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Combined assessment of genetic variability of *Coregonus albula* (L.) populations in Latvia based on allozymes and RAPD markers

Jelena Oreha*,

Aleksandra Morozova,

Natalja Shkute

Institute of Ecology,
Daugavpils University,
Vienības str. 13,
Daugavpils LV-5400,
Latvia

The vendace (*Coregonus albula* L.) inhabits several Latvian lakes. In Latvia this species is included into the list of specially protected species with limited use. Taking into consideration the high variability of vendace and the fact that it belongs to valuable and marketable fish, there arises a scientific interest to reveal possible reasons influencing its variability. There is no precise scientific data on the local vendace populations and its biology and this hampers the rational usage of this fish and possibilities of its reproduction in Latvian lakes.

In the present work the genetic polymorphism and the structure of vendace populations in Rāznas, Nirzas and Sventes lakes in Latvia have been defined based on the analysis of isoenzyme systems and RAPD markers. The genetic variability and population structure both within and among vendace populations have been studied. The activity of eight isoenzyme systems was studied and 16 loci were selected for genetic analysis. Eight of them were polymorphic and used for the statistical analysis. Also, 60 decanucleotide (RAPD) primers were tested. For the analysis, eight decanucleotide primers were used.

The average level of isoenzyme polymorphism in vendace populations from three lakes was 41.67%. The diversity of alleles based on allozyme data in the studied populations was approximately the same. Data of RAPD analysis showed that *Coregonus albula* populations from the studied lakes were highly polymorphic.

The results of this study indicate that the estimated intraspecific variation may be more pronounced with RAPD markers than with allozymes when the two approaches were applied to the same populations.

Key words: genetic variability, allozymes, RAPD markers, *Coregonus albula*, genetic structure, polymorphism

* Corresponding author. E-mail: jelena.oreha@du.lv

INTRODUCTION

At the beginning of the last century the vendace (*Coregonus albula* L.) was introduced into more than 30 lakes of Latvia from Peipus (Estonia) and Ladoga (Russia) lakes. In the 1930s commercial catch of vendace reached 13 tons per lake. However, in the 1990s the vendace was observed only in five Latvian lakes (Plikšs, Aleksejevs, 1998). Presently it can be found in eight Latvian lakes (unpublished data). Its share in the fishery is not large as the catch is insignificant and unstable. This species is included in the list of specially protected species with restricted use in Latvia (Regulation No. 396 of the Cabinet of Ministers of the Republic of Latvia, 14 November 2000).

Taking into consideration the high variability of vendace and the fact that it belongs to valuable and marketable fish, it is a matter of scientific interest to reveal possible reasons which influence the present alterations. The absence of precise scientific data on the local vendace populations and its biology hampers the rational usage of this fish and possibilities of its reproduction in Latvian lakes.

The research of the genetic structure of populations is necessary for the understanding of biogeographic processes occurring within these populations (Johnson, Taylor, 2004; Fraser, Bernatchez, 2008). The estimation of genetic variability is the first step in the determination of the status of this species for its long-term protection in its natural habitat. It is especially significant for the species with low population sizes, with a high risk of inbreeding and genetic drift.

Allozyme markers are widely used for the study of the genetic structure of different species populations. Thus, for example, on the basis of allozymes, spawning stocks of whitefish (*Coregonus lavaretus* L.) in one of Norwegian lakes were differentiated according to ecotypes (Næsje et al., 2004). The authors also noticed a pronounced genetic differentiation among the studied stocks; in order to avoid the loss of genetic potential of individual spawning stocks it was also recommended to build on genetic data when developing the programme of commercial catch (Næsje et al., 2004).

The screening of anonymous genome sequences (RAPD markers) provides valuable information for the study of species population structure (Parker, 1998). The authors of this method (Williams et al., 1993) have demonstrated that the RAPD analysis provides a more precise estimation of closely related populations. Since the moment of its development, the RAPD method has been widely used for the analysis of genetic variability and structure of different fish species populations (Dinesh et al., 1993; Bardakci, Skibinski, 1994; Naish et al., 1995). Recently this method has been also applied in the studies of whitefish (Bockarev, Zyikova, 2008) and salmon (Melnikova et al., 2008). It has also been shown that private DNA fragments obtained as a result of the RAPD analysis provide a possibility to develop and optimise the markers specific for these populations (Melnikova et al., 2008).

The aim of the present work is to analyse the genetic structure of *Coregonus albula* populations from Latvian lakes Nirzas, Sventes and Rāznas. The genetic analysis of vendace populations was made using allozymes and RAPD markers.

MATERIALS AND METHODS

The material was collected in autumn of 2007. Samples of vendace were taken from vendace populations of three Latvian lakes: Nirzas, Rāznas and Sventes (Table 1, Fig. 1). As the proportion of vendace in monitoring catches is small and irregular, the size of vendace samples is also relatively

Table 1. Parameters of lakes

Lake	Location	Area (ha)	Depth (m)	
			max	average
Rāznas	Ludzas region 56°23'Z; 27°54'A	5756.4	17.0	7.0
Nirzas	Rēzekne region 56°19'Z; 27°27'A	552.0	21.0	8.2
Sventes	Daugavpils region 55°51'Z; 26°21'A	734.8	38.0	7.8

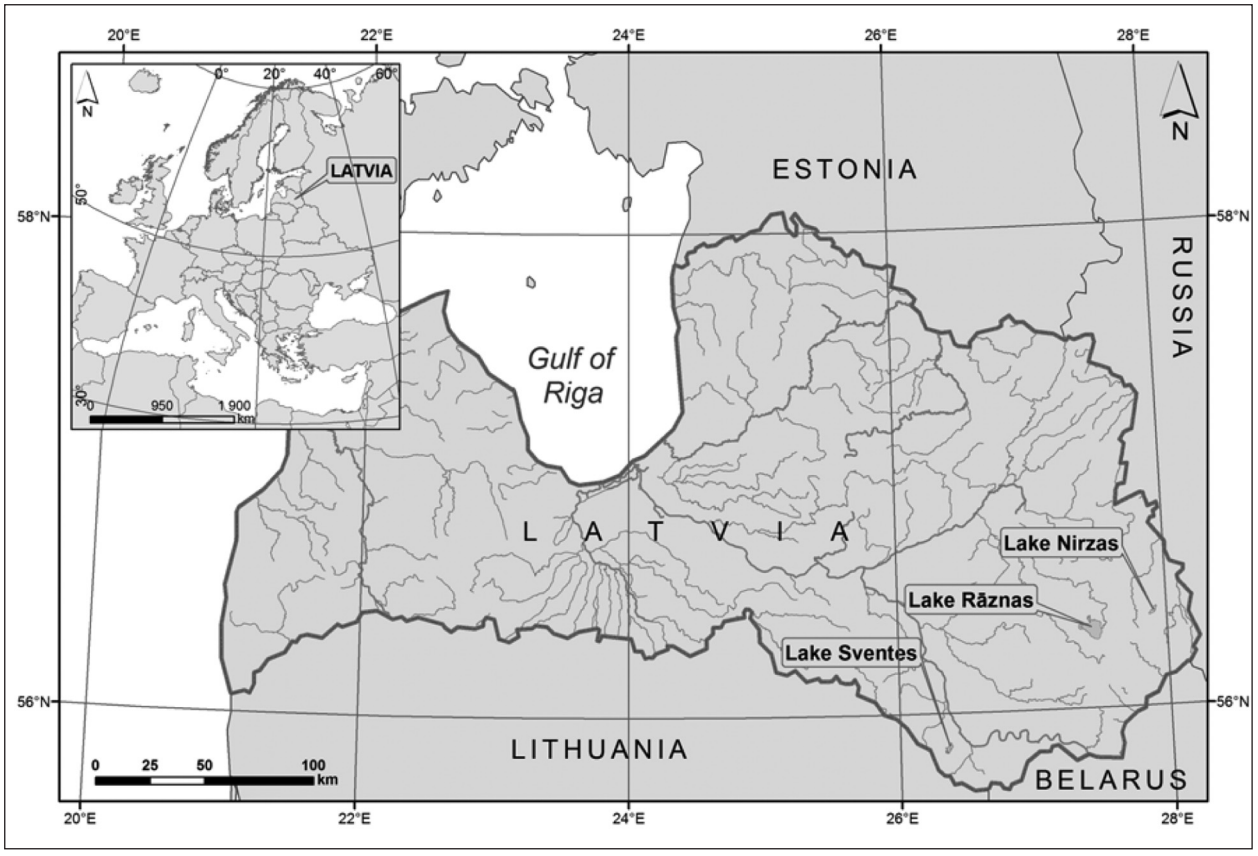

Fig. 1. The location of sampling sites of *Coregonus albula* in Latvia

small (Lake Sventes n = 9; Lake Nirzas n = 13; Lake Rāznas n = 9).

For the genetic analysis a collection of samples of liver and skeletal muscles of each individual was made. Prior to the analysis the collected material was kept under the temperature of –20 °C.

The homogenate of vendace liver and skeletal muscles has been used for the allozyme analysis. Defrosted tissues were homogenised in buffer (0.2 M Tris-HCl, pH 8.0; 0.01 mg/ml Triton X – 100; 4 mg/ml $MgCl_2$; 0.2 mg/ ml NADP (Paulauskas, Tubelytė-Kirdienė, 2002)) in ratio 1:2 (tissue: buffer).

For separation of allozymes the polyacrylamide gel of different concentration (5–10%) was used depending on analysed isoenzyme systems according to the protocol (Paulauskas, Tubelytė-Kirdienė, 2002). For separation of allozymes in the polyacrylamide gel two buffer systems: (A) Tris–H3BO3, pH 8.3–8.4 (Paulauskas, Tubelytė-Kirdienė, 2002), (B) Tris–Glicin, pH ~ 8.3 (Pobezimova et al., 2004) were used. Table 2 summarises the data of analysed isoenzyme loci, their tissue expression and used buffer systems. Histochemical staining was made according to standard methods (Korockin et al., 1977).

The loci and allelic nomenclature are denoted according to recommendations of Shaklee (1990). Abbreviations of proteins and genes which encode them were used in compliance with recommendations (Nomenclature Committee of the International Union of Biochemistry and Molecular Biology, http://www.chem.qmul.ac.uk/iupac/jcbn/).

Isoenzyme loci were identified according to the established schemes for whitefish (Vuorinen, 1984; Sendek, 2000). All alleles for each locus were ranked by electrophoretic mobility of their allozymes from the smallest to the largest. The most frequent allele was named allele A; other alleles were named according to their frequency, accordingly B, C, etc. For statistical analysis of the obtained data the computer program "GeneAlex" (Peakall, Smouse, 2006) was used.

The genetic differentiation between populations was estimated according to standard parameters of genetic diversity, as a percent of polymorphic loci ($P_{99\%}$), allele diversity and average expected heterozygosity (*He*), gene flow estima-

Table 2. Isoenzyme systems, loci, their expression in tissues and used buffer systems

Isoenzyme systems	Loci	Tissues	Buffer systems
Aspartat Aminotransferase (AAT, E.C. 2.6.1.1)	*AAT1,2*	M	A
	AAT4	L	A
Superoxide dismutase (SOD, E. C. 1.15.1.1)	*SOD1*	L	B
	SOD2	L	B
Alcohol dehydrogenase (ADH, E.C. 1.1.1.1)	*ADH*	L	A
Lactate dehydrogenase (LDH, E.C. 1.1.1.27)	*LDHB1,2*	M	A
Malic enzyme (ME, E. C. 1.1.1.40)	*ME3,4*	L	B
Malate dehydrogenase (MDH, E.C. 1.1.1.37)	*MDH3,4*	L	B
	MDH1,2	M	B
Esterase D (EstD, E.C.3.1.1.1)	*ESTD*	M(L)	A
Esterases (EST, E.C.3.1.1.-)	*EST2*	M(L)	A
	EST4	M(L)	A
Glucose-6-phosphate dehydrogenase (G6PDG, E.C. 1.1.1.49)	*G6PDH*	L	A
Glycerol-3-phosphate dehydrogenase (G3PDH E.C. 1.1.1.8)	*G3PDH1*	M	A
	G3PDH2	M	A

(M – muscle tissue, L – liver; buffer (A) Tris–H3BO3, pH 8.3–8.4 (Paulauskas, Tubelytė-Kirdienė, 2002), (B) Tris–Glicin, pH ~ 8.3 (Pobezimova et al., 2004)

tion (*Nm*), genetic distances (*D*). Homogeneity of allele frequency distribution was estimated by indexes of genetic differentiation *Fst* (Wright, 1978).

For the RAPD analysis DNA was purified from skeletal muscle tissue according to a salt-extraction method by Aljanabi et al. (1997). The extracted DNA was stored at –20 °C until the analysis. For the analysis DNA was diluted to 10 ng/µl concentration. The amplification was carried out in a thermocycler ABI 9700 programmed for 35 cycles. The RAPD-PCR reaction was performed in volume of 12 µl (PCR reaction buffer with KCl, 25 mM $MgCl_2$, 0.7U Taq DNA polymerase (recombinant from *Thermus aquaticus*, (MBI Fermentas, Vilnius, Lithuania)), 0.1% Triton X –100, 2 mM dNTP mix, template DNA).

DNA-amplified fragments were separated on 1.4% agarose gel (Agarose, Sigma) in TBE buffer (0.045 M Tris-borate and 0.001 M EDTA). The gels were run at 4.3 V/cm^{-1} for 15 min and 6.5 V/cm^{-1} for 2 h and stained with ethidium bromide. DNA fragments were visualized using a UVP Imaging system. The size of DNA fragments was assessed by comparison with GeneRulerTM 100 bp DNA Lader Plus (MBI Fermentas, Vilnius, Lithuania) using VisionWorksLS Software.

In order to confirm the reproducibility of polymorphic fragments the analysis of samples was made in three replications. Each series of amplifications included a negative control (PCR reaction without the addition of DNA). For the analysis of three vendace populations only clear, well reproducible and informative DNA fragments were selected. The presence (1) and absence (0) of fragments in each sample were assessed and the data was assembled in a binary matrix. DNA fragments with the same mobility were accepted as identical. The genetic parameters of the populations were calculated from the band presence–absence matrix by the Computer program POPGENE v 1.31 (Yeh et al., 1999).

Genetic differentiation among the populations was estimated using Nei's gene diversity statistics (Nei, 1973; 1978). The total gene diversity (*Ht*), the gene diversity within populations (*Hs*) and the genetic differentiation coefficient (*Gst = (Ht-Hs)/Ht*) were also calculated. The level of gene flow among these populations was estimated as *Nm = (1/GST -1)/4* (Slatkin, Barton, 1989).

RESULTS

Allozymes

In the studied isoenzyme systems 20 isoenzyme loci were established (Table 3). Nine of them were monomorphic in all samples. Other nine loci also were polymorphic in all studied samples. There was established one polymorphic locus in system of superoxide dismutase in vendace samples from

Table 3. Polymorphism of enzymatic loci in the studied vendace populations

Isoenzyme systems	Loci	Sventes	Nirzas	Rāznas
Aspartat Aminotransferase (AAT, E.C. 2.6.1.1)	*AAT1,2*	2M	2M	2M
	AAT4	1P	1P	1P
Superoxide dismutase (SOD, E. C. 1.15.1.1)	*SOD1*	1M	1P	1P
	SOD2	1M	–	–
Alcohol dehydrogenase (ADH, E.C. 1.1.1.1)	*ADH*	1M	1M	1M
Lactate dehydrogenase (LDH, E.C. 1.1.1.27)	*LDHB1,2*	2M	2M	2M
Malic enzyme (ME, E. C. 1.1.1.40)	*ME3,4*	2M	2M	2M
Malate dehydrogenase (MDH, E.C. 1.1.1.37)	*MDH3,4*	2M	2M	2M
	MDH1,2	2P	2P	2P
Esterase D (EST D, E.C. 3.1.1.1.)	*EST D*	1P	1P	1P
Esterases (EST, E.C.3.1.1.-)	*EST2*	1P	1P	1P
	EST4	1P	1P	1P
Glucose-6-phosphate dehydrogenase (G6PDG, E.C. 1.1.1.49)	*G6PDH*	1P	1P	1P
Glycerol-3-phosphate dehydrogenase (G3PDH E.C. 1.1.1.8)	*G3PDH1*	1P	1P	1P
	G3PDH2	1P	1P	1P
Total		11M/9P	9M/10P	9M/10P
Percentage of polymorphic loci (%)		37.50	43.75	43.75
Mean protein polymorphism (%)			41.67	

(M – monomorphic locus; P – polymorphic locus)

Rāznas and Nirzas lakes, which was monomorphic in vendace sample from Lake Sventes. Moreover, one slower monomorphic zone was obtained on zymograms of superoxide dismutase of vendace sample from Lake Sventes.

In general, the level of polymorphism in the studied isoenzyme loci was 37.5% in vendace sample from Lake Sventes and 43.75% in samples from Rāznas and Nirzas lakes. The average level of isoenzyme polymorphism in the studied vendace samples from three lakes was 41.67%.

The diversity of alleles in the studied samples was approximately the same (Table 4). However, it should be emphasised that in the system of aspartate aminotransferase (locus *AAT4*, liver samples) in the population of Lake Sventes a private allele was obtained.

In general, the level of heterozygosity in the studied samples varies in different loci. The level of observed and expected heterozygosity (according to Hardy-Weinberg) significantly differs only in Lake Sventes vendace sample in *ESTD* locus ($p < 0.01$) and in Lake Rāznas vendace sample in *EST4* locus ($p < 0.05$) (Table 5). Thus, the average level of observed heterozygosity in the studied vendace samples is high and ranges from 0.17 (Lake Rāznas) to 0.22 (Lake Sventes) (Fig. 2). As it is seen from Fig. 2, the values of observed and expected (according to Hardy-Weinberg) heterozygosity differ insignificantly.

Paired comparison of the studied vendace samples has shown quite high values of genetic differentiation (*Fst*) (Wright 1978). The lowest *Fst* value has been obtained for vendace samples

Table 4. The average allele diversity in the vendace samples from lakes Nirzas, Rāznas and Sventes

Name of population	Nirzas	Sventes	*Rāznas*
	Mean value ±SE	Mean value ±SE	Mean value ±SE
Number of alleles per locus	1.438 ± 0.128	1.438 ± 0.128	1.438 ± 0.155
Effective number of alleles per locus	1.364 ± 0.111	1.354 ± 0.121	1.318 ± 0.100
Number of private alleles per locus	0.00	0.063 ± 0.063	0.00

Table 5. Values of X^2 criterion and validity of differences obtained during the comparison of observed and expected heterozygosity (according to Hardy-Weinberg) by isoenzyme loci in the studied vendace samples

Loci / Populations	*AAT4*	*SOD1*	*MDH1*	*MDH2*	*ESTD*	*EST2*	*EST4*
Nirzas	2.3	0.6	0.07	0.01	0.7	1.2	0.15
Rāznas	0.2	0.63	0.5	0.03	0.63	0.7	6*
Sventes	1.75	M	2.25	0.09	9.00 **	2.78	0.26

(M – monomorphic locus; * – $p < 0.05$; ** – $p < 0.01$)

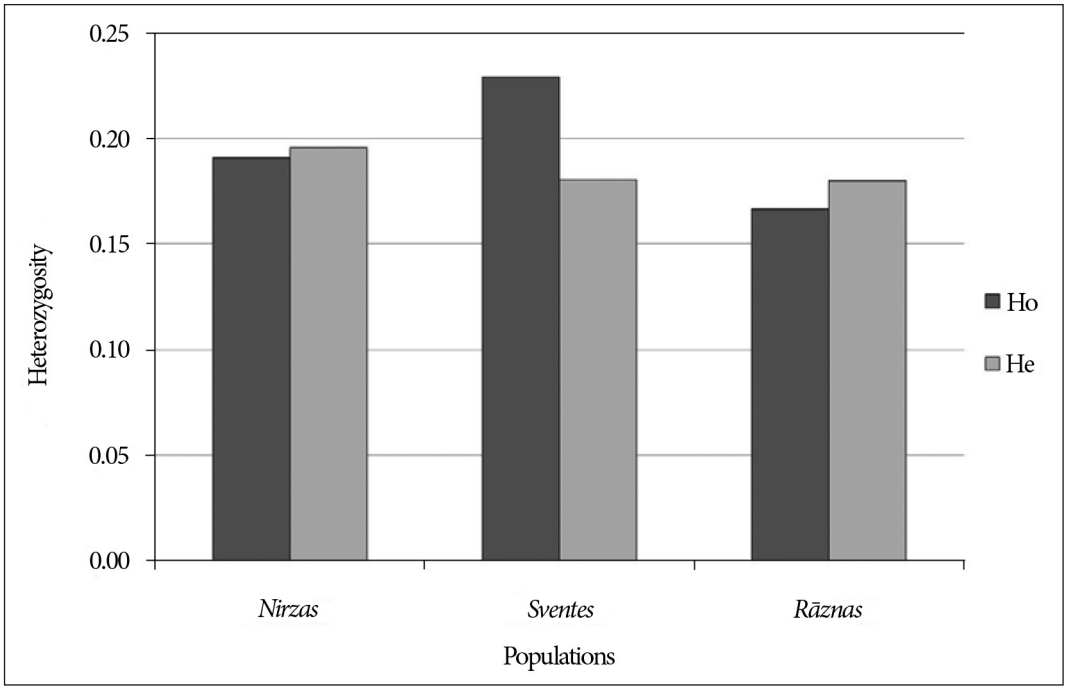

Fig. 2. The average level of heterozygosity in vendace samples from the studied lakes (*Ho* – observed heterozygosity, *He* – expected heterozygosity (according to Hardy-Weinberg))

Rāznas–Nirzas (0.042), when the highest *Fst* value has been obtained for vendace samples from Sventes–Nirzas lakes (0.088) (Table 6).

Values of genetic distances (*D*) (Nei, 1978) correlate with the obtained *Fst* values. Among the studied samples the genetic distance also was the biggest for vendace samples from lakes Sventes–Nirzas (0.0346), and the smallest for vendace samples from Sventes–Rāznas lakes (0.0078) (Table 7).

Table 6. *Fst* values obtained during the pair comparison of vendace samples from the studied lakes

	Nirzas	Sventes	Rāznas
Nirzas	0.000		
Sventes	0.088	0.000	
Rāznas	0.051	0.042	0.000

(*Fst* = 1 – two populations differ significantly (i. e. there is no gene-flow); *Fst* = 0 – populations do not differ)

According to the allozyme data, the inter-population component of genetic diversity was 9.42% of the general variability, when the intra-population variability was 90.58% (Fig. 3).

RAPD

For the RAPD analysis, 60 RAPD primers were tested (CarlRoth). For the present analysis eight decanucleotide primers from sets A and B were used (Table 8).

In general, from 2 to 14 bands in each primer were obtained (in average 9.6 bands per each primer) in the course of amplification of the above mentioned primers, the size of the obtained bands (fragments) varied from 300 to 1 800 bp (Table 9).

Table 7. Nei's genetic distances and genetic similarity indexes (Nei, 1978) for vendace samples from lakes Nirzas, Rāznas and Sventes (according to the data of isoenzyme analysis)

Allozymes	Nirzas	Sventes	Rāznas
Nirzas	–	0.9660	0.9808
Sventes	0.0346	–	0.9923
Rāznas	0.0194	0.0078	–

(Above the diagonal – genetic similarity, below the diagonal – genetic distances)

There were revealed 77 loci (zones), 60 were polymorphic.The amplification with the primer B10 has revealed only two monomorphic loci, when the amplification with other seven primers has shown well reproducible polymorphic zones (Table 9).

Summarising the data obtained during the amplification there were revealed 58 loci characterizing all three vendace samples; 23 of them were polymorphic. In all studied vendace samples 16 equally monomorphic zones were revealed. Other 19 loci in different samples were either monomorphic or polymorphic. It should be noted that private

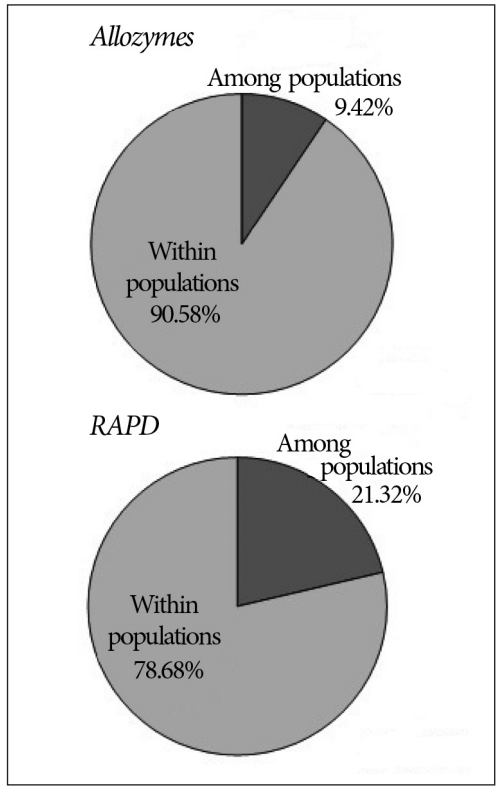

Fig. 3. Genetic differentiation of the studied populations according to the data of allozyme and RAPD analysis

Table 8. The name and sequences of RAPD primers used in order to reveal polymorphism in *Coregonus albula* samples from three Latvian lakes

Primer set	Primer name	Sequences 5' → 3'
B	B08	GTC CAC ACG G
	B10	CTG CTG GGA C
A	A02	TGC CGA GCT G
	A09	GGG TAA CGC C
	A03	AGT CAG CCA C
	A04	AAT CGG GCT G
	A05	AGG GGT CTT G
	A08	GTG ACG TAG G

Table 9. Characterisation of primer productivity, the number and size of the obtained DNA fragments

Primer set	Primer name	Number of loci	Number of polymorphic loci	DNA fragment size (bp)
B	B08	3	2	600–400
	B10	2	-	800–400
A	A02	10	7	1100–430
	A09	14	9	1800–400
	A03	11	9	1100–300
	A04	11	10	1200–400
	A05	11	9	1200–400
	A08	15	14	1600–300
Total	8	77	60	1800–300

Table 10. Number of polymorphic RAPD loci and the level of polymorphism in the vendace samples in Nirzas, Rāznas and Sventes lakes

Population	Number of loci	Number of polymorphic loci	Level of polymorphism (%)	Number of private loci
Sventes	68	38	49.35	6
Rāznas	69	43	55.84	2
Nirzas	67	47	61.04	–
Mean	68	42.7	55.41	2.6
Total	77	60	77.92	8

Table 11. Genetic diversity parameters of *Coregonus albula* populations from the studied lakes by RAPD markers

Population	Sample size	*h*	*I*	*Hs*	*Ht*	*Gst*	*Nm*
Sventes	9	0.1869	0.2755				
Rāznas	9	0.2147	0.3140				
Nirzas	13	0.2233	0.3308				
Total	31	0.2641	0.3965	0.2083	0.2625	0.2065	1.92

(*h* – Nei's genetic diversity (1973); *Nm* – estimation of gene-spread; *Gst* – coefficient of differentiation of populations; *Hs* – gene diversity within populations; *Ht* – total gene diversity; *I* – Shannon information index (Lewontin, 1972))

loci were revealed in vendace samples from lakes Sventes (6 loci) and Rāznas (2 loci) (Table 10).

Thus, in the studied vendace samples the percent of polymorphic loci varied from 49.35% in Lake Sventes to 61.04% in Lake Nirzas vendace populations.

Generally, the average level of RAPD polymorphism of vendace populations in the studied lakes was 55.41% (Table 10).

The general genetic diversity (*Ht*) and genetic diversity within populations (*Hs*) (Nei, 1987) was 0.2625 and 0.2083, respectively. The coefficient of genetic differentiation between vendace samples (*Gst*) was 0.2065 (Table 11).

Furthermore, it was found that the genetic diversity is larger in the vendace sample from Lake Nirzas (I = 0.3308), while in the vendace sample from Lake Sventes this parameter was the smallest (I = 0.2755). The genetic diversity index (h), according to Nei (1973), was also the highest in vendace sample from Lake Nirzas (0.2233) and the lowest in vendace sample from Lake Sventes (0.1869) (Table 11). In this case it should be mentioned that there were no private loci in RAPD markers in vendace sample from Lake Nirzas.

Values of genetic distances and genetic similarity of the analysed vendace samples by RAPD markers are shown in Table 12. The range of genetic distances varied from 0.0812 (between vendace samples from Nirzas and Rāznas lakes) to 0.1153 (between vendace samples from Nirzas and Sventes lakes).The inter-population components constituted 21.32% of the general variability, while the intra-population variability constituted 78.68% (Fig. 3).

Table 12. Values of genetic distances and Nei's genetic similarity (Nei, 1978) for the vendace samples from lakes Nirzas, Rāznas and Sventes according to the data of RAPD markers

RAPD	Nirzas	Sventes	Rāznas
Nirzas	–	0.8911	0.9220
Sventes	0.1153	–	0.9183
Rāznas	0.0812	0.0852	–

(Above the diagonal – genetic similarity, below the diagonal – genetic distances)

DISCUSSION

Initially, it had been supposed that there were significant size fluctuations of vendace population in Latvian lakes as a result of some external factors (intensive catch, unacceptable habitat conditions, etc). It had been also supposed that vendace population fluctuations in Latvian lakes would influence their genetic structure. Thus, the aim of this research was to define the genetic structure of vendace populations in Nirzas, Sventes and Rāznas lakes in Latvia and to discover the influence of small size of populations on their genetic structure.

Allozymes

Some authors anticipate that isoenzyme loci are selectively neutral (Nei, Graur, 1984; Skibinski et al., 1993) and alterations in their heterogeneity can be caused only by serious population fluctuations. The level of polymorphism (i. e. % of polymorphic loci) and the average heterozygosity level of individuals in populations are the key parameters of genetic variability.

The level of polymorphism in the studied vendace samples varies from 37.50 to 43.75%. Recent studies have shown that the level of polymorphism for different whitefish species varies significantly. For example, in some whitefish populations (*Coregonus lavaretus* L.) it varies from 17 to 45% (Sendek, 2004). For vendace populations this parameter differs in various populations from 17% (Pak, 2004) to 39–50% (Sendek, 2002).

It is assumed that narrowly endemic species are less variable than those with quite broad limits of adaptation (Altukhov et al., 2000). This probably explains a quite high level of obtained polymorphism in the studied vendace populations, because vendace is known as the most flexible whitefish species.

Meanwhile, the level of polymorphism directly depends on the number of isoenzyme systems included into the analysis. It is supposed that with the greater number of isoenzyme systems included into the analysis, the level of polymorphism in studied vendace samples will change.

The observed level of heterozygosity in the studied vendace populations varies from 0.1667 (Lake Rāznas) to 0.2292 (Lake Sventes), while the expected level of heterozygosity (according to Hardy-Weinberg) in the studied samples differs insignificantly (0.1912–0.1945). It should be noted that for vendace population in Obj–Taz river basin this parameter is merely 0.0489 (Pak, 2004), but in the vendace population in Lake Ladoga it varies from 0.095 to 0.099 (Sendek, 2002). Hence, despite the small size of the studied vendace samples in Latvian lakes, there is quite high level of heterozygosity.

The average allele diversity in the studied samples is quite identical. The only difference is the presence of the private allele in the locus *AAT4* in vendace sample of Lake Rāznas. It is difficult to determine the status of this allele because these are initial studies of the vendace population in this lake and at the present moment it is not possible to define whether this allele is a new variant of the gene or it remains in the population since the time of introduction (from the donor population). It should be emphasized that the defined difference can be just a consequence of a small sample size taken for analysis. However, taking into consideration the status of this species in Latvia and a small number of vendace individuals in Latvian lakes populations, currently a larger sample size for studies is not available.

For genetic differentiation determination, a comparison of paired vendace samples was performed. The obtained genetic distances varied from 0.0078 to 0.0346. Such values of genetic distances anticipate the genetic isolation of the studied populations that can be explained by different environmental conditions influencing the gene pool of the studied vendace populations in Rāznas, Nirzas and Sventes lakes.

Thus, for example, in the last century vendace fry was released into one of Scandinavian lakes. After 92 years, a comparison of biochemical variability of donor and artificially created populations was made (Vuorinen et al., 1991). Significant differences between donor and artificially created populations were observed, namely the level of heterozygosity in artificially created population had significantly increased, and an allele absent in the donor population was found. It seems that time periods for such changes should be completely different. Moreover, according to the founder, effect in the isolated population some rare variants of genetic variability due to genetic drift and other processes can appear and homozygosity can increase. The authors had some doubts anticipating that the obtained facts can be explained by adaptive processes, because environmental condition differed in both lakes: an artificially inhabited lake is colder.

At the same time the obtained genetic distances in the studied vendace populations in Nirzas, Sventes and Rānas lakes can be a consequence of a small number of individuals in samples. Nevertheless, the genetic distance between Nirzas and Sventes lakes (0.0346) is more typical for distant populations or for related species. For example, the genetic distance between populations of European and American whitefish is 0.038 (Bodaly et al., 1991).

It is known that the decrease of population size can cause the bottleneck effect; as a consequence,

in the process of genetic drift population genetic structure changes can occur. Alterations of genetic structure of populations can also cause the increase of genetic distances (Hedrik, 2000). It is also known that the genetic drift changes the frequencies of alleles and reduces genetic variability within populations. Populations that recently suffered significant fluctuations of their number are more liable to such adverse processes as inbreeding, fixation of bad alleles, etc. As a result of unbalanced distribution of gametes, the shift of allele frequencies is possible (for example, excess of heterozygotes).

In the light of this, it should be noted that Lake Sventes vendace population probably recently had significant population size fluctuations (bottleneck effect). This was confirmed by superoxide dismutase monomorphic locus, which is polymorphic in Nirzas and Rāznas lakes vendace populations; and by *ESTD* locus in Lake Sventes vendace population, where no homozygotic genotypes were defined (i. e. excess of heterozygotes) (Table 3).

However, we cannot make any serious conclusions, we can only make assumptions.

Therefore, in order to scan anonymous sequences of nuclear genome for a more detailed study of the available biologic material the RAPD method was applied.

RAPD

The RAPD analysis can be useful as an express-method for revealing a genetic polymorphism. New mechanism of origin of RAPD markers still has not precisely been found. Some authors suppose that probably one of mechanisms of origin is nucleotide substitutions in priming regions (Naish et al., 1995); the second possible reason of the RAPD polymorphism can be the changes in DNA fragment length in the priming region during such chromosome rearrangements as insertions and deletions (Lynch, Milligan, 1994; Bardakci, 2001). With the help of RAPD markers a more clearly pronounced genetic polymorphism between populations can be determined compared with the isoenzyme analysis.

For example, Mamuris and co-authors (1998a) had studied intra-species genetic variability of eight Mediterranean Sea mullet populations and compared the results defined by the RAPD method with the data of isoenzyme analysis (Mamuris et al., 1998b). Both methods have shown good results in determining heterogeneity of individuals, but have not shown a sufficient number of specific markers for differentiation of the studied population. In another work, Mamuris with co-authors (1999) note that the RAPD technique allows to study a greater part of genome than the analysis of isoenzyme systems.

The RAPD polymorphism in the studied vendace samples varied from 49.35 (Lake Sventes) to 61.04% (Lake Nirzas). As it is seen, this parameter is higher than the level of polymorphism obtained in the allozyme analysis. Furthermore, in samples from Lake Sventes the level of polymorphism remains the lowest among studied vendace samples (both based on the allozyme data – 37.5%, and on RAPD markers – 49.35%). A quite high level of RAPD polymorphism in studies of other fish species was also obtained by some other authors. For example, in studies of the genetic variability of several trout populations, the level of RAPD polymorphism varied from 37.0 to 80.0% (Cagigas et al., 1999), but the level of RAPD polymorphism in cultivated populations of Korean catfish (*Silurus asotus*) varied from 56.4 to 59.6% (Yoon, Kim, 2001). For *Oreochromis niloticus* populations these values varied from 49.04 to 60.1% (Rashed et al., 2008). In available sources of literature, however, there is no data on the level of RAPD polymorphism of whitefish populations. Thus, it can be assumed that the level of RAPD polymorphism in the studied vendace populations in Sventes, Rāznas and Nirzas lakes is quite high. However, the lowest level of polymorphism in Lake Sventes vendace population (both by the allozyme data and the data of RAPD markers) makes it possible to suppose that in Lake Sventes significant fluctuations of vendace population size (some unfavourable genetic processes) occurred.

The presence of six private RAPD loci in the vendace sample from Lake Sventes confirms the assumption about the presence of processes that influence its genetic structure. It is noteworthy that only two private loci were defined in vendace sample from Lake Rāznas, but in vendace sample from Lake Nirzas private loci were absent.

However, Nei's genetic diversity parameter (h) (Nei, 1973) in Lake Sventes vendace sample (0.1869) is lower than in two other vendace samples (0.2147–0.2233).

Genetic distances (Nei, 1978) showing the degree of genetic differentiation of the studied vendace populations varied from 0.0812 to 0.1153. Genetic distances defined on the RAPD markers are larger than those defined in the allozyme analysis (Tables 7, 12). The largest genetic distance was determined (also on the allozyme data) between vendace samples from Sventes and Nirzas lakes. The genetic isolation of the studied vendace populations is confirmed that can be explained by the impact of different environmental conditions on genetic material of vendace populations in Rāznas, Nirzas and Sventes lakes.

Probably the scanning of anonymous sequences (based on RAPD markers) discovers some hidden genetic processes in the studied vendace populations, which have no obvious external expression. It is noteworthy that the genetic distances defined on the basis of RAPD markers correlate with geographical distances (83%; $p < 0.05$). It is noteworthy that values of genetic distances defined on the RAPD markers are more homogeneous than those defined on allozyme data. Possibly, it is caused by a small number of the analysed isoenzyme loci and a quite large number of RAPD loci included into the analysis. It can also be supposed that some stochastic processes occur in all three studied vendace populations. As a result of the mentioned processes the studied vendace population genetic structure changes insignificantly and remains in a relatively stable condition.

Such genetic distances perhaps are determined by the absence of genetic exchange between these populations which is defined by geographical factors.

Table 13 summarises the values of basic parameters of vendace population genetic structure defined on allozyme and RAPD data.

The values of estimation of gene-flow (*Nm*) by allozymes and RAPD data differ (Table 13). Theoretically, such number of migrants per generation (3.12 by allozyme data and 1.92 by RAPD data) is sufficient to prevent such undesirable processes in populations as genetic drift and inbreeding in the natural population habitat environment (Hedrick, 2000).

However, it should be noted that the possibility of migrations of vendace individuals from other lakes is limited and the studied vendace populations are geographically isolated. Moreover, genetic differentiation, in general, grows up with the increase of geographical isolation (Ferguson, 1995).

Table 13. Genetic variability basic parameters of *Coregonus albula* according to the data of allozymes and RAPD markers

Variability	Allozymes (mean)	RAPD (mean)
Ht	0.185	0.263
Gst	0.09	0.20
D	0.027	0.094
Nm	3.12	1.92
Level of polymorphism (%)	41.67	55.41

However, the data defined by RAPD markers and allozymes shows that with absence of gene-flow the differentiation of the studied vendace populations is not so high.

It is known that in terms of natural undisturbed reproduction of species gene pools, the ratio of intra- and inter-population components of genetic diversity remains unchanging at different levels of species population structure. Unfortunately, at the present stage of research it is not possible to estimate the alteration of intra- and inter-population components of genetic diversity of the studied vendace populations in time. However, it can be assumed that the values of intra- and inter-population components of genetic diversity are the result of adaptation of the studied vendace populations to environmental conditions.

In general, it should be noted that the RAPD technique used to establish systematic features has some limitations, and in taxonomic interpretation it needs comparison and application of the data by other markers. However, once optimised the RAPD method is a fast and reproducible way for analysis and estimation of genetic structure of species and populations and can be a basic method for the development of new specific primers.

The RAPD method was also used in the study of genetic differentiation of sympatric whitefish species in Lake Teletskoe (Bockarev, Zyikova, 2008). The authors studied anonymous sequences (RAPD) after the analysis of definite regions of the mitochondrial DNA (Bockarev, Zyikova, 2006). Unfortunately, while using mitochondrial DNA markers no significant differences between two supposed species were revealed. However, in the study of anonymous sequences by random

primers there were revealed fragments specific to one or another group of sympatric whitefish from Lake Teletskoe, which indicated that these species are genetically differentiated (Bockarev, Zyikova, 2008). In genetic differentiation studies of *Oncorhynchus mykiss* population (Kamchatka), the derived information was not sufficient for differentiation of these populations on the basis of nuclear and mitochondrial DNA markers by PCR-RFPL method (Melnikova et al., 2008). Consequently, the analysis of polymorphism of anonymous sequences was applied, during which DNA fragments enabling these populations to differ were defined. For further application of the revealed markers the sequencing of specific fragments was made and specific primers were developed (the method SCAR-markers (Sequence Characterized Amplified Region)).

It is assumed that the analysis of microsatellite sequences can provide more data on the conditions of vendace population in Sventes, Nirzas and Rāznas lakes because of its high variability. The data on microsatellite sequences can provide information on genetic processes that occurred in populations in the recent past.

CONCLUSIONS

The present research reveals the genetic structure of vendace populations in Nirzas, Rāznas and Sventes lakes in Latvia. The vendace population genetic differentiation can be influenced by ecological, evolutionary and historical factors. It is supposed that gene drift or natural selection is the basic factor influencing genetic differentiation of the studied vendace populations. Probably, the vendace population of Lake Sventes was subject and at the present moment is subject to a stronger influence from outside; and this is revealed as evident differences in its genetic structure.

Still, at the present stage of research it is not possible to precisely determine the basic factors that influenced and are influencing the genetic variability of the studied vendace populations. In different lakes, varied factors can differently influence the genetic structure of populations. The discovered genetic variability in vendace populations of Nirzas, Rāznas and Sventes lakes, probably is a result of vendace adaptation to habitat conditions after introduction.

However, a larger set of markers would allow to study the genetic variability of these populations in detail. There are plans to continue the analysis of vendace populations of the above mentioned and other Latvian lakes using a larger set of markers (allozymes, RAPD and microsatellites).

Further studies are necessary in order to discover the probable factors influencing the genetic variability of vendace populations in Latvian lakes.

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Jelena Oreha, Aleksandra Morozova, Natalja Shkute

COREGONUS ALBULA (L.) POPULIACIJŲ LATVIJOJE GENETINIO KINTAMUMO BENDRAS ĮVERTINIMAS PAGAL IZOFERMENTINIUS IR AAPD ŽYMENIS

Santrauka

Seliava (*Coregonus albula* L.) aptinkama keliuose Latvijos ežeruose. Ši rūšis Latvijoje įrašyta į specialų riboto naudojimo saugomų rūšių sąrašą. Atsižvelgiant į didelę seliavų įvairovę ir tai, kad ji yra vertinga komercinė žuvis, iškyla būtinybė įvertinti galimas šios įvairovės priežastis. Tikslių duomenų apie seliavų vietines populiacijas ir jų biologiją nebuvimas trukdo racionaliam jų panaudojimui ir reprodukcijai Latvijos ežeruose. Šiame darbe pagal izofermentinius ir AAPD žymenis buvo įvertinta Latvijos Rāznas, Nirzas ir Sventes ežerų seliavų polimorfizmas ir genetinė struktūra, kintamumas populiacijos viduje ir tarp populiacijų. Genetinei analizei panaudotos 8 izofermentinės sistemos su 16 lokusų. Aštuoni iš jų buvo polimorfiniai ir panaudoti statistikos analizei. Iš 60 išanalizuotų dekanukleotidų AAPD pradmenų genetinės įvairovės analizei panaudoti aštuoni. Vidutinis seliavų izofermentinis polimorfizmas tirtuose ežeruose buvo 41,67 %, alelių dažnis panašus. AAPD analizė rodo, kad *Coregonus albula* populiacijos tirtuose ežeruose pasižymi dideliu polimorfizmu. Tyrimų rezultatai atskleidė, kad tiriant tą pačią seliavų populiaciją skirtingais metodais, AAPD metodas yra tinkamesnis nei izofermentų tyrimas.

Raktažodžiai: genetinis kintamumas, izofermentai, AAPD žymenys, *Coregonus albula*, genetinė struktūra, polimorfizmas