

Intraspecific allozyme diversity of *Gobio gobio* in Czech and Slovak rivers

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Abstract. Samples of *Gobio gobio* from 30 localities in the Czech Republic and Slovak Republic were studied. Starch gel electrophoresis was used for evaluation of variability of allozyme patterns in 16 active protein systems comprising 26 loci. Products of 17 loci were considered to study the genetic structure of its populations. From this, genetic variability was found in ten loci, occurrence of rare alleles was detected in next four loci. Percentage of polymorphic loci ranged between 6 and 41%; mean occurrence of alleles was between 1.1 and 2.0 per locus; and mean heterozygosity was observed at 0.013 to 0.248. The genetic distances among populations were 0.002 to 0.175 (Nei 1972) and 0.063 to 0.385 (Cavalli – Sforza & Edwards 1967). Differences in occurrence and frequencies of alleles in some loci among the sea basins were evident. Higher genetic variability identified in populations from the Danube river system (Black Sea basin) is probably connected with the fact that this basin served as a refuge for ichthyofauna during glaciations.

Key words: common gudgeon, genetic variability, population differences

Introduction

Knowledge on the population structure and history of species is a prerequisite of efficient measures for its protection and to identify the original structure before changes by the human activities. Although common gudgeon does not belong to commercially important species, its transfers can be realized non-intentionally with other fish species. Thus mixing of populations even from different sea basins can occur and eliminate original, interpopulation differences. On the other hand, bottleneck and founder effects can bring “artificial” differences not corresponding to reality.

The native range of common gudgeon (*Gobio gobio*) extends across most of Europe and Asia. Different authors described many subspecies and forms mostly based on the variability of different external morphometric characters (Kottelat 1997, Bănărescu et al. 1999). As these characters are variable by nature and moreover, some of them can show geographic clines, their applicability for distinguishing of individual taxons of common gudgeon could be limited.

Insufficient data on gudgeon population structure and genetics has been collected until recently, including protein / allozyme (Valenta et al. 1986, Dobrovoldov 1994, Manaresi et al. 2001, Schreiber 2002) and DNA variability (Gilles et al. 2001,

Wolter et al. 2003, Callejas et al. 2004). These studies have revealed basic a pattern linked to main river basins, with reflections on the colonization history of the species.

The present study tries to map genetic population structure of common gudgeon in rivers of the Czech and Slovak republics using the allozyme data. Rivers in this area belong to three sea basins (North, Baltic, Black) and basic structure according these regions is supposed.

Material and Methods

Specimens were collected by electrofishing from 33 localities in the Czech Republic and Slovak Republic (Table 1). In total, 772 individuals were sampled for trunk muscle and liver tissues. Starch gel electrophoresis of clarified tissue extracts (Valenta et al. 1971), followed by enzyme-specific staining, was used to evaluate the variability of allozyme patterns in 11 active enzyme / protein systems, comprising 17 loci (Table 2). Details of method are described in Šlechtová et al. (1998). The nomenclature of loci and alleles followed that recommended by Shalee et al. (1990). Statistical evaluation of genetic data was performed using the BIOSYS-1 software (Swofford & Selander 1981), PHYLIP (Felsenstein 1993) and GENETIX (Belkhir et al. 1996).

Results

Electrophoretic analysis revealed the presence of genetic polymorphism in 15 loci; two loci (*AK** and *m-IDHP**) were monomorphic. The most variable loci were *SOD**, *6PGDH** and *G3PDH-2**, which showed polymorphism nearly in all populations studied. The loci with maximal number of alleles were *GPI-1**, *GPI-2** and *PGM** with six, five and five alleles, respectively. The loci with lowest variability were two LDH and two sMDH loci, *mAAT-1** and *mMDH** locus. The mean number of alleles per locus was between 1.1 and 2.0, and the degree of polymorphism (in percentage of polymorphic loci) varied from 5.9 to 41.2 (Table 3). The observed heterozygosities ranged between 0.038 and 0.248 and the expected heterozygosities between 0.034 and 0.180 (Table 3). Nei (1972) genetic distance among studied populations ranged from 0.002 to 0.175. The phenogram constructed using these data expressed no marked grouping of populations nor did bootstrapping reveal any branching support. The differentiation among different localities was rather low.

After samples were grouped by river basin (Table 1) further discrimination could be observed. Populations from the Elbe River basin clustered together with those from the Odra River basin (with support 66.8%), then with the Vistula River basin (support 71.2%). Populations of the Danube River basin clustered together with Tisza River basin samples. The Nei (1972) genetic distance (G_{st}) was much lower between the Elbe River and Odra River basins than between the Elbe and Danube groups. Maximum genetic differentiation was calculated for the Elbe-Tisza group pair and the minimum was between the Elbe and Odra river basins (see Table 4a). Danube group showed the highest number of alleles per locus and highest heterozygosities, whereas the Vistula samples possessed the highest proportion of polymorphic loci (Table 3b).

Grouping together of population samples from the same sea basins revealed that fishes from the Black Sea basin showed the highest number of alleles per locus and expected heterozygosity, whereas the highest percentage of polymorphic loci was found in Baltic Sea basin populations (see Table 3c). The Nei (1972) genetic distance between North and Baltic Sea basins was the lowest whereas between North and Black Sea basins it was maximal (Table 4b).

Table 1. Locations from which common gudgeon, *Gobio gobio*, were collected.

Pop. no.	Population name	River basin	Sea basin	No of fish
1	Plaveč	Vistula	Baltic	12
2	Hromoš	Vistula	Baltic	8
3	Marjánka	Elbe	North	8
4	Lužická Nisa	Odra	Baltic	7
5	Jevišovka	Danube	Black	8
6	Laus Neisse	Odra	Baltic	60
7	Bystřice	Elbe	North	15
8	Olše	Odra	Baltic	40
9	Dyje (Vranov)	Danube	Black	12
10	Planá	Elbe	North	15
11	Divoká Orlice	Elbe	North	50
12	Blanice S.	Elbe	North	26
13	Blanice V.	Elbe	North	21
14	Odra	Odra	Baltic	25
15	Stěna	Odra	Baltic	25
16	Svratka	Danube	Black	23
17	Rokytná	Danube	Black	16
18	Haná	Danube	Black	20
19	Bečva	Danube	Black	101
20	Bystrička	Danube	Black	26
21	Vlára	Danube	Black	47
22	Váh	Danube	Black	39
23	Hron	Danube	Black	13
24	Ida	Tisza	Black	14
25	Belžanský p.	Tisza	Black	16
26	Olšava	Tisza	Black	56
27	Revištia	Tisza	Black	7
28	Ublianka	Tisza	Black	14
29	Soutok	Danube	Black	5
30	Liběchovka	Elbe	North	43
Total				772

Table 2. Enzyme loci analysed in common gudgeon, *G. gobio*, for the presence of variability.

Enzyme	Loci		
aspartate aminotransferase AAT	<i>sAAT*</i>	<i>mAAT-1*</i>	<i>mAAT-2*</i>
adenylate kinase AK	<i>AK*</i>		
glucosephosphate isomerase GPI	<i>GPI-1*</i>	<i>GPI-2*</i>	
glycerol-3-phosphate dehydrogenase G3PDH	<i>G3PDH-2*</i>		
isocitrate dehydrogenase IDH	<i>mIDHP*</i>		
lactate dehydrogenase LDH	<i>LDH-A*</i>	<i>LDH-B*</i>	
malate dehydrogenase MDH	<i>sMDH-1*</i>	<i>sMDH-2*</i>	<i>mMDH*</i>
mannosephosphate isomerase MPI	<i>MPI*</i>		
phosphoglucomutase PGM	<i>PGM*</i>		
superoxide dismutase SOD	<i>SOD*</i>		
6-phosphogluconate dehydrogenase 6PGDH	<i>6PGDH*</i>		

Table 3. Genetic variability in common gudgeon, *G. gobio* (standard errors in parentheses).

Population	Mean no. of alleles/ locus	% of loci polymorphic*	Mean heterozygosity	
			H _o	H-W H _e **
a) among population samples				
1. Plaveč	1.5 (0.2)	35.3	0.109 (0.042)	0.142 (0.051)
2. Hromoš	1.5 (0.2)	41.2	0.154 (0.053)	0.180 (0.057)
3. Marjánka	1.4 (0.1)	29.4	0.093 (0.047)	0.095 (0.046)
4. L. Nisa	1.1 (0.1)	11.8	0.038 (0.030)	0.034 (0.026)
5. Jevišovka	1.5 (0.2)	41.2	0.248 (0.089)	0.177 (0.061)
6. Laus Neisse	1.4 (0.1)	23.5	0.069 (0.033)	0.074 (0.034)
7. Bystřice	1.3 (0.1)	17.6	0.099 (0.053)	0.080 (0.040)
8. Olše	1.6 (0.1)	35.3	0.085 (0.034)	0.107 (0.039)
9. Dyje (Vranov)	1.8 (0.2)	35.3	0.129 (0.047)	0.165 (0.055)
10. Planá	1.5 (0.2)	29.4	0.129 (0.057)	0.125 (0.052)
11. Divoká Orlice	1.6 (0.2)	29.4	0.106 (0.049)	0.095 (0.040)
12. Blanice S.	1.4 (0.1)	29.4	0.075 (0.036)	0.088 (0.037)
13. Blanice V.	1.5 (0.2)	29.4	0.113 (0.051)	0.140 (0.052)
14. Odra	1.6 (0.2)	35.3	0.131 (0.048)	0.137 (0.051)
15. Stěňava	1.5 (0.2)	23.5	0.084 (0.034)	0.076 (0.030)
16. Svratka	1.4 (0.1)	29.4	0.124 (0.058)	0.113 (0.044)
17. Rokytná	1.6 (0.2)	41.2	0.112 (0.043)	0.121 (0.046)
18. Haná	1.5 (0.2)	23.5	0.131 (0.056)	0.121 (0.052)
19. Bečva	2.0 (0.3)	35.3	0.129 (0.045)	0.138 (0.047)
20. Bystrička	1.5 (0.2)	29.4	0.072 (0.034)	0.076 (0.034)
21. Vlára	1.5 (0.2)	35.3	0.075 (0.027)	0.095 (0.038)
22. Váh	1.8 (0.2)	35.3	0.081 (0.027)	0.109 (0.034)
23. Hron	1.4 (0.1)	23.5	0.052 (0.021)	0.085 (0.035)
24. Ida	1.2 (0.1)	5.9	0.013 (0.009)	0.012 (0.009)
25. Belžanský p.	1.2 (0.1)	17.6	0.051 (0.029)	0.064 (0.034)
26. Olšava	1.5 (0.2)	17.6	0.052 (0.027)	0.054 (0.025)
27. Revištia	1.3 (0.1)	29.4	0.118 (0.054)	0.115 (0.049)
28. Ublianka	1.5 (0.2)	29.4	0.042 (0.020)	0.106 (0.044)
29. Soutok	1.4 (0.1)	35.3	0.094 (0.042)	0.125 (0.050)
30. Liběchovka	1.8 (0.2)	29.4	0.119 (0.057)	0.104 (0.041)
F_{ST} = 0.3036				
b) among populations grouped according river basins				
1. Elbe	2.2 (0.3)	35.3	0.109 (0.046)	0.107 (0.039)
2. Odra	2.1 (0.2)	35.3	0.085 (0.031)	0.100 (0.034)
3. Tisza	1.9 (0.2)	23.5	0.051 (0.019)	0.086 (0.036)
4. Danube	2.6 (0.3)	35.3	0.108 (0.036)	0.129 (0.045)
5. Vistula	1.6 (0.2)	41.2	0.126 (0.041)	0.158 (0.050)
F_{ST} = 0.2144				
c) among populations grouped according sea basins				
North Sea	2.2 (0.3)	35.3	0.109 (0.046)	0.107 (0.039)

Baltic Sea	2.2 (0.3)	41.2	0.090 (0.031)	0.109 (0.035)
Black Sea	2.9 (0.2)	35.3	0.093 (0.031)	0.120 (0.043)
	F_{ST} = 0.1992			

* A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95

** Unbiased estimate (see Nei, 1978)

H_O = observed heterozygosity; H-W H_e = heterozygosity expected according to Hardy-Weinberg law

In nine loci (i.e. *sAAT**, *mAAT-2**, *GPI-2**, *G3PDH-2**, *m-IDHP**, *LDH-B**, *sMDH-2**, *MPI** and *6PGDH**), 13 alleles specific for *G. gobio* were found (Table 5, marked by exclamations), which allowed us to classify gudgeon individuals as to their species pertinence (these conclusions are based on our previous studies and unpublished data). Also introgression of genes from other gudgeon species is presumed. Alleles found in common gudgeon in this study and supposed to originate from other gudgeon species are marked by section sign (§) in Table 5. Their usage allows us to determine interspecific introgression or hybrids.

Table 4. Nei (1972) genetic distances, genetic differentiation (**G_{ST}**) and estimated gene flow (*Nm*) in *G. gobio* from area studied.

a) among river basin

Population		Odra	Tisza	Danube	Vistula
Elbe	distance	0.007	0.069	0.080	0.031
	G_{ST}	0.0276	0.2402	0.2237	0.0940
	<i>Nm</i>	<i>17.62</i>	<i>1.58</i>	<i>1.74</i>	<i>4.82</i>
Odra	distance		0.053	0.063	0.019
	G_{ST}		0.2036	0.1921	0.0616
	<i>Nm</i>		<i>1.96</i>	<i>2.10</i>	<i>7.62</i>
Tisza	distance			0.007	0.026
	G_{ST}			0.0306	0.0891
	<i>Nm</i>			<i>15.84</i>	<i>5.11</i>
Danube	distance				0.034
	G_{ST}				0.0916
	<i>Nm</i>				<i>4.96</i>

a) among sea basins

Population		Baltic Sea	Black Sea
North Sea	distance	0.007	0.077
	G_{ST}	0.0050	0.0308
	<i>Nm</i>	<i>99.5</i>	<i>15.7</i>
Baltic Sea	distance		0.055
	G_{ST}		0.0222
	<i>Nm</i>		<i>22.0</i>

Table 5. Appearance of alleles in individual loci.

<i>sAAT*</i>	<i>mAAT-1*</i>	<i>mAAT-2*</i>	<i>AK*</i>	<i>GPI-1*</i>	<i>GPI-2*</i>
100	100	-100	100	100	175 !
080 !	080	-200 !		112	100
120 (2)	120	-050 §		098	250 !
				086 §	025 !
				074 §	020 §
				115 §	
<i>G3PDH-2*</i>	<i>m-IDHP*</i>	<i>LDH-A*</i>	<i>LDH-B*</i>	<i>sMDH-1*</i>	<i>sMDH-2*</i>
100	100 !	100	100 !	100	100 !
135 !		200 (1)	080 §	120 (1)	050 (2)
081 §			090 §	075 §	126 (1)
117					
<i>mMDH*</i>	<i>MPI*</i>	<i>PGM*</i>	<i>6PGDH*</i>	<i>SOD*</i>	
100	100 !	100	100 !	100	
170	090 (1)	082	125 !	072	
112 (1)	105	108 (2)	075 !	050	
		118 (2)			
		091 §			

! = allele specific to *G. gobio*

() = rare allele (no. of appearances)

§ = probable introgression from other *Gobio* species

Discussion

Genetic variability of common gudgeon populations in the studied area was found to be rather high. When compared with results of Schreiber (2002), most of our populations showed higher mean number of alleles per locus and higher proportion of polymorphic loci. Also the observed heterozygosities were usually higher than in the study mentioned.

Populations of the Danube River basin (except those of easternmost, rather isolated Tisza River) belong to those possessing highest mean number of alleles per locus. Drainage area of Danube River is the biggest in the region studied; moreover, it is supposed to have served as a refuge during glacial periods, so such diversification could be anticipated. Populations from the Tisza River basin showed also high allelic variability. The highest values as to the number of variable loci and heterozygosity were found in Vistula populations. Interpopulation differences are the main reason for the genetic divergences among both the river systems and sea basins (F_{ST} was 0.2144 and 0.1992, respectively; F_{ST} was 0.3036 when all population samples were calculated separately).

Genetic differentiation (GST) seems to be congruent with geographic distances. The lowest value, for Elbe and Odra river basins, could be explained by their close proximity, with possible river capture events at some locations in the geological past. The Tisza and Danube basins are connected, although the Tisza was relatively isolated system prior to river regulation (the upland

reaches were separated from the main Danube by a vast marshlands). On the other hand, some of high migration rates could be explained by fish transfers, which were non-intentional in the case of gudgeons – this species very probably was transported along with commercially important fish.

Several alleles in some loci can be used for species recognition (unpublished data). Alleles marked as such in Table 5 allowed us to distinguish gudgeon species pertinence in questionable cases and also to determine the genomic composition in hybrids. Using these allelic markers, we revealed gene introgression in seven loci at geographic locations where coincidence of *G. gobio* with other gudgeon species was detected.

Our results showed rather high genetic differentiation and relatively clear geographic structure of gudgeon populations. Using allozyme markers, however, can hardly describe the history of populations. Other markers with better predicative power ought to be used for more detailed description of species structure (e.g. microsatellites) and history (mitochondrial DNA markers).

A c k n o w l e d g e m e n t

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LITERATURE

- BĂNĂRESCU P., ŠORIĆ V. & ECONOMIDIS P. 1999: *Gobio gobio* (Agassiz, 1828). In: Bănărescu P. (ed.), The Freshwater Fishes of Europe. Cyprinidae 2/I. *Aula, Wiesbaden*: 81–134.
- BELKHIR K., BORSA P., CHIKHIL., RAUFASTE N. & BONHOMME F. 1996–2002: GENETIX 4.04, logiciel sous Windows TM pour la génétique des populations. *Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier (France)*.
- CALLEJAS C., LUSKOVÁ V. & OCHANDO M.D. 2004: A contribution to the genetic characterisation of some species of the genus *Gobio* (Cyprinidae). *Folia Zool.* 53: 433–436.
- CAVALLI-SFORZA L. L. & EDWARDS A. W. F. 1967: Phylogenetic analysis: models and estimation procedures. *Evolution* 21: 550–570.
- DOBROVOLOV I. S. 1994: [Electrophoretic investigations of protein from gudgeons (genus *Gobio*, Pisces) in Bulgaria with regard to their taxonomy]. *Izv. Inst. Ribni Resursi, Varna* 22: 117–134 (in Bulgarian).
- FELSENSTEIN J. 1993: PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author. *Department of Genetics, University of Washington, Seattle*.
- GILLES A., LECOINTRE G., MIQUELIS A., LOERSTCHER M., CHAPPAZ R. & BRUN G. 2001: Partial combination applied to phylogeny of European cyprinids using the mitochondrial control region. *Mol. Phylogenet. Evol.* 19: 22–33
- KOTTELAT M. 1997: European Freshwater Fishes. *Biologia, Bratislava* 52 (Suppl. 5): 1–271.
- MANARESI S., MANTOVANI B. & ZACCANTI F. 2001: Egg to adult identification of 13 freshwater fishes from Italy: a biochemical-genetic key. *Aquat. Sci.* 63: 182–190.
- NEI M. 1972: Genetic distances between populations. *Amer. Nat.* 106: 283–292.
- NEI M. 1978: Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583–590.
- SCHREIBER A. 2002: Differences in levels of heterozygosity in populations of the common gudgeon (*Gobio gobio*, Cyprinidae) among adjacent drainages in Central Europe: an effect of postglacial range dynamics? *Heredity* 89: 163–170.
- SHAKLEE J. B., ALLENDORF F. W., MORIZOT D. C. & WHITT G. S. 1990: Gene nomenclature for protein-coding loci in fish. *Trans. Amer. Fish. Soc.* 119: 2–15.

- SWOFFORD D. L. & SELANDER R. B. 1981: BIOSYS-1: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J. Hered.* 72: 281–283.
- ŠLECHTOVÁ V., ŠLECHTA V., LUSKOVÁ V., LUSK S. & BERREBI P. 1998: Genetic variability of common barbel, *Barbus barbus*, populations in the Czech Republic. *Folia Zool.* 47 (Suppl. 1): 21–34.
- VALENTA M., HYLDGAARD-JENSEN J. & JENSEN E. S. 1971: Interaction of veronal, pyrophosphate, citrate and protein with lactate dehydrogenase isozyme determination and kinetics. *Acta Vet. Scand.* 12: 15–35.
- VALENTA M., ŠLECHTOVÁ V., KAŇKOVÁ J., ŠLECHTA V. & STRATIL A. 1986: The polymorphism and kinetic-properties of liver form of lactate-dehydrogenase (LDH-C – EC 1.1.1.27.) in common carp (*Cyprinus carpio*) and gudgeon (*Gobio gobio*). *Živ. Výroba* 31: 865–876.
- WOLTER C., KIRSCHBAUM F. & LUDWIG A. 2003: Sub-population structure of common fish species in the Elbe River estimated from DNA analysis. *J. Appl. Ichthyol.* 19: 278–283.