VYTAUTAS MAGNUS UNIVERSITY

Irma PŪRAITĖ

GENETIC EFFECTS OF ALIEN CERVIDS ON THE NATIVE SPECIES, BIOLOGICAL DIVERSITY AND STABILITY OF THE COMMUNITY

Summary of Doctoral Dissertation
Biomedical sciences, Ecology and Environmental (03 B)

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VYTAUTO DIDŽIOJO UNIVERSITETAS

Irma PŪRAITĖ

SVETIMKRAŠČIŲ ELNINIŲ GENETINIS POVEIKIS VIETINĖMS RŪŠIMS, ĮTAKA BENDRIJŲ BIOLOGINEI ĮVAIROVEI IR STABILUMUI

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INTRODUCTION

Lithuanian fauna of hoofed animals formed after the Ice Age. The changes were determined by natural and climatic conditions, later by direct and indirect human activities. After the dissolution of the last glacier in the territory of Lithuania in the cold climate, animals of the tundra – the reindeer, the bison, the red deer – lived there. Later, when the climate became warmer, flocks of these animals disappeared or fled further to north (Baleišis et al., 2003).

Today the Lithuanian *Cervidae* populations are abundant and spread in almost all the forests. However, around the world, Lithuania being no exception, the gene pool of animals of various deer species is affected or influenced by the reintroduction, transfer of animal, keeping them in enclosures, environmental fragmentation and hunting (Wang and Schreiber, 2001; Hartl et al., 2003, Kuehn et al., 2003; Coulon et al., 2004). In recent decades, ungulate biology, morphology and ecology, as well as the importance of a cultural landscape in Lithuania have been investigated (Baltrūnaitė 1999; Pėtelis and Brazaitis, 2004; Balčiauskas, 2004; Narauskaitė et al., 2011); the genetic diversity studies of the different deer species, however, have been launched only recently.

The methods of molecular investigations are applied in studying genetic differences between separate populations and individuals, in carrying out identification of species and subspecies. The studies of the molecular analysis of genetic variability of the cervids is an important issue, it is necessary to examine genetics of these animals and to monitor changes occurring in their populations, obtain knowledge of breeding and restoring the species. In the world, particularly in Western Europe, the problem of alien cervids is examined intensively – cervids are introduced almost in all Europe (Bartoš, 2009; McDevitt et al., 2009, Senn and Pemberton, 2009), and in most American countries, New Zealand, South Africa (Koubek and Zima, 1999; Shackell et al., 2003).

Genetic variability may vary due to mutations, different chromosomal reorganisations or hybridization with other related species. The genetic integrity of various taxa may be affected by differences in the genetic background of the animals being introduced seeking to improve and preserve the endangered populations; however, the genetic condition of stable populations into which animals of different

genetic types were being introduced can be affected by alien genes. During the past decade breeding of the red deer and the sika deer in enclosures, releasing them and their escape to freedom has increased considerably in Lithuania therefore animals of unsuitable origin might themselves in freedom. According to the Order on the Approval of the Regulations of the Use of Wild Animals (30 June 2011, No. D1-533/B1-310) wild animals of non-native and invasive species cannot be released into the wild in Lithuania. Their owners must immediately inform the police and the Department of Regional Environmental Protection if the animals escaped from the enclosures to freedom. Hybrids, animals of invasive species and non-resident wild animals should be euthanized or caught within the shortest possible term, and no longer than within one month. The tagged animals would be found faster and easier in the wild nature.

Despite the risk of hybridization the introduced ungulate species can have an effect on biodiversity - there is a threat of a competition with the local ungulate populations and damage done to vegetation (Spear and Chown, 2009).

The aim of the study was to investigate genetic diversity of the sika deer, the ed deer and the roe deer and to assess the impact of the alien mammal sika deer on biological diversity and stability.

Main objectives of the study:

1. To apply molecular genetic markers of spieces identification to the sika deer, the red deer and the roe deer species identification and to assess genetic diversity.
2. To evaluate the sika deer effect on the red deer populations in freedom and enclosures
3. To evaluate the Siberian roe deer effect on the European roe deer population in Lithuania.

Scientific innovation of the work. Molecular research of the *Cervidae* family is new in Lithuania. There is a multitude of morphological and ecological studies, but so far no data on the genetic diversity of the *Cervidae* in Lithuania have been published.

The most important result of this study is a set of microsatellite markers used for the first time in the genetic studies of the sika deer population which has been used in research of the reindeer, the roe deer, the fallow deer and the red deer thus far. Hybrid animals have been found in the sika deer and the red deer populations (in nature and in captivity). We identified 20 mtDNA D-loop sequence fragments of the roe deer and

placed them in the "GenBank" sequence database (identification numbers:KM215767-KM215786). Three new haplotypes and eight mtDNA sequences in the Lithuanian roe deer population were unique and have no equivalents in "GenBank" deposited sequences. The results indicated introgression of the Siberian roe deer (*C. pygargus*) mtDNA in the European roe deer genome; the introgressed individuals accounted for 20% of the roe deer studied.

Approval of the work. The main results of this work were presented at the following conferences and meetings: "6th International Conference „Research and conservation of biological diversity in Baltic Region" (Daugavpils, Latvia, 2011); "8th International conference on behaviour, physiology and genetics of wildlife" (Berlin, Germany); "8th Baltic Theriological Conference" (Palanga, Lithuania, 2011); "5th Baltic Congress of Genetics" (Kaunas, Lithuania, 2012); "7th International Conference „Research and conservation of biological diversity in Baltic Region" (Daugavpils, Latvia, 2013); "2nd International Symposium on Hunting" (Novi Sad, Serbia, 2013); "6th International Symposium Dynamics of game animals populations in Northern Europe" (Karelia, Russia, 2014); "Žmogaus ir gamtos sauga 2014", (Kaunas, Lithuania, 2014); "3rd International Symposium on Hunting" (Zemun-Belgrade, Serbia, 2014).

The results of this research have been published in 4 publications in reviewed international scientific journals.

The structure and scope of dissertation. The dissertation is written in Lithuanian and consists of the Introduction, Literature Review, Materials and Methods, Results, Discussion, Conclusions, List of author's publications, Acknowledgements and List of References. References include 200 sources, the thesis has 113 pages. The research data are presented in 52 figures and 27 tables.

MATERIALS AND METHODS

Sampling and DNA extraction

In this study a total of 174 animals (30 sika deer, 39 red deer, 105 roe deer) were analysed. Tissue samples were obtained from legally hunted animals from 2005 till 2013 in Lithuania (Fig. 1). Genomic DNA was extracted from small pieces of muscle tissue using a „Genomic DNA Purification Kit" („Thermo Scientific",

Lithuania) and from blood using „GeneJET Whole Blood Genomic DNA Purification Mini Kit“ („Thermo Scientific“, Lithuania) and stored at -20 ºC.

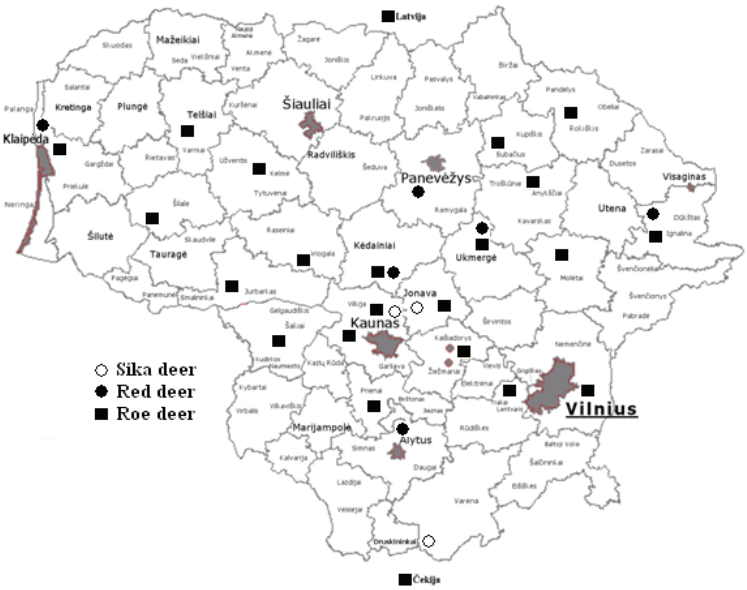

Figure 1. Map of the study area with sampling sites.

Amplification and analysis of microsatellites

Sika deer, red deer and roe deer individuals were genotyped via polymerase chain reaction at 6 microsatellites locus (Table 1). The PCR protocol consisted of 20 µl reaction mix containing approximately 50 ng DNA, 0,3 µM each primer, 1,5-2,0 mM $MgCl_2$, 0,1 mM dNTPs, 1 U of Taq polymerase (“Thermo Scientific”, Lithuania). The PCR consisted of an initial denaturation step at 95°C for 10 min followed by 30 cycles of 30 s at 95°C, 1 min at 52-54°C, 30 s at 72°C, with final elongation step of 10 min at 72°C after the last cycle. All polymerase chain reaction products were run on an ABI 3130 Genetic Analyzer (“Applied Biosystems”) and sized with internal lane standard LIZ500 using program Genemapper version 4.0 (“Applied Biosystems”) (Fig. 2).

Expected (H_e) and observed (H_o) heterozygosities as well as significant deviations from Hardy-Weinberg Equilibrium (HWE) for single loci were calculated,

effective number of alleles, allele size range, unbiased expected heterozygosity (uH_e), Shannon's information index (I), private alleles and frequency were calculated using GenAlEx 6.501 (Peakall and Smouse, 2012)

Table 1. Microsatellite locus primer sequences, repeat motif and references

Locus.	Primer sequence	Repeat motif	References
RT1	5'-TGCCTTCTTTCATCCAACAA-3' 5'-CATCTTCCCATCCTCTTTAC-3'	$(GT)_{22}$	Wilson et al., 1997
RT23	5'-GGCCATTGGGTAGTCTCC-3' 5'-AGCCTCCCTGAGTGCTCT-3'	$(GT)_{22}$	Wilson et al., 1997
NVHRT16	5'-ATTCTAAGCCCAAATAATCTT-3' 5'-TCTAAGGGGTCTGTGTCTT-3'	$(CA)_5TA(CA)_5(TG)_2CG(CA)_{19}$	Roed and Midthjell, 1998
NVHRT21	5'-GCAGCGGAGAGGAACAAAAG-3' 5'-GGGGAGGAGCAGGGAAATC-3'	$(GT)_{16}(GC)_4GT$	Roed and Midthjell, 1998
NVHRT48	5'-CGTGAATCTTAACCAGGTCT-3' 5'-GGTCAGCTTCATTTAGAAAC-3'	$(GT)_2ATGTAT(GT)_6AT(GT)_{12}$	Roed and Midthjell, 1998
NVHRT73	5'-CTTGCCCATTTAGTGTTTTCT-3' 5'-TGCGTGTCATTGAATAGGAG-3'	$(CT)_4GT(CT)_3GCCTGT(CT)_2C$ $CTT(CT)_3TT(CT)_{13}CACT$ $(CA)_8TA(CA)_3$	Roed and Midthjell, 1998

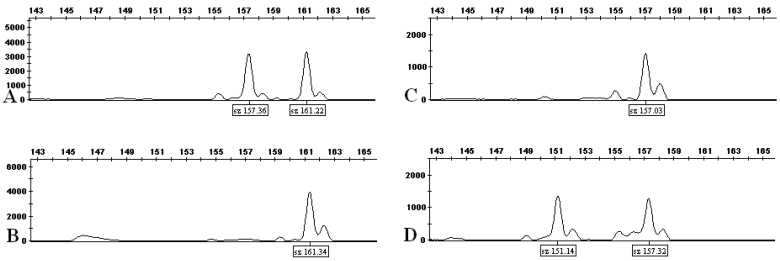

Fig. 2. Electrophoregram of capillary electrophoresis of NVHRT16 locus

GenAlEx 6.501 software was also used for calculating the overall fixation index F_{ST}, yielding the proportion of the total genetic diversity accounted for by the differentiation among populations. Population differentiation was further quantified with Nei's (1978). The dendograms were constructed by UPGMA (Unweighted Pair–Group Method with Arithmetic Mean) method using MEGA6 software. Analysis of population and individual admixture using the microsatellite multilocus genotypes were carried oput with a Bayesian clustering algorithm implemented in STRUCTURE 2.3.4 (Pritchard et al., 2000; Falush et al., 2003; Falush et al., 2007). This models assumes thera are K populations, each of which is characterized by set of allele frequencies at each locus.

Amplification, sequencing and analysis of mitochondrial DNA

For sequencing and analysis of mtDNA 20 samples of roe deer were used. Speciments were collected from 11 different Lithuania regions; any reintroductions have never been documented. Amplification of the mitochondrial control region (457 bp) was performed using primers pairs: L-Pro and H-493 (Table 2). The PCR protocol consisted of 20 µl reaction mix containing approximately 50 ng DNA, 0,2 µM each primer, 2 mM $MgCl_2$, 0,2 mM dNTPs, 0,2 U of Taq polymerase ("Thermo Scientific", Lithuania). The PCR consisted of an initial denaturation step at 94°C for 5 min followed by 35 cycles of 30 s at 94°C, 30 s at 60°C, 1 min at 72°C, with final elongation step of 10 min at 72°C after the last cycle.

Products were separated on 1.5% agarose gel and visualized by etidium bromide (Fig. 3). PCR amplification products of D-loop were purified by "GeneJET Gel Extraction Kit" ("Thermo Scientific", Lithuania) and sequenced with "BigDye® Terminator v3.1 Cycle Sequencing Kit" ("Applied Biosystems") following the manufacturer's recommendations, which run on 3130xl Genetic Analyzer ("Applied Biosystems"). Sequences were aligned using CLUSTAL W (Thompson et al., 1994), BioEdit 7.2.5 (Hall, 1997) and MEGA 6 (Tamura et al., 2013) software, haplotypes were identified by DnaSP v.5 (Librado and Rozas, 2009) program. Haplotype network were constructed using the median joining (MJ) algorithm (Bandelt et al., 1999) in the Network 4.6.1.2 program (fluxus-engineering.com).

2 table. PCR primers used for roe deer mtDNA analysis

Primer	Primer sequence	Amplicon size	Position
L-Pro	5'-CGTCAGTCTCACCATCAACCCCCAAAGC-3'	457 bp	15740 $tRNA^{Pro}$
H-493	5'-TGAGATGGCCCTGAAGAAAGAACC-3'		420 $tRNA^{Phe}$

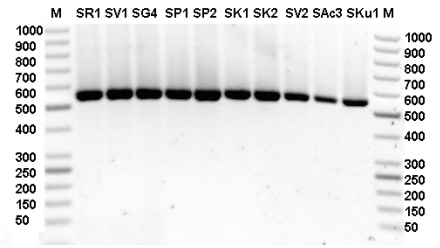

Figure 3. PCR amplified products of mtDNA D-loop fragment. Lane M: 50 bp DNA ladder, other lanes (SR1, SV1, SG4 and other) amplified products (550 bp)

RESULTS AND DISCUSSIONS

Analysis of microsatellites of sika deer and red deer

Sika deer appeared in Lithuania 60 years ago, when animals were introduced in Dubrava forest, Kaunas district in 1954. The original herd of these animals consisted of 24 individuals, by 1970 herd increased and amounted for about 60 -70 individuals. Later number of animals decreased to 48 individuals, although these animals were not hunted. Sika deer did not spread to other forests. A constant number of 50-60 animals were recorded prior 1991, but the reasons why the increase of these animals stopped, still not clear. It is believed that red deer spread in Dubrava and two species began hybridize. Since 1992 fauna of game records of these animals haven't been recorded (Baleišis et al., 2003).

Since 1989 human have started to keep sika deer in enclosures, animals were brought from the Chechen-Ingush, Kaliningrad and Vladivostok. There were about 750 sika deer in enclosures in Lithuania (Baltrūnaitė, 1999). According to data of Ministry of the Environment about 2000 sika deer are kept in enclosures of Lithuania now.

Red deer distribution around the world has led human. From the 17 to the 19 century numbers of red deer population have decreased in many European countries (Hartl et al., 2003, Kuehn et al., 2003, 2004; Hmwe et al., 2006; Nielsen et al., 2008), and in some central and eastern parts of Europe, this species total disappeared (Baleišis and Škerys, 1984; Baleišis, 1988). Since then, many red deer have been moved or migrated to these areas from the areas where the population was abundant (Baleišis and Škerys, 1984; Baleišis 1988; Hmwe et al., 2006; Nussey et al., 2006). In Europe red deer were transferred from Germany, Austria and Russia in the north-eastern Poland, the central, northern and western regions of Poland in the east of Poland, from Voronezh Reserve to Lithuania and Belarus (Baleišis and Škerys, 1984; Baleišis 1988; Niedziałkowska, 2008). In Lithuania red deer migrated naturally from Latvia and northern Russia (St. Petersburg) (Vereshchagin and Rusakov, 1979; Baleišis and Škerys, 1984; Baleišis, 1988). Red deer from the Germany were transferred to Latvia and Voronezh Reserve in 19th century end (Barabash-Nikiforov and Pavlovskii, 1949; Baleišis, 1988). Red deer from the Caucasus Mountains and Poland were also released in Latvia (Baleišis, 1988). Many red deer of the Middle Ages were moved from Germany to many other countries (eg. Russia, the United Kingdom, Austria, Hungary

and Poland) (Niethammer, 1963). In eastern Europe the local population has remained only in a few areas in the north-eastern Poland (Niedziałkowska, 2008), Kaliningrad, Latvia (Vereshchagin and Rusakov, 1979) and the Carpathian region of Ukraine (Tatarinov, 1973).

Estimates of genetic diversity of both species were obtained. Genetic variation expressed as mean H_o was 0,69 (range 0,37 – 0,97) in sika deer and 0,63 (range 0,03 – 1,00) in red deer population. All loci were polymorphic yielding between 3 and 17 alleles in sika deer population and between 3 and 18 alleles in red deer population. The smallest number of alleles was detected in RT23 locus (3 alleles), 17 and 18 alleles were determined in NVHRT73 locus (SD population) and NVHRT21 locus (RD population). The total number of alleles ranged from 55 (SD) to 63 (RD) (Table 3). The numbers of private alleles were 17 in both sika and red deer populations (Table 4).

3 table. Locus specific diversity measures estimated for sika deer and red deer. Na – number of alleles, Ne – effective numberof alleles, Al. size - allele size range, H_o – Observed heterozygosity, H_e – Expected heterozygosity, uH_e - Unbiased Expected Heterozygosity, I – Shannon's Information Index, F – Fixation index

		Loci					
Population		**NVHRT48**	**NVHRT73**	**NVHRT21**	**RT1**	**RT23**	**NVHRT16**
Sika deer (SD)	**N**	30	30	30	30	30	30
	Na	6	17	12	13	3	4
	Al.size	85-97	211-249	141-173	205-235	137-141	157-179
	Ne	4,306	9,677	4,196	5,844	1,484	2,406
	I	1,600	2,522	1,873	2,065	0,558	1,007
	Ho	0,967	0,933	0,533	0,767	0,367	0,600
	He	0,768	0,897	0,762	0,829	0,326	0,584
	uHe	0,781	0,912	0,775	0,843	0,332	0,594
	F	-0,259	-0,041	0,300	0,075	-0,124	-0,027
Red deer (RD)	**N**	39	39	39	39	39	39
	Na	9	13	18	13	3	7
	Al.size	81-99	207-245	141-185	209-239	137-169	151-169
	Ne	6,196	5,805	10,711	8,289	1,053	1,653
	I	1,935	2,064	2,586	2,260	0,137	0,901
	Ho	1,000	0,897	0,769	0,872	0,026	0,205
	He	0,839	0,828	0,907	0,879	0,050	0,395
	uHe	0,849	0,838	0,918	0,891	0,051	0,400
	F	-0,192	-0,084	0,152	0,009	0,490	0,481

In order to accurately assess the genetic diversity of red deer in Lithuania, the total population was divided into two: the wild red deer (W) and red deer kept in enclosures (E). Allelic patterns across populations are given in Fig. 4. Small amount of

number of alleles and private alleles in red deer population from enclosures could be due to the small number of samples in this population.

4 table. The list of private alleles and alleles frequency in sika deer and red deer populations

Population	Locus	Allele	Allele frequency	Population	Locus	Allele	Allele frequency
Sika deer	NVHRT48	85	0,083	Red deer	NVHRT48	81	0,141
Sika deer	NVHRT73	211	0,067	Red deer	NVHRT48	83	0,064
Sika deer	NVHRT73	219	0,017	Red deer	NVHRT48	91	0,154
Sika deer	NVHRT73	237	0,017	Red deer	NVHRT48	99	0,013
Sika deer	NVHRT73	241	0,033	Red deer	NVHRT73	207	0,013
Sika deer	NVHRT73	243	0,017	Red deer	NVHRT73	213	0,013
Sika deer	NVHRT73	247	0,033	Red deer	NVHRT73	245	0,051
Sika deer	NVHRT73	249	0,033	Red deer	NVHRT21	157	0,090
Sika deer	NVHRT21	149	0,017	Red deer	NVHRT21	167	0,077
Sika deer	NVHRT21	153	0,133	Red deer	NVHRT21	175	0,090
Sika deer	RT1	205	0,017	Red deer	NVHRT21	177	0,064
Sika deer	RT1	207	0,033	Red deer	NVHRT21	179	0,051
Sika deer	RT1	219	0,017	Red deer	NVHRT21	181	0,013
Sika deer	RT23	139	0,183	Red deer	NVHRT21	183	0,013
Sika deer	RT23	141	0,017	Red deer	NVHRT21	185	0,026
Sika deer	NVHRT16	173	0,067	Red deer	RT1	213	0,090
Sika deer	NVHRT16	179	0,033	Red deer	RT1	221	0,128

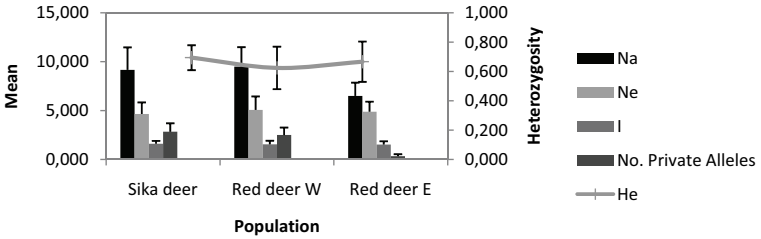

Figure 4. Allelic patterns across studied sika deer, red deer (W) and red deer (E) populations

In order to accurately assess the genetic diversity of red deer in Lithuania, the total population was divided into two: the wild red deer (W) and red deer kept in enclosures (E). Allelic patterns across populations are given in Fig. 4. Small amount of number of alleles and private alleles in red deer population from enclosures could be due to the small number of samples in this population.

Significant deviations from HWE occurred in whole red deer population for all loci, in sika deer population significant deviations occurred in 3 loci (NVHRT48, NVHRT73, NVHRT73) (Table 5).

5 table. HWE in populations of sika deer and red deer according loci

Population	Locus	DF	χ^2	Probability	Significant
Sika deer	NVHRT48	15	40,229	0,000	***
	NVHRT73	136	168,262	0,031	*
	NVHRT21	66	123,554	0,000	***
	RT1	78	68,483	0,771	ns
	RT23	3	5,620	0,132	ns
	NVHRT16	6	6,950	0,325	ns
Red deer	NVHRT48	36	110,600	0,000	***
	NVHRT73	78	173,002	0,000	***
	NVHRT21	153	246,085	0,000	***
	RT1	78	142,120	0,000	***
	RT23	3	78,000	0,000	***
	NVHRT16	21	53,139	0,000	***
ns=non significant, * P<0.05, ** P<0.01, *** P<0.001					

Pairwise F_{ST} were highest for comparisons among sika deer and wild red deer population (Table 6). Average meaning of F_{ST} accros locus was 0,078, the average number of migrants during the generation – 3,537 (Table 7). The factorial correspondence analysis (FCA) was performed to visualize the relationships between individuals from different species and populations to test possible admixtures between them (Fig. 5). FCA was computed using GENETIX program (Belkhir et al., 2001).

6 table. Pairwise F_{ST} values calculated from microsatellite. Red deer (W) – animals culled in wild, red deer (E) – animals from enclosures

Sika deer	Red deer (W)	Red deer (E)	
0,000			**Sika deer**
0,075	0,000		**Red deer (W)**
0,045	0,052	0,000	**Red deer (E)**

7 table. F-values in all populations for each loci

	NVHRT48	NVHRT73	NVHRT21	RT1	RT23	NVHRT16	Mean	SE
F_{is}	-0,236	-0,100	0,202	0,030	-0,030	0,268	**0,022**	0,077
F_{it}	-0,174	-0,038	0,250	0,078	0,080	0,374	**0,095**	0,080
F_{st}	0,050	0,056	0,060	0,049	0,107	0,146	**0,078**	0,016
Nm	4,752	4,188	3,893	4,838	2,088	1,463	**3,537**	0,581

The results of 10 independent runs of STRUCTURE simulations at each value of K (Length of burnin period 150000; number of MCMC Reps after Burnin 200000) produced consistent results, and division of the sika deer data set into 2 clusters (Fig. 6), red deer data – 2 clusters (Fig. 7) and sika deer with red deer data – 2 clusters (Fig. 8).

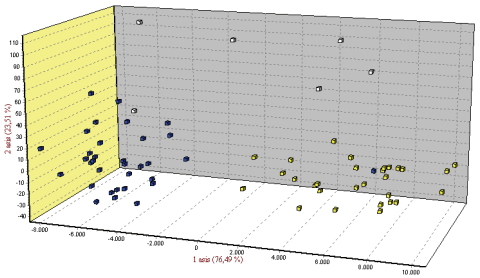

Figure 5. Factorial correspondence analysis (FCA) of sika deer (yellow box) and red deer from enclosures (white box) and from wild (blue box) populations multilocus scores computed using GENETIX

Figure 6. Assigment probabilities of sika deer individuals to putative population clusters (K=2) using the program STRUCTURE 2.3.4.

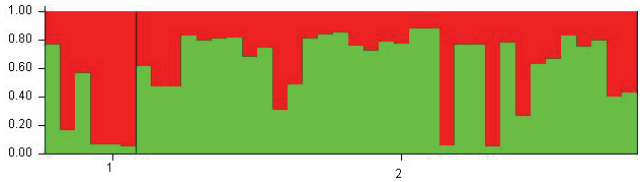

Figure 7. Assigment probabilities of red deer individuals to putative population clusters (K=2) using the program STRUCTURE 2.3.4. Populations which individuals were collected are indicated below the graph: 1 – red deer from enclosures, 2 – wild red deer

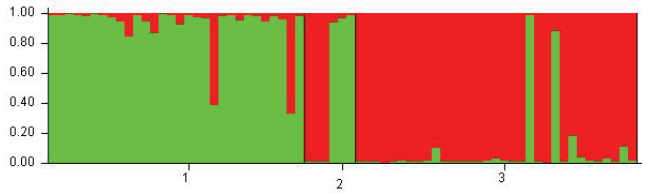

Figure 8. Assigment probabilities of sika deer and red deer individuals to putative population clusters (K=2) using the program STRUCTURE 2.3.4.
Populations which individuals were collected are indicated below the graph:
1 – sika deer, 2 - red deer from enclosures, 3 – wild red deer

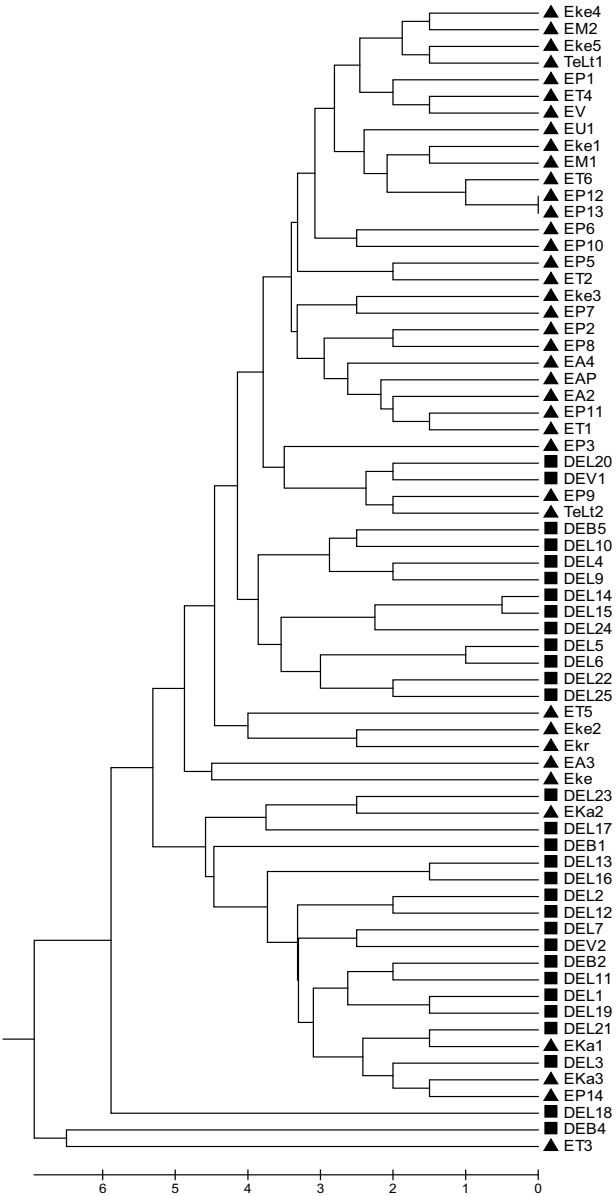

Figure 9. UPGMA dendrogram according to Nei's (1978) genetic distance between sika deer (■) and red deer (▲) individuals

Based on the Bayesian analysis found that the sika deer grown in enclosures of Lithuania was derived from the two different locations. According to literature data Lithuanian red deer population should consist of naturally migrated and imported animals, Bayesian analysis confirmed that studied Lithuanian red deer samples formed 2 separate clusters (see Fig. 7). The dendrogram of sika deer and red deer was constructed by UPGMA (Unweighted Pair–Group Method with Arithmetic Mean) method according to Nei's (1978) genetic distances (Fig. 9).

Hybrid animals were found in sika deer population and in red deer population, although these animals were kept in enclosures, but the possibility of hybridization always remain. There are many cases, not only in Lithuania, but also all around the world, animals from enclosures can escape or are intentionally released into the wild, some of them returned to the enclosures, while others remain at wild. In our investigation we identified and wild red deer animals as hybrids.

The obtained results in genetic diversity of sika deer studies were compared with results of foreign authors (Table 8). Population of sika deer in Lithuania characterized by same values of observed and expected heterozygosity 0,69, although lower number of alleles were established in this population compared with red deer population. Studies of sika deer in Japan and Ireland showed similar results that the observed heterozygosity is very close to the expected heterozygosity ~0,6 and ~0,4, respectively (Okada et al., 2005; McDevitt et al., 2009). Low observed 0,14 and expected 0,15 heterozygosity were determined in Scotland (Senn and Pemberton, 2009).

Table 8. Genetic diversity of sika deer from different countries(No.L. - number of loci, Na - the total number of alleles, Ave.Na - average locus alleles, Ho - observed heterozygosity, He. - expected heterozygosity)

Location	No. L.	Na	Ave. Na	H_o	H_e	References
Lithuania	6	55	9,17	0,69	0,69	This study
Vietnam	9	61	5,70	-	0,60	Thevenon et al., 2003
Japan (resident pop.)	13	-	-	0,60	0,58	Okada et al., 2005
Japan (non resident pop.)	13	-	-	0,58	0,59	
Scotland	22	67	3,04	0,14	0,15	Senn and Pemberton, 2009
Ireland	8	61	7,63	0,40	0,39	McDevitt et al., 2009
Poland, Kaliningrad, Lithuania	14	86	6,14	-	0,48	Biedrzycka et al., 2012
China	14	83	5,93	0,57	0,69	Shen-Jin et al., 2014

-data not available

Expected heterozygosity 0,65 value established in red deer population from Lithuania was slightly greater than the observed heterozygosity 0,63 value. Similar results have shown in most European countries: Serbia, Romania, Spain, Italy, Sardinia, Italy, Bulgaria, Scotland (Zachos et al., 2003; Feulner et al., 2004; Senn and Pemberton, 2009).

Sika deer and red deer are different species according to morphological features (weight, antlers, fur discoloration) and karyotype, however these two related species can crossbreed with each other. Hybridization problem of sika deer and red deer is particularly relevant in Europe, investigations launched more than 30 years ago in Czech Republic (Bartoš and Žirovnicky, 1981), Scotland (Goodman et al., 1999, Senn et al., 2010), Ireland (McDevitt et al., 2009), Poland and Russia (Biedrzycka et al., 2012), but in Lithuania hybridization have not been studied till now. Our result showed that topic of hybridization is important in Lithuania; hybrid animals have been found in the wild and in the herds of enclosures.

Analysis of microsatellites of roe deer

Genetic diversity studies of roe deer started before 20 years in Europe (Kurt et al., 1993; Lorenzini et al., 1993) and continue to this day (Vorobiev et al., 2011; Lorenzini et al., 2014; Matosiuk et al., 2014, Olano-Marin et al., 2014). First studies of genetic diversity of Lithuanian roe deer published in 2013 (Pūraitė et al., 2013), previously only the ecological and morphometric studies had been carried out (Pėtelis and Brazaitis, 2003; Balčiauskas, 2004, Narauskaitė et al., 2011).

In order to assess the genetic diversity of roe deer in Lithuania 6 pairs of microsatellite markers were used. We tested a total of 103 roe deer from Lithuania and 2 animals from Latvia and Czech Republic. Roe deer individuals were divided into separate populations according to Lithuania region where were hunted – west LT (20 individuals), north LT (19 individuals), east LT (31 individuals) and south LT (33 individuals).

Genetic diversity on the analysed data set was assessed computing the expected (H_e) and observed (H_o) heterozygosity. Genetic variation expressed as mean H_o was 0,82 (range 0,21 – 1,00), H_e was 0,69 (range 0,19 – 0,88). All loci were polymorphic yielding between 2 and 12 alleles in roe deer population. The smallest number of alleles

was detected in RT23, NVHRT73 loci (2 alleles), 12 alleles were determined in NVHRT21 locus (East LT population). The total number of alleles ranged from 34 (North LT) to 43 (East LT and South LT) in population (Table 9). The numbers of private alleles were from 3 to 7 in Lithuanian roe deer populations (Table 10). Any unique locus was determinated in the North LT population.

9 table. Locus specific diversity measures estimated for roe deer. Na – number of alleles, Ne – effective numberof alleles, allele size range, H_o – Observed heterozygosity, H_e – Expected heterozygosity, uH_e - Unbiased Expected Heterozygosity, I – Shannon's Information Index, F – Fixation index

Population	Locus	N	Na	Ne	I	Ho	He	uHe	F
West LT	NVHRT48	20	5,000	3,113	1,305	1,000	0,679	0,696	-0,473
	NVHRT73	20	5,000	3,347	1,365	0,550	0,701	0,719	0,216
	NVHRT21	20	8,000	6,250	1,932	0,950	0,840	0,862	-0,131
	RT1	20	10,000	5,333	1,928	0,850	0,813	0,833	-0,046
	RT23	20	4,000	2,305	0,952	1,000	0,566	0,581	-0,766
	NVHRT16	20	5,000	3,175	1,255	0,650	0,685	0,703	0,051
North LT	NVHRT48	19	6,000	2,597	1,163	1,000	0,615	0,632	-0,626
	NVHRT73	19	2,000	1,232	0,336	0,211	0,188	0,193	-0,118
	NVHRT21	19	11,000	7,934	2,203	0,947	0,874	0,898	-0,084
	RT1	19	9,000	4,846	1,849	0,895	0,794	0,815	-0,127
	RT23	19	2,000	2,000	0,693	1,000	0,500	0,514	-1,000
	NVHRT16	19	4,000	3,780	1,355	0,632	0,735	0,755	0,141
East LT	NVHRT48	31	6,000	3,718	1,459	0,806	0,731	0,743	-0,103
	NVHRT73	31	4,000	2,152	0,916	0,484	0,535	0,544	0,096
	NVHRT21	31	12,000	8,144	2,257	1,000	0,877	0,892	-0,140
	RT1	31	11,000	7,040	2,104	0,968	0,858	0,872	-0,128
	RT23	31	4,000	2,124	0,834	0,968	0,529	0,538	-0,829
	NVHRT16	31	6,000	4,470	1,589	0,710	0,776	0,789	0,086
South LT	NVHRT48	33	6,000	3,129	1,334	0,970	0,680	0,691	-0,425
	NVHRT73	33	3,000	2,218	0,902	0,515	0,549	0,558	0,062
	NVHRT21	33	11,000	6,958	2,111	0,848	0,856	0,869	0,009
	RT1	33	11,000	7,049	2,125	0,970	0,858	0,871	-0,130
	RT23	33	5,000	2,444	1,066	1,000	0,591	0,600	-0,692
	NVHRT16	33	7,000	4,109	1,580	0,879	0,757	0,768	-0,161
no LT	NVHRT48	2	2,000	2,000	0,693	1,000	0,500	0,667	-1,000
	NVHRT73	2	3,000	2,667	1,040	0,500	0,625	0,833	0,200
	NVHRT21	2	4,000	4,000	1,386	1,000	0,750	1,000	-0,333
	RT1	2	4,000	4,000	1,386	1,000	0,750	1,000	-0,333
	RT23	2	2,000	2,000	0,693	1,000	0,500	0,667	-1,000
	NVHRT16	2	2,000	1,600	0,562	0,500	0,375	0,500	-0,333

10 table. The list of private alleles and alleles frequency in roe deer populations.

Population	Locus	Allele	Allele no.	Allele frequency
West LT	NVHRT73	233	3	0,050
	RT1	227		0,025
	NVHRT16	163		0,025
East LT	NVHRT48	95	7	0,032
	NVHRT73	221		0,016
	NVHRT73	229		0,065
	NVHRT21	177		0,016
	RT1	213		0,032
	RT1	215		0,048
	NVHRT16	165		0,032
South LT	NVHRT21	153	5	0,015
	RT1	234		0,015
	RT23	167		0,030
	NVHRT16	155		0,015
	NVHRT16	161		0,030
no LT	NVHRT21	181	2	0,250
	RT1	217		0,250

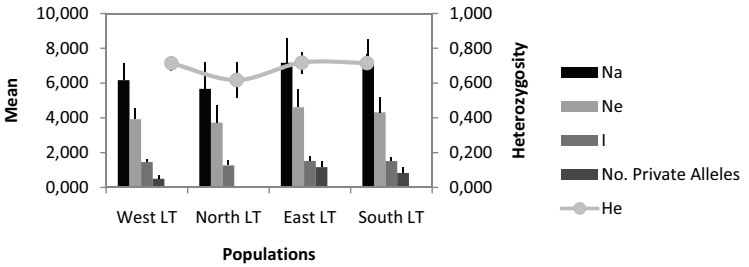

Figure 10. Allelic patterns across studied roe deer populations

Allelic patterns across roe deer populations are given in Fig. 10. The lowest value of expected heterozygosity (H_e) was found in the North LT, also no private alleles were found in this population.

Significant deviations from HWE occurred in all Lithuanian roe deer population for NVHRT48 loci. Pairwise F_{ST} were highest for comparisons among roe deer of North LT and West LT populations 0,038; lowest – 0,009 among East LT and South LT populations (Table 11). Average meaning of F_{ST} accros locus was 0,061, the average number of migrants during the generation – 12,426 (Table 12). The factorial correspondence analysis (FCA) was performed to visualize the relationships between

individuals from different populations to test possible admixtures between roe deer populations in Lithuania (Fig. 11).

11 Table. Pairwise F_{ST} values calculated from microsatellite in roe deer population

West LT	North LT	East LT	South LT	
0,000				**West LT**
0,038	0,000			**North LT**
0,013	0,025	0,000		**East LT**
0,010	0,026	**0,009**	0,000	**South LT**

12 Table. . F-values in all populations for each loci

	NVHRT48	NVHRT73	NVHRT21	RT1	RT23	NVHRT16	Mean	SE
Fis	-0,490	0,131	-0,131	-0,150	-0,849	-0,013	-0,250	0,146
Fit	-0,448	0,263	-0,084	-0,068	-0,840	0,057	-0,187	0,161
Fst	0,028	0,152	0,042	0,071	0,005	0,069	0,061	0,021
Nm	8,658	1,394	5,772	3,283	52,063	3,387	12,426	7,993

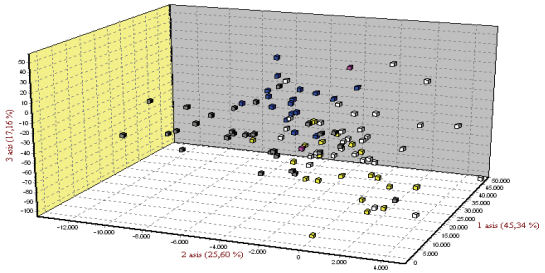

Figure 11. Factorial correspondence analysis (FCA) of roe deer populations multilocus scores computed using GENETIX. ■ – West LT; ■ – East LT, ■ – North LT, ■ – South LT; ■ – no LT

The results of the STRUCTURE analyses showed that a partitioning of the individual genetic variation into three genetic clusters was the most probable (K=3) (Fig. 12). The roe deer populations in Lithuania have high degree of genetic diversity.

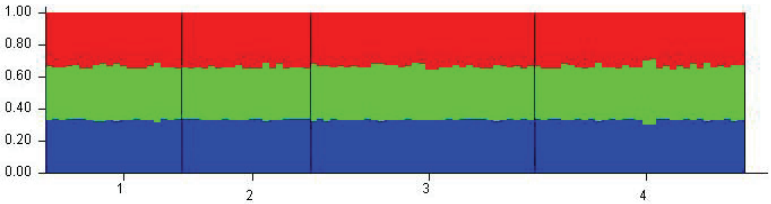

Figure 12. Assigment probabilities of roe deer individuals to putative population clusters (K=3) using the program STRUCTURE 2.3.4. Populations which individuals were collected are indicated below the graph: 1 – West LT, 2 – North LT, 3 – South LT, 4 - East LT

Based on the Nei's (1978) genetic distances between roe deer individuals and using UPGMA clustering method, dendrogram were plotted (Fig. 13). Dendrogram shows analyzed DNA of roe deer similarities and distribution of individuals in two-dimensional space. According to the results it was concluded that Lithuania roe deer genetic diversity is large, individuals from separate populations are mixed together in groups, but consists of 3 major clusters. All populations are grouped in clusters A and B, but individuals from North LT population aren't found in cluster C.

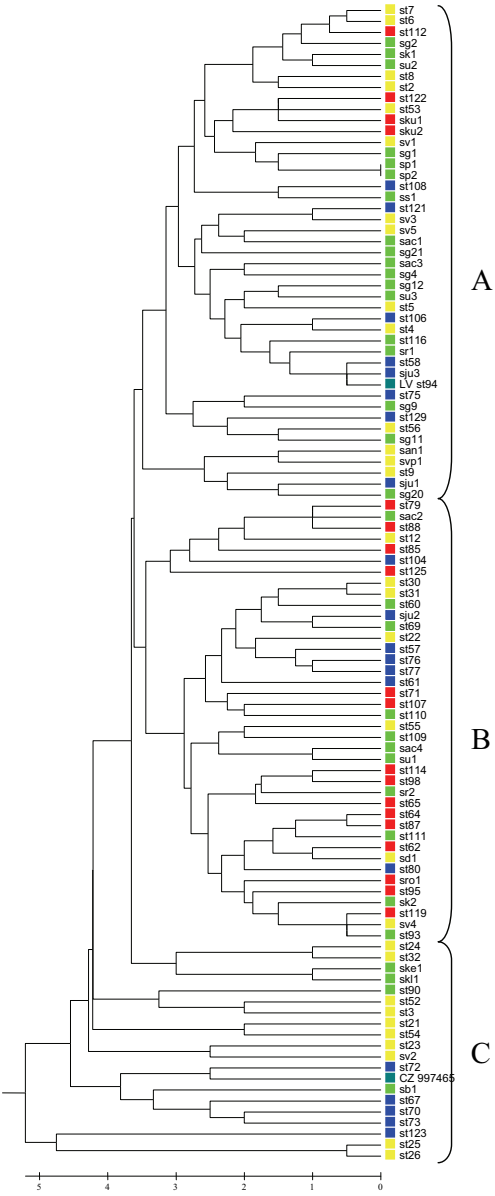

Figure 13. UPGMA dendrogram according to Nei's (1978) genetic distance between roe deer individuals. ■ – North LT ■ – West LT, ■ – South LT, ■ – East LT, ■ – no LT

Results of PCoA analysis, FCA, STRUCTURE and UPGMA clustering showed that Lithuania roe deer population is mixed, and has high genetic diversity.

Genetic diversity of sika deer, red deer and roe deer populations was investigated using six microsatellite markers. These markers are suitable for evaluation of cervids genetic diversity and identification of these species (Fig. 14).

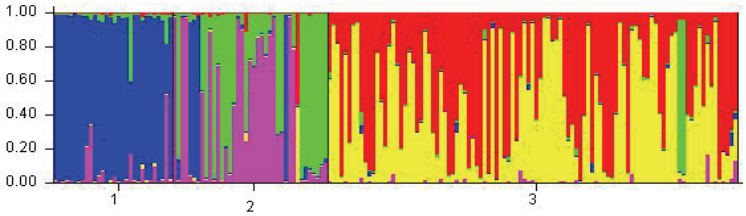

Figure 14. Assigment probabilities of sika deer, red deer and roe deer individuals to putative population clusters (K=5) using the program STRUCTURE 2.3.4. Populations which individuals were collected are indicated below the graph:
1 – sika deer, 2 – red deer, 3 – roe deer

Analysis of mtDNA sequences

The 457 bp mtDNA control region sequence and 38 variable nucleotide sites were determined from 20 individuals. Six haplotypes and two haplogroups were identified in population of roe deer in Lithuania (GenBank accession numbers KM215767-KM215786) (Table 13). Haplotypes Hap_1 and Hap_2 were the most common in Lithuania roe deer population. Comparative analysis of the data was performed using homologous mtDNA control region sequences uploaded in GenBank database. Hap_2, Hap_3 and Hap_4 haplotypes were specific in roe deer from Lithuania. Haplotypes Hap_1 and Hap_5 were identified roe deer populations from Russia (Zvychalnaya et al., 2011) and Lithuania. The phylogenetic tree indicated that the mtDNA haplotypes of the roe deer are split into two well divergent clades (European roe deer and Siberian roe deer) (Fig. 15). Analyses of control region mtDNA sequences indicated widespread introgression of Siberian roe deer (*C. pygargus*) mtDNA in the European roe deer genome, introgressed individuals constituted 20% of the deer studied.

Table 13. GenBank accession number of examined speciments, district and haplotype

Accession number in GenBank	Individual code	District	Haplotype
KM215767	SR1	Raseiniai dis.	Hap_3
KM215768	SV1	Moletai dis.	Hap_3
KM215769	SKel64	Kelmes dis.	Hap_2
KM215770	SG4	Jonava dis.	Hap_2
KM215771	SK1	Kaunas dis.	Hap_2
KM215772	SJu1	Jurbarkas dis.	Hap_2
KM215773	SKe1	Kedainiai dis.	Hap_5
KM215774	SK2	Kaunas dis.	Hap_5
KM215775	SKu1	Kupiskis dis.	Hap_1
KM215776	SKel65	Kelme dis.	Hap_1
KM215777	SRiet80	Rietavas dis.	Hap_1
KM215778	SRiet104	Rietavas dis.	Hap_1
KM215779	SAc3	Jonava dis.	Hap_1
KM215780	SV2	Moletai dis.	Hap_1
KM215781	ST22	Ukmerge dis.	Hap_1
KM215782	ST24	Ukmerge dis.	Hap_1
KM215783	SP1	Prienai dis.	Hap_4
KM215784	SP2	Prienai dis.	Hap_4
KM215785	SRok122	Rokiskis dis.	Hap_6
KM215786	SRok79	Rokiskis dis.	Hap_6

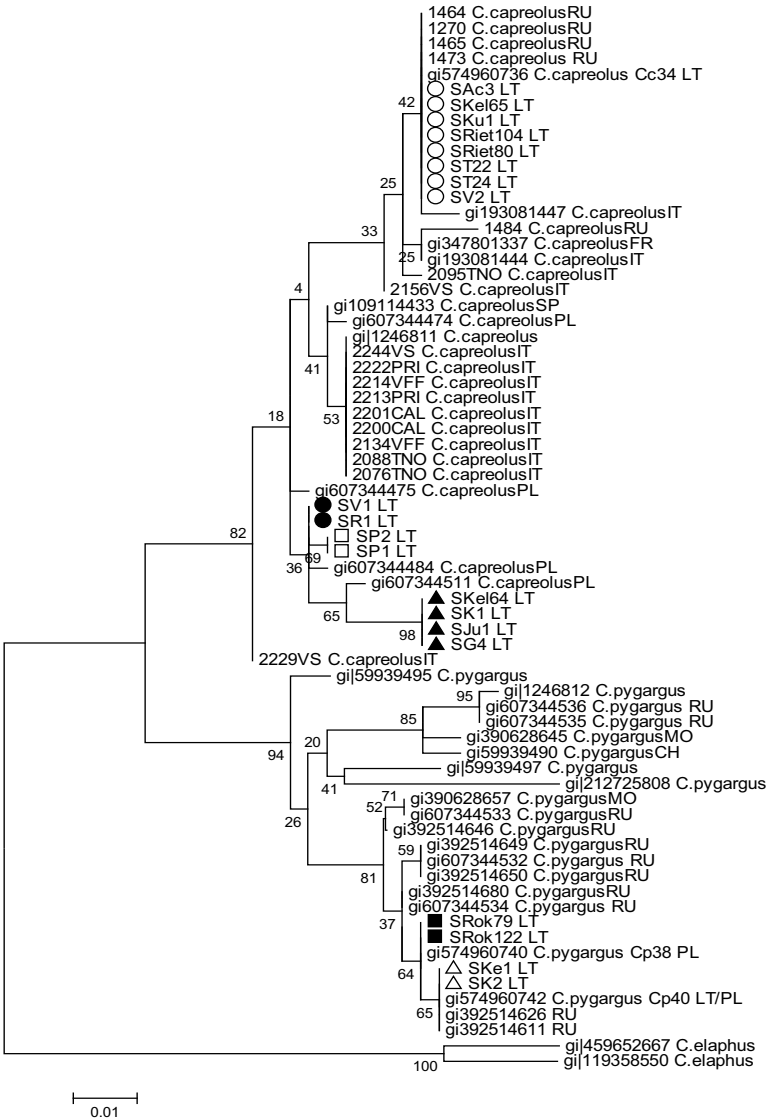

Figure 15. Neighbour-joining tree based on pairwise nucleotide divergence of roe deer mtDNA haplotypes. Numbers at the nodes show support from 1000 bootstrap replicates, *Cervus elaphus* used as an outgroup. Six haplotypes identified in roe deer population of Lithuania: ○ – Hap_1, ▲ – Hap_2, ● – Hap_3, □ – Hap_4, Δ - Hap_5, ■ – Hap_6

According DnaSP v5 results haplotype diversity was H_d - 0,800, variance of haplotype diversity - 0,00445, nucleotide diversity - 0,03031, average number of nucleotide differences k - 13,853, sequence conservation C - 0,917.

The roe deer individuals belonging to the first haplotype (Hap 1) were found in western, northeastern, eastern and central part of Lithuania. Haplotype Hap 2 was established in the west, southwest and central part of Lithuania. Haplotype Hap 3 was identified in individuals from the western and eastern Lithuania, Hap 4 - southern Lithuania. Haplotype Hap 5 was determined in individuals from the central part of Lithuania and Hap 6 - north-east Lithuania part (Fig. 16).

MP (*Maximum Parsimony*) network were constructed in order to assess the roe deer intraspecific genetic diversity and evolution of intraspecific haplotypes consisting of 50 European and Siberian roe deer mtDNA D-loop region sequences (Fig. 17). The network shows 20 haplotypes and two haplogoups of foreign countries (Russia, Mongolia, China, Italy, France, Spain) and Lithuania.

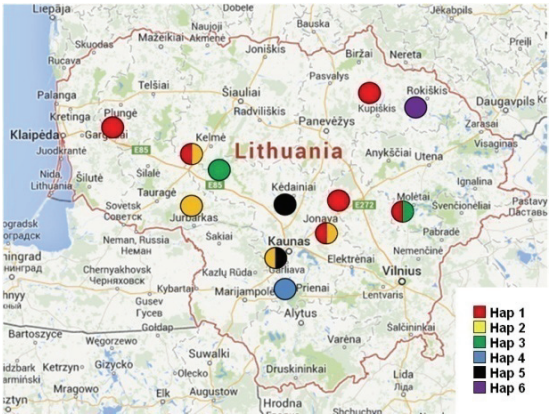

Figure 16. Distribution of haplotypes in roe deer population in Lithuania

Analysis of mtDNA control sequences indicated introgression of Siberian roe deer (*C.pygargus*) mtDNA in the European roe deer genome, current distribution range of Siberian roe deer is 2000 km from Lithuania. The European roe deer and Siberian roe deer have different morphological appearance and karyotype (*C.pygargus* characterized by additional B chromosomes) (Danilkin, 1985, Sokolov et al., 1978; Graphodatsky 1990; Vorobiev et al., 2011). In 19th century Siberian roe deer has been introduced in

the European roe deer habitats for hunting purposes (Germany, Slovakia, Ukraine, and other areas) (Danilkin, 1996), however Danilkin and Hewison (2001) reported that the gene introgression of Siberian roe deer and survival in wild is hardly possible due to the formed strong barriers of reproductive during the formation of the species and after. It is considered that current introduction of other species animals will not affect native species (Lorenzini et al., 2014). Siberian roe deer gene introgression into European roe deer genome is the results of post glacial migration (Matosiuk et al., 2014). In our opinion it could be the results of species formation and migration before glacial period.

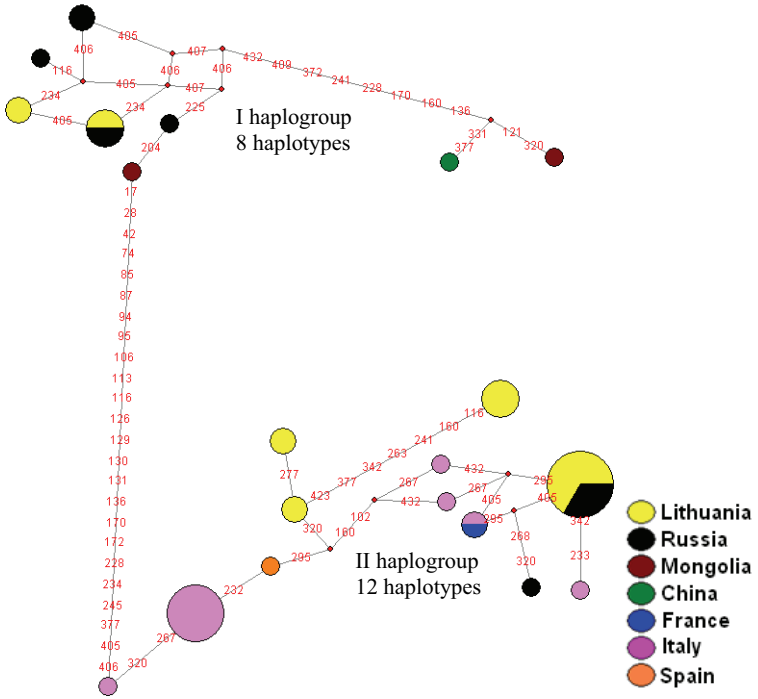

Figure 17. Median-joining network of total 20 mtDNA haplotypes and 2 haplogroups of roe deer from Lithuania, Russia, France, Italy, Spain, China and Mongolia

CONCLUSIONS

1. Six microsatellite markers were used in the studies of genetic diversity of the sika deer, the red deer and the roe deer population. These markers are suitable for the identification of these species.
2. These molecular markers were used for the first time in the sika deer studies, established that average number of the alleles in the locus was 9.20, the number of effective allele was 4.65 in the sika deer population and their number in the samples of the red deer constituted 10.50 and 5.62, respectively. The values of the average observed heterozygosity H_o under investigation and the expected heterozygosity H_e in the samples of the sika deer were equal 0.694, and those in the samples of the red deer H_o 0.628 were somewhat smaller than H_e 0.650.
3. The population of the sika deer has an impact on the Lithuanian red deer population. The biological pollution has been found in the samples of wild and farmed red deer, such as hybrid animals.
4. Having assessed genetic diversity of the Lithuanian roe deer it was established that the population was heterogeneous, consisting of 96% of the variety of the inside population and 4% of the variety of the interpopulation. The average number of the alleles in the roe deer population was 6.54, and the number of affective alleles totaled 4.14; the determined heterozygosity H_o 0.825 in the population of the Lithuanian roe deer is higher than H_e 0.691; 38 variable nucleotide sites were determined in the 457 bp mtDNA control region sequences: six haplotypes and two haplogroups were identified in the population of the roe deer in Lithuania. Three of six haplotypes derived from the Lithuanian roe deer were unique.
5. Mitochondrial DNA D-loop sequences of the roe deer showed a different distribution of nucleotide frequencies. The largest number of T nucleotides was detected in the roe deer from Kedainiai, Kaunas and Rokiskis - 32.8%, the content of C nucleotides in the studied subjects ranged from 20.4% to 21.2%. The maximum amount of 32.6% of A nucleotides was found in the sequences of the individuals from Rokiskis district, mainly 15.3% of G nucleotide were identified in the roe deer sequences from Prienai district, where unique haplotype was found.

6. The results obtained indicated introgression of the Siberian roe deer (*C. pygargus*) mtDNA in the European roe deer genome, the introgressed individuals accounted for 20% of the roe deer studied in Lithuania. Siberian roe deer gene introgression into European roe deer genome could be the results of the migration and formation of the species before glacial period.

SANTRAUKA

Temos aktualumas. Lietuvos kanopinių žvėrių faunos, susiformavusios po ledynmečio, kitimas buvo nulemtas gamtos ir klimato sąlygų pokyčių, vėliau tiesioginės ir netiesioginės žmonių veiklos. Ištirpus paskutiniam ledynui, šalto klimato sąlygomis, Lietuvos teritorijoje gyveno tundros ir miškatundrės žvėrys – šiauriniai elniai, stumbrai, taurieji elniai ir avijaučiai, vėliau, atšilus klimatui, šių žvėrių kaimenės išnyko arba pasitraukė toliau į šiaurę (Baleišis ir kt., 2003).

Šiandien Lietuvoje gyvenantiems elniniams pavojus išnykti negresia, jų populiacijos gausios ir paplitusios beveik visuose miškuose. Tačiau visame pasaulyje, ne išimtis ir Lietuvoje, elninių žvėrių genų fondas yra paveiktas arba įtakojamas reintrodukcijos, gyvūnų perkėlimo, laikymo aptvaruose, aplinkos fragmentacijos bei medžioklės (Wang ir Schreiber, 2001; Hartl ir kt., 2003; Kuehn ir kt., 2003; Coulon ir kt., 2004). Pastaraisiais dešimtmečiais Lietuvoje buvo tiriama kanopinių biologija, morfologija bei ekologija ir jų reikšmė kultūriniam kraštovaizdžiui (Baltrūnaitė, 1999; Pėtelis ir Brazaitis, 2004; Balčiauskas, 2004; Narauskaitė ir kt., 2011), tačiau elninių genetinės įvairovės tyrimai pradėti vykdyti neseniai.

Molekulinių tyrimų metodai taikomi tiriant genetinius skirtumus tarp atskirų populiacijų ir individų, atliekant rūšių ir porūšių identifikaciją. Elninių genetinio kintamumo tyrimai – aktuali tema, būtina nagrinėti šių žvėrių genetiką, stebėti pokyčius vykstančius populiacijose, gautas žinias panaudoti veisiant bei atkuriant rūšis. Įvairių elninių, kaip svetimkraščių gyvūnų, genetiniai tyrimai ir poveikis aborigeninėms Lietuvos populiacijoms iki šiol nebuvo vykdyti. Pasaulyje, o ypač Vakarų Europoje, problema nagrinėjama labai intensyviai - elniniai yra įveisti beveik visose Europos (Bartoš, 2009; McDevitt ir kt., 2009; Senn ir Pemberton, 2009) ir daugelyje Amerikos šalių, Naujojoje Zelandijoje, Pietų Afrikoje (Koubek ir Zima 1999; Shackell ir kt., 2003).

Genetinis kintamumas gali kisti dėl mutacijų, įvairių chromosomų persitvarkymų ar hibridizacijos su kitomis giminingomis rūšimis. Įvairių taksonų genetinis vientisumas gali būti paveiktas introdukuojant skirtingą genetinį foną turinčius gyvūnus, siekiant pagerinti ir išlaikyti nykstančias populiacijas, tačiau stabilių populiacijų genetinė būklė, į kurias buvo introdukuojami skirtingų genetinių tipų gyvūnai, galėjo būti paveikta svetimų genų. Rūšių reintrodukcija vykdoma

neatsižvelgiant į paleidžiamų individų genetinę kilmę, taip sukeliant nenuoseklų genetinį pasiskirstymą (Linnell ir Zachos, 2011). Lietuvoje per paskutinį dešimtmetį elninių auginimas aptvaruose, žvėrių paleidimas bei jų pabėgimas į laisvę labai išaugo, todėl laisvėje gali atsidurti visiškai netinkamos kilmės gyvūnai. Remiantis įsakymu "Dėl laukinių gyvūnų naudojimo taisyklių patvirtinimo" (2011 m. birželio 30 d. Nr. D1-533/B1-310) aptvaruose, voljeruose ar kituose statiniuose išauginti Lietuvos gamtoje natūraliai gyvenančių laukinių gyvūnų rūšių gyvūnai gali būti perkeliami tik laikantis „Introdukcijos, reintrodukcijos ir perkėlimo tvarkos", tačiau aptvaruose, voljeruose ar kituose statiniuose išauginti gyvūnų hibridai, svetimžemių ir invazinių laukinių gyvūnų rūšių gyvūnai bei Lietuvos teritorijoje natūraliai negyvenę laukiniai gyvūnai negali būti išleidžiami į laisvę. Ištrūkus į laisvę laukiniams gyvūnams, aptvarų, voljerų ar kitų statinių naudotojai privalo nedelsdami pranešti atitinkamam RAAD ir policijai apie kiekvieną nelaisvėje laikytų ir į laisvę ištrūkusių laukinių gyvūnų pabėgimo atvejį. Gyvūnų hibridai, svetimžemių ir invazinių laukinių gyvūnų rūšių gyvūnai bei Lietuvos teritorijoje natūraliai negyvenę laukiniai gyvūnai turi būti sugauti arba numarinti per trumpiausią įmanomą terminą, bet ne ilgesnį kaip vienas mėnuo, tačiau ne visuomet tai pavyksta padaryti. Aptvaruose laikytus ir sužymėtus, bet į laisvę ištrūkusius gyvūnus pagal ženklinimo žymes gamtoje būtų galima aptikti greičiau ir paprasčiau.

Nepaisant hibridizacijos pavojaus, introdukuotos kanopinių rūšys gali turėti poveikį ir biologinei įvairovei – kyla konkurencijos grėsmė vietinėms kanopinių gyvūnų populiacijoms ir žala augalijai (Spear ir Chown, 2009).

Darbo tikslas: ištirti dėmėtųjų elnių, tauriųjų elnių ir stirnų genetinę įvairovę ir įvertinti svetimkraščių gyvūnų poveikį bendrijų biologinei įvairovei ir stabilumui.

Darbo uždaviniai:

1. Pritaikyti molekulinius genetinius žymenis dėmėtųjų elnių, tauriųjų elnių ir stirnų rūšių identifikacijai, įvertinti genetinę įvairovę.
2. Įvertinti dėmėtųjų elnių poveikį tauriųjų elnių auginamų aptvaruose ir laukinėms populiacijoms.
3. Įvertinti sibirinės stirnos genetinį poveikį europinės stirnos populiacijai Lietuvoje.

Mokslinis darbo naujumas

Cervidae šeimos gyvūnų molekuliniai tyrimai - nauji Lietuvoje. Atlikta nemažai morfologinių ir ekologinių tyrimų, tačiau iki šiol jokių duomenų apie elninių genetinę įvairovę Lietuvoje, nebuvo publikuota (išskyrus mūsų publikacijas: Pūraitė ir kt., 2011. „Genetic diversity of Norwegian and Lithuanian red deer (*Cervus elaphus*) populations"; Pūraitė ir kt., 2013. „Analysis of genetic diversity of roe deer (*Capreolus capreolus* L.) in Lithuania using RAPD and allozyme systems", Pūraitė ir kt., 2014 „Genetic analysis of roe deer (*Capreolus capreolus* L.) using DNA markers" ir Pūraitė ir kt., 2014 „Mitochondrial DNA variation in roe deer populiation from Lithuania).

Svarbiausi šio tyrimų rezultatai – pirmą kartą genetiniuose dėmėtųjų elnių populiacijos tyrimuose panaudotas mikrosatelitinių žymenų rinkinys, kuris iki šiol buvo naudojamas šiaurinių elnių, stirnų, tauriųjų elnių ir danielių tyrimuose. Dėmėtųjų ir tauriųjų elnių (auginamų aptvaruose ir laukinių) imtyse aptikti hibridiniai gyvūnai.

Tyrimo metu identifikuota 20-ies stirnų mtDNR D-kilpos fragmento sekos, kurios patalpintos į „Genų banko" sekų duomenų bazę, joms suteikti identifikaciniai numeriai: KM215767-KM215786. Nustatyti trys nauji haplotipai bei aštuonios mtDNR sekos, kurios Lietuvos stirnų populiacijoje yra unikalios ir neturi atitikmenų „Genų banke" esančioms sekoms. Nustatyta, kad 20% tirtų Lietuvos europinės stirnos (*Capreolus capreolus*) individų mtDNR genome įvykusi sibirinės stirnos (*Capreolus pygargus*) genų introgresija.

Darbo rezultatų aprobacija ir publikacijos. Šio darbo rezultatai buvo pristatyti 13-oje tarptautinių ir nacionalinių konferencijų: 3-ame tarptautiniame medžiotojų simpoziume (Serbija, 2014), tarptautinėje konferencijoje "Žmogaus ir gamtos sauga" (Lietuva, 2014), 6-ame tarptautiniame šiaurės Europos medžiojamųjų gyvūnų dinamikos simpoziume (Karelija, Rusija, 2014), 7-oje ir 8-oje tarptautinėse konferencijose "The Vital Nature Sign" (Lietuva, 2013, 2014), 6-oje nacionalinėje doktorantų mokslinėje konferencijoje (Lietuva, 2013), 6-oje ir 7-oje tarptautinėse konferencijose "Baltijos regiono bioįvairovės tyrimai ir išsaugojimas" (Latvija, 2011, 2013), 5-ame "Baltijos šalių genetikos kongrese" (Lietuva, 2012 m.), 8-oje "Baltijos teriologų konferencijoje" (Lietuva, 2011), 8-oje tarptautinėje konferencijoje "Laukinių gyvūnų elgsena, fiziologija ir genetika" (Vokietija, 2011)

Šios disertacijos rezultatai buvo paskelbti 4 publikacijose recenzuojamose Lietuvos ir užsienio moksliniuose žurnaluose.

IŠVADOS

1. Dėmėtųjų elnių, tauriųjų elnių ir stirnų populiacijų genetinės įvairovės įvertinimui panaudoti 6 mikrosatelitiniai (RT1, RT23, NVHRT16, NVHRT21, NVHRT48, NVHRT73) molekuliniai žymenys. Šie žymenys yra tinkami atskirų elninių žvėrių rūšių identifikacijai.
2. Pirmąjį kartą panaudojus šiuos molekulinius žymenys dėmėtųjų elnių tyrimuose nustatyta, kad Lietuvos dėmėtųjų elnių imtyje vidutinis alelių skaičius lokuse yra 9,20, efektyvių alelių sk. 4,65, o tauriųjų elnių imtyje – 10,50 ir 5,62 atitinkamai. Vidutinio stebimo H_o ir tikėtino heterozigotiškumo H_e reikšmės dėmėtųjų elnių imtyje yra lygios 0,694, o tauriųjų elnių imtyje vid. H_o 0,628 šiek tiek mažesnis už H_e 0,650.
3. Lietuvoje aptvaruose auginamų dėmėtųjų elnių populiacija turi poveikį Lietuvos tauriųjų elnių populiacijai. Laukinių ir aptvaruose auginamų tauriųjų elnių imtyse nustatyta biologinė tarša – hibridiniai gyvūnai.
4. Įvertinus Lietuvos stirnų genetinę įvairovę nustatyta, kad populiacija yra heterogeniška, vidupopuliacinė įvairovė sudaro 96%, tarppopuliacinė įvairovė – 4%: vidutinis alelių skaičius tirtų stirnų populiacijose – 6,54, efektyvių alelių skaičius – 4,14; Lietuvos stirnų populiacijoje nustatyta H_o reikšmė 0,825 yra aukštenė už H_e 0,691. Stirnų mtDNR D-kilpos sekose aptikti 38 variabilūs nukleotidai, 419 nukleotidų buvo konservatyvūs, nustatyti 6 haplotipai, 3 iš jų unikalūs.
5. Stirnų mitochondrinės DNR D-kilpos sekų analizė parodė skirtingą nukleotidų pasiskirstymo dažnį. Gausiausiai T nukleotidų buvo aptikta stirnų sekose iš Kėdainių, Kauno ir Rokiškio rajonų - 32,8%, C nukleotidų kiekis nustatytas tirtuose individuose svyravo nuo 20,4 % iki 21,2 %; didžiausias A nukleotidų kiekis 32,6% aptiktas individų sekose iš Rokiškio rajono, o G nukleotidų 15,3 % – stirnų sekose iš Prienų raj., šiame rajone buvo nustatytas ir vienas iš unikalių haplotipų.
6. Remiantis mtDNR D-kilpos sekų duomenimis Lietuvoje europinės stirnos (*Capreolus capreolus*) populiacijoje aptinkama sibirinės stirnos (*Capreolus pygargus*) genų introgresija. Introgresijos paveikti gyvūnai sudarė 20% tirtų individų. Sibirinės stirnos genų introgresija Europinės stirnos rūšyje galėjo įvykti dar prieš ledynmetį besiformuojant atskiroms rūšims.

Disertacijos tema publikuoti straipsniai

1. **Pūraitė I.**, Paulauskas A., Rosef O., Sruoga A. 2011. Genetic diversity of Norwegian and Lithuanian red deer (*Cervus elaphus*) populations. Biologija. 2011, vol. 57, Nr. 1. ISSN 1392-0146 p. 15-19.

2. **Pūraitė I.**, Paulauskas A., Sruoga A. 2013. Analysis of genetic diversity of roe deer (*Capreolus capreolus* L.) in Lithuania using RAPD and allozyme systems. Biologija 2013, vol. 59, Nr. 1,. ISSN 1392-0146 p. 29-37.

3. **Pūraitė I.**, Paulauskas A., Sruoga A. 2014. Genetic analysis of roe deer (*Capreolus capreolus* L.) using DNA markers. Game Management Issues, priimtas spaudai.

4. **Pūraitė I.**, Paulauskas A., Sruoga A. 2014. Mitochondrial DNA variation in roe deer population from Lithuania. Balkan Journal of Wildlife Research, priimtas spaudai.

Kiti publikuoti straipsniai

1. **Pūraitė I**., Griciuvienė L., Paulauskas A., Sruoga A., Gedminas V., Butkauskas D., 2011. Genetic variability of raccoon dogs and their impacts on the environment in Lithuania. Julius-Kühn-Archiv, 432, 2011.

Konferencijų tezės:

1. **Pūraitė I.**, Paulauskas A., Sruoga A. Mitochondrial DNA variation in roe deer population from Lithuania. The 3rd International Symposium on Hunting, 26-28 September, Zemun-Belgrade, Serbia, 2014.

2. **Pūraitė I**., Paulauskas A., Radzijevskaja J., Rosef O., Sruoga A. Detection of *Bartonella* DNA in wild cervids. The vital nature sign: 8th international scientific conference, 15-17 May, Kaunas, Lithuania, 2014.

3. Gibiežaitė J.M., **Pūraitė I.**, Mažeika V., Paulauskas A. Parasites of sika deer (*Cervus nippon*). The vital nature sign: 8th international scientific conference, 15-17 May, Kaunas, Lithuania, 2014

4. **Pūraitė I.**, Paulauskas A., Sruoga A. Genetic analysis roe deer (*Capreolus capreolus*) in Lithuania. "Dynamics of game animals population in Northern Europe": 6th International Symposium, 31 March – 4 April, Republic of Karelia, Russia, 2014.

5. Gibiežaitė J.M., **Pūraitė I**., Mažeika V., Paulauskas A. *Fasciola hepatica* parasitisms among isolated populations of sika deer. The vital nature sign: 7th international scientific conference, Kaunas, Lithuania, 2013.

6. Juškaitė E., Paulauskas A., Radzijevskaja J., **Pūraitė I**. Molecular detection and characterization of *Anaplasma phagocytophilum* strains in *Ixodidae* ticks and reservoir host. The vital nature sign: 7th international scientific conference, Kaunas, Lithuania, 2013.

7. **Pūraitė I**., Radzijevskaja J., Paulauskas A., Sruoga A. *Babesia* spp. in questing ticks and ticks parasitizing cervids. The vital nature sign: 7th international scientific conference, Kaunas, Lithuania, 2013.

8. **Pūraitė I**., Paulauskas A., Radzijevskaja J., Juškaitė E., Sruoga A. Elninių žvėrių ektoparazitai ir jų pernešami patogenai. Mokslas - sveikatai : VI nacionalinė doktorantų mokslinė konferencija, 2013 m. balandžio 5 d., Kaunas.

9. **Pūraitė I**., Paulauskas A., Sruoga A. Investigation of genetic diversity of roe deer (Capreolus capreolus L.) population in Lithuania. Research and conservation of biological diversity in Baltic region: 7th international conference, 25-27 April, Daugavpils, Latvia, 2013.

10. Radzijevskaja J., Paulauskas A., **Pūraitė I**., Galdikaitė E. Identification of Babesia species in Lithuania. XII international Jena symposium on tick-borne diseases, Weimar, Germany, 2013.

11. **Pūraitė I**., Gibiežaitė J.M., Paulauskas A., Sruoga A., Rosef O. Microsatellite analysis of the genetic status of red deer (*Cervus elaphus*). 5th Baltic congress of genetics, 19-22 October, Kaunas, Lithuania, 2012.

12. **Pūraitė I**., Paulauskas A., Sruoga A. Genetic diversity of roe deer (*Capreolus capreolus*) in the Lithuanian population. 5th Baltic congress of genetics, 19-22 October, Kaunas, Lithuania, 2012.

13. Radzijevskaja J., Paulauskas A., **Pūraitė I**., Juselytė I., Rosef O. Molecular identification of Babesia species in Lithuania. MEEGID XI : 11th international conference on molecular epidemiology and evolutionary genetics of infectious diseases, October 30 - November 2, New Orleans, USA, 2012.

14. **Pūraitė I**., Paulauskas A., Sruoga A. Genetic diversity of roe deer (Capreolus capreolus) based on RAPD and microsatellites analysis. 8th Baltic theriological conference, October 7-9, Palanga, Lithuania, 2011.

15. **Pūraitė I**., Paulauskas A., Rosef O., Sruoga A. Genetic diversity of Norwegian and Lithuanian red deer (Cervus elaphus) populations. Research and conservation of biological diversity in Baltic region: 6th international conference, 28–29 April, Daugavpils, Latvia, 2011.

16. **Pūraitė I**., Paulauskas A., Rosef O., Sruoga A. Genetic diversity of red deer (Cervus elaphus). Contributions to the 8th international conference on behaviour, physiology and genetics of wildlife, 14-17 September, Berlin, Germany, 2011.

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GENETIC EFFECTS OF ALIEN CERVIDS ON THE NATIVE SPECIES, BIOLOGICAL DIVERSITY AND STABILITY OF THE COMMUNITY

Summary of Doctoral Dissertation

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