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Morphological and Genomic Differences Between Cutting and Racing Lines of Quarter Horses

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ABSTRACT

To investigate morphological and genomic differences between cutting and racing lines of Quarter Horses, 120 racing and 68 cutting animals of both sexes, registered at the Brazilian Association of Quarter Horse Breeders, were used. Blood samples were collected, and the following physical traits were measured: weight; height at withers; body length; length of the shank, pastern, rump, head, and neck; and chest, shank, and hoof circumference. For analysis of genomic differences, 54,602 single-nucleotide polymorphisms (SNPs) were genotyped using the Equine SNP50 BeadChip, and the quality of individual and SNP genotype data were evaluated. The fixation index, F_{ST}, was used to identify genome regions that were altered in the lines by selection. The results showed significant differences between the lines in all physical traits. Quality control led to the exclusion of four cutting animals with a call rate of <0.95. After filtering, 12,544, 13,815, and 13,370 SNPs were excluded for the whole population ($n = 184$), the 120 racing animals, and the 64 cutting animals, respectively. The number of informative polymorphisms detected in each line and in the whole population indicated that the Equine SNP50 BeadChip can be used in genetic studies of Quarter Horses. The fixation index, F_{ST}, identified 2,558 genome regions that may have been modified by divergent selection.

1. Introduction

As a breed of global importance, corresponding to 52% of all horses, Quarter Horses are important because of their great versatility in different equestrian events [1]. Quarter Horses were developed in North America in the 17th century from Arabian and Turkish horses brought by European settlers. The major development of this breed occurred during westward expansion when pioneers needed robust and versatile horses, fit for the saddle and for traction, in view of the difficulty to keep a varied stock of animals to satisfy diverse necessities [1].

The Quarter Horse breed is subdivided into different lines according to skills resulting from distinct selection objectives, including cutting and racing horses. The cutting line is destined for functional tests, exploring skills such as agility and obedience, which are important for cattle management in the field. The racing line is characterized by great sprinting speed over short distances on straight tracks. The cutting type is shorter and more compact and has muscular hindquarters, whereas the racing type is taller and has longer legs and a less prominent musculature.

The simultaneous study of thousands of DNA polymorphisms spread across the genome, known as genome-wide association analysis, has permitted the study of

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different populations of various domestic animal species [2-4], as well as the estimation of genetic divergence within and between populations [5]. During the process of domestication and breed formation, domestic animals were subjected to natural and artificial selection. These selection pressures led to an increase in the frequency of some mutations in specific regions of the genome, which generated more adapted animals or provided them with favorable characteristics to meet human needs. At the same time, the frequency of other polymorphisms decreased or they were completely eliminated [6]. In this respect, the comparison of allele frequencies between selected and unselected populations or between populations selected for different objectives provides insights into the regions of the genome that have been modified by selection.

According to Chowdhary and Raudsepp [7], one of the highlights from the analysis of the horse genome is its complete sequencing from a Thoroughbred animal (EquCab2.0) and, from this, the identification of 1,162,753 single-nucleotide polymorphisms (SNPs) in different breeds [8]. Designed to identify SNPs and genes that contribute to traits of interest in the major horse breeds raised today in the world, the Equine SNP50 BeadChip developed by Illumina, Inc., (San Diego, California, USA) represents a powerful platform for genetic studies of this species, permitting researchers to perform a variety of experiments that require the genotyping of DNA polymorphisms.

In view of these considerations, the aims of the present study were to investigate the genomic differences, using the Equine SNP50 BeadChip, and morphological differences between cutting and racing lines of Quarter Horses as a result of selection for different objectives.

2. Materials and Methods

2.1. Animals and Phenotypic Data

One hundred eighty-eight Quarter Horses of both sexes born between 1985 and 2009, including 120 racing horses and 68 cutting horses, registered at the Brazilian Association of Quarter Horse Breeders, were studied. All experimental procedures were conducted in accordance with the Brazilian legislation on animal welfare.

The following physical traits were measured according to Torres and Jardim [9]: weight; height at withers; body length; length of the shank, pastern, rump, head, and neck; and chest, shank, and hoof circumference. The measurements were performed by the same person using a tape measure and measuring stick, always on the right side of the animal, with the horse standing with front and rear legs perpendicular to the ground. For genotyping, a 5-mL sample of whole blood was collected from each animal by puncture of the left jugular vein in the neck region into vacuum tubes containing 7.5 mg ethylenediaminetetraacetic acid.

The animals of the racing line (18 male and 102 female horses), born to 48 stallions and 107 mares, belonged to five farms in the countryside of the State of São Paulo, Brazil. The animals of the cutting line (26 male and 42 female horses), born to 44 stallions and 64 mares, belonged to three other farms in the countryside of the State of São Paulo. In both lines, full sibs were avoided.

2.2. Genotyping of SNPs

Genomic DNA was extracted from the blood samples of Quarter Horses using the Illustra Blood Genomicprep Mini Spin kit (GE Healthcare, Little Chalfont, Buckinghamshire, UK) according to manufacturer instructions. DNA integrity was analyzed using 0.8% agarose gel electrophoresis, and DNA was quantified using a NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The DNA concentration in the samples was adjusted to 40-60 ng/μL.

SNPs were genotyped on the HiScan system (Illumina Inc.) using the Illumina Equine SNP50 BeadChip at the Faculty of Agricultural and Veterinary Sciences, UNESP, Jaboticabal, São Paulo, Brazil. The chip contains 54,602 SNPs uniformly distributed across the entire genome of 15 horse breeds. The SNPs are distributed across the 31 autosomes and X chromosome. The mean interval between SNPs is 43,200 bp. This content is derived from the EquCab2.0 SNP Collection compiled by the Broad Institute's Equine Genome Sequencing Project, which identified >940,000 SNPs in Arabian, Andaluz, Akhal-Teke, Islandesa, Standardbred, Thoroughbred, and Quarter Horses.

2.3. Analysis of Morphological Differences and Differences in Inbreeding between Cutting and Racing Lines

Morphological differences between the two Quarter Horse lines (cutting and racing) were evaluated using a model that included the effects of sex and line, and age at recording as covariate. The general linear model (GLM) procedure of the Statistical Analysis System v.9.1 program (SAS Institute Inc., Cary, North Carolina, USA) [10] was used for statistical analysis, and means were compared using the Tukey test at a level of significance of 5%.

The inbreeding coefficient was calculated for each animal of the two lines based on pedigree records using the Relax2 program (MTT Agrifood Research Finland, Biometrical Genetics, Jokioinen, Finland) [11]. The relationship matrix contained 762 animals, with a depth of ancestry of four generations. The average inbreeding of consanguineous animals and of all animals of each line was estimated using the coefficients of inbreeding.

2.4. Quality of Genotype Data

The quality of individual and SNP genotype data was investigated using the Genome Studio program, version 2011.1 (Illumina Inc.). For individuals, call rate, heterozygosity, and gender estimation were determined. Animals with a call rate <0.95, heterozygosity of ±3 standard deviations from the mean, and errors in gender estimation were excluded from the sample. In addition, agreement between four replicates and parentage concordance (allele sharing) between four stallion/progeny and three stallion/mare/progeny pairs were evaluated.

With respect to the quality of SNP genotypes in the whole population and in each line, SNPs located on the X chromosome were excluded (filtered). SNPs with low genotyping quality (cluster separation <0.3), a call frequency <0.9, a minor allele frequency (MAF) <0.05 (including fixed alleles), and a Hardy–Weinberg $P < .001$ were also excluded.