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Analysis of genetic diversity of roe deer (*Capreolus capreolus* L.) in Lithuania using RAPD and allozyme systems

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In recent decades, ungulate biology, morphology and ecology as well as the significance of roe deer in the cultural landscape have been studied but data on the genetic diversity of roe deer in Lithuania is still sparse. To determine the genetic diversity of roe deer in Lithuania RAPD (random amplified polymorphic DNA) and enzyme studies were performed. DNA from the total of 39 roe deer individuals were extracted. Five ROTH-180 primers were used and fifty-seven RAPD polymorphic loci ranging from 150 to 3 000 base pair were found. The evaluation of different locations roe deer genetic variability was different: the genetic distances according to Nei ranged from 0.04 to 0.76. Using four isoenzyme systems (NSP, EST, MDH and ME) between roe deer of three districts were analysed. Observed average heterozygosity was 0.468 and 9 polymorphic loci: Est-2, Est-3, MDH-1, MDH-2, Me-1, Me-2, NSP -1, NSP-2, NSP-3 were detected.

Key words: roe deer, *Capreolus capreolus* L., genetic diversity, allozymes, RAPD

INTRODUCTION

Presently, the roe deer occurs throughout the West Palearctic region, from the Iberian Peninsula to South Scandinavia, and eastwards to the Caucasus. This small ungulate is a generalist browser, which can also make use of grasses and sedges (Duncan et al., 1998). The origin of this species is unclear. The oldest relatives of modern roe deer appeared all over Europe as early as at the beginning of the Middle Pleistocene (900 000–800 000 years ago), while forms closely related to extant species may have been presented in southern Europe already close to the end of the Middle Pleistocene, approximately 250 000 years ago (Lister et al., 1998).

With glacier retreating to the north, roe deer along with other representatives of the fauna

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forest-steppe spread in the current territory of Lithuania. It is believed that this was 8 000–9 000 thousand years ago (Baleišis et al., 2003). Lithuanian fauna of ungulates which was formed after the Ice-Age to the present day had changed significantly. This was due to natural and climatic variations, later to human direct and indirect activities. After last glacier melted Lithuanian territory was inhabited by tundra animals – musk oxen, reindeer which later due to climate warming disappeared or moved further north characterized by cold climate. In the sixteenth and seventeenth centuries bison and red deer became extinct in Lithuania (later these animals started to be reared in our country again) (Baleišis et al., 2003).

The current Lithuanian roe deer population is abundant, consisting of approximately 110 000 individuals (http://www.stat.gov.lt/). Studies on the genetic diversity of roe deer have only been launched recently. Consequently, data on the genetic variability of roe deer in Lithuania is still sparse. The aim of this study was to investigate the levels of genetic diversity of roe deer in Lithuania using allozyme and RAPD analysis.

MATERIALS AND METHODS

Sampling and laboratory procedures

Samples of 39 roe deer specimens from different localities of Lithuania (Fig. 1.) were collected by hunters in 2006 and 2009.

Isoenzymes

Isoenzyme is one of the multiple forms in which an enzyme may exist in an organism, different species, various forms differing chemically, physically, or immunologically, but catalyzing the same reaction.

Muscle and liver tissues homogenates of roe deer collected from three localities (Kupiškis, Žiežmariai and Taujėnai) were used for electrophoretic investigations of four isoenzyme systems (nonspecific protein (NSP), esterase (EST), malate dehydrogenase (MDH), malic enzyme (ME)) according to Glazko and Sozinov, 1993.

The defrosted tissues were homogenised in a mortar using buffer 0.2 M Tris-HCl, pH 8.0. To prevent enzyme degradation, the prepared homogenates were kept at temperature of –20 °C.

The vertical gel electrophoresis apparatus was used for isoenzyme electrophoresis in a vertical

Fig. 1. Roe deer collecting places. ■ – samples for RAPD analysis, ▲ – samples for isoenzyme analysis

polyacrilamide gel (PAAG) block. The one-layer 5% polyacrilamide gel was used for separation of ME and MDH. To separate EST and NSP the two-layer 5% and 7.5% PAA gel was used. The concentrating layer made up 1/3–1/4 and the separating layer 2/3–3/4 of the total gel amount in a double layer polyacrilamide gel. Electrophoresis was performed by three stages: pre-electrophoresis (voltage 150 V, duration 30 min), samples exit from wells (80 V, 40 mA, 20 min), operating mode (250 V, 110 mA, 1–2 h).

After electrophoresis, gels were incubated in stain at 37 °C for about 2–3 hours. Dying mixtures for the separation of enzyme activity zones were prepared according to standart protocols (Harris and Hopkinson, 1976; Shaw et al., 1970) with some modifications (Paulauskas, Tubelytė-Kirdienė, 2002).

Random amplification of polymorphic DNA (RAPD)

Genomic DNA was extracted from muscles using a "Genomic DNA Purification Kit # K0512" with NaCl, lysis and precipitation solutions (Thermo Scientific (Fermentas), Lithuania) according to the manufacturer's instructions. The quality and concentration of DNA were estimated with "Eppendorf BioPhotometer" spectrophotometer.

Samples of thirty roe deer individuals corresponding to eight different localities were analysed by random amplification of polymorphic DNA (RAPD) polymerase chain reaction (PCR). Roe deer DNA was amplified with nine ROTH-180 primers: ROTH-180-01, ROTH-180-03, ROTH-180-04, ROTH-180-05, ROTH-180-06, ROTH-180-07, ROTH-180-08, ROTH-180-09, ROTH-180-10 (Thermo Scientific (Fermentas), Lithuania). PCR was performed on a Mastercycler gradient thermal cycler (Eppendorf) in 25 µl reaction mix containing 10 pmol/µl ROTH-180 primer, 0.1 mM dNTP, 1X Taq buffer with KCl and $MgCl_2$, 1U Taq-polymerase, 25–50 ng template DNA. Thermocycling parameters after predenaturation step at 94 °C for 1 min, annealing step at 49.9–56.4 °C for 1 min, elongation step at 72 °C for 1 min and the final elongation step at 72 °C for 2 min.

PCR products were sorted according to their size by electrophoresis in 1.7% agarose gel with TrisBorate-EDTA as a running buffer and using a molecular mass marker GeneRuler TM 100 bp DNALadder Plus (Thermo Scientific (Fermentas), Lithuania). DNA stripes were stained with ethidium bromide and photographed under the UV light (Easy Win 32, Herolab, Germany).

Data analysis

The enzyme systems data was analysed using computer program BIOSYS-2 (Swofford and Selander, 1997). Genotypes, alleles frequencies and average observed heterozygosity were calculated, also the most common and the rarest genotypes were identified.

A binary matrix reflecting specific RAPD stripe presence (1) or absence (0) of a given amplification product in each genotype were generated for data analysis (Nei, Li, 1979). TREECON for Windows software (Van de Peer, Watcher, 1994) was used for estimating the genetic diversity. The relationships among individuals were represented in a UPGMA cluster tree.

RESULTS AND DISCUSSION

Analysis of enzyme systems

The four isoenzyme systems (NSP, EST, MDH, ME) detected 10 loci (Table 1): 9 polymorphic loci and 1 monomorphic locus, which was revealed by the EST system.

Esterases (EST, E. C. 3.1.1.). Esterases are enzymes belonging to hydrolases. Analysis of roe deer individuals revealed two polymorphic and one monomorphic zones of EST: the most mobile zone Est-1, the slower zone Est-2 and the slowest zone Est-3. Three genotypes were detected at the loci Est-2 and Est-3: AA, AB, BB. Only one genotype was detected at the locus Est-1: AA.

Malate dehydrogenase (MDH, E. C. 1.1.1.37). The enzyme that reversibly catalyzes the oxidation of malate to oxaloacetate using the reduction of NAD^+ to NADH. The MDH enzyme in roe deer was polymorphic and two zones, Mdh-1 and Mdh-2, were detected. Three identical genotypes were detected at the MDH loci: AA, AB, BB.

Malic enzyme (ME, E. C. 1.1.1.40). The enzyme that catalyzes the oxidative decarboxylation of (S)-malate to pyruvate, with the concominant release of carbon dioxide and conversion of $NADP^+$ to NADPH. The activity of this enzyme was found in two zones. At the Me-1 locus two genotypes

Table 1. Genotypes and alleles distribution of the studied roe deer from Lithuania

Locus	Genotype	Genotype frequencies	The most common genotype	The rarest genotype	Allele	Allele frequencies	Ho
Est-1	AA	1.000	AA	–	A	1.00	0.000
Est-2	AB	0.600	AB	BB	A	0.30	0.600
	BB	0.400			B	0.70	
Est-3	AA	0.170			A	0.42	
	AB	0.500	AB	AA	B	0.58	0.500
	BB	0.330					
Mdh-1	AA	0.125			A	0.44	
	AB	0.625	AB	AA	B	0.56	0.625
	BB	0.250					
Mdh-2	AA	0.110			A	0.39	
	AB	0.670	AB	AA	B	0.61	0.670
	BB	0.220					
Me-1	AB	0.290			A	0.14	
	BB	0.710	BB	AB	B	0.86	0.290
Me-2	AA	0.750			A	0.81	
	AB	0.125	AA	AB, BB	B	0.19	0.125
	BB	0.125					
Nsp-1	AA	0.556			A	0.78	
	AC	0.111	AA	AC	C	0.06	0.444
	AD	0.333			D	0.16	
Nsp-2	AA	0.143					
	AB	0.143			A	0.50	
	AC	0.571	AC	AA, AB, BB	B	0.21	0.714
	BB	0.143			C	0.29	
Nsp-3	AB	0.143			A	0.36	
	AC	0.571	AC	AB	B	0.07	0.714
	CC	0.286			C	0.57	
			Average				0.468

were established: AB and BB, at the Me-2 locus, three genotypes: AA, AB, BB were established.

Non-specific protein system (NSP). A protein substance that elicits a response not mediated by specific antigen-antibody reaction. The electrophoresis revealed three polymorphic zones. In Lithuanian roe deer four different genotypes: AA, AB, AC, BB were determined in the zone coded by the Nsp-2 locus. At the loci Nsp-1 and Nsp-3 the following three genotypes AA, AC, AD and AB, AC, CC were detected, respectively.

Cervids are among the few groups of large mammals which have been extensively studied by electrophoretic multilocus investigations to evaluate genetic diversity within and between populations and species (Hartl, Reimoser, 1988; Hartl 1990a). The first multilocus investigations to estimate the amount of genetic variability present in roe deer compared with other cervids were made by Baccus et al., (1983) and, using a more representative sample of individuals, populations and loci, by Hartl, Reimoser (1988). Hartl et al. (1991) studied genetic variability and differentiation of roe deer. Tissue samples of 239 roe deer from 13 regions were collected during hunting seasons of 1988–1989 and 1989–1990. The 27 enzyme systems were screened and for completion, data from previously studied roe deer (160 individuals) were included in that paper. Three enzyme systems described by Hartl et al. (1991) were used in the study of Lithuanian roe deer. While comparing the results of our study with those of Hartl et al. (1991) it was found that enzime system of esterases of Lithuanian roe deer had two loci while roe deer from Hungary, Austria and Switzerland had only one locus.

One of the most important problems in the comparison of biochemical-genetic variation between different studies is very unequal evolutio-

Table 2. Primers used in the study

Primer	Number of fragments	Size of fragments
ROTH-180-04	14	300–3000
ROTH-180-05	5	500–2000
ROTH-180-08	15	250–3000
ROTH-180-09	15	150–3000
ROTH-180-10	8	300–2000
Total:	57	150–3000

nary rate among proteins (Nei, 1978; Hartl, 1990b; Hartl et al., 1990b). Therefore, unless much the same set of enzymes is examined in all taxa concerned, genetic diversity may be seriously under- or overestimated (Hartl et al., 1991).

Analysis of RAPD

After roe deer DNA amplification with five ROTH-180 primers (ROTH-180-04, ROTH-180-05, ROTH-180-08, ROTH-180-09, ROTH-180-10) fifty seven fragments ranging from 150 to 3000 base pairs were found (Table 2). Other ROTH primers (such as ROTH-180-01 – ROTH-180-03 and ROTH-180-06 and ROTH-180-07) were unsuitable to assess genetic diversity of Lithuanian roe deer.

According to TREECON for Windows software analysis, genetic distance among roe deer individuals varied from 0.04 to 0.76 (Table 3). The largest genetic distance was between individuals from Ukmergė and Alytus regions. Genetically closest individuals were from Ukmergė locality.

According to RAPD results, the roe deer dendrogram was constructed (Fig. 2). This dendrogram was composed of three clusters. "A" cluster is formed of two groups of animals, one of them samples from Ukmergė and Molėtai localities and the genetic distance between individuals was less than 0.16, in another group were samples from Ukmergė region. This cluster included

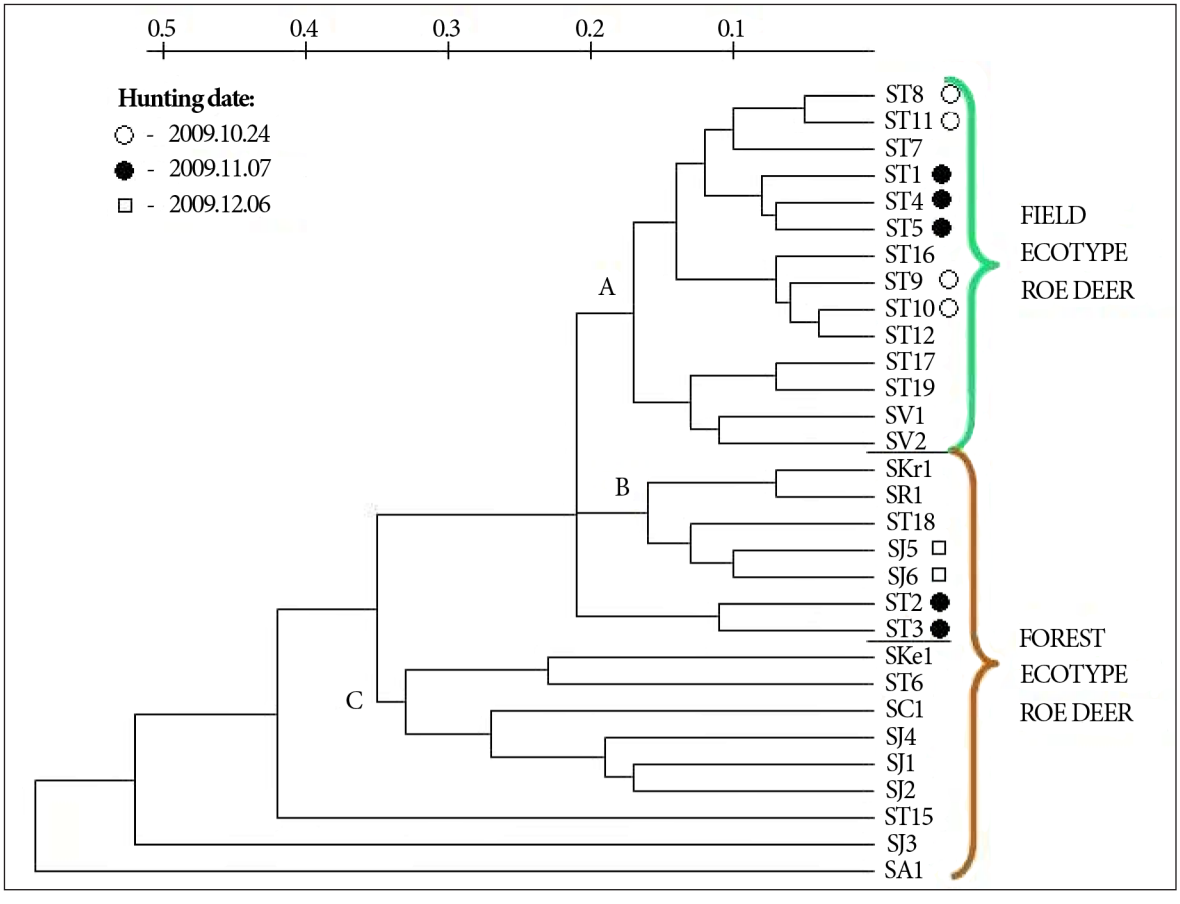

Fig. 2. Phylogenetic tree of the analysed roe deer individuals (SA – Alytus district; SV – Vilkaraistis, Molėtai district; ST – Taujėnai, Ukmergė district; SKr – Kryžkalnis, Raseiniai district; SR – Raseiniai district; SJ – Jonava district; SKe – Kėdainiai district; SC – Cinkiškiai, Kaunas district)

Table 3. Genetic distances of roe deer according to TREECON programme

Ind.	SA1	SC1	SJ1	SJ2	SJ3	SJ4	SJ5	SJ6	SKe1	SKr1	SR1	ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST8	ST9	ST10	ST11	ST12	ST15	ST16	ST17	ST18	ST19	SV1	SV2
SA1	0.00	0.64	0.58	0.49	0.76	0.57	0.54	0.58	0.61	0.56	0.56	0.64	0.53	0.54	0.61	0.56	0.49	0.55	0.59	0.60	0.61	0.53	0.58	0.61	0.61	0.65	0.54	0.66	0.54	0.65
SC1	0.64	0.00	0.22	0.35	0.53	0.23	0.32	0.33	0.33	0.45	0.40	0.44	0.42	0.47	0.42	0.45	0.40	0.43	0.45	0.44	0.51	0.50	0.47	0.44	0.50	0.50	0.45	0.51	0.51	0.50
SJ1	0.58	0.22	0.00	0.17	0.50	0.19	0.24	0.30	0.35	0.38	0.38	0.37	0.40	0.40	0.30	0.33	0.32	0.36	0.38	0.37	0.44	0.39	0.40	0.51	0.43	0.42	0.38	0.44	0.40	0.42
SJ2	0.49	0.35	0.17	0.00	0.37	0.18	0.18	0.20	0.41	0.27	0.31	0.25	0.30	0.33	0.24	0.23	0.29	0.29	0.27	0.33	0.28	0.28	0.28	0.45	0.35	0.29	0.27	0.30	0.28	0.33
SJ3	0.76	0.53	0.50	0.37	0.00	0.50	0.50	0.46	0.55	0.54	0.59	0.49	0.55	0.61	0.57	0.54	0.54	0.53	0.49	0.49	0.46	0.50	0.46	0.48	0.56	0.50	0.56	0.52	0.57	0.56
SJ4	0.57	0.23	0.19	0.18	0.50	0.00	0.16	0.18	0.29	0.25	0.25	0.31	0.24	0.31	0.25	0.28	0.27	0.31	0.32	0.39	0.37	0.33	0.37	0.42	0.40	0.35	0.28	0.36	0.33	0.39
SJ5	0.54	0.32	0.24	0.18	0.50	0.16	0.00	0.10	0.29	0.13	0.17	0.22	0.24	0.27	0.18	0.21	0.27	0.23	0.21	0.31	0.29	0.22	0.29	0.42	0.32	0.26	0.16	0.28	0.22	0.35
SJ6	0.58	0.33	0.30	0.20	0.46	0.18	0.10	0.00	0.30	0.15	0.19	0.16	0.22	0.29	0.19	0.19	0.20	0.21	0.19	0.24	0.23	0.24	0.23	0.43	0.22	0.15	0.10	0.21	0.19	0.23
SKe1	0.61	0.33	0.35	0.41	0.55	0.29	0.29	0.30	0.00	0.33	0.38	0.36	0.36	0.40	0.35	0.38	0.23	0.45	0.42	0.45	0.43	0.43	0.48	0.50	0.51	0.41	0.33	0.43	0.39	0.41
SKr1	0.56	0.45	0.38	0.27	0.54	0.25	0.13	0.15	0.33	0.00	0.07	0.12	0.18	0.17	0.11	0.11	0.27	0.16	0.14	0.19	0.15	0.16	0.19	0.38	0.21	0.18	0.13	0.16	0.11	0.18
SR1	0.56	0.40	0.38	0.31	0.59	0.25	0.17	0.19	0.38	0.07	0.00	0.15	0.18	0.14	0.11	0.14	0.27	0.13	0.18	0.23	0.22	0.19	0.22	0.38	0.25	0.27	0.17	0.24	0.15	0.22
ST1	0.64	0.44	0.37	0.25	0.49	0.31	0.22	0.16	0.36	0.12	0.15	0.00	0.19	0.19	0.08	0.08	0.29	0.10	0.08	0.13	0.08	0.13	0.12	0.36	0.14	0.11	0.18	0.09	0.12	0.16
ST2	0.53	0.42	0.40	0.30	0.55	0.24	0.24	0.22	0.36	0.18	0.18	0.19	0.00	0.11	0.15	0.11	0.23	0.17	0.18	0.23	0.22	0.13	0.22	0.43	0.24	0.30	0.28	0.27	0.19	0.26
ST3	0.54	0.47	0.40	0.33	0.61	0.31	0.27	0.29	0.40	0.17	0.14	0.19	0.11	0.00	0.14	0.14	0.30	0.16	0.17	0.26	0.25	0.15	0.25	0.48	0.27	0.29	0.27	0.27	0.14	0.22
ST4	0.61	0.42	0.30	0.24	0.57	0.25	0.18	0.19	0.35	0.11	0.11	0.08	0.15	0.14	0.00	0.07	0.32	0.13	0.15	0.20	0.15	0.16	0.19	0.43	0.22	0.19	0.22	0.17	0.12	0.19
ST5	0.56	0.45	0.33	0.23	0.54	0.28	0.21	0.19	0.38	0.11	0.14	0.08	0.11	0.14	0.07	0.00	0.27	0.13	0.11	0.15	0.11	0.09	0.15	0.42	0.17	0.18	0.21	0.16	0.11	0.18
ST6	0.49	0.40	0.32	0.29	0.54	0.27	0.27	0.20	0.23	0.27	0.27	0.29	0.23	0.30	0.32	0.27	0.00	0.25	0.31	0.29	0.32	0.28	0.28	0.50	0.31	0.33	0.22	0.39	0.32	0.29
ST7	0.55	0.43	0.36	0.29	0.53	0.31	0.23	0.21	0.45	0.16	0.13	0.10	0.17	0.16	0.13	0.13	0.25	0.00	0.09	0.10	0.13	0.11	0.09	0.36	0.12	0.21	0.23	0.18	0.17	0.21
ST8	0.59	0.45	0.38	0.27	0.49	0.32	0.21	0.19	0.42	0.14	0.18	0.08	0.18	0.17	0.15	0.11	0.31	0.09	0.00	0.12	0.11	0.05	0.11	0.29	0.13	0.18	0.21	0.16	0.15	0.22
ST9	0.60	0.44	0.37	0.33	0.49	0.39	0.31	0.24	0.45	0.19	0.23	0.13	0.23	0.26	0.20	0.15	0.29	0.10	0.12	0.00	0.08	0.13	0.04	0.36	0.06	0.20	0.31	0.17	0.24	0.20
ST10	0.61	0.51	0.44	0.28	0.46	0.37	0.29	0.23	0.43	0.15	0.22	0.08	0.22	0.25	0.15	0.11	0.32	0.13	0.11	0.08	0.00	0.13	0.04	0.35	0.10	0.15	0.25	0.13	0.19	0.19
ST11	0.53	0.50	0.39	0.28	0.50	0.33	0.22	0.24	0.43	0.16	0.19	0.13	0.13	0.15	0.16	0.09	0.28	0.11	0.05	0.13	0.13	0.00	0.13	0.35	0.15	0.24	0.26	0.22	0.13	0.24
ST12	0.58	0.47	0.40	0.28	0.46	0.37	0.29	0.23	0.48	0.19	0.22	0.12	0.22	0.25	0.19	0.15	0.28	0.09	0.11	0.04	0.04	0.13	0.00	0.35	0.06	0.19	0.29	0.17	0.23	0.19
ST15	0.61	0.44	0.51	0.45	0.48	0.42	0.42	0.43	0.50	0.38	0.38	0.36	0.43	0.48	0.43	0.42	0.50	0.36	0.29	0.36	0.35	0.35	0.35	0.00	0.42	0.51	0.47	0.43	0.43	0.51
ST16	0.61	0.50	0.43	0.35	0.56	0.40	0.32	0.22	0.51	0.21	0.25	0.14	0.24	0.27	0.22	0.17	0.31	0.12	0.13	0.06	0.10	0.15	0.06	0.42	0.00	0.13	0.24	0.11	0.18	0.13
ST17	0.65	0.50	0.42	0.29	0.50	0.35	0.26	0.15	0.41	0.18	0.27	0.11	0.30	0.29	0.19	0.18	0.33	0.21	0.18	0.20	0.15	0.24	0.19	0.51	0.13	0.00	0.13	0.07	0.15	0.14
ST18	0.54	0.45	0.38	0.27	0.56	0.28	0.16	0.10	0.33	0.13	0.17	0.18	0.28	0.27	0.22	0.21	0.22	0.23	0.21	0.31	0.25	0.26	0.29	0.47	0.24	0.13	0.00	0.19	0.14	0.22
ST19	0.66	0.51	0.44	0.30	0.52	0.36	0.28	0.21	0.43	0.16	0.24	0.09	0.27	0.27	0.17	0.16	0.39	0.18	0.16	0.17	0.13	0.22	0.17	0.43	0.11	0.07	0.19	0.00	0.13	0.12
SV1	0.54	0.51	0.40	0.28	0.57	0.33	0.22	0.19	0.39	0.11	0.15	0.12	0.19	0.14	0.12	0.11	0.32	0.17	0.15	0.24	0.19	0.13	0.23	0.43	0.18	0.15	0.14	0.13	0.00	0.11
SV2	0.65	0.50	0.42	0.33	0.56	0.39	0.35	0.23	0.41	0.18	0.22	0.16	0.26	0.22	0.19	0.18	0.29	0.21	0.22	0.20	0.19	0.24	0.19	0.51	0.13	0.14	0.22	0.12	0.11	0.00

individuals from field ecotype. "B" and "C" clusters were composed of individuals from forest properties in Raseiniai, Ukmergė and Jonava regions. "C" cluster is formed from separate individuals SA1, SJ3, ST15 and a group of other individuals from Jonava, Kėdainiai, Kaunas and Ukmergė regions. These localities are geographically close to each other, thus the roe deer genetic similarity may be influenced by migration, however, these individuals were the most genetically distant from other tested individuals. Subpopulations of roe deer grouped in clusters according to their living areas and the analysis showed that roe deer of field ecotype and roe deer of forest ecotype were different.

It is known that most RAPD markers have a dominant Mendelian mode of inheritance (Lynch, Milligan, 1994; Zhivotovsky, 1999; Williams et al., 1990; Scott et al., 1992; Cheah et al., 1994; Cheah, Paigen, 1996). If an allele of a RAPD locus is amplified, then the marker / marker homozygotes do not differ from the marker / null heterozygotes (Petrosyan et al., 2002). To quantitatively analyse the genetic parameters in populations of Siberian and European roe deer, a database of RAPD markers for 111 loci was created (Tokarskaya et al., 2000). In general, the results of comparative analysis of Nei's distances demonstrate substantial evolutionary differences between the Far Eastern and Trans-Ural populations (at the subspecies level) and between the populations of Siberian and European roe deer (at the species level). Samples from local groups of roe deer in the Trans-Ural region did not differ significantly in Nei's genetic distances 0.0056; 0.0273; 0.0218 (Petrosyan et al., 2002). According to our results based on RAPD markers, Nei's distances were from 0,040.

The roe deer population is suffering a variety of anthropogenic influences such as selective hunting, translocations and habitat fragmentation (Zachos et al., 2006). It can have a huge impact on the genetic diversity of roe deer. Largest roe deer living in Western Siberia and males can weigh about 59 kg and females up to 52 kg. In Lithuania, body mass and height of roe deer is very different. In 1967–1970 in Lithuania roe deer mass was investigated and the enormity of more than a thousand roe deer hunted in various areas of Lithuania was determined. The data suggested that the weight of roe deer varied within 15–40 kg. Roe deer mass and height may significantly differ not only in remote habitat areas but also in adjacent areas. This phenomenon has been observed in European countries long time ago and is interpreted in the frame of local populations inherited characteristics, different roe deer densities, effects of dietary conditions and other factors (Baleišis et al., 2003).

In Lithuania as in many European countries (Poland, Germany, Czech Republic, Slovakia, Hungary, Romania, Denmark) two roe deer ecotypes can be distinguished: forest roe deer and field roe deer (Raesfeld et al., 1985). The field roe deer can be described as an ecological form of this species adapted to live in an open agricultural landscape (Pėtelis, Brazaitis, 2003). The formation process of this ecotype in Southwest Lithuania began in 1965–1967 (Pėtelis, 1997; 1998), but no genetic analysis was done. According to RAPD results in dendrogram (Fig. 2), subpopulations of roe deer grouped in clusters according to their living ecotypes: field roe deer and forest roe deer.

CONCLUSIONS

For allozyme analysis, samples of roe deer individuals were collected from three localities. Tissues homogenates were used for electrophoretic investigations of four isoenzyme systems: NSP, EST, MDH, ME. These isoenzyme systems detected 10 loci: 9 polymorphic loci and 1 monomorphic locus, which were revealed by the EST system. Observed heterozygosity in Lithuanian roe deer population was 0.468.

Samples of thirty roe deer individuals from seven different regions were analysed by RAPD. Genetic distance according to Nei in Lithuanian roe deer population ranged between 0.04 and 0.76. Roe deer individuals grouped in clusters according to their living areas and the analysis showed that the roe deer of field ecotype and roe deer of forest ecotype were different.

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LIETUVOS STIRNŲ (*CAPREOLUS CAPREOLUS* L.) GENETINIS KINTAMUMAS REMIANTIS ATSITIKTINAI PAGAUSINTOS POLIMORFINĖS DNR IR IZOFERMENTŲ ANALIZE

Santrauka

Pastaraisiais metais buvo tiriama kanopinių žvėrių biologija, morfologija ir jų poveikis kraštovaizdžiui, tačiau duomenų apie Lietuvoje gyvenančių stirnų genetinę įvairovę beveik nėra. Norint nustatyti Lietuvos stirnų genetinę įvairovę, buvo atlikti APPD (atsitiktinai pagausintos polimorfinės DNR) ir izofermentų tyrimai. Iš 39 stirnų raumenų ir kepenų pavyzdžių išskirta DNR. Naudojant penkis ROTH-180 molekulinius žymenis Lietuvos stirnų populiacijoje nustatyti 57 polimorfiniai APPD lokusai, kurių fragmentų dydžiai svyravo nuo 150 iki 3 000 bazių porų. Įvertinus įvairių vietovių stirnas, genetinis kintamumas buvo skirtingas: genetinės distancijos pagal Nei svyravo nuo 0,04 iki 0,76. Panaudojant keturias izofermentų sistemas (NSP, EST, MDH ir ME) tarp trijų rajonų stirnų nustatytas 0,468 heterozigotiškumas ir 9 polimorfiniai lokusai: Est-2, Est-3, Mdh-1, Mdh-2, Me-1, Me-2, Nsp-1, Nsp-2, Nsp-3.

Raktažodžiai: stirna, *Capreolus capreolus* L., genetinis kintamumas, izofermentai, APPD