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Temporal Genetic Variation of Brown Trout *Salmo trutta* L. from Vorobiev Creek (White Sea) Based on Allozyme Analysis

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Abstract—We performed an analysis of allozyme variation in brown trout from Vorobiev creek. Seventeen allozyme loci encoding glycerol-3-phosphate dehydrogenase (G3PDH), aspartate aminotransferase (AAT), malate dehydrogenase (MDH), lactate dehydrogenase (LDH), superoxide dismutase (SOD), and esterase D (EST-D) were studied. We found statistically significant differences in allele frequencies for the AAT-1,2*, G3PDH-2,3*, LDH-5*, and MDH-2* loci between brown trout samples collected in 1981–1982 and/or 1992–1995. We suggest that temporal changes of allele frequencies in brown trout from Vorobiev Creek are associated with gene drift.

Keywords: population biology, population genetics, polymorphism, population structure.

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INTRODUCTION

Brown trout (*Salmo trutta* L.) is a long-standing object of intensive population genetic studies. This interest is caused by its huge geographical range and its high ecological and morphological plasticity. In addition, there is considerable genetic differentiation between the populations from different sea basins as well as between populations within basins. Since the brown trout is a promising object of fish breeding, its gene pool undergoes significant changes caused by human activity. The White Sea is one of the few regions of the brown trout geographical range where native populations still persist, which can become an excellent object for genetic monitoring of brown trout populations. Long-term studies of brown trout genetic variation are of considerable interest, because, in addition to the information on the constant allele frequencies of protein loci in natural brown trout populations [1], there is data for the White sea basin, as well as for other basins, that there are differences in allele frequencies between samples taken in different years from a single water basin [2–4].

MATERIAL AND METHODS

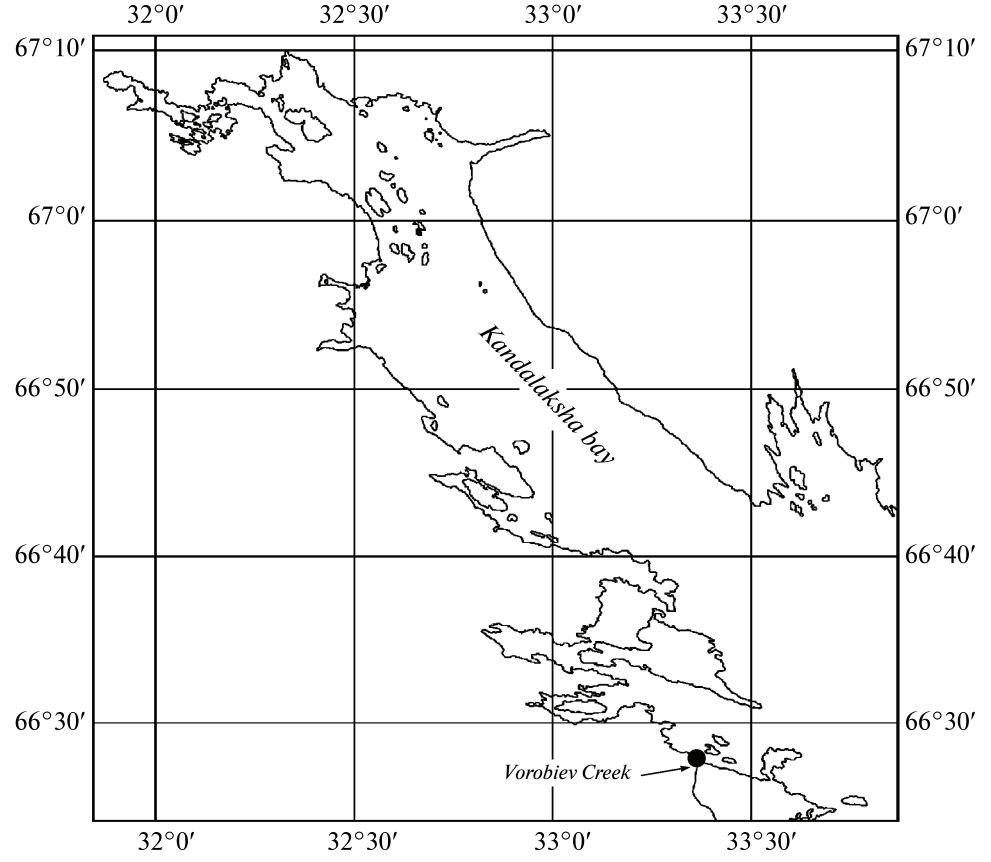
Brown trout samples were collected in June 2006 and 2007 (41 and 39 specimens, respectively) in Vorobiev Creek, a small creek ~5 km long located on the Karelia shore of the White sea (Fig. 1). Fish were caught using electrical and pole-and-lining fishing devices. Body length (AC), weight, sex, and sex gland maturity state were determined for all specimens; scales were used for age determination. The studied specimens were mostly young. Separate tissues (eyes, muscles, liver) or whole specimens were frozen at –70°C and delivered to the laboratory where they were stored at –20°C. Electrophoretic protein studies were performed in polyacrylamide gel using standard techniques [5, 6]. Allozyme loci were denoted according to the nomenclature accepted for salmonids [7] taking some recommendations into account [8].

Gene loci encoding aspartate aminotransferase (AAT), esterase D (EST-D), glycerol-3-phosphate dehydrogenase (G3PDH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), and superoxide dismutase (SOD) were studied.

Genetic interpretation of the observed variation in the studied loci was performed according to the guidelines described in the literature. For the *EST-D**, *LDH-5**, *mSOD-1**, *sSOD-2**, and *sSOD-3** loci, simple codominant inheritance was assumed. For the *AAT-1,2** loci, the model assuming equal allele frequencies for both loci was used. Calculations were performed according to [9]. For the duplicated loci *MDH-1,2**, *LDH-1,2**, and *G3PDH-2,3**, the model of inheritance assuming that only a single locus is polymorphic was adopted [10, 11].

The consistency of the observed genotypes frequencies with the Hardy-Weinberg distribution was tested using the χ^2 test. Significance of the differences of allele frequencies was estimated using Fisher's *F*-criterion [12] and the χ^2 homogeneity test in the CHIRXC program [13].

Studies of brown trout allozyme polymorphism in this river were performed earlier in the 1980s and 1990s [2, 11]. In this connection, this study of brown trout



Collection sites of brown trout samples.

from Vorobiev Creek is of considerable interest and allows us to estimate temporal changes of the brown trout populations over 20 years. Some data on the brown trout sample collected in 2006 were published earlier [14].

RESULTS AND DISCUSSION

The loci *EST-D**, *LDH-1,2**, *LDH-3,4**, *MDH-1**, *TO-1**, *TO-2**, and *TO-3** were monomorphic in the studied sample. For polymorphic loci, electrophoretic mobility of one allele was taken as 100, and the mobilities of other alleles were: *AAT-1,2*, 120, G3PDH-2,3*, 75, LDH-5* 90, MDH-2* 142.* Electrophoretic mobility of several alleles differed from that reported by A.G. Osinov and L. Bernache [11] and A.A. Makhrov et al. [2]. In addition, Osinov and Bernache [11] found *LDN-1* 0Q* allele to be present in brown trout populations from Vorobiev Creek with low frequency, whereas, according to Makhrov et al. [2] and this study, only the *LDH-1* 100* allele is present. This may be caused by different buffer systems used in these studies.

Allele frequencies of all loci were distributed according to the Hardy-Weinberg equation, except for the *AAT-1,2** ($\chi^2 = 16.25$, *d.f.* = 3, $p < 0.001$ in the 2006 sample and $\chi^2 = 8.66$, *d.f.* = 3, $p < 0.05$ in the 2007 sample). Allele frequencies of polymorphic loci in 1982–2007 are given in Table 1.

The χ^2 test comparison of brown trout samples of different years demonstrated that the heterogeneity of allele frequencies is significant for all analyzed loci (Table 1). However, the more correct and broad pairwise comparison using Fisher's F-criterion found no significant differences for many samples (Tables 2, 3).

For example, no significant differences between brown trout samples were found in the *LDH-5** locus; only the differences between the 1992 and 1994 samples are significant for the *AAT-1,2** locus; for the *MDH-2**, it is only the 2006 sample that differs from all other samples. The main statistically significant differences were found for the *G3PDH-2,3** locus between the 1981–1982 and 1992–1994 samples, i.e., within the 10 year interval. However, for the sample collected in the other 10 years (2006–2007), no significant differences were found either with the 1980s or with the 1990s samples.

Thus, although allele frequencies have changed for several loci, overall genetic parameters of the brown trout did not change during over 20 years. This suggests the absence of directed temporal changes of allele frequencies in Vorobiev Creek brown trout and

Table 1. Allele frequencies (*100) of polymorphic loci in brown trout samples from Vorobiev Creek collected in different years

Collection year	*n*	Loci			
		*AAT-1,2**	*G3PDH-2,3**	*LDH-5**	*MDH-2**
1981	60	–	0.908	0.328	0.857
1982	30	0.880	0.883	0.367	0.850
1992	99	0.879	0.995	0.237	–
1993	53	0.774	1.000	0.217	–
1994	99	0.673*	1.000	0.203	0.798*
1995	11	0.727	0.909	0.455	–
2006	41	0.780	0.988	0.351	0.611
2007	39	0.859	0.987	0.270	0.855
d.f.		6	7	7	4
χ^2		17.55	54.98	18.70	23.49
p		<0.01	<0.001	<0.05	<0.001

Note: Data on 1981 and 1982 were taken from [11]; data on 1992–1995 were taken from [2]; * $n = 25$.

Table 2. Pairwise comparison of the samples for the *AAT-1,2** (below diagonal) and *G3PDH-2,3** (above diagonal) loci using Fisher's criterion

Year	Year							
	1981	1982	1992	1993	1994	1995	2005	2007
1981		–	**	**	***	–	–	–
1982	n.d.		**	**	***	–	*	–
1992	n.d.	–		–	–	–	–	–
1993	n.d.	–	–		–	–	–	–
1994	n.d.	–	*	–		–	–	–
1995	n.d.	–	–	–	–		–	–
2005	n.d.	–	–	–	–	–		–
2007	n.d.	–	–	–	–	–	–	

Note: n.d. is no data; "–" difference is not statistical significant; * difference is significant for $p < 0.05$; ** difference is significant for $p < 0.01$; *** difference is significant for $p < 0.001$.

Table 3. Pairwise comparison of the samples for the *LDH-5** (below diagonal) and *MDH-2** (above diagonal) loci using Fisher's criterion

Year	Year							
	1981	1982	1992	1993	1994	1995	2005	2007
1981		–	n.d	n.d	–	n.d	**	–
1982	–		n.d	n.d	–	n.d	*	–
1992	–	–		n.d	n.d	n.d	n.d	n.d
1993	–	–	–		n.d	n.d	n.d	n.d
1994	–	–	–	–		n.d	–	–
1995	–	–	–	–	–		n.d	n.d
2005	–	–	–	–	–	–		*
2007	–	–	–	–	–	–	–	

Note: n.d. is no data; "–" difference is not statistical significant; * difference is significant for $p < 0.05$; ** difference is significant for $p < 0.01$.

does not support the opinion of several researchers on the existence of natural selection on certain loci for these populations [2]. We should note that the authors suggested natural selection on two loci: *MPI*, which was not studied by us, and *LDH-5**. It seems most probable that allele frequency changes in brown trout populations are caused by gene drift [15]. Given the low number of sires in this small creek, highly significant differences in allele frequencies between their descendants may be observed even in the absence of reproductive isolation [16]. In addition, migration from other rivers may also contribute to this process. For example, the *110 allele was fixed in the *G3PDH-2,3** locus in 1993–1994 but the alternative allele reappeared from 1995 on.

Therefore, our data demonstrate the absence of significant changes in allele frequencies of polymorphic loci after a decade since previous studies and corroborate the authors' opinion that allele frequency changes are caused mainly by gene drift.

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