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THE ROE DEER CLASSIFICATION TO ECOTYPES IN MOSAIC LANDSCAPE OF LITHUANIA

The roe deer is the most numerous large wild herbivore in Europe and Lithuania. In Lithuania, roe deer population is continuously increasing and consists of more than 115000 individuals. The diverse landscape of the country causes differentiation of roe deer into two ecotypes: regular forest ecotype and field ecotype – as ecological forms adapted to specific environmental conditions. Classification of Roe deer into ecotypes was started in 1960s, unfortunately based on low scientific evidence. We have studied roe deer variation among ecotypes in morphological traits and DNA markers: skull traits, roebuck antler trophic value comparison and nuclear microsatellite polymorphism at five loci. Totally, 603 roe deer skulls and 228 roebuck antlers, also 79 individuals for the DNA study were sampled at different parts of the country. The ANOVA ad AMOVA analyses were carried for each age class and sex separately. The results showed that only few morphological skull traits at particular age classes showed significant differences among ecotypes. However, there were significant differences among ecotypes in the roebuck anther trophic value. We have not found significant genetic differences among ecotypes based on AMOVA. These findings suggest that the ecotypes are closely related owing to evolutionary recent separation from the common ancestor and geneflow among the ecotypes and have not reached the level of subspecies.

Keywords: ecotype, skull traits, polymorphism, microsatellites, geneflow.

INTRODUCTION

Capreolus Gray, 1821 is one of the world‘s most widespread Artiodactyls genera. *Capreolus* species are found in 50 countries including North America where they were introduced (Danilkin, 1999). At present, two roe deer species and four basic subspecies are acknowledged (Sheremetyeva and Sheremetyev 2007). The independence of *Capreolus capreolus Linnaeus*, 1758 and *Capreolus pygargus Pallas*, 1771 was supported using molecular markers of

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DNA-fingerprinting, DNA taxoprint analysis, random amplification pf polymorphic DNA polymerase chain reaction, and sequencing of the control region of the mitochondrial genome (Sheremetyeva and Sheremetyev, 2008).

The European roe deer *Capreolus capreolus* is a typical faunal element of the Holocene. It was already present in Europe at least 600 000 years ago and it has been known from both glacial and interglacial phases since then. The combined pattern of genetic data and fossil records of European roe deer suggests several regions in Iberian peninsula, southern France, Italy and Balkans as well as in the Carpathians and/or eastern Europe as glacial refugia. In further suggests that *C. capreolus* might have recolonized most parts of central-northern Europe out of one or more eastern European (not Balkan) and/or Carpathian refugia (Sommer et al., 2009).

The roe deer is the most numerous large wild herbivore in Europe, and it is one of the most numerous big game species in Lithuania. According to official census report by The Environmental Ministry of Lithuania (2014), total roe deer population consist from more than 110000 individuals in all the country (www.am.lt…). Roe deer preferable habitat is the mosaic of forests and fields, because it can find a foraging ground and shelter tree (Brzuski et al., 1998). In Lithuania, as in many European countries, two roe ecotypes can be distinguished: "forest" roe deer and "field" roe deer. These ecotypes vary in many important traits. The field roe deer is recognized as the ecological form of the adaptation of a species for living in an open agricultural landscape (Kałuzinski, 1982). The landscape in Lithuania varies a lot in its mosaic prevalence, as well as forest land and open areas are spread differently. Various environment conditions formed separate roe deer populations in the country. After case study in Poland the Brzuski et al. (1997), concluded that the weight and body morphological measurements of roe deer shows a considerable variation under various environmental conditions. Roe deer are flexible species and easily adapt to various site conditions, so they occupy many different habitats, from large forest complexes, through small in-field tree stands and shrubs, to treeless grounds and field monocultures.

The aim of our study was to define two roe deer ecotypes in morphometric and genetic aspects.

MATERIAL AND METHODS

We have studied roe deer variation among ecotypes in morphological traits, DNA markers and roebuck antler morphometric traits.

For the morphometric variation in roe deer skull, 13 different traits of 603 roe deer skulls were measured (154 females and 449 males). The measured skulls were assigned to ecotypes: 365 to forest and 238 to field ecotypes, based on the culling location. The populations were assigned into the ecotypes based on their living environment. The forest cover percentage in a living environment (range) of each roe deer population, was evaluated by a GIS program using the State Forest Cadastre data from 2011. The populations of roe deer were assigned

into the field ecotype if the forest cover in its living area were less than 20 %. Both male and female skulls were divided into age classes: I age class (juvenile individuals from 6 up to 18 months), II age class (sub-adult individuals from 24 up to 36 months), III age class (individuals older than 36 months). 13 different skull morphology traits used for analysis: Total length of the skull; Condylobasal length; Total breadth; Inter-orbital distance; Zigomatic breadth; Nasal length; Neuro-cranium breadth; Maxilla tooth raw length; Mandible tooth raw length; Diastema length; Mandible length; Mastoidic width; The height of second molar tooth.

For genetic variation analysis the culled roe deer muscle tissue samples from 79 individuals were sampled in different regions in Lithuania during the 2010-2012 hunting seasons and stored in the freezer at - 70°C. The sex ratio was more or less equal with 43 females and 36 males, 43 individuals of field ecotype and 36 individuals of forest ecotypes were sampled. Five nuclear SSR loci were used (for rain deer Roed and Midthjell1998, for roe deer by Postma et al, 2001, Lorenzini et al. 2003): NVHRT30, NVHRT71, NVHRT48, NVHRT16 and NVHRT24 (further abbreviated as N30, N71, N48, N16 and N24). We used two multiplex reactions for the PCR amplification – NVHRT30 and NVHRT71 as multiplex No 1, and NVHRT48, NVHRT16 and NHRT24 as multiplex No 2. The capillary electrophoresis was carried out with a genetic analyzer (Applied Biosystems), allele sizing was performed using the GeneMapper program (Applied Biosystems version 4.0).

For roe buck antler comparative analysis we have measured roebuck antlers of forest and field ecotype populations. Totally, we have measured 292 individuals: 156 individuals were assigned as field ecotype and 136 individuals as forest ecotype. We have measured: length of right and left buck, diameter and circumstance of bucks, diameter and circumstance of roses, weight of antlers (together with a skull weight) and span between bucks in their biggest distance. We have used ANOVA statistical analysis to compare roe deer antlers among regions, ecotype, subpopulations and age classes.

RESULTS AND DISCUSSION

From the practical field observations during the hunting, was believed in the existence of two roe deer ecotypes, differing from each other in their morphology and behaviour. Our studies of roe deer skulls from different subpopulations of field and forest ecotype indicated very small differentiation between ecotypes. In pooled gender, comparative analysis, significant difference between ecotypes was found in diastema length at 3rd age class, mastoidic width at 1st and 3rd age classes, maxilla tooth raw length and zigomatic breadth at 2nd age class (fig 1), but not in the other traits or age classes.

Body and cranium size is the most important instrument to distinguish two separate roe deer ecotypes. Field ecotype roe deer are adopted to live in open landscape and are larger and heavier then forest ecotype roe deer (Narauskaitė & Pėtelis, 2010). Body weight is changing together with the age of an animal. In

roe deer, the highest growth rate observed between 1 - 3 year of their life, when roebucks increase their body weight by 4,2 kg (forest roe deer), and 2,5 kg (field roe deer), and does 4,5 and 1,2 kg respectively (Fruziński et al., 1982).

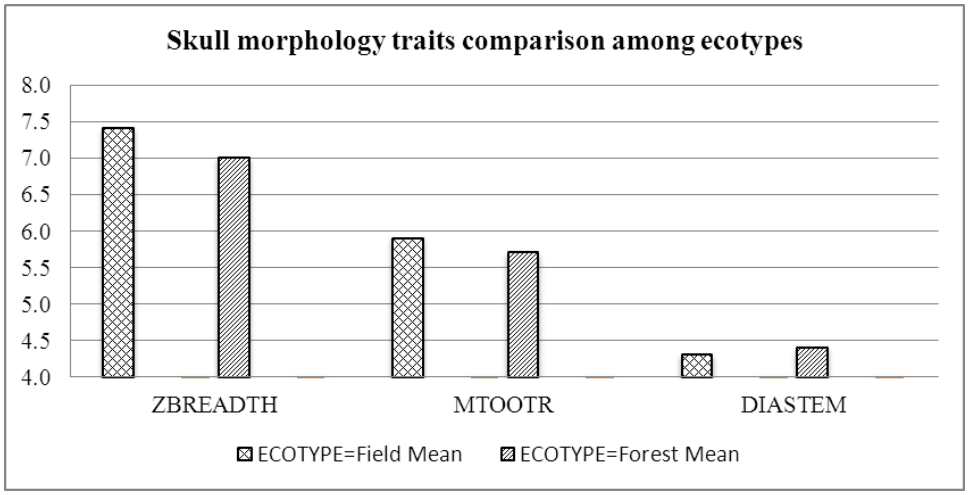

Fig. 1. Roe deer skull morphometric traits (zygomatic breadth, maxilla tooth raw length and diastema length) comparison among ecotypes.

The skull is the most complex bone structure in the body and is high variable in shape, reflecting variable in genetic origin. Performed craniometrical data analysis in Southwest Lithuania has confirmed that cranium is growing through all life span of roe deer (Petelis and Brazaitis, 2003). These authors had compared field and forest ecotype roe deer in their morphometric traits in southwest Lithuania. They have found that field ecotype roe deer are significantly larger that forest ecotype roe deer in the same region. They found significant differences in the total cranial length, length of nasals and length of the teeth row in maxilla. Other measures were not significantly different. Narauskaite et al. (2011) has found that roe deer living in Silute region, is slightly smaller in comparison to field and forest ecotype roe deer from southwest Lithuania. It was observed that there are tendentious differences between male and female craniums in Silute region.

According to Kałuziński (1978) a comparison of body measurements of roe deer in relation to ecotypes is reliable only when the data come from not too distant areas, permanently inhabited by typical populations of both forest and field roe deer. The research of Janiszewski and Kolasa (2007) shows that field roebucks, compared with forest animals, are characterized by longer carcasses and achieve higher values as regards height at withers and sacrum. The index of massiveness calculated for them also confirms their bigger size.

The formation of field ecotype of roe deer started in the south-western part of Lithuania during 1965 – 1967, when the peak of roe deer population abundance was the highest in the country as well as in the south-western

Lithuania (Pėtelis, 1998). Baleišis et al. (1989) found that the field ecotype of roe deer formed in rich soils on open landscape, where its abundance increased drastically under pressure to find other habitats than forest by other cervidae animals (red deer *Cervus elaphus* and fallow deer *Dama dama*).

In spite of the body size difference between the field and forest ecotypes (Pėtelis and Brazaitis, 2003), our study indicates no genetic differentiation between field and forest ecotype. Field ecotype possessed slightly more private alleles, higher number of alleles and higher observed heterozigosity. Other parameters including the expected heterozigosity were rather uniform (table 1). Apparently, the morphological differences reflect high phenotypic plasticity in roe deer (Kurt, 1991). This low differentiation between the ecotypes may, primarily, be explained by strong gene-flow in shared habitats in the absence of mating barriers (Kurt et al. 1993). The sampling error may also have an effect, where an individual culled in the field could come from the forest habitat. However, we believe the within-ecotype sample size of 38 individuals is high enough considering the sessile habitats of roe deer in Lithuania. Slightly lower genetic diversity of the forest ecotype in our study suggests a tendency for a stricter herd structure, stable male-female relationships in the forest ecotype with fewer migrants, causing relatively more frequent mating among relatives and higher inbreeding than for the field ecotype (Strandgaard 1972; Ellenberg 1978; Kurt et al., 1993). Our finding that the main differences in the genetic diversity between the ecotypes were at the cost low diversity of the males in the field ecotype supports the migration related cause of the low genetic diversity in the forest ecotype.

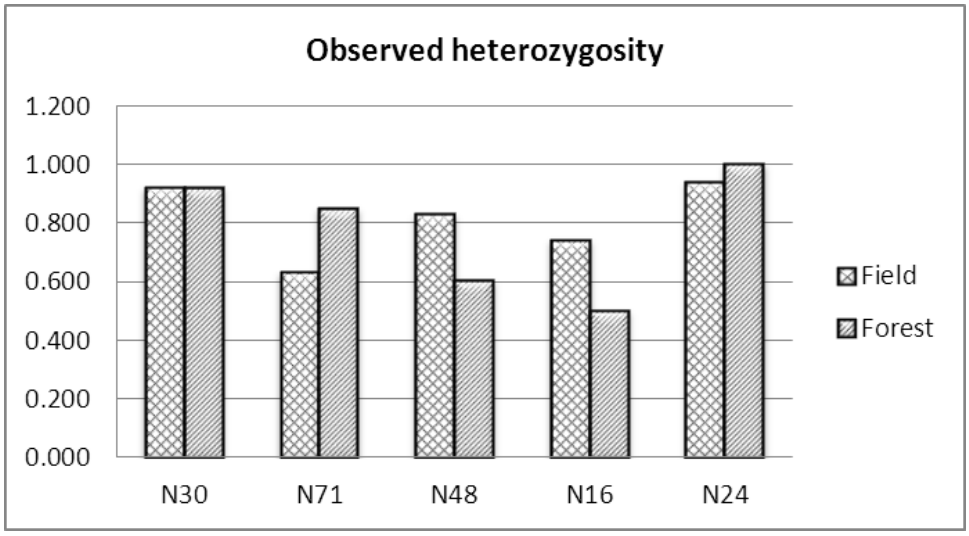

Fig. 2 Observed heterozygosity for by ecotype and at five locus.

Table 1. Comparison of genetic diversity parameters between the ecotypes.

Population		N	Na	Ne	I	Ho	He	UHe	F
Field	**Mean**	36.000	6.800	3.162	1.275	0.813	0.650	0.660	-0.290
	SE	0.837	1.428	0.529	0.183	0.058	0.051	0.052	0.160
Forest	**Mean**	38.400	5.800	3.100	1.246	0.775	0.655	0.664	-0.244
	SE	0.400	1.158	0.364	0.160	0.095	0.047	0.048	0.229

In a pooled sample data analysis of roebuck antler comparison among ecotypes shows significant differences mainly in length of antlers ($p = 0.0037$; $p = 0.0002$, right and left antler, respectively) diameter of pedicle ($p = 0.0449$), diameter of roses ($p = 0.0700$), weight ($p = 0.0572$) and the span between antlers ($p = 0.0273$). However, the circumstance of pedicle and circumstance of roses were not significantly different. In comparison of mean values, the length of bucks is higher in forest ecotype in all age classes.

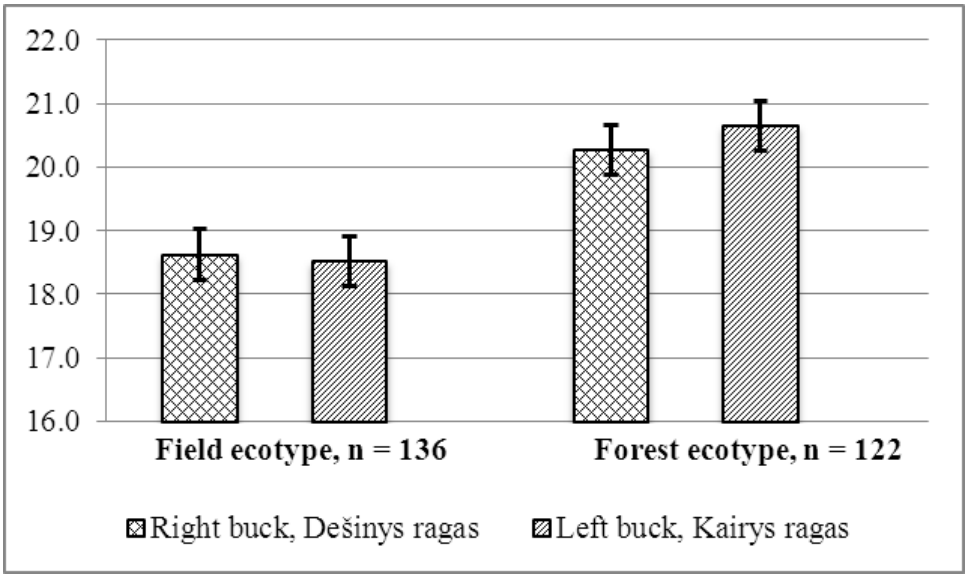

Fig. 3. The comparison of the length of right and left buck among ecotypes, (cm)

CONCLUSIONS

In conclusion, only some skull morphometric traits in certain age classes showed significance between forest and field roe deer ecotypes. Adult males tend to vary more among ecotypes, then younger males or females. The reason could be a higher male home range and migration distance in reaching for suitable living and reproducing areas.

Our study indicates no genetic differentiation between field and forest ecotype, although field ecotype possessed slightly more private alleles, higher

number of alleles and higher observed heterozigosity. Other parameters including the expected heterozigosity were rather uniform.

In roebuck antler comparison analysis among ecotypes, the significant differences were found in many measured traits, indicating the roe deer morphological adaptability to environmental conditions.

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