Electronic Journal of Biotechnology 17 (2014) 317–321

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

Application of microsatellite markers for breeding and genetic conservation of herds of Pantaneiro sheep

Bruno do Amaral Crispim [a], Leonardo de Oliveira Seno [b], Andréa Alves do Egito [c], Fernando Miranda de Vargas Junior [b], Alexéia Barufatti Grisolia [a,*]

[a] Faculdade de Ciências Biológicas e Ambientais, Universidade Federal da Grande Dourados, Mato Grosso do Sul, Brasil
[b] Faculdade de Ciências Agrárias, da Universidade Federal da Grande Dourados, Mato Grosso do Sul, Brasil
[c] Embrapa Gado de Corte, Campo Grande, MS, Brasil

ARTICLE INFO

Article history:
Received 10 February 2014
Accepted 30 September 2014
Available online 12 October 2014

Keywords:
Animal genetic resources
Genetic diversity
Kinship
Locally adapted breed
STR

ABSTRACT

Background: The aim of the present study was to assess the genetic diversity of Pantaneiro sheep, using microsatellite markers, in order to assist maintenance and management plans, enhance mating systems and reduce the inbreeding rate. A total of 127 animals were genotyped at eight microsatellite loci. They belonged to populations from the Experimental Farm of the Universidade Federal da Grande Dourados (UFGD) (Dourados/MS/Brazil) and Embrapa Pantanal (Corumbá/MS/Brazil).
Results: The population of Pantaneiro sheep from the UFGD exhibited a high mean number of alleles (11.13) and allelic richness (10.66). The polymorphic information content was highly informative in the locus studied, resulting in a mean value of 0.71. Observed heterozygosity was lower than expected for all molecular markers assessed. The analysis of molecular variance showed a differentiation rate of 5.2% between populations.
Conclusions: The results of the statistical parameters indicated that populations of Pantaneiro sheep require special attention on herd management, and it's further necessary to implement breeder exchange programs in order to preserve the genetic variability of these populations. Furthermore, the maintenance of those populations in their typical habitats is rather required to allow different responses from the herds to the interactions between genotype and environment.

1. Introduction

The genetic group of sheep adapted to the conditions of the Pantanal of the state of Mato Grosso do Sul in Brazil [1] is known as the Pantaneiro sheep. They can be used as an animal genetic resource to improve sheep production for meat and milk in this state. Females of this breed have no reproductive seasonality and are an important role in the performance of their lambs during the period from birth to weaning [2,3]. Lambs have a satisfactory productive potential, in terms of carcass traits and meat quality [3,4]. In addition, Pantaneiro sheep produce wool, which can be used as feedstock in regional craftwork.

Native Brazilian sheep breeds are characterized by rusticity and adaptability to tropical and subtropical areas within Brazil. Gomes et al. [1] stated that the Pantaneiro sheep exhibit a combination of alleles of wool and woolless sheep breeds from southern and north-eastern regions of Brazil. These animals are phenotypically similar to each other, but differ from other breeds bred in Brazil. Currently, the Pantaneiro sheep is widely diffused in several isolated farms in the state of Mato Grosso do Sul. They live for a long time in Pantanal region and surely faced natural selection mechanism without going through breeding programs. This fact confirms that these sheep are locally adapted [2].

Molecular genetic markers, such as microsatellites, can complement morphological and productive information about genetic resources, contributing to an increase in the efficiency of processes of genetic diversity and genetic purity analysis. In addition, they are able to generate information for the planning of crossings and the selection of genotypes in genetic breeding programs [5]. Microsatellites are suitable for studies of both genetic variability and parentage tests. These markers are co-dominant and frequently have an expected

* Corresponding author at: Laboratório de Biotecnologia Aplicada a Produção Animal, Universidade Federal da Grande Dourados, Rua João Rosa Góes, 1761 - Vila Progresso. Caixa Postal 322 - CEP: 79.825-070, Dourados, Brazil. Tel.: +556734102223.
E-mail addresses: brunocrispim.bio@gmail.com (B.A. Crispim), alexeiagrisolia@ufgd.edu.br (A.B. Grisolia).
Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.ejbt.2014.09.007
0717-3458/

heterozygosity above 0.7, which enables discrimination between individuals. Due to the specificity of the PCR assays associated with the high polymorphic content of these markers, it is possible to determine the identity of individuals based on estimates derived from allelic frequencies.

Assessments of genetic diversity using microsatellite markers have become an important tool for conservation programs and those involved in the genetic breeding of animal herds in conservation nuclei. The aim of the present study was to assess microsatellite markers, in terms of characterizing and determining genetic diversity for conservation and management, in order to enhance the mating system and reduce consanguinity in populations of Pantaneiro sheep.

2. Materials and methods

2.1. Animals

Blood samples were collected from 127 animals by jugular venipuncture from each animal using 4.5 mL collection tubes (Vacutainer®) for the genomic DNA extraction, from two conservation nuclei of Pantaneiro Sheep. The conservation nuclei were located on the Experimental Farm of the Universidade Federal da Grande Dourados (UFGD) in the city of Dourados/MS (64 female and 5 male), using a herd formed approximately eight years previously with animals from the ANHANGUERA-UNIDERP Sheep Technological Center in Campo Grande/MS. The other was located at Embrapa Pantanal (47 female and 11 male), using a herd formed approximately 5 years previously, with animals from different places of the Pantanal plain of Corumbá/MS, Brazil.

2.2. Microsatellite loci

Genomic DNA was extracted from blood using 300 μL of blood which were incubated in microtubules at 60°C with 3 μL of proteinase K (20 mg = μL) and 500 μL of 20% SDS (sodium dodecyl sulfate); chloroform (800 μL) and a protein precipitation solution (350 μL) were subsequently added. The microtubules were centrifuged (14,000 rpm) for 10 min and the supernatant transferred to another microtube. One mL of 100% ethanol was added to the pellet and it was centrifuged again, followed by another washing of the precipitate in 70% alcohol. After drying the pellet, 50 μL of TE buffer (pH 8.7) with RNase (10 ng = μL) was added. The material was incubated at 37°C for 1 h and stored in a freezer at 20°C. The quantity and purity of the genomic DNA samples was determined using a spectrophotometer (NanoDropND-2000 UV–vis).

PCR reactions were performed for 8 microsatellite loci (CSRD247, HSC, OarAE129, MAF214, OarFCB304, OarCP49, SPS113, and D5S2) including those proposed by the Ministry of Agriculture Herds and Provisions of Brazil (MAPA) [6] and markers recommended by the International Society for Animal Genetics (ISAG) [7]. Reactions were performed using a multiplex fluorescent system, with all markers included simultaneously. The PCR was performed in a final volume of 10 μL, containing: 3.6 μL of ultrapure water; 1.5 μL of 10× PCR buffer; 1.5 μL mix of primers; 50 mM $MgCl_2$; 10 mM dNTPs; 0.4 μL Platinum® Taq DNA Polymerase (Invitrogen) and 3.0 μL of template DNA (50–100 ng). Negative controls were used to monitor the reactions. The PCR were realized in thermocycler (Applied Biosystems®), and the thermal profile used was initial denaturing for 7 min at 95°C, followed by 40 cycles of 30 s at 95°C, annealing at 63°C for 90 s and elongation at 72°C for 60 s. A final extension step was performed at 72°C for 30 min. At the end of the amplification, the samples were stored at 4°C. Denatured amplicons were subjected to capillary electrophoresis in a MegaBACE™ 1000 DNA Analysis System (GE Healthcare, USA). Then, a solution with TWEEN and molecular weight marker ET-400 (GE Healthcare) was prepared. Each sample subjected to electrophoresis was composed of 0.3 μL ROXsize standard, 7.7 μL TWEEN 20 a 0.1% and 2 μL of the amplified product. Samples were denatured for 3 min at 94°C and cooled on ice. Sample injection was performed at 3 kV for 80 s and the electrophoresis run was performed at 8 kV for 80 min. Genotyping results for allele discrimination were visualized in the Fragment Profiler program, version 1.2 (GE Healthcare).

2.3. Data analysis

Allele frequency, private alleles and parameters of locus diversity (expected heterozygosity (He), observed heterozygosity (Ho), polymorphic information content (PIC), Hardy–Weinberg equilibrium (HWE) and allelic richness (AR)) were estimated for all microsatellites using the CERVUS 3.0 [8], Microssatelite Tookit, GenAlEx [9] and FSTAT [10] software programs.

Estimates for the inbreeding coefficient (F_{IS}) and population structure were assessed by analysis of molecular variance (AMOVA), using the Arlequin program [11].

The pairwise genetic distances between all individuals were estimated by the logarithm proportion of shared alleles (Dps) [12] using the MICROSAT program [13]. The Neighbor joining method (NJ) [14] was used to build a phylogenetic tree based on the genetic distance matrix, with the aid of the PHYLIP computational package [15] and TreeExplorer 2.1.2.

Based on the results of the genotypes of eight microsatellites, the animals were grouped in a given number of populations and probabilistically placed into groups inferred by Bayesian analysis, using the STRUCTURE program [16]. The tests were performed using an admixture model, in which the allelic frequencies were correlated. The programs were set to distinguish samples from two different populations. In order to select the appropriate number of inferred populations, several analyses were conducted with K (number of populations inferred) ranging from 2 to 5, a total of 300,000 interactions (burn-in period of 3000) and three independent replications for each analysis. The real K values were inferred from the magnitude of ΔK and given as a function of K, using the Structure Harvester program [17], according to the model proposed by Evanno et al. [18].

3. Results

Table 1 displays the descriptive statistical analysis of the eight microsatellites for the populations of Pantaneiro sheep studied (127 genotyped animals). All loci exhibited polymorphism resulting in a total number of 100 alleles. The mean number of alleles per locus was 12.5 (ranging from 7 to 21 for the SPS113 and OarCP49 markers, respectively). The loci OarAE129 and SPS113 were in the Hardy–Weinberg equilibrium when populations were analyzed together. However, sheep from Pantaneira Corumbá had shown greater number of markers in equilibrium (D5S2, OarFCB304, OarAE129 e MAF214), when compared to those from Pantaneira UFGD (MAF214 e OarCP49).

The polymorphic information content was highly informative for all loci in the studied populations (overall mean of 0.71). The population of Pantaneiro sheep from the UFGD recorded a higher mean number of alleles per locus (11.13) and private alleles (4.50), as well as greater allelic richness (10.66), genetic diversity (0.73) and observed heterozygosity (0.67) than the population from Embrapa Pantanal. Considering that F_{IS} values are associated with higher homozygosity, the results of the present study indicate that the inbreeding coefficient (F_{IS}) was higher for the Embrapa Pantanal population (0.11) than for the UFGD population (0.09) (Table 2). Among the eight analyzed loci, the population from Embrapa Pantanal showed greater number of loci (4) compared to those from UFGD population (2).

The AMOVA revealed differences (5.2%) between the populations of Pantaneiro sheep studied. The estimates of genetic differentiation based on F_{ST} were significant ($P < 0.002$). The individual dendrogram of both populations was built using the Neighbor joining method, based on