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Genetic and morphological variability in roach *Rutilus rutilus*, from Austria

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Abstract

Seven samples of roach (*Rutilus rutilus* L.) from the Austrian section of the Danube river, the Drau river and lake Wallersee were examined to study possible population genetic effects of anthropogenic habitat modification and subdivision. Genetic variability was assessed using electrophoretic variation of 11 enzyme systems coded by 14 gene loci. Genetic variation of roach was remarkably high, and was mainly located within samples while differences between them were weak. Morphometric analyses of conventional external measurements revealed considerable morphological variation within samples. Significant differences between samples mainly involved body depth and fin size, but did not allow adaptive ecomorphological interpretations in several cases. The comparison of morphological and genetic variation showed no significant correlations but was limited by the narrow range of heterozygosity estimates.

Introduction

Environments of riverine freshwater fish have been changed dramatically in this century under human influence. River regulation, construction of hydroelectric dams, straightening the river channels and cutting off lateral river branches strongly modified the ecological character of large rivers like the Danube, made environments more uniform, and induced changes in fish communities (Persat, 1988; Schiemer & Waidbacher, 1992). Dams, dikes and transverse weirs restricted habitats of freshwater fish to smaller compartments with reduced structural diversity and opportunity for migration. This could cause partitioning of formerly connected fish populations. In such subdivided populations with small effective sizes, genetic drift and inbreeding may reduce genetic diversity, and thus impair viability and adaptability.

Roach, *Rutilus rutilus*, seemed well suited as target species to study possible population genetic effects of habitat modification and subdivision: this ecologically flexible cyprinid fish is widespread and common in most Austrian freshwater habitats. Local genetic divergence among roach populations was described in lake Vishtynetskoe by Evlanov (1986) and in the Upper Rhone system by Bouvet et al. (1991). Bouvet et al. (1995) reported differences in genetic diversity among roach populations of Rhone and Saone, corresponding to the structural diversity and hydrological characteristics of these contrasted large rivers.

Five samples were taken along a lateral transect from the mainstream to isolated backwaters of the Danube river. To allow comparison in a wider context, two more remote and ecologically distinct sampling sites were also included: a fast flowing section of the river Drau and the eutrophic subalpine lake Wallersee. Genetic variability within and between these samples was studied by allozyme electrophoresis. Morphometric analyses were carried out to assess habitat specific differentiation and to test for a possible relationship between morphological and genetic variability. The theory of genetic homeostasis (Lerner, 1954) predicts a negative correlation between variability of morphological traits and heterozygosity at the individual level,

caused by a greater ability of heterozygous individuals to stay within the norms of canalised development. If this prediction is extrapolated to higher levels of organisation, one would expect to observe this negative correlation also among populations with different degrees of heterozygosity. On the other hand, if morphological and genetic variance are only two independent estimates of total genetic variation, the observed correlation should be positive (Strauss, 1991). The results of empirical work to study this association are inconsistent and include nonexisting as well as moderately significant positive and negative correlations (Zink et al., 1985).

Materials and methods

Description of the sampling stations (Figure 1)

Three samples were taken from Roßkopfarm near Stopfenreuth (STR-1, STR-2, STR-3), a strongly isolated backwater 35 km downstream of Vienna on the left bank of the Danube. The connection with the Danube at river kilometre 1886 only exists at annual flood events for approximately three days a year. Sampling point STR-1 is situated near the confluence area while STR-2 is situated two km upstream and is isolated from the confluence area by a transverse weir. The last sampling point in this backwater (STR-3) is isolated from the two others by two more transverse weirs and by a dam constructed in the late 19th century (Marchfelddamm).

The second backwater east of Vienna, the Regelsbrunner-Haslauer-Arm situated 25 km downstream of Vienna near Regelsbrunn, is characterised by its dynamic hydrological situation and is connected with the Danube at its downstream end (river km 1895) at mean water -0.4 m. Sampling station REG is situated upstream to a transverse weir with three through-lets which enable connection with the downstream part of the backwater except in the event of a long-term low water situation.

Sample TUL was captured at a backwater near Tulln, 30 km upstream of Vienna on the right bank of the Danube, connected with the mainstream at mean water +1 m.

Sample DRA was collected from a free-flowing section of the Drau river, a tributary of the Danube, which flows into the Danube at river km 1382. Sample WAL was captured at the eutrophic subalpine lake Wallersee (48° 55′ N 13° 10′E) situated at an altitude of 506 m (a.A).

Electrophoresis

All fish were captured using gill nets or electrofishing. They were taken to the laboratory, where they were sacrificed with an overdose of MS222 and dissected immediately. For further morphological analysis specimens were stored frozen at $-20\,^{\circ}$C. Pieces of liver and lateral trunk muscle were homogenised in an approximately equal volume of Tris-EDTA-NADP homogenation buffer, and after centrifugation at 15 000 g at 2 °C for 20 minutes, the supernatant was stored in capillary tubes at $-80\,^{\circ}$C.

Multi-locus genotypes of individuals were scored by starch gel electrophoresis using extracts of skeletal muscle and liver. Allelic designations follow the usage of Bouvet et al. (1991). Genetic variation was assessed using BIOSYS (Swofford & Selander, 1989). Allelic frequencies, observed and expected heterozygosity were computed for each locus as well as the observed and expected values for average heterozygosity (Hartl & Clark, 1989; Nei, 1978). We analysed genetic structure at the polymorphic loci by means of Wright's (1978) F-statistics, handling the single samples as subpopulations, whereas the overall population was the ensemble of the seven samples. To avoid problems with multiallelic loci, which make chi-square tests suspect if samples are small and some alleles are rare, we pooled genotypes in three classes, and calculated exact significance probabilities for deviation from Hardy-Weinberg equilibrium, following Fisher's test. An UPGMA dendrogram was constructed using Nei's (1978) genetic distance.

Morphometry

Since sexual dimorphism is negligible in roach (Stangenberg, 1947; Libosvarsky & Ruban, 1985), sexes were pooled together for morphological analysis. The following measurements were taken to the nearest 0.1 mm using digital electronic callipers (abbreviations are given in parentheses): (HW) head width; (HL) head length; (IOW) interorbital width; (POL) preorbital length; (GBD) greatest body depth; (LBD) least body depth; (LVF) length of the ventral fin; (LPF) length of the pectoral fin; (BDF) base of the dorsal fin; (BPF) base of the pectoral fin; (BVF) base of the ventral fin; (BAV) base of the anal fin; (LCP) length of the caudal peduncel; (PD) predorsal distance.

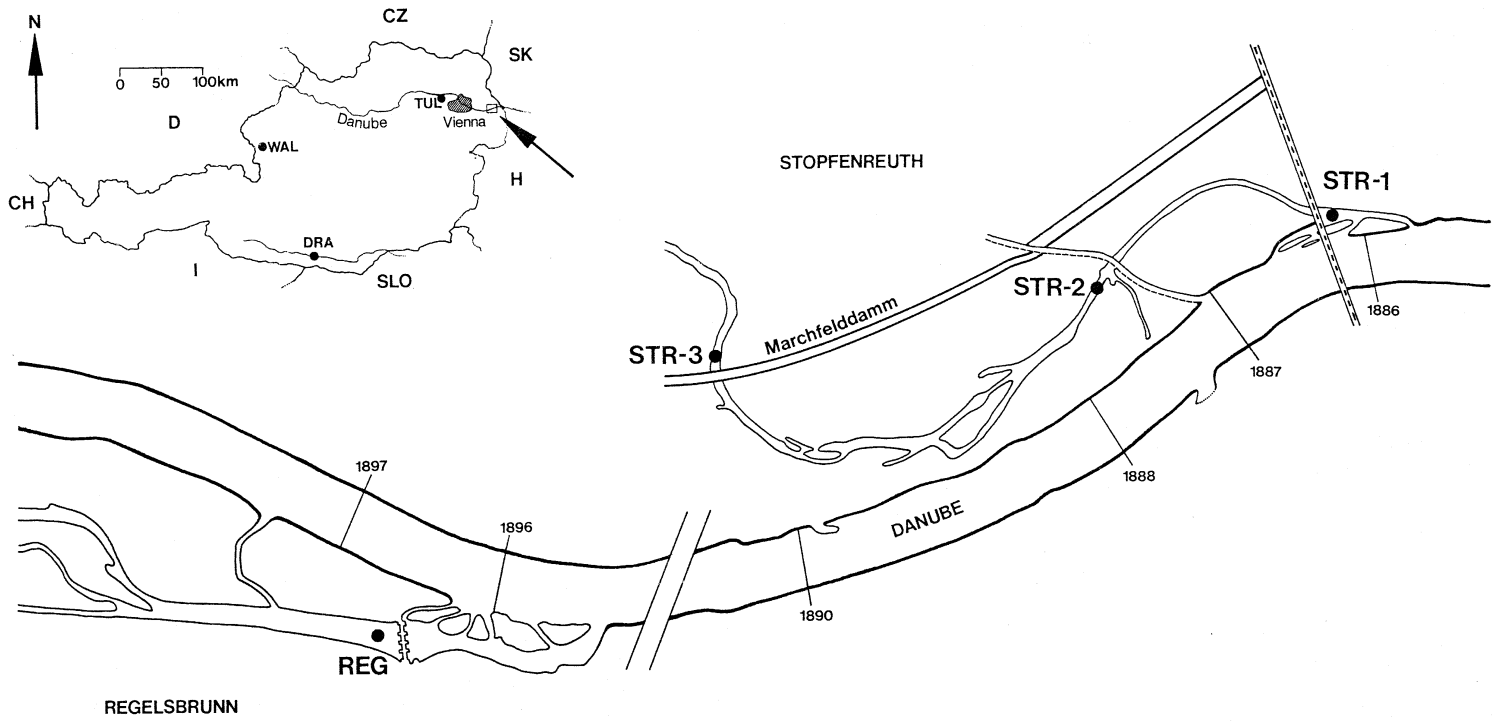

Figure 1. Collection sites of roach. The arrow in the map of Austria (above) points to the region east of Vienna shown in the detail map (below). Broken lines in the detail map indicate roads and bridges, a scale is provided by the river kilometres.

Raw data were converted to $\log_{10}$ to homogenise variance and linearize allometric relationships. Since size heterogeneity was probably caused by sampling bias we decided to adjust for size. The following allometric formula was used because it allows for the complete removal of size variation whereas effects on the correlation and covariance structure of the data are only minimal (Reist, 1985).

$Y_{adj.ij} = Y_{ij}\text{-}b_{\text{within}} * (X_{ij} - X_m)$.

$Y_{adj.ji}$ = predicted measurement for each individual for each variable.

Y_{ij} = $\log_{10}$ transformed unadjusted measurement.

X_{ij} = $\log_{10}$ transformed standard length of the individual.

b_{within} = pooled within group slope between X and Y.

X_m = $\log_{10}$ transformed mean of the standard length of all individuals.

The adjusted measurement is a prediction of what an individual's value for a particular variable would be if the individual had the overall mean standard length.

To screen the data for normality and homogeneity of variances univariate statistics were computed (Kolmogorov-Smirnov-test, Leven's test). We performed principal component analysis (PCA) based on the correlation matrix of the fourteen morphological characters. The extracted varimax rotated principal components (PCs) were interpreted in accordance with the loadings of the original characters. As character loadings represent correlation coefficients between the scores of the original characters and the factor scores of the PCs, significance of the loadings of those characters which were used to interpret PCs was tested using the randomisation routine COVRAN (Nemeschkal, 1991). With the resulting factor scores of the varimax rotated solution we calculated analysis of variance (ANOVA), using sampling sites as grouping factor, to assess the distribution of variance between and within samples. Scheffe's multiple range tests were used to compare means of factor scores of PC1 to PC4 between the seven samples of roach in a stepwise fashion.

Since character coupling represented in similarity matrices is suitable for characterising OTUs (Nemeschkal, 1991) we calculated correlation matrices also for each sample. Significance of pattern similarity between these matrices was tested using quadratic assignment procedures (QAP). The QAP routine first calculates an initial index of matrix correlation, an element-wise Pearson product moment correlation between identical elements of two matrices, which is termed the observed coefficient of matrix correspondence (R_{obs}). A highly positive matrix correlation indicates a strong similarity of correlation patterns. The statistical significance of R_{obs} was tested by randomisation (Edington, 1987). One of the initial matrices remained unchanged for the whole procedure while the other matrix was changed by permuting rows simultaneously with the columns 5000 times. A correlation coefficient (R_{ran}) was calculated after each permutation. The significance value (P) of R_{obs} is given as the proportion of R_{ran} values in the resulting null distribution of R values, which are equal or greater than R_{obs}. If P exceeds 0.05 the null hypothesis assuming no similarity between matrices is supported (Nemeschkal, 1993).

To assess the level of morphological variation, we calculated the determinant of the variance-covariance matrix (D-varcov) of the fourteen characters for each sample. This measure is affected both by the variance and the covariance among the characters and avoids redundant information which comes from intercorrelations of characters. The lower the determinant the smaller is the overall level of variation (Cheverud et al., 1989). To test the predicted inverse relationship between morphological and genetical variation we calculated Pearson correlation coefficients between D-varcov and the direct count estimates of heterozygosity per locus and sample (*H* d.c.) and average heterozygosity (*H* d.c) respectively. The statistical significance of the resulting correlation coefficients was tested with the randomisation procedure COVRAN.

Results

Genetic variation

Fourteen enzyme loci were resolved satisfactorily (Table 1). Six of them were polymorphic at the 95% level in all samples. In sample STR-1 the common allele (**100*) of *GPI-A** slightly exceeded the frequency of 0.950 (Table 2); therefore the percentage of polymorphic loci was lower (42.86%) in this sample than in all the others (50%). Two more loci, *LDH-A** and *AAT**, showed little variability (Table 2). No allelic variation could be detected at the five remaining loci.

The maximum number of alleles (seven) was found at *PGM-2**, but only two of them (**102*, **95*) appeared in all samples; allele **100* showed frequencies ranging from 0.145 to 0.290 in samples STR-1 to TUL but was not present in WAL. The four other alleles were rare. Patterns of allozyme frequencies showed high similar-

Table 1. Enzyme systems analysed and electrophoretic buffers used. The gene nomenclature follows the recommendations of Shaklee et al. (1990). Tissues: L = liver; M = muscle. Buffers: TC6.7 = Tris-citrate pH 6.7 gel buffer / pH 6.3 electrode buffer (Pasteur et al., 1988); TC7 = Tris-citrate pH 7.0 buffer (Shaw & Prasad, 1970); TC8 = Tris-citrate pH 8.0 buffer (Pasteur et al., 1988).

Enzymes	IUBNC Number	Locus	Tissue	Buffer
Adenylate Kinase	2.7.4.3	*AK**	M	TC6.7
Creatine Kinase	2.7.3.2	*CK**	M	TC6.7
Glucose-6-phosphate Isomerase	5.3.1.9	*GPI-A**	M	TC7
		*GPI-B**	M	TC7
Aspartate Aminotransferase	2.6.1.1	*AAT**	M	TC6.7
Phosphoglucomutase	5.4.2.2	*PGM-2**	L	TC7
Phosphogluconate Dehydrogenase	1.1.1.44	*PGDH**	L	TC7
Malic Enzyme (NADP+)	1.1.1.40	*MEP-1**	M	TC8
Malate Dehydrogenase	1.1.1.37	*MDH-1**	L,M	TC7,TC8
		*MDH-2**	M	TC7,TC8
L-Lactate Dehydrogenase	1.1.1.27	*LDH-A**	M	TC7
		*LDH-B**	M	TC7
Guanine Deaminase	5.3.4.3	*GDA**	L	TC7
Mannose-phosphate Isomerase	5.3.1.8	*MPI**	L,M	TC7

ity among samples with the exception of *PGM-2** and *GPI-B**; at *GPI-B** the allele **100* prevailed with frequencies ranging from 0.859 to 0.952 in all samples except WAL where it occurred with a frequency of only 0.071 (Table 2). The directly observed estimates (*H* d.c.) of average heterozygosity were rather high in all samples, ranging from 0.187 for STR-1 to 0.209 for TUL whereas the values for expected heterozygosity (unbiased estimate, *H* unb.) were slightly higher in five cases and ranged from 0.195 for WAL to 0.224 for REG (Table 2).

Genotype frequencies showed significant deviations from Hardy-Weinberg expectations in only two of 49 tested cases (TUL: *PGM-2**, REG:*GDA**). Fixation indices (F_{is}) for these loci indicated deficiency of heterozygotes at the *GDA** locus in sample REG but a heterozygote excess at the *PGM-2** locus in sample TUL. Results of Wright's hierachical F-statistics indicated that genetic variability was mainly located within samples while differences between them were only weak (Table 3). The high F_{st} and F_{it} values found at the *GPI-B** locus are caused by the allele frequency differences described above. High F_{it} values for the *GDA** locus correspond with high fixation indices (F_{is}) in five of the seven samples (Table 3). The UPGMA dendrogram based on genetic distances (Nei, 1978) highlights the close genetic similarity of the roach samples from the Danube backwaters and the Drau river (Figure 2). The higher distance of WAL was mainly due to the allele frequency differences at *GPI-B** and *PGM-2** loci.

Morphometry

All characters used in the following analysis satisfied the assumption of normality and homogeneity of variance. According to PCA, calculated from the pooled size adjusted data set, five principal components showing eigenvalues higher than one explained 69.6% of the total variance (Table 4). We interpreted the new variables (PCs) in accordance to the loadings of the single characters on each PC. Characters of the head were strongly correlated with PC1 whereas characters mainly describing body depth showed high loadings on PC2. PC3 and PC4 summarised information due to the length and the base of the pectoral, ventral and anal fin. PC5 was dominated by the characters describing the length axis of the fish. The randomisation procedure (COVRAN) testing the significance of the factor loadings confirmed that the factor loadings of the characters used to interpret principal components were significant in all cases (boldface values of Table 4).

One-way ANOVA (Table 5) calculated with the z-standardised scores of the five principal components and sampling site as grouping factor showed highly significant sample effects for the first four principal components but not for PC5. Leven's test confirmed that the assumption of homogeneity of variance was

Table 2. Allele frequencies at variable loci and genetic variability measures in seven samples of roach.
P: Percentage of polymorphic loci (95% criterion).
H(d.c.): Average heterozygosity (direct-count estimate).
H(unb.): Average heterozygosity (unbiased estimate).

Locus	Allele	STR-1	STR-2	STR-3	DRA	REG	TUL	WAL
n		31	32	44	32	30	30	35
*PGDH**	**100*	0.339	0.313	0.341	0.297	0.283	0.333	0.143
	**90*	0.419	0.422	0.534	0.250	0.450	0.450	0.543
	**70*	0.242	0.266	0.102	0.453	0.233	0.217	0.314
	**60*	0.000	0.000	0.023	0.000	0.033	0.000	0.000
*PGM-2**	**105*	0.000	0.078	0.045	0.113	0.000	0.000	0.000
	**103*	0.000	0.000	0.000	0.016	0.000	0.000	0.000
	**102*	0.339	0.375	0.443	0.210	0.532	0.452	0.757
	**100*	0.290	0.141	0.170	0.161	0.145	0.194	0.000
	**96*	0.000	0.000	0.011	0.000	0.000	0.000	0.000
	**95*	0.371	0.375	0.307	0.484	0.290	0.355	0.200
	**92*	0.000	0.031	0.023	0.016	0.032	0.000	0.043
*MPI**	**100*	0.726	0.797	0.709	0.766	0.694	0.661	0.743
	**88*	0.274	0.203	0.291	0.234	0.306	0.339	0.257
*MDH-1**	**120*	0.016	0.000	0.000	0.000	0.000	0.016	0.000
	**100*	0.855	0.859	0.830	0.797	0.887	0.839	0.743
	**75*	0.129	0.141	0.170	0.203	0.113	0.145	0.257
*GDA**	**105*	0.290	0.266	0.250	0.219	0.488	0.258	0.456
	**100*	0.710	0.734	0.750	0.781	0.512	0.742	0.544
*GPI-A**	**105*	0.032	0.078	0.114	0.031	0.109	0.016	0.143
	**100*	0.952	0.922	0.852	0.938	0.859	0.919	0.829
	**95*	0.016	0.000	0.034	0.031	0.031	0.064	0.029
*GPI-B**	**115*	0.258	0.281	0.216	0.188	0.234	0.355	0.929
	**112*	0.016	0.000	0.023	0.000	0.016	0.000	0.000
	**100*	0.726	0.719	0.761	0.813	0.750	0.645	0.071
*LDH-A**	**105*	0.000	0.016	0.000	0.000	0.016	0.000	0.000
	**100*	1.000	0.984	1.000	1.000	0.984	1.000	0.986
	**95*	0.000	0.000	0.000	0.000	0.000	0.000	0.014
*AAT**	**100*	0.000	0.000	0.011	0.000	0.016	0.016	0.000
	**90*	1.000	1.000	0.989	1.000	0.984	0.984	1.000
	P	42.9	50.0	50.0	50.0	50.0	50.0	50.0
	H(d.c.)	0.187	0.203	0.209	0.202	0.198	0.209	0.207
	S.E.	0.061	0.072	0.062	0.069	0.058	0.070	0.062
	H(unb.)	0.209	0.209	0.216	0.201	0.224	0.219	0.195
	S.E.	0.069	0.068	0.066	0.067	0.068	0.068	0.059

satisfied for PC1 to PC4. Multiple range tests (Scheffe, significance level 0.05) detected significant differences between factor score means for at least three (PC1) up to twelve (PC2) of twenty-one possible pairwise comparisons (Figure 3). Sample WAL differed from all other samples for PC2 factor scores showing a mean of -1.3669 standard deviations. When scores of PC1 were plotted against those of PC2, polygons enclosing all data points of an individual sample overlapped widely. Only WAL was partly separated according to the lower average body depth (Figure 4).

The initial indices of matrix correlation (R_{obs}) calculated for each pair of samples ranged from 0.768 (STR-1/WAL) up to 0.889 (STR-2/STR-3). In all test-

Table 3. Summary of F-statistics at all loci and F_{is} values for *GDA** locus for each sample.

Locus	F_{is}	F_{it}	F_{st}
*PGDH**	−0.016	0.019	0.034
*PGM-2**	0.036	0.095	0.061
*MPI**	0.038	0.047	0.009
*MDH-1**	−0.010	0.005	0.015
*GDA**	**0.147**	**0.186**	0.046
*GPI-A**	0.001	0.024	0.023
*GPI-B**	−0.037	**0.217**	**0.245**

	STR-1	STR-2	STR-3	DRA	REG	TUL	WAL
F_{is} GDA*	0.217	0.119	−0.091	0.269	0.350	0.158	−0.008

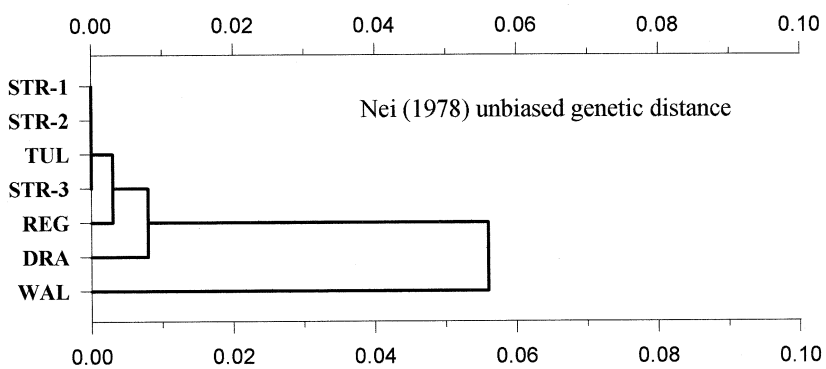

Figure 2. UPGMA dendrogram based on genetic distances, according to Nei (1978).

ed cases P values did not exceed 0.001, therefore the null hypothesis assuming no similarity had to be rejected in all cases; patterns of intercorrelation were similar between all samples.

Pearson correlation coefficients between total morphological variation (D-varcov) and genetic variation assessed as the direct count estimates of average heterozygosity and heterozygosity per locus showed rather high heterogeneity. As COVRAN turned out none of the calculated values ranging from −0.382 for *PGDH** to 0.384 for *GPI-A**was significant (Table 6).

Discussion

Allozyme variation in Austrian roach populations was remarkably high. Average heterozygosity was almost four times as large as the mean values of 183 freshwater teleost fish species published by Nevo et al. (1984) or of 60 North American cyprinids examined by Avise (1977) and also higher than values published for other European cyprinids (Coelho, 1992; Alves & Coelho, 1994; Coelho et al., 1995; Luskova et al., 1995). Estimates of heterozygosity depend strongly on the set of loci investigated and the electrophoretic conditions of each study, and thus have to be compared with caution (Buth, 1984; Ward et al., 1992). However, Bouvet et al. (1991) and Wagner (1992) obtained similar high levels of genetic variation in roach populations from French rivers and German lakes, respectively. Mitton & Lewis (1989) found high heterozygosity in fish species able to cope with temporal variation in the environment through life-history features allowing rapid population increase. *Rutilus rutilus* seems to be another example of this trend; probably high effective population sizes are also responsible for the maintenance of high genetic variability.

Genetic variation was similar in all samples, no reduction of variability was evident in the population from an isolated backwater. Several factors possibly contribute to the absence of observed genetic effects of habitat modification: changes in the environment

Table 4. The loadings of the original characters for the first five principal components and the percentage of explained variance.

Characters	Factor loadings				
	PC1	PC2	PC3	PC4	PC5
	25.1%	14.7%	13.6%	8.3%	7.9%
HW	**0.7990**	0.2305	−0.1520	0.0592	0.0667
HL	**0.7310**	−0.0462	0.3519	0.0360	0.2274
IOW	**0.7215**	0.2559	0.1808	0.1151	−0.1288
POL	**0.5144**	−0.2013	0.0924	0.3352	0.4357
GBD	0.3574	**0.7426**	−0.0677	0.0325	0.1531
BDF	−0.0962	**0.7392**	0.1814	0.1400	−0.0649
LBD	0.2079	**0.6998**	−0.0774	0.1615	0.0838
LVF	0.1214	0.1401	**0.9117**	0.0460	0.0015
LPF	0.0617	−0.0806	**0.8911**	0.1158	−0.0139
BPF	0.2305	0.2104	−0.0696	**0.7870**	0.0449
BVF	0.1541	−0.0159	0.3221	**0.7452**	−0.0604
BAF	−0.2006	0.4193	0.0196	**0.6080**	0.0388
LCP	0.1020	−0.2328	0.0701	−0.1375	**−0.8325**
PD	0.3847	−0.0688	0.0605	−0.2889	**0.6724**

Table 5. One-way ANOVA test for significant differences between means of factor scores of the first five principal components (PC1 to PC5).

	STR-1	STR-2	STR-3	DRA	REG	TUL	WAL	F-value	P level of F
PC1	−0.5894	0.2444	0.2214	0.4958	−0.2160	0.1251	−0.4139	5.4592	<0.0001***
PC2	0.4640	0.2691	−0.2800	0.2659	0.9876	−0.3906	−1.3669	29.7486	<0.0001***
PC3	−0.1082	0.2661	0.1493	−1.0942	−0.3277	0.6220	0.5978	15.0943	<0.0001***
PC4	−0.2493	−0.0457	−0.7231	0.5601	−0.2394	0.5223	0.5497	11.0413	<0.0001***
PC5	−0.1428	−0.2035	0.0937	0.1741	0.0974	−0.2253	0.1534	0.9278	0.4758 n.s.
n	31	31	43	32	30	27	28		

Table 6. Pearson product moment correlation coefficients (r) between morphological (D-varcov) and genetic variation. Note that for correct values of D-varcov the table entries of the first row have to be multiplied by 10^{-50}.

		STR-1	STR-2	STR-3	DRA	REG	TUL	WAL	
	D-varcov	0.02516	0.25341	0.48389	0.63434	0.01648	0.09735	0.01024	r
PGDH∗	H (d.c.)	0.581	0.750	0.545	0.750	0.633	0.633	0.600	0.384
PGM-2∗	H (d.c.)	0.548	0.750	0.523	0.645	0.516	0.774	0.429	0.231
MPI∗	H (d.c.)	0.419	0.281	0.488	0.344	0.355	0.355	0.400	0.066
MDH-1∗	H (d.c.)	0.226	0.219	0.250	0.406	0.226	0.194	0.457	0.237
GDA∗	H (d.c.)	0.323	0.344	0.409	0.250	0.325	0.323	0.500	−0.364
GPI-A∗	H (d.c.)	0.097	0.094	0.205	0.125	0.281	0.161	0.343	−0.382
GPI-B∗	H (d.c.)	0.419	0.375	0.477	0.313	0.375	0.452	0.143	0.187
TOTAL	H (d.c.)	0.187	0.203	0.209	0.202	0.198	0.209	0.207	0.315

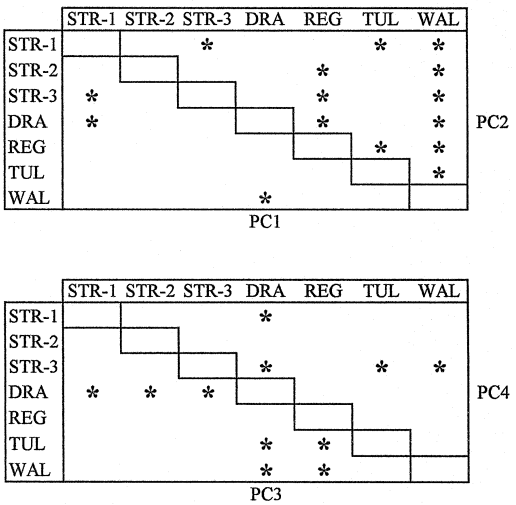

Figure 3. Scheffe test with significance level 0.05 for multiple comparison of means (Table 5). Factor scores means of PC1 to PC4 were compared between the seven samples of roach. Fig 3a shows results from PC1 (left triangle) and PC2 (right triangle) respectively, whereas 3b illustrates the situation at PC3 and PC4. Asterisks indicate significant differences between samples.

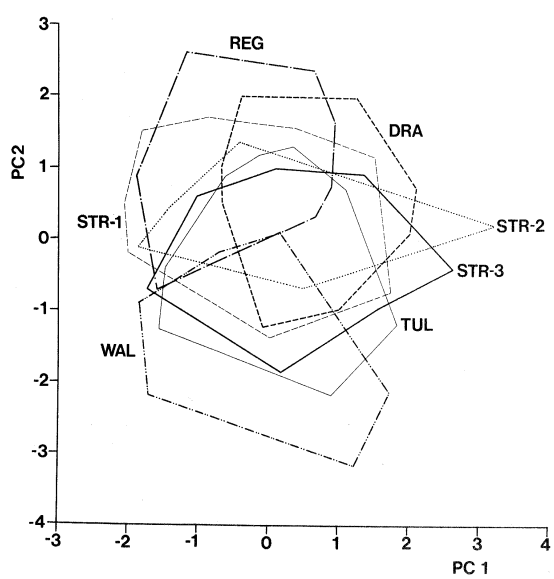

Figure 4. Scatterplot of the factor scores of the first two principal components derived from the correlation matrix of fourteen morphological characters. Polygons enclose all data points of the indicated sample. For character loadings and interpretation of the principal components see Table 4 and text respectively.

may have been established too recently to affect population structures; migration barriers may be permeable during episodic floods; effective population sizes may be high. Most of the genetic variability was contained within populations, a result in agreement with the findings of Bouvet et al. (1991, 1995) and Wagner (1992). High fixation indices at *GDA** in most samples, notably in all samples from backwaters connected with the mainstream of the Danube, may be due to the Wahlund effect. However, if these samples represent mixtures of subpopulations differing in allele frequencies, it is unclear why only *GDA** and not also other loci showed this pattern of variation; alternative explanations such as selection against heterozygotes, atypical gene expression in heterozygotes, or the presence of null alleles, cannot be excluded at present.

The close genetic similarity between samples from the Danube and the one from the Drau river is striking (Figure 2), considering their separation by approximately 1000 river kilometers. Populations of the rheophilic cyprinid nase (*Chondrostoma nasus*) from the same areas show comparable genetic likeness (Gollmann, 1995). These congruent patterns suggest high levels of gene flow along the Danube and her tributary Drau, at least until the construction of hydroelectric dams in the recent past. Roach from lake Wallersee, which is geographicaly much closer to the Danube (Figure 1), had distinct allele frequencies at the *GPI-B** locus (Tables 2, 3). Since WAL was the only sample from a lake, we cannot answer the question whether this divergence reflects adaptation, regional differentiation or random drift. Nevertheless, these differences indicate reduced gene flow between roach from lake Wallersee and the Danube river.

Morphological variation in roach mainly involves two features: body depth and fin size (Stangenberg, 1947; Köhler, 1992). We observed considerable variation concerning those traits within populations, but obtained also significant differences between them (Table 5, Figure 3). Low body and broad fins have been described as characteristic for habitats with faster water current (Köhler, 1992). Specimens from the Drau river showed the largest mean values for the principal components describing head size (PC1) and base width of the paired fins (PC4), and they had the lowest values for PC3 which represents the length of these fins (Tables 4, 5). This association of characters can be viewed as an adaptation to their lotic habitat; however, the differences to the samples from the Danube were only partly significant (Figure 3), and patterns of correlations among characters were similar in all samples, as demonstrated by the QAP analysis. The most distinctive morphological feature of any of our samples was the low body depth (PC2) of fish from the lake Wallersee; this finding defies an adaptive ecomorpho-

logical interpretation, and may result from proximal environmental factors such as food supply or temperature (Holcik & Skorepa, 1971; Libosvarsky & Ruban, 1985). The comparison of morphological and genetic variation was limited by the similar levels of genetic variability in our samples: the narrow range of heterozygosity estimates made it highly unlikely to find significant correlations (Table 6).

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