

## Suitability of BovineSNP50 BeadChip for the evaluation of the *Cervidae* family diversity

R. KASARDA<sup>1</sup>, N. MORAVČÍKOVÁ<sup>1</sup>, V. ŠIDLOVÁ<sup>1</sup>, A. TRAKOVICKÁ<sup>1</sup>, O. KADLEČÍK<sup>1</sup>, J. POKORÁDÍ<sup>2</sup>, R. ŽIDEK<sup>3</sup>

<sup>1</sup>Department of Animal Genetics and Breeding Biology, Slovak University of Agriculture in Nitra, Nitra, Slovak Republic

<sup>2</sup>Xcell Slovakia Breeding Services s.r.o., Bratislava, Slovak Republic

<sup>3</sup>Department of Food Hygiene and Safety, Slovak University of Agriculture in Nitra, Nitra, Slovak Republic

**ABSTRACT:** Polymorphic SNPs were identified using BovineSNP50 BeadChip in three groups of cervids: farmed Red deer ( $n = 3$ ), and free range Red deer ( $n = 5$ ) and Fallow deer ( $n = 2$ ). From the total of 54 609 SNPs, 53.85% could be genotyped. Out of 28 502 successfully genotyped autosomal SNPs only 5.3% were polymorphic. The average minor allele frequency within cervids was 0.23 (number of polymorphic SNPs ranged from 467 to 686). Results of the molecular variance analysis showed that 67.38% of variation occurred within individuals and the rest was explained by a species difference ( $F_{ST} = 0.32$ ). The value of  $F_{IT}$  (0.33) indicated a higher proportion of homozygote genotypes in the analyzed dataset. Pairwise  $F_{ST}$  values showed very clearly the genetic differentiation between Red and Fallow deer which ranged from 0.06 (farmed and free range deer) to 0.74 (farmed Red and Fallow deer). A similar result was found for Nei's genetic distances that ranged from 0.01 (among Fallow deer) to 0.79 (among farmed Red and Fallow deer). The genetic differentiation of the analyzed cervid species was evaluated also by the principal component analysis with the involvement of 6 other species from the family *Cervidae*, which showed a division of the *Cervidae* cluster into 7 subpopulations. The panels of SNPs primarily produced for a model species are becoming the marker of choice for the application in other species, but the best methods of their discovery, validation, and genotyping in non-model species need further investigations.

**Keywords:** cross-species genotyping; non-model species; Fallow deer; Red deer

### INTRODUCTION

The determination of population genetics parameters can be based on different data, but genome-wide markers are more advantageous over morphological or biochemical data, because they show genetic differences on a more detailed level without interferences of environmental factors. Single nucleotide polymorphisms (SNPs) are very useful to scan large and separate regions of a genome due to their abundance in both coding and non-coding regions, their co-dominant nature, and lack of ambiguity (Williams et al. 2010). The

development of high-throughput SNP genotyping methods has led to the increase of their use as molecular genetic markers. Currently, based on their abundance in animal genomes and the increased throughput of SNP arrays, SNPs provide an exceptional insight into the phylogenetic relationship, migration, and evolution of natural populations (Morin et al. 2004; Lepoittevin et al. 2010). SNPs have gained wide use in humans and model species and are becoming the marker of choice for applications in other "non-model" species, e.g. in population genetics, to determine kinship and parentage, individuals or population

Supported by EU funds (Project No. ITMS 26240220080, operational programme "Research and Development") and by the Slovak Research and Development Agency (Contracts No. APVV-0636-11 and No. APVV-14-0054).

structure (Williams et al. 2010, Hauser et al. 2011). In addition to the necessary mining of factors affecting the distribution and abundance of taxa in the wild, the ability to bring together high-throughput genotyping with robust comparative data sets that can be readily augmented will promote their use in conservation and management of populations (Seeb et al. 2011). The panel of SNPs including neutral loci and loci under selection could be valuable in studies of non-model organisms, but loci under selection may not be optimal for calculating population parameters (Manel et al. 2010). The loci under selection can be detected based on differences in the levels of genetic variation and divergence among samples or on the basis of linkage disequilibrium among loci (Vasemagi and Primmer 2005).

To date, commercially developed livestock genotyping arrays have been used in several studies to identify novel SNPs in closely evolutionarily related non-model species (Miller et al. 2011, Wu et al. 2013), including those from the family *Cervidae* (Bixley et al. 2009, Decker et al. 2009, Haynes and Latch 2012). The successful detection of novel SNPs applicable for population studies of non-model organisms using cross-species genotyping depends on the genetic divergence among species (Miller et al. 2012). Bixley et al. (2009) and Decker et al. (2009) have begun a large scale genomic sequence and SNP discovery programme to progress towards genome-wide studies in different species from the family *Cervidae*. Haynes and Latch (2012) have already used BovineSNP50 BeadChip for the genomic study in mule and black-tailed (*Odocoileus hemionus*) and white-tailed (*Odocoileus virginianus*) deer.

The aim of this study was to evaluate the suitability of Illumina BovineSNP50 BeadChip for cross-species SNP genotyping, the comparison of SNPs distribution and variation, and the analysis of genetic diversity based on polymorphic loci detected in the *Cervidae* family species.

## MATERIAL AND METHODS

**Animal data collection.** Two genotype datasets of cervids were used to study the suitability of bovine genotyping array for cross-species application: the original unpublished dataset of 10 males (dataset I) and the public dataset of 56 cervids published by Decker et al. (2009) (dataset II).

Genotyping data in the dataset I was obtained for cervids originating from two species, Red deer (*Cervus elaphus*) and Fallow deer (*Dama dama*). Ten samples of cervids semen were collected from three unrelated farmed Red deer, male progeny of sires from New Zealand and dams from Hungary, and from five free range Red and two Fallow deer, which were trophy animals from Slovakia. Genomic DNA for all the samples was genotyped at a commercial lab using an Illumina BovineSNP50 Genotyping BeadChip.

The dataset II (Decker et al. 2009) consisted of data from 56 animals in total: 7 species from the *Cervidae* family, 10 North American mooses (*Alces alces*), 8 Axis deer (*Axis axis*), 8 Wapiti (*Cervus canadensis*), 8 Sika deer (*Cervus nippon*), 8 Fallow deer (*Dama dama*), 8 White-tailed deer (*Odocoileus virginianus*), and 6 Caribou (*Rangifer tarandus*). This genotyping data was used just to better describe the genetic relatedness based on Neighbor-Joining tree and principal component analysis (PCA) within animals included in dataset I.

**Quality control of SNP data.** The genotype datasets I and II were obtained using genotyping arrays with different numbers of SNPs. The BovineSNP50 BeadChips (Illumina) consisted of 54 609 SNPs (dataset I) and 54 693 (dataset II). The loci in dataset II were previously selected based on strict quality criteria described in Decker et al. (2009). After the selection, dataset II contained 40 843 autosomal SNPs suitable for the study.

The quality controls and computation for SNP data were run using the software tool PLINK (Version 1.07, 2007), separately for dataset I and for the dataset resulting from the merger of datasets I and II. All loci with unknown position or chromosome assignment and loci assigned to chromosomes X and Y were excluded. The SNPs with more than 10% missing genotypes, minor allele frequency (MAF) lower than 0.05, and Hardy-Weinberg equilibrium (HWE) test limit of 0.0001 were also removed. After quality control the analysis of genetic diversity was prepared including data from 1530 and 1168 autosomal SNPs in dataset I and in the merged dataset, respectively. The merged dataset that included genotyping information on 8 cervid species (66 individuals) has been used only for evaluating genetic relatedness based on Nei's genetic distances and PCA analysis.

**Genetic diversity.** In total, 1530 SNPs localized on autosomes were used to evaluate population ge-

doi: 10.17221/8457-CJAS

netic indices of the analyzed cervids after applying quality control in dataset I. For cervid population and locus combination, the departure from HWE was estimated with the Fisher's exact test using GENETIX software (Version 4.05, 2004). Observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosity were calculated using Arlequin software (Version 3.0, 2005) to evaluate the genetic diversity among cervids. The genetic differentiation within and among cervids and pairwise  $F_{ST}$  were measured also by the analysis of molecular variance (AMOVA) with 1000 permutations.

The genetic relatedness among cervids was analyzed based on 1168 polymorphic autosomal SNPs that were obtained after quality control application in the merged dataset. The genetic distances across and within individuals were calculated from allelic frequency and quantified by Nei's D distance (Nei 1982) with 100 bootstrap replications. The Neighbor-Joining tree based on the genetic distance between pairwise combinations of individuals was applied under the model of Nei (1982) using PowerMarker software (Version 3.25, 2006) and visualized using FigTree (Version 1.4.2, 2009).

The principal component analysis (PCA) was carried out to evaluate the population structure pertaining to the individuals and species. PCA was applied over the whole study population and performed according to Zheng (2013). To determine the principal components, genetic covariance

matrix was calculated based on genotype data, and correlation coefficient between sample loadings and genotypes was computed for each SNP across all species. PCA was performed using the R software packages SNPRelate and gdsfmt (2009).

## RESULTS AND DISCUSSION

**Distribution and variation of SNPs.** Of the total 54 609 SNPs on the BovineSNP50 BeadChip in dataset I, 28 502 autosomal loci were successfully genotyped in at least 90% of individuals. Call rates for free range Red, farmed Red, and Fallow deer ranged from 60.75 to 61.73%. The analysis of cervids screening data showed lower genotype call rate across autosomal SNPs (61.26%) compared to *Bos taurus* or other species from the family *Bovidae*. The genotype call rate is useful as a screening tool for data quality and genomic evaluations and is related to genotype accuracy on a SNP and animal basis (Cooper et al. 2013). The worldwide threshold ranges from 80 to 90% and genotypes with lower threshold are mostly eliminated from evaluation (Cooper et al. 2013), but cross-species call rate decreases by about 1.5% with each million year divergence between species (Miller et al. 2012). Despite of the 25.1–35.1 million years divergence between the families *Bovidae* and *Cervidae* (Hassanin and Douzery 2003), the call rate was relatively high. Results of cross-species geno-

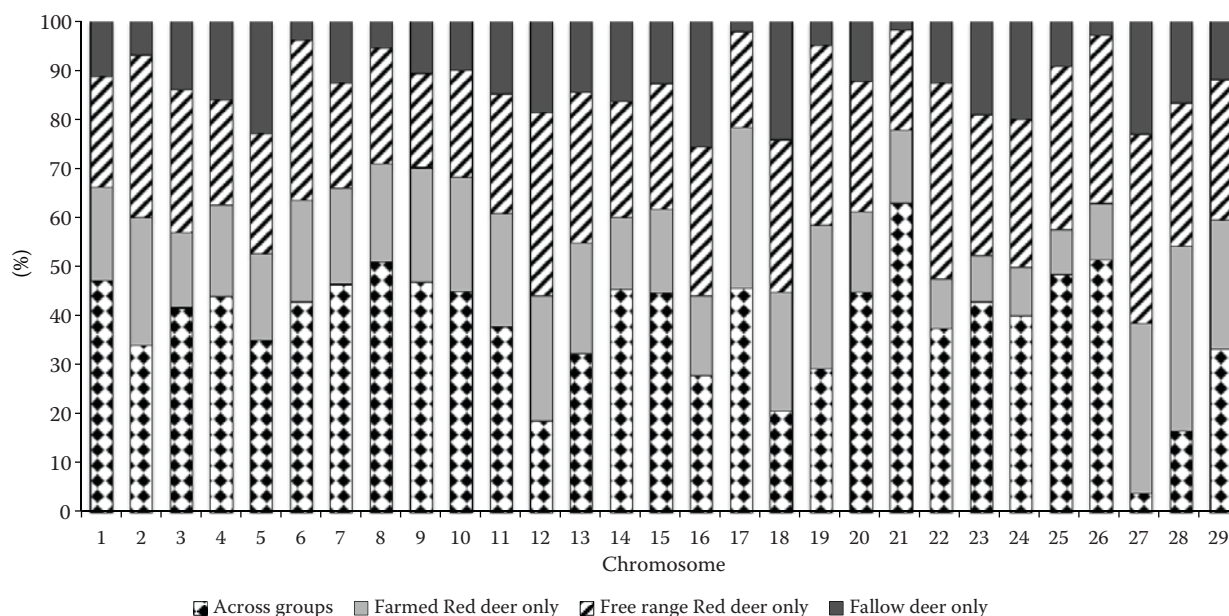


Figure 1. Proportion of SNPs (in %) from the total of 1530 autosomal loci by polymorphic status across cervid groups

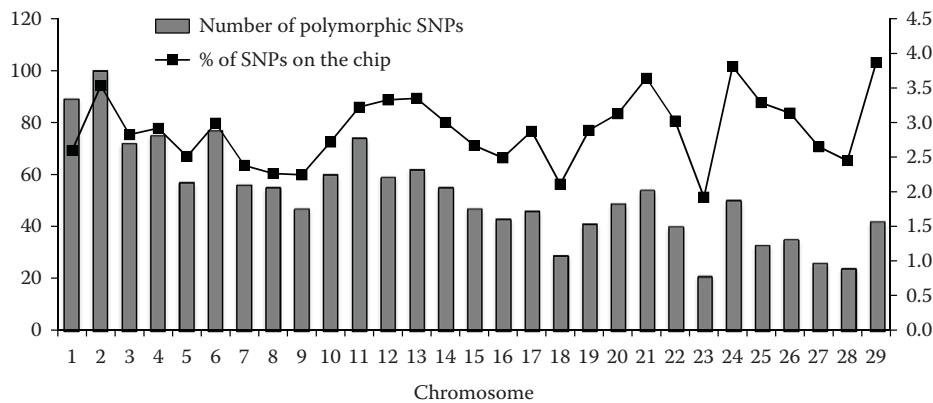


Figure 2. Distribution of the 1530 polymorphic SNPs within each autosome and their proportion to the total number of SNPs on the BovineSNP50 BeadChip

typing must be described with caution, because the genotyping array prepared for *Bos taurus* is not fully representative to the cervids genome. Moreover, the karyotypes of cervids and bovids are very different (cattle  $n = 30$ , cervids  $n = 34$ ).

Even though 53.89% of autosomal SNPs from the bovine chip could be genotyped, most of them were monomorphic in each cervids group. In total 94.63% of the successfully genotyped autosomal loci had only one allele in all individuals, and 1530 SNPs were polymorphic. Subsequently analyzed individuals will have a total of 20 alleles at a locus so with MAF of 5.37% there needs to be only one alternate allele to survive that threshold. Figure 1 shows the number of polymorphic SNPs across all individuals that are shared by each group vs SNPs private to one group. Fallow deer showed the highest proportion of polymorphisms in the analyses of separate groups, but differences were low (710–735). In comparison, Haynes and Latch (2012) successfully genotyped 5.1% of polymorphic SNPs in deer using the same chip. Despite the fact

that the water buffalo is evolutionarily closer to cattle than is the deer, Michelizzi et al. (2011) and Wu et al. (2013) identified only 1.87% and 2.65% polymorphic SNPs, respectively.

Most of polymorphic loci (100) were localized on chromosome 2 and only 21 SNPs were assigned to chromosome 23. The number of fixed SNPs in each group was high and ranged from 98.64 to 98.69%. Proportion of the retained loci after quality control of data to the total number of SNPs on the bovine chip is shown in Figure 2. The distribution of polymorphic SNPs over the entire genome of all autosomes across cervids was not uniform. A significant positive correlation ( $P < 0.0001$ ) was found between the total number of SNPs in the genotyping array and the number of retained loci. Across and within cervids groups each autosome consisted of a variable number of polymorphic SNPs (Figure 3). Free range and farmed Red deer displayed a comparative pattern of SNP distribution over autosomal chromosomes. Relatively different levels of polymorphisms on all chromosomes were observed in Fallow deer.

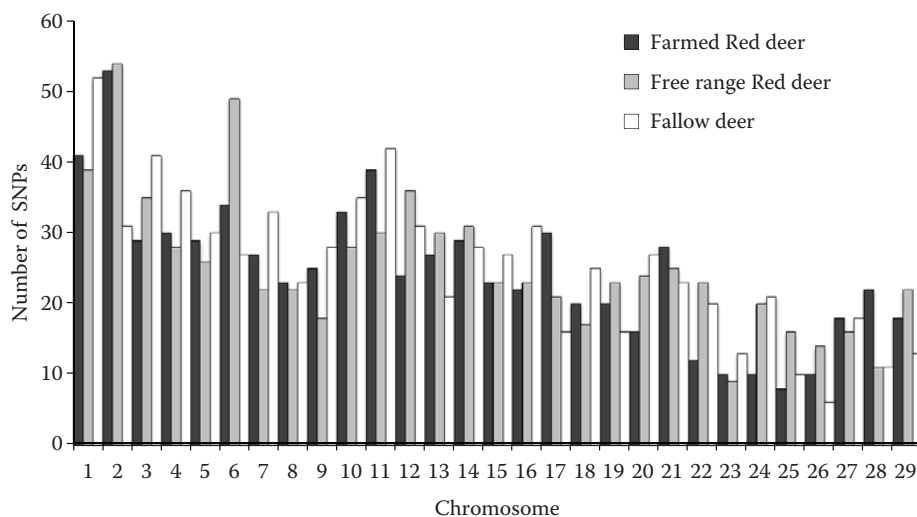


Figure 3. Distribution of polymorphic SNPs on autosomes of free range Red, farmed Red, and Fallow deer



doi: 10.17221/8457-CJAS

Table 1. Minor allele frequencies (MAF) for individual cervid groups

MAF	Animals in total ( $n = 10$ )		Free range Red deer ( $n = 5$ )		Farmed Red deer ( $n = 3$ )		Fallow deer ( $n = 2$ )	
	loci $n$	%	loci $n$	%	loci $n$	%	loci $n$	%
< 0.1	402	26.27	845	55.23	984	64.31	1063	69.48
0.1–0.2	666	43.53	201	13.14	121	7.91	0	0.00
0.2–0.3	80	5.23	85	5.56	20	1.31	30	1.96
0.3–0.4	71	4.64	39	2.55	46	3.01	65	4.25
0.4–0.5	311	20.33	360	23.53	359	23.46	372	24.31
Average	$0.23 \pm 0.13$		$0.33 \pm 0.17$		$0.40 \pm 0.13$		$0.45 \pm 0.06$	
Total SNPs	1530		685		546		467	

Minor allele frequencies for each cervids group calculated from genotyping data are presented in Table 1. Average common values of MAF were  $0.23 \pm 0.13$ . The analysis of all the 1530 autosomal SNPs revealed the average MAF of  $0.33 \pm 0.17$ ,  $0.40 \pm 0.13$ , and  $0.45 \pm 0.06$  for free range Red, farmed Red, and Fallow deer, respectively. The average value for Fallow deer was by 12 and 27% higher than the values for free range and farmed Red deer, respectively. Common variants of MAFs ( $\geq 0.1$  and  $\leq 0.5$ ) in free range and farmed Red deer accounted for 44.78 and 35.69% of the total polymorphic SNPs. A lower proportion of common variants SNPs showed Fallow deer (30.52%). Comparison between the three groups of cervids revealed a highly significant ( $P < 0.0001$ ) difference between Red and Fallow deer for minor allele frequencies of polymorphic loci. Small size of the evaluated groups could provide a relevant bias on the obtained values of MAF.

**Genetic diversity.** The evaluation of genetic structure and variability across the cervids groups in dataset I was carried out by estimating the heterozygosity level and  $F$  statistics using 1530 SNPs localized on autosomes. The number of polymorphic SNPs between Red and Fallow deer significantly differed ( $P < 0.0001$ ). Departures from HWE were non-significant ( $P > 0.05$ ) in dataset I. The average expected and observed heterozygosities across the

cervids groups were 0.499 and 0.587, respectively. The average values of estimated  $F_{IS}$  across cervids (within population inbreeding estimates) and  $F_{IT}$  (total inbreeding) were  $-0.097$  and  $0.326$ , respectively. The value of  $F_{IS}$  close to zero indicated a sufficient proportion of heterozygotes within the particular population, but the positive value of  $F_{IT}$  indicated a higher proportion of homozygous genotypes in all evaluated animals (Table 2).

The AMOVA revealed that most of the variation was distributed within individuals (67.38%), while less of the variation was attributed to variation among the cervids species (subpopulation). The remaining 0.88% of variations was explained by differences among individuals within subpopulations (Table 2).

The pairwise  $F_{ST}$  value ranged from 0.06 (between free range and farmed Red deer) to 0.74 (between Fallow and farmed Red deer) and identified Fallow and free range Red deer to be more close populations than those of Fallow and farmed Red deer. Similar results were obtained with Nei's genetic distance matrix. The highest genetic distance was found between farmed Red and Fallow deer (0.79), while farmed and free range Red deer were the closest to each other (0.56). The intra-population distance among free range Red deer was 0.04 and among farmed Red deer 0.03 on average. The genetic distance among Fallow deer was low (0.01).

Table 2. Analysis of molecular variance (AMOVA) based on 1530 autosomal SNPs among three cervid subpopulations

Source of variation	d.f.	Sum of squares	Variance components	Fixation indices	Percentage of variation
Among subpopulation	2	1106.30	63.02	$F_{ST} = 0.31743^1$	31.74
Among individuals within subpopulations	7	960.75	1.75	$F_{IS} = 0.01292^2$	0.88
Within individuals	10	1337.50	133.75	$F_{IT} = 0.3262^3$	67.38
Total	19	3404.55	198.51515		

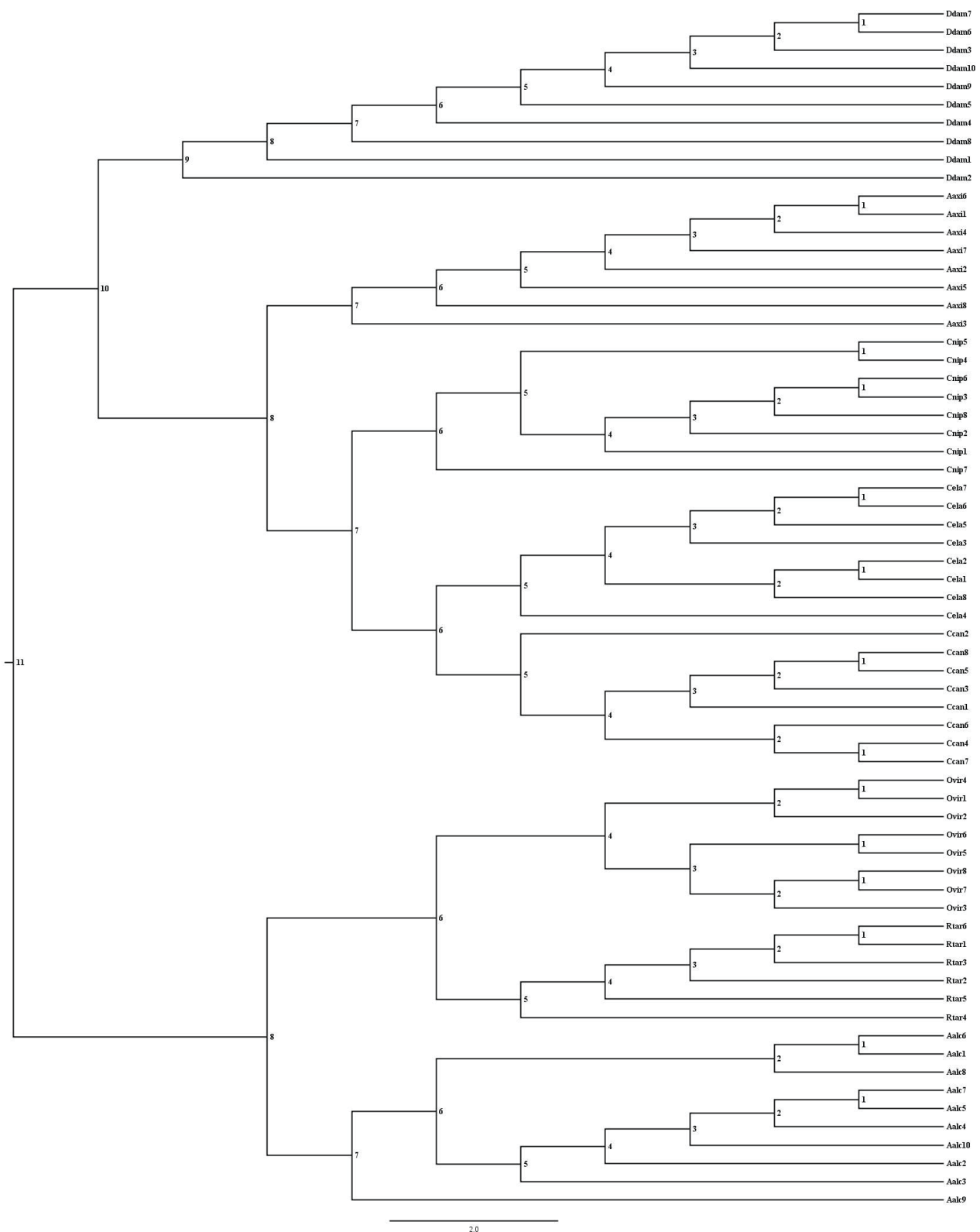


Figure 4. Neighbor-Joining tree based on genetic distances among cervids calculated using 1168 autosomal SNPs according to Nei (1972) with dataset II by Decker et al. (2009)

Aalc = North American moose, Aaxi = Axis deer, Ccan = Wapiti, Cnip = Sika deer, Ddam = Fallow deer, Ovir = White-tailed deer, Rtar = Caribou, Cela = Red deer

doi: 10.17221/8457-CJAS

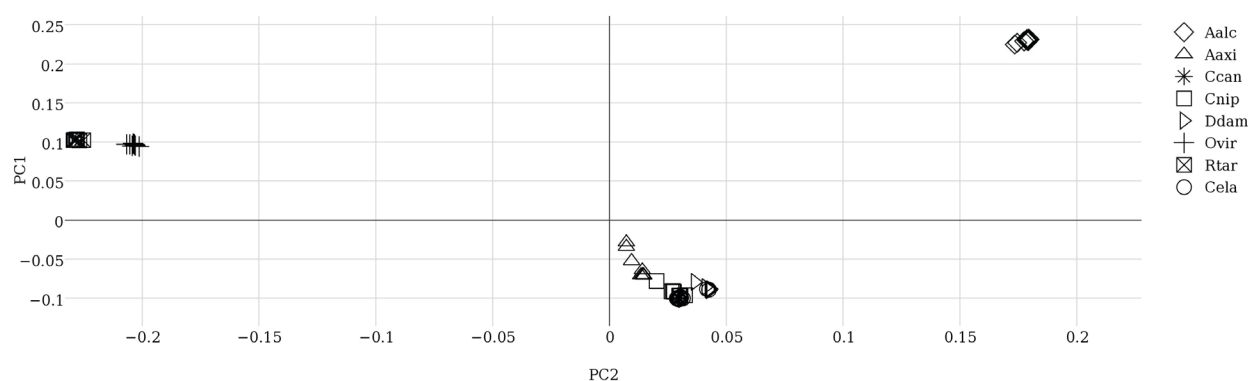


Figure 5. Principal component analysis of the genetic relationship between the analyzed species from the family *Cervidae* and those from the dataset II by Decker et al. (2009)

PC1 = first principal component, PC2 = second principal component, Aalc = North American moose, Aaxi = Axis deer, Ccan = Wapiti, Cnip = Sika deer, Ddam = Fallow deer, Ovir = White-tailed deer, Rtar = Caribou, Cela = Red deer

The resultant phylogenetic tree was constructed by the Neighbor-Joining method using dataset II to illustrate better the genetic relationships. In addition to Red and Fallow deer, 6 other cervid species were included in the final analysis that was based on 1168 polymorphic autosomal SNPs observed across all individuals (66). The phylogenetic tree shows the clear separation of all analyzed species from the family *Cervidae* (Figure 4).

The PCA was performed on the basis of dataset II. It was prepared based on the computation of the genetic covariance matrix using genotype data (overall 1168 autosomal polymorphic loci) and the correlation coefficient between sample and genotypes for each SNP across all species. The cervid species were clearly differentiated by the first two principal components which were sufficient to explain the genetic structure of evaluated individuals (Figure 5). The first principal component (PC1) explained 23.89% of the SNP variation, while the second component (PC2) explained 13.94% of the variances of each species. The PCA results were very similar to those provided by the Neighbor-Joining tree (Figure 4). The analysis showed the division of the *Cervidae* cluster into 7 subpopulations. Red deer, Wapiti, and Sika deer were very tightly clustered, whereas Fallow and Axis deer were located in a separate and looser cluster. American moose was very clearly segregated from other species. Similarly, White-tailed deer and Caribou were separated from each other and located in individual subpopulations.

Our results indicate that the commercially developed SNP arrays can be successfully applied also

in the case of evolutionarily related species. The small sample size used in our study greatly affects the level of genetic diversity that can be seen at the given locus. For non-model organisms any process of SNPs discovery brings about some risk of ascertainment bias, the systematic deviation from the expected allele frequency distribution (Haynes and Latch 2012), which may occur if the SNPs are generally identified in a small panel of individuals from a part of the species range (Heylar et al. 2011). Ascertainment bias may compromise the analyses based on diversity measures (Seeb et al. 2011), it can lead to wrong inferences of genetic diversity and population structure, and also can be introduced when subsequently applied to a larger sample of individuals (Albrechten et al. 2010). The ascertainment bias is introduced when comparing the genetic variability in cattle and cervids, because the loci on the bovine chip may not be representative of the evolutionary changes in the *Cervidae* family (Haynes and Latch 2012). However, high-density SNP assays have still been used for cross-species genotyping because genome-wide data of many wild species are not available. Genome-wide SNP genotyping assays developed for one species were successfully used for a rapid phylogenomic analysis across a broad taxonomic range and are powerful tools for population and evolutionary studies (Decker et al. 2009, Miller et al. 2012, Wu et al. 2013).

## CONCLUSION

The BovineSNP50 BeadChip represents a valuable resource for genomic research across several

evolutionarily related species. The present study examined its transferability to species from family *Cervidae*. Among 52 886 bovine autosomal SNPs on the chip, only 2.89% displayed polymorphism and were useful for subsequent genetic diversity study of evaluated cervids. Genetic differentiation between cervid species was demonstrated using the analysis of genetic distance based on autosomal SNPs. Only low differentiation was found between Red deer groups. Free range Red deer showed higher level of genetic identity to Fallow deer than farmed Red deer. The clear separation of the analyzed cervid species from each other was observed also by the principal component analysis with the involvement of 6 other species from the *Cervidae* family. The AMOVA showed that most of the genetic variation in the three analyzed cervid groups was distributed within individuals. Our results showed that the commercially developed genotyping array is a valuable tool for genetic diversity evaluation of wild animal populations. Another benefits of using SNP chips developed for a model species to identify novel SNPs in non-model organisms is the availability of information which can link SNP variation and distribution to DNA sequences and finally to map fitness related genes by genome-wide association studies.

## REFERENCES

- Albrechtsen A., Nielsen F.C., Nielsen R. (2010): Ascertainment biases in SNP chips affect measures of population divergence. *Molecular Biology and Evolution*, 24, 1–20.
- Bixley M.J., Ward J.F., Brauning R., Archer J.A., Fisher P.J. (2009): Building a deer SNP chip. *Proceedings of the Association for the Advancement of Animal Breeding and Genetics*, 18, 300–303.
- Cooper T.A., Wiggans G.R., VanRaden P.M. (2013): Short communication: Relationship of call rate and accuracy of single nucleotide polymorphism genotypes in dairy cattle. *Journal of Dairy Science*, 96, 3336–3339.
- Decker J.E., Pires J.C., Conant G.C., McKay S.D., Heaton M.P., Chen K., Cooper A., Vilkki J., Seabury C.M., Caetano A.R., Johnson G.S., Brennenman R.A., Hanotte O., Eggert L.S., Wiener P., Kim J.J., Kim K.S., Sonstegard T.S., Van Tassell C.P., Neibergs H.L., McEwan J.C., Brauning R., Coutinho L.L., Babar M.E., Wilson G.A., McClure M.C., Rolf M.M., Kim J., Schnabel R.D., Taylor J.F. (2009): Resolving the evolution of extant and extinct ruminants with high-throughput phylogenomics. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 18644–18649.
- Hassanin A., Douzery E.J.P. (2003): Molecular and morphological phylogenies of Ruminantia and the alternative position of the *Moschidae*. *Systematic Biology*, 52, 206–228.
- Hauser L., Baird M., Hilborn R., Seeb L.W., Seeb J.E. (2011): An empirical comparison of SNPs and microsatellites for parentage and kinship assignment in a wild sockeye salmon (*Oncorhynchus nerka*) population. *Molecular Ecology Resources*, 1, 150–161.
- Haynes G.D., Latch E.K. (2012): Identification of novel single nucleotide polymorphisms (SNPs) in deer (*Odocoileus spp.*) using the BovineSNP50 BeadChip. *PLoS One*, 7, e36536.
- Helyar S.J., Hemmer-Hansen J., Bekkevold D., Taylor M.I., Ogden R., Limborg M.T., Cariani A., Maes G.E., Diopere E., Carvalho G.R., Nielsen E.E. (2011): Application of SNPs for population genetics of nonmodel organisms: new opportunities and challenges. *Molecular Ecology Resources*, 11, 123–136.
- Lepoittevin C., Frigerio J.M., Garnier-Gere P., Salin F., Cervera M.T., Vornam B., Harvengt L., Plomin C. (2010): *In vitro* vs *in silico* detected SNPs for the development of a genotyping array: What can we learn from a non-model species? *PLoS One*, 5, e11034.
- Lewontin R.C. (1964): The interaction of selection and linkage. I. General considerations; heterotic models. *Genetics*, 49, 49–67.
- Manel S., Joost S., Epperson B.K., Holderegger R., Storer A., Rosenberg M.S., Scribner K., Bonin A., Fortin M.J. (2010): Perspectives on the use of landscape genetics to detect genetic adaptive variation in the field. *Molecular Ecology*, 19, 3760–3772.
- Michelizzi V.N., Wu X., Dodson M.V., Michal J.J., Zambano-Varon J., McLean D.J., Jiang Z. (2011): A global view of 54,001 single nucleotide polymorphisms (SNPs) on the Illumina BovineSNP50 BeadChip and their transferability to water buffalo. *International Journal of Biological Sciences*, 7, 18–27.
- Miller J., Poissant J., Kijas J., Coltman D. (2011): A genome-wide set of SNPs detects population substructure and long range disequilibrium in wild sheep. *Molecular Ecology Resources*, 11, 314–322.
- Miller J.M., Kijas J.W., Heaton M.P., McEwan J.C., Coltman D.W. (2012): Consistent divergence times and allele sharing measured from cross-species application of SNP chips developed for three domestic species. *Molecular Ecology Resources*, 12, 1145–1150.
- Morin P.A., Luikart G., Wayne R.K., SNP Workshop Group (2004): SNPs in ecology, evolution and conservation. *Trends in Ecology and Evolution*, 19, 208–216.
- Nei M. (1982): Evolution of human races at the gene level. In: Bonne-Tamir B. (ed.): *Human Genetics, Part A*:



doi: 10.17221/8457-CJAS

- The Unfolding Genome. Alan R. Liss, New York, USA, 167–181.
- Seeb J.E., Carvalho G., Hauser L., Naish K., Roberts S., Seeb L.W. (2011): Single-nucleotide polymorphism (SNP) discovery and applications of SNP genotyping in nonmodel organisms. *Molecular Ecology Resources*, 1, 1–8.
- Vasemagi A., Primmer C.R. (2005): Challenges for identifying functionally important genetic variation: the promise of combining complementary research strategies. *Molecular Ecology*, 14, 3623–3642.
- Williams L.M., Ma X., Boyko A.R., Bustamante C.D., Oleksiak M.F. (2010): SNP identification, verification, and utility for population genetics in a non-model genus. *BMC Genetics*, 11, 32.
- Wu J.J., Song L.J., Wu F.J., Liang X.W., Yang B.Z., Wathes D.C., Pollott G.E., Cheng Z., Shide S., Liu Q.Y., Yang L.G., Zhang S.J. (2013): Investigation of transferability of BovineSNP50 BeadChip from cattle to water buffalo for genome-wide association study. *Molecular Biology Reports*, 40, 743–750.
- Zheng X. (2013): A Tutorial for the R Package SNPRelate. University of Washington, Washington, USA.

Received: 2014–07–25

Accepted after corrections: 2015–03–12

---

*Corresponding Author*

doc. Ing. Radovan Kasarda, PhD., Slovak University of Agriculture in Nitra, Department of Animal Genetics and Breeding Biology, Tr. A. Hlinku 2, Nitra, 949 76, Slovak Republic  
Phone: +421 376 414 292, e-mail: Radovan.Kasarda@uniag.sk

---