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DISTRIBUTION PATTERNS AND GENETIC VARIABILITY OF THREE STREAM-DWELLING FISH SPECIES

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Present study established correlations between the spatial distribution of three stream-dwelling fish species (chub, stone loach and gudgeon), the environmental variables which affect their distribution, and the genetic structure of their assemblages, in a North Hungarian drainage system. The spatial distribution of gudgeon and stone loach (which have more specific habitat needs) was affected by elevation, slope and distance from the mouth. Chub occurred frequently in the wider and deeper streams of the hills with higher velocity, but was repeatedly caught in lowland situated sections close to the mouth as well. Genetic data obtained with AFLP were correlated with distribution and as a result higher genetic distances were revealed among gudgeon and stone loach than among chub assemblages. The assemblages of the former two species show a clear pattern of isolation by distance. Results suggest that lowland sections of the studied streams might act as ecological barriers for stone loach and gudgeon assemblages, but not for the chub. Hence, in this drainage system the stone loach and gudgeon appear with separated populations while the chub is characterized by a metapopulation structure.

Key words: AFLP, stone loach, gudgeon, chub, distribution, isolation by distance

INTRODUCTION

There are a number of European freshwater fish species which are widely distributed, but require more specialized habitats (BĂNĂRESCU 1990). The small, rheophylic, bottom-dwelling species living in hilly streams represent this group. These fish show strong site fidelity, and because of the mosaic pattern of their habitats, gene flow among their populations is more difficult than among the populations of those species that do not insist on special habitats (FUMAGALLI *et al*. 2002, PAŚKO & MAŚLAK 2003). Isolated spatial distribution can lead to genetic subdivision (RUNDLE & NOSIL 2005).

It is a general trend that the genetic diversity of Nearctic and Palearctic species – not only fish – is in inverse relation to geographical latitude (HILLEBRAND 2004, MITTELBACH *et al.* 2007). In terms of this trend, the Quaternary glaciations had been of outstanding importance confining the formerly widely distributed spe-

cies to southern refugia. Interglacial recolonization of North and West Europe took place mainly from the Iberian and Balkan refugia. Because of the bottleneck effect, genetic variability is usually lower in the newly colonized area then in the never glaciated refugia (BERNATCHEZ & WILSON 1998). Large-scale studies revealed that those species which had survived the ice ages in Northern or Western European refugia have genetically more segregated populations than those species which had disappeared then recolonized these areas after the ice ages (CULLING *et al.* 2006, DURAND *et al.* 1999, HEWITT 2000, EMERSON & HEWITT 2005, SCHREIBER 2002).

Besides these large-scale effects, the characteristics of the habitats may also influence the level of the genetic substructuring as showed in fine-scale studies. Publications (BARLUENGA & MEYER 2005, HÄNFLING & WEETMAN 2006, NERAAS & SPRUELL 2001) suggest that for species with special habitat requirements a deeper, open water body or a man-made dam may serve as a significant ecological barrier.

Generally, fine-scale studies investigate only one species (MACHRODOM *et al.* 1999, BARLUENGA 2006, HÄNFLING *et al.* 2002, KOSKINEN *et al.* 2000), while the number of publications which compare genetic diversities of some characteristic fish species in certain running water types are still limited (WOLTER 1999, WOLTER *et al.* 2003).

A previous survey (TAKÁCS & NAGY 2005) revealed remarkable differences in the composition of fish assemblages of hilly and lowland sections of streams at a North Hungarian drainage system. In addition, the disjunct distribution of three rheophylic fish species – the stone loach (*Barbatula barbatula* (LINNAEUS, 1758)), the gudgeon (*Gobio gobio* LINNAEUS, 1758) and the chub (*Leuciscus cephalus* (LINNAEUS, 1758)) – as characteristic species of hilly streams in Hungary (ERŐS 2001, ERŐS *et al.* 2003, HARKA & SALLAI 2004) were found.

Two of these species, the gudgeon and the stone loach, have strong site fidelity, partially because they require relatively specific habitats (WHEELER 1983, GERKING 1953). These are small, bottom-dwelling species and their life strategy often correlates with strong genetic subdivision (MARKERT *et al.* 1999). The chub is opportunistic and common in most running waters in Hungary (HARKA & SALLAI 2004), and Europe (BĂNĂRESCU 1991).

For genetic investigations amplified fragment length polymorphism (AFLP), a PCR based molecular genetic method, was used. This technique combines the advantages of restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) which are preferred methods in fish studies (ZHANG 2004). However, compared with these methods, AFLP is much more reproducible, reveals higher level of polymorphism, and also a high level of shared

fragments which are required to investigate relatedness among populations (CADRIN *et al.* 2005).

This fine scale investigation aims to address the following questions: What kind of relationships can be established between the spatial distribution and the environmental variables, and between the spatial distribution and genetic variability of the assemblages? What kind of population structure can be observed for the assemblages of the three species in the drainage system studied? Do lowland sections of streams act as a barrier resulting in genetic isolation of assemblages of the studied species?

MATERIALS AND METHODS

Fish sampling and study sites

The studied system is situated in the foothills of the Bükk Mountains (Bükkalja), and in a lowland region called Borsodi-mezőség. Except for the Kulcsárvölgyi stream all streams studied belong to the catchment area (1379 km^2) of the Rima stream which empties into the Kisköre Reservoir built on the River Tisza. The Kulcsárvölgyi stream also empties into the River Tisza, through the Hejő stream and the River Sajó. Consequently, despite the geographical proximity, the Kulcsárvölgyi stream is widely separated from the other streams (Fig. 1). Eighteen sampling sites were chosen for collecting distribution data of the three fish species. Faunistic surveys were performed over 2 years on 6 occasions in 2004–2005 (three surveys in spring, summer, and autumn in both years). Each fishing section was 100 m long.

For genetic investigation, fish were caught by electrofishing in eight upstream sites on five streams (R1, K1, K3, S1, G1, G2, Ku1, Ku2) during the faunistic survey in summer of 2005. Gudgeons were collected at six sampling sites (R1, K1, K2, S1, G1, KU1), while stone loach and chub were sampled in five sites (K1, K2, S1, G1, KU1; and R1, K1, K2, G2, KU2, respectively). To compare hydrographical and genetic distances of the studied assemblages, the distances of the sampling sites were derived from a hydrological map (1:10 000). Five individuals of each species were collected from all sampling sites respectively and the fish were frozen at –20 °C until further use.

From the seven measured environmental factors, stream slope (m·km^{-1}), and distance from the mouth into the Sajó and the Tisza (km) were derived from a hydrological map (1:10 000). The other five variables: the cover of aquatic vegetation (%), velocity (m·s^{-1}), elevation (m), mean width (m) and depth (m) were measured directly during the field surveys.

Genetic investigation

Skeletal muscle was sampled from each fish avoiding contamination. DNA was extracted using phenol-chlorophorm-isoamylalcohol (25:24:1, Sigma-Aldrich, USA) extraction after proteinase K (Sigma-Aldrich) digestion. In order to eliminate RNA RNase (Sigma-Aldrich) was applied. After ethanol precipitation the DNA was dissolved in TE buffer (10 mM TRIS and 0.1 mM EDTA, pH8). The DNA concentration and purity was determined using a GeneQuantIII DNA/RNA Calculator (Pharmacia Biotech, USA).

AFLP was carried out as described by VOS *et al*. (1995) with modifications. 500 ng DNA was digested with 5 U *Eco*RI (Fermentas, Canada) for 1 h at 37°C then with 5 U *Mse*I (Fermentas) at 65 °C

for 1 h in a total volume of 40 µl containing 2X Buffer Tango (Fermentas). Ligation of digested DNA at 22 °C for 16 h with adapters was performed by adding 1 U T4 DNA ligase (Fermentas), 5 pmol *Eco*RI- and 50 pmol *Mse*I-adapters (Integrated DNA Technologies, USA) in 1X Ligation Buffer (Fermentas).

Five µl of ligation mixture was preamplified in a total volume of 25 µ containing 12.5 µl Taq Ready Mix PCR Reaction Mix (Sigma-Aldrich), 10 pmol *Eco*RI+A primer and 10 pmol *Mse*I+C primer (Integrated DNA Technologies). DNA amplification was started with an initial denaturation at 94 °C for 3 min, followed by 20 cycles of denaturation for 30 sec at 94 °C, annealing for 1 min at 56 °C and 1 min extension at 72 °C and then completed with a final elongation at 72 °C for 5 min in an ABI PRISM 2700 Thermo Cycler (Applied Biosystems, USA).

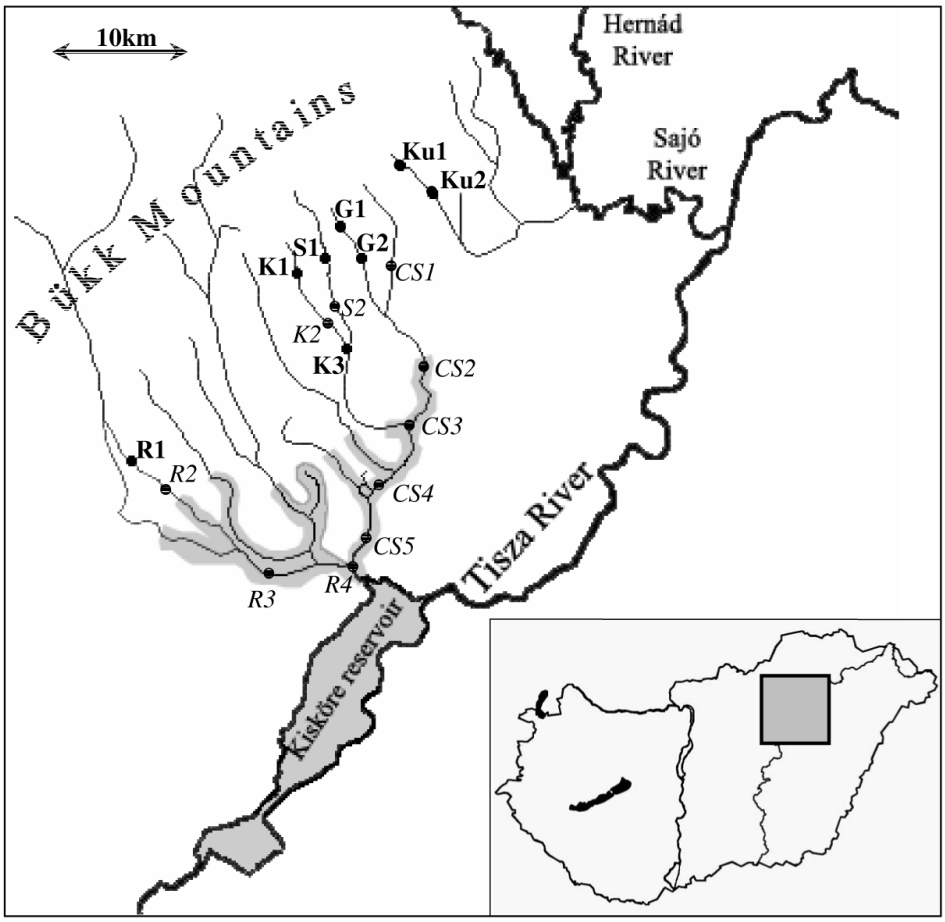

Fig. 1. Map of sampling sites. (bold = sampling sites of genetic surveys) (drawn with grey: lowland sections of streams) Latitudes and longitudes: top (of box): 48°05'N; bottom: 47°21'N; left: 20°16'E; right: 21°17'E

Preamplified DNA was 10 fold diluted and 5 µl was amplified in a total volume of 25 µl containing 12.5 µl Taq Ready Mix PCR Reaction Mix (Sigma-Aldrich), 10 pmol *Mse*I+CAG primer (Integrated DNA Technologies) and 10 pmol 5' NED labelled *Eco*RI+ACC (Applied Biosystems). Touch down PCR was carried out in a MyCycler Thermo Cycler (Bio-Rad Laboratories, USA) with cycling profile as described by VOS *et al*. (1995).

Amplified fragments were analyzed by an automated ABI PRISM 3100 Genetic Analyzer and sized with a GeneScan HD400 ROX standard. AFLP patterns were analyzed by GeneScan software 3.1 version (Applied Biosystems).

Statistical analyses

Relationships between the environmental factors and the relative abundances were analysed by Canonical Correspondence Analysis (CCA). The effect of the extreme values was minimised by data transformation, using the formula log(x+1). For the analysis the SYN-TAX 2000 (PODANI 2001) statistical software was used.

On the basis of the electropherograms binary data were generated. To investigate genetic similarities, binary data sets were converted to similarity matrices (values) using the Dice similarity coefficient (CADRIN *et al.* 2005). Intrapopulation analysis was carried out according to NEI and LI (1979). Genetic distances between population-pairs were calculated by the equation suggested by LYNCH (1991) and the results were used for Principal Component Analysis (PCA, with Euclidean distance) with the SYN-TAX 2000 software (PODANI 2001). Genetic distances among groups were tested for each species by exact test (RAYMOND & ROUSSET, 1995) using the TFPGA 1.3 statistical software (MILLER 1997) (2000 dememorization steps, 10 batches, with 2000 permutations per batch) (CADRIN *et al.* 2005). In order to reveal correlations between hydrographical and genetic distances a Mantel-test (MANTEL 1967) was performed by the zt 1.0 statistical software (BONNET & VAN DE PEER 2002).

RESULTS

Table 1 shows the "frequency of occurrence" (how many times a given species was caught in a studied section during the six surveys), the mean number and the relative abundances of the species. At upstream sections fish assemblages mostly consist of gudgeon, stone loach and chub. At lowland sections – except for the Cs2 site – only chub was present but with a lower abundance.

Distribution of the stone loach in the drainage system correlated positively with the distance from mouth and the cover of macrophytes, however elevation and slope were also correlated with distribution. The highest relative abundance of the gudgeon was found in those sections which were characterized by high elevation and velocity, although the wide riverbed and high descent were also favourable. At the same time, the chub was abundant in the wide, deep and fast flowing sections (Fig. 2).

To investigate the genetic diversities of the studied fish assemblages percentage of polimorphic loci (P_{95}) and unbiased heterozygosity (H_u) were determined

Table 1. Number of occurrence from the six surveys (F), specimen numbers (N) (mean per 100m ± SD), and relative abundance data (Ab.) (mean ± SD) of the species at the 18 sampling sites, based on a total of six surveys. (Bold: sites of genetic surveys; Cs2–Cs5 and R3–R4 are lowland situated sites)

Code	Sone loach			Gudgeon			Chub		
	F	N	Ab.	F	N	Ab.	F	N	Ab.
R1	6/0	0.0±0.0	0.000±0.00	**6/6**	**81.8±42.9**	**0.446±0.11**	**6/6**	**81.0±23.7**	**0.441±0.07**
R2	6/0	0.0±0.0	0.000±0.00	6/6	19.7±14.4	0.199±0.11	6/6	61±31.4	0.616±0.22
K1	**6/6**	**12.8±10.6**	**0.086±0.05**	**6/6**	**30.0±16.5**	**0.200±0.09**	**6/6**	**57.2±14.5**	**0.382±0.12**
K2	6/5	3.6±3.5	0.063±0.06	6/6	6.8±4.4	0.123±0.08	6/6	13.7±5.2	0.246±0.08
K3	**6/6**	**19.7±14.2**	**0.151±0.07**	**6/6**	**14.0±7.7**	**0.107±0.05**	**6/6**	**24±15.4**	**0.184±0.05**
S1	**6/6**	**11.0±9.2**	**0.059±0.05**	**6/6**	**112.2±64.8**	**0.602±0.25**	6/0	0.0±0.0	0.000±0.00
S2	6/4	10.8±10.5	0.056±0.06	6/6	11.7±11.6	0.060±0.06	6/5	5.2±4.3	0.027±0.02
G1	**6/6**	**31.0±8.0**	**0.496±0.06**	**6/6**	**30.2±5.8**	**0.483±0.06**	6/5	1.2±1.0	0.019±0.02
G2	6/4	8.3±10.0	0.185±0.22	6/5	11.0±15.7	0.244±0.15	**6/5**	**22.2±24.5**	**0.491±0.34**
Cs1	6/0	0.0±0.0	0.000±0.00	6/1	0.2±0.4	0.003±0.01	6/2	0.8±1.3	0.017±0.02
Ku1	**6/4**	**4.2±4.9**	**0.044±0.05**	**6/6**	**25.0±18.4**	**0.265±0.15**	6/1	0.2±0.4	0.002±0.00
Ku2	6/6	14.7±19.3	0.084±0.13	6/6	61.5±20.6	0.351±0.11	**6/3**	**2.7±3.8**	**0.015±0.02**
Cs2	6/4	1.8±2.14	0.015±0.04	6/1	0.7±1.6	0.005±0.01	6/5	7.7±5.4	0.062±0.06
Cs3	6/0	0.0±0.0	0.000±0.00	6/0	0.0±0.0	0.000±0.00	6/0	0.0±0.0	0.000±0.00
Cs4	6/0	0.0±0.0	0.000±0.00	6/0	0.0±0.0	0.000±0.00	6/0	0.0±0.0	0.000±0.00
Cs5	6/0	0.0±0.0	0.000±0.00	6/0	0.0±0.0	0.000±0.00	6/1	0.2±0.4	0.001±0.00
R3	6/0	0.0±0.0	0.000±0.00	6/0	0.0±0.0	0.000±0.00	6/5	3.5±3.1	0.028±0.02
R4	6/0	0.0±0.0	0.000±0.00	6/0	0.0±0.0	0.000±0.00	6/2	0.5±0.8	0.004±0.01

Table 2. Genetic diversities of stone loach, gudgeon and chub samples; number of specimens analysed (N), unbiased heterozygosity (H_u); percentage of polymorphic loci at 95% criterion (P_{95}); intrapopulation similarity ratio (S)

Code	Stone loach				Gudgeon				Chub			
	N	H_u	P_{95}	S	N	H_u	P_{95}	S	N	H_u	P_{95}	S
R1	–	–	–	–	5	0.081	20.20	0.918	5	0.108	26.04	0.882
K1	5	0.090	20.81	0.916	5	0.047	12.12	0.955	5	0.134	31.25	0.845
K3	5	0.121	26.17	0.889	5	0.059	14.14	0.944	5	0.160	40.10	0.822
S1	5	0.057	14.09	0.943	5	0.093	20.20	0.915	–	–	–	–
G1	5	0.060	14.09	0.950	5	0.126	28.28	0.899	–	–	–	–
G2	–	–	–	–	–	–	–	–	5	0.170	36.98	0.812
Ku1	5	0.144	32.89	0.884	5	0.115	28.28	0.901	–	–	–	–
Ku2	–	–	–	–	–	–	–	–	5	0.257	55.21	0.719

(Table 2). In the case of stone loach 168 AFLP fragments (loci) were found. The percentage of polymorphic loci (P_{95}) varied between 14.09 and 32.89%, and the unbiased heterozygosity (H_u) between 0.057 and 0.144. In gudgeon assemblages

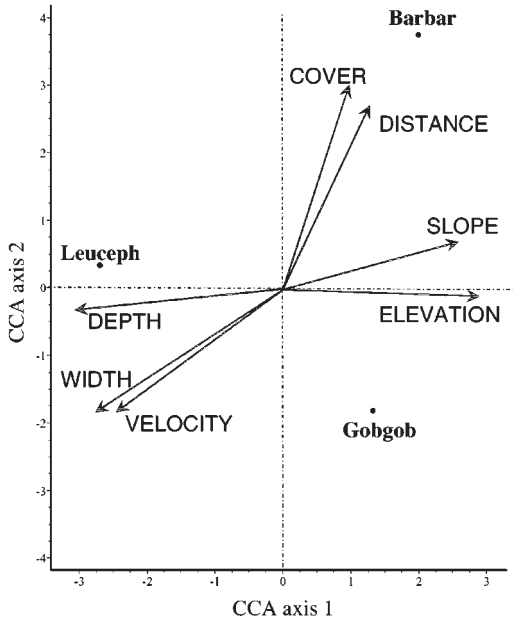

Fig. 2. Canonical Correspondence Analysis (CCA) ordination diagram showing the effect of environmental factors on the relative abundances of studied species. The first axis explains 29.93% and the second axis 9.79% of variability

Table 3. Variation in AFLP DNA fragments between sampling sites for the studied species. Diagonal (bold): total number of bands; above: dissimilarity ratios; below: p values of exact tests (*=significant difference)

stone loach	K1	K3	S1	G1	Ku1	
K1	**102**	0.036	0.085	0.124	0.181	
K3	0.999	**108**	0.082	0.109	0.175	
S1	0.024*	0.0026*	**92**	0.110	0.192	
G1	< 0.001*	< 0.001*	< 0.001*	**108**	0.145	
Ku1	< 0.001*	< 0.001*	< 0.001*	< 0.001*	**125**	
gudgeon	R1	K1	K3	S1	G1	Ku1
R1	**42**	0.109	0.107	0.105	0.167	0.142
K1	0.001*	**52**	0.098	0.052	0.148	0.189
K3	0.005*	0.053	**52**	0.075	0.114	0.119
S1	0.001*	0.003*	0.240	**50**	0.143	0.168
G1	< 0.001*	< 0.001*	< 0.001*	< 0.001*	**55**	0.071
Ku1	< 0.001*	< 0.001*	< 0.001*	< 0.001*	0.003*	**49**
chub	R1	K1	K3	G2	Ku2	
R1	**123**	0.057	0.032	0.043	0.074	
K1	0.881	**122**	0.023	0.088	0.086	
K3	0.999	1	**137**	0.059	0.045	
G2	0.985	< 0.001*	0.270	**135**	0.071	
Ku2	< 0.001*	< 0.001*	0.060	< 0.001*	**156**	

12.12–28.28% loci were polymorphic, and H_u ranged from 0.047 to 0.126. The analyzed fragments of this species were 99. The highest level of genetic diversity was found in chub assemblages where 192 fragments were observed. P_{95} varied between 26.04 and 55.21%, while H_u between 0.108 and 0.257. Heterozygosities were significantly correlated with the number of polymorphic loci in all species (stone loach: $p = 0.0002$, $r = 0.99$; gudgeon: $p = 0.00039$, $r = 0.98$; chub: $p = 0.0023$, $r = 0.98$).

P_{95} and H_u values of the three species were analyzed by paired samples t-test. The results demonstrate a significantly higher level of polymorphism and heterozygosity in the chub compared to the stone loach (P_{95} $p = 0.029$; H_u $p = 0.046$) and also the gudgeon (P_{95} $p = 0.011$; H_u $p = 0.016$). Nevertheless, there was no significant difference between the gudgeon and the stone loach in P_{95} ($p = 0.816$) nor H_u ($p = 0.726$) values.

The intrapopulation similarity ratios varied between 0.884 and 0.950; 0.899 and 0.955, and 0.719 and 0.882 for the stone loach, gudgeon and chub, respectively

(Table 2). Paired sample t-tests showed that the intrapopulation similarities were significantly higher for stone loach and gudgeon assemblages than for chub assemblages ($p = 0.02$; and $p = 0.009$, respectively), while there was no significant difference between gudgeon and stone loach assemblages ($p = 0.77$).

The genetic distances of stone loach assemblages ranged from 0.036 to 0.192, from 0.052 to 0.189 between gudgeon assemblages, and from 0.032 to 0.088 for chub assemblages (Table 3). Lower genetic distances were observed between those assemblages of all species which were hydrographically closer to each other (Fig. 3). Whereas higher genetic distances between hydrographically distant assemblages were revealed for gudgeon and stone loach, but lower for chub assemblages. To investigate the rate of the genetic distances between the fish assemblages, exact test was performed. Except for the K1 and K3 assemblages, signifi-

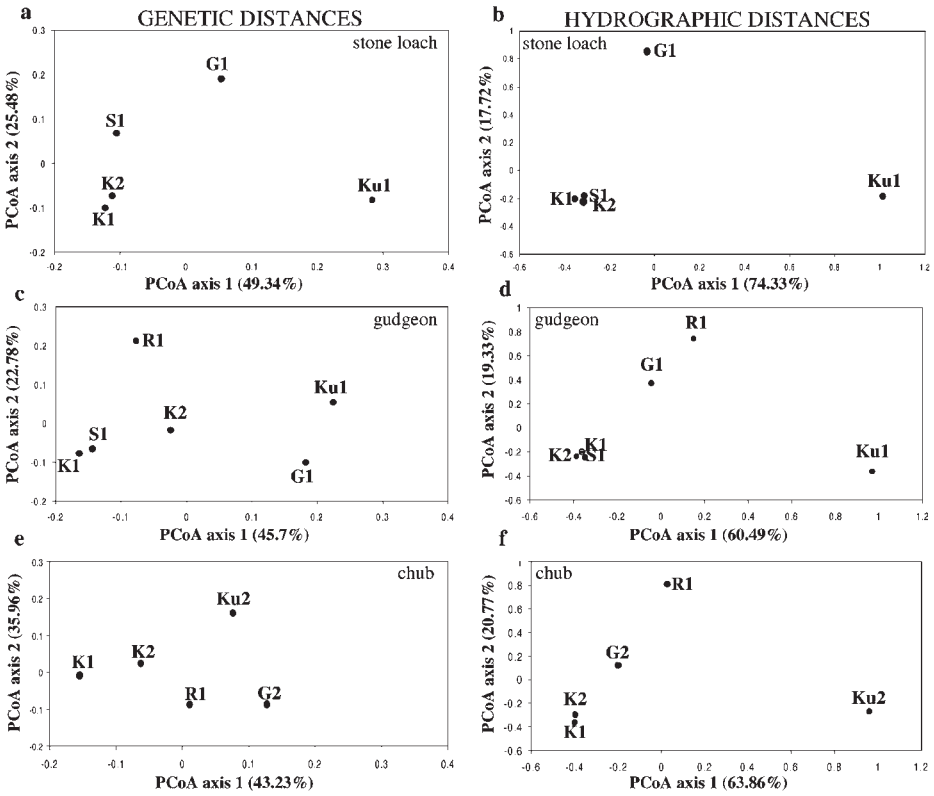

Fig. 3. Principal Coordinates Analysis (PCoA) of the genetic and hydrographic distances of the sampled stocks (a, c, e: genetic distances; b, d, f: hydrographic distances)

cant genetic distances were found between stone loach assemblages, suggesting that K1 and K3 are genetically not distinct assemblages (Table 3).

In the case of gudgeon significant differences were revealed between almost all assemblages. However, the K3 assemblage did not show genetic difference from S1 and K1 assemblages from the upper part of the confluent streams. At the same time, significant genetic distance was observed between K1 and S1 (Table 3). This significant difference may be due to single directional movement of fish from upstream to downstream caused e.g. by floods. There was no significant genetic difference between most of the chub assemblages in the catchment area of the Rima stream except for the K1 and G2 assemblages. However, the only population (Ku2), originated from the catchment area of the Sajó, was significantly different from most of the assemblages in the Rima area (Table 3). A significant correlation was found with Mantel-test between the genetic and the hydrographic distance of stone loach ($p = 0.025$, $r = 0.90$) and gudgeon assemblages ($p = 0.038$, $r = 0.417$). There was no significant correlation for chub assemblages ($p = 0.058$, $r = 0.483$).

DISCUSSION

Our survey shows that the stone loach and the gudgeon chiefly occurred in the hilly sections in high numbers. The chub was frequent in the hilly sections, but appeared in the lowland sections as well. Among the three studied species the chub had the widest range in the drainage system. These results agree with the wider tolerance of this species (ARLINGHAUS & WOLTER 2003).

In accordance with this fact, environmental factor analyses revealed that the stone loach and the gudgeon were abundant at those sites of the streams which had higher elevation, slope and distance from the mouth. Similarly to other publications (SANTOUL *et al*. 2005, HYSLOP 1982, WELTON *et al.* 1983) strong positive correlation was found between the covering and the abundance of the stone loach. In case of the gudgeon the velocity and the width also influence the distribution. Although other articles described that habitat tolerance of the gudgeon permits its continuous and abundant occurrence (SCHREIBER 2002, BĂNĂRESCU *et al.* 1999), disjunct distribution of this species was found in this drainage system.

Results from the genetic investigations agree well with the distribution data. Among the three species studied the highest interpopulation genetic diversity was observed for stone loach which prefers chiefly the uppermost sections of the streams. The interpopulation similarities were higher in case of the chub than for the two benthic species. The high interpopulation dissimilarities among the stone loach and the gudgeon assemblages correspond to previous studies of these species

(BARLUENGA & MEYER 2005, SCHREIBER 2002, MENDEL *et al.* 2005). At the same time, chub assemblages are less separated just like its other European populations (HÄNFLING & BRANDL 1998*a*). The interpopulation genetic diversity was similarly high for the two benthic species studied than for other species (eg. Iberian chub or European bullhead) with narrow tolerance and specialized habitat requirements (BRITO & COELHO 1999, HÄNFLING & BRANDL 1998*b*). However, the assemblages of the two benthic species studied are not separated extremely (FUMAGALLI *et al.* 2002), since it was a fine scale study, the genetic dissimilarities observed are considerable. Intrapopulation similarities were higher for the stone loach and the gudgeon than for the chub. The relatively high intrapopulation similarities compared to other investigations (MENDEL *et al.* 2005, WOLTER *et al.* 2003) suggest that the gudgeon populations may be strongly separated in the studied drainage system. Different levels of genetic dissimilarities of the three species may result from the different environmental requirements and also from the different migration behaviour.

The higher level of the genetic diversity and the interpopulation similarities of the chub may be due to the fact that the chub is a migratory species. Migration is chiefly determined by spawning (HLADIK & KUBECKA 2003, HOHAUSOVA *et al.* 2003), however, it is also motivated by flood (STOTT 1967). In other experiments, as a result of the site fidelity, most gudgeon and stone loach individuals which were removed from their habitats returned to the original home range (STOTT 1967, BRUNKEN 1988). The relatively high intrapopulation similarities, and the low level of heterozigosity of the stone loach and the gudgeon may be the consequences of the inbreeding in the small assemblages. The size and the patchiness of the available habitats have the strongest effect on the size and the genetic variability of the assemblages for a benthic species (HÄNFLING *et al.* 2002).

Nevertheless, the fact that the Carpathian Basin thus the studied drainage system had not been glaciated during the ice ages (EMERSON & HEWITT 2005, TABERLET *et al*. 1998), might explain the high interpopulation genetic diversities, since the assemblages of the species might have been separated for a long time. Our results suggest that beside the general latitudinal diversity gradient (HILLEBRAND 2004) there is another trend. Owing to the habitat fragmentation, higher genetic diversity develops in case of benthic, non migratory species then in case of migratory fish which do not require special habitats.

To summarize the results of this study, we found correlation among the spatial distribution of the three studied fish species and the genetic substructuring of the assemblages, respectively. The two benthic species show a clear pattern of isolation by distance. Our results suggest that the lowland sections of the studied streams may act as ecological barriers for the stone loach and the gudgeon popula-

tions, but do not for the chub. Therefore, the lowland sections separate the assemblages of the two benthic species into distinct populations, while the chub shows metapopulation structure in the drainage system studied.

In case of the species with disjunct distribution area the genetic diversity mainly originates from the interpopulation genetic differences (MEFFE & VRIJENHOEK 1988), therefore, the conservation of the separated populations is essential. Our genetic results confirm the importance of the conservation of the habitats in natural state. As a result of the habitat degradation, the decreasing size of the populations may result in bottle neck effect, thus lower genetic diversity. A low level of genetic variability may effect on fitness, which may lead to extinction of these assemblages (KNAEPKENS *et al.* 2002; MENDEL *et al.* 2005). As a result of this, species might lose valuable genestocks.

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