Introgression and fixation of Arctic char (*Salvelinus alpinus*) mitochondrial genome in an allopatric population of brook trout (*Salvelinus fontinalis*)

Louis Bernatchez, Hélène Glémet, Chris C. Wilson, and Roy G. Danzmann

Abstract: Although mitochondrial introgression between taxa has been increasingly documented, interspecific replacement of mtDNA is rare, particularly when the donor species is absent. We document evidence for a population of brook trout (*Salvelinus fontinalis*) in which all individuals possess the mitochondrial genome of Arctic char (*S. alpinus*) despite the present-day absence of the latter species in the watershed where the population is located. The mitochondrial genotype of 48 brook trout from Lake Alain (Québec) was characterized by RFLP analysis performed over the entire mtDNA molecule and/or a 2.5-kb PCR-amplified segment of the ND-5/6 region. Although the fish examined were morphologically indistinguishable from typical brook trout and homozygous for the diagnostic alleles characteristic of brook trout, the mtDNA of all individuals was identical to the Québec Arctic char haplotype. Together, these results indicate that the mtDNA haplotype observed in Lake Alain brook trout has resulted from ancient introgression with Arctic char rather than ancestral polymorphism or convergent evolution. They also demonstrate that introgressive hybridization between those two species can have significant and long-term effects on their genetic composition.

Résumé : On rapporte de plus en plus l'introgression mitochondriale entre des taxons, mais l'échange d'ADNmt entre deux espèces est rare, notamment lorsque l'espèce donneuse est absente. Nous présentons une population d'Ombles de fontaine (*Salvelinus fontinalis*) dont tous les individus possèdent le génome mitochondrial de l'Omble chevalier (*S. alpinus*) sans que cette dernière espèce soit présente actuellement dans le bassin où la population d'Ombles de fontaine a été localisée. Le génome mitochondrial de 48 Ombles de fontaine provenant du lac Alain (Québec) a été caractérisé par une analyse des polymorphismes au niveau de toute la molécule d'ADNmt et/ou d'un segment de 2,5 kb de la région ND-5/6 amplifié par la PCR. Les poissons examinés étaient indifférenciables morphologiquement d'un omble de fontaine typique et étaient homozygotes pour les caractères allèles de diagnostic de l'Omble de fontaine, mais l'ADNmt de tous les individus était identique à l'haplotype d'ADNmt de l'Omble chevalier du Québec. Tous ces résultats indiquent que l'haplotype d'ADNmt observé chez l'Omble de fontaine du lac Alain provient d'une ancienne introgression avec l'Omble chevalier plutôt que d'un polymorphisme ancestral ou d'une convergence. Ces résultats montrent également qu'une hybridation introgressive entre ces deux espèces peut avoir des effets importants et à long terme sur leur composition génétique.

[Traduit par la Rédaction]

Introduction

There is little consensus regarding the evolutionary significance of hybridization and introgression, although there is increasing evidence for their occurrence. Traditionally, the transfer of genes across species boundaries has been thought to have little or no evolutionary importance (Mayr 1963; Heiser 1973). The main argument supporting this view is that introgressive hybridization among animal taxa is rare and that documented cases leading to persistent,

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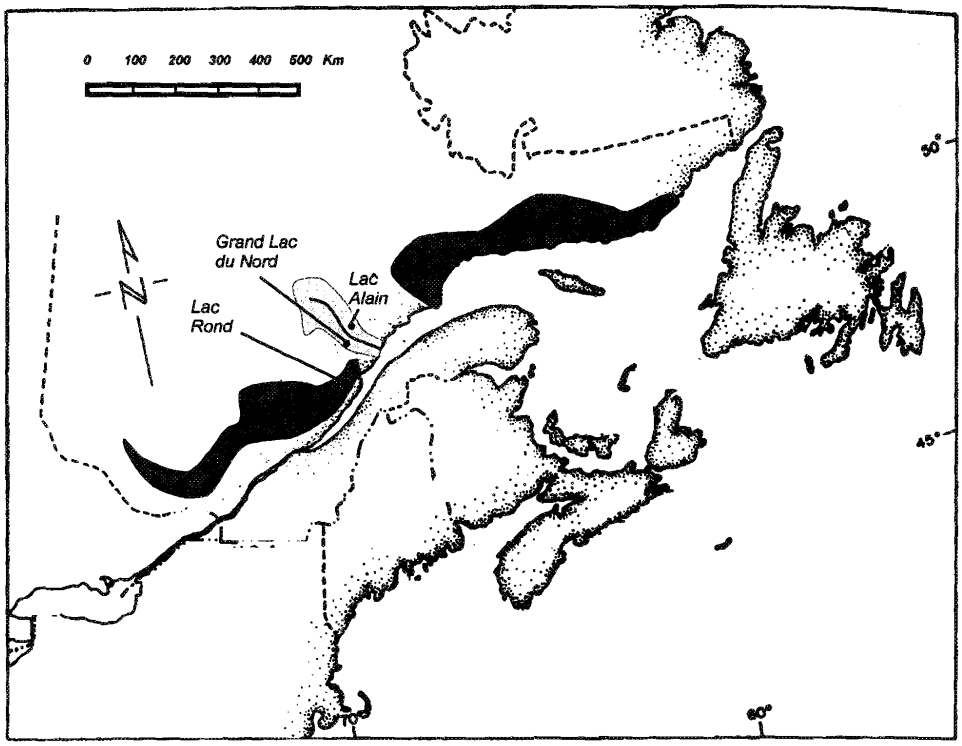
L. Bernatchez[1] and H. Glémet. Institut National de la Recherche Scientifique (INRS-Eau), C.P. 7500, 2800, rue Einstein, Sainte-Foy, QC G1V 4C7, Canada.
C.C. Wilson and R.G. Danzmann. Department of Zoology, University of Guelph, Guelph, ON N1G 2W1, Canada.

[1] Author to whom correspondence should be addressed. Present address: Département de biologie, Université Laval, Ste-Foy, QC G1K 7P4, Canada.

Fig. 1. Location map of Lake Alain, Lac Rond, and Grand Lac du Nord showing present-day distribution of land-locked Arctic char populations on the north shore of the St. Lawrence River, as well as the limits of the Portneuf River watershed.



long-term incorporation of one species' genes into another are even rarer. Recent molecular and ecological studies, however, support the prevalence of introgressive hybridization in several species complexes and argue that even rare introgressive events may be more important than mutation as a source of new genetic variability within taxa (reviewed in Arnold 1992).

Introgressive hybridization could be particularly important in fish, which, as a group, appear to hybridize fairly readily (Hubbs 1955). Hybridization beyond the first generation has been reported for many species (reviewed in Dowling et al. 1989; Dowling and Hoeh 1991; Verspoor and Hammar 1991; Carmichael et al. 1993), suggesting the potential for introgression.

One well-documented case of potential interspecific introgression is the natural hybridization between Arctic char (*Salvelinus alpinus*) and brook trout (*Salvelinus fontinalis*) reported by Hammar et al. (1991). Protein electrophoresis detected repeated hybridization between these species in northern Labrador. Furthermore, isozyme phenotypes suggested the presence of second-generation hybrids and/or backcrosses to both species. However, there was no evidence of persistent incorporation of genes from one species into the other, precluding any determination of the evolutionary impact of hybridization for these species.

In this paper, we report the fixation of Arctic char mitochondrial DNA (mtDNA) in an allopatric population of brook trout from an inland lake in Québec, outside the present-day contact zone of the two species. This shows that introgressive hybridization as described by Hammar et al. (1991) can have significant and long-term effects on the genetic composition of brook trout. This case of mitochondrial introgression represents one of the very few documentations of complete interspecific replacement of mitochondrial genome and the first known occurrence in salmonid fishes.

Material and methods

Sample description and location

Brook trout were collected from Lake Alain (48°48′00″, 69°35′30″) and Grand Lac du Nord (48°42′00″, 69°40′00″), which are in the Portneuf River system (Fig. 1), in the context of a macrogeographic survey of brook trout in eastern North America (R.G. Danzmann et al., unpublished data). Sixteen fish were collected from Lake Alain in August 1992, and 32 additional fish were collected between January and July 1993. Arctic char does not occur in Lake Alain or its river system and the nearest reported population is approximately 50 km away (Dumont 1982; M. Brault, Ministère du Loisir, de la Chasse et de la Pêche, Québec, personal communication). Arctic char were collected from Lake Rond (48°15′00″, 70°37′30″) and obtained from a local pisciculture. Arctic char obtained from the pisciculture were of Fraser River (Labrador) origin. Liver was sampled from each fish and either processed immediately or stored at −80°C for later analysis.

Morphological identification

Upper and lower gill rakers were counted on the first left gill arch, and external features which are classically used to distinguish Arctic char and brook trout were recorded on all freshly killed fish (Scott and Crossman 1974). The

diagnostic features examined were tail shape (square versus forked), coloration of lower fins (presence/absence of a black stripe following the white border), presence/absence of vermiculations on the back, caudal, and dorsal fins, and presence/absence of red spots with a blue halo on the sides of the body.

Allozyme analysis

Protein electrophoresis was carried out on cellulose acetate using liver tissue homogenates as described by Hebert and Beaton (1989). Fish were screened at several loci known to be polymorphic for Arctic char and brook trout (Hammar et al. 1991). Four diagnostic enzymes representing six loci were utilized to distinguish brook trout from Arctic char and included isocitrate dehydrogenase (IDH, 1.1.1.42), lactate dehydrogenase (LDH, 1.1.1.27), sorbitol dehydrogenase (SDH, 1.1.1.14), and superoxide dismutase (SOD, 1.15.1.1). Loci were designated as recommended by Shaklee et al. (1989). All enzymes examined were resolved by using a Tris glycine buffer system of pH 8.5 and the staining recipes outlined by Hebert and Beaton (1989). Allelles were identified by their relative electrophoretic mobilities as measured from the gels. The most common allelle in the brook trout population (Grand Lac du Nord) was designated a standard mobility of 100.

Characterization of mtDNA

For the first 16 individuals analyzed from Lake Alain, mtDNA was extracted and purified from fresh liver by the rapid isolation method of Chapman and Powers (1984) with modifications of Danzmann et al. (1991*a*). Intact mtDNA was digested with eight restriction enzymes (*Acc*I, *Bam*HI, *Bcl*I, *Bst*EII, *Nco*I, *Nhe*I, *Pst*I, *Xba*I). Digested mtDNA fragments were separated on 0.8% agarose gel run overnight at 30 V, visualized by UV irradiation after ethidium bromide staining, and photographed. All of these enzymes provided diagnostic fragment patterns between Arctic char and brook trout throughout their distributions (Grewe et al. 1990; Danzmann et al. 1991*a*, 1991*b*; Ferguson et al. 1991; Bernatchez and Danzmann 1993; Wilson and Hebert 1993; C. Wilson, unpublished results). Digested samples were subsequently compared against mtDNA digests from Québec Arctic char (Lake Rond) and brook trout (Grand Lac du Nord).

Total DNA was extracted from the remaining 32 individuals of Lake Alain as described previously by Bernatchez et al. (1992). A 2.5-kb fragment of the mitochondrial genome encompassing the ND-5/6 region was amplified by the polymerase chain reaction (PCR) with the primers published by Cronin et al. (1993). PCR reactions were those described by these authors and the amplification conditions consisted of a first denaturation step of 95°C for 1 min followed by 35 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 3 min. Amplified DNA was digested with three additional restriction enzymes (*Ava*I, *Hae*III, and *Hin*cII) which generated diagnostic fragment patterns in the ND-5/6 segment between Arctic char and brook trout. The DNA fragments were separated on 1.2% agarose gels, run for 5 h at 85 V, stained with ethidium bromide, and photographed with polaroid under UV light.

Table 1. Allele frequencies at diagnostic loci for Arctic char (SA) and brook trout (SF). Introgressed char and trout are from Lake Alain and are represented as LA. The number of fish examined at each locus is given in parentheses.

Locus and allele	SA (20)	LA (30)	SF (24)
*IDH-3,4**			
(*100/100*)	0	1.00	1.00
(*130/130*)	1.00	0	0
*LDH-3**			
(*0[a]/0[a]*)	0	1.00	1.00
(*100/100*)	1.00	0	0
*SDH-1,2**			
(*100/100*)	0	1.00	1.00
(*40/40*)	1.00	0	0
*SOD**			
(*100/100*)	0	1.00	1.00
(*260/260*)	1.00	0	0

[a]The allele present in *S. alpinus* was assigned a value of *100*, as the *S. fontinalis* allele remained at the origin.

Results

Morphological identification

Based on gill raker counts and external features, all Lake Alain fish collected were unambigiously identified as brook trout. Gill raker counts (LGR range 6-7, UGR range 10–12, total range 15–19) were typical of brook trout populations when compared with overall ranges reported for that species (UGR range 4–7, LGR range 10–15, total range 14–22) and for Arctic char (UGR range 7–13, LGR range 12–19, total range 19–32). All 48 individuals analyzed had a square tail, vermiculated patterns on the back, dorsal, and caudal fins, a white border followed by a black stripe on lower fins, and red spots with a blue halo on the body sides. These morphological characteristics are all unambiguously typical of brook trout.

Allozymes

Identification of char and trout from Lake Alain (LA) was based on diagnostic fixed alleles between *S. alpinus* (SA) and *S. fontinalis* (SF) at the *IDH*-3,4*, *LDH*-3*, *SDH*-1,2*, and *SOD** loci (Table 1). Alleles at all loci in the char and trout from Lake Alain were found to be identical to those characteristic of *S. fontinalis*, with no evidence of shared alleles with *S. alpinus*.

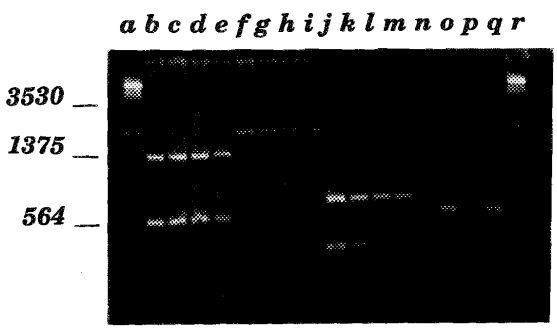
mtDNA characterization

Restriction analysis performed either over the entire molecule or on the ND-5/6 segment clearly showed that 100% of the 48 individuals characterized possessed only mtDNA of Arctic char origin (Table 2, Fig. 2). Only one mtDNA genotype was observed in Lake Alain which matched the genotype of *S. alpinus* from Lac Rond, which is also the dominant genotype observed among many populations of

Table 2. List of mtDNA fragments generated by restriction digests of Québec brook trout, Lake Rond (SA), Lake Alain fish (LA), and Québec brook trout, Grand Lac du Nord (SF). Presence (1) or absence (0) of different fragment patterns is indicated. Arctic char (SA) samples have fragment patterns typical of Arctic char from southeastern Québec. Brook trout samples (SF) are also representative. The number of fish examined is given in parentheses.

Enzyme	Fragment sizes						SA	LA	SF
Total mtDNA digests							(25)	(16)	(24)
*Acc*I	6450	5040	2080	1700	1000	500	1	1	0
	8600	3420	2980	1700	480	330	0	0	1
*Bam*HI	15520	1270					1	1	0
	14350	2450					0	0	1
*Bcl*I	8100	7850	1000				1	1	0
	8100	4060	3610	1020			0	0	1
*Bst*EII	9350	4500	2380	580			1	1	0
	7090	4850	4250	560			0	0	1
*Nco*I	7200	6700	1800	1180			1	1	0
	8980	7720	1180				0	0	1
*Nhe*I	7470	7470	1870				1	1	0
	7700	6400	3300				0	0	1
*Pst*I	16800						1	1	0
	16490	310					0	0	1
*Xba*I	13600	3200					1	1	0
	6150	5190	3200	2420			0	0	1
ND-5/6 segment							(25)	(32)	(24)
*Ava*I	2000	520					1	1	0
	1450	520	520				0	0	1
*Hae*III	600	600	560	350	350		1	1	0
	730	730	380	380	220		0	0	1
*Hin*cII	1050	900	560				1	1	0
	1050	950	520				0	0	1

Fig. 2. Photograph of an ethidium bromide stained 1.2% agarose gel showing restriction fragments produced by digestions of the ND-5/6 amplified segment with *Ava*I (lanes *b* to *i*) and *Hae*III (lanes *j* to *q*). For both enzymes, the first two lanes correspond to individuals from Lake Alain and the next two of Arctic char from Lake Rond (Québec). The four others are of brook trout from Grand Lac du Nord (Québec). Lanes *a* and *r* are lambda phage cut with *Hin*dIII and *Eco*RI (double digest).



Arctic char in eastern North America (C. Wilson, unpublished data). This genotype was also similar to the dominant genotype reported for Icelandic char (Danzmann et al. 1991*b*), with the exception of the fragment pattern for *Pst*I. The large number of fish sampled from Lake Alain and the absence of any brook trout mtDNA genotypes suggest that brook trout mtDNA has been eliminated from this population.

Discussion

Interspecific mtDNA exchanges have been reported in many animal groups including invertebrates (e.g., Powell 1983; Solignac and Monnerot 1986; Harrison et al. 1987; Aubert and Solignac 1990), amphibians (e.g., Spolsky and Uzzell 1984; Lamb and Avise 1986), reptiles (e.g., Wright et al. 1983), and mammals (e.g., Tegelstrom 1987; Lehman et al. 1991). In fish, interspecific transfer of mtDNA has been documented for several species (reviewed in Billington and Hebert 1991). However, definite cases of introgression are rare and have generally been observed at very low frequencies (Billington et al. 1988; Bernatchez et al. 1989; Avise et al. 1990; Bernatchez and Dodson 1991; Wilson and Hebert 1993). To our knowledge, complete interspecific replacement of the mitochondrial genome in natural populations has been described in only one fish species complex, *Notropis cornutus/chrysocephalus* (Dowling et al. 1989; Dowling and Hoeh 1991). Therefore,

the fixation of mitochondrial genome in Lake Alain brook trout is unusual and so far unique in salmonids.

Alternative hypotheses to introgression might explain the distinct mtDNA composition of this population. First, ancestral polymorphic mtDNA may have been retained in different taxa since they diverged from a common ancestor. Second, identity with the Arctic char mitochondrial genome may have resulted from convergent evolution. Generally speaking, it is often very difficult to distinguish which factor (or combination of factors) is responsible for the sharing of genetic variants in different taxa (discussed in Verspoor and Hammar 1991). However, alternative hypotheses to introgression can easily be ruled out in the present case. mtDNA variation observed in all available studies (Grewe et al. 1990; Danzmann et al. 1991*a*, 1991*b*; Ferguson et al. 1991; Bernatchez and Danzmann 1993; Wilson and Hebert 1993; C. Wilson, unpublished results) dealing with *S. alpinus* and *S. fontinalis* showed that no mtDNA genotypes are shared by both species over their range of distribution, which is inconsistent with a symplesiomorphic scenario. Secondly, net mtDNA sequence divergence between genotypes of both species is relatively high, estimated at 3.0% by Grewe et al. (1990).

This implies that even the most parsimonious scenario would require a high number of homoplastic events to explain a confusion of mtDNA variants in brook trout from Lake Alain with those of Arctic char, making the hypothesis of convergence very improbable. Therefore, there is no doubt that the mtDNA genotype observed in all brook trout from Lake Alain has been incorporated through introgression with Arctic char. Because Arctic char has never been found in any lake of the Portneuf River system despite intensive sport fishing, and biological surveys conducted over the past 30 years, it is most likely that these introgressive events are ancient and that their effect has persisted for a long time. Therefore, the present case clearly illustrates that introgressive hybridization may have a permanent effect on the genetic makeup of animal species. We are currently extending our survey to verify if introgressed populations of brook trout are also present in other river systems.

The fact that fish from Lake Alain were otherwise indistinguishable from brook trout based on morphological and allozyme criteria substantiates that nuclear introgression has not occurred or has long been diluted. Natural hybridization between sympatric Arctic char and brook trout populations in Labrador has revealed both morphological and nuclear intermediacy in the hybrids, supporting the latter scenario. It is possible that the present situation observed in char and trout from Lake Alain arose by repeated backcrossing of female hybrids with male brook trout until the Arctic char nuclear genome eventually disappeared. The lack of nuclear introgression in brook trout from Lake Alain further corroborates the hypothesis that complete interspecific replacement of the mitochondrial genome is an ancient event.

The evolutionary impact that the permanent mitochondrial replacement may potentially have on that population is directly related to the neutral or selective nature of variation between the mitochondrial genome of brook trout and that of Arctic char. Most if not all intraspecific variation detected in the mitochondrial genome is generally accepted to be neutral. This concept of mitochondrial neutrality has been extended to explain interspecific transfer of mtDNA where introgression has occurred (e.g., Tegelstrom 1987; Dowling and Hoeh 1991). It has also been reinforced by theoretical work demonstrating that mitochondrial gene flow across species boundaries is likely, unless the fitness of resulting offspring is very low (Takahata and Slatkin 1984). However, the acceptance of neutrality without further consideration of adaptibility has hampered our understanding of the relative roles of deterministic and stochastic processes as driving forces of gene exchange among different taxa (Arnold 1992). Indeed, a strict test of neutralist versus selectionist hypotheses will require an experimental approach that can substantiate predictions of differential response between introgressed and pure populations (Powers et al. 1991).

The permanent replacement of brook trout mtDNA with that of Arctic char could be selectively significant. Differences in the two mitochondrial genomes may be manifested physiologically, since several mitochondrial enzymes, central to intermediary metabolism, are partly encoded by mitochondrial genes. For example, metabolic thermosensitivity in ectotherms has been directly linked to the thermosensitivity of a mitochondrial enzyme, cytochrome oxydase (Blier and Guderley 1994). The activity of this enzyme, therefore, may reflect the organism's capacity towards thermal adaptation. Genetic differences between the mitochondrial genomes of introgressed and nonintrogressed populations could conceivably translate to differences in the metabolic capacity of the different mitochondria and, hence, their adaptiveness to environmental temperature. Indeed, the Arctic char is typically more adapted to cold environments than is brook trout, as exemplified by its more northern distribution and lower thermal optimum (Beamish 1980; Johnson 1980). In such a case, we can hypothesize that under low temperature constraints, there is a selective advantage imparted towards introgressed brook trout in having the mitochondrial genome of Arctic char. We are currently testing this prediction by experimentally assessing differences in thermosensitivity between introgressed and pure brook trout individuals at the biochemical, cellular, and organism levels of organization.

Clearly, experimentation is needed to determine if the fixation of Arctic char mtDNA in this population has resulted from deterministic (selection) or stochastic (historical) events. Together, experimentation and observation at several levels could potentially provide new insights into selection of both nuclear and mitochondrial gene flow.

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