Genetic Analysis of Red Deer (*Cervus elaphus*) and Sika Deer (*Cervus nippon*) to Evaluate Possible Hybridisation in Lithuania

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Abstract

Genetic diversity of the red deer *Cervus elaphus* (6 individuals from enclosures and 33 individuals living in the wild) and the sika deer *Cervus nippon* (30 individuals from enclosures) species was studied by using tissue samples from the legally hunted animals in Lithuania. Genotyping was based on seven microsatellites loci (STR) of nuclear DNA. The STR loci were variable, with 1-17 alleles and higher than intermediate values of heterozygosity (sika deer: H_o=0.695, H_e=0.694; wild red deer: H_o=0.626, H_e=0.624; red deer from enclosures: H_o=0.639, H_e=0.667). Among 69 individuals phenotypically designated as red deer and sika deer by hunters, based on Bayesian method 31 individuals had *Q*-values of 0.95-1.0 ("pure red deer"), 3 individuals had *Q*-values of 0.75–0.95 (hybrid of the "red type"), 2 individuals had values of $0.25 < Q \leq 0.75$ (intermediate hybrid), 6 individuals had *Q*-values of 0.05–0.25 (hybrid of the "sika type"), and 27 individuals had *Q* values of 0–0.05 ("pure sika").

Keywords: *Cervus nippon*, the sika deer, *Cervus elaphus*, the red deer, genetic variability, hybridisation, microsatellites

Introduction

The sika deer (*Cervus nippon*) are considered to be an invasive species in Europe. They were raised for European zoos and enclosures in China, Japan, Korea, Taiwan, Russia and Vietnam (Wilson and Mittermeier ed.; Mattioli (Family Cervidae (Deer) chapter) 2011). They were released or escaped from enclosures or other structures in the late 19th and early 20th centuries. Separate populations established themselves in different countries, such as the United Kingdom, Austria, the Czech Republic, Denmark, Finland, France, Germany, Poland, Western Russia and Ukraine (Bartoš 2009, Perez-Espona et al. 2009). The main problem that the European free-living sika deer cause significant damage to forests; moreover, s their hybridisation with the local red deer (Harrington 1982, McDevitt et al. 2009, Lowe and Gardiner 1975, Goodman et al. 1999) in captivity (Harrington 1973) and in the wild commonly occurred in the UK, the Czech Republic and New Zealand (Davidson 1973, Lowe and Gardiner 1975, Harrington 1979, Bartoš et al. 1981, Goodman et al. 1999).

For the first time, the sika deer was introduced to Lithuania in 1954 from the Gorno-Altaysk area, the Shabalin deer farm, and released into the Dubrava forest close to Kaunas. This sika deer herd consisted of 6 males and 18 females. In 1954-1992, the sika deer were found in Liepkalnis forests in Jonava region; however, since 1992 the sika deer was not observed and recorded in the wild (Baleišis et al. 2003). Since 1988, the sika deer were raised in captivity in Kaunas region, Didžiosios Lapės. During 1997-1998, almost 750 sika deer were kept in enclosures in Lithuania. Data from the Ministry of Environment indicate that currently, approximately two thousand sika deer are raised in enclosures in Lithuania.

The red deer first appeared in Pleistocene Europe during the Cromerian interglacial period; approximately half a million years ago and fossil records suggest a subsequent distribution that changed with the glacial cycles (Lister 1993). During the first year of the Lithuanian wildlife census in 1934, a total 18 red deer were found in Žagarė forest. It was believed that these red deer came from Latvia. The other version is that the red deer dispersed into the forests of Žagarė from the enclosure of Earl Naryshkin. By the year 1982, the red deer from Žagarė forest had spread to other eight administrative regions (Akmenė, Joniškis, Kelmė, Mažeikiai, Pakruojis, Radviliškis, Šiauliai and Telšiai). Small groups of red deer have been observed in other districts too. In order to facilitate the dispersal of the red deer, in 1969 the red deer were started to be caught and transferred to new locations

(Figure 1) (Baleišis et al. 2003). Currently, the red deer are widely spread throughout most parts of Lithuania (Ministry of Environment of the Republic of Lithuania, 2014).

In the wild, the sika deer cross-breed with the local red deer (Lammertsma et al. 2012), and it is difficult to phenotypically distinguish the hybrids. Hybridisation has been reported amongst the sika deer males and the red deer females. The first-generation offspring (F1) retained the features characteristic of both species. However, even hunters often overlook a deer-hybrid, in which case, confirmation using DNA testing is necessary (Bartoš 2009). Genetic testing helps to identify genetic differences among deer species populations and assess the identity of different populations as well as to identify individual animals. When a population becomes very small and isolated, the genetic problem becomes very important. Inbreeding (mating between related individuals) can cause a decrease in heterozygosity and an increase in the number of deleterious alleles. The reduction of genetic variability can influence a loss of evolutionary flexibility and increase susceptibility to different diseases (Frankham 1995, Higgins and Lynch 2001).

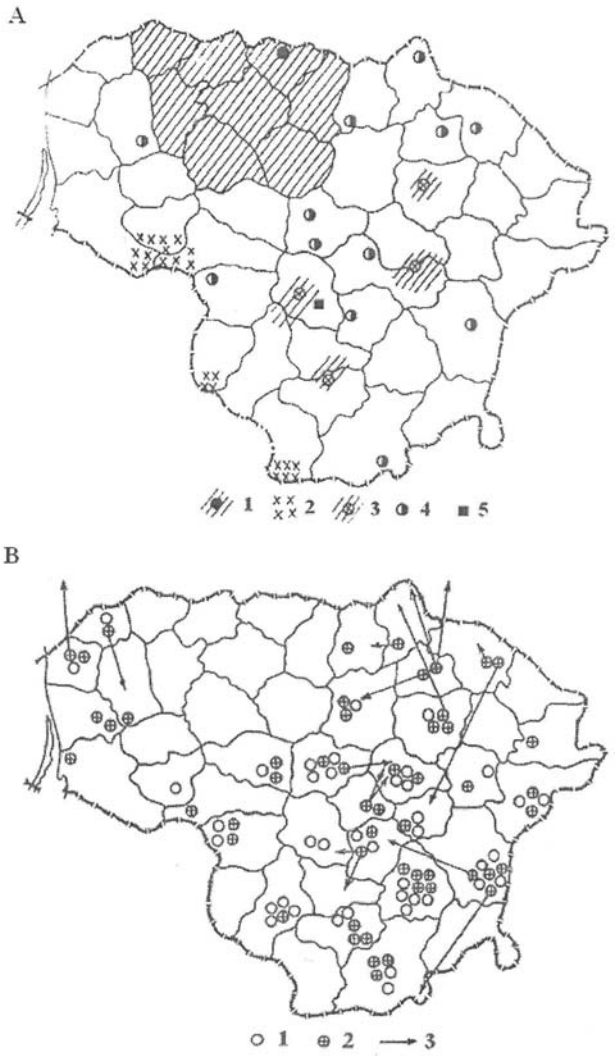

Figure 1. (A) Prevalence of the red deer introduced and the red deer immigrated to Lithuania in 1951-1982. 1 – the primary location of the red deer introduction; 2 – the localization of the red deer herd comprised of immigrated animals; 3 – the release and prevailing locations of the red deer imported from the Voronezh Reserve; 4 – the locations where single red deer or their small groups were observed; 5 – the locations of introduced sika deer.
(B) Red deer transfer to Lithuania in 1969-1987; 1 – the location, where group of 10 individuals were released; 2 – the location, where group of 10 and more individuals were released; 3 – movement directions of the labelled red deer (Baleišis et al. 2003)

The earlier genetic diversity studies of the sika deer and red deer farmed in enclosures, in zoos, and those living in the wild have been carried out employing Random Amplified Polymorphic DNA (RAPD) markers (Tamate et al. 1995), Restriction Fragment Length Polymorphism (RFLP) markers (Goodman et al. 1999), Amplified Fragment Length Polymorphism (AFLP) markers (Zhao et al. 2011), mitochondrial D-loop and cytochrome B gene sequences (Kuznetsova et al. 2012, Rosvold et al. 2012), microsatellite/Simple Sequence Repeat (SSR) markers (Okada and Tamate 2000, Biedrzycka et al. 2012, Mukesh et al. 2013). The genetic diversity of the red deer living in Lithuania has not been exhaustively investigated.

The aim of this study was to analyse the genetic diversity of the sika deer and the red deer living in enclosures and those living in the wild, and to evaluate hybridization of the sika deer and the red deer in Lithuania.

Materials and Methods

Sampling

Thirty-nine red deer samples were collected (6 individuals from the enclosures A and B, 33 wild individuals from the following districts: Kėdainiai, Molėtai, Panevėžys and Ukmergė). Thirty sika deer samples were collected in 2012 and 2013 from three enclosures in different localities in Lithuania.

Molecular analysis

DNA was extracted from the muscle tissue and the spleen by using the Genomic DNA Purification Kit K#0512 ("Thermo Fisher Scientific Baltics", Lithuania) according to the manufacturer's instructions. The DNA concentration and purity of the samples was measured by using the Nano Drop 2000 system.

The microsatellite analysis was used to compare the genetic profiles of individual sika and red deer. Genotyping was based on seven microsatellites: RT1, RT23 (Wilson et al. 1997); NVHRT16, NVHRT21, NVHRT48, NVHRT73 (Roed and Midthjell 1998); BM888 (Bishop et

al. 1994). The total reaction volume of 20 µl contained 1X Taq buffer with 50 mM KCl, 0.2 µM of each primer, 0.2 mM dNTPs, 2 mM $MgCl_2$, 0.2-unit (U) Taq DNA polymerase, 50 ng of the tested DNA and water. PCR mixture without DNA was used as a negative control for each reaction. The DNA amplification was performed to one cycle at 95 °C for 10 minutes, this was followed by 30 cycles at 95 °C for 30 seconds, at 54 °C or 52 °C (depending on the primer annealing temperature) for one minute and at 72 °C for 90 seconds, the final extension was held at 72 °C for 10 minutes. SSR fragments were visualised in 2% agarose and 8% polyacrylamide gels to confirm the amplification. The resulting fragments were visualised under UV light by using GelDoc-It 310 (United Kingdom). The PCR products were separated by capillary electrophoresis using the ABI3130 Genetic Analyser (Applied Biosystems Ltd., Germany). Prior to capillary electrophoresis, the PCR products were diluted 10 times with deionized formamide, and the DNA length standard GeneScan-500 LIZ was added to the solution.

Statistical analysis

Electrophoregram peak signal strength was measured, which was followed by genotyping with the Gene Mapper 4.0 programme (Applied Biosystems, Ltd., Germany). Evidence for null alleles, stutter-errors and large allele drop-out were checked using Micro-Checker v.2.2.3 with 95% confidence interval (van Oosterhout et al. 2004). After the allele sizes had been identified, the data was entered and processed with GenAlEx6.501 (Peakall and Smouse, 2012), individuals were classified by the principal coordinate analysis (PCoA). In order to obtain the measures of gene diversity and allelic richness values per locus were calculated with F_{STAT} (Goudet 1995). Coefficient F_{IS} was calculated according to Weir and Cockerham (W&C) and for the Hardy-Weinberg equilibrium estimation we followed the probability test approach using the program GENEPOP, version 4.2 (Rousset 2008).

The analysis of the population and individual admixture was performed and hybrid scores (Q) was assigned with the Bayesian clustering algorithm presented in STRUCTURE 2.3.4 (Falush et al. 2003, Hubisz et al. 2009). This model proposes that each K population is described by a set of allele frequencies at each locus. In STRUCTURE, for each different number of genetic clusters (K from 1 to 7), 500000 burn-in cycles and 500000 Markov Chain Monte Carlo (MCMC) iterations were run 10 times. The average posterior probability between the runs and the standard error was calculated for each K value. The main genetic structure was interpreted from ΔK, which is negatively related to the often-increasing variance between the runs and higher posterior probabilities of higher K values and which can be used to identify major breakpoints (Evanno et al. 2005). The probable number of clusters was determined by the ΔK criterion with the STRUCTURE HARVESTER, Web version 0.6.93 software (Earl and Holdt 2012). The hybrid score is the estimated proportion of genes a putative hybrid has inherited from each parental species, calculated using the six microsatellite markers. Each of the 69 individuals was ascribed to either the sika deer cluster (when membership probability is $0 < Q \leq 0.05$), red deer cluster ($0.95 < Q \leq 1$), interspecific hybrid of red type ($0.75 < Q \leq 0.95$), sika type ($0.05 < Q \leq 0.25$), or hybrids of equal ancestry ($0.25 < Q \leq 0.75$) that are the probable 1st-generation hybrids.

Results

Of the seven microsatellite polymorphisms tested, 6 could be amplified in both species. Only the microsatellites BM888 gave no product in the sika deer species. All microsatellite loci were polymorphic, only one RT23 were monomorphic in red deer E population. Null alleles were determined in three loci (NVHRT21, in the sika deer and the red deer W populations; RT23 and NVHRT16, in the red deer W population). No evidence for stutter-errors and large allele drop-out were detected. The observed heterozygosity (H_o) in the sika deer species in 4 (NVHRT48, NVHRT73, RT23, NVHRT16) out of 6 loci was higher than the expected heterozygosity (H_e) (Table 1).

Table 1. Comparison of the observed (H_o) and expected (H_e) heterozygosity in sika deer and red deer in the wild (W) and kept in enclosures (E)

Species		NVHRT48	NVHRT73	NVHRT21	RT1	RT23	NVHRT16	Average
Sika deer	*N*	30	30	30	30	30	30	
	Na	6	17	12	13	3	4	9.167
	Ae	4.306	9.677	4.196	5.844	1.484	2.406	4.652
	Ho	0.967	0.933	0.533	0.767	0.367	0.600	0.695
	He	0.768	0.897	0.762	0.829	0.326	0.584	0.694
Red deer W	*N*	33	33	33	33	33	33	
	Na	7	11	17	12	3	7	10.000
	Ae	5.762	4.961	9.723	7.459	1.063	1.427	5.066
	Ho	1.000	0.879	0.788	0.879	0.030	0.182	0.626
	He	0.826	0.798	0.897	0.866	0.059	0.299	0.624
Red deer E	*N*	6	6	6	6	6	6	
	Na	7	9	9	9	1	4	7.000
	Ae	5.143	7.200	6.000	7.200	1.000	2.769	4.885
	Ho	1.000	1.000	0.667	0.833	0.000	0.333	0.639
	He	0.806	0.861	0.833	0.861	0.000	0.639	0.667

N is sample size, N_a is number of alleles at the locus, A_e is effective alleles, H_o is observed heterozygosity, H_e is expected heterozygosity (W is wild red deer, E is red deer kept in enclosures)

The observed heterozygosity (H_o) in the red deer living in wild in 3 (NVHRT48, NVHRT73 and RT1) out of 6 loci was found to be higher than the expected heterozygosity (H_e), and of the red deer living in the enclosures in 2 loci (NVHRT48, NVHRT73) out of 6 loci. An average richness of alleles per locus in the sika deer was 5.004, in the red deer living in enclosures – 5.005 and in red deer living in the wild – 5.659 (Table 2). Gene diversity per locus and population were calculated using F_{STAT}. The lowest values of gene diversity were determined in RT23 locus in all populations (from 0 to 0.33), the highest values in NVHRT73 loci (from 0.81 to 0.93). F_{is} estimation of exact P-Values by the Markov chain method was calculated. The lowest values of F_{is} were calculated in NVHRT48 locus in all populations: -0.2432 in sika deer, -0.1538 in red deer from enclosures and -0.1952 in wild red deer population (Table 3).

Table 2. Allelic Richness per locus and population

Locus	Sika deer	Red deer E	Red deer W
NVHRT48	4.826	5.621	5.956
NVHRT73	7.995	5.701	7.558
NVHRT21	5.779	7.885	7.881
RT1	6.327	6.686	7.308
RT23	2.134	1.364	1.908
NVHRT16	2.963	2.772	3.345
Average	5.004	5.005	5.659

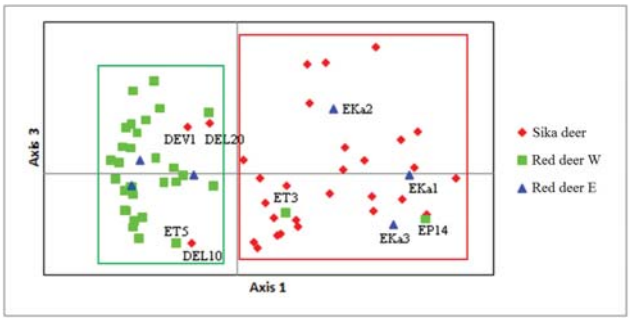

Figure 2. The results of genetic similarities in the sika and red deer individuals according to the principal coordination analysis (*W* is wild red deer, *E* is red deer in enclosures)

Inbreeding coefficient (Fis) and probability of significant deviation from the Hardy-Weinberg equilibrium (P) are presented in Table 3. F_{is} ranged between -0.229 and 0.205 (mean = - 0.025, *SE* = 0.093).

The STRUCTURE 2.3.4 analysis was based on 6 microsatellite loci, with parameters *K,* was from 1 to 7 clusters. The highest hierarchical level of STRUCTURE, as indicated by *"K*, was *K*=2. Seven bars represent the following hybrids: the sika deer - DEL17, DEV1; the red deer from wild - EP14, ET3; the red deer from enclosures - EKa1, EKa2, EKa3. For each individual, the proportion of ancestry from each cluster (*Q*) was obtained. Among 69 individuals phenotypically designated as the red deer and the sika deer by hunters, 31 individuals had *Q*-values of 0.95–1.0 ("pure red deer"), 3 individuals had *Q*-values of 0.75–0.95 (hybrid of the "red type"), 2 individuals had values of $0.25 < Q \leq 0.75$ (intermediate hybrid), 6 individuals had *Q*-values of 0.05–0.25 (hybrid of the "sika type"), and 27 individuals had *Q* values of 0–0.05 ("pure sika").

Table 3. Hardy-Weinberg test. F_{is} estimation of exact *P*-Values by the Markov chain method

Locus	Sika deer			Red deer E			Red deer W		
	Fis	*P*	*S.E.*	*Fis*	*P*	*S.E.*	*Fis*	*P*	*S.E.*
NVHRT48	-0.2432	0.0000	0.0000	-0.1538	0.7556	0.0159	-0.1952	0.0000	0.0000
NVHRT73	-0.0240	0.0130	0.0047	-0.0714	0.3779	0.0289	-0.0854	0.0000	0.0000
NVHRT21	0.3151	0.0000	0.0000	0.2857	0.0391	0.0152	0.1369	0.0030	0.0023
RT1	0.0919	0.0653	0.0140	0.1228	0.3874	0.0289	0.0005	0.1349	0.0179
RT23	-0.1076	0.1352	0.0044	-	-	-	0.5000	0.0154	0.0014
NVHRT16	-0.0097	0.4360	0.0075	0.5455	0.0758	0.0051	0.4056	0.0092	0.0054

F_{is} – inbreeding coefficient; *P* – probability of significant deviation from Hardy-Weinberg equilibrium.

The principal coordinate analysis (PCoA) (Figure 2) was used to examine the red deer and the sika deer species distribution in two-dimensional space. Three PCoA axes describe the major part of genetic diversity of the analysed samples: the first axis is 14.29%, the second axis (not specified in the picture) is 22.28% and the third axis is 29.86%. The PCoA analysis showed that the sika deer samples differed from the red deer samples: however, five red deer individuals (three individuals from enclosures: Eka1, Eka2, Eka3 (these samples were obtained from the A enclosure, where both the red deer and the sika deer animals were reared); two from the wild population: EP14, ET3 (Panevėžys and Ukmergė regions) were identified, which mix with the individuals of the sika deer. Although according to the phenotype, these animals were attributed to the red deer; however, according to a fixed genotype, they are closer to the sika deer. Three sika deer individuals (DEV1, DEL20, DEL10) mixed in a 2D space with the red deer individuals. Thus, it can be concluded that all these animals are the hybrids of the red deer and the sika deer.

Discussion

Our analysis of 7 unlinked loci in a sample of 69 deer reveals that whilst most of the sample set can be clearly divided into the red deer or the sika deer, and there was an appreciable proportion of hybrids. All the loci used in this study were used previously by Poetsch et al. 2001 in the study of genetic diversity of red deer, roe deer, fallow deer and reindeer. These authors showed that observed

heterozygosity (H_o) in the red deer population using the same 6 loci were from 0.13 (RT23) to 0.70 (NVHRT73). In our study the lowest observed heterozigosity was observed in the same RT23 locus (values: H_o=0.37 in sika deer, H_o=0.00 in red deer from enclosures and H_o=0.03 in wild red deer). The highest meanings of observed heterozigosity were determined in NVHRT48 and NVHRT73 loci as well as Poetsch et al. 2001.

Also, these loci used in this study had previously been used by other authors: Wilson et al. (1997), Roed and Midthjell (1998), Soriguer and Rico (2008), Haanes et al. (2011). These authors showed that loci were polymorphic in *Cervus elaphus, Cervus elaphus hispanicus* and *Dama dama* species. The results of our studies as well as those carried out by McDevitt et al. (2009), Senn and Pemberton (2009), Biedrzycka et al. (2012) reflect higher number of alleles at the locus than those documented in Poetsch et al. (2001) and Haanes et al. (2005, 2008) (Table 4). In the populations where hybrids had been identified a higher number of alleles was determined, this could be due to hybridisation between the red deer and the sika deer. It is important to mention that the gene pool of various deer species is affected or can be influenced by many factors, including reintroduction, transfer of animals, breeding and keeping them in enclosures, environmental fragmentation and hunting (Wang and Schreiber 2001, Hartl et al. 2003, Kuehn et al. 2003; Coulon et al. 2004). Hence, lower levels of genetic variation observed in the Scandinavian populations suggest that genetic variation has been lost since the postglacial colonisation. This could be the result of either a strong genetic drift during founder events or one or more subsequent bottlenecks (Haanes et al. 2011).

The sika deer are assumed to be one of the most invasive introduced species in Europe (DAISIE 2009). Therefore, it has been argued that immediate actions should be taken to impede the quick expansion of this species (Nentwig et al. 2010). Hybridisation within the invasive species is capable of altering the phenotype and, consequently, ecology of a native counterpart.

Increased phenotypic resemblance between the two species very often leads to further hybridisation. Ecological consequences of this phenomenon are not easy to predict. If the native and invader deer have distinct phenotypes, their hybrids will have a mixture of genes derived from the two parental populations. The presence of intervening hybrid phenotypes facilitates a further gene flow between the two populations, thus creating a positive feedback that finally results in complete blending of the two populations (Pinto et al. 2005). Hybridisation of

Table 4. Comparison of genetic indicators between the red deer and the sika deer populations in different countries (H_o is observed heterozygosity, H_e is expected heterozygosity)

Locality	Red deer (*Cervus elaphus*) Number of loci	*Number of alleles*	*Average N_a*	H_o	H_e	References
Lithuania	6	63	10.50	0.63	0.65	**This study**
Ireland	9	122	13.55*	0.61	0.72	McDevitt et al. 2009
Poland, Kaliningrad, Lithuania	14	178	12.71*	-	0.79	Biedrzycka et al. 2012
Belgium, Germany, France Luxembourg	13	120	9.23	0.69	0.74	Frantz et al. 2006
Scotland	22	165	7.50*	0.52	0.56	Senn and Pemberton 2009
Tunisia	13	73	5.62	0.46	0.78	Hajji et al. 2007
Southern Spain	7	29	5.80	0.65	0.80	
Val di Susa, Italy	7	40	8.00	0.75	0.85	
Tarvis, Italy	7	35	7.00	0.76	0.81	Zachos et al. 2003
Sardinia	7	16	3.20	0.36	0.52	
Bulgaria	7	30	6.00	0.74	0.85	
Sweden	14	67	4.10	-	-	
Norway	14	63	4.10	-	-	
Lithuania	14	99	6.00	-	-	Haanes et al. 2011
Scotland	14	120	6.90	-	-	
Hungary	14	121	7.00	-	-	
Germany	11	50	4.55	0.50	-	Poetsch et al. 2001
Norway	25	105	4.20	0.48	-	Haanes et al. 2005
Norway	14	74	4.00	-	-	Haanes et al. 2008
Canada, Alaska (enclosures)	10	-	4.10	0.55	0.55	
North-western America, Asia	10	-	3.90	0.48	0.54	Cronin et al. 2009
Sika deer (*Cervus nippon*)						
Lithuania	6	55	9.17	0.69	0.69	**This study**
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
Vietnam	9	61	5.70	-	0.60	Thevenon et al. 2004
Japan (local pop.)	13	-	-	0.60	0.58	Okada et al. 2005
Japan (non-local pop.)	13	-	-	0.58	0.59	
Scotland	22	67	3.04*	0.14	0.15	Senn and Pemberton 2009

* Hybrids were identified in these studies

these two species could alter their nutritional ecology because of changes in the body size and dentition (Bell 1971, Geist 1974, Jarman 1974, Gordon and Illius 1988), which, in turn, might alter the competitive interactions between the two species. Originally from Japan, the sika deer are strongly genetically differentiated from the native red deer with which they hybridise (Goodman et al. 1999). The red deer are larger than sika, typically around 30 cm taller at the shoulder and whilst the red stags can grow antlers of 12 points or more, sika antlers rarely exceed eight points (Whitehead 1964, Harrington 1973). Although there are obvious interspecific differences between these two species, however, for hunters it is difficult to identify the hybrids of the red deer and the sika deer. The differences are most evident among adult males, but females and juveniles are most commonly hunted.

Goodman et al. (1999) described the hybridization process in Scottish populations as a bimodal hybrid zone, with deer falling into 2 distinct classes. These classes are red deer–like and sika-like. The authors concluded that occasional hybridization was followed by introgression through backcrossing into the 2-parental species, resulting in red deer and sika populations containing a large number of individuals with a small number of introgressed alleles. In another study, an overall 6.9% mixed ancestry hybrids including sika deer and red deer were estimated by Senn and Pemberton (2009). A comparable study in Ireland (McDevitt et al. 2009) also revealed similar levels of hybridization, with 9.4% of hybrids among red deer-like individuals and 10.6% among sika-like ones.

Twenty samples of the red deer from Lithuania were compared with those of Norwegian, Swedish, Hungarian and Scottish red deer populations (Haanes et al. 2011). Hybridisation between native and introduced species of the red deer in Eastern Europe was investigated (Biedrzycka et al. 2012), including 39 samples of the red deer from Lithuania, however, it is not known where these samples were collected (from the animals reared in enclosures or those living in freedom). During the investigation, only one red deer from Lithuania was identified as a hybrid using microsatellite and mitochondrial markers. In our study we determined 2 “intermediate hybrid” class animals. Among 30 individuals assigned phenotypically to the sika deer, 4 appeared to be “sika type” hybrids, 2 were intermediate hybrids. Among 39 individuals assigned to red deer, 3 appeared to be “pure sika deer”, 2 “sika type” hybrids and 3 “red type” hybrids. All three “pure red deer” individuals were sampled in the same A enclosure. One “sika type” hybrid and two “pure sika deer” individuals were sampled in the B enclosure. Among 33 wild red deer individuals 1 “pure sika” and 1 “sika type” hybrid were identified. This study presents hybridisation between the red deer and the sika deer living in enclosures and in the wild in Lithuania.

It is a problem in Europe in terms of the areas, in which free-living red deer cross-breed with the sika deer. Therefore, this is a debatable issue, which attracts a lot of attention from scientists (Goodman et al. 1999, Diaz et al. 2006, Bartoš 2009, McDevitt et al. 2009, Senn and Pemberton 2009, Senn et al. 2010, Zachos and Hartl 2011).

Conclusions

In this study we identified hybridisation between the red deer and the sika deer living in enclosures in Lithuania where two species were kept together at the beginning of farming.

Although since 1992 sika deer were not recorded in the wild in Lithuania but our research has shown that the hybrids are detected in the wild red deer population. There are two possibilities of hybrids: the first one - the offspring of a previously introduced sika deer individuals to the wild, and the second one - sika deer escaped from enclosures mate with wild red deer individuals.

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