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GENETIC AND STATISTICAL ANALYSIS OF FOUNDER LINES IN RED DEER POPULATION

GENETICKO-ŠTATISTICKÁ ANALÝZA ZAKLADATEĽSKÝCH LÍNIÍ V POPULÁCII JELEŇOVITÝCH

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Deer (Cervidaei) belong to the most important species and are used as farm animals as well as game animal. When implementing management of farm animals, understanding of population genetic structure is important. Population and genetic analysis has been performed on deer population originating from New Zealand and Hungary. The population was examined by using 13 microsatellites. The allele frequencies of 13 microsatellite loci were analyzed in 53 deer from two sources. A total of 96 alleles combined in 201 genotypes have been observed in New Zealand population and 118 alleles combined in 254 genotypes in Hungarian deer population. By using PowerMarker and GENETIX software we create a graphical view of relationships among father, mothers and their offspring upon which we assess the genetic similarity of offspring to parents

Key words: genetic distance, genetic marker, red deer

Deer (Cervidaei) belong to the most important species and is used as a farm animal as well as game animal.

When implementing management of farm animals, understanding of population genetic structure is important. Recent developments in molecular biology and statistics have opened the possibility of identifying and using genomic variation and major genes for the genetic improvement of livestock. It is interesting to know, in terms of breeding animals, the genetic similarity of a founder line and its descendants and if there are characteristics transferred by founder of line.

Microsatellite DNA has become a very powerful genetic marker. The detection of the microsatellite marker can indicate the genotype or productivity of specific individual (Xu Yan-chun et al., 2001). Microsatellite or simple sequence repeat (SSR) marker-type maps have been constructed in many organisms (Crawford et al., 1995; Jacob et al., 1995; Crooijmans et al., 1996; Dib et al., 1996; Dietrich et al., 1996; Kappes et al., 1997) and have been used to help locate genes for hereditary diseases and quantitative trait loci (QTL) controlling traits of economic importance (Andersson et al., 1994; Georges et al., 1995; Grobet et al., 1997; Knott et al., 1998).

To date, about 200 microsatelitte loci were found in cervids by transferring microsatellite PCR primers derived in bovine and ovine to cervids, as well as a few loci derived directly from deer microsatellite library. These loci have been used in parentage determination, genetic diversity and population structure, population introgression (Xu Yan-chun et al., 2001).

Microsatellites are genomic sequences comprised of tandem repeats of short nucleotide motifs (1 to 6bp) (Leclercq, Rivals and Jarne, 2007). SSR markers are largely codominantly expressed, evenly distributed throughout the genome, and surveyed rapidly in many individuals using PCR techniques data (Lee and Kocher, 1996; Slettan et al., 1997; Knapik et al., 1998). Based on the linkage of microsatellite and QTL or corelationship of microsatellite and phenotype, identification of target character of breeding population will shorten the time and improve the efficiency of breeding. For adults/breeders, microsatellite markers help to predict the character of offspring (Xu Yan-chun et al., 2001).

Knowledge of the genes located at QTL could greatly assist in estimation of animal's true genotype. Most QTL known today can only be targeted by genetic markers. We cannot actually observe inheritance at the QTL itself, but we observe inheritance at the marker, which is close to the QTL. When map distances are small (<10 cM), the map distance equals the recombination frequency. However, this relationship does not apply for map distances that are greater than 10 cM (Hartl and Jones; 2009). The closer a marker is from a QTL, the lower the chance of recombination occurring between marker and QTL. Therefore, the QTL marker will be usually inherited together in the progeny, and the mean of the group with the tightly-linked marker will be significantly different ($P < 0.05$) to the mean of

Table 1 Primers list of mastermix one

BM888	VIC-ACTAGGAGGCCATATAGGAGGC AGCTCAAAACGAGGGACAGGG
OarFCB5	6FAM-AAGTTAATTTTCTGGCTGGAAAACCCCAG ACCTGACCCTTACTCTCTTCACTC
RM188	VIC-GCACTATTGGGCTGGTGATT GGTTCACAAAGAGCTGGAC
RT1	VIC-CATATGGCTAACTACCTAGCTTGCC GAGTCCCAAAGATTTCAGCCCTAC
RT13	NED-GCCCAGTGTTAGGAAAGAAGA CATCCCAGAACAGGAGTGAG
T26	6FAM-TGCCATAGTTTTTCCTACCTTC GAAGTTCCAATAGACACGCTC
T156	6FAM-ATGAATACCCAGTCTTGTCTG TCTTCCTGACCTGTGTCTTG
T501	PET-CTCCTCATTATTACCCTGTGA ACATGCTTTGACCAAGACCC

Tabuľka 1 Zoznam primerov reakčnej zmesi jedna

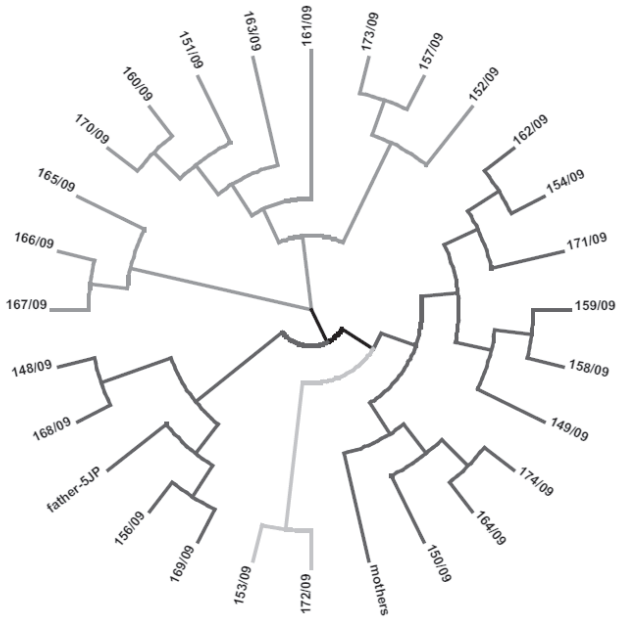

Figure 1 Genetic structure of F1 generation based on New Zealand population
Obrázok 1 Genetická štruktúra F1 generácie Novozélandskej populácie

the group without the marker (Collard et al.; 2005). Since the localization of microsatellites, which we used on chromosome, is known, we can observe the transfer of part of chromosome from generation to generation.

The aim of this study is the evaluation of genetic structure of farm deer populations and stock assessment of founder lines. This work is using standard population statistic methods of cluster analysis to identify individuals similar to founder line as well as to pool of mothers genotypes.

Table 2 Primers list of mastermix two

IOBT965	6-FAM-GGGGTTGTGGGTAAGCGGAGTT GATCTAGCGCCAGACAGACGTGTCAT
Haut14	VIC-CCAGGGAAGATGAAGTGACC TGACCTTCACTCATGTTATTAA
ETH225	NED-ACATGACAGCCAGCTGCTACT GATCACCTTGCCACTATTTCCT
CSSM19	PET-TTGTCAGCAACTTCTTGTATCTTT TGTTTTAAGCCACCCAATTATTTG
BM1818	VIC-AGTGCTTTCAAGGTCCATGC AGCTGGGAATATAACCAAAGG

Tabuľka 2 Zoznam primerov reakčnej zmesi dva

Material and methods

DNA was isolated from hair roots from a total of 53 deer originating from New Zealand from Hungarian region (27 animals). DNA isolation followed the protocol of NucleoSpin® Tissue Isolation Kit (Macherey-Nagel). We used 13 microsatellite markers for evaluation of population. PCR reaction was based on two multiplexes using 8 (mix1) and 5 (mix2) microsatellites markers. Total of 8 microsatellite markers of mix 1 (table 1) runs in modified multiplex according to Ernst (2008). Additional 5 microsatellites of mix 2 (table 2) runs in the second multiplex. Mastermix 1 and 2 contains 1 μl of DNA sample (50–100 ng of DNA); 1x Go Taq® Flexi Buffer (Promega, Medison USA); 0.34 mM dNTP (Applied Biosystems); 1.8 mM MgCl2 (Promega, Medison USA); 0.5 U of GoTaq® Hot Start Polymerase (Promega, Medison USA); 3% of DMSO and 80–400 nM of each primer (table 1) diluted by redestilated water up to total value 10 μl. The PCR step-cycle condition (PTC-150

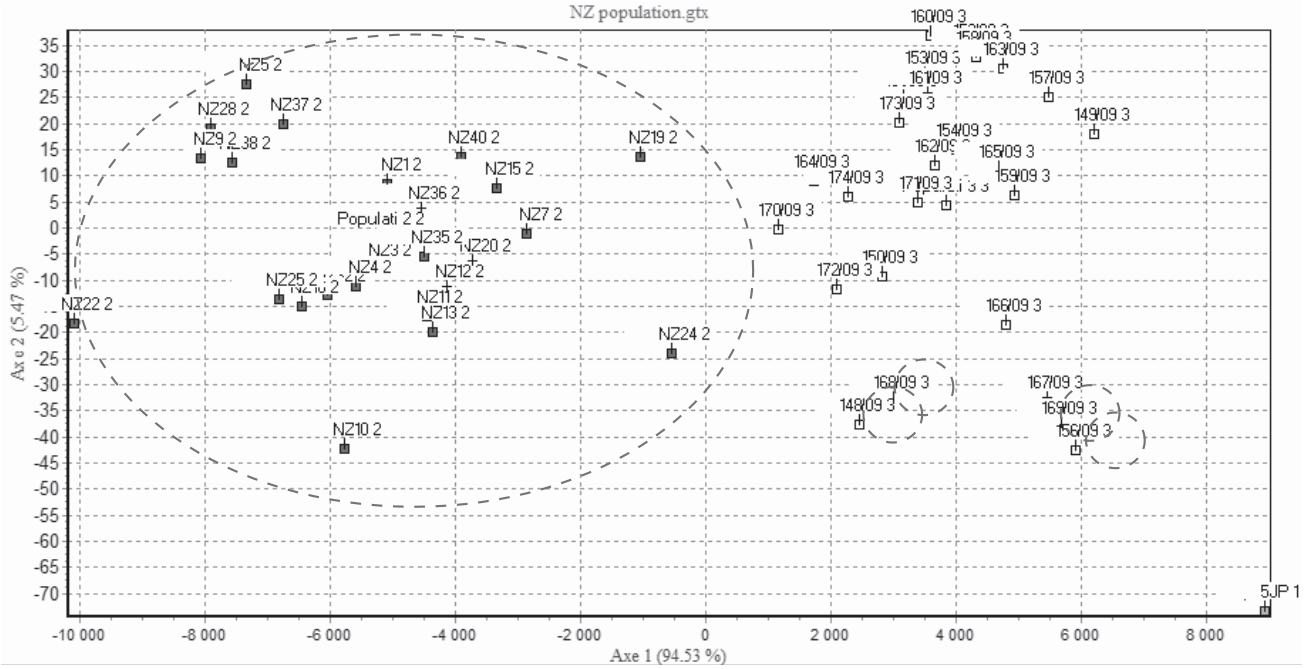

Figure 2 Principal component analysis based on New Zealand population
Obrázok 2 Analýza základných komponentov vychádzajúca z Novozélandskej populácie

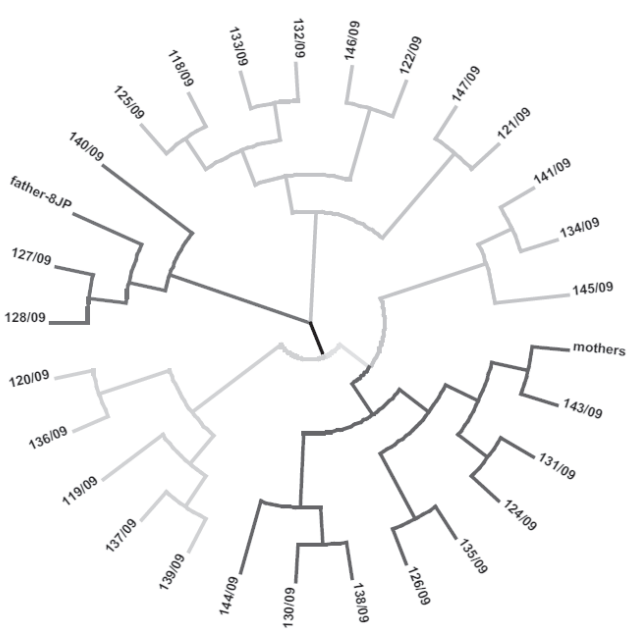

Figure 3 Genetic structure of F1 generation based on Hungarian population
Obrázok 3 Genetická štruktúra F1 generácie Maďarskej populácie

Minicycler™, MJ Reasearch) was as follows: pre-incubation at 95 °C for 5 min, 30 cycles consisting of denaturation at 95 °C for 30 s, annealing at 59 °C for 90 s, and extension at 72 °C for 90 s, followed by a final extension at 72 °C for 60 min PCR products have been tested by ABI 310 Genetic Analyzer and Genemapper software. Line structures have been calculated in POWERMARKER 3.23 (Liu and Muse, 2003) using allelic frequency data. Principal component analyses have been computed in GENETIX 4.05 software.

Results and discussion

The allele frequencies of 13 microsatellite loci were analyzed in 53 deer from two sources. A total of 96 alleles combined in 201 genotypes were observed in population based on New Zealand mothers and 118 alleles combined in 254 genotypes in population based on Hungarian mothers. Selected microsatellite markers are more polymorph than a marker used in Frantz, Hamman and Klein (2008), number of alleles in this one ranged from 2 to 14.

Figure 1. is graphical view of F1 generation based on New Zealand females and New Zealand stag. Mothers' genotypes has been pooled and computed as one individual to get transparent results. Cluster analysis separate the F1 generation into 3 groups. In the first group there are the individuals in the same branch as the mothers. In this group there are individuals with similar allele composition as mother allele pool. The second group is in the same cluster as the Father, and represents individuals with similar genotype structure as the line founder. Third group represents individuals without relevant similarity to father or mothers population. From a total of 11 animals that are genetically similar to the mother, 9 of them are genetically very close to the mother. On the other hand only 4 animals are genetically very close to the founder. We used principal component analysis based on allele frequencies as a second observing method. Results presented in figure shows the gene similarity structure of analyzed population. Big circle represents the genetic variability among mothers. Small circles represent F1 individuals proved as closely related with father genotype using cluster method as well as PCA method.

Figure 3. is a graphical view of F1 generation based on Hungarian mothers. On this figure we can see many branches which show the animals that are more genetically related to

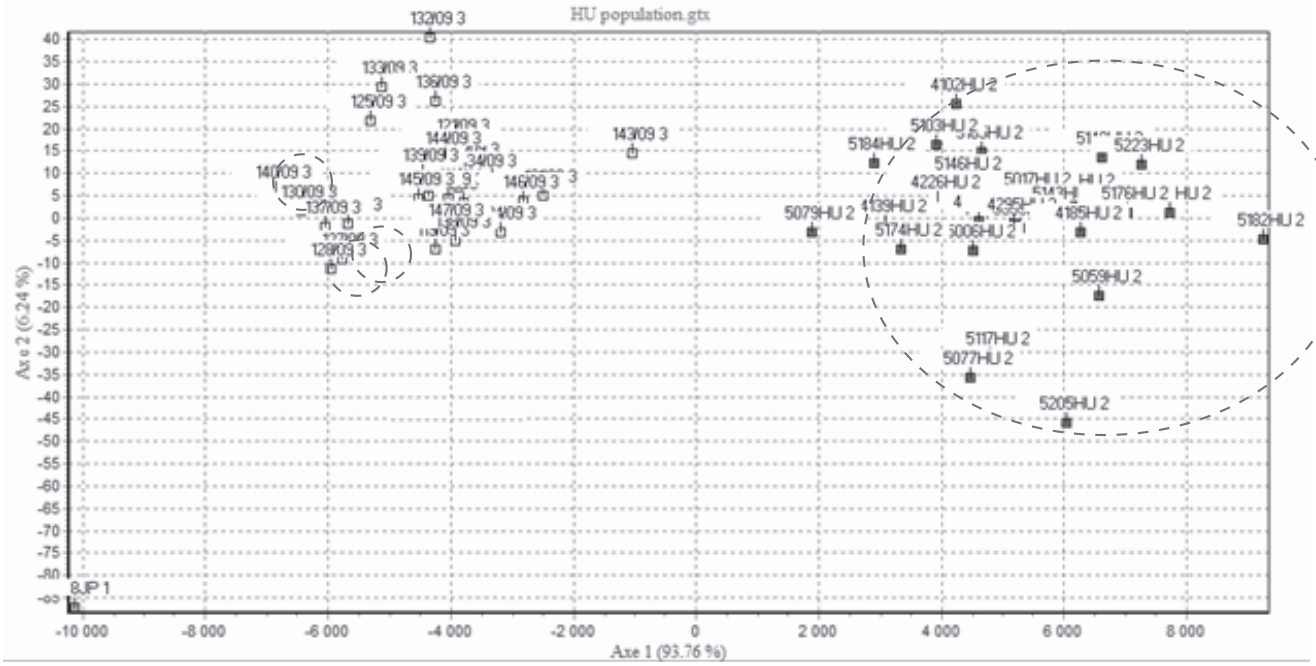

Figure 4 Principal component analysis based on Hungarian population
Obrázok 4 Analýza základných komponentov F1 generácie Maďarskej populácie

each other then to the pool of mother alleles or father (8 JP). From a total of 11 animals that are genetically similar to the mother, 8 of them are genetically very close to the mother. On the other hand, from a total of 11 animals that are genetically similar to the founder line, only 3 animals are genetically very close to founder. Of the entire population, 5 animals are genetically similar both to father and mother. It means that they did not inherit alleles typical for father or mothers population. Another view is available by PCA analysis. Results presented in figure 4 pointed out the lower level of diversity among Hungarian group of mothers. The diversity among F1 individuals was low, too. Big circle represents female ancestors. Small circles represent F1 individuals marked as most genetically similar to father.

Genetic structure of population could be a powerful tool in breeding strategy implemented on a small population without relevant pedigree data. Molecular genetic data combined with observed relevant traits could lead to complex population view.

Conclusion

Population genetic analysis has been performed on deer population originating from New Zealand and Hungary. We can affirm the selected microsatellite markers are polymorph and suitable for this kind of evaluation. We are able to analyze first filial generation according to microsatellite data and to describe similarities between fathers and their offsprings. Given data could be used in breeding strategies for maximization of heterosis effect as well as for gene fixation of founder line. According to known chromosome position of microsatellite markers we are also able to trace specific parts of chromosomes. It is interesting to know, in terms of breeding animals, the genetic similarity of a founder line and its descendants and if there are characteristics transferred by founder of line.

Súhrn

Jelene (*Cervidaei*) patria medzi najdôležitejšie druhy, ktoré sa využívajú ako farmové zvieratá a rovnako ako lovná zver. Pochopenie genetickej štruktúry populácií, je veľmi dôležité pri manažmente farmových zvierat. Populačno-genetická analýza bola vykonaná na skupine jeleňov pochádzajúcich z Nového Zélandu a Maďarska. Na hodnotenie bolo použitých 13 mikrosatelitných systémov. Alelová štruktúra bola analyzovaná pri 53 zvieratách. V populácii z Nového Zélandu bolo pozorovaných 96 alel, ktoré vytvorili 201 genotypov a v Maďarskej populácii bolo pozorovaných 118 alel tvoriacich 254 genotypov. Použitím programov PowerMarker a GENETIX sme vypracovali rôzne grafické náhľady na genetickú podobnosť medzi otcom, matkami a potomkami s označením potomkom s rovnakou alelovou skladbou ako zakladateľ línie.

Kľúčové slová: genetická vzdialenosť, genetický marker, jeleň

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References

ANDERSSON, L. – HALEY, C. S. – ELLEGREN, H. – KNOTT, S. A. – JOHANSSON, M. et al. 1994. Genetic mapping of quantitative trait loci for growth and fatness in pigs. In: Science, vol. 263, 1994, p. 1771–1774.

BELKHIR, K. – BORSA, P. – CHIKHI, L. – RAUFASTE, N. – BONHOMME, F. 1996–2004 GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier (France).

COLLARD, B.C.Y. – JAHUFER, M.Z.Z. – BROUWER, J.B. – PANG E.C.K. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. In: Euphytica, vol. 142, 2005, p. 169–196.

CRAWFORD, A.M. – DODDS, K.G. – EDE, A.J. – PIERSON, C.A. – MONTGOMERY, G.W., et al., 1995. An autosomal genetic linkage map of the sheep genome. In: Genetics, vol. 140, 1995, p. 703–724.

CROOIJMANS, R.P.M.A – VAN KAMPEN, A.J.A. – VAN DER POEL, J.J – GROENEN, M.A.M.1996. Preliminary linkage map of the chicken (*Gallus domesticus*) genome based on microsatellite markers : 77 new markers mapped. In: Poultry Science, vol. 75, 1996, p. 746–754.

DIB, C. – FAURE, S. – FIZAMES, C. – SAMSON, D. – DROUOT, N. et al. 1996. A comprehensive genetic map of the human genome based on 5,264 microsatellites. In: Nature, vol. 380, 1996, p. 152–154.

DIETRICH, W.F. – MILLER, J. – STEEN, R. – MERCHANT, M.A. – DAMRON-BOLES, D. et al. 1996. A comprehensive genetic map of the mouse genome. In: Nature, vol. 380, 1996, p. 149–152.

ERNST, M. – KLIMENT, J. – LEVÝ, E. – KOURKOVÁ, L. – STEJSKAL, M. 2008 Populace bílých jelenů – Využití mikrosatelitních analýz při šlechtění populace bílých jelenů u LČR, s. p. Hradec Králové : Edice Grantové služby LČR, 2008. 28 s. 03/07. ISBN 978-80-86945-01-9

FRANTZ, A. C. – HAMANN, J. L. – KLEI, N. F. 2008. Fine-scale genetic structure of red deer (*Cervus elaphus*) in a French temperate forest. In: Eur J Wildl Res, vol. 54, 2008, p. 44–52.

GEORGES, M. – NIELSEN, D. – MACKINNON M. – MISHRA, A. – OKIMOTO, R. et al. 1995. Mapping quantitative trait loci controlling milk production in dairy cattle by exploiting progeny testing. In: Genetics, vol. 139, 1995, p. 907–920.

GROBET, L. – R.MARTIN, L.J. – PONCELET, D. – PIROTTIN, D. – BROUWERS, B. et al. 1997 A deletion in the bovine myostatin gene causes the double-muscled phenotype in cattle. In: Nature Genetics, vol. 17, 1997, p. 71–74.

HARTL, D. – JONES, E. 2009. Gnetics: Analysis of Genes and Genomes. Sudbury, MA : Jones and Bartlett Publishers, 2009. 763 p. ISBN 978-0-7637-5868-4

JACOB, H. J. – BROW, D.M. – BUNKER, R.K. – DALY, M. J. – DZAU, V.J. et al. 1995. A gentic linkage map of the laboratory rat, Rattus norvegicus. In: Nature Genetics, vol. 9. 1995, p. 63–69.

KAPPES, S.M. – KEELE, J.W. – STONE, R. T. – MCGRAW, R. A. – SONSTEGARD, T. S. et al. 1997. A second-generation linkage map of the bovine genome. In: Genome Research. vol. 7, 1997, p. 235–249.

KNAPIK, E. W. – GOODMAN, A. – EKKER, M. – CHEVRETTE, M. – DELGADO, J. et al. 1998. A microsatellite genetic linkage map for zebrafish (*Danio rerio*). In: Nature Genetics, vol. 18, 1998, p. 338–343.

KNOTT, S. A. – MARKLUND, L. – HALEY, C.S. – ANDERSSON, K. – DAVIES, W. et al. 1998. Multiple marker mapping of quantitative trait loci in a cross between outbred wild boar and large white pigs. In: Genetics, vol. 149, 1998, p. 1069–1080.

LECLERCQ, S. – RIVALS, E. – JARNE, P. 2007. Detecting microsatellites within genomes: significant variation among algorithms. In: BMC Bioinformatics, vol. 8, 2007, p. 125.

LEE, W.J. – KOCHER, T.D. 1996. Microsatellite DNA markers for genetic mapping in Oreochromis niloticus. In: Journal of Fish Biology, vol. 49, 1996, p. 169–171.

LIU, K. – MUSE, S. V. 2005 PowerMarker: Integrated analysis environment for genetic marker data. In: Bioinformatics, vol. 21, 2005, p. 2128–2129.

PÉREZ-ESPONA, S. – PÉREZ-BARBERÍA, F. J. – MCLEOD, J. E. – JIGGINS, C. D. – GORDON, I. J. – PEMBERTON, J. M. 2008. Landscape features affect gene flow of Scottich Highland red deer

(*Cervus elaphus*). In: Molecular Ecology, vol. 17, 2008, no. 4, p. 981–996.
SLETTAN, A. – OLSAKER, I. – LIE, O. 1997. Segregation studies and linkage analysis of Atlantic salmon microsatellites using haploid genetics. In: Heredity, vol. 78, 1997, p. 620–627.
XU, YAN-CHUN – PAN, ZI-CHEN – XU, ZHI-RU – YANG, SHU-HUI – JIN, YU – BAI, SU-YING 2001. Status of microsatellites as genetic markers in cervids. In: Journal of Forestry Research, vol. 12, 2001, no. 1, p. 55–58.

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