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Brief Communication

A Study of Applicability of SNP Chips Developed for Bovine and Ovine Species to Whole-Genome Analysis of Reindeer *Rangifer tarandus*

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Abstract

Two sets of commercially available single nucleotide polymorphisms (SNPs) developed for cattle (BovineSNP50 BeadChip) and sheep (OvineSNP50 BeadChip) have been trialed for whole-genome analysis of 4 female samples of *Rangifer tarandus* inhabiting Russia. We found out that 43.0% of bovine and 47.0% of Ovine SNPs could be genotyped, while only 5.3% and 2.03% of them were respectively polymorphic. The scored and the polymorphic SNPs were identified on each bovine and each ovine chromosome, but their distribution was not unique. The maximal value of runs of homozygosity (ROH) was 30.93 Mb (for SNPs corresponding to bovine chromosome 8) and 80.32 Mb (for SNPs corresponding to ovine chromosome 7). Thus, the SNP chips developed for bovine and ovine species can be used as a powerful tool for genome analysis in reindeer *R. tarandus*.

Subject areas: Conservation genetics and biodiversity
Key words: reindeer *Rangifer tarandus*, SNP, whole-genome analysis

Rangifer tarandus has a circumpolar distribution in the tundra and taiga zones of northern Europe, Siberia, and North America (Corbet 1978; Hall 1981; Koubek and Zima 1999; Wilson and Ruff 1999). It represents one of the most interesting large mammal species of Russia, which plays an important role in the economy of the northern part of the country and Siberia, providing employment and prosperity to indigenous people of these vast regions. Reindeer are distributed across the Russian tundra from the Kola Peninsula to Chukotka including Siberia, and in the mountain-taiga zone. Because little is known about the genetic diversity and genetic structure of populations of reindeer inhabiting Russia, research in this field is undoubtedly significant at present.

Over the last 2 decades, advances in molecular genetics have introduced a new generation of molecular markers for the genetic characterization of livestock. Single nucleotide polymorphisms (SNPs) have become increasingly popular tools for the genetic study of natural populations; on the other hand, it can be difficult to develop them into nonmodel organisms (including deer) due to a paucity of genomic information (Seeb et al. 2011). However, recent studies have shown that medium-density SNP arrays (containing more than 50 000 markers) developed for domestic animal species including cattle, horse, pig, sheep, and dog (Fan et al. 2010) can be successfully applied to closely related nonmodel species. In fact, they can yield

a large number of markers for a relatively modest technical effort and expenditure (vonHoldt et al. 2012; Hoffman et al. 2013). For example, Pertoldi et al. (2010) identified 2209 polymorphic SNPs in European (*Bison bonasus*) and American bison (*Bison bison*) using Bovine SNP50 BeadChip developed for cattle (*Bos taurus*). Miller et al. (2011) identified 868 SNPs in bighorn (*Ovis canadensis*) and thinhorn sheep (*Ovis dalli*) applying the OvineSNP50 BeadChip developed for domestic sheep (*Ovis aries*). Several studies extend this approach to species, which are phylogenetically more distant from those in which the arrays were originally developed, including deer species. Ogden et al. (2012) described the application of the Bovine SNP50 BeadChip to discover SNPs in 2 antelope species, which yielded 148 polymorphic markers in the scimitar-horned oryx (*Oryx dammah*) and 149 in the Arabian oryx (*Oryx leucoryx*) from a total of 54 001 SNPs. Bixley et al. (2009) have mapped 43.9 Mbp genomic sequences of New Zealand red deer (*Cervus elaphus*) to unique positions in the bovine genome and selected 768 SNPs to be placed in a Golden Gate (IlluminaTM) SNP chip. Selected SNPs were aligned to all 30 bovine chromosomes. Haynes and Latch (2012) applied Bovine SNP50K BeadChip in order to characterize 2 reindeer species of Cervidae family (*Odocoileus hemionus* and *Odocoileus virginianus*); this led to the discovery of 1068 polymorphic SNPs. Despite the proportion of polymorphic SNPs is expected to decline rapidly with phylogenetic distance, dropping to around 5% for species that diverged 3 million years ago (Miller et al. 2012), the information obtained from these experiments has an essential value in genetic characteristic of species for which own DNA arrays are lacking.

The objective of our study was to trial 2 sets of commercially available chips developed for cattle (*B. taurus*) and sheep (*O. aries*) to whole-genome analysis of semi-domesticated reindeer *R. tarandus* inhabiting Russia.

Materials and Methods

In the present study, the tissue samples were collected from reindeer inhabiting the southeast region of Siberia. Four female samples were randomly selected and genomic DNA was extracted using Nexttec column (Nexttec Biotechnologie GmbH) according to the manufacturer's instructions. Genotyping was performed using 2 sets of commercially available chips (Illumina, Inc.) developed for cattle (Bovine SNP50 v2 BeadChip) and sheep (Ovine SNP50 BeadChip). Basic information including SNP name, chromosome, and map location was downloaded from the Illumina website (Illumina, Inc.). PLINK v1.07 (Purcell et al. 2007) was used to generate summary statistics for each arrays including individual and locus-specific call rates, assessment of the number of polymorphic sites. The SNPs, which were successfully scored on all 4 reindeer animals, were used for analysis. In addition, PLINK was used to calculate runs of homozygosity (ROH), which takes a window of 10 000 kb (100 SNPs) and slides it across the genome, determining homozygosity on each window. In compliance with data archiving guidelines (Baker 2013), we have deposited the primary data sets underlying these analyses with Dryad.

Results

Genotyping 4 reindeer *R. tarandus* using the Bovine SNP50 v2 BeadChip revealed that 23 481 out of 54 609 SNPs (43.0%) were successfully scored on all 4 reindeers, while 24 240 (44.4%) had no calls. The remaining 6771 SNPs (12.6%) were partially genotyped on various numbers of animals, including 2621 SNPs (1342 SNPs or 51.2% of which were polymorphic) on 1 animal, 2092 SNPs (1043 SNPs or 49.9%) on 2 animals and 2175 SNPs (930 SNPs or 42.7%) on 3 animals (Table 1). Only 1257 SNPs (2.3% of the total; 5.3% of fully genotyped loci) were found to be polymorphic in *R. tarandus*. The most frequent nucleotide substitution was A/G (987 SNPs that corresponds to 79.5% of polymorphic SNPs). The other substitutions were distributed between A/C (209 SNPs or 16.8%), C/G (25 SNPs or 2.0%), and A/T (21 SNPs or 1.7%). Thousand sixty-seven SNPs were characterized by the presence of only one of the homozygous genotypes, whereas 175 SNPs had both the heterozygous and the homozygous genotypes. The observed heterozygosity of polymorphic SNPs was 0.91 ± 0.01 and the expected heterozygosity was 0.48 ± 0.143. The values of ROH varied between animals from 13.96 to 15.17 Mb. The maximal value of ROH was 30.93 Mb for SNPs on BTA8. The linkage disequilibrium (LD) was $r^2 = 0.41$.

Genotyping 4 reindeer *R. tarandus* using the Illumina Ovine SNP50 BeadChip resulted in successfully scoring 25 512 of 54 241 SNPs (47.0%) on all 4 animals, while 24 487 (45.1%) had no calls. The others 4242 SNPs (7.96%) had calls on various numbers of animal samples, including 1585 SNPs (406 or 25.6% of which were polymorphic) on 1 animal, 1133 SNPs (275 or 24.7%) on 2 animals and 1524 SNPs (271 or 17.8%) on 3 animals (Table 1). The number of polymorphic SNPs was 519, which represents only 1.0% of the

Table 1. Statistics for different subsets of the BovineSNP50 BeadChip and the Ovine SNP50 BeadChip grouped by number of reindeer with a scored genotype

Number of samples	Number of scored SNPs	Heterozygosity		H-W, *P*-value	Number of polymorphic SNPs	Heterozygous SNPs, %
		Observed	Expected			
BovineSNP50 BeadChip						
4	23 481	0.04	0.03	0.97	1257	86
3	2175	0.41	0.21	0.77	930	91
2	2092	0.47	0.24	0.99	1043	89
1	2621	0.51	0.26	1.00	1342	100
0	24 240	—	—	—	—	—
Ovine SNP50 BeadChip						
4	25 512	0.02	0.01	0.99	519	70
3	1524	0.16	0.08	0.91	271	86
2	1133	0.23	0.12	0.99	275	87
1	1585	0.26	0.13	1.00	406	100
0	24 487	—	—	—	—	—

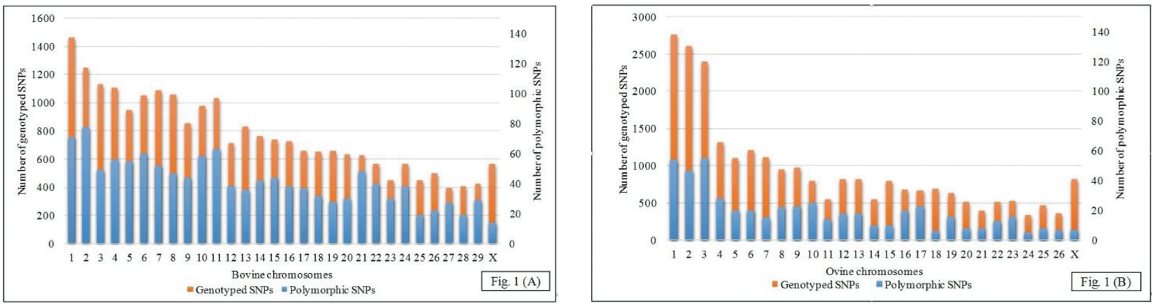

Figure 1. Overall distribution of polymorphic and genotyped SNPs among bovine (**A**) and ovine (**B**) chromosomes. *Note:* 14 bovine SNPs (A) and 3 ovine SNPs (B) with unknown chromosomal localization are not shown.

total number of SNPs and 2.03% of fully scored loci. The nucleotide substitution A/G was the most frequent (418 SNPs or 82.4% of polymorphic SNPs). The other substitutions were represented by A/C (80 SNPs or 15.8%), C/G (6 SNPs or 1.2%), and A/T (3 SNPs or 0.6%). Only one of homozygous genotypes was observed for 350 SNPs and 157 SNPs had both the heterozygous and the homozygous genotypes. The observed heterozygosity of polymorphic SNPs was 0.79 ± 0.01 and the expected heterozygosity was 0.44 ± 0.004. The ROH values varied from 29.45 to 37.80 depending on the animals. The maximal value of ROH was 80.32 Mb for SNPs on chromosome 7. The LD was $r^2 = 0.54$.

Evaluation of the chromosomal distribution of SNPs showed that both scored and polymorphic SNPs were identified on each bovine and each ovine chromosome, but their distribution was not unique. The number of genotyped bovine SNPs varied from 395 SNPs on BTA27 to 1463 loci on BTA1, while the lowest density of polymorphic SNPs was observed on BTA30 (14 SNPs) and the highest density was found on BTA2 (77 SNPs) (Figure 1A). The number of genotyped ovine SNPs varied from 337 SNPs on ovine chromosome 24 to 2764 SNPs on chromosome 1. The chromosomal distribution of the polymorphic ovine SNPs was characterized by the minimal value on chromosome 24 (5 SNPs) and had the maximal value on chromosome 1 (52 SNPs) (Figure 1B).

Conclusion

During the last decade, the results of the majority of studies relating to genetic diversity and population genetic structure of reindeer have been obtained considering variation in mitochondrial DNA and microsatellite DNA (Cronin et al. 2005; Ball et al. 2010; Røed et al. 2011; Colson et al. 2014). In the recent years, SNPs have increasingly been applied for these purposes. In nonmodel organisms, the SNPs can be used instead of microsatellite markers (or in tandem with them) in order to investigate genetic diversity, parentage identification and populations' structure (Tokarska et al. 2009; Garvin et al. 2010; Haynes and Latch 2012). Generally 2–3 SNPs are required to reach a level of effectiveness similar to the one normally obtained using a microsatellite marker (Schopen et al. 2008; Fernández et al. 2013), which might be very cost-effective and time consuming.

The SNPs proposed in the present study would permit the simultaneous genotyping of thousands of loci for an array of reindeer (or other deer species) and thereby create new opportunities and new challenges for scientists when trying to obtain broader genome coverage along with higher quality data on the structure of populations.

The application of SNP chips developed for cattle (Bovine SNP 50K v2 BeadChip) and sheep (Ovine SNP 50K BeadChip) on reindeer *R. Tarandus* revealed the call rates for fully scored SNP at 43.0 and 47.0%, respectively, but only 5.3% (1257 SNPs) and 2.0% (519 SNPs) of them were found to be polymorphic. By genotyping 2 other cervids species (*O. hemionus* and *O. virginianus*) using the Bovine SNP 50K BeadChip Haynes and Latch (2012) were able to score 38.7% loci (at the call rate of 90%), 5.1% of which were polymorphic. This data indicated that SNPs (but not the polymorphic SNPs) are relatively conserved between the Bovidae and Cervidae families which diverged between 25.1 and 30.1 million years ago (Hassanin and Douzery 2003). Taking into consideration a very wide distribution of reindeer genus *Rangifer*, the application of SNP analysis to genome-wide range will be highly useful for the characterization of genetic diversity patterns of local reindeer population.

Our study confirms that SNP chips developed for domestic animal species can be used as a tool for SNP discovery even in widely diverged nonmodel species including unique species such as *R. tarandus*.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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