

toward the sustainability of the livestock industry.

**Key Words:** recombination, selection, variation

**P4062 DNA sequencing and genetic polymorphism discovery in the canine monoamine oxidase A (MAOA) gene.** J. Sacco, A. Ruplin, P. Skonieczny, and M. Ohman (Drake University, Des Moines, IA)

Monoamine oxidase type A (MAOA) is an enzyme that degrades neurotransmitters. In humans, reduced activity of the MAOA enzyme due to genetic polymorphisms within the *MAOA* gene leads to increased neurotransmitter levels in the brain which may result in aggressive behavior. Our overall hypothesis is that, in dogs, aggression, a common behavioral problem, is influenced by variation within the canine *MAOA* gene. Therefore, the aim of this preliminary study was to identify novel alleles in functionally important regions of the canine *MAOA* gene, located on chromosome X. Genomic DNA was collected via cheek swabs from 50 non-aggressive pure-bred dogs (22 females, 28 males) representing diverse genetic clusters (ancient, herding, mastiffs, modern European, mountain). Following DNA purification, target regions of the canine *MAOA* gene were amplified, sequenced, and screened for polymorphisms. All genetic polymorphisms that were found have not been reported previously. Seven were single nucleotide polymorphisms (SNPs; two exonic, two intronic and three in the promoter) and four were repeat intronic variations. Two synonymous coding SNPs, c.1254 C > T and c.1567 C > T, were discovered in exons 12 and 15, respectively. Two highly heterozygous microsatellites (ATTT and TTTA repeats) were found in introns 1 and 10, respectively. The microsatellite region in intron 10 was represented by three alleles, representing variable numbers of TTTA repeats (10, 11, or 12). The polymorphism in intron 1 was an ATTT sequence inserted within a short interspersed nuclear element (SINE) that is unique to canids. Haplotype analysis indicated strong evidence of recombination between several alleles. Comparative genomic analysis demonstrated that a proximal promoter SNP (-212A > G), which was found in only two of the dogs sequenced, is the major allele in wolves and other related mammalian species not subject to domestication. This -212G allele is predicted to alter the binding of several transcription factors in the MAOA promoter. These novel MAOA polymorphisms provide information for follow-up behavioral genetic studies in aggressive dogs.

**Key Words:** canine, MAOA, aggression

**P4063 Genotyping of ApaLI RFLP at heat shock transcription factor 1 in Creole and Holstein cattle differing in coat type and associations with molecular breeding value and DHI traits.**

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Heat stress in dairy cattle is one of the major problems that contribute to farmer's economical losses in tropical climates. Dairy cattle under heat stress show lower milk yields, deficiency in reproductive traits and immune depression. Genetic selection of animals that present higher thermotolerance in high temperatures helps offsets the adverse effect of heat stress. In this study genotyping of ApaLI RFLP reported at the Heat Shock Transcription Factor 1 (HSF1) gene was performed in Puerto Rican Creole ( $n = 21$ ) and Holstein [Slick (SH;  $n = 36$ ) vs. Wild-Type (WT;  $n = 56$ )] cattle. Segregation of the three genotypes (TT, TC and CC) associated with this RFLP was observed in the three cattle groups. Allelic frequencies were 0.79 T/0.21 C and 0.68T/0.32 C for SH and WT Holsteins, respectively. In Creole, similar allelic frequencies were observed (0.69 T/0.31 C; Chi Square  $P > .05$ ). A significant association of this HSF1-RFLP with a molecular breeding value (Igenity, Neogen Corp.) for milk (TT, CT > CC;  $P < .05$ ) but was not phenotypically confirmed with milk adjusted at 305d (DHI;  $P > .05$ ). Conversely, an interaction between HSF1 and coat type was observed in calving interval ( $P = 0.0358$ ). In that regard, SH-TT presented a calving interval 52 d lower than WT-CT ( $P = 0.0240$ ). Also, WT-CC has 72.5 d lower calving intervals when compared with SH-CT ( $P = 0.0475$ ). These results suggest that the reproductive usefulness of this HSF1 polymorphism is dependent on the type of coat in Holstein cattle and that both natural (Creole) and artificial selection (SH/WT Holstein) favors the T allele.

**Key Words:** HSF1, slick, Creole

**P4064 Genetic diversity and population structure of wild and semi-domesticated reindeer (*Rangifer tarandus*) inhabited in northeastern Siberia based on single nucleotide polymorphism markers.**

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Reindeer is one of the most important species of farm animals in the northern parts of Russia. Wild populations are also tightly associated with the life of indigenous people. The study of reindeer genetic diversity has always interested scientists worldwide. One of the modern approaches for biodiversity assessment in non-model species (species whose genomes have not been sequenced yet) is screening for single nucleotide polymorphisms using DNA BeadChips, designed for their related model species. Our study aimed at characterizing genetic diversity and population structure of wild and semi-domesticated reindeer using commercially available SNP chips, developed for cattle. A total of 47 of wild and 38 of semi-domesticated reindeer individuals were included in this study. The samples of wild reindeer were collected in 14 different sites in northeastern Siberia (the Republic Sakha-Yakutia) and the samples of semi-domesticated reindeer were taken from two farms of the same region. DNA was extracted from tissue samples using Nexttec column (Nexttec Biotechnology GmbH, Germany) according to recommendation of manufacture. Genotyping was performed using Bovine SNP50 v2 BeadChip (Illumina Inc., San Diego, CA). Statistical analysis was performed with PLINK 1.07, Arlequin 3.5.2.2, HP-Rare 1.1 and GenAEx 6.5.1 software. In total, 625 SNPs were selected after quality filtering as the set of markers for further analyses. Pairwise  $F_{st}$  value between wild and semi-domesticated reindeer was 0.0497 ( $p < 0.001$ ). ANOVA indicated that the genetic variation mainly occurred within populations (95.06%) and the variance among populations was only 4.94%. The average number of effective alleles was  $1.942 \pm 0.009$  for wild reindeer in comparison with  $1.781 \pm 0.017$  alleles for semi-domesticated reindeer, the number of informative alleles was  $1.285 \pm 0.012$  and  $1.288 \pm 0.013$ , respectively. Allelic richness was significantly higher in wild population as compared to semi-domesticated population:  $1.92 \pm 0.01$  and  $1.78 \pm 0.02$ , respectively. The observed heterozygosity was  $0.178 \pm 0.006$  in wild reindeer and  $0.181 \pm 0.007$  in semi-domesticated reindeer. Insignificant differences in the levels of expected heterozygosity were obtained:  $0.184 \pm 0.006$  and  $0.181 \pm 0.007$  in wild and

semi-domesticated reindeer, respectively. Inbreeding coefficients were higher in wild reindeer ( $F = 0.029 \pm 0.008$ ) than in semi-domesticated reindeer ( $F = 0.014 \pm 0.008$ ). The group of wild reindeer was characterized by minor heterozygote deficiency, whereas no differences between observed and expected heterozygosity values were observed in semi-domesticated reindeer. Our study demonstrates that the commercially available DNA chip designed for cattle might be successfully applied for biodiversity assessment of reindeer (*Rangifer tarandus*). The study was supported by the Russian Science Foundation within Project no. 14-36-00039.

**Key Words:** biodiversity, SNP, reindeer  
*Rangifer tarandus*

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#### **P4065 MicroGBS: High-throughput microsatellite genotyping using Illumina sequencing platforms.**

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The use of microsatellite markers in animal agriculture is less frequent with the advent of high-throughput SNP genotyping platforms. Though available for genomic selection in channel and blue catfish, these SNP platforms are not cost effective for the thousands of animals that require genotyping to resolve parentage and sibship because catfish cannot be efficiently tagged for identification. Therefore we expanded on a genotyping-by-sequencing (GBS) protocol for high-throughput SNP genotyping to perform high-throughput microsatellite genotyping. We designed 400–500 bp amplicons that contained microsatellite loci known to be polymorphic in channel and blue catfish based on resequencing analysis. Amplicons were multiplex amplified in 96-well format for 10 cycles based on locus-specific primers that were tailed with Illumina sequencing primers. Diluted products were then amplified with primers that matched the Illumina primer sequences and were tailed with 8 bp barcodes and the Illumina binding sequences. Barcoded amplicons were pooled from each plate, purified from lower molecular weight contaminants using magnetic beads, quantified for each 96-well plate, then equimolar plate pools were re-quantified and sequenced on the MiSeq platform using a single 300 bp read with dual index reads. Sequences from individual samples were demultiplexed using bcl2fastq. A bash script extracted locus-specific reads from each sample, defined the microsatellite repeat region, and determined the length of the repeat. We genotyped 37 loci each in 576 samples on the MiSeq platform. These loci contained di-, tri-, tetra-, or penta-nucleotide repeats. The 300 bp read lengths eliminated allele sizing errors due to non-template adenylation