

Genetic structure and relationships among anadromous and landlocked populations of rainbow smelt, *Osmerus mordax*, Mitchill, as revealed by mtDNA restriction analysis

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Larvae of anadromous rainbow smelt originating in various spawning tributaries are retained in the St Lawrence estuary. We proposed that these smelt represent one population genetically differentiated from adjacent populations characterized by geographically distinct larval retention areas. We also analysed four landlocked populations to evaluate the phylogenetic basis of dwarf and normal-sized phenotypes and their relation to anadromous smelt. A phylogenetic distinction was revealed between anadromous and landlocked smelt, with only one of the two mtDNA phylogenetic groups of anadromous fish observed among landlocked smelt. Significant geographical heterogeneity in the distribution of mtDNA genotypes was observed among landlocked smelt, suggesting that dwarfism in smelt may be polyphyletic in origin. St Lawrence smelt were genetically identical but distinct from adjacent populations, supporting the proposition that population genetic structure reflects the number of larval retention zones rather than spawning sites.

Key words: rainbow smelt; mtDNA; population structure; member/vagrant hypothesis; dwarf-normal phenotypes.

I. INTRODUCTION

The member/vagrant hypothesis proposes that the number of populations of a fish species is defined at the early life-history stages and is a function of the number of geographical settings within which the species' life-cycle may be completed (Sinclair, 1988). An extension of this hypothesis is that in cases where the distribution of larvae is different from the distribution of spawning sites, population structure reflects the number of larval retention zones rather than that of spawning sites. In Québec waters, anadromous rainbow smelt, *Osmerus mordax* (Mitchill), have been proposed to form three regional populations based on variations in morphometrics, meristics and the occurrence of parasites (Fréchet *et al.*, 1983*a, b*). One population is associated with the St Lawrence estuary where, following spring spawning, larvae from a number of spawning tributaries remain in a maximum turbidity zone by using active vertical tidal migrations (Dodson *et al.*, 1989; Laprise & Dodson, 1989). These observations are consistent with the propositions of the member/vagrant hypothesis. Thus, the first objective of the present study was to obtain genetic evidence, based on the analysis of restriction fragment length polymorphisms in mitochondrial DNA (mtDNA), that smelt from two spawning tributaries situated at the upstream and downstream end of the St Lawrence retention zone represent one population genetically differentiated from two adjacent populations characterized by geographically distinct larval retention areas.

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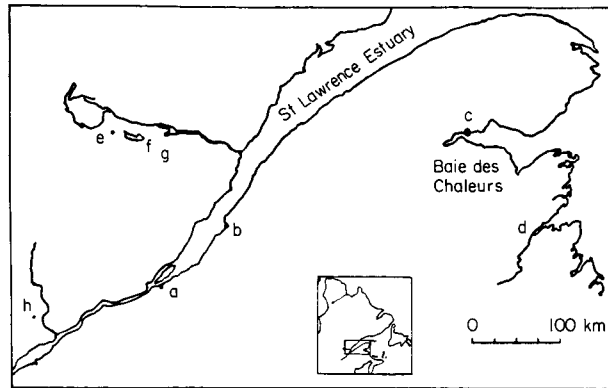


FIG. 1. Location of sampling sites for populations of anadromous (a–d) and landlocked (e–h) smelt. (a) Beaumont (Ruisseau de l'église), St Lawrence estuary; (b) Rivière Ouelle, St Lawrence estuary; (c) Rivière Carleton, Baie des Chaleurs; (d) Miramichi River, New Brunswick; (e) Lac Vert, allopatric, normal-sized phenotype; (f) Lac Kénogami, sympatric, normal-sized phenotype; (g) Lac Kénogami, sympatric, dwarf phenotype; (h) Lac à la Perchaude, allopatric, dwarf phenotype.

Many salmonid species are characterized by the occurrence in both sympatry and allopatry of dwarf and normal-sized phenotypes specialized for different life-histories. This is also the case among landlocked smelt and the dwarf phenotype from four eastern North American lakes has been awarded species status (pygmy smelt, *Osmerus spectrum* Cope, 1870; Lanteigne & McAllister, 1983). In the case of lake whitefish, *Coregonus clupeaformis* (Mitchill), Bernatchez & Dodson (1990) demonstrated that the presence of sympatric pairs of dwarf and normal-sized whitefish in the Allegash basin, northern Maine, resulted from the secondary contact of two monophyletic mtDNA phylogenetic assemblages of whitefish that evolved allopatrically in separate refugia during the last glacial events. Thus, the second objective of the study was to test the hypothesis that dwarf and normal-sized landlocked smelt belong to different mtDNA phylogenetic assemblages.

II. MATERIALS AND METHODS

Anadromous and landlocked smelt were obtained in spring 1990 and winter 1991 from four spawning tributaries and three lakes, respectively (Fig. 1, Table I). Mature ovaries or livers were transported on wet ice and mtDNA extracted within 10 days of capture. mtDNA was purified according to Bernatchez *et al.* (1988). Aliquots of mtDNA were digested separately with six hexameric (*Apa* I, *Bgl* I, *Bst*E II, *Dra* I, *Eco*R V, *Pst* I), four multi-hexameric (*Ava* I, *Ban* I, *Ban* II, *Hinc* II) and two multi-pentameric restriction endonucleases (*Ava* II, *Nci* I). Mitochondrial DNA fragments were electrophoretically separated on 0.8 or 1.2% agarose gels for 4 h at 75 V.

Ethidium bromide staining revealed restriction fragments when mtDNA was obtained in sufficient quality and quantity. In cases of low or poor-quality yield, mtDNA was transferred to nylon membranes under alkaline conditions and hybridized with a purified radiolabelled total smelt mtDNA probe prepared with the multiprime (Amersham) DNA labelling reaction. Membranes were autoradiographed using an intensifying screen for 5–16 h at -70°C .

Size estimates of fragments were made by running simultaneously into the agarose gel digests of phage lambda DNA with *Hind* III and *Eco*R I-*Hind* III double digest. Fragments

TABLE I. Sample locations, ecotype, sample sizes, number of mtDNA genotypes and estimated nucleon diversity for smelt populations sampled in Northeast North America

| Population | Type | Sample size | Number of genotypes | Nucleon diversity |
|---------------|--------------|-------------|---------------------|-------------------|
| (a) Beaumont | Anadromous | 36 | 13 | 0.73 |
| (b) Ouelle | Anadromous | 36 | 14 | 0.75 |
| (c) Carleton | Anadromous | 36 | 24 | 0.95 |
| (d) Miramichi | Anadromous | 36 | 22 | 0.83 |
| (e) Vert | Normal/fresh | 12 | 3 | 0.51 |
| (f) K enogami | Normal/fresh | 5 | 1 | 0.00 |
| (g) K enogami | Dwarf/fresh | 24 | 8 | 0.70 |
| (h) Perchaude | Dwarf/fresh | 24 | 7 | 0.70 |

Normal/fresh = normal-sized landlocked smelt; dwarf/fresh = dwarf landlocked smelt. Letters (a)–(h) correspond to sampling sites illustrated in Fig. 1.

less than 200 base pairs in length were not scored. Distinct single endonuclease patterns were identified by a specific letter. Each fish was assigned a multi-letter code which described its composite mtDNA genotype.

Sequence divergence between genotypes was estimated according to Upholt's (1977) fragment method. The resulting distance matrix was clustered by UPGMA (Sneath & Sokal, 1973) using the average linkage algorithm of the SAS statistical package. Intra-population diversity of mtDNA lineages was estimated with Nei & Tajima's (1981) nucleon diversity index. As a measure of inter-population genetic diversity, we computed the maximum likelihood estimate of the average number of nucleotide substitutions per restriction site between populations (Nei, 1987). Site differences between genotypes were estimated from changes in fragment patterns. Geographical heterogeneity in the frequency of genotypes among populations was analysed using the likelihood ratio chi-squared test (G^2 , SAS statistical package).

III. RESULTS

A total of 71 mtDNA genotypes were observed among the 209 smelt analysed. mtDNA diversity varied between the populations sampled, lowest values occurring among normal-sized landlocked forms and highest values in the anadromous Miramichi and Carleton populations (Table I). The UPGMA phenogram revealed two major mtDNA assemblages separated by a mean sequence divergence of 0.83% (Fig. 2). Among the 111 fish belonging to group A, 45.9% (51 specimens) were characterized by one mtDNA genotype (genotype 71, Fig. 3). Among the 98 fish belonging to group B, 53.6% (52 specimens) were characterized by one mtDNA genotype (genotype 27, Fig. 3). The remaining genotypes of group A and B were no more than eight mutation-step variants of the group's most common genotype.

A major phylogenetic split was observed between anadromous and landlocked forms (Fig. 4). All landlocked smelt (65 fish) were characterized by group B genotypes. In contrast, 77% (111 fish) of anadromous smelt were characterized by group A genotypes, the remaining 23% (33 fish) characterized by group B genotypes (Fig. 2). Therefore, dwarf smelt were genetically more similar to landlocked normal-sized smelt than to anadromous populations (Fig. 4).

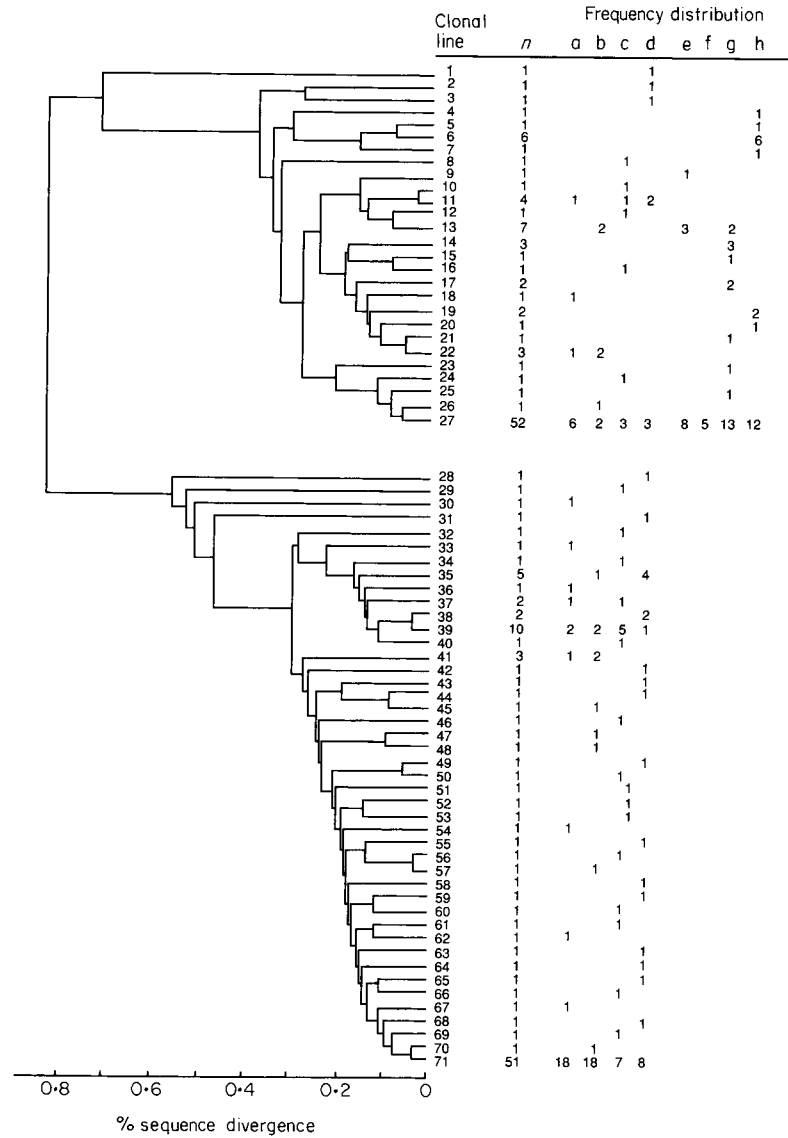


FIG. 2. UPGMA dendrogram clustering the distance matrices of sequence divergence estimates calculated for rainbow smelt, and the geographical distribution of genotypes. n = absolute abundance; (a-h) sampling sites illustrated in Fig. 1.

Significant geographical heterogeneity in the distribution of mtDNA genotypes among landlocked smelt was revealed by analysing the distribution of the most common freshwater genotypes 6, 13, 27 and all remaining genotypes pooled as a fourth group ($P=0.002$, d.f. = 9). Pairwise comparisons of the distributions of all mtDNA genotypes among landlocked groups (K enogami normal-sized fish excluded owing to small sample size) revealed that the dwarf forms of Lac   la Perchaude differed from Lac Vert normal phenotypes ($P=0.008$, d.f. = 7) and K enogami dwarf phenotypes ($P<0.001$, d.f. = 12). No difference was observed between the latter two populations ($P=0.19$, d.f. = 8). Significant geographical

| <i>Apa</i> I | | <i>Ava</i> I | | <i>Ava</i> II | | <i>Ban</i> I | | <i>Ban</i> II | | <i>Bgl</i> I | |
|--------------|----|--------------|---|---------------|----|--------------|---|---------------|----|--------------|---|
| 71 | 27 | 71,27 | | 71 | 27 | 71,27 | | 71 | 27 | 71,27 | |
| 7900 | — | 2700 | — | 2100 | — | 4000 | — | 2850 | — | 5000 | — |
| 6300 | — | 2500 | — | 1700 | — | 4000 | — | 2100 | — | 4300 | — |
| 3800 | — | 2350 | — | 1650 | — | 3500 | — | 1500 | — | 3400 | — |
| 1800 | — | 2250 | — | 1650 | — | 1900 | — | 1350 | — | 1450 | — |
| 1600 | — | 1450 | — | 1600 | — | 1200 | — | 900 | — | 1000 | — |
| 1025 | — | 1200 | — | 1500 | — | 470 | — | 700 | — | 1000 | — |
| 750 | — | 1175 | — | 1400 | — | 370 | — | 680 | — | 580 | — |
| 740 | — | 830 | — | 1175 | — | 350 | — | 680 | — | — | — |
| 700 | — | 750 | — | 760 | — | 340 | — | 630 | — | — | — |
| | | 710 | — | 725 | — | | | 600 | — | — | — |
| | | 550 | — | 725 | — | | | 580 | — | — | — |
| | | 350 | — | 620 | — | | | 580 | — | — | — |
| | | | | 525 | — | | | 550 | — | — | — |
| | | | | 420 | — | | | 550 | — | — | — |
| | | | | 380 | — | | | 440 | — | — | — |
| | | | | 370 | — | | | 440 | — | — | — |
| | | | | 370 | — | | | 300 | — | — | — |
| | | | | 310 | — | | | 300 | — | — | — |
| | | | | 300 | — | | | — | — | — | — |
| | | | | 300 | — | | | — | — | — | — |
| | | | | 250 | — | | | — | — | — | — |
| | | | | 200 | — | | | — | — | — | — |

| <i>BstE</i> II | | <i>Dra</i> I | | <i>EcoR</i> V | | <i>Hinc</i> II | | <i>Nci</i> I | | <i>Pst</i> I | |
|----------------|---|--------------|---|---------------|---|----------------|----|--------------|----|--------------|---|
| 71,27 | | 71,27 | | 71,27 | | 71 | 27 | 71 | 27 | 71,27 | |
| 6600 | — | 9300 | — | 14000 | — | 4200 | — | 3100 | — | 9500 | — |
| 5600 | — | 3500 | — | 2800 | — | 4200 | — | 2500 | — | 5500 | — |
| 4300 | — | 2250 | — | | | 3400 | — | 2200 | — | 1850 | — |
| | | 1150 | — | | | 1750 | — | 1550 | — | | |
| | | 820 | — | | | 1750 | — | 1075 | — | | |
| | | | | | | 1600 | — | 900 | — | | |
| | | | | | | 1100 | — | 750 | — | | |
| | | | | | | 680 | — | 570 | — | | |
| | | | | | | | | 570 | — | | |
| | | | | | | | | 520 | — | | |
| | | | | | | | | 490 | — | | |
| | | | | | | | | 490 | — | | |
| | | | | | | | | 430 | — | | |
| | | | | | | | | 390 | — | | |
| | | | | | | | | 380 | — | | |
| | | | | | | | | 320 | — | | |
| | | | | | | | | 250 | — | | |
| | | | | | | | | 210 | — | | |
| | | | | | | | | 200 | — | | |

FIG. 3. Fragment size estimates (in base pairs) of the restriction fragment patterns (produced following digestion with each of 12 enzymes) characterizing the most common mtDNA genotype observed in smelt mtDNA assemblage A (genotype 71) and those characterizing the most common mtDNA genotype observed in smelt mtDNA assemblage B (27).

heterogeneity in the distribution of mtDNA genotypes among anadromous populations was revealed by analysing the distribution of the most common anadromous genotypes 27, 39, 71 and all remaining genotypes pooled as a fourth group ($P=0.0009$, d.f. =9). Pairwise comparisons of the distribution of all mtDNA genotypes among anadromous populations revealed significant differences between all sites except for the two St Lawrence spawning tributaries (Table II). This population structure was also reflected by the clustering of nucleotide divergence among anadromous and freshwater populations (Fig. 4).

IV. DISCUSSION

A phylogenetic distinction was revealed between anadromous and landlocked smelt, with only one of the two phylogenetic groups of anadromous fish observed

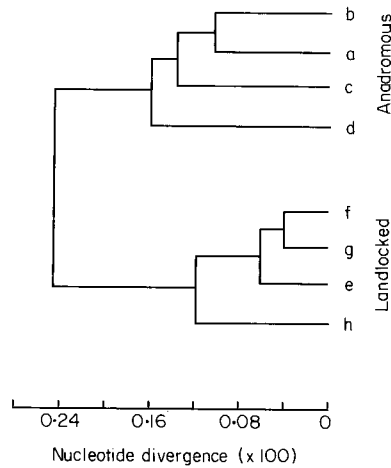


FIG. 4. UPGMA dendrogram clustering anadromous and landlocked populations of rainbow smelt according to the distance matrix resulting from maximum likelihood estimation of the average number of nucleotide substitutions per site between populations (nucleotide divergence). (a-h) Sampling sites illustrated in Fig. 1.

TABLE II. Probability level of the likelihood ratio chi-squared test used to analyse in a pairwise fashion the geographical heterogeneity in the frequency of all mtDNA genotypes observed among four samples of anadromous smelt

| | (a) Beaumont | (b) Ouelle | (c) Carleton | (d) Miramichi |
|---------------|--------------|------------|--------------|---------------|
| (a) Beaumont | | 0.15 | 0.05 | 0.02 |
| (b) Ouelle | 20 | | 0.01 | 0.01 |
| (c) Carleton | 31 | 34 | | 0.02 |
| (d) Miramichi | 29 | 31 | 41 | |

Probability level, P , presented above the diagonal, degrees of freedom presented below the diagonal. All comparisons are significantly different except for the Beaumont/Ouelle comparison.

among landlocked smelt. Thus, anadromous and landlocked populations of smelt appear far more genetically distinct than such populations of salmonids (Wilson *et al.*, 1985; Ståhl, 1987; Guyomard, 1989). We may only speculate whether the colonization of fresh water by one phylogenetic group is due to some physiological advantage in fresh water or simply the result of differential recolonization of the two habitats.

Dwarf landlocked smelt possess many more unique type B mtDNA genotypes than do the normal-sized landlocked forms suggesting some genetic difference between dwarf and normal-sized smelt. However, heterogeneity in the distribution of mtDNA genotypes among landlocked smelt conformed more to sampling site than phenotype, indicating that, unlike the case of lake whitefish (Bernatchez & Dodson, 1990), dwarfism may be polyphyletic in origin. The fact that dwarf landlocked populations from Lakes Kénogami and Perchaude are genetically distinct

supports this proposition. In such a case, not all small-sized smelt may be considered as pygmy smelt (Lanteigne & McAllister, 1983). The highly significant discrimination of pygmy smelt from normal-sized landlocked and anadromous smelt based on mensural and meristic data (Copeman, 1977) suggests that more divergent dwarf lineages than found in this study may exist in other eastern North American lakes.

Smelt from two spawning tributaries in the St Lawrence estuary are genetically identical but significantly differentiated from adjacent populations characterized by geographically distinct larval retention areas. St Lawrence estuary smelt thus appear to form one genetic stock. This stock is associated with one common estuarine retention zone, with no evidence of retention of larvae in the vicinity of the spawning tributaries (Ouellet & Dodson, 1985). In addition, these observations are consistent with the absence of any evidence of homing to spawning tributaries of smelt from the St Lawrence estuary based on tagging studies (Magnin & Beaulieu, 1965). The Miramichi River is also characterized by typical estuarine circulation permitting retention of larval smelt at the head of the estuary (Rogers, 1940), suggesting that all smelt spawning in the Miramichi system form one genetic stock. The results of Fréchet *et al.* (1983a, b) revealed that smelt from four spawning tributaries in the Baie des Chaleurs were different from the St Lawrence group but similar to one another indicating the existence of another regional genetic stock. The pattern of genetic structure exhibited by anadromous smelt is consistent with the proposition of the member/vagrant hypothesis that the number of populations of a fish species is defined at the early life-history stages and is a function of the number of larval retention zones.

We thank Françoise Colombani, Caroline Berger, Marie Giroux, Hélène Gouin, Anne-Marie Savaria, Raymond Bossé, Roger Picard and Paul Beauchemin for assistance in the field and the laboratory. This project was funded by Le Ministère du Loisir, de la Chasse et de la Pêche du Québec, FCAR (Québec) and NSERC (Canada). This paper is a contribution to the program of GIROQ (Groupe Interuniversitaire de Recherches Océanographiques du Québec).

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