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Short communication

Application of genomic data to assist a community-based breeding program: A preliminary study of coat color genetics in Morada Nova sheep

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ABSTRACT

The Brazilian Sheep Breeders' Association recognizes two varieties of the Morada Nova hair breed, white and red. However, the black variety and/or animals with a pigmented nose has frequently been disqualified from genealogical records. Previous studies suggest that this genetic group might be similar to the red variety. Thus, the aim of this study was to conduct a Genome-wide association study (GWAS) to identify genomic regions related to hair color and confirm the position of black relative to other Morada Nova varieties. After quality controls, 48 animals were genotyped for 45.982 SNPs using the OvineSNP50k BeadChip. Estimated Fst values between white and red animals, white and black, and red and black were 10.78% ($p < 0.00001$), 9.23% ($p < 0.00001$), and 2.93% ($p < 0.00001$), respectively. The comparison between white and red (n=30) versus black (n=18) animals revealed 10 highly significant SNPs, most located in a 6.8 Mb window in *Oar*14 which contains the *MC1R* gene. Differences between black and red coats are the result of the expression of different alleles of the same gene without directly affecting productive/reproductive characteristics. These two varieties showed low genetic variation, insufficient to define them as different groups, and to increase the breeding herd, the animals with black hair and/or pigmentation of the nose should be used breeding purposes. The results of this study contribute to the discussion of the importance in reconciling conservation, traditional breed standards and breeding of farm animals.

1. Introduction

Brazil has more than 17 million sheep (IBGE, 2013) and more than 60% of the flock can be classified as hair-sheep, being a majority distributed in the Northeast region (Hermuche et al., 2013; McManus et al., 2014). The Morada Nova hair sheep (Fig. 1) is an important locally adapted breed from Brazil used for lamb production, which shows high prolificacy and good maternal ability (Facó et al. 2008).

A Morada Nova community-based participatory breeding program was created in 2007 and has since been coordinated by Embrapa Goats and Sheep with major contributions from the Breeder's Association, Universities and other government agencies (Shiotisuki and Facó, 2013). The program provides alternative solutions to maximize the conservation and use of this locally adapted breed, while applying and integrating contemporary animal breeding methods with popular knowledge to support the socio-economic development of regional communities (Mirkena et al., 2012). According to the Brazilian Association of Sheep Breeders (ARCO), the breed is composed of 151 registered purebred flocks distributed across 102 municipalities (McManus et al., 2013) mainly in Northeastern Brazil (ARCO, 2014). A census conducted by Ribeiro et al. (2014) across five Brazilian States identified

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Fig. 1. Brazilian Morada Nova hair sheep coat color variations. A=White; B=Black; C=Red. Photographs: PhD Débora Andrea Evangelista Façanha. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3145 animals in 36 flocks. These numbers suggest that Morada Nova breed is therefore not in any FAO risk status category.

ARCO only accepts registries of Morada Nova sheep with two distinct coat colors: red or white (ARCO, 2014). A significant number of lambs with black coats, born from mating of registered rams and ewes, are routinely observed and consequently discarded from registered flocks, posing a major challenge to the breeding and conservation program. Annual culling based on coat color and low pigmentation of muzzle/nose and hooves has been estimated to reach up to 45% (Shiotsuki et al., 2016), posing a major negative impact on the breed's genetic variability levels and drastically reducing genetic improvement rates of production traits, since most of the remaining registered animals can barely support flock replacement rates.

The use of SNP (Single Nucleotide Polymorphism) panels containing thousands of molecular markers has greatly improved the speed and success rate of studies aiming to identify genes affecting production traits in livestock, especially when few genes control the trait of interest (Jonhstonn et al., 2011; Kijas et al., 2013). The present study was performed to estimate levels of genetic differentiation between Morada Nova sheep with different coat colors and to search for underlying factors affecting this trait, and to provide information for a critical evaluation of current practices and future objectives in the breed's breeding program.

2. Material and methods

2.1. Sampling, DNA processing and genotyping

A total of 61 animals (21 red, 20 white and 20 black) were randomly sampled from 10 farms in three states in Brazil's Northeastern region (Supplemental Material 1). DNA was extracted from lymphocytes with a salting out protocol modified from Miller et al. (1988). Samples were genotyped with the Illumina OvineSNP50 Bead Chip at a commercial lab (Neogen-GeneSeek, Nebraska, USA).

2.2. Data quality control and genetic structure and association analyses

Individual SNPs with call rates < 90% and MAF values < 0.0001 were excluded from the dataset. SNPs located on sex chromosomes and with undetermined map positions were also excluded from the final dataset. Overall autosomal heterozygosity rates were obtained to eventually identify contaminated samples with an over or under-abundance of autosomal heterozygous SNPs. Genomic relationships between individuals were estimated by IBD (Identity by descent) analysis to identify closely related animals, that needed to excluded from the dataset.

The final dataset was used in the population structure and genome-wide association studies (GWAS). Fst (Wright, 1978) estimates between all coat color groups were obtained, and principal component analysis (PCA) was performed on the Fst matrix generated (Price et al., 2006). GWAS was performed using a "case-control" model where individual animals were coded in binary form according to the coat color, and both dominant and additive inheritance models were tested. Chi-square tests were applied with Bonferroni correction for multiple tests. In addition, the correction for population structure was performed using the inflation factor (Lambda). The following contrasts were performed: (1) black (cases) vs red and white (controls); (2) black (cases) vs red animals (controls). Cutoff p values were empirically determined by identification of SNPs with most significant effects, and according to previously published methods (Kijas et al., 2013), where the 10 most significant SNPs were chosen for further investigation. Genome map positions and gene annotations from Oar_v3.1and Ensembl were used for gene identification in genomic regions observed to be associated with tested contrasts. All analyses were performed using the SNP & Variation Suite software – SVS (Golden Helix, Inc.).

2.3. Resequencing of MC1R gene fragments

Previously published primers were used to amplify and sequence 954 bp of the whole coding domain sequence of the Melatonin receptor 1 (MC1R) gene from all animals included in the study, according to published protocols (Vage et al., 1999). PCR products were purified with Exosapit™ and sequenced with BigDye terminator chemistry in an ABI3100 automated sequencer, with standard protocols. Sequencing data was processed using SeqScape Software v.2.6 (Life Technologies) for identification of polymorphisms.

3. Results

SNP data from a total of 48 individuals were considered for further analysis after data pruning (Supplemental Material 2). A total of 16% of the markers on the OvineSNP50 Bead Chip were discarded (Supplemental Material 3). Mean call rate on the final dataset was 98%.

Estimated Fst values between White and Red animals, White and Black, and Red and Black were 10.78% ($p < 0.00001$), 9.23% ($p < 0.00001$), and 2.93% ($p < 0.00001$), respectively. PCA results showed that tested animals clustered within two main groups. White animals were clustered in a distinct group, while Red and Black animals were clustered in the same group and therefore could not be fully differentiated (Fig. 2), when considering the first two principal components, which together explained 6.31% of the total observed variation.

GWAS between white and red (n=30) versus Black (n=18) animals revealed a total of 10 highly significant SNPs located in a