27** (2)					
71. UCR—B		**27** (4)					
72. BLK	**20** (9)	**1** (1), **3** (4), **27** (23)					
73. STR	**6** (1)	**1** (3), **27** (1), **66** (3)					
Lake Erie drainage							
74. BIG [2]	**18** (17)	**1** (3)					Danzmann et al. 1991*b*
75. KNT	**18** (26)	**1** (1), **2** (1)					
76. PAT	**18** (17)	**1** (2), **7** (21)					
77. SPR	**18** (2)		**21** (8)				
78. WDE	**18** (1)		**21** (3)				
Maine							
79. BTP		**1** (8)					
80. TRF		**1** (9), **7** (1)					
81. EDR		**1** (8)					
Maryland (Chesapeake Bay drainage)							
82. WIN		**7** (10)					
83. PAN	**6** (2)	**1** (5), **2** (1), **4** (1), **7** (1)					
84. KEL	**6** (8)						
85. TIM		**1** (9), **27** (1)					
86. TUR	**6** (8), **15** (1), **63** (1)						
87. FIS	**6** (10)						
Maryland (Ohio River drainage)							
88. BEA				**51** (10)			
89. MIL				**51** (5), **52** (7)			
West Virginia (Ohio River drainage)							
90. SHA			**54** (6), **55** (2)				
91. CRF						**53** (8)	
92. SUT						**58** (8)	
93. WAL	**59** (3)					**58** (5)	

Table 3 (*concluded*).

Population	Assemblage A	B	C	D	E	F	References
West Virginia (Potomac/Chesapeake Bay drainage)							
94. TET	**6** (1), **56** (4), **57** (3)	**1** (1)					
95. HOR	**6** (6), **56** (1)						
Virginia (James/Chesapeake Bay drainage)							
96. SPY (isolated underground/Atlantic drainage)	**6** (1)	**27** (9)					
97. DRY	**64** (9), **65** (1)						
Tennessee (Ohio drainage)							
98. IND					**50** (24)		

Note: Haplotypes are in boldface type, with sample sizes in parentheses. Numbers in square brackets denote the number of populations sampled within certain drainages.

C haplotype fish sampled from the Ohio River drainage were unique to this region and not present further north. In addition, most of the fish in this region had D and F assemblage haplotypes. These two facts suggest that fish from the Monongahela, Youghiogheny, and Kanawha/New drainages of the Ohio River in West Virginia and Maryland may have contributed only minimally, if at all, to northern recolonization movements of brook trout. Northern connections to the Laurentian (= St. Lawrence) drainage via the lower, middle, and upper Allegheny drainages that had existed since the Pleiocene were not considered extant past the Illinoian ice age (Hocutt et al. 1986), making this route less tenable for northern post-Wisconsinan dispersal.

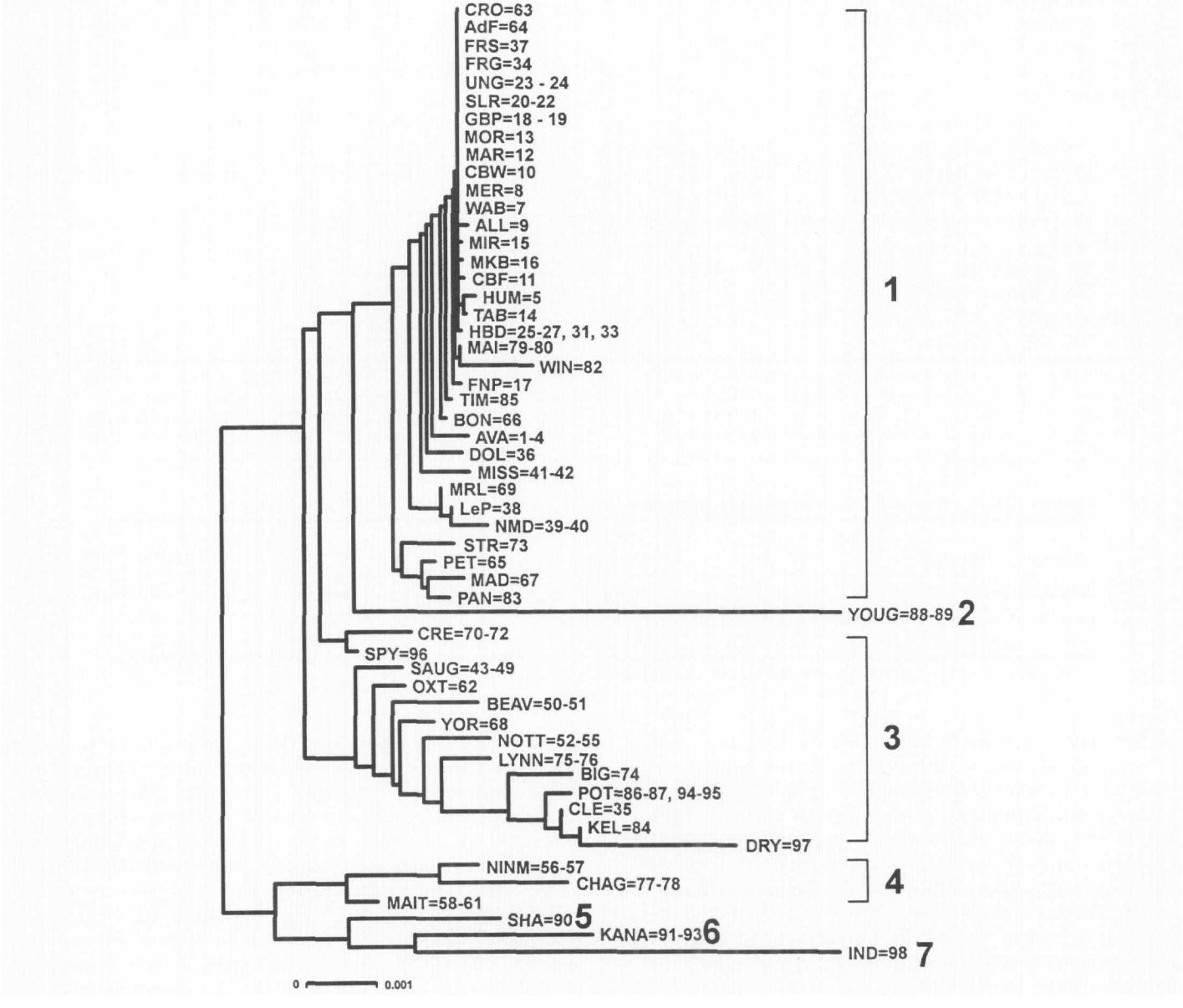
Regions south of the Wisconsinan glacial maximum are a possible refugial source for fish recolonizing the lower Great Lakes. These fish would have access to this region during the time period when glacial Lake Maumee (= Lake Erie) and Lake Chicago (= southern Lake Michigan) were connected to the Mississippi River drainage through the Maumee/Wabash and Illinois River outlets, respectively (approximately 14 000 – 13 000 years BP) (Underhill 1986). Up to approximately 11 000 years BP, access to the Lake Erie basin was still possible for fish from the Mississippi basin, as outflows from Lake Erie were both eastward (through Lake Iroquois (= Lake Ontario) to the Champlain Sea or south through the Hudson River) and westward (through Lake Chicago to the Mississippi) (Fig. 5). Since brook trout south of the Great Lakes in the region of glacial intrusion (i.e., approximate confluence of the Ohio and Missouri rivers) are no longer present in the United States, the exact refugial origin of these fish may only be surmised.

It should be noted that the Shavers Run fish (Monongahela River drainage) were the only C clade fish sampled from the Ohio River drainage. This suggests a more recent connection between Lake Erie fish and fish in the upper portion of the Ohio River drainage. If fish with haplotypes 21 and 22 (C clade) or 18 and 20 (A clade) are present in the upper portion of the Allegheny River drainage, then a post-Illinoian connection of the upper Allegheny to the Great Lakes drainage would be supported. This may have occurred by localized impoundments of headwater sections of the Allegheny River drainage.

Atlantic

Brook trout sampled from the Lake Ontario watershed appear to have originated solely from an Atlantic refugium. These fish have a high frequency of haplotype 6 (A assemblage) and B assemblage haplotypes (e.g., 1, 2, 7, and 27). These haplotypes are also present in the fish sampled from the Atlantic coastal region. This indicates that migration up the Hudson River and Susquehana/Genesee valley from the Atlantic coastal zone into glacial Lake Iroquois was a likely route for recolonization of the Lake Ontario basin. However, further northward expansion of brook trout beyond this region is only partially compatible with the observed distribution of haplotypes in fish from the Canadian Maritime Provinces, Ontario, and Quebec. Haplotype 1 predominates in all these regions (Fig. 4). Therefore, if recolonization of northern regions was solely from an Atlantic refugium, then strong selection against fish possessing haplotypes other than haplotype 1 must be accepted.

Fig. 3. Neighbor-joining tree of the phylogenetic distances (*D*) of brook trout found in the different drainages sampled in North America. Distances are additive according to the scale shown. Sampling site abbreviations are given in Table 1. Populations sampled from each drainage are referenced by the numbers given following the drainage designation and refer to the numbers listed in Table 1. Population groupings possessing similar haplotypic complements are numbered on the right-hand side of the figure.



Allozyme data also indicate that fish from the Hudson and Delaware rivers and the Raquette River drainage (flowing into eastern Lake Ontario) are more similar to one another than they are to fish sampled from northern New York State and Quebec drainages (flowing into the St. Lawrence River) (McGlade 1981; Perkins et al. 1993). Despite being in the St. Lawrence drainage, the Raquette River appears to have been colonized by fish using the Hudson–Mohawk corridor.

Northeastern coastal or "Acadian"

The restricted distribution of certain B assemblage haplotypes (37, 38, 39, 40, 41, 43, 44, 49, 60, and 61) to east coast populations and the lack of haplotype 2 fish in this region support a more restricted dispersal origin for these fish than one that is compatible with an Atlantic or Mississippian origin. Fish from a more northerly coastal Atlantic refugium may have reinvaded habitats northward and westward and thus recolonized most of the Canadian Maritimes. This refugium could have extended along the southeast shore of Newfoundland to the Georges Bank region of the Atlantic coast (Pielou 1991) and was designated as the northeastern coastal refugium (Schmidt 1986) or "Acadian" refuge (Bernatchez and Dodson 1991; Bernatchez 1997).

Dispersal routes and contact zones

Two alternative hypotheses regarding recolonization routes for haplotype 1 fish exist and these alternatives are not necessarily mutually exclusive. As mentioned, haplotype 1 predominates throughout almost all the northern deglaciated range of the species. Since haplotype 1 occurs at high frequency in both western and eastern populations, it is possible that expansion of haplotype 1 fish may have been

Table 4. Partitioning of drainages among geographic regions.

Region	Drainage[a]
1. Avalon Peninsula	1, 2, 3, 4
2. Ungava Bay	23, 24
3. St. Lawrence River and Maritimes	5, 7, 10, 11, 12, 13, 14, 18, 19, 20, 21, 22, 15a, 15b, 15c
4. Eastern Nova Scotia	8
5. Bay of Fundy	9, 17a, 17b, 17c, 17d, 17e, 17f, 17g
6. Hudson Bay	25, 26, 27, 31, 33
7. Lake Superior/Glacial Lake Duluth	34, 35, 36, 37, 38, 39, 40
8. Northern Lake Huron	41, 42
9. Southern Lake Huron	(43–49), (50–51), (52–55), (56–57), (58–61), 62
10. Lake Erie/Glacial Lake Maumee	74, (75–76), (77–78)
11. Ottawa River and Lake Ontario/glacial Lake Iroquois	63, 64, (65–66), (67–68), 69, (70–72), 73
12. Maine	79, 80
13. Chesapeake Bay	82, 83, 84, 85, (86–87), (94–95), 96
14. Ohio River (North)	(88–89), 90, 91, (92–93)
15. Isolated	97
16. Ohio River (South)	98

[a]Drainage numbers refer to the rivers and lakes listed in Table 1. In instances where a number is followed by a letter, the drainages are as follows: 15a, North Branch of the North-West Miramichi; 15b, Catamaran Brook; 15c, Little South-West Miramichi; 17a, Peticodiac River; 17b, Quiddy River; 17c, Goose Creek; 17d, Point Wolfe River; 17e, Upper Salmon River; 17f, Dickson Brook; 17g, Goose River. The numbers in parentheses refer to populations within drainages that have been combined for the analysis. Refer to the reference(s) cited in Table 1 for details on these drainages. Numbers preceding the region designations refer to the numbered regions depicted in Fig. 4.

Table 5. Results from AMOVA partitioning variation by drainages and by populations within drainages.

Variance component	df	Variance	% of total	P^a	ϕ statistic
Among drainages	59	0.69490	38.79	<0.001	$\phi_{CT} = 0.388$
Among populations/drainage	91	0.36735	20.50	<0.001	$\phi_{SC} = 0.335$
Within populations	2115	0.72936	40.71	<0.001	$\phi_{ST} = 0.593$

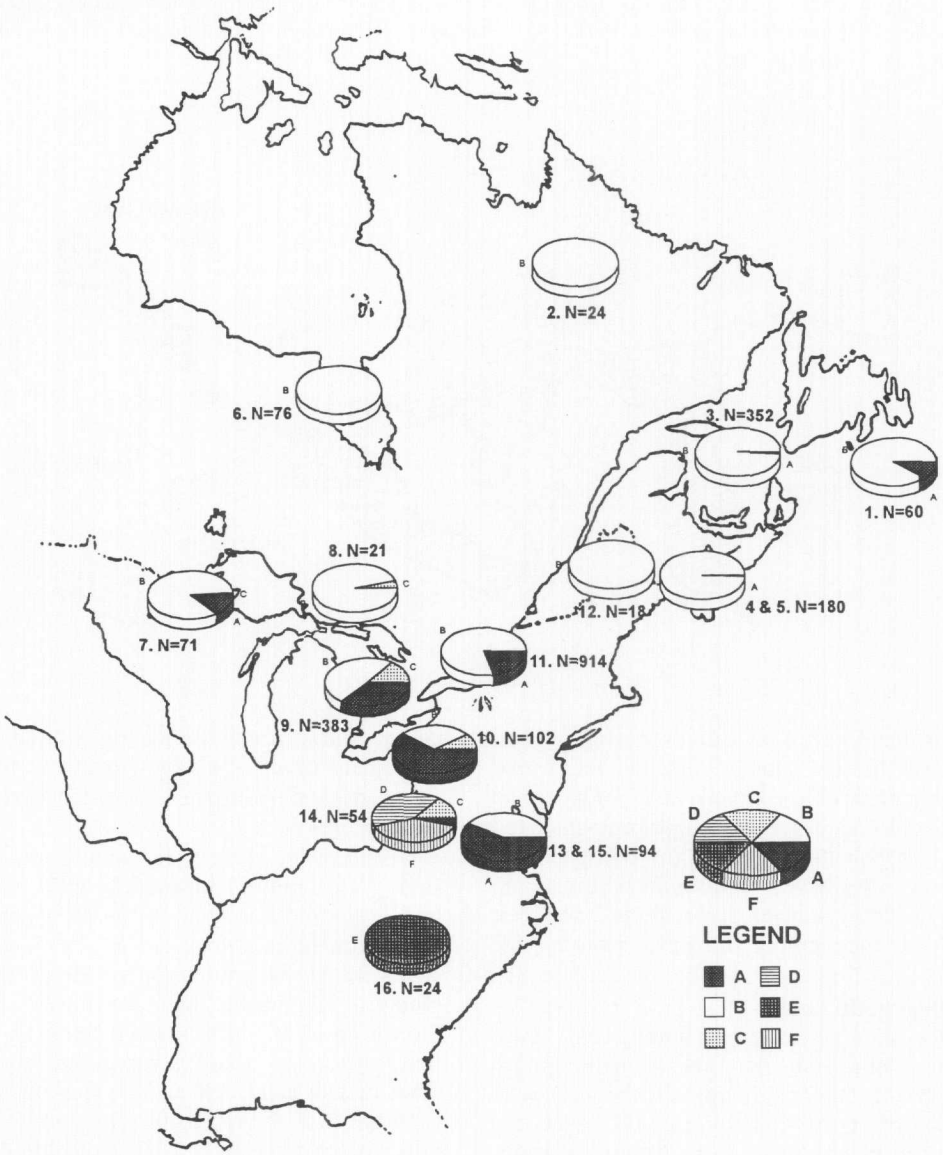
[a]Probability of more extreme random values from 1000 permutation tests.

predominantly northeastward (from a Mississippian source) or westward (from a northeastern source) as well as northward from an Atlantic refugium. Since glacial outflows were predominantly from west to east (Hough 1958; Bailey and Smith 1981), it likely that dispersal predominated in this direction as well. However, it is also possible that westward migration could have occurred as the Labrador ice cap receded. This ice dome formed a barrier to migration to more northern regions of Quebec long after more southernly migration corridors were established (i.e., approximately 7–6.5 thousand years ago) (Pielou 1991). As these northern regions opened, fish from a northeastern refugium may have had more opportunity to invade than fish from a more southerly Mississippian source.

Based upon the mtDNA phylogeographic data from rainbow smelt (*Osmerus mordax*), it appears that fish derived from the Acadian refuge colonized the Maritime Provinces prior to those derived from the Atlantic refugium (Bernatchez 1997). If brook trout had similar temporal and spatial recolonization routes, then fish possessing haplotypes derived from a northeastern refugium should predominate in the Maritimes regions, while fish colonizing the upper St. Lawrence River should primarily be derived from the Atlantic refugial zone. The colonization of the Ottawa River drainage by Atlantic refugial fish is supported in this study, while the presence of many B assemblage haplotypes one mutational step removed from haplotype 1, but not present in the Atlantic refugial zone, also supports the origin of haplotype 1 fish from a separate northeastern refugial zone. It also appears that haplotype 1 fish were present in the Mississippian refugium, as evidenced by the presence of this haplotype in Lawrence and Soper Creek populations in Wisconsin (northern Mississippi drainage) and populations in the central Great Lakes basin.

Resolution of eastward versus westward recolonization routes and secondary contact zones for B assemblage fish may be obtained by more detailed study of rarer B assemblage haplotypes. Jones et al. (1996) have documented an additional number of B assemblage haplotypes in Maritimes fish that were not examined in this study. The distribution of these haplotypes in more western populations would provide evidence for the dispersal of east coast fish into these regions and aid in the identification of a possible contact zone in Ontario or Quebec. However, the localization of these east coast haplotypes to populations within watersheds and their low sequence divergence (<0.1%) suggest a relatively recent origin for these lines, which may be compatible with their genesis after the last glacial period. The geographic distribution of rare B assemblage haplotypes identified as belonging to one refugial source will help to discriminate hypotheses regarding their time of divergence and will "weight" the use of such haplotypes in defining refugial dispersal routes. Haplotypes with low sequence divergence but widely scattered (i.e., two or more nonadjacent drainages)

Fig. 4. Representative distribution of the six major phylogenetic clades of brook trout in North America in the 16 major regions sampled (see Table 4). Sample sizes are indicated.



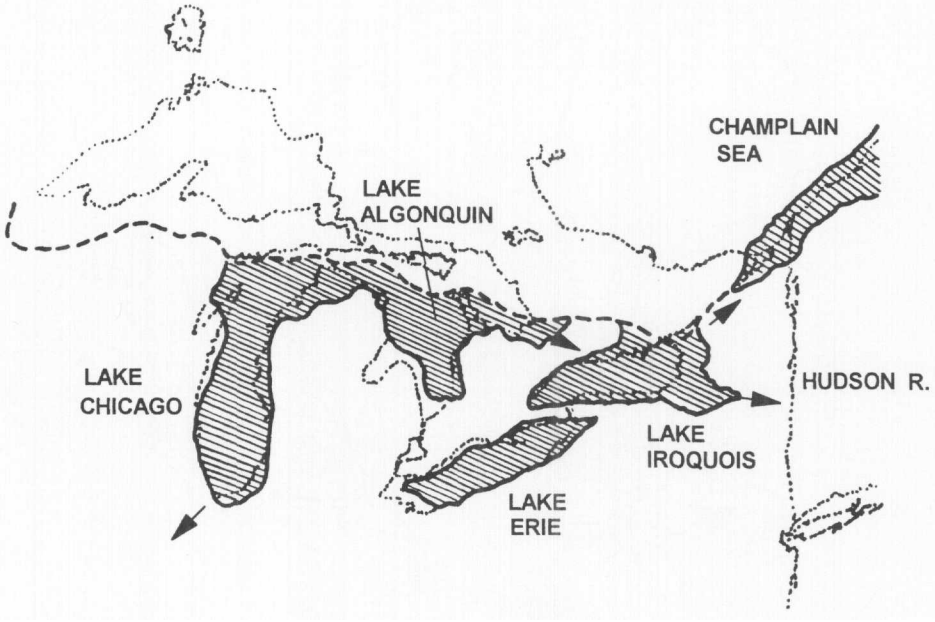
were likely present in a refugial grouping prior to recolonization.

There does appear to be evidence for a contact zone between fish arising from a northeastern coastal refuge and inland Atlantic refugial populations in the Chesapeake Bay region. The majority of the B assemblage fish sampled in the putative Atlantic refugial zone were restricted to the lowland Atlantic coastal populations (Winch Run, Panther Branch, and Timber Run) in Maryland. This suggests a possible secondary contact of these fish from the northeastern coastal lowland refugium. Dispersal may have been up the Susquehanna River drainage when ocean levels were lower.

Evidence of extensive contact in the southern Great Lakes region between eastern and western refugial races of several freshwater fish species has been reported (Billington and Hebert 1988; Grewe and Hebert 1988; Murdoch and Hebert 1997). An exception to this pattern occurs in lake whitefish (*Coregonus clupeaformis*), which appear to have recolonized the central part of their range solely from a Mississippian refugium (Bernatchez and Dodson 1991), although evidence for a separate Acadian glacial race for this species also exists. These Acadian lake whitefish are restricted to upper New England and the Canadian Maritime Provinces. Arctic char appear to have dispersed throughout most of northern North America from two sources: an Arctic or Beringian refugial zone and an Atlantic source. The Beringian source appears to have colonized most of the Canadian Arctic, although the Atlantic grouping recolonized as far northwestward as Ungava Bay (Wilson et al. 1996).

All the species (including brook trout) that show evidence for extensive contact from different refugial zones in the southern Great Lakes region have a more southerly distribution than lake whitefish and Arctic char. Populations of these species still exist in regions that were unimpacted by the last glacial advance. Thus, these groupings may reflect very ancient clades that have become established in allopatry and

Fig. 5. Major outflows of glacial Lakes Chicago (= Michigan), Algonquin (= Huron), Erie, and Iroquois (= Ontario) approximately 11 500 years BP. The ice margin is indicated by a broken line. Recolonization of fish into the lower Great Lakes region was possible from a Mississippian refugium (via Lake Chicago) or from the Atlantic region (via Hudson–Mohawk valley). Previous invasions from Mississippi drainages were also possible as early as 13 000 – 14 000 years BP through Lake Chicago and Lake Maumee (= Erie) connections. (Adapted from Underhill 1986.)



may have served as recolonization sources during several previous glacial ages. Species such as lake whitefish and Arctic char, which are more cold adapted, may not have had the physiological capabilities of taking refuge in unglaciated southern regions. Consequently, population bottlenecking during glacial advances may have greatly reduced the extant genetic variability in these species, as reflected by their present-day mtDNA phylogeographic dispersal patterns.

Divergence of phylogenetic clades

Assessment of the divergence times among the major mtDNA phylogenetic clades indicates that all major groupings were extant prior to the beginning of the last Wisconsinan glacial period (approximately 100 000 years BP) but diverged within the Pleistocene epoch. Assuming a molecular clock estimate of 1% divergence per million years for salmonids (Smith 1992), the two most closely related phylogenetic clades, A and B, diverged approximately 6.4×10^5 years ago compared with the two most distantly related clades, D and E (approximately 1.62×10^6 years ago).

Both category I and category V phylogeographic distributions (sensu Avise et al. 1987) were evident in brook trout. C clade fish appear restricted to a Mississippian refugium (category I distribution), but their apparent absence in large parts of the Ohio drainage suggests that the historical distribution of fish in this clade was limited. Thus, the observed type I patterning may be due to drift following population founding events. Type V patterning is evident with both A and B assemblage haplotypes. Both haplotype 1 (B clade) and haplotype 6 (A clade) are widespread throughout the species range, and both haplotypes are plesiomorphic within their respective assemblages. This suggests widespread gene flow since the divergence of these two assemblages. However, several recently derived haplotypes exhibit a phylogeographic structure indicating limited gene flow in recent geological time. A likely scenario is that these haplotypes arose in glacial refugia during the past Wisconsinan glaciation.

Evolutionary differences among clades detected in this study are quite substantial and suggest that certain lineages/populations should be recognized as evolutionarily significant units (Moritz et al. 1995). Separate subspecific status for brook trout from the Great Smoky Mountains region of Tennessee has previously been suggested by Stoneking et al. (1981) based upon an allozyme survey of fish in this region and is supported by recent allozyme data (McCracken et al. 1993). The present mtDNA survey indicates that fish in this region (Indian Camp Creek population) differ on average by 1.27% sequence divergence from fish with the most common haplotypes in the other clades.

The phylogeographic distinctness of fish in the D clade is also evident from this study. D assemblage fish appear localized to the Youghiogheny drainage and help constitute the first (1.61%), third (1.31%), and fourth (1.29%) most divergent interassemblage relationships detected with fish from the E, F, and C assemblages, respectively. Thus, these fish appear phylogenetically more closely related to fish that predominate in more northern regions (i.e., A and B assemblage fish). This may be due to the stream capture of the Monongahela and Youghiogheny rivers by the Old Lower Allegheny River, which flowed northward to the Greater Laurentian River basin (= St. Lawrence River) (Hocutt et al. 1986). Hocutt et al. (1986) indicated that the Youghiogheny portion of the drainage was especially isolated because of high cataracts and surrounding montane conditions and reported that the drainage experienced an impoundment during the Illinoian glacial advance. Thus, the ancestors of Youghiogheny drainage fish may come from the Laurentian

system rather than the Teays/Mississippian system. Also, the fact that these fish do not possess the B phenotype restriction fragment patterns at *Bam*HI, *Dra*I, and *Pvu*II that appear to characterize fish from more southerly distributions (Bernatchez and Danzmann 1993; Danzmann and Ihssen 1995; Hayes et al. 1996) suggests a more northerly origin for D clade fish.

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References

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.

Avise, J.C., Arnold, J., Ball, R.M., Bermingham, E., Lamb, T., Neigel, J.E., Reeb, C.A., and Saunders, N.C. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annu. Rev. Ecol. Syst. **18**: 489–522.

Bailey, R.M., and Smith, G.R. 1981. Origin and geography of the fish fauna of the Laurentian Great Lakes basin. Can. J. Fish. Aquat. Sci. **38**: 1539–1561.

Bernatchez, L. 1997. Mitochondrial DNA analysis confirms the existence of two glacial races of rainbow smelt *Osmerus mordax* and their reproductive isolation in the St. Lawrence River estuary (Quebec, Canada). Mol. Ecol. **6**: 73–83.

Bernatchez, L., and Danzmann, R.G. 1993. Congruence in control-region sequence and restriction site variation in mitochondrial DNA of brook charr (*Salvelinus fontinalis* Mitchell). 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., and Wilson, C.C. 1998. Comparative phylogeography of Nearctic and Palearctic fishes. Mol. Ecol. **7**: 431–452.

Billington, N., and Hebert, P.D.N. 1988. Mitochondrial DNA variation in Great Lakes walleye (*Stizostedion vitreum*) populations. Can. J. Fish. Aquat. Sci. **45**: 643–654.

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.

Danzmann, R.G. 1998. MTDIS: a computer program to estimate genetic distances among populations based upon variation in single copy DNA. J. Hered. **89**: 283–284.

Danzmann, R.G., and Ihssen, P.E. 1995. A phylogeographic survey of brook charr (*Salvelinus fontinalis*) in Algonquin Park, Ontario based upon mitochondrial DNA variation. Mol. Ecol. **4**: 681–697.

Danzmann, R.G., Ferguson, M.M., Skulason, S., Snorrason, S.S., and Noakes, D.L.G. 1991*a*. Mitochondrial DNA diversity among four sympatric morphs of Arctic charr, *Salvelinus alpinus* L., from Thingvallavatn, Iceland. J. Fish Biol. **39**: 649–659.

Danzmann, R.G., Ihssen, P.E., and Hebert, P.D.N. 1991*b*. Genetic discrimination of wild and hatchery populations of brook charr, *Salvelinus fontinalis* (Mitchell), in Ontario using mitochondrial DNA analysis. J. Fish Biol. **39**(Suppl. A): 69–77.

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. 1989. PHYLIP — phylogeny inference package. Cladistics, **5**: 164–166.

Ferguson, M.M., Danzmann, R.G., and Hutchings, J. 1991. Incongruent estimates of population differentiation among brook charr, *Salvelinus fontinalis*, from Cape Race, Newfoundland, Canada, based upon allozyme and mitochondrial DNA variation. J. Fish Biol. **39**(Suppl. A): 79–85.

Grewe, P., 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.

Hayes, J.P., Guffey, S.Z., Kriegler, F.J., McCracken, G.F., and Parker, C.R. 1996. The genetic diversity of native, stocked, and hybrid populations of brook trout (*Salvelinus fontinalis*) in the southern Appalachians. Conserv. Biol. **10**: 1403–1412.

Hocutt, C.H., Jenkins, R.E., and Stauffer, J.R., Jr. 1986. Zoogeography of the fishes of the central Appalachians and central Atlantic coastal plain. *In* The zoogeography of North American freshwater fishes. *Edited by* C.H. Hocutt and E.O. Wiley. John Wiley & Sons, Toronto, Ont. pp. 161–211.

Hough, J.L. 1958. Geology of the Great Lakes. University of Illinois Press, Urbana.

Jones, M.W. 1995. Conservation genetics and life-history variation in brook charr (*Salvelinus fontinalis*) in eastern Canada. M.Sc. thesis, University of Guelph, Guelph, Ont.

Jones, M.W., Clay, D., and Danzmann, R.G. 1996. Conservation genetics of brook trout (*Salvelinus fontinalis*): population structuring in Fundy National Park, New Brunswick, and eastern Canada. Can. J. Fish. Aquat. Sci. **53**: 2776–2791.

Kumar, S., Tamura, K., and Nei, M. 1993. MEGA: molecular evolutionary genetics analysis, version 1.01. Pennsylvania State University, University Park.

Mandrak, N.E., and Crossman, E.J. 1992. Postglacial dispersal of freshwater fishes into Ontario. Can. J. Zool. **70**: 2247–2259.

McCracken, G.F., Parker, C.R., and Guffey, S.Z. 1993. Genetic differentiation and hybridization between stocked hatchery and native brook trout in Great Smoky Mountains National Park. Trans. Am. Fish. Soc. **122**: 533–542.

McGlade, J. 1981. Genotypic and phenotypic variation in brook trout, *Salvelinus fontinalis* (Mitchell). Ph.D. thesis, University of Guelph, Guelph, Ont.

Moritz, C., Lavery, S., and Slade, R. 1995. Using allele frequency and phylogeny to define units for conservation and management. Am. Fish. Soc. Symp. No. 17. pp. 249–262.

Murdoch, M.H., and Hebert, P.D.N. 1997. Mitochondrial DNA evidence of distinct glacial refugia for brown bullhead (*Ameirus nebulosus*) in the Great Lakes. Can. J. Fish. Aquat. Sci. **54**: 1450–1460.

Neigel, J.E., and Avise, J.C. 1993. Application of a random walk model to geographic distributions of animal mitochondrial DNA variation. Genetics, **135**: 1209–1220.

Neigel, J.E., Ball, R.M., Jr., and Avise, J.C. 1991. Estimation of single generation migration distances from geographic variation in animal mitochondrial DNA. Evolution, **45**: 423–432.

Perkins, D.L., Krueger, C.C., and May, B. 1993. Heritage brook trout in northeastern USA: genetic variability within and among populations. Trans. Am. Fish. Soc. **122**: 515–532.

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

Power, G. 1980. The brook charr, *Salvelinus fontinalis*. *In* Charrs: salmonid fishes of the genus *Salvelinus*. *Edited by* E.K. Balon. Dr. W. Junk Publishers Ltd., The Hague, the Netherlands. pp. 141–203.

Radforth, I. 1944. Some considerations on the distribution of fishes in Ontario. R. Ont. Mus. Zool. Paleontol. Contrib. **25**: 1–116.

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

Schmidt, R.E. 1986. Zoogeography of the northern Appalachians. *In* The zoogeography of North American freshwater fishes. *Edited by* C.H. Hocutt and E.O. Wiley. John Wiley & Sons, Toronto, Ont. pp. 137–159.

Scott, W.B., and Crossman, E.J. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. No. 184.

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

Stoneking, M., Wagner, D.J., and Hildebrand, A.C. 1981. Genetic evidence suggesting subspecific differences between northern and southern populations of brook trout (*Salvelinus fontinalis*). Copeia, **1981**: 810–819.

Underhill, J.C. 1986. The fish fauna of the Laurentian Great Lakes, the St. Lawrence lowlands, Newfoundland and Labrador. *In* The zoogeography of North American freshwater fishes. *Edited by* C.H. Hocutt and E.O. Wiley. John Wiley & Sons, Toronto, Ont. pp. 105–136.

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. 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**: 187–197.