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Incongruent estimates of population differentiation among brook charr, *Salvelinus fontinalis*, from Cape Race, Newfoundland, Canada, based upon allozyme and mitochondrial DNA variation

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We determined the amount and temporal stability of genetic differentiation among brook charr sampled from five rivers on Cape Race, Newfoundland, with an electrophoretic analysis of 42 protein coding loci. Fish from four of these rivers were analysed for restriction fragment length polymorphisms in mitochondrial DNA (mtDNA). A single mtDNA clone was observed in all rivers sampled, except one, where 47% of fish were from a different and relatively divergent clone (0·31% sequence divergence). In contrast, Cape Race brook charr show large amounts of genetic differentiation at six enzyme coding loci; Nei's genetic distance ranged between 0·020 and 0·048. This differentiation is relatively stable as no significant differences in allele frequencies were detected between fish sampled from two rivers over two consecutive years. The most divergent population based on protein polymorphism is not that with two mtDNA clonal lineages. In contrast to the commonly held view, mtDNA analyses do not necessarily provide greater resolution of population structure than allozyme analyses.

Key words: population structure; life-history; mtDNA; allozymes; salmonids; brook charr; *Salvelinus fontinalis*.

I. INTRODUCTION

An understanding of genetic population structure is essential for the determination of genetically meaningful management units and the conservation of genetic resources (Ryman & Utter, 1987). Despite the large number of studies on natural populations, studies of unexploited populations are rare. Therefore, it is often difficult to attain a clear picture of population structure and past evolutionary divergence because many populations have been manipulated through practices such as introductions.

Non-anadromous brook charr, *Salvelinus fontinalis* (Mitchill), on Cape Race Newfoundland, provide the opportunity to examine genetic differentiation among unexploited populations that have been separated since the late-Wisconsinan glaciation or longer (Rogerson, 1981). Despite proximity of adjacent rivers (2–3 km), these populations show marked life-history variation in growth, age and size at maturity, egg size, fecundity and relative allocation of tissue to gonads (Hutchings, 1990). This variation cannot be ascribed solely to environmental heterogeneity because Cape Race covers a relatively small geographical area and physical heterogeneity is minimal between rivers. We determined the amount and the temporal stability of genetic differentiation among fish sampled from five rivers

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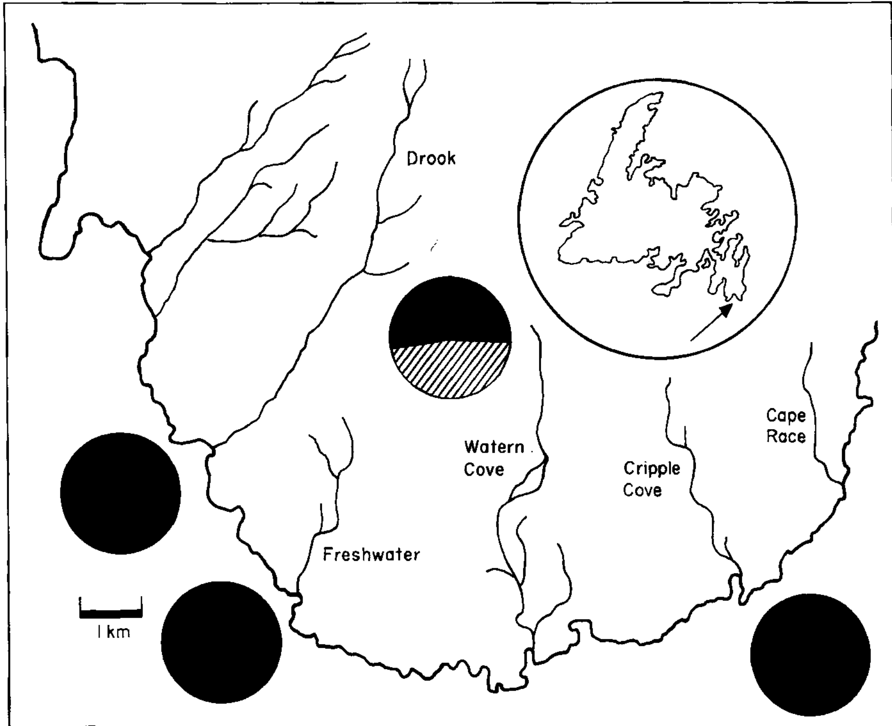

FIG. 1. mtDNA clonal diversity in brook charr from four rivers on Cape Race, Newfoundland. See text for explanation. Black, clone 1; hatching, clone 6.

with an electrophoretic analysis of 42 protein-coding loci. Fish from four of these rivers were analysed for restriction fragment length polymorphisms in mitochondrial DNA (mtDNA).

II. MATERIALS AND METHODS

Brook charr were collected from Drook, Freshwater, Watern Cove, Cripple Cove and Cape Race Rivers on Cape Race, Newfoundland (an area of 120 km^2 bounded by 53°16'W, 46°45'N, 53°04'E and 46°38'S; Fig. 1) in October 1987. Freshwater and Cripple Cove Rivers were resampled in October 1988. Fish were frozen at −20° C and stored until July and August 1990 when they were analysed for polymorphisms at 42 protein coding loci. We searched for polymorphisms at the following enzymes: *N*-acetyl-beta-glucosaminidase (bGLUA; E.C. 3.2.1.30), acid phosphatase (ACP; E.C. 3.1.3.2), adenylate kinase (AK; E.C. 2.7.4.3), alcohol dehydrogenase (ADH; E.C. 1.1.1.1), creatine kinase (CK; E.C. 2.7.3.2), dipeptidase (PEPA; E.C. 3.4.-.-), glucose-6-phosphate isomerase (GPI; E.C. 5.3.1.9), beta-glucuronidase (bGUS; E.C. 3.2.1.31), glycerol-3-phosphate dehydrogenase (G3PDH; E.C. 1.1.1.8), L-iditol dehydrogenase (IDDH; E.C. 1.1.1.14), isocitrate dehydrogenase (sIDHP; E.C. 1.1.1.42), L-lactate dehydrogenase (LDH; E.C. 1.1.1.27), malate dehydrogenase (MDH; E.C. 1.1.1.37) malic enzyme (sMEP; E.C. 1.1.1.40), mannose-6-phosphate isomerase (MPI; E.C. 5.3.1.8), phosphoglucomutase (PGM; E.C. 5.4.2.2), phosphogluconate dehydrogenase (PGDH; E.C. 1.1.1.44), superoxide dismutase (SOD; E.C. 1.15.1.1), tripeptide aminopeptidase (PEPB; E.C. 3.4.-.-), and xanthine dehydrogenase (XDH; E.C. 1.1.1.20). Enzymes requiring high pH buffers (Tris glycine pH 8·5) were resolved on cellulose acetate gels (Hebert & Beaton, 1989) whereas starch gels (Leary & Booke, 1990) were used for those requiring low pH buffers (amine citrate pH 6·6).

TABLE I. Allozyme variation in brook charr collected from five rivers on Cape Race, Newfoundland

Locus	Allele	DR87	FW87	FW88	WC88	CC87	CC88	CR87
*ADH**	−100	0·299	0·453	0·386	0·784	0·926	0·945	0·118
	−29	0·701	0·547	0·614	0·216	0·074	0·055	0·882
*G3PDH**	100	0·896	0·381	0·332	0·816	0·866	0·808	0·288
	167	0·104	0·619	0·668	0·184	0·134	0·192	0·712
*IDHP-4**	100	0·478	0·833	0·851	0·432	1·000	1·000	1·000
	80	0·067	0·004	0·010	0·384	0·000	0·000	0·000
	65	0·455	0·163	0·139	0·184	0·000	0·000	0·000
*LDH-3**	100	0·209	0·269	0·282	0·795	0·000	0·000	0·905
	72	0·791	0·731	0·718	0·205	1·000	1·000	0·095
*MEP-1**	100	0·933	0·439	0·485	0·758	0·630	0·577	0·856
	50	0·000	0·014	0·005	0·005	0·359	0·423	0·144
	0	0·067	0·547	0·510	0·237	0·011	0·000	0·000
*PGM-1**	100	0·137	0·782	0·701	0·477	0·000	0·000	0·272
	null	0·863	0·218	0·299	0·523	1·000	1·000	0·728
n		67	147	101	95	142	91	153

DR, Drook; FW, Freshwater; WC, Watern Cove; CC, Cripple Cove, CR, Cape Race.

The mtDNA analysis was based on fish sampled from Drook, Freshwater, Watern Cove, and Cripple Cove Rivers in October 1990. Fifteen fish were electrofished from each river and transported to Memorial University on wet ice. Livers were removed and placed in cold sterile TEK buffer (Chapman & Powers, 1984), with the addition of 0·25 M sucrose, placed in wet ice, and shipped unfrozen to the University of Guelph. Upon arrival, mtDNA was isolated (within 48 h after death) with the rapid isolation method of Chapman & Powers (1984) and the modifications of Danzmann *et al.* (1991*b*).

Partially purified mtDNA was digested with nine hexanucleotide restriction endonucleases (*Acc* I, *Ban* I, *Ban* II, *Hin*d III, *Nco* I, *Nhe* I, *Nsi* I, *Pst* I and *Nde* I). A more extensive analysis with 58 restriction endonucleases (Danzmann *et al.*, 1991*b*) has shown that this subset detects most of the clonal variation observed so far in this species. mtDNA fragments were separated on 0·8% agarose (Boehringer, Mannheim) gels and visualized by UV irradiation after ethidium bromide staining.

III. RESULTS

Six restriction enzymes (*Ban* I, *Ban* II, *Nde* I, *Nhe* I, *Nco* I and *Pst* I) showed variation in mtDNA fragments while three (*Acc* I, *Hin*d III and *Nsi* I) produced identical patterns in all 60 individuals. These polymorphisms have been grouped into two major and divergent clonal lines (1 and 6) that show 0·31% sequence divergence (Danzmann *et al.*, 1991*a, b*). Cripple Cove, Freshwater and Drook were fixed for clone 1 (Fig. 1). In Watern Cove, 8 of 15 fish (53%) belonged to clone 1 and while the remainder (47%) belonged to clone 6.

Polymorphism was detected at *ADH**, *LDH-3**, *G3PDH**, *MEP-1**, *IDHP-4** and *PGM-1** (Table I). No significant differences in allele frequencies (contingency *G*-tests of allele counts) were detected within Cripple Cove and Freshwater Rivers between 1987 and 1988. However, fish from different rivers show significant heterogeneity in allele counts. For example, fish in Cripple Cove and Cape Race Rivers are almost fixed for alternate alleles at *ADH** and *LDH-3**. The

TABLE II. Nei's genetic identity (above diagonal) and distance ($D \times 10^{-2}$ below diagonal) among brook trout collected in 1987 and 1988 from five rivers on Cape Race, Newfoundland based on an electrophoretic analysis of 42 loci

	DR87	FW87	FW88	WC88	CC87	CC88	CR87
DR87	0·045	0·973	0·975	0·980	0·980	0·978	0·970
FW87	2·651	0·059	0·999	0·977	0·966	0·966	0·974
FW88	2·427	0·013	0·060	0·977	0·967	0·967	0·978
WC88	2·039	2·328	2·373	0·059	0·970	0·969	0·974
CC87	1·984	3·475	3·405	3·057	0·020	0·999	0·953
CC88	2·171	3·443	3·372	3·157	0·007	0·022	0·953
CR87	2·899	2·651	2·180	2·679	4·822	4·815	0·023
n	67	147	101	95	142	91	153

Average expected heterozygosities are given on the diagonal.
DR, Drook; FW, Freshwater; WC, Watern Cove; CC, Cripple Cove; CR, Cape Race.

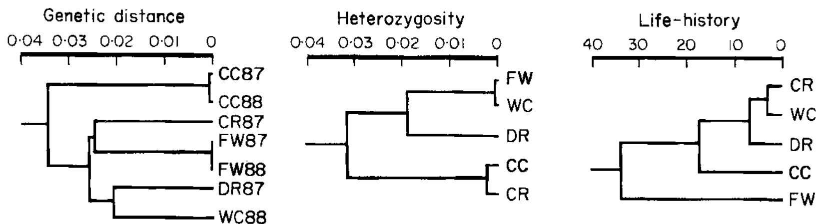

FIG. 2. Dendrograms (average linkage method) of the relationships among brook charr from Cape Race, Newfoundland collected in two years in genetic distance (Nei's), average heterozygosity, and life-history (growth rate, GSI, egg size, fecundity from Hutchings, 1990). Life-history variables were corrected to unit equivalence with growth rate which ranged from 93 to 139 mm, i.e. 46 units. The maximum difference between any two measures for the other variables was, therefore, divided by 46. This value was then used as a denominator to standardize the absolute difference in measurements between any two populations by dividing the difference by the denominator for that life-history measure. Rivers: DR, Drook; FW, Freshwater; WC, Watern Cove; CC, Cripple Cove; CR, Cape Race.

Nei's genetic distances (Nei, 1978) range between 0·02 and 0·048 based on 42 loci (Table II). The dendrogram constructed from these values (UPGMA) was composed of six branches (Fig. 2). A hierarchical series of *G*-tests based on numbers of alleles at the six polymorphic loci was used to determine which branches were significant. For example, at node 3, the allele counts of Cape Race-87 were compared with the combined counts of Freshwater-87 and Freshwater-88. All branches of the dendrogram were significant except those composed of collections from the same river (Cripple Cove-87 *v*. Cripple Cove-88 and Freshwater-87 *v*. Freshwater-88). The populations differed three-fold in average expected heterozygosity ranging from 2% in Cripple Cove-87 to 6% in Freshwater-88 (Table II).

IV. DISCUSSION

GENETIC POPULATION STRUCTURE

Variation at protein coding loci and restriction fragment length polymorphisms in mtDNA provide incongruent estimates of differentiation among populations and variation within populations of Cape Race brook charr. Despite the marked

heterogeneity in allele frequencies at protein coding loci among populations, the mtDNA clonal diversity is remarkably limited. For example, Freshwater and Cripple Cove Rivers differ in allele counts at all six enzyme loci but are fixed for the same mtDNA clonal line. Therefore, a genetic analysis restricted to mtDNA variation would have failed to detect the genetic diversity of Cape Race brook charr.

The paucity of mtDNA variation in brook charr from this region of Newfoundland suggests that only two major mtDNA clonal lines (clone 1 and 6) re-invaded or remained in this region of North America subsequent to the Pleistocene glacial periods. These clonal lines are the most common in natural populations of brook charr from Ontario (unpubl. obs.). Clone 1 appears to have a more northerly distribution than clone 6 (Danzmann *et al.*, 1991*a*). Re-colonizations after glacial retreat or initial colonizations of new river systems by a few founder individuals could result in random lineage extinctions. Thus, if the frequency of clone 1 was historically higher in northern parts of the range, such as Cape Race, the probability of founder events fixing this clone would be greater. Alternatively, differences in fitness between clones leading to selection against clone 6 during or following the Pleistocene glaciations could also explain the present distribution.

LIFE-HISTORY AND POPULATION DIFFERENTIATION

Empirically-based model simulations support the hypothesis that life-history variation within populations is evolutionarily stable (Charlesworth, 1980), i.e. observed reproductive strategies maximize the intrinsic rate of natural increase (Hutchings, 1990). As adaptive responses to differences in growth rate (Hutchings, unpubl. obs.), brook charr life-histories have evolved in association with marked differentiation at enzyme coding loci as evidenced by the large differences in Nei's genetic distances among populations. However, the concomitant evolution of allozyme and life-history differentiation is not evidence of a direct cause and effect relationship. Allozymes may simply serve as markers that also reflect the likelihood of greater divergence in genes associated with the expression of fitness differences. Unless allozymes are linked directly to genes that influence fitness, the observed associations are likely to be random among populations.

Congruence between similarity in life-history and average heterozygosity (but not genetic distance) has been observed among six hatchery strains of rainbow trout (Danzmann *et al.*, 1989). Therefore, we determined if the patterns of life-history differentiation among brook charr from Cape Race corresponded to the degree of genetic differentiation (Nei's genetic distance and average heterozygosity). An UPGMA dendrogram from mean life-history values (growth rate: length at the end of 3 years; GSI: standardized for a 130 mm fish; egg size: standardized to mean length of mature females; fecundity: standardized to length of mature females; Hutchings, 1990), was constructed so that similarities among rivers could be compared directly to a dendrogram of genetic distances and similarities in average heterozygosity (Fig. 2). Freshwater and Cripple Cove brook charr are the most extreme in life-history (Hutchings, 1990). Freshwater females grow slower, mature earlier at a smaller size, produce more eggs of a larger size per unit body mass, and allocate a greater proportion of body tissue to gonads than Cripple Cove females. Cripple Cove and Freshwater are also divergent for the two genetic measures; fish from these rivers cluster on different sides of the first major branch in

each dendrogram. The populations are, in fact, the most divergent in regards to average heterozygosity and second most divergent in genetic distance. However, the life-history patterns among the other populations do not correspond to similarities in heterozygosity and genetic distance. Thus, any associations between allozyme divergence and life-history is only detectable at the broadest level and may be in fact due to chance.

In contrast, the patterns of mtDNA variation do not reflect the genetic differentiation that has accompanied the divergence among rivers in life history. Brook charr from Freshwater and Cripple Cove Rivers are the most extreme in life-history but are from the same mtDNA clonal lineage. Similarly, Watern Cove is the only population composed of two clonal lineages but is not the most extreme with regards to life-history. Allozyme variation is expected to be a better marker of life-history variation because most genes affecting these traits are most likely nuclear and any nuclear variation may be in linkage disequilibrium with genes controlling life history. Furthermore, even though mtDNA has a faster nucleotide substitution rate compared to nuclear DNA (Moritz *et al.*, 1987), the mtDNA molecule will show less variation because it is approximately 180 000 times smaller than the nuclear genome in salmonids (Ohno, 1972; Li & Grauer, 1991) and shows no recombination. However, because of the scarcity of studies, it is premature to preclude mtDNA as a marker of fitness differences, especially if those fitness components have a maternal component that results from the clonal inheritance of imprinting genes.

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