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# 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 (Québec, Canada)

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## Abstract

**A phylogeographic analysis of mitochondrial DNA variation was performed in order to test the hypothesis that north-eastern North America has been postglacially recolonized by two races of rainbow smelt *Osmerus mordax*. This was accomplished by documenting the geographical distribution of two major mtDNA phylogenetic clades among 1290 smelt from 49 lacustrine and anadromous populations covering most of the species' native range. The data set was built by combining previously published results with those generated in this study. The two mtDNA clades showed a geographical dichotomy, independent of life-history types, whereby the more eastern populations were either fixed or largely dominated by one clade and western populations for the other. Such geographical pattern implying a phylogenetic discontinuity provided strong evidence for the persistence of smelt in two distinct glacial refugia as well as their differential postglacial dispersal. The most likely refugium for the so-called Atlantic race was the Atlantic coastal plains, whereas that of the so-called Acadian race was the exundated Grand Banks area. Patterns of postglacial dispersal interpreted from palaeogeographic events suggested that the Atlantic race recolonized northern regions about 5000 years prior the Acadian race. Both races came into contact in the St Lawrence River estuary. While gene flow has been possible, the sympatric occurrence in the estuary of anadromous populations alternatively dominated by one mtDNA clade or the other indicated that reproductive isolation mechanisms between the two races developed within this contact zone. This represents the first evidence of secondary intergradation among distinct races of aquatic organisms in an estuarine environment.**

*Keywords*: Smelt, *Osmerus*, phylogeography, mitochondrial DNA, postglacial dispersal

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## Introduction

The rainbow smelt *Osmerus mordax* represents a population complex native to watersheds of the north-western Atlantic Ocean, from northern New Jersey USA, to southern Labrador Canada (Scott & Crossman 1973). Its distribution has, however, been considerably extended by introductions during the twentieth century (Rubec 1975; Bergstedt 1983). Rainbow smelt exhibits extensive life-history diversity, being composed of anadromous and lacustrine populations (Nellbring 1989). Lacustrine populations are classified as normal- and dwarf-sized ecotypes, which characteristically differ in several ecological and morphological attributes (Lanteigne & McAllister 1983; Taylor & Bentzen 1993). Both ecotypes are found in sympatry and remain reproductively isolated, thus fulfilling the major criterion of the biological species concept (BSC, Dobzhansky 1937). Consequently, the dwarf-sized ecotype has been recognized by some as a distinct taxon, the pigmy smelt *O. spectrum* (Cope 1870; Lanteigne & McAllister 1983; McAllister 1985; Mayden *et al.* 1992), whereas phenotypic dissimilarity between the two ecotypes has been judged insufficient by others to justify their taxonomic distinction (Robins *et al.* 1991).

Further understanding of smelt population origins and

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radiation has more recently been obtained by the analysis of mitochondrial DNA variation. Two independent studies (Baby *et al.* 1991; Taylor & Bentzen 1993) provided genetic evidence for the polyphyletic origins of lacustrine dwarf and normal ecotypes which led the authors to conclude that taxonomic distinction of the two ecotypes was unwarranted (discussed in Bernatchez 1995). These studies were, however, conflicting on other issues which remained unresolved since then. Baby *et al.* (1991) identified two major mtDNA phylogenetic groups (named A and B) distinguished by six apomorphic restriction sites and diverging by a net sequence divergence estimate of 0.7%, a relatively high intraspecific value among north temperate fishes (Billington & Hebert 1991). These groupings were differentially associated with anadromous (possessing both mtDNA groups) and lacustrine populations (fixed for B group), indicating that there was a phylogenetic split between the two smelt life-history types. It remained unresolved, however, whether the apparent colonization of fresh water by only one phylogenetic group was due to some physiological advantage or instead, the result of differential recolonization of the two habitats by smelt that survived in different glacial refugia. In their study that covered a more eastern part of smelt distribution range, Taylor & Bentzen (1993) identified the same two mtDNA phylogenetic groups found by Baby *et al.* (1991). They did not, however, find any association of mtDNA lineages with either anadromous or lacustrine populations. The anadromous and lacustrine populations they surveyed were either fixed or largely dominated by mtDNA group A, whereas the more west distributed lacustrine populations surveyed by Baby *et al.* (1991) were fixed for the mtDNA group B. Taylor & Bentzen (1993) concluded that a phylogenetic distinction between both smelt life-history types was unwarranted and that the geographical variation in the distribution of the two mtDNA groups should be attributed to a sampling artefact, rather than a differential postglacial recolonization by two ancestral genetic groups. This conclusion was substantiated by the general belief that contemporary rainbow smelt populations originated from a single coastal Atlantic refugium (Behnke 1972; Rubec 1975; Legendre & Legendre 1984; Schmidt 1986; Mandrak & Crossman 1992).

There are, however, several arguments in favour of the possible postglacial recolonization of north-eastern North America by more than one genetic group of smelt. Recent phylogeographic studies in other north temperate fishes provided strong evidence for the existence of distinct glacial races that were unsuspected from available biogeographical and morphological evidence (Bernatchez & Dodson 1991; Bernatchez & Dodson 1994; Murdoch & Hebert in press; Wilson & Hebert 1996). More important, a detailed analysis of mtDNA variation of anadromous rainbow smelt from the St Lawrence River estuary (Québec, Canada) revealed that adult fish sampled in vicinity of spawning grounds on both shores of the middle estuary represented genetically distinct populations alternatively dominated by mtDNA groups A and B, despite the absence of physical barriers, apparent lack of phenotypic dimorphism (such as dwarf and normal ecotypes), and population admixture of larval stages (Bernatchez & Martin 1996; D. Pigeon, unpublished data). This suggested that these populations could belong to distinct genetic races that evolved in allopatry in glacial times. Such reproductive isolation between intraspecific groups derived from allopatric divergence have previously been documented in other fishes, namely in whitefish, *Coregonus* sp. (Bernatchez & Dodson 1990; Bernatchez *et al.* 1996).

In this paper, previously published smelt mtDNA data is combined with those generated from the analysis of new samples that extends the geographical coverage of the species over most of its native range in order to (i) test the hypothesis that north-eastern North America has been recolonized by two smelt races that used differential dispersal routes following their isolation in allopatry during Pleistocene glaciations, and (ii) test the hypothesis that the St Lawrence River estuary represents a zone of secondary intergradation where the presumed glacial races remained reproductively isolated. Evidence for the existence of two glacial races would be provided by the demonstration of a dichotomy in the geographical distribution of both mtDNA groups, independent of ecotypic differentiation among populations. Finally, a modified biogeographical hypothesis for the postglacial dispersal of the species is proposed.

## Materials and methods

### Samples

First, mtDNA data published by Baby *et al.* (1991), Taylor & Bentzen (1993) and Bernatchez & Martin (1996) was combined. This resulted in the analysis of 1077 fish representing 39 populations from the eastern distribution range of smelt. An additional 213 fish from 10 localities were added, covering western and southern parts of the species native range (Table 1). The inclusion of introduced populations was deliberately avoided, such as those from the Laurentian Great Lakes area (Mandrak & Crossman 1992), although the possibility that some of the chosen native sites have been stocked with nonindigenous smelt cannot entirely be ruled out.

### Analysis

Analytical procedures for previously published data are provided in the original references. Briefly, these three studies involved the use of RFLP analysis performed over

**Table 1** Sampling sites, life history types (N, lacustrine normal-sized; D, lacustrine dwarf-sized; A, anadromous), sample sizes, relative frequency distribution of mtDNA groups A and B, number of haplotypes, and nucleon diversity index (*h*) for 49 native rainbow smelt populations

| Population | Form | Coordinates | *n* | A | B | hapl | h | Reference |
|---|---|---|---|---|---|---|---|---|
| Champlain L. | N | 44°54′, 73023′ | 20 | 0.00 | 1.00 | NA | NA | this study |
| Muskrat L. | N | 45°38′, 77°06′ | 17 | 0.00 | 1.00 | NA | NA | this study |
| Richelieu R. | N | 45°06′, 73°14′ | 24 | 0.00 | 1.00 | NA | NA | this study |
| Memphremagog L. | N | 45°00, 72°14′ | 24 | 0.12 | 0.88 | NA | NA | this study |
| Winnipesaukee L. | N | 43°35′, 71°18′ | 24 | 0.96 | 0.04 | NA | NA | this study |
| Town R. | A | 42°32′, 71°00′ | 20 | 0.95 | 0.05 | NA | NA | this study |
| Squam L. | N | 43°45′, 71°30′ | 24 | 1.00 | 0.00 | NA | NA | this study |
| Hudson R. | A | 41°30′, 74°00′ | 21 | 0.90 | 0.10 | NA | NA | this study |
| Great Bay | A | 43°05′, 70°54′ | 21 | 0.57 | 0.43 | NA | NA | this study |
| Heney L. | D | 46°02′, 75°55′ | 18 | 0.00 | 1.00 | NA | NA | E. Taylor ( pers. comm.) |
| Perchaude L. | D | 46°31′, 72°58′ | 24 | 0.00 | 1.00 | 7 | 0.70 | Baby *et al.* (1991) |
| Vert L. | N | 48°58′, 69°16′ | 12 | 0.00 | 1.00 | 3 | 0.51 | Baby *et al.* (1991) |
| Kénogami L. | N | 48°20′, 71°23′ | 5 | 0.00 | 1.00 | 1 | 0.00 | Baby *et al.* (1991) |
| Kénogami L. | D | 48°20′, 71°23′ | 24 | 0.00 | 1.00 | 8 | 0.70 | Baby *et al.* (1991) |
| Beaumont | A | 46°50′, 71°01′ | 36 | 0.75 | 0.25 | 13 | 0.73 | Baby *et al.* (1991) |
| Ouelle R. | A | 47°25′, 70°02′ | 36 | 0.81 | 0.19 | 14 | 0.75 | Baby *et al.* (1991) |
| Carleton | A | 48°06′, 66°08′ | 36 | 0.75 | 0.25 | 24 | 0.95 | Baby *et al.* (1991) |
| Miramichi R. | A | 47°10′, 65°00′ | 36 | 0.88 | 0.22 | 22 | 0.83 | Baby *et al.* (1991) |
| Rivière-du-Loup | A | 47°50′, 69°32′ | 36 | 0.83 | 0.17 | 14 | 0.69 | Bernatchez & Martin (1996) |
| Île Verte | A | 48°03′, 69°16′ | 36 | 0.86 | 0.14 | 12 | 0.71 | Bernatchez & Martin (1996) |
| Matane | A | 48°51′, 67°32′ | 36 | 0.70 | 0.30 | 13 | 0.73 | Bernatchez & Martin (1996) |
| Grande-Rivière | A | 48°24′, 64°30′ | 36 | 0.78 | 0.22 | 19 | 0.83 | Bernatchez & Martin (1996) |
| Miguasha | A | 48°04′, 66°18′ | 36 | 0.70 | 0.30 | 24 | 0.91 | Bernatchez & Martin (1996) |
| Caraquet | A | 47°47′, 64°57′ | 36 | 0.72 | 0.28 | 17 | 0.87 | Bernatchez & Martin (1996) |
| Forestville | A | 48°44′, 69°05′ | 36 | 0.92 | 0.08 | 15 | 0.83 | Bernatchez & Martin (1996) |
| Baie Comeau | A | 49°13′, 68°09′ | 36 | 0.92 | 0.08 | 11 | 0.78 | Bernatchez & Martin (1996) |
| Beauport | A | 46°52′, 71°11′ | 36 | 0.17 | 0.83 | NA | NA | Bernatchez & Martin (1996) |
| Cap-Brûlé | A | 47°06′, 70°43′ | 30 | 0.17 | 0.83 | 12 | 0.74 | Bernatchez & Martin (1996) |
| Saint-Siméon | A | 47°50′, 69°53′ | 33 | 0.27 | 0.73 | 13 | 0.87 | Bernatchez & Martin (1996) |
| Saint-Fulgence | A | 48°27′, 70°54′ | 35 | 0.06 | 0.94 | 11 | 0.71 | Bernatchez & Martin (1996) |
| Conne R. | A | 47°52′, 55°45′ | 17 | 1.00 | 0.00 | 5 | 0.58 | Taylor & Bentzen (1993) |
| Gambo R. | A | 48°46′, 54°14′ | 16 | 1.00 | 0.00 | 2 | 0.53 | Taylor & Bentzen (1993) |
| Margaree R. | A | 46°20′, 61°05′ | 20 | 0.90 | 0.10 | 15 | 0.96 | Taylor & Bentzen (1993) |
| East R. | A | 46°09′, 63°00′ | 22 | 0.77 | 0.23 | 9 | 0.61 | Taylor & Bentzen (1993) |
| Musquodoboit H. | A | 44°47′, 63°09′ | 21 | 1.00 | 0.00 | 5 | 0.71 | Taylor & Bentzen (1993) |
| Annapolis R. | A | 44°45′, 65°31′ | 19 | 0.95 | 0.05 | 12 | 0.87 | Taylor & Bentzen (1993) |
| Penobscot R. | A | 44°34′, 69°12′ | 25 | 0.96 | 0.04 | 10 | 0.78 | Taylor & Bentzen (1993) |
| Grand L. | N | 44°54′, 63°35′ | 16 | 1.00 | 0.00 | 4 | 0.47 | Taylor & Bentzen (1993) |
| Lochaber L. | N | 45°25′, 62°02′ | 17 | 1.00 | 0.00 | 3 | 0.58 | Taylor & Bentzen (1993) |
| Lochaber L. | D | 45°25′, 62°02′ | 37 | 1.00 | 0.00 | 9 | 0.82 | Taylor & Bentzen (1993) |
| Skiff L. | D | 45°50′, 67°33′ | 20 | 1.00 | 0.00 | 6 | 0.57 | Taylor & Bentzen (1993) |
| North L. | D | 45°50′, 67°44′ | 31 | 0.94 | 0.06 | 10 | 0.81 | Taylor & Bentzen (1993) |
| Utopia L. | D | 45°09′, 66°46′ | 36 | 1.00 | 0.00 | 7 | 0.56 | Taylor & Bentzen (1993) |
| Utopia L. | N | 45°09′, 66°46′ | 31 | 1.00 | 0.00 | 5 | 0.56 | Taylor & Bentzen (1993) |
| Onawa L. | D | 45°23′, 69°22′ | 32 | 1.00 | 0.00 | 9 | 0.66 | Taylor & Bentzen (1993) |
| Onawa L. | N | 45°23′, 69°22′ | 11 | 1.00 | 0.00 | 2 | 0.18 | Taylor & Bentzen (1993) |
| Floods P. | D | 45°19′, 68°26′ | 18 | 1.00 | 0.00 | 8 | 0.79 | Taylor & Bentzen (1993) |
| George L. | D | 45°06′, 69°40′ | 30 | 1.00 | 0.00 | 7 | 0.59 | Taylor & Bentzen (1993) |
| Green L. | D | 44°34′, 68°34′ | 25 | 0.00 | 1.00 | 4 | 0.19 | Taylor & Bentzen (1993) |

the entire mtDNA molecule with 12 restriction enzymes. Baby *et al.* (1991) and Bernatchez & Martin (1996) used the same enzymes, among which nine were common to the 12 by Taylor & Bentzen (1993). This allowed the comparisons of all data sets. The proportion of haplotypes belonging to either mtDNA groups A and B in each population was compiled as well as their nucleon diversity indices (Nei 1987).

Total DNA from new samples was purified from ethanol-preserved tissues as described in Bernatchez *et al.* (1992). As many samples were of poor quality, we did not perform a detailed RFLP analysis of the entire mtDNA molecule. Instead, a 2.4-kb segment of the molecule encompassing the ND5 and ND6 subunits of the NADH dehydrogenase was amplified by the polymerase chain reaction (PCR) using the primers C-Leu-3 and C-Glu designed by Park *et al.* (1993). The amplified segment was digested with two enzymes (*Apa*I and *Dde*I) that generate diagnostic restriction sites for the two mtDNA groups A and B. Technical procedures of amplification, restriction digests and electrophoresis are detailed in Bernatchez *et al.* (1995). This analysis was sufficient to assess the geographical distribution and the proportion of the two mtDNA groups among populations and therefore, test the hypothesis for the existence of two glacial races. However, it precluded finer investigations of intrapopulation diversity and local population differentiation which have been fully addressed in previous studies.

## Results

The combined studies screened 1290 fish from 27 anadromous and 22 lacustrine (11 dwarf and 11 normal) populations covering most of the native range of rainbow smelt (Table 1). Populations analysed previously were generally highly polymorphic, with a mean of 10 haplotypes (range = 1–24) per population and a mean haplotype diversity (*h*) of 0.675 (range 0.00–0.95). These studies resolved 218 different haplotypes which all clustered into the two major mtDNA groups A and B. Both mtDNA groups were equally polymorphic, as shown by their respective nucleon diversity index of 0.862 and 0.821. Both mtDNA groups A and B were observed among the additional 213 fish analysed in the present study (Table 1).

Globally, the two mtDNA groups showed a strong geographical pattern of distribution, independent of life-history types (Fig. 1). Group A was fixed or largely dominated (75% or more) among the most eastern populations whereas group B dominated the most western ones. The St Lawrence River middle estuary was the only

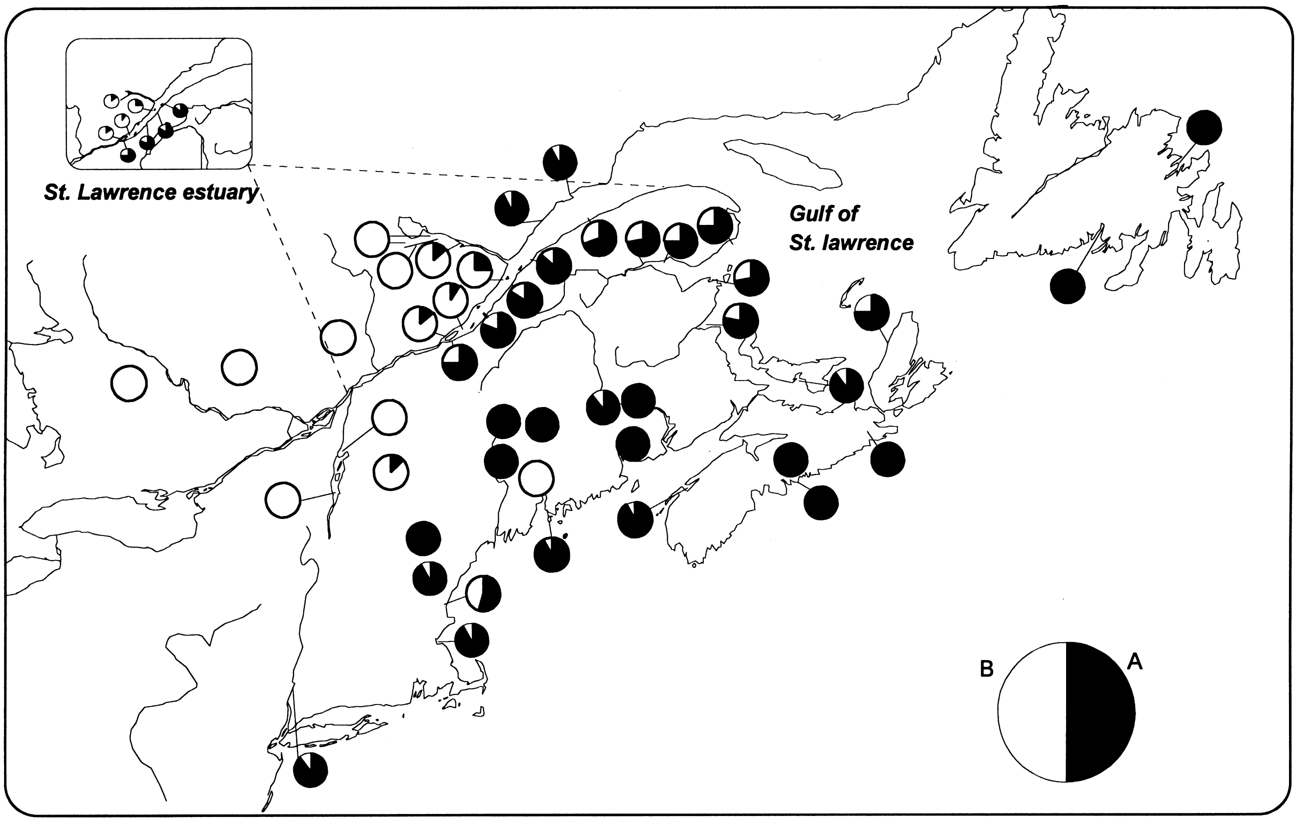

**Fig. 1** Frequency distribution of mtDNA groups A and B among 49 native rainbow smelt populations.

site where populations alternatively dominated by either group A or B were found in sympatry.

The geographical pattern of distribution of the two mtDNA groups differed among lacustrine and anadromous populations (Fig. 2, Fig. 3). The geographical dichotomy in the genetic composition of freshwater populations was determined by the Appalachian mountains which separate the St Lawrence River from other Atlantic watersheds (Fig. 2a). This separation followed a longitudinal trend, as illustrated by the steep cline in the frequency distribution of mtDNA group B which strongly shifted around the 71th parallel (Fig. 2b). An exception to this was the Green L. population, which was fixed for mtDNA group B despite its eastern distribution.

There was no obvious longitudinal pattern in the genetic composition of anadromous populations smelt, except that populations dominated by the B group were only found in the St Lawrence River middle estuary, all others being highly dominated or fixed for the A group (Fig. 3a). The extent of admixture of A and B groups within those latter populations varied latitudinally along the coastline. This was illustrated by the plot of a nucleon diversity index estimated from the frequency of A and B groups for populations dominated by the A group as a function of their latitudinal rank (Fig. 3b). Thus, most populations within the estuary and Gulf of St Lawrence had nucleon diversity values above 0.25. Values dropped sharply in populations outside the Gulf of St Lawrence, and increased again among more southern populations.

The extent of admixture of mtDNA groups A and B was very different between lacustrine and anadromous populations. Nineteen out of 22 lacustrine populations were fixed for either A or B group, whereas the two groups intermixed among 25 out of 27 anadromous popu-

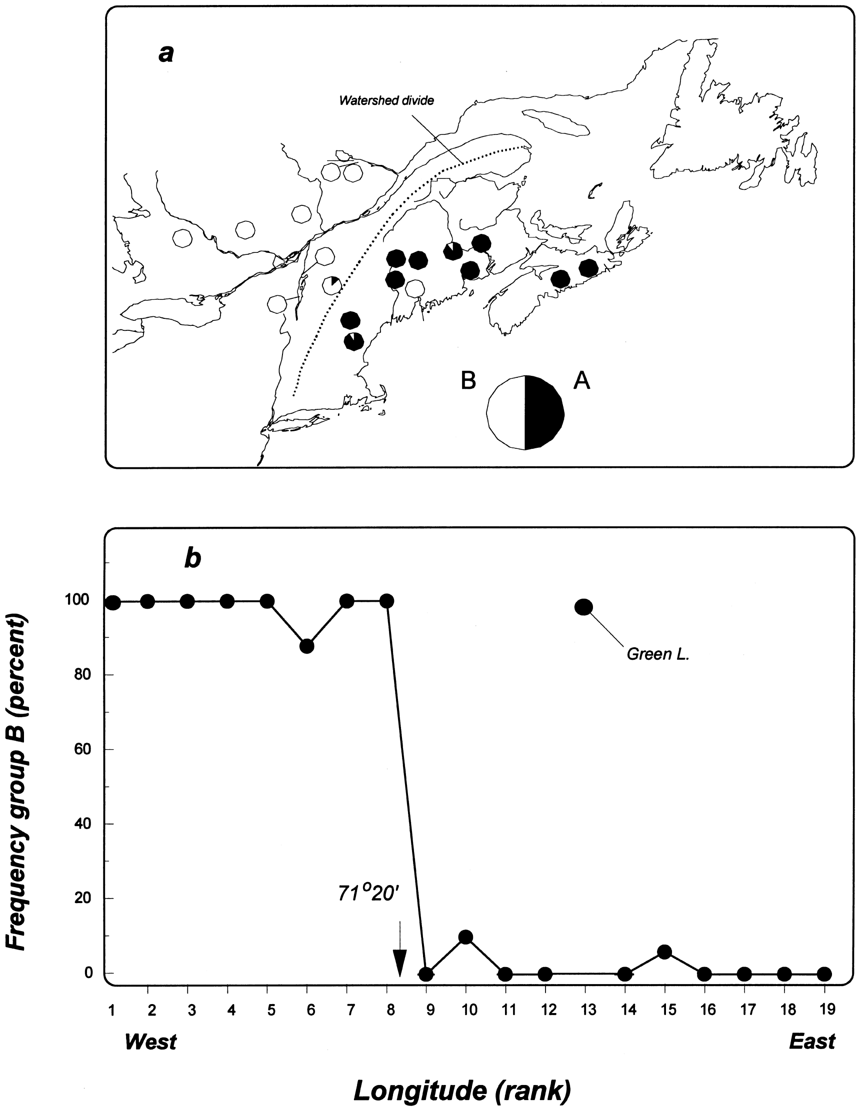

**Fig. 2** (a) Frequency distribution of mtDNA groups A and B among rainbow smelt from 19 lacustrine collecting sites (b) frequency distribution of mtDNA group B among lacustrine rainbow smelt populations as a function of their relative ranked longitude.

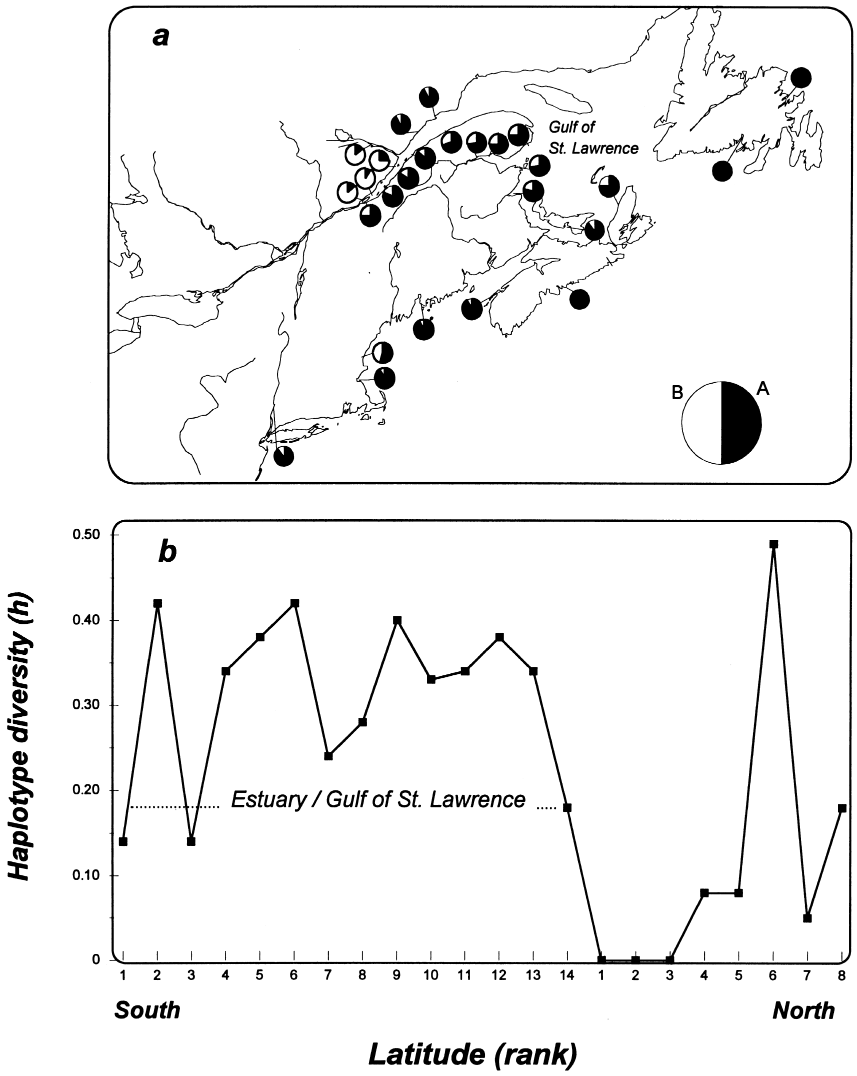

**Fig. 3** (a) Frequency distribution of mtDNA groups A and B among native anadromous rainbow smelt from 27 sampling sites; (b) nucleon diversity index (*h*) estimated from the proportion of mtDNA groups A and B among anadromous rainbow smelt populations dominated by the group A as a function of their relative ranked latitude within and outside the estuary and Gulf of St Lawrence.

lations. This difference in admixture of mtDNA groups between the two life-history types was highly significant ($\chi^2 = 25.99$, d.f. = 2, $P < 0.0001$). The extent of admixture was also quantified by the estimate of an interpopulation gene diversity index ($G_{ST}$, Nei 1987) based on the composition of A and B groups. The overall $G_{ST}$ value among lacustrine populations was 0.96, a value more than twice higher than that of 0.40 computed for anadromous populations.

## Discussion

### *Evidence for two genetic races of rainbow smelt*

The distribution analysis of two phylogenetically distinct mtDNA groups throughout the native range of smelt revealed a strong geographical dichotomy independent of life-history types, whereby the more eastern populations were either fixed or largely dominated by one group and western populations for the other. This was best exemplified by lacustrine populations which showed an almost fixed longitudinal difference between populations found in the St Lawrence River watershed and others draining directly to the Atlantic basin, independently of whether they were of dwarf- or normal-sized ecotypes. Such a phylogeographic pattern implying a phylogenetic discontinuity concomitant with a strong spatial partitioning can best be explained by an allopatric origin of two monophyletic groups (Avise 1989). The alternative hypothesis implying a single origin for smelt and differential stochastic lineage extinction of phylogenetically distinct mtDNA genotypes according to longitude appears much less parsimonious and untenable in the present case. These observations thus provide strong evidence for the persistence of smelt in two distinct glacial refugia during the Pleistocene glaciations events, as well as their differential postglacial dispersal. Evidence for the existence of two genetic races is in contrast with earlier hypotheses of a single post-Pleistocene origin of rainbow smelt based on biogeographicalal data (Rubec 1975; Legendre & Legendre 1984; Schmidt 1986; Mandrak & Crossman 1992) and morphological analyses (Behnke 1972). The case of smelt thus adds to the growing evidence that, in terms of understanding patterns of intraspecific genetic diversity in fishes, we still are at the basic step of identifying the most important natural assemblages of populations (e.g. Birmingham & Avise 1986; Billington & Hebert 1991; Bernatchez & Dodson 1994; Bernatchez & Osinov 1995; Dodson *et al.* 1995; Richardson & Gold 1995; Wilson & Hebert 1996; Murdock & Hebert in press). The fact that all three life-history types were observed among populations that were alternatively fixed (lacustrine dwarf and normal ecotypes) or largely dominated (anadromous) by distinct phylogenetic mtDNA groups depending on their geographical location re-emphasized that phenotypic diversity of rainbow smelt populations has a polyphyletic origin (Baby *et al.* 1991; Taylor & Bentzen 1993). This study also supports Taylor & Bentzen's (1993) conclusions that there is no phylogenetic distinction between freshwater and anadromous populations. Finally, it reinforces the view that replicate radiation of life-history types adapted to occupy distinct ecological niches is a common feature among members of the north temperate fish fauna (Hindar *et al.* 1986; McPhail 1993; Taylor & Bentzen 1993; Bernatchez *et al.* 1996).

### *Biogeographical hypothesis of origins and postglacial dispersal*

Patterns of genetic diversity along with current knowledge of the Wisconsinian and Holocene palaeogeographic history of north-eastern North America suggests a modified biogeographical hypothesis for the origins and distribution of contemporary rainbow smelt populations. The mtDNA groups that characterized the two genetic races diverged by a net sequence divergence estimates of 0.7%. A mitochondrial 'molecular clock' for salmoniforms has been estimated at 1% divergence per million years (Smith 1992). If applicable to smelt, the two races last shared a common ancestor 700 000 years ago, well before the beginning of the Wisconsinian glaciation. The fact that both mtDNA groups were fixed among all but three freshwater populations from different watersheds suggested that the two races remained completely isolated until the last glacial retreat. The nucleon diversity within both smelt mtDNA groups was higher that reported for most north temperate freshwater and anadromous fishes (Billington & Hebert 1991; but see Murdock & Hebert in press). In fact, the values observed for smelt (0.82 and 0.86) were more in the range of those usually reported among species from more southern latitudes that were not, or only partially, glaciated during the Pleistocene. This suggests that unlike several other northern fishes, rainbow smelt did not suffer major reduction of mtDNA genetic diversity due to population bottlenecks and reduced habitat availability during the last glacial advances (Billington & Hebert 1991).

While there is little doubt that smelt survived Pleistocene glaciations within two geographical isolates, locating these refugia is complicated by the fact that they must have been outside the contemporary distribution range of smelt which was completely glaciated several times. Furthermore, there are still debates around the precise extent of glacial advances which makes the existence of potential refugia contentious (e.g. Dyke & Prest 1987; Piper *et al.* 1990). Whatever the exact locations of refugia, the absence of natural occurrence of smelt in inland waters, such as the Laurentian Great Lakes (Mandrak & Crossman 1992), makes it impossible that the species survived in the Mississipian glacial refugium. Clearly, both smelt refugia must have been associated with unglaciated coastal waters east of the Appalachian mountains.

It is most likely that smelt surviving during the Pleistocene were anadromous since anadromy is the ancestral life-history form for smelt, whereas the lacustrine form was postglacially derived, as for many salmonids (Benhke 1972; Scott & Crossman 1973). This implies that unlike freshwater fishes, smelt did not have to be strictly confined to freshwater refugia. Freshwater habitat was only essential for reproduction which may have occurred either in tributaries or bottoms of lakes or large rivers. It is likely that suitable habitat for smelt survival existed along the exundated Atlantic coastal plain that extended from Georges Bank (41° latitude:) to North Carolina (36 ° latitude). At the Wisconsinian glacial maximum, coastal plains extended 100 km seaward and cool climates extended southward to North Carolina which was covered by spruce-pine forests at the end of Wisconsinian glacial advance, 18 000 years ago (Schmidt 1986). Namely, the possibility that Georges Bank may have acted as a refugium for smelt is reinforced by evidence for the persistence of large freshwater streams due to melting caused by the relatively mild climate that characterized the area, even during glacial maxima (Schmidt 1986). These may have provided suitable spawning habitat for smelt. Reproductive habitat may also have been available in more southern parts of the Atlantic coastal plains, as evidenced by the Pleistocene persistence of several north temperate freshwater and anadromous fishes, such as lake whitefish, *Coregonus clupeaformis* (Bernatchez & Dodson 1991), walleye, *Stizostedion vitreum* (Billington *et al.* 1992), lake trout, *Salvelinus namaycush* (Wilson & Hebert 1996), and brook charr, *S. fontinalis* (R.G. Danzmann *et al.* unpubl. data). Its overall more western distribution, and the possibility of inland dispersal northward from this refugium via the Hudson River valley (see below), indicate that the smelt genetic race characterized by the mtDNA group B survived along the Atlantic coastal plains, including Georges Bank. Because of this location, and for continuation with previous studies, the name of 'Atlantic race' should be kept for identifying this group of smelt.

The refugium for the second genetic race must have been north of the Atlantic coastal plains. It has been hypothesized by some from palaeogeographic evidence that the continental shelf has been entirely glaciated during the Wisconsinian maximum, apparently making the possibility of a north-eastern refugium unlikely (Piper *et al.* 1990). This, however, appears untenable from a biogeographical point of view. There are several fishes for which the contemporary distribution range cannot readily be explained as a result of dispersal from a southern refugium. For instance, relict landlocked Arctic charr (*S. alpinus*) populations in northern New England and eastern Canada implies the species' persistence in a north-eastern refugium (Schmidt 1986). Survival in a north-eastern refugium must also be inferred to explain the actual distribution of freshwater copepods in northern New England (Stemberger 1995). Finally, a mtDNA phylogeographic analysis of the lake whitefish identified a north-eastern genetic race (named Acadian) which was diagnostically distinct from a more southern Atlantic race (Bernatchez & Dodson 1991). The most likely location for this refugium where smelt associated with mtDNA group A survived was the exundated Grand Banks, near Newfoundland, which are now part of the submerged continental shelf (Schmidt 1986). It is noteworthy that anadromous populations fixed for group A were only found in adjacent locations of Newfoundland and south-eastern Nova Scotia. To follow with the identification of the lake whitefish race from the same region, the name of 'Acadian race' should be used for the smelt that persisted in that area.

### *Dispersal routes and recolonization of contemporary distribution range*

Table 2 lists the major palaeogeographic events from which we can infer a postglacial dispersal hypothesis for both the Atlantic and Acadian races of smelt. The retreat of ice began 18 000 years ago, marking the end of the Wisconsinian glaciation. Northward dispersal, however, must have been very limited until 13 000 years ago due to glaciers still covering most of the continent, and to the arctic oceanic conditions that prevailed in the Goldthwait Sea, precursor to the Gulf of St Lawrence (Pagé 1992; de Vernal *et al.* 1993). At that time, lowlands of coastal Maine, New Hampshire and New Brunswick became ice-free and were inundated by marine intrusions that provided an opportunity for smelt to disperse inland (Larson & Stone 1982; Schmidt 1986). The local predominance of mtDNA group A indicates that the Acadian race was likely the first and most abundant to recolonize this area, although the presence mtDNA group B (such as in Green L.) indicates that it was also recolonized by the Atlantic race. Marine intrusions retreated 12 000 years ago, establishing the age of landlocked smelt populations in this area. Most of Nova Scotia also deglaciated about 13 000 years ago which made its colonization possible. Southward coastal dispersal of the Acadian race was also possible at that time.

During that same period, inland dispersal of the Atlantic race was potentially feasible due to the development of ice-damned Lake Vermont (13 000–12 000 years ago) in the Hudson River valley. However, there are reasons to believe that smelt did not use this dispersal route. During its so-called Belleville-Fort Ann phase (12 500–12 000 years ago), Lake Vermont connected to lake Iroquois (precursor of lake Ontario). This should have provided ample possibility for smelt dispersal into the Laurentian Great Lakes system which they apparently never recolonized naturally (Mandrak & Crossman 1992). It is possible that northward dispersal of smelt has been limited by cold water temperatures, approaching lower arctic conditions, that prevailed at this time in Lake Vermont (McPhail 1963; Lacasse & Magnan 1993).

It is likely not until 12 000 years ago, with the intrusion of the Champlain Sea, that the Atlantic race dispersed northward via the Hudson River valley. The Champlain Sea developed due to breaking of the ice damn that persisted near Quebec City and separated Lake Vermont from

**Table 2** Major palaeogeographic events related to the postglacial dispersal of rainbow smelt. Data compiled from Prest 1970, Larson and Stone 1982, Fulton and Andrews 1987

| Time (years ago) | Event |
|---|---|
| 18 000 | Maximum advance of the Wisconsinian glaciation: exundated Atlantic coastal plains, George Bank and Grand Banks |
| 13 000–12 000 | Marine invasion of coastal Maine, New Hampshire and New Brunswick |
| 12 900–12 200 | Connections between proglacial Laurentian Great Lakes and the Hudson River valley via the Mohawk River outlet |
| 12 500–12 000 | Connection of Lake Vermont with proglacial lake Iroquois during the Belleville-Fort Anne phase |
| 12 000–9800 | Development of Champlain Sea and connections with the Hudson River and the Goldwaith Sea |
| 9800–7800 | Development of Laflamme Sea, connection with the St Lawrence River estuary |

the Goldthwait Sea. It covered lowlands surrounding the St Lawrence River, Ottawa River and Champlain Lake, reaching an altitude of about 210 m above today's sea level (Rubec 1975). Environmental conditions of the Champlain Sea were likely favourable for smelt survival, with surface salinity varying between 5 and 20‰. depending on time and location, and surface water temperature averaging 10–12 °C in August (Hilaire-Marcel 1981). Freshwater runoffs draining to the Champlain Sea may also have provided suitable spawning habitats (Rubec 1975). Indeed, the presence of smelt in the Champlain Sea was confirmed by findings of bone remnants in the sea deposits (McAllister *et al.* 1988). The Champlain Sea retreated about 9800 years ago which probably establishes the age of lacustrine populations in the St Lawrence River, Ottawa River and Champlain Lake system. The fact that smelt possessing mtDNA group A (Acadian race) has not been observed among those populations indicates that, unlike reported in all previous studies (Delisle & Veilleux 1969; Scott & Crossman 1973; Rubec 1975; Legendre & Legendre 1984; Underhill 1986; Mandrak & Crossman 1992), smelt did not recolonize this area by dispersal from the Goldwaith Sea despite its direct connections to the Champlain Sea. The most likely explanation for this is that environmental conditions, such as high salinities (30‰. at the surface) and cold water temperatures (averaging 4 °C in August), that prevailed in the Goldthwait sea until 10 000 years ago (de Vernal *et al.* 1993) limited smelt dispersal via this route. In fact, it is probably not until 8000–7500 years ago that the Acadian race was able to invade the estuary and upper Gulf of St Lawrence. Evidence for this was provided by the fixation of mtDNA group B (Atlantic race) among smelt from lacustrine populations of Lake St Jean area, such as Lake Vert and Lake Kénogami (Table 1). The colonization of these lakes was only possible during the development of the brackish Laflamme Sea which had direct connections with the St Lawrence estuary, and inundated the area between 9800 and 7800 years ago. The absence of mtDNA group A in these lakes is therefore suggestive that the Acadian race penetrated into the St Lawrence estuary following the retreat of Laflamme Sea.

### *Secondary contact and reproductive isolation in the St Lawrence estuary*

Around 7800 years ago, sea levels and oceanographic conditions in the estuary and Gulf of St Lawrence became pretty much the same as today (Fulton & Andrews 1987; de Vernal *et al.* 1993). This may be the time when both the Atlantic and Acadian races of smelt could come into contact in the St Lawrence River estuary. As illustrated by the admixture of both mtDNA groups A and B among anadromous populations, gene flow was possible between both races, which allowed the dispersal of the Atlantic race from the St Lawrence River estuary into the Gulf of St. Lawrence. On the other hand, the sympatric occurrence of anadromous populations alternatively dominated by one mtDNA group or the other in the St Lawrence River middle estuary despite the absence of physical barrier to gene flow, and population admixture at young life-history stages indicate that reproductive isolation mechanisms between the two races developed within this contact zone (Bernatchez & Martin 1996). This represents to our knowledge the first evidence of a secondary intergradation among distinct races of aquatic organisms within an estuarine environment. This situation however, resembles those reported previously in freshwater fishes, such as whitefish, *Coregonus* sp. (Bernatchez & Dodson 1990; Bernatchez *et al.* 1996), and brown trout, *Salmo* sp. (Giuffra *et al.* 1996), for which reproductive isolation has been documented among sympatric or parapatric populations originating from diagnosable genetic races still capable of hybridizing. In contrast, extensive admixture following secondary contact of distinct races without apparent restriction to gene flow has been reported in other species, namely bluegill sunfish *Lepomis macrochirus* (Avise & Saunders 1984), walleye *Stizostedion vitreum* (Billington *et al.* 1992), and lake trout *Salvelinus namaycush* (Wilson & Hebert 1996). The elucidation of mechanisms responsible for differential patterns in the extent of achievable reproductive isolation among races of different species represents a challenging avenue of research that may increase our understanding of species formation in fishes.

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