Molecular Ecology (1998) 7, 1655–1662

Geographical extent of Arctic char (*Salvelinus alpinus*) mtDNA introgression in brook char populations (*S. fontinalis*) from eastern Québec, Canada

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Abstract

The geographical extent of Arctic char (*Salvelinus alpinus*) mitochondrial DNA introgression into brook char (*Salvelinus fontinalis*) populations found in eastern Québec was determined by analysing a total of 598 fish from 29 lakes. The nuclear genome was analysed by protein electrophoresis, whereas the ND-5,6 portion of the mitochondrial genome was analysed by restriction fragment length polymorphism. This survey revealed that introgressed *S. fontinalis* populations are restricted to only one river subdrainage of the Portneuf basin, where Arctic char is completely absent. Elsewhere, nonintrogressed pure *S. fontinalis* populations populate the lakes. These findings suggest that the initial hybridization event between the species is ancient and probably occurred shortly after recolonization of the area. At that time, the species would have been in contact and the chances of reproductive isolation mechanisms breaking down would have been high. We discuss the possibility that a combination of biogeographical conditions coupled with positive selection for mtDNA introgression led to the present-day distribution of introgressed *S. fontinalis* in northeastern North America.

Keywords: Arctic char, brook char, geographical distribution, introgression, mitochondrial DNA, *Salvelinus*

Received 3 March 1998; revision received 17 June 1998; accepted 17 June 1998

Introduction

The potential for natural hybridization in freshwater fish is elevated as a result of several factors including external fertilization, unequal abundance of parental species, competition for limited spawning habitat, and susceptibility to secondary contact between recently evolved forms (Hubbs 1955). Introgressive hybridization of foreign mitochondrial DNA (mtDNA) into a species may eventually lead to complete mtDNA replacement. In such a case, all individuals of an entire population possess the foreign mtDNA. This has been documented for various species of animals including grasshoppers, voles, fruit flies, crickets, treefrogs, mice, fish, deer and frogs (see Avise 1994 for review). For fish, complete mitochondrial DNA fixation is relatively rare having been documented only for minnows occurring in midwestern USA from the *Notropis cornutus/chrysocephalus* species complex (Dowling *et al.* 1989; Dowling & Hoeh 1991; Duvernell & Aspinwall 1995) and recently for *Salvelinus fontinalis/alpinus* (Bernatchez *et al.* 1995), and for *S. namaycush/alpinus* (Wilson & Bernatchez 1998) in eastern Québec, Canada. The evolutionary significance of mitochondrial introgressive hybridization is not clear; however, it appears that it can have a significant and long-term effect on the genetic composition of the species involved (Bernatchez *et al.* 1995) and can thereby contribute to genetic diversity (Dowling & DeMarais 1993). In fact, Arnold (1992) argues that mtDNA exchange through introgressive events may be more important than mutations as a source of new genetic variability within taxa.

In fish, the extent of introgression and the geographical distribution of introgressed populations are not so well characterized in detected cases of introgressive hybridation. The extent of mtDNA introgression has only been

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studied for *Luxilus cornutus* and *L. chrysocephalus* populations along transects of their hybrid zone (Dowling & Hoeh 1991; Dowling *et al.* 1997) and more recently for populations in Missouri and Arkansas, USA (Duvernell & Aspinwall 1995). Defining the extent of geographical introgression is potentially important for understanding evolutionary processes such as reproductive isolation and speciation.

In North America, the geographical distribution of *S. alpinus* and *S. fontinalis* ranges throughout northern Canada and eastern USA with *S. alpinus* having a more northern distribution and *S. fontinalis* a more southern distribution (Fig. 1). Freshwater populations of both species may occur in sympatry where their distributions overlap at the southern extreme of the range of *S. alpinus* and northern end of the range of *S. fontinalis* (Scott & Crossman 1973). South of 58°N, *S. alpinus* is generally replaced by *S. fontinalis* (Saunders & Power 1969). In northeastern North America, the known distribution of land-locked *S. alpinus* is limited to approximately a hundred lakes, most of them in southern Québec, Canada. These populations are thought to be vestiges of anadromous populations that lived in the Champlain sea and Atlantic ocean about 12 000 years ago (Power *et al.* 1973).

In the Côte-Nord region of southeastern Québec, a brook char population (*S. fontinalis*) completely introgressed with the mtDNA of Arctic char (*S. alpinus*) was discovered in lac Alain which belongs to the Portneuf river drainage (Bernatchez *et al.* 1995). All individuals from this lake were morphologically indistinguishable from typical brook char, homozygous for diagnostic brook char alleles at enzymatic loci, and only possessed microsatellite alleles characteristic of brook char (Bernatchez *et al.* 1995; Glémet 1997). As Arctic char are presently absent from the Portneuf watershed, it is thought that the original hybridization event between the species probably occurred during the postglacial recolonization of the area (Bernatchez *et al.* 1995).

In this study we determine the geographical distribution of introgressed populations in lakes from the drainages of the Côte-Nord region of southeastern Québec and examine the extent of mtDNA introgression in these lakes. Results are discussed in the context of historical and evolutionary factors that may have been responsible for shaping the present-day distribution of introgressed populations.

Materials and methods

Study area and samples

Lac Alain, where the original introgressed brook char population was first discovered, is located in the Rocheuse river, a tributary of the Portneuf river watershed on the north shore of the St Lawrence river (Fig. 2, location 17 on map; Table 1). Initial sampling efforts were focused on lakes surrounding the Rocheuse river. Populations upstream and downstream of the Portneuf river drainage were also sampled. All populations were sampled either by angling or gill netting. Sampling was extended to populations from all neighbouring major drainages (Fig. 2; Table 1). A total of 598 fish from 29 lakes was thus sampled. All fish collected were identified as brook char (*S. fontinalis*) based on external appearance. Generally, Arctic char are not known to occur in the river systems of the Côte-Nord region (Dumont 1982; M. Braut: Minstère de l'Environnement et de la Faune, Québec, personal communication), except in the Aux Anglais river drainage where

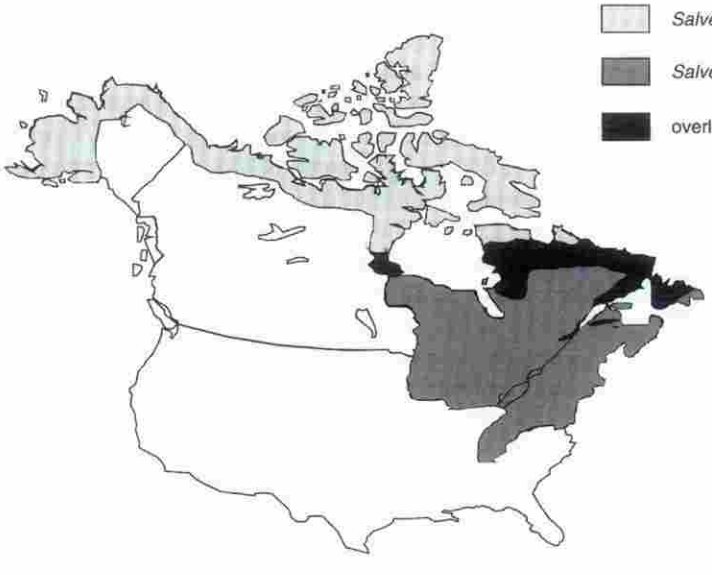

Fig. 1 Geographical distribution of *Salvelinus alpinus* and *S. fontinalis* and the present overlap zone between the two species in North America.

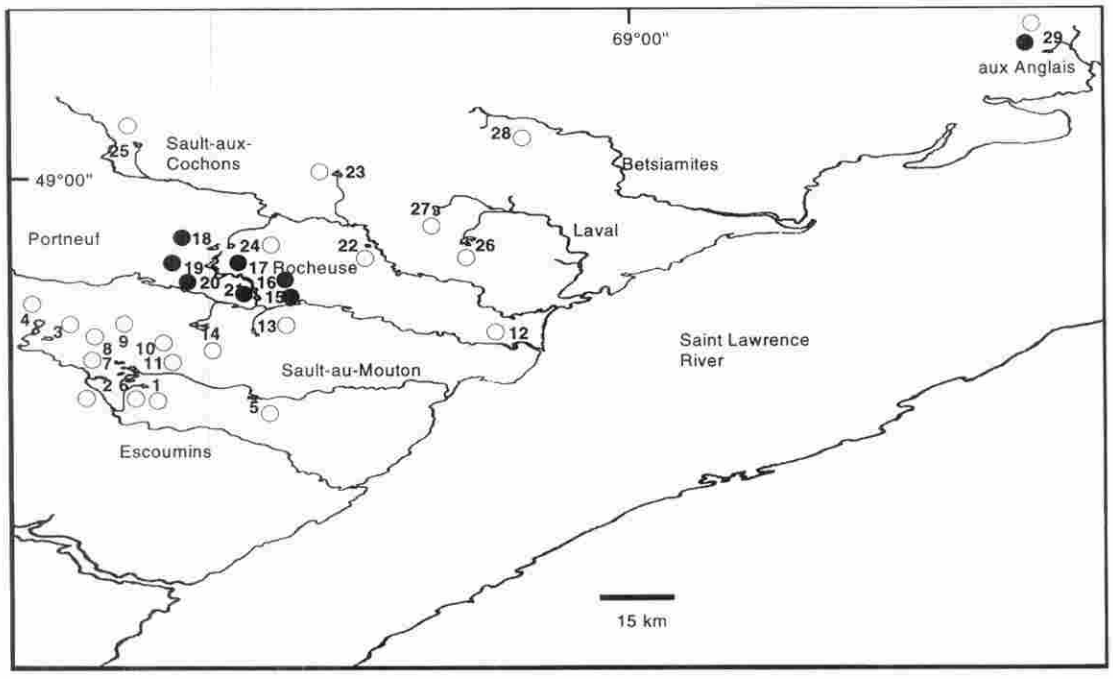

Fig. 2 Location of watersheds and the sites where brook char populations *Salvelinus fontinalis* were sampled in the Côte-Nord region of southeastern Québec. Open circles represent the brook char mtDNA haplotype, whereas closed circles represent the Arctic char mtDNA haplotype. For names of lakes which are here denoted by numbers, refer to Table 1.

both Arctic and brook char are known to occur sympatrically (Fig. 2; location 29 on map). From the same lake of this latter drainage, seven Arctic char and 10 brook char were collected by gill netting and identified based on external morphological characteristics. In the field, all specimens were either frozen whole or only the digestive organs were conserved and frozen. The liver tissue was later dissected and stored at – 80 °C until genetic analysis was performed.

Nuclear DNA analysis

Protein electrophoresis was carried out on cellulose acetate using liver tissue homogenates as described by Hebert & Beaton (1989). Four diagnostic enzymes having alleles previously found to be fixed for either brook char or Arctic char were used to distinguish the species' nuclear genomes (Bernatchez *et al.* 1995). These enzymes included isocitrate dehydrogenase (IDH), EC 1.1.1.42; lactate dehydrogenase (LDH), EC 1.1.1.27; sorbitol dehydrogenase (SDH), EC 1.1.1.14; and superoxide dismutase (SOD), EC 1.15.1.1. All enzymes examined were resolved by using a Tris-glycine buffer system at pH 8.5 and the staining recipes of Hebert & Beaton (1989).

Mitochondrial DNA analysis

Total DNA was extracted from liver tissue using a phenol–chloroform method (Bernatchez *et al.* 1992). A 2.5-kb portion of the mitochondrial ND5/6 region was amplified by the polymerase chain reaction (PCR) using primers by Cronin *et al.* (1993). Amplification conditions are those described in Bernatchez *et al.* (1995). Amplified DNA was digested with three restriction enzymes (*Ava*I, *Hae*III and *Hin*cII) which generated haplotypes found to be diagnostic between Arctic char and brook char (Bernatchez *et al.* 1995). Based on fragment patterns generated by the aforementioned restriction enzymes, a composite mtDNA haplotype was designated for Arctic char as (AAA), and for brook char as (BBB). The DNA fragments were separated on 1.2% agarose gels, run for 5 h at 85 V, stained with ethidium bromide, and photographed under UV light.

Results

Allozymes

Based on diagnostic loci having alleles fixed for either brook char or Arctic char, all individuals from each of the populations sampled revealed a nuclear genome of brook char (Table 2). In no cases were there shared alleles with Arctic char, suggesting that introgression by Arctic char at these nuclear genes had not occurred in any of the individuals. For the lac Sans Baie population, where both Arctic char and brook char were captured, both species were fixed for alternative diagnostic mtDNA haplotypes or alleles at the four enzymatic loci.

Table 1 Geographical location of populations sampled from various drainage basins in the eastern Québec region

Drainage basin and lakes	Location on map	Longitude	Latitude	*n*
Escoumin				
Cormier	1	48°31'00"	69°43'30"	20
Belanger	2	48°35'00"	69°50'30"	31
Loup	3	48°40'15"	69°56'00"	37
des Coeurs	4	48°42'00"	69°58'00"	36
Sault-au-mouton				
des Pilliers	5	48°32'15"	69°35'00"	30
des Passes	6	48°33'45"	69°46'15"	7
Brûlé	7	48°35'30"	69°48'15"	24
la Roche	8	48°37'00"	69°48'30"	30
Roger	9	48°36'15"	69°47'45"	4
Féfane	10	48°34'30"	69°47'45"	22
de la Petite Montange	11	48°35'00"	69°47'00"	15
Portneuf				
Noir	12	48°43'30"	69°15'45"	12
Boucher	13	48°40'45"	69°35'15"	22
Grand lac du Nord	14	48°41'45"	69°41'00"	26
Rocheuse branch				
Savard	15	48°45'15"	69°35'00"	27
Bourbeau	16	48°46'30"	69°35'15"	12
Alain	17	48°47'45"	69°36'00"	30
Manon	18	48°52'00"	69°39'00"	22
Docile	19	48°51'00"	69°39'15"	12
Micheal	20	48°49'45"	69°39'30"	12
Ruth	21	48°48'00"	69°38'00"	14
Sault-aux-cochons				
Machoire du Diable	22	48°51'45"	69°23'00"	6
de la Main	23	49°02'15"	69°26'45"	22
Pipe	24	48°51'45"	69°37'45"	7
la Corne	25	49°05'15"	69°47'30"	30
Laval				
Croche	26	48°52'15"	69°13'15"	21
Ouellette	27	48°56'45"	69°16'30"	30
Betsiamites				
Mille 45	28	49°08'45"	69°14'45"	20
Aux anglais				
Sans Baie	29	49°17'30"	69°13'30"	17
Total				598

mtDNA characterization

Restriction analysis performed on the ND-5/6 segment of mtDNA generated diagnostic haplotypes which were either characteristic of Arctic char (AAA) or brook char (BBB) (Table 2). mtDNA analysis of populations from the Côte-Nord region of eastern Québec revealed that introgressed populations were restricted geographically to the Rocheuse river branch of the Portneuf river drainage basin. All individuals from these seven populations were fixed for Arctic char mtDNA. Coupled with the allozyme analysis of the nuclear genome of these fish, the results show that brook char populations are completely introgressed with the mtDNA of Arctic char, and that hybrids and pure parentals are apparently absent from these lakes. No intermediate forms resulting from partial introgression were observed. Upstream and downstream of the Portneuf River drainages, populations were found to

Table 2 Allele and mtDNA haplotype frequencies for populations from various drainage basins found in the eastern Québec region

		Diagnostic loci and alleles				Diagnostic mtDNA haplotype
Drainage basin and lakes	Location on map	IDH-3,4* (100/100) (130/130)	LDH-3* (0/0) (100/100)	SDH-1,2* (100/100) (40/40)	SOD* (100/100) (260/260)	mtDNA (BBB) (AAA)
Escoumin						
Cormier	1	1.0	1.0	1.0	1.0	1.0
		0	0	0	0	0
Belanger	2	1.0	1.0	1.0	1.0	1.0
		0	0	0	0	0
Loup	3	1.0	1.0	1.0	1.0	1.0
		0	0	0	0	0
des Coeurs	4	1.0	1.0	1.0	1.0	1.0
		0	0	0	0	0
Sault-au-mouton						
des Pilliers	5	1.0	1.0	1.0	1.0	1.0
		0	0	0	0	0
des Passes	6	1.0	1.0	1.0	1.0	1.0
		0	0	0	0	0
Brûlé	7	1.0	1.0	1.0	1.0	1.0
		0	0	0	0	0
la Roche	8	1.0	1.0	1.0	1.0	1.0
		0	0	0	0	0
Roger	9	1.0	1.0	1.0	1.0	1.0
		0	0	0	0	0
Féfane	10	1.0	1.0	1.0	1.0	1.0
		0	0	0	0	0
de la Petite Montange	11	1.0	1.0	1.0	1.0	
		0	0	0	0	0
Portneuf						
Noir	12	1.0	1.0	1.0	1.0	1.0
		0	0	0	0	0
Boucher	13	1.0	1.0	1.0	1.0	1.0
		0	0	0	0	0
Grand lac du Nord	14	1.0	1.0	1.0	1.0	1.0
		0	0	0	0	0
Rocheuse branch						
Savard	15	1.0	1.0	1.0	1.0	1.0
		0	0	0	0	0
Bourbeau	16	1.0	1.0	1.0	1.0	1.0
		0	0	0	0	0
Alain	17	1.0	1.0	1.0	1.0	1.0
		0	0	0	0	0
Manon	18	1.0	1.0	1.0	1.0	1.0
		0	0	0	0	0
Docile	19	1.0	1.0	1.0	1.0	1.0
		0	0	0	0	0
Micheal	20	1.0	1.0	1.0	1.0	1.0
		0	0	0	0	0
Ruth	21	1.0	1.0	1.0	1.0	1.0
		0	0	0	0	0
Sault-aux-cochons						
Machoire du Diable	22	1.0	1.0	1.0	1.0	1.0
		0	0	0	0	0
de la Main	23	1.0	1.0	1.0	1.0	1.0
		0	0	0	0	0
Pipe	24	1.0	1.0	1.0	1.0	1.0
		0	0	0	0	0

Table 2 *Continued*

Drainage basin and lakes	Location on map	Diagnostic loci and alleles				Diagnostic mtDNA haplotype
		IDH-3,4* (100/100) (130/130)	LDH-3* (0/0) (100/100)	SDH-1,2* (100/100) (40/40)	SOD* (100/100) (260/260)	mtDNA (BBB) (AAA)
la Corne	25	1.0 0	1.0 0	1.0 0	1.0 0	1.0 0
Laval						
Croche	26	1.0 0	1.0 0	1.0 0	1.0 0	1.0 0
Ouellette	27	1.0 0	1.0 0	1.0 0	1.0 0	1.0 0
Betsiamites						
Mille 45	28	1.0 0	1.0 0	1.0 0	1.0 0	1.0 0
Aux anglais						
Sans Baie (brook char)	29	1.0 0	1.0 0	1.0 0	1.0 0	1.0 0
Sans Baie (Arctic char)	29	1.0 0	1.0 0	1.0 0	1.0 0	1.0 0

have the mtDNA haplotype of typical brook char (Fig. 2; location 13 and 14 on map). Similarly, for neighbouring drainages, all individuals tested from these populations had a brook char mtDNA haplotype.

Discussion

Geographical distribution of introgressed brook char: historical and ecological considerations

A salient finding from the present survey is that introgressed brook char populations are restricted to the Rocheuse river subdrainage of the Portneuf basin where all fish analysed possessed the mtDNA genome of Arctic char. In contrast, not a single fish from these lakes ($n = 129$) was found to possess Arctic char alleles at enzymatic loci. Elsewhere in the Portneuf drainage and in neighbouring drainage basins, nonintrogressed pure brook char populate the lakes.

The presence of introgressed brook char in only the Rocheuse branch of the Portneuf river system and nowhere else is unusual as Arctic char and brook char are known to have occurred elsewhere in sympatry (Lacasse & Magnan 1994). In fact, we observed pure parental forms of both species in lac Sans Baie although it could be argued that introgressed fish could exist there at low frequencies because our sample size was limited. One hypothetical explanation for the absence of introgression elsewhere is that the necessary demographic conditions that would have led to this distribution did not occur. This hypothesis cannot, however, be tested. Apparently, introgressed brook char have not dispersed out of the Rocheuse branch. To our knowledge, there are no obvious geographical barriers restricting gene flow from lakes of the Rocheuse branch to the rest of the drainage downstream. However, the discrepancy is striking as not a single introgressed fish has been found elsewhere within the connected Portneuf river drainage and populations from the Rocheuse branch do not contain a single nonintrogressed pure brook char.

The present-day distribution of brook char is widespread in eastern Québec (Bernatchez & Giroux 1991). This observation, coupled with a high genetic diversity for the species observed from allozyme data (McGlade 1981) compared to that observed in Arctic char (Kornfield *et al.* 1981; Anderson *et al.* 1983), suggests that historical population sizes of brook char were probably larger than those of Arctic char. The occurrence of natural hybridization is thought to be related to a disparity in size of one of the parental populations (Hubbs 1955; Avise *et al.* 1988). Presumably, if the proper mates are not available, the species that is locally rare will probably out-cross (Hubbs 1955). This has been observed in the Fraser River, Labrador (Canada), where natural hybrids between Arctic char and brook char occur, but where Arctic char numbers largely predominate (Hammar *et al.* 1991). Thus, the historical presence of Arctic char in comparatively smaller numbers may have favoured the occurrence of

hybridization. A subsequent change in habitat or interspecific competition with brook char may have led to the eventual decline and disappearance of Arctic char in the watershed.

mtDNA introgression patterns in other animal groups and the selectionist hypothesis

There are several examples in the literature illustrating recent or more ancient unidirectional mtDNA transfers among vertebrates, e.g. rodents (Ferris *et al.* 1983; Tegelstrom 1987), deer (Carr *et al.* 1986; Carr & Hughes 1993), canids (Lehman *et al.* 1991), and fishes (Avise & Saunders 1984; Dowling *et al.* 1989; Dowling & Hoeh 1991; Wilson & Bernatchez 1998). Two cases in the literature where the extent of introgression has been studied within and beyond the contact zone of the species are for pocket gopher (Ruedi *et al.* 1997) and cyprinid fish of the genus *Luxilus* (Dowling & Hoeh 1991; Duvernell & Aspinwall 1995) species. These systems have some features in common with the brook char system we describe. First, mtDNA introgression is not necessarily limited to the present-day contact zone of the species. In both cases, mtDNA replacement is observed outside the range of contact where the mtDNA donor species is presently absent. Second, the species in question have different ecological requirements or opposing geographical distributions, which suggest that the species either have different temperature preferenda or did in the past. For example, with pocket gophers, *Thomomys bottae ruidosae* is restricted to high-elevation coniferous forest zones, whereas *T.b. actuosus* occupies lower-elevation woodland areas (Ruedi *et al.* 1997). Similarily, for the cyprinid species, *Luxilus cornutus* is distributed in the north, while *L. chrysocephalus* has a more southern distribution (Duvernell & Aspinwall 1995). As with the introgressed brook char, in these examples the 'cold-adapted' species is the mtDNA donor species. Finally, mtDNA introgression is unidirectional such that reciprocal introgression is generally not observed.

It could be argued that the observed distribution of introgressed brook char is because introgressed brook char are at a selective advantage in the Rocheuse river watershed. Hypotheses implicating selection as a force for shaping the geographical distribution of introgressed populations have been proposed in the past by population geneticists (Dowling *et al.* 1989; Arnold 1992; Duvernell & Aspinwall 1995). However, the selectionist hypothesis has often been dismissed for the traditional neutralist explanation. Given that the mitochondrial genome encodes for several units of enzymes involved in oxidative phosphorylation, a functional influence by mtDNA in respiratory metabolism is not unimaginable. In an experiment specifically designed to investigate the influence of the mitochondrial genome on physiological performance in introgressed and nonintrogressed brook char, nonequivalence was demonstrated for the thermal sensitivity of cytochrome *c* oxidase and pyruvate oxidation by whole red muscle mitochondria at only low temperatures (Glémet 1997). At the individual level, a selective advantage was not identified in juvenile fish based on swimming performance and the metabolic scope for aerobic activity; however, an advantage may still exist at other stages of development (Glémet 1997).

In early postglacial times, a physiological basis at low temperature would have been advantageous for introgressed brook char as the mean water temperature during the growing season was presumably lower than it is presently (Pagé 1992). As observed for other animal systems displaying mtDNA introgression, Arctic char which donate the mitochondrial genome are distributed more northerly than brook char. Although the selective value of differences observed at the molecular and submolecular level remains to be firmly tested, it is plausible that through introgressive hybridization, an Arctic char mitochondrial genome in a nuclear brook char background was more desirable for survival at cold temperatures. If the is correct, we propose a combination of both historical demographic conditions and selection for mtDNA introgression, rather than pure stochastic processes, as a more plausible mechanism which could have produced the present-day geographical distribution observed for introgressed brook char in eastern Québec. As cases are increasingly being uncovered that suggest that the mitochondrial genome may be subject to selective forces (see Ballard & Kreitman 1995 for review) and may even evolve under thermal constraints (Rand 1994), it is becoming important to consider carefully the alternative selectionist hypothesis.

Acknowledgements

The authors wish to thank Pierre Landry and Angelo Chouinard for their help in obtaining samples. This work was funded by a *Fonds pour la Formation de Chercheurs et l'Aide à la Recherce* FCAR *(Québec) and* an *Institut National de la Recherche Scientifique* postgraduate scholarships awarded to H.G., and by Natural Sciences and Engineering Research Council of Canada and FCAR (Québec) operating grants to L.B., and by a *Fonds de Développement Académique du Réseau (Université du Québec)* grant to L.B. and P.B.

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