



Investigation of the genetic distances of bovids and cervids using BovineSNP50k BeadChip

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Abstract. This study presents the application of BovineSNP50 BeadChip for genome-wide screening of two taurine breeds (*Bos primigenius taurus*) and Zebu (*Bos primigenius indicus*), and two species from the family Cervidae: red deer (*Cervus elaphus*) and fallow deer (*Dama dama*). The aim of the paper was to evaluate the use of bovine array for cross-species genotyping and analyse single nucleotide polymorphism (SNP) distribution, diversity within groups of animals and genetic distance among analysed species. The number of polymorphic SNPs decreased with the increase of phylogenetic distance between species, which also reflected a decrease in call rate (from 99.54 to 61.19 %). The minor allele frequency (MAF) values were significantly different between species and ranged from 0.18 ± 15 (Zebu) to 0.26 ± 0.14 (Pinzgau). The subsequent analyses of genetic diversity were based on the polymorphic loci detected in cervids. Differences in the expected heterozygosity was low (0.06), on average 0.34. In analysed groups the F_{IS} values were close to zero, which suggested low SNP variance within them. The value of F_{IT} indicated homozygote excess in evaluated individuals. Analysis of molecular variance revealed that most of the variability was distributed within all individuals. Observed genetic distances within and across groups of animals suggested that taurine cattle and cervids were more distant. The study results showed that genotyping array prepared for model species can be applied not only to organisms for which was developed, but can be also successfully used in closely related and more phylogenetically divergent species.

1 Introduction

The families Bovidae and Cervidae belong to the Pecorans (higher ruminants, order Artiodactyls), the most important group of large terrestrial herbivorous mammals. A 25.1–30.1 million year divergence was found between these two families. The richness in species and geographical spread of ruminants are reasons for the general interest in much phylogenetic and diversity research (Hassanin and Douzery, 2003; Fernández and Vrba, 2005; Decker et al., 2009). Bovidae and Cervidae represent the greatest degree of taxonomic and geographical diversity among the ruminants, with 48 bovid genera in Africa, most of Eurasia, and North America and 16 cervid general from mainly North and South America and Eurasia (Hassanin and Douzery, 2003). Traditionally, the

phylogenetic relationships of the majority of groups have been estimated using morphological data from extant and fossil taxa; however, the use of molecular markers and genomic data provide an unparalleled tool for the evaluation of genetic relationships among organisms and has exploded in recent years (Marcot, 2007; Bibi, 2013). The discovery of molecular markers usable for phylogenetic and population research incorporates markers derived from both mitochondrial DNA and nuclear genomes. The sequencing of complete mitochondrial DNA (mtDNA) of many species enabled the determination of the evolutionary history and phylogenetic relationship in more detail and promotes research on molecular evolution of these animal groups. Currently, more than 500 complete mtDNA genomes from vertebrates have been determined, including livestock and free-living

species such as those from the family Cervidae (Wada et al., 2010). Because of complete genome-wide sequences not being available for all species, much of the analyses in the discovery of nuclear DNA markers of non-model organisms are based on comparative work with already sequenced genomes of other “model” species (Hall, 2009). Nevertheless, the phylogenetic relationships among Cervidae and Bovidae species have been until now investigated using both mitochondrial and nuclear markers (Decker et al., 2009; Wada et al., 2010; Zhang and Zhang, 2012). Moreover, the availability and popularity of single nucleotide polymorphisms (SNPs) as useful nuclear markers for investigating not only evolutionary processes but also genetic diversity parameters are growing. The genomic programmes in livestock species have enabled the building and application of large SNP panels containing more than 50 000 markers (Bixley et al., 2009). The application of SNPs in population genetics studies of non-model organisms can be difficult due to the absence of genomic resources (Seeb et al., 2011). However recent studies have shown that the high-density SNP array development for livestock species such as cattle, sheep and horse can be successfully used in closely related and also phylogenetically more distant non-model species, in which they can yield large numbers of markers for relatively modest technical efforts and expenditure (Pertoldi et al., 2010; Haynes and Latch, 2012; Miller et al., 2012; Hoffman et al., 2013).

The aim of this study was to evaluate the transferability of bovine SNP array for cross-species genotyping in closely related and more evolutionary distant species and analyse variation and distribution of useful polymorphic SNPs for the determination of genetic diversity parameters and the relationship between species based on genetic distance and pairwise F_{ST} .

2 Material and methods

Samples of semen were collected from in total 89 males creating four different groups (of that 19 Pinzgau, 30 Brown Swiss, 30 Zebu and 10 cervids) and used for analysis. Ten cervid samples were obtained from unrelated farmed three red deers (male progeny of New Zealand sires and dams from Hungary) and free range four red and three fallow deer. Genotyping of samples was carried out in a commercial lab using Illumina BovineSNP50 BeadChip. A 50K SNP array was constructed with 54 609 SNPs in Pinzgau and cervids, 54 001 in Brown Swiss and 48 734 in Zebu.

Quality control of genotype data was performed according to Purcell et al. (2007). The proportion of usable SNPs for analyses of genetic diversity and relatedness between animals was computed by identifying polymorphic SNPs within the arrays used in all analysed groups. The quality control was arranged to remove any SNPs with more than 10 % of missing genotype, SNPs with less than 0.05 minor allele frequency (MAF) and Hardy–Weinberg equilibrium test with

limit of 0.0001. The variations and distribution of remaining SNPs across and within all chromosomes for each group were evaluated in the first step. Subsequently, for the analysis of genetic structure within and across all animal groups, only polymorphic loci localized on autosomal chromosomes were selected.

After quality control application, only autosomal loci based on cervids polymorphic SNPs from all data sets were selected in order to infer genetic diversity and relationships among the analysed groups. The Hardy–Weinberg equilibrium (HWE) within each analysed group was tested using Fisher’s exact test (Raymond and Rousset, 1995). Animal groups were evaluated by the F statistic (Wright, 1965) and inbreeding-like effects within subpopulations (F_{IS}), among subpopulations (F_{ST}) and across the entire analysed animal group (F_{IT}). Level of observed (H_o) and expected (H_e) heterozygosity and the F_{IS} indices (Weir and Cockerham, 1984) were calculated within each group according to Excoffier et al. (2005) and Belkhir et al. (2004). The genetic differentiation within and among groups and pairwise F_{ST} were measured by analysis of molecular variance (AMOVA) with 1000 permutations (Excoffier et al., 2005). The pairwise Nei’s genetic distances between individuals used for construction of neighbour-joining tree were calculated from allelic frequencies under the Nei 1982 model (Liu and Muse, 2001) with 100 bootstrap replications. A consensus phylogenetic tree was obtained by Phylip software package (Felsenstein, 2005).

3 Results and discussion

The number of successfully genotyped SNPs in least 90 % of individuals ranged from 98 % (taurine breeds) to 53.85 % (cervids). The call rate had a decreasing tendency from taurine breeds (99.15–99.54 %) to cervids (61.19 %) due to phylogenetic distances between analysed species and reflects the degree of diversity between their genomic regions. Genetic relatedness between *Bos primigenius taurus* and *Bos primigenius indicus* compared to cervids confirms only a slight decrease in detected value of call rate (98.91 %). By cross-species genotyping using bovine, ovine and equine SNP50 array, a consistent linear decrease in call rate was found of about 1.5 % per million years and retention polymorphisms showed an exponential decay (Miller et al., 2012).

A total of 37 256 (70.29 %) and 43 120 (79.79 %) of SNPs were found to be polymorphic (MAF > 0.05) in Pinzgau and Brown Swiss, respectively. The number of successfully genotyped SNPs in Zebu (47 502) was high compared to cervids (29 408). More than half of the SNPs in Zebu were monomorphic and 21 841 SNPs were polymorphic (45.98 %). Within analysed groups the lowest proportion of polymorphisms was found in species from the family Cervidae (red deer and fallow deer, order Cetartiodactyla), when only one allele was detected in 94.76 % of successfully

Table 1. MAF distribution of BovineSNP50 BeadChip in analysed groups.

Population	N	Average MAF	Fixed (0)		Rare (>0 and <0.05)		Intermediate (≥ 0.05 and <0.10)		Common (≥ 0.10 and ≤ 0.50)	
			SNP	%	SNP	%	SNP	%	SNP	%
Brown Swiss bulls	30	0.24 ± 0.15	11 298	21.31	4337	8.18	4582	8.64	32 790	61.86
Pinzgau bulls	19	0.26 ± 0.14	8480	15.69	2540	4.70	4792	8.87	38 230	70.74
Zebu cattle	30	0.18 ± 0.15	19 632	41.33	5964	12.56	4655	9.80	17 251	36.32
Cervids	10	0.23 ± 0.16	27 866	94.76	0	0.00	289	0.98	1253	4.26

genotyped SNPs, and 5.24 % (1542) of SNPs were polymorphic. Rapid decrease in the proportion of polymorphic SNPs in cervids can be related to the phylogenetic distance between them and the species for which was the array optimized. Miller et al. (2012) showed that only about 5 % of amplified loci remain polymorphic after application of genotyping array to species which diverged 3 million years ago. Only few studies so far have utilized commercially developed SNP arrays for genotyping more phylogenetically distant species (Decker et al., 2009; Haynes and Latch, 2012; Miller et al., 2012; Hoffman et al., 2013). Since the initial genotyping array has a large enough number of loci, even a low proportion of cross-amplifying SNPs may represent a useful set of markers for species which lack genomic resources (Hoffman et al., 2013). The distribution of SNPs over entire genomes of all chromosomes was not uniform and varied among the analysed groups. The proportion of polymorphic SNPs to the total number of SNPs for each chromosome on the genotyping arrays is shown in Fig. 1. The highest proportion of polymorphisms and lowest level of fixed SNPs within analysed groups showed Pinzgau and Brown Swiss with proportions of monomorphic loci of only 20.21 and 29.71 %, respectively. Conversely the highest level of fixed SNPs showed cervids and across all analysed groups was the number of fixed SNPs highest on chromosome 1 (ranging from 709 to 1777). Only 1.18 % of SNPs were on average polymorphic across all evaluated groups. Figure 2 shows the proportion of common SNP variants and SNPs exclusive for bovids and cervids groups to the total number of markers. Most of polymorphic SNPs were localized on chromosome 1 (2694 in taurine cattle and 1364 in Zebu) and chromosome 2 (100 in cervids). In all analysed groups, the lowest number of polymorphic SNPs was found on chromosome X (ranging from 16 to 777). Chromosome Y has not been evaluated because the 50K bovine chip used in this study included only one Y specific SNP, which was additionally monomorphic in the cervids group.

The average value and distribution of minor allele frequency (MAF), which refers to the frequency of least common SNP allele, in analysed groups shown in Table 1. The values of MAF varied across analysed groups and ranged from 0.18 (Zebu) to 0.26 (Pinzgau). Significant differences were observed between different groups of animals

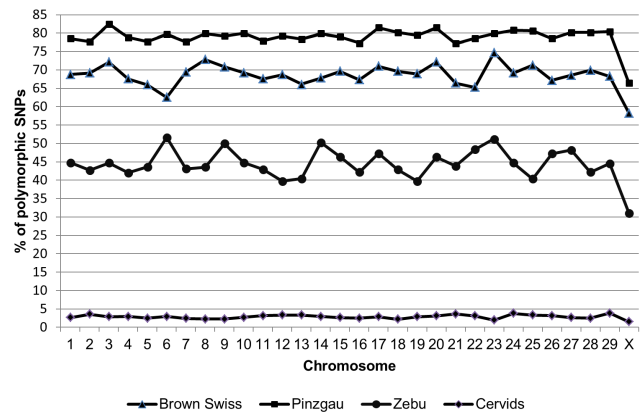


Figure 1. Proportion of polymorphic SNPs to the total number of SNPs for each chromosome on the BovineSNP50 BeadChip.

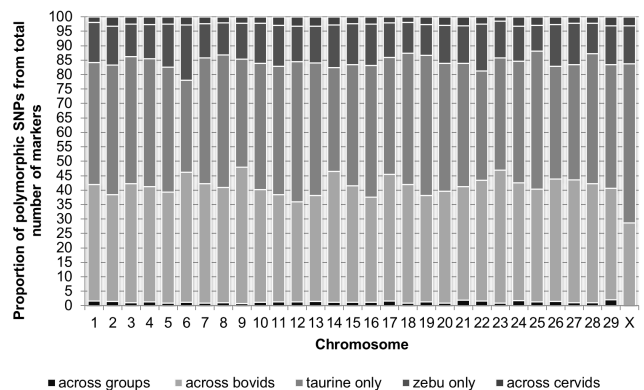


Figure 2. Proportion of SNPs by polymorphic status of the BovineSNP50 BeadChip across bovids and cervids.

and MAFs ($P < 0.0001$). The highest proportion of common MAF variants were found in taurine cattle (61.86 and 70.74 %) and the lowest in cervids (4.26 %). Taurine and Zebu cattle had an excess of rare MAF SNPs compared to cervids. The average values of MAF for both analysed cattle breeds were lower than the mean values reported for taurine breeds (Boichard et al., 2012; Wiggans et al., 2012). The value found for Zebu and cervids was comparable with results reported by Mustafa et al. (2014) and Haynes and Latch (2012), respectively. The variations in values of MAFs in

Table 2. HWE p values, expected (H_e) and observed (H_o) heterozygosity and fixation indexes (F_{IS}).

	Brown Swiss bulls	Pinzgau bulls	Zebu cattle		Cervids
HWE	0.995	0.997	0.999	P value – highly significant	< 0.05
H_e	0.37 ± 0.13	0.38 ± 0.13	0.32 ± 0.14		0.32 ± 0.15
H_o	0.37 ± 0.14	0.38 ± 0.15	0.32 ± 0.16		0.28 ± 0.37
F_{IS}	-0.02 ± 0.0001	0.003 ± 0.0001	-0.01 ± 0.0001		0.13 ± 0.0006

Table 3. Analysis of molecular variance (AMOVA) based on selected SNPs.

Source of variation	Degrees of freedom	Sum of squares	Variance components	Fixation indices	Percentage of variation
Among subpopulation	3	10 533.07	79.72	$F_{ST} = 0.33407$	33.41
Among individuals within subpopulations	85	13 599.21	2.52	$F_{IS} = 0.01589$	1.06
Within individuals	89	13 793	156.39	$F_{IT} = 0.34465$	65.53
Total	177	37 925.28	238.64		

presented results and previous studies could be caused by different densities of SNPs on the used genotyping arrays. Moreover the MAF value can be affected in the application of cross-species genotyping arrays by ascertainment bias, the systematic deviations from the expected allele frequency distribution leading to downstream biases in the evaluation of many indices (Satkoski Trask et al., 2011). Probability that a SNP is identified in ascertainment panel with small size compared to the population is a function of its MAF and the SNP discovery process, favours the most variable sites and eliminates rare SNPs (Garvin et al., 2010; Helyar et al., 2011). The evaluation of population genetic parameters such as diversity can be biased mainly because the SNPs on the chip optimized for model species are not completely representative for non-model animals. The number and variability of individuals used in SNP discovery panel in non-model species can also lead to some degree of ascertainment bias because the panel consists of only a small subset of the genotyped individuals (Albrechtsen et al., 2010). The degree of ascertainment bias depends on the number in which the SNPs were originally found and results in a skewing of the allele frequency spectrum towards common alleles (De Donato et al., 2013). In our data set, some level of ascertainment bias could be introduced because bovine genotyping array was used to determine polymorphic SNPs in closely related (*Bos primigenius indicus*) and more phylogenetically distant species from the family Cervidae, and therefore the comparison should be interpreted with caution.

Genetic variability and phylogenetic relationship within and between analysed groups was evaluated based on selected polymorphic loci ($MAF \geq 0.05$) from cervids (Table 1). The level of expected heterozygosity was relatively comparable in all evaluated groups of animals. The highest H_e was observed in Pinzgau and lowest in cervids and Zebu, but differences were very low (0.06). The observed heterozy-

gosity ranged from 0.28 (cervids) to 0.38 (Pinzgau). In taurine and Zebu groups, HWE equilibrium was found (Table 2). In cervids, the variation in proportion of SNPs led to the deviation from the Hardy–Weinberg equilibrium ($P < 0.05$). Because of the low number of cervids and the fact that this group consists of two different species, the determination of HWE in this case is a modelled computation to obtain an overall view of the data. The within population fixation index (F_{IS}) had negative values in Brown Swiss and Zebu, suggesting relatively higher proportion of heterozygote genotypes and conversely positive values in Pinzgau and cervids. All F_{IS} values were close to zero (Table 2), suggesting low SNP variance within separately evaluated groups. The average estimated F_{IS} (within group inbreeding estimates) and F_{IT} (total inbreeding) values were positive (Table 3), indicating a higher proportion of homozygote genotype on the level of particular groups and all evaluated animals as well.

The AMOVA and ascertained value of F_{ST} revealed that the most of the variance was distributed within all evaluated individuals (Table 3). A lesser part of the genetic variation (33.41 %) was attributed to variation among the groups. Only a very low proportion of variation explained differences among individuals within groups.

The genetic relationship between analysed species was examined using pairwise F_{ST} and Nei's genetic distances. The pairwise F_{ST} estimated between analysed groups based on total of 1530 autosomal SNPs shown in Table 4. The F_{ST} values from pairwise comparisons between different groups ranged from 0.11 (Pinzgau vs. Zebu) to 0.47 (Brown Swiss vs. cervids). As expected, the most genetically differentiated group of those compared was cervids. The higher observed F_{ST} value between Pinzgau and Brown Swiss compared to Pinzgau vs. Zebu was unexpected, because Zebu groups are phylogenetically more distant, which also suggests the observed pairwise genetic distance among them (0.21). The

Table 4. The pairwise F_{ST} values between analysed groups.

Group/Population	Brown Swiss bulls	Pinzgau bulls	Zebu cattle	Cervids
Brown Swiss bulls	0			
Pinzgau bulls	0.31738	0		
Zebu cattle	0.36378	0.10622	0	
Cervids	0.47166	0.36689	0.40346	0

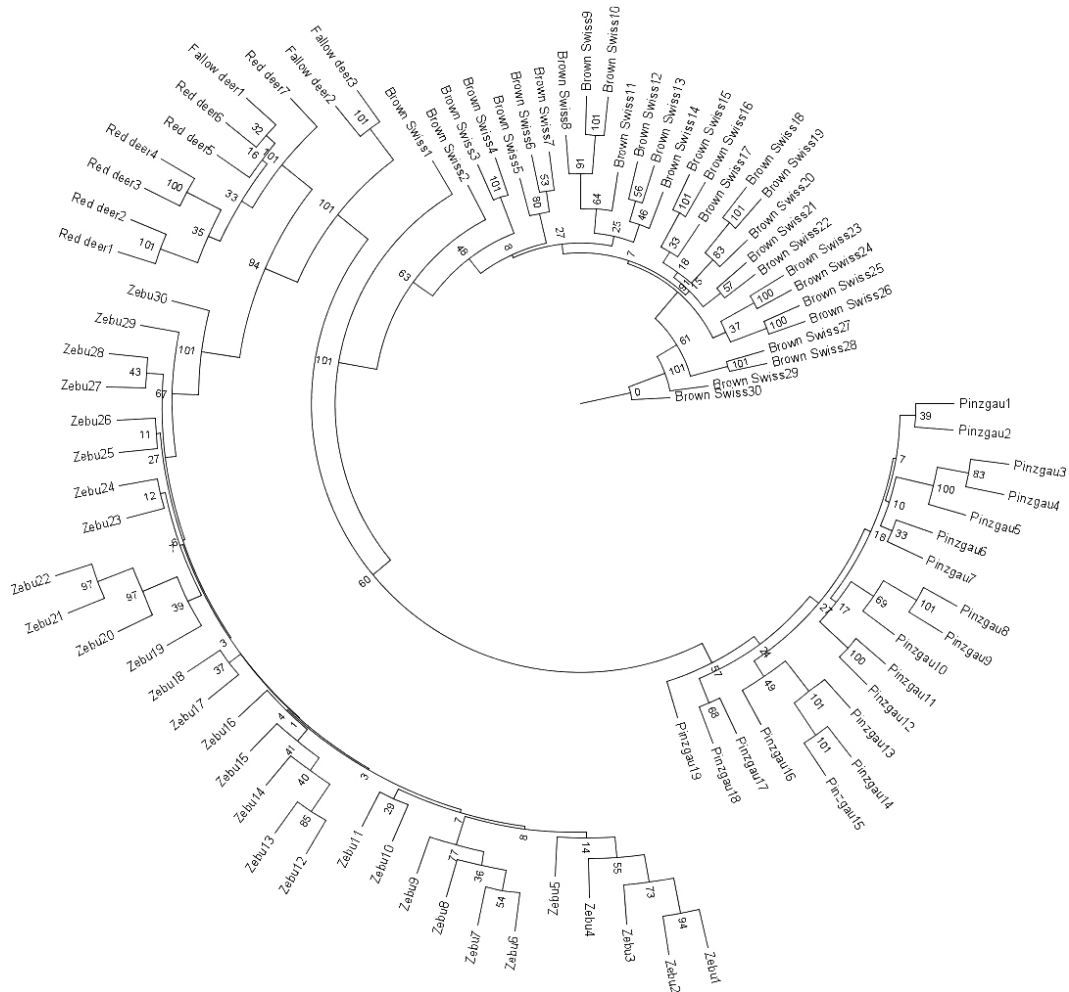


Figure 3. Neighbour-joining tree clustering genetic distances and bootstraps estimates among zebu, two taurine breeds and cervids.

average genetic distances between pairs of individuals from the same analysed groups was lowest between Pinzgau and Brown Swiss (0.07).

The greatest genetic distance between pairs of analysed groups showed Brown Swiss and cervids ($D = 0.31$). Similar distances between cervids were found also for Pinzgau ($D = 0.28$) and Zebu ($D = 0.30$). Pinzgau were relatively genetically closer to cervids than Zebu, but the difference was only 0.02. The genetic distance between Zebu and Pinzgau were lower than between Brown Swiss and Zebu (0.21 vs. 0.24). Overall the results showed that the most genetic

distant from all other evaluated groups were cervids, and the closest were the taurine breeds. The neighbour-joining tree (Fig. 3) created from allelic frequencies of polymorphic SNPs, selected based on data set from cervids, showed clear differentiation between the two taurine breeds, Zebu and cervids.

Nevertheless, though the genotyping array used in our study was primarily produced for application in bovine cattle, the results showed that it can be successfully used in analyses of diversity and genetic relationship in phylogenetically close and also distant species. However, the results for any

discovery process of SNPs in non-model organisms based on a genotyping array designed for phylogenetically distant species must be interpreted with caution because it may be affected by ascertainment bias. However, if the genotyping arrays for non-model species are not commercially available, utilization of these arrays is one possible approach for genetic research in this area.

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