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Genetic variability of Roe deer populations (*Capreolus capreolus* L.) from northeast Yugoslavia

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Abstract

Tissue samples of 94 roe deer (*Capreolus capreolus* L.) from five populations in Yugoslavia were examined for genetic variability and differentiation at 33 presumptive structural loci by means of horizontal starch gel electrophoresis. The proportion of polymorphic loci varied between 3.3% and 12.1 %. Average heterozygosity per locus varied between 0.2% and 2%. Estimates of standardized variance of gene frequencies (F_{ST}) varied between 0.015 and 0.204 with a mean of 0.110. Indirect estimates of gene flow among populations based on the degree of population subdivision, F_{ST}, was 2.70 migrants per generation, whereas the "private alleles method" showed a gene flow level of 12.26 migrants per generation. Significant heterogeneity of gene frequencies existed between the highland populations south of the Danube. The data on polymorphism, heterozygosity, and gene flow rates are within the range of those reported by other researchers for Hungarian and Bulgarian populations.

An overall assessment of the factors determining the genetic structure of the analysed populations in this part of the roe deer range reveals no evidence of genetic drift, implying that selection or non-random mating are more important determining factors. Our data, together with that in the literature, suggest the existence of a clinal North-South gradient in basic population genetic parameters.

Introduction

In recent years the study of genetic variability of roe deer populations from central and southeastern Europe has been extensively reported by Hartl and Reimoser (1988) and Hartl et al. (1991, 1993). With the exception of one population from Slovenia (Hartl et al. 1993) and three from Central Serbia (Milošević-Zlatanović et al. 1994), the whole range of the roe deer in the former Yugoslavia is poorly represented.

In an attempt to contribute to the knowledge of genetic structure and variability in this part of the species range, we present in this study the results of our analysis of genetic variability in five populations of roe deer from northeast Yugoslavia – three from the lowland area north of the Danube, two from the mountainous region south of the Danube (Fig. 1). Our data are relevant to several hypotheses concerning roe deer population structure: the extent of genetic differentiation within the species range; the possibility of subspecies differentiation, including the proposed existence of north-south clinal variation and the existence and strength of population barriers (Hartl et al. 1991); the existence

and magnitude of ecotypic differentiation within the species (HARTL et al. 1993), and the impact of game management techniques on genetic structure (RYMAN et al. 1980).

Fig. 1. Sampling sites and localities for the five roe deer populations from Yugoslavia. The insert depicts the Kozara enclosure. (1) Bački Monoštor; (2) Kozara; (3) Zrenjanin; (4) Severni Kučaj; (5) Negotin.

Material and methods

A detailed exposition of sampling areas and localities is presented in this first account.

Samples were taken from several localities (Fig. 1), within five hunting areas defined by game management plans. The "Bački Monoštor" area is situated on the left bank of the Danube, from the Hungarian border to the 1407th km of the Danube (45°42′ N, 45°56′ E) on an area of 11,764 hectares. It lies completely within the alluvial plain of the Danube on alluvial pararendzinas and sandy chernozem soils, and is completely fenced. The area is predominantly forested (>50%), with a typical turnover of floodplain forests (ass. *Salicetum albae – Ulmeto-fraxinetum quercetosum – Populetum albae et nigrae – Quercetum roboris*). Grasslands (30%) are represented by pastures (20%) and meadows (10%). The remainder are ecotonal semiwooded habitats. The area is predominantly a red deer reserve with a planned red deer density of 12 individuals per 100 ha. Roe deer are present in an estimated density of 2.3 ind. per 100 ha. A total of 30 roe deer was sampled from the area. To facilitate management, the area is divided arbitrarily into 8 districts. One of these, "Kozara", was separately analysed (15 individuals). It is an area of 900 ha of pastures on solonec soils. It has an estimated abundance of 36 roe deer with a sex ratio of 1.5 : 1 in favor of males. In most characteristics it differs from the remaining areas, i. e, around 500 male red deer typically reside and graze in this area, which was the main reason for distinguishing this locality as a separate sample.

The "Zrenjanin" area is a composite sample (25 individuals) obtained from 6 game districts lying within 40 km from "Zrenjanin" (45°20′ N, 20°50′ E) between the Tisa and Tamiš Rivers. The roe deer is dominant on an area of approximately 175,000 ha, with an estimated density of 3 ind. per 100 ha. Around 70% of the area is comprised of arable land under various cultures on chernozem soils, with mixed broad-leaved forests (variously degraded) comprising a minority of 10% within the alluvial plains of rivers.

Both the "Bački Monoštor" and "Zrenjanin" areas are under 90 m altitude. Both areas are under the influence of the pannonian semicontinental climate.

The "Severni Kučaj" area (44°30′ N, 21°50′ E) is a hunting ground of 21,507 ha, completely forested (>95%), its altitude ranging from 200–600 m, and under the influence of a continental climate – a hilly landscape with well-developed soils (brown forest soils and podzols) and a dominance of submontane beech forests (ass. *Fagetum submontanum*). Roe deer are present with an estimated density of 0.3 ind. per 100 ha with a sex ratio of 1 : 2 in favour of females. The estimated capacity of 300–500 heads is never reached due to over-exploitation by poaching. Red deer are also present at a comparable density, and are, in contrast to the roe deer, close to the projected capacity as a result of more effective management. 21 individuals were obtained from this area.

The "Negotin" hunting area (44°15′ N, 22°20′ E) is a hilly mountainous area of 96,423 ha on an altitude range under 1,100 m and under the influence of a continental climate. Soil types vary from brown acid soils on silicates to rendzinas on limestone. About 50% of the area is arable land, with pastures and meadows (25%) and forests (25%) covering the rest. Forests are mostly oak and hornbeam-ash forests (ass. *Quercetum frainettocerris, Carpinetum orientalis serbicum*) in lower, and montane oak and beech forests in higher elevations (ass. *Quercetum montanum, Fagetum montanum serbicum*). Roe deer are present at an estimated density of 4 ind. per 100 ha and are well managed. Red deer are present at low densities in adjacent regions. A total of 18 individuals was taken from this area.

In sum, a total of 94 specimens (188 genomes) has been analysed. The samples were taken during the regular hunting season in 1993 and 1994. Small samples of liver, kidney and muscle were removed immediately during field dressing, or very shortly after the animal was shot, and stored adequately labelled on ice. The samples were frozen prior to the analyses and kept at –25 °C to –30 °C. Techniques of horizontal starch gel electrophoresis and protein staining techniques were performed according to the procedures of Selander et al. (1971) and Ayala et al. (1972), with minor modifications. The 33 protein loci examined and buffer conditions used are listed in table 1. The most common allele was designated

Table 1. Survey of protein loci and electrophoretic conditions analysed in the roe deer.

No.	Protein*	Loci;	Enzyme commission number*	Electroforetic conditions**
1.	Alcohol dehydrogenase	Adh^+	1.1.1.1.	6
2.	α-Glycerophosphate dehydrogenase	α-Gpd, α-Gpd2	1.1.1.8	6
3.	Sorbitol dehydrogenase	Sdh^+, Sdh^-	1.1.1.14	6
4.	L-Lactate dehydrogenase	Ldh-1, Ldh-2	1.1.1.27	5
5.	Malate dehydrogenase	Mdh-1, Mdh-2	1.1.1.37	4
6.	Malic enzyme	Me-1, Me-2	1.1.1.40	2
7.	Isocitrate dehydrogenase	Idh-2	1.1.1.42	2
8.	6-Phosphogluconate dehydrogenase	6-Pgd	1.1.1.44	4
9.	Octanol dehydrogenase	Odh^-	1.1.1.73	5
10.	Xanthine dehydrogenase	Xdh	1.2.2.37	3
11.	NADH-diaphorase	Dia	1.6.2.2	5
12.	Superoxide dismutase	Sod-1, Sod-2	1.15.1.1	2
13.	Creatine kinase	Ck	2.7.3.2	2
14.	Adenylate kinase	Ak	2.7.4.3	2
15.	Phosphoglucomutase	Pgm-1, Pgm-2	2.7.5.1	5
16.	Esterase	Est-1, Est-2, Est-3	3.1.1.1	1
17.	Peptidase	Pep-1, Pep-2, Pep-3, Pep-4	3.4.1.1	4
18.	Carbonic anhydrase	Ca	4.2.1.1	3
19.	Mannosephosphate isomerase	Mpi	5.3.1.8	3
20.	Glucophosphate isomerase	Gpi	5.3.1.9	4
21.	Protein	Pt	–	1

* Nomenclature Commitee of the International Union of Biochemistry (1984).
** (1) Lithium hydroxide; (2) Tris-citrate pH 8; (3) Tris-versene-borate;
(4) Phosphate-citrate; (5) Tris-maleate pH 7.4; (6) Tris-boric acid for dehydrogenase pH 9.

as 100 and other alleles were assigned numbers corresponding to the relative mobility of their respective allozymes. All variants having mobilities similar enough to preclude consistent separation were conservatively scored as the same allele. No electrophoretic differences between males and females have been found and hence no record on the sex ratio in the samples has been kept.

Allozyme data were analysed with the statistical package BIOSYS-1 (SWOFFORD and SELANDER 1981), using single-locus genotypes as input data for estimating parameters of genetic structure and the extent of genetic differentiation between populations. NEI's (1978) distance coefficient was clustered by the unweighted pair-group method (UPGMA: SNEATH and SOKAL 1973) to provide an overview of the genetic relationships among the samples.

In addition to WRIGHT's (1965, 1978) standard F-statistics, used to describe genetic structure of the analysed populations, indirect estimates of gene flow among populations were obtained using the procedures described in GONZÁLES-CANDELAS et al. (1992): WEIR and COCKERHAM's (1984) modification of F-statistics estimates; WRIGHT's (1943) method for estimate the gene flow level based on F_{ST} coefficient values and the "private alleles" method (SLATKIN 1985).

Results

Screening of 21 enzyme systems (a total of 33 presumptive structural loci) revealed polymorphism at the following 12 loci: Sdh+, Mdh-1, Me-1, Idh-2, 6-Pgd, Gpd-1, Ak, Pgm-1,

Table 2. Allele frequencies for five populations at 12 polymorphic loci in the roe deer. 1 = Bački Monoštor; 2 = Kozara; 3 = Zrenjanin; 4 = Severni Kučaj; 5 = Negotin.

	Population				
Locus (N)	1 (15)	2 (15)	3 (25)	4 (21)	5 (18)
Sdh 100	1.000	0.800	1.000	1.000	0.861
110	0.000	0.200	0.000	0.000	0.139
Mdh-1 90	0.000	0.000	0.080	0.000	0.000
100	1.000	0.733	0.920	1.000	1.000
110	0.000	0.267	0.000	0.000	0.000
Me-1 100	1.000	1.000	0.980	1.000	0.972
102	0.000	0.000	0.020	0.000	0.028
Idh-2 90	0.000	0.267	0.000	0.000	0.028
100	1.000	0.733	1.000	1.000	0.972
6-Pgd 95	0.033	0.000	0.000	0.000	0.000
100	0.933	1.000	0.920	1.000	1.000
110	0.033	0.000	0.080	0.000	0.000
α-Gpd 90	0.000	0.000	0.042	0.071	0.056
95	1.000	1.000	0.958	0.857	0.889
100	0.000	0.000	0.000	0.000	0.000
105	0.000	0.000	0.000	0.071	0.056
Ak 100	1.000	0.733	0.980	1.000	1.000
105	0.000	0.267	0.020	0.000	0.000
Pgm-1 94	0.033	0.000	0.060	0.000	0.000
100	0.967	1.000	0.940	1.000	1.000
Pgm-2 92	0.067	0.000	0.020	0.000	0.000
100	0.933	1.000	0.980	1.000	1.000
Ca 95	0.100	0.033	0.040	0.000	0.000
100	0.900	0.967	0.960	1.000	1.000
Mpi 100	0.933	1.000	0.960	1.000	1.000
106	0.067	0.000	0.040	0.000	0.000
Gpi 95	0.000	0.000	0.000	0.048	0.000
100	1.000	1.000	0.860	0.952	0.833
108	0.000	0.000	0.140	0.000	0.167

Pgm-2, Ca, Mpi and Gpi (Tab. 2). In all cases heterozygote band patterns were consistent with the known quaternary structure of the enzyme concerned (Harris and Hopkinson 1976; Harris 1980). The following 21 loci were monomorphic: Adh, Sdh$^-$, Ldh-1, Ldh-2, Mdh-2, Me-2, Odh$^-$, Gpd-2, Xdh, Dia, Sod-1, Sod-2, Est-1, Est-2, Est-3, Pep-1, Pep-2, Pep-3, Pep-4 and Pt.

The deviation of genotype frequencies from Hardy-Weinberg equilibrium was estimated by the Chi-square test, modified by Levene's (1949) correction for small samples. Statistically significant deviations from equilibrium were obtained for the "Kozara" and "Severni Kučaj" populations. Eight loci showed statistically significant deviations from the Hardy-Weinberg distribution in at least one sample (Gpd-1 – in all samples except "Bački Monoštor"; Gpi – "Severni Kučaj", "Zrenjanin"; Pgm-1 – "Zrenjanin"; Sdh, Mdh-1, Idh-2, Ak, 6-Pgd – "Kozara").

Parameters of genetic variation are given in table 3. The proportion of polymorphic loci varied between 3.0 per cent and 12.1 per cent. The populations south of the Danube seem to be less polymorphic (3.0 per cent and 9.1 per cent) than the populations north of the Danube (all populations have 12.1 per cent polymorphic loci). Average heterozygosity per locus varied between 0.2 per cent and 2.0 per cent. The lowest genetic variability level characterized the sample from "Kozara", a subarea of "Bački Monoštor".

Table 3. Genetic variability at 33 loci in five populations of roe deer. (standard errors in parentheses)

Population	Mean sample size per locus	Mean no. of alleles per locus	Percentage of loci polymorphic*	Mean heterozygosity	
				Directcount	HdyWbg expected**
1. BAČKI MONOŠTOR	15.0 (.0)	1.2 (.1)	12.1	0.020 (.009)	0.019 (.009)
2. KOZARA	15.0 (.0)	1.2 (.1)	12.1	0.002 (.002)	0.049 (.022)
3. ZRENJANIN	25.0 (.0)	1.3 (.1)	12.1	0.016 (.005)	0.031 (.010)
4. SEVERNI KUČAJ	21.0 (.0)	1.1 (.1)	3.0	0.004 (.004)	0.011 (.008)
5. NEGOTIN	18.0 (.0)	1.2 (.1)	9.1	0.015 (.008)	0.026 (.013)

*A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95
** Unbiased estimate (see Nei 1978)

Nei's (1978) unbiased genetic distance coefficients varied between 0.000 and 0.007 (Tab. 4). The largest genetic distance was obtained between population "Kozara" and all the others and the least was noted between the two rather distant populations from Vojvodina (Pannonian plain). The genetic distances between our four populations (except "Kozara") correspond to their geographical distribution.

Table 4. Nei's unbiased genetic similary (above diagonal) and distance (below diagonal) for five roe deer populations.

Population	1	2	3	4	5
1. BAČKI MONOŠTOR	*****	0.993	0.999	0.999	0.998
2. KOZARA	0.007	*****	0.993	0.993	0.994
3. ZRENJANIN	0.001	0.007	*****	0.999	0.999
4. SEVERNI KUČAJ	0.001	0.007	0.001	*****	0.999
5. NEGOTIN	0.002	0.006	0.001	0.001	*****

Table 5. Contigency chi-square analysis among all analysed roe deer populations from north-eastern Yugoslavia. n. s = non-significant, *P < 0.05, **P < 0.01, ***P < 0.001.

Locus	allele	All five populations		
		χ^2	df	P
Sdh	2	22.359	4	***
Mdh-1	3	54.976	8	***
Me-1	2	2.518	4	n. s.
Idh-2	2	37.960	4	***
6-Pgd	3	13.822	8	n. s.
α-Gpd	3	11.628	8	n. s.
Ak	2	37.790	4	***
Pgm-1	2	6.158	4	n. s.
Pgm-2	2	6.716	4	n. s.
Ca	2	7.180	4	n. s.
Mpi	2	6.158	4	n. s.
Gpi	3	23.541	8	**
Total		230.796	64	***

The results of UPGMA clustering showed that the populations north of the Danube River cluster together with respect to the populations south of the Danube and that is in accordance with the existence of a geographical barrier represented by the Danube River (Fig. 2). The distribution of allele frequencies differed significantly among populations for 45% of analysed polymorphic loci (Tab. 5) indicating significant overall genetic heterogeneity.

When the "Kozara" sample was excluded from the analysis due to its specific position in the dendrogram, the distribution of allele frequencies among the remaining four populations did not differ significantly among samples for 75% of the polymorphic loci, but showed a total significant departure from randomness (Tab. 6). When only samples from area north of the Danube River ("lowland" populations) were subjected to analysis, the overall distribution of allele frequencies suggested absence of spatial heterogeneity. For the subset of samples south of the Danube River ("highland" populations), an overall significant genetic heterogeneity was again noted (Tab. 6).

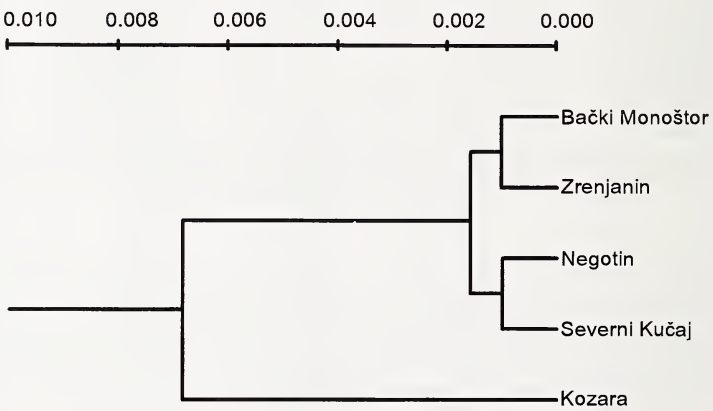

Fig. 2. UPGMA dendrogram of Nei's genetic distance for five roe deer populations.

The measure of the amount of genetic heterogeneity at all loci among the five sampling localities was calculated using WRIGHT's (1965, 1978) F-statistics (F_{ST}, F_{IS} and F_{IT}), quantifying the amount of inbreeding at different levels of nesting (Tab. 7). F_{IS} indicated statistically significant heterozygote deficiency (positive F_{IS} values) for most loci. For four loci (Me-1, Pgm-2, Ca and Mpi) there were negative F_{IS} values (excess heterozygosity) but without statistical significance. The highest positive value of F_{IS} was obtained for the Ak locus (0.907) and the highest negative value was for the Me-1 locus (–0.025). The mean value of F_{IS} for all loci was 0.564. The high positive value of F_{IS} for all loci suggests

Table 6. Contigency chi-square analysis among four roe deer populations from north-eastern Yugoslavia. ("Kozara" sample excluded).
n. s = non-significant, *P < 0.05, **P < 0.01, ***P < 0.001.

Locus	allele	Four populations			Lowland populations			Highland populations		
		χ^2	df	P	χ	df	P	χ^2	df	P
Sdh	2	10.363	3	*	/	/	/	3.640	1	n. s.
Mdh-1	2	8.864	3	*	2.526	1	n. s.	/	/	/
Me-1	2	1.797	3	n. s.	0.608	1	n. s.	1.182	1	n. s.
Idh-2	2	3.410	3	n. s.	/	/	/	1.182	1	n. s.
6-Pgd	3	10.666	6	n. s.	2.324	2	n. s.	/	/	/
α-Gpd	3	7.762	6	n. s.	1.283	1	n. s.	0.175	2	n. s.
Ak	2	2.174	3	n. s.	0.608	1	n. s.	/	/	/
Pgm-1	2	4.542	3	n. s.	0.281	1	n. s.	/	/	/
Pgm-2	2	5.174	3	n. s.	1.131	1	n. s.	/	/	/
Ca	2	7.237	3	n. s.	1.152	1	n. s.	/	/	/
Mpi	2	4.542	3	n. s.	0.281	1	n. s.	/	/	/
Gpi	3	17.308	6	**	4.608	1	*	9.020	2	*
Total		83.839	45	***	14.795	11	n. s.	15.199	7	*

the possibility that either selection or non-random mating or both caused an excess of homozygosity. Estimates of standardized variance of gene frequencies (F_{ST}) varied between 0.015 and 0.204 with a mean of 0.110. This value was also obtained by Hartl et al. (1991) for all populations from Hungary, Austria and Switzerland.

Indirect estimates of gene flow among populations obtained according to Weir and Cockerham's (1984) procedure (based on the degree of subdivision between populations, F_{ST}) was 2.70 migrants per generation (Tab. 8). By using their modification for loci, its value varied between 2.38 and 2.73, while using the modification for populations, the average number of migrants per generations varied between 3.06 and 4.14. The "private alleles" method (Slatkin 1985) showed a gene flow level of 12.26 migrants per generation. Using various modifications for loci its value varied between 17.62 and 46.78, while using modifications for populations the average number of migrants per generations varied between 22.31 and 27.95 (for commentary about modifications see Gonzáles-Candelas et al. 1992). All estimates of Nm were larger than 1, indicating that gene flow keeps populations from drifting to fixation.

Table 7. Summary of F-statistics at all loci for five roe deer populations.

Locus	F_{IS}	F_{IT}	F_{ST}
SDH	0.702	0.736	0.115
MDH-1	0.851	0.878	0.177
ME-1	–0.025*	–0.010*	0.015*
IDH-2	0.875	0.900	0.197
6-PGD	0.221	0.255	0.043*
α-GPD	0.735	0.747	0.046*
AK	0.907	0.926	0.204
PGM-1	0.398	0.418	0.032*
PGM-2	–0.059	–0.018*	0.039*
CA	–0.079	–0.036*	0.040*
MPI	–0.060	–0.022*	0.036*
GPI	0.570	0.605	0.083
Mean	0.564	0.612	0.110

*-coefficient is significant at P < 0.05

Discussion

Genetic variability in the roe deer from northeast Yugoslavia is lower than in populations from central Europe (Switzerland, Austria, Hungary), reported by Hartl et al. (1991). Also, genetic variability in Bulgarian, Slovenian and Slovakian roe deer is high when compared to the populations from Austria, Switzer-

Table 8. Estimates of Nm and their variances using two different methods for roe deer for 12 loci and 5 populations.

Estimate	loci/populations	p(1)	F_{ST}
Direct		12.26	2.70
Jackknife	loci	17.62	2.73
	populations	22.31	4.14
Variance	loci	442.81	0.10
	populations	401.77	16.10
Less biased	loci	46.78	2.38
	populations	27.95	3.06

land, France, and Hungary (HARTL et al. 1993). J. ERNHAFT (pers. comm.) reported for the Hungarian roe deer populations a proportion of polymorphic loci of 11.27 per cent and expected average heterozygosity of 3.84 percent. MILOŠEVIĆ (1986) obtained a value of mean heterozygosity of 10.0 per cent and polymophism level of 31.0 percent for the populations of roe deer from central Yugoslavia. Those values are higher than in the present study. Apart from computational differences, different loci were sampled which contributed to this difference.

Our values of the proportion of polymorphic loci and expected average heterozygosity for the populations from Vojvodina, the southern part of the Pannonian plain, are of the order presented for the populations from Hungary which is the closest neighboring area (P = 12.1 per cent v. s. 13.0 percent; Hmean = 3.3 per cent v. s. 3.7 per cent; reported by HARTL et al. 1991, 1993; and P = 12.1 per cent v. s. 11.3 per cent; Hmean = 3.3 per cent v. s. 3.8 percent; reported by J. ERNHAFT, pers. comm.). Compared with Bulgarian populations, the values for the populations from the eastern part of Serbia are lower (P = 6.1 percent v. s. 17.5 percent; Hmean = 1.9 per cent v. s. 6.5 per cent; HARTL et al. 1993).

For the samples from "Kozara" and "Severni Kučaj" we obtained low genetic variability compared to the other analysed populations. Our explanation could support the hypothesis that genetic variability level is mostly dependent on parameters of the population structure such as population density and effective population size. According to SOULÉ (1976), heterozygosity is largely determined by population size; more precisely, SIMANEK (1978) argued that effective population size determines the level of heterozygosity. Those arguments are well reflected in our data, i. e., the populations from "Kozara" and "Severni Kučaj". In these two populations we believe that game management techniques (enclosure, competition, overexploitation) directly influence effective population size and lead to observed loss of genetic variability. These factors do not operate in the other populations of this study which are more effectively managed. Similar to the study by HARTL et al. (1993), sample groups belonging to different ecotypes ("field" vs. "forest") did not show genetic distance higher than those typical for local populations.

Our results on the level of gene flow, based on both F_{ST} and "private" alleles estimates, are of the same order, especially when compared to values reported for subdivision of population groups of the same species (2.66, 3.92, 1.67; HARTL et al. 1991). They are, however, higher than the values for the Hungarian populations ("Eastern group"), 2.70:1.67, but lower than those reported for four Bulgarian populations (5.43, HARTL et al. 1993). Our examined populations lie geographically in the transect Hungary-Bulgaria and are pannonian ("Bački Monoštor" and "Zrenjanin") and perirhodopic ("Severni Kučaj" and "Negotin"). We note that the transect Hungary, Yugoslavia, Bulgaria has growing rates of gene flow (1.67, 2.70, 5.43).

This could be relevant to HARTL's et al. (1991) discussion on the existence of, broadly speaking, a north-south gradient in roe-deer population differentiation (Hungary, Yugoslavia, Bulgaria). We would argue that this gradient is not only geographical (longitudinal) but also reflects the transition between lowland (predominantly agricultural) to highland landscapes with, we believe, an adequate shift in game management activities. On the local level, the Danube River does not represent a strong migration barrier, although we observed a difference in the average number of migrants per generation between lowland "Bački Monoštor" – "Zrenjanin"and highland "Severni Kučaj" – "Negotin" subgroups. The spatial distribution of allele frequencies also has a different pattern among lowland (sample from "Kozara" excluded) compared to highland samples. Allele frequencies for most polymorphic loci are randomly distributed among localities north of the Danube River, while in the highland area (south of the Danube River) less loci are in polymorphic condition and the overall spatial distribution of their allele frequencies is significantly non-random. Nevertheless, with genetic distance values of up to 0.007 and the lack of clear differences in allele types on homologous loci, there is no evidence of subspecific differentiation on the level of genic-enzymatic systems (see: HARTL et al. 1991).

An overall assessment of the factors determining the genetic structure of roe deer populations in this part of the range is that there is no evidence of genetic drift, implying selection or non-random mating as importànt factors. Our data provide some evidence for the existence of a north-south selection gradient, clinal in nature. Spatial heterogeneity exists south of the Danube River barrier and is located within the highland areas. Inadequate game management significantly alters population structure in two populations ("Kozara", "Severni Kučaj"). Our data suggest that non-random mating (strengthened by game management-exploitation, reproductive behavior and territoriality, adaptation to semiurban habitat complexes) is probably more important than selection in influencing population genetic structure.

The small number of populations analysed in this study and their heterogeneity in regard to effective population size, game management, and habitat type do not give us adequate opportunity to analyse in greater detail the genetic structuring of populations in this part of species range and the influence of various factors on possible differentiation within species. However, additional samples from both lowland and highland regions of Yugoslavia will make possible the testing of various hypotheses about roe deer population structure. As THORPE (1980) suggested, for recognizing the possible subtle racial differences within species one should investigate also the variation of external morphology. We suppose that a combination of biochemical, craniometric and morphometric analyses, will give a clearer impression of the status of Yugoslavian roe deer populations.

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Zusammenfassung

Genetische Variabilität von Rehpopulationen (Capreolus capreolus L.) aus dem nordöstlichen Jugoslawien

Gewebeproben von 94 Rehen (*Capreolus capreolus* L.) aus fünf Populationen in Jugoslawien wurden mittels horizontaler Stärkegelelektrophorese auf genetische Variabilität und Differenzierung an 33 hypothetischen Strukturgenloci untersucht. Die Polymorphierate schwankte zwischen 3,3% und 12,1%, der durchschnittliche Heterozygotiegrad zwischen 0,2% und 2%. Die Schätzwerte für die standardisierte Varianz der Genfrequenzen (F_{ST}) reichten von 0,015 bis 0,204, mit einem Mittelwert von 0,110. Indirekte Schätzungen des Genflusses zwischen Subpopulationen bewegten sich zwischen 2,7 (nach der F_{ST}-Methode) und 12,26 (nach der „Private-Allele-Methode") Migranten pro Generation. Die Populationen im Hochland südlich der Donau zeigten signifikante Unterschiede in den Allelfrequenzen. Die Angaben über Polymorphieraten, Heterozygotiergrade und Genflußraten liegen innerhalb des von anderen Autoren bei ungarischen und bulgarischen Populationen gefundenen Bereichs. Nach unseren Daten wird die genetische Struktur der Populationen im Untersuchungsgebiet weniger durch genetische Drift als durch Selektion oder Abweichungen von der Zufallspaarung bestimmt. Unter Berücksichtigung publizierter Daten läßt sich beim Reh eine Nord-Süd-Kline in den populationsgenetischen Grundparametern erkennen.

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