Potravinarstvo, vol. 8, 2014, no. 1, p. 15-19
doi:10.5219/320
Received: 18 December 2013. Accepted: 19 December 2013.
Available online: 24 April 2014 at www.potravinarstvo.com

ISSN 1337-0960 (online)

GENETIC DIVERSITY AND RELATEDNESS AMONG SEVEN RED DEER (*CERVUS ELAPHUS*) POPULATIONS

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ABSTRACT

Deer (*Cervidae*) recently belongs to the most important species. The aim of presenting study was evaluation of genetic diversity and relationship within and among seven red deer populations from different origins - Czech Republic, Hungary, hybrids Hungary x New Zealand, Lithuania, New Zealand, Poland and Slovak Republic. This study was conducted to determine the levels of genetic variability and relationships among deer populations from a total of 637 animals originating from seven countries Czech Republic (50), Hungary (35), Hungary x New Zealand hybrids (67), Lithuania (26), New Zealand (82), Poland (347) and Slovak Republic (30). We used the hair bulbs as a source of DNA. In total, 213 alleles were observed from the 10 loci surveyed. The number of alleles per locus ranged from 11 (IOBT965) to 35 (T156, RT13). Genetic diversity and relatedness among red deer populations has been performed on a total of 637 animals. A panel of 10 microsatellite markers used in deer were optimized. On the basis of this panel of microsatellites we were investigated genetic variability and relationships by using statistical and graphical programmes. We evaluated how close populations are to each other and their genetic admixture. Molecular genetic data combined with evaluation in statistical programmes could lead to a complex view of populations.

Keywords: genetic variability; relatedness; microsatellite loci; red deer (*Cervus Elaphus*)

INTRODUCTION

This study was conducted to determine the levels of genetic variability and relationships among seven red deer populations.

Deer (*Cervidae*) belong to the most important species, which are used as a farm animal as well as hunting wild animal. For long-term conservation and development purposes, it therefore appears compulsory to manage wildlife to maintain both species survival and within species genetic diversity **(Franklin, 1980)**.

Since the 1980s genetic markers have been used, based on examination of DNA variability, for identification of the populations and individuals within it. In recent years, the importance of microsatellites for population genetics, linkage mapping, and parentage studies has increased significantly **(Poetsch et al., 2001)**. To date, about 200 microsatellite loci have been found in cervids by transferring microsatellite PCR primers derived in bovine and ovine to cervids, as well as a few loci derived directly from the deer microsatellite library **(Xu Yan-chun et al., 2001)**. The usefulness of microsatellite markers for the estimation of genetic diversity and relationships among individual populations has been documented in numerous studies **(Buchanan et al., 1994; Saitbekova et al., 1999; Schmid et al., 1999, Xu Yan-chun et al. 2001)**.

Two classes of markers are used: the first, termed type I loci, are widely conserved across species but are frequently invariable within species, the second, termed type II loci, are usually polymorphic within a species, but tend to be less widely conserved (e.g., microsatellites) **(Slate et al., 2002)**. Furthermore, primers for microsatellites isolated in other ruminants frequently amplify their deer orthologs **(Slate et al., 1998)**.

Recent developments in molecular biology and statistics have opened up the possibility of identifying and using genomic variation. It is interesting to know the genetic similarity between individuals and populations. Several different software programmes, which use molecular genetic data, have been developed for evaluating relationships among populations.

The objectives of this study were to quantify and compare levels of genetic variability and relatedness among seven red deer populations representing different uses and population sizes. The populations were analysed using by 10 microsatellite markers and genotypes of each population were used for statistical evaluation of relationships between populations.

MATERIAL AND METHODOLOGY

Samples were collected from a total of 637 red deer originating from Czech Republic (50), Hungary (35), Hungary x New Zealand hybrids (67), Lithuania (26), New Zealand (82), Poland (347) and Slovak Republic (30). We used the hair bulbs as a source of DNA.

Genomic DNA we obtained as a whole-cell lysate from 10 hair roots using PCR buffer with Tween 20. 10 microsatellite markers were runed in two optimized multiplex PCR reactions. The 8 microsatellite markers of mix 1 (Table 1) were amplified in modified multiplex-polymerase chain reaction according to **Ernst et al. (2008) (Maršálková et al., 2010)** using fluorescently labelled primers. Additional 2 microsatellites markers were amplified in the second multiplex (Table 1). PCR

Tab. 1 List of primers (name, fluorescent dye, sequences)

Primers list of master mix one:

BM888	*VIC* -	ACTAGGAGGCCATATAGGAGGC AGCTCAAAACGAGGGACAGGG	**(Talbot et al., 1996)**
OarFCB5	*6FAM* -	AAGTTAATTTTCTGGCTGGAAAACCCCAG ACCTGACCCTTACTCTCTTCACTC	**(Buchanan et al., 1994)**
RM188	*VIC* -	GCACTATTGGGCTGGTGATT GGTTCACAAAGAGCTGGAC	**(Barendse et al., 1994)**
RT1	*VIC* -	CATATGGCTAACTACCTAGCTTGCC GAGTCCCAAAGATTTCAGCCCTAC	**(Wilson et al., 1997)**
RT13	*NED* -	GCCCAGTGTTAGGAAAGAAGA CATCCCAGAACAGGAGTGAG	**(Wilson, et al., 1997)**
T26	*6FAM* -	TGCCATAGTTTTTCCTACCTTC GAAGTTCCAATAGACACGCTC	**(Jones et al., 2002)**
T156	*6FAM* -	ATGAATACCCAGTCTTGTCTG TCTTCCTGACCTGTGTCTTG	**(Jones et al., 2002)**
T501	*PET* -	CTCCTCATTATTACCCTGTGA ACATGCTTTGACCAAGACCC	**(Jones et al., 2002)**

Primers list of master mix two:

IOBT965	*6FAM* -	GGGGTTGTGGGTAAGCGGAGTT GATCTAGCGCCAGACAGACGTGTCAT	**(Kuehn et al., 2003)**
BM1818	*VIC* -	AGTGCTTTCAAGGTCCATGC AGCTGGGAATATAACCAAAGG	**(Cosse et al., 2007)**

was carried out in 10 µl volumes consisting of 1 µl of whole-cell lysate containing DNA; 1.2x Go Taq® Hot Buffer (Promega, Madison USA); 1.8 mM $MgCl_2$ (Promega, Madison USA); 0.34 mM dNTP (Applied Biosystems); concentrations of the individual primers varied from 80 to 400 nM; 0.5 U of GoTaq® Hot

Start Polymerase (Promega, Madison USA). Samples were initially heated to 95 °C for 5 minutes and then subjected to 30 cycles of PCR amplification at 95 °C for 30 s, 59 °C for 90 s, 72 °C for 90 s, and followed by a final extension at 72 °C for 60s (PTC-150 Minicycler™, MJ Research). Fragments of interest were genotyped by ABI Prism® 310 Genetic Analyzer (Applied Biosystems) and genotypes of individuals were evaluated by GeneMapper® software.

From the output of the statistical software program Tanagra was identified significant difference ($p < 0.001$) between the sample A and the sample F for shear strange textural parameter (Table 2). Therefore, we can state that storage at 12 °C two days significantly affecting the strength of chicken breast. Another significant difference ($p < 0.05$) for strength was found between the sample A and the sample B. In the case of work in shear (Table 3), we have identified a significant difference ($p < 0.001$) between the sample A and the sample F.

Genetic diversity of specific populations has been assessed on the base of allele number (NA), genotype number, gene diversity (GD), heterozygosity (Ho) and Hardy-Weinberg equilibrium (HWE). These values have been calculated from genotypes using the POWERMARKER 3.23 programme **(Liu et al., 2003)**. Distance measures were estimated by the method of **Nei et al. (1983)**. The neighbour-joining tree topology was obtained with the PHYLIP-3.69 **(Felsenstein, 1993)** software using the Cavalli-Sforza distance and the neighbour-joining tree construction was carried out with the Dendroscope software **(Huson et al., 2007)**.

Results and discussion

This study was conducted to determine the levels of genetic variability and relationships among deer populations from a total of 637 animals originating from seven countries. In total, 213 alleles were observed from the 10 loci surveyed. The number of alleles per locus ranged from 11 (IOBT965) to 35 (T156, RT13). Selected microsatellite markers are more polymorphic than the marker used in **Frantz et al. (2008)**, number of alleles ranged from 2 to 14, and are more polymorphic than microsatellite loci used for the analysis of paternity in **Walling et al. (2010)**, where the number of alleles ranged from 6 to 15. The number of alleles of two bovine microsatellite loci (BM888, RM188), which we used in our study, is greater than in **Slate et al. (1998)**.

He per locus overall population was between 0.716 (RT1) and 0.917 (RT13) (Table 2) and, on average, **0.831** ±0.019. No locus had a significant ($P < 0.05$) deviation from HWE. Ho per locus ranged from 0.638 (RT13) to 0.854 (BM888) and mean number of Ho was **0.738 ±0.017**, which is comparable with the mean number of Ho per locus in **Perez-Espona et al. (2008)** and **Walling et al. (2010)** and is greater than mean number of Ho in the population of Java Rusa Deer in **Webley et al. (2004)**. Values of He and Ho are relatively high and values of He are greater than Ho for all markers. He and Ho of markers are greater than expected and observed heterozygosity of markers in the Bavarian red deer population used in **Kuehn et al. (2003)**.

Tab. 2 Genetic diversity per alleles

Marker	GenotypeNo	SampleSize	AlleleNo	He	Ho
OarFCB5	74	637	16	0.845	0.794
T156	118	637	35	0.874	0.751
BM888	137	637	31	0.907	**0.854**
RT1	82	637	20	**0.716**	0.673
RT13	141	637	35	**0.917**	**0.638**
T501	55	637	15	0.817	0.740
T26	67	637	18	0.872	0.791
RM188	57	637	16	0.759	0.701
IOBT965	35	637	11	0.738	0.693
BM1818	63	637	16	0.853	0.745
Mean	82.9	637	21.3	**0.830**	**0.738**

P <0.05 in all observed loci
He- expected heterozygosity
Ho- observed heterozygosity

Tab. 3 Genetic diversity per populations

Population	GenotypeNo	SampleSize	AlleleNo	He	Ho
CR	19.3	50	11	0.774	0.768
HU	17.4	35	9.2	0.788	0.680
HUxNZ	22.4	67	10.2	0.781	0.788
LTV	16	26	9.7	0.793	0.746
NZ	22	82	9.7	0.748	0.720
PL	59.8	347	18.4	0.815	0.737
SR	14.6	30	8.3	0.777	0.703

He- expected heterozygosity
Ho- observed heterozygosity

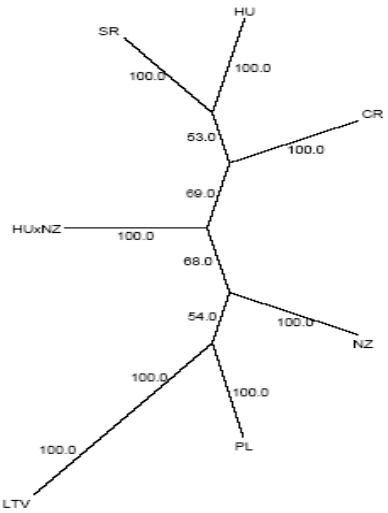

Fig. 1 Genetic distances among seven red deer populations

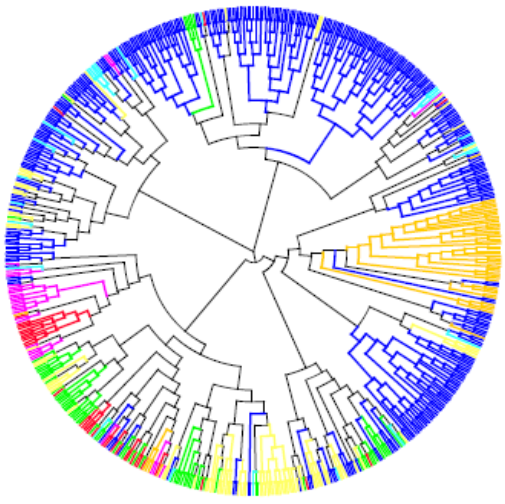

Fig. 2 Cluster diagram of all evaluated red deer individuals from different populations.

A total of 11 alleles combined in 19.3 genotypes were observed in population from CR, 10.2 alleles combined in 22.4 genotypes in hybrids HUxNZ, 9.7 alleles combined in 16 genotypes in LTV population, and 9.7 alleles combined in 22 genotypes in NZ population. The largest number of alleles (18.4) combined with the largest number of

genotypes (59.8) were found in a population from PL. The Hungarian population (9.2 alleles, 17.4 genotypes) and population from Slovak Republic (8.3 alleles, 14.6 genotypes) have the smallest number of alleles that forms the smallest number of genotypes (Table 3). Allele number in HU and SR population is higher than mean number of alleles in all populations in **Maudet et al. (2009)**.

He per population was between 0.748 ±0.033 (New Zealand) and 0.815 ±0.022 (Poland). Ho per population ranged from 0.680 ±0.032 (Hungary) to 0.788 ±0.026 (hybrids HUxNZ) (Table 3), which is slightly lower than lowest and greatest value of observed heterozygosity in **Pérez-Espona et al. (2008)**. The values of He and Ho are relatively high in all populations and therefore populations appear to be sufficiently heterogeneous. The values of He are greater than Ho also in all populations. For correct output, it is necessary to analyse at least 30 individuals, otherwise the results cannot provide relevant insight into the test group.

As we seen in Figure 1, Hungary and Slovak Republic populations are the closest of all. CR population is on the same main branch with SR and HU population and create first group. The LTV population is genetically farthest from the SR and HU populations. The PL and NZ population with LTV population create another group that is genetically distant from the first group. HUxNZ hybrids have approximately the same distance from these two groups of populations.

Figure 2 is more detailed view and represents the mixing of individuals from populations. It is cluster analysis where each branch represents an individual. Data source for constructing cluster diagrams is genetic distance. Matrix of genetic distance is calculated with the observed allele frequencies for each individual. The graphical view is divided into four main branches. Individuals of each population mostly tend to cluster together. The first main branch is created by individuals from Polish (blue) population and a few individuals from New Zealand (yellow) and Lithuanian (light blue) population. The second main branch is mainly created by individuals from Polish (blue) population. The third main branch is created by individuals from Czech and Polish population. The Polish population (blue) is mixed with individuals from New Zealand population (yellow) and hybrids HUxNZ (green). The fourth main branch is created by individuals from New Zealand population (yellow), hybrids HUxNZ (green), Hungarian (red) and Slovak population (pink).
The most mixed populations are Hungarian population and HUxNZ hybrids. The least clustered individuals are from the Lithuanian population and the most clustered are individuals from Czech and Polish population.

Based on these graphical views we can see the genetic distances among seven populations as well as distances among individuals within these populations. Molecular genetic data combined with evaluation in statistical programmes could lead to complex view of populations.

CONCLUSION

Genetic variability and relationship analyses have been performed on seven red deer populations from a total of 637 animals of different origin (Czech Republic, Hungary, Hungary x New Zealand hybrids, Lithuania, New Zealand, Poland and Slovak Republic). We can affirm that optimized panel of microsatellite markers are polymorphic and suitable for this kind of evaluation. We were able to evaluate and visualise genetic diversity and relatedness between populations on the basis of this panel of microsatellites by using statistical programmes. Neighbour-joining trees showed that individuals from each population tend to cluster together, except Lithuanian population, which have the least clustered individuals. Most of those clustered are individuals from CR. The results indicate the genetic uniformity of individual groups of animals and we can conclude that populations have enough distance from each other so they have sufficient genetic diversity.

ACKNOWLEDGEMENTS

This work was supported by grant VEGA No. 1/1074/11. This project is being co-financed by the European Union, ITMS 26240220080. We support research activities in Slovakia

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