IJAS Iranian Journal of Applied Animal Science

Microsatellite Analysis DNA for Controlled Breeding of *Cervidae* and Genetic Breeding of White Colored *Cervus elaphus* in Czech Republic

Research Article

M. Emst[1*], L. Putnova[2], J. Pokoradi[3], J. Matouskova[1], R. Zidek[4], L. Marsalkova[4] and J. Lamka[5]

[1] Department of Forest Protection and Game Management FFWT, Mendel University, Brno, Czech Republic
[2] Department of Animal Morphology, Physiology and Genetics FA, Mendel University, Brno, Czech Republic
[3] Xcell Slovakia Breeding Services, Slovak Republic
[4] Department of Hygiene and Food Safety, Faculty of Biotechnology and Food Science, Slovak University of Agriculture, Slovak Republic
[5] Department of Pharmaceutical Chemistry and Drug Control, Faculty of Pharmacy in Hradec Králové, Charles University, Prague, Czech Republic

Received on: 1 Nov 2011
Revised on: 28 Nov 2011
Accepted on: 30 Nov 2011
Online Published on: Sep 2012

*Correspondence E-mail: ernst@email.cz

Online version is available on: www.ijas.ir

ABSTRACT

A territorial isolation is a danger to the game population, causing deterioration called the inbreeding depression. The typical signs are anomalous body proportions, such as short lower jaw, shortened lifetime, weak physique and lower birth rate. This is caused by low number of game in the community. White colored deer is in the location of the Czech Republic bred in separation since 18th century. For their preservation, it was necessary to refresh the population by 'wild colored' *Cervus elaphus*. By this way, it was reduced the inbreeding depression and population was saved. Simultaneously white colored, beige colored, red colored, combined colored and spotted individuals started to appear. For further breeding development (retention of good health condition, fertility, inbreeding reduction and increase of white colored deer population), practical exploitation of molecular genetics methods were made by deer identification and verification of family relationships. Proven results were used for compilation of breeding groups with target of gradual reduction in: inbreeding, negative consequences of inbreeding depression and increase of white deer population (the origin from Kashmir or Persia). These breeding mechanisms are considered as breeding with controlled reproduction. This project is researching genetic variances within separated white deer population in game-preserve Žleby from 2004 to 2010. The idiotypes have been determined for thirteen microsatellite frequencies (BM888, OarFCB5, RM188, RT1, RT13, T26, T156, T193) and the genetic diversity, heterozygote contribution, polymorphic information content (PIC) and inbreeding factor (f) have been assessed during the project elaboration. Software Power Marker V 3.25 was used for elaboration of obtained results. The project results demonstrated the exploitation of molecular genetic methods for controlled game reproduction.

KEY WORDS breeding, *Cervus elaphus*, microsatellite, white colored deer.

INTRODUCTION

A furher understanding of population genetic structure is important for species management as genetically isolated populations with limited diversity are often associated with inbreeding and reduced reproductive fitness. Population bottlenecks can have a pronounced effect on genetic diversity and can result in reduced mean number of alleles and heterozygosity (Webley *et al.* 2007). The consequences of a bottleneck have been widely documented, with historical

records often congruent with molecular data, as it was shown by several authors (Broders *et al.* 1999; Lenny Williams *et al.* 2002; Webley *et al.* 2004). Deer (*Cervidae*) are, nowadays as well as in ancient times, among the most important species.

Thus, not unexpectedly, people have been translocating deer all over the world. Deer are used as farm animals as well as hunting wild animals. Actually, two main methods are preferred for analyzing genetic structure of population. Nine polymorphic microsatellite loci and mitochondrial DNA control region have been analysed in northern Germany population (Zachos *et al.* 2007).

Both methods have been also verified by Hmwe *et al.* (2006) on samples from 69 British red deer (*Cervus elaphus scoticus*). Similar work based on microsatellite markers focused on admixture in deer population and disequilibrium in a selected group have been presented by Slate and Pemberton (2007).

Another view on genetic biodiversity was provided by Pérez-Espona *et al.* (2008), these authors analysed gene flow in deer population originated from Scottish Highlands by 21 microsatellite markers. Biodiversity in French deer population has been described by Frantz *et al.* (2008). White colored deer is in the location of the Czech Republic bred in separation since 18th century and it is presented as game-preserve Žleby from the second half of the 20th century. Literature sources indicate that white deer came to middle Europe about year 1780 most likely from India (Kashmir) or Persia where these animals had lived free in the nature. The leucism causes that these deer individuals have pigmented skin and white pelage while their irides are blue coloured. For their preservation it was necessary to refresh the population by 'wild colored' *Cervus elaphus*. This reduced the inbreeding depression and population was saved. Simultaneously white colored, beige colored, red colored, combined colored and spotted individuals started to appear. For further breeding development (retention of good health condition, fertility, inbreeding reduction and increase of white colored deer population) practical exploitation of molecular genetics methods were made by deer identification and verification of family relationships. Proven results were used for compilation of breeding groups with target of gradual reduction in: inbreeding, negative consequences of inbreeding depression and increase of white deer population.

These breeding mechanisms are considered as breeding with controlled reproduction. The aim of this study was to evaluate the genetic structure of a farm deer population originated from New Zealand and Hungary and of a white coloured deer population from Czech Republic focussing our attention in the analysis of population biodiversity and genetic similarity within and between animals.

MATERIALS AND METHODS

DNA was isolated from peripheral blood from a total of 31 deer originated from: New Zealand region (n=11; red coloured animals), Hungarian region (n=16; red coloured animals) and Czech Republic (n=4; white coloured animals).

DNA extraction was made by following the protocol of Promega (Wizard Genomic DNA Purification Kit).Genetic analysis was realised using 13 microsatellite markers: OarFCB5, BM888, RT1, RT13, T501, T26, RM188 and T193 IOBT965, BM1818, ETH225, CSSM19 and Haut14 (Tullová, 2008). PCR products have been tested by ABI 310 Genetic Analyzer and their fragment size was performed by Genescan software. Basic population genetic analysis have been calculated in Genetics software. To represent geometric relationships among the deer populations, a principal component analysis (PCA) was applied using gene frequencies of all variable loci by Genetics software package (Belkhir *et al.* 1996).

Dendrogram based on genetic distances between individuals was computed using Powermarker 3.23 (Liu and Muse, 2005). The other genetic indices for deer population from Žleby deer preserve (n=13; white colored, beige colored, red colored, combined colored and spotted animals) were computed using above mentioned software as well. These individuals were tested with microsatellite panel. The results coming out of these analysis were used to show the utilization of testing procedure for establishing relationships and consequently to set together the stud of individuals.

RESULTS AND DISCUSSION

The allele frequencies of 13 microsatellite loci were analyzed in 31 deer from three sources. The overall F_{IS} (Specify the meaning of this acronym) values per locus ranged in deer populations from 0.081 (CSSM19) to 0.125 (OarFCB5), showing an overall F_{IS} of 0.103 (Table 1). The F_{ST} (Specify the meaning of this acronym) values ranged from 0.112 (T193) to 0.132 (Haut14). The mean F_{ST} value of 0.123 from all the loci indicated that 88.7% of the genetic variation was caused by differences among individuals and 12.3% only due to differentiation among the origin of animals.

Different results were observed by Frantz *et al.* (2008) in isolated French deer population (female F_{IT} 0.045 and male F_{IT} 0.003). In the present work, it was observed variability between populations (F_{ST} 0.123), being the values obtained higher than those computed by Pérez-Espona *et al.* (2008) for Scottish Highlands deer (F_{ST} 0.019). Graphical view of both analysed populations has been designed by PCA method (Figure 1).

Table 1 Genetic diversity, heterozygosity, Polymorphism Information Content (PIC) and inbreeding coefficient (f) of 13 white deer tested with Ernst's *et al*. (2008) microsatellite panel

Locus	Genetic diversity	Heterozygosity	PIC	f
OarFCB5	0.6657	0.7692	0.6198	-0.1163
T156	0.7544	0.9231	0.7274	-0.1852
T26	0.6391	0.7692	0.5659	-0.1651
BM888	0.7101	0.6154	0.6731	0.1724
RM188	0.7337	0.7692	0.6914	-0.0084
RT1	0.6746	0.6924	0.6112	0.0137
RT13	0.6923	0.5385	0.6611	0.2599
T193	0.6834	0.6923	0.6345	0.0270
Average	0.6942	0.7212	0.6481	0.0011

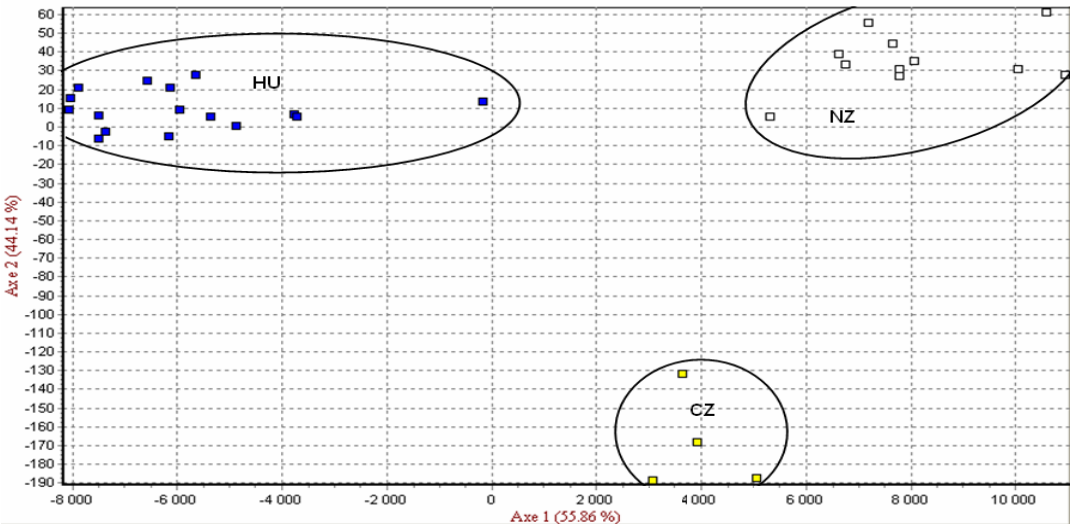

Figure 1 Scatter diagram showing relative position of 31 individuals defined by principal component factor scores based on correlation matrix from allele frequency of the 13 microsatellites

Principal component analysis split analysed population into three groups. Differences between Hungarian and New Zealand populations were only visible on x axis in which these differences represented by 55.86% of variability. Czech population of White deer difference was marked on y axis in which it represented by 44.14% of variability. According to computed F_{ST} 0.119, graphical view presents only 11.90% of differences in analysed deer population. Similar results were obtained using the phylogenetic tree constructed on the base of Nei's DA genetic distance computed for each possible combination of animals separately (Figure 2). All group of animals were separated to branches according to their origin. The results of genetic indices for deer population from Czech deer preserve Žleby are showed in Table 1. These individuals were tested with microsatellite panel. The presumption of prosperity in the Czech population shows highly genetic diversity. The value of genetic diversity was 0.69 in this population. It is also heterozygosity that points out the quality of a population.

In this group of individuals the rate of heterozygotes was ascertained at 72% and it showed lower value than that observed on free-living deer in Scotland, where the rate of heterozygotes was stated at 75%. Even bigger difference can be observed on value of Polymorphism Information Content (PIC), where our research obtained an average value of 0.65, while stated an average value of 0.81 for the same locuses. All the computed values are neither critical nor optimal. Therefore it is necessary to treat this particular population in order to heighten all above mentioned genetic indices. The condition of the population also shows the coefficient of inbreeding 0.11%. The population does not appear to be in inbreeding depression, although, the wrong game management can lead to it. Table 2 shows practical utilization of microsatellite analyzes (testing of relationships) in reserved breeds or small populations. It is evident from this table that red coloured deer No. BJ64 is the offspring of spotted deer No. white coloured, there white coloured, there is a higher presumption that the deer No. BJ34

Table 2 Identification card of red coloured deer No. BJ64 and spotted deer No. BJ34

Locus	**BJ64** (2005)	**BJ34** (2000)
OarFCB5	79 / 87	79 / 87
T156	152 / 160	160 / 178
T26	352 / 358	352 / 358
BM888	194 / 194	194 / 238
RM188	137 / 139	131 / 139
RT1	260 / 276	260 / 260
RT13	303 / 307	301 / 303
T193	192 / 204	168 / 204
T501	237 / 259	237 / 265

Possibilities of alleles succession are written red, the year of birth is featured in brackets.

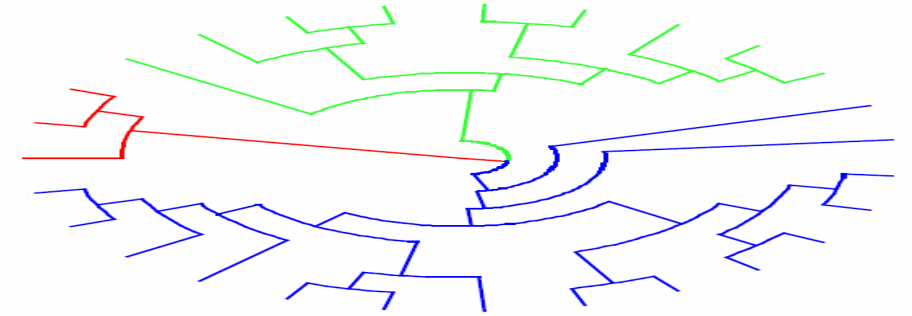

Figure 2 Dendrogram based on genetic distances between deer individuals
Red color (White deer), Blue color (Hungarian deer), Green color (New Zealand deer)

Figure 3 Color variations in individuals of *Cervus elaphus* from game preserve Žleby

would be red coloured. This will be tested on other experiments and if the research confirms the previous hypothesis No. BJ34 will be no longer used in breeding.

CONCLUSION

Genetic variability of three populations of deer has been analysed using microsatellite markers. The allele frequencies of 13 microsatellite loci were computed and analysed using different statistical methods. PCA was performed by splitting population under study into three separate groups. Similar results were obtained by using phylogenetic tree constructed on the base of Nei's DA genetic distance confirming the PCA results. All group of animals were separated into branches according to their origin. Our results show differences between European deer, New Zealand deer and White coloured deer. A territorial isolation is a danger to the game population, causing deterioration called the inbreeding depression. The typical signs are anomalous body proportions, such as short lower jaw, shortened lifetime, weak physique and lower birth rate. This is caused by low number of game in the community. There have been several researches engaged in possible utilization of molecular and genetic methods for inbreeding diagnosis and its possible reduction. In our study, it was investigated the presence of inbreeding within the white red deer population (*Cervus elaphus* L.), which has been breeded in the location of the Czech Republic since the end of the 18th century. The method of DNA separation has been elaborated through the blood samples and it has been applied by using the method PCR and by fragmentation analysis. Furthermore, the genetic diversity, heterozygote contribution, PIC and inbreeding factor [f] have been assessed with 13 white red deers. The idiotypes have been determined for eight microsatellite frequencies and factors of genetic variability and diversity have been calculated for white red deer population during the project elaboration. The quality of observed white red deer population in game preserve Žleby seems to be good. The average heterozygote contribution is 72.12% and the average inbreeding factor amounts 0.11%. However, considering the low amount of examined subjects we can not be certain that observed deer population is not in inbreeding depression. The project results demonstrate the exploitation of molecular genetic methods for controlled game reproduction.

ACKNOWLEDGEMENT

This work was supported by a grant to the Faculty of Forestry and Wood Technology, from the Czech Ministry of Education, Youth and Sports (No. MSM 6215648902) and it was also funded by the Grant Agency of Forestry of the Czech Republic, State Enterprise (No. OS 450301).

REFERENCES

Belkhir K., Borsa P., Chichi L., Raufaste N. and Bonhomme F. (1996). Genetics 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, DNRS UMR 5171, Université de Montpellier II, Montpellier France. Available: http://www.univ-montp2.fr/~genetix/ genetix/genetix.htm.

Broders H.G., Mahoney S.P., Montevecchi W.A. and Davidson W.S. (1999). Population genetic structure and the effect of founder events on the genetic variability of moose, *Alces alces,* in Canada. *Mol. Ecol.* **8,** 1309-1315.

Frantz A.C., Hamann J.L. and Klein F. (2008). Fine-scale genetic structure of red deer (*Cervus elaphus*) in a French temperate forest. *Eur. J. Wildl. Res.* **54,** 44-52.

Hmwe S.S., Zachos F.E., Sale J.B., Rose H.R. and Hartl G.B. (2006). Genetic variability and differentiation in red deer (*Cervus elaphus*) from Scotland and England. *J. Zoology*. **270(3),** 479-487.

Lenny Williams C., Sarfass T.L., Cogan R. and Rhodes O.W. (2002). Microsatellite variation in the reintroduced Pennsylvania elk her. *Mol. Ecol*. **11,** 1299-1310.

Liu K. and Muse S.V. (2005). Power Marker: Integrated analysis environment for genetic marker data. *Bioinformatics*. **21,** 2128-2129.

Pérez-Espona S., Pérez-Barbería F.J., McLeod J.E., Jiggins C.D., Gordon I.J. and Pemberton J.M. (2008). Landscape features affect gene flow of Scottich Highland red deer (*Cervus elaphus*). *Mol. Ecol*. **17(4),** 981-996.

Slate J. and Pemberton J.M. (2007). Admixture and patterns of linkage disequilibrium in a free-living vertebrate population. *J. Evol. Biol*. **20(4),** 1415-1427.

Tullová M. (2008). Genetická premenlivosť populácií jeleňa lesného (*Cervus Elaphus* L.) na Slovensku a v Európe. *Act. Fac. Forest. Zvolen*. **50,** 7-18.

Webley L.S., Zenger K.R., English A.W. and Cooper D.W. (2004). Low levels of genetic variation within introduced Javan rusa deer (*Cervus timorensis russa*) in Australia. *Eur. J. Wildl. Res.* **50,** 137-140.

Webley L.S., Zenger K.R., Hall G.P. and Cooper D.W. (2007). Genetic structure of introduced European fallow deer (*Dama dama dama*) in Tasmania, Australia. *Eur. J. Wildl. Res.* **53,** 40-46.

Zachos F.E., Althoff C., Von Steynitz Y., Eckert I. and Hartl G.B. (2007). Genetic analysis of an isolated red deer (*Cervus elaphus*) population showing signs of inbreeding depression. *Eur. J. Wildl. Res.* **53,** 61-67.