African Journal of Biotechnology Vol. 11(90), pp. 15617-15625, 8 November, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.2275
ISSN 1684–5315 ©2012 Academic Journals

Review

Molecular markers for genetic diversity and phylogeny research of Brazilian sheep breeds

Bruno do Amaral Crispim[1]*, Márcia Cristina Matos[2], Leonardo de Oliveira Seno[3] and Alexéia Barufatti Grisolia[1]

[1]Faculdade de Ciências Biológicas e Ambientais, Universidade Federal da Grande Dourados – Dourados/MS, Brazil.
[2]Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista – Jaboticabal/SP, Brazil.
[3]Faculdade de Ciências Agrárias, Universidade Federal da Grande Dourados – Dourados/MS, Brazil.

Accepted 2 October, 2012

Brazilian sheep descended from several breeds brought to the New World by Portuguese and Spanish colonists, and they have evolved and adapted to local climatic variations and acquired tolerance or resistance to many diseases. Molecular markers are widely used in analyzing genetic variability, and markers such as amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), microsatellite, mtDNA and single nucleotide polymorphism (SNP) have facilitated the characterization of genetic diversity and population structure, and in the investigation of the natural history, behavior, and evolution of several sheep breeds. In this context, we present here a review of the uses of molecular markers in ecological and conservation research of Brazilian sheep breeds.

Key words: DNA polymorphism, genetic conservation, *Ovis aries*.

INTRODUCTION

Brazilian sheep originated from Portuguese and Spanish breeds that were introduced into the New World by colonists. Over the years, these breeds were subjected to natural selection of local environmental and edapho-climatic conditions, resulting in breeds that are currently considered naturalized, locally adapted, or native (Mariante et al., 1999). Agricultural census data collected by the Brazilian Institute of Geography and Statistics put the Brazilian sheep population at more than 17 million animals, center mainly in the southern and northeastern regions of the country.

Ovinoculture constitutes a sound economic option due to demands for meat (Aro et al., 2007). Naturalized breeds are often preferred due to their rustic characteristics and adaptability in tropical and subtropical climates, giving these breeds important attributes and offering future genetic resources.

The naturalized Brazilian breeds are usually small animals that have been subjected to only weak levels of artificial selection and sustainable genetic improvement, and they are little specialized for intensive milk and/or meat production (Paiva et al., 2005a). The general traits of well-known naturalized breeds in Brazil are described in Table 1.

The survival and preservation of naturalized breeds with important genetic heritages were threatened during the 20th century, by indiscriminate crossbreeding with exotic breeds (mainly from Africa and Europe) (Morais, 2001). It should be noted that these naturalized animals have many adaptive traits that make them useful for breeding and production, such as: tolerance or resistance to diseases and parasites, and extensive adaptations related to the availability and quality of food resources and water – as the animals that were better adapted and/or more resistant survived and reproduced. Naturalized breeds therefore represent the results of

*Corresponding author. E-mail: brunocrispim.bio@gmail.com.

Abbreviations: SNP, single nucleotide polymorphism; **PCR,** polymerase chain reaction; **AFLP,** amplified fragment length polymorphism; **PCR/RFLP,** restriction fragment length polymorphism-polymerase chain reaction; **RAPD,** random amplified polymorphic DNA; **STR,** simple sequence repeats; **SRY,** sex-determining region Y; **COI,** cytochrome oxidase I.

Table 1. Origins, sites of occurrence, and phenotypic traits of naturalized Brazilian sheep breeds.

Breed	Origin	Site of occurrence	Phenotypic trait	Reference
Crioula Lanada	Crossbreeding between local and exotic breeds: Spanish Lacha, Romney Marsh and Corriedale, as well as herds introduced by Portuguese colonizers	Rio Grande do Sul State; South America (from Peru to Uruguay)	Face and extremities uncovered. Wool ranges from white to black, medium length, suitable for wool production; Resistant to endoparasites.	Mariante et al., 2003; ARCO, 2011
Santa Inês	Crossbreeding between Bergamacia, Morada Nova and mixed races from Northeastern Brazil (possibly of African origin)	Northeastern Brazil (Ceará, Maranhão, Sergipe, among other states)	Woolless, short hair, small animals. High quality meat with low fat content. Of economic importance due to their sizes and environment adaptations.	Mariante et al., 2003; Paiva, 2005a
Morada Nova	Crossbreeding between African and Portuguese Bordaleiro	Northeastern Brazil (Ceará, Piauí, among other states)	Woolless; no horns; red, white or cream wool; with or without earrings. Suitable for meat production and high quality leather.	Oklahoma State University, 2007; ARCO, 2011
Brazilian Bergamacia	Originally from Italy	Northeastern Brazil (Bahia) and temperate climate states in the central-western region	Large size, white wool Rustic with multiple uses (meat, wool and milk)	ARCO, 2011
Brazilian Somalis	Somalia and Ethiopia	Northeastern Brazil (Ceará and Rio Grande do Norte states)	High fertility, fat rump, with some wool on the body, good meat and leather production	ARCO, 2011
Damara (Rabo Largo)	Northwestern Namibia and southern Angola*	Northeastern Brazil (arid regions)	Medium size, good meat and leather production ,woolless animals	ARCO, 2011
Black belly	African origin	Northeastern Brazil and a conservation nucleus in Roraima State	High fertility and reproductively efficient, woolless animals	Oklahoma State University, 2007

*The hypothesis that Rabo Largo is derived from Damara was not confirmed by microsatellite marker analyses (Paiva et al., 2005a).

long-term natural selection processes.

Conservation and genetic improvement programs focusing on naturalized animals are important to avoid both their inbreeding and indiscriminate crossbreeding, so that pure native breeds can be conserved. In this context, it would be necessary to design production systems that allow producers to use local breeds more efficiently for better financial returns (Notter, 1999). Research on the adaptive traits of different breeds are important for supporting livestock production systems based on native breeds, reducing environment impacts, and obtaining better products for commercial consumption.

Recent technological developments and new molecular tools have dated researchers discovering the origins and domestication processes of a wide variety of species. These tools have aided our understanding of the evolutionary relationships, taxonomies, and demographics of a wide variety of species and provided support for identifying priority areas for preservation programs and for analyzing genetic diversity in both domestic species and wild and endangered species (Rosa and Paiva, 2009; Grisolia and Moreno-Cotulio, 2012).

Single nucleotide polymorphism (SNP) markers have begun to provide new perspectives for genomic studies, particularly in investigations of the diversities of the genomes of individuals and populations, in the search for genes that cause diseases, and in the identification of selection signatures (Kijas et al., 2012; Pariset et al., 2012).

This review discusses the evolution of the use of molecular markers in analyzing the genetic diversity and

phylogeny of naturalized Brazilian sheep breeds.

MOLECULAR MARKERS

Molecular markers can be considered as any molecular phenotype derived from a specific DNA segment, and correspond to regions that are expressed (or not) in the genome (Ferreira and Grattaplaglia, 1996). Isozyme markers were initially developed, which are direct products of gene expression (Oliveira et al., 2002), but were followed by molecular markers that amplify DNA chains using polymerase chain reaction (PCR).

Research focusing on patterns of genetic variation of certain loci markers within a population make it possible to minimize the impacts generated by crossbreeding between naturalized animals, ensuring the conservation of their genetic diversity. These studies provide information about loss of intra-population genetic variability as a consequence of reductions in the effective size of populations – leading to increases in consanguinity and genetic drift (Kantanen et al., 1999).

The use of nuclear DNA molecular markers can increase the efficiency of genetic breeds programs through selection and by avoiding crossbreeding within the same generation (Melo et al., 2008). The polymorphic markers currently used in genetic diversity analyses are: amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism-polymerase chain reaction (PCR/RFLP), random amplified polymorphic DNA (RAPD), microsatellites or simple sequence repeats (STR), sex-determining region Y (SRY) and SNP.

The AFLP technique is based on PCR amplification of a subset of fragments obtained from the site-specific cleavage of genomic DNA by type II restriction enzymes (Lopes et al., 2002). This technique has been found to be efficient in studies of genetic diversity in sheep, such as those of Xiao et al. (2009) who analyzed this marker in six breeds of sheep in China.

PCR/RFLP detects patterns of polymorphisms among different individuals and is based on the differences in the sizes of restriction fragments generated by endonuclease cleavage of amplified DNA regions. PCR-RFLP analysis of molecular markers in phylogenetically related species has been used to elucidate ambiguous taxonomic classifications. Paiva et al. (2005a), examining this marker in part of the cytochrome oxidase I (COI) mitochondrial gene (1052 base pairs fragment) was able to show that the main breeds of naturalized Brazilian sheep were of European origin (such as Santa Ines and Bergamacia), although other naturalized breeds (such as Somalis and Morada Nova) were of African origin.

RAPD studies are based on polymerase chain reactions, using primers homologous to target sites in the genome. This marker aids in deciphering patterns of genetic variation and can be used to direct conservation measures for threatened or endangered species.

Although there are many advantages to the use of this marker, there are also some disadvantages in terms of low reproducibility (Paiva et al., 2005b) and the formation of heteroduplex is a characteristic of dominance, but a number of researchers have demonstrated the efficiency of this technique in genetic diversity studies of sheep breeds in Pakistan (Qasim et al., 2011), Indian (Kumar et al., 2003), and Turkey (Elmaci, 2007).

Another technique widely used for characterizing genetic variability, individual identifications, paternity testing, the construction of genetic maps, and studies of population genetics employs microsatellites. Microsatellites have high mutation rates, abundances, and distributions throughout the genome, and show neutrality and co-dominance, and the easy automation of analytical procedures allow their use in estimating genetic diversity between and within breeds (Ligda et al., 2009). Microsatellite markers show good reproducibility and high degrees of polymorphism (Cañon et al., 2000; Grisolia et al., 2007) and are widely used in characterizing the genetic diversity of sheep breeds (Ramey II et al., 2000; Arranz et al., 2001).

SRY molecular markers are extremely sensitive probes of paternal inheritance and are used to elucidate genetic histories, the processes of breed domestication, population relationships, and male gene-flow. The use of SRY markers combined with the Y chromosome can provide specific details of the gene-flow in males. The numbers of research projects in sheep using the Y chromosome have been quite limited, however, when compared to other domestic animals such as cattle (Hanotte et al., 2000; Pérez-Pardal et al., 2009) and goats (Pidancier et al., 2006; Sechi et al., 2009). Only one microsatellite locus (SRYM18) and eight SNP, located in the region 5' of the promoter of the sex-determining gene (SRY), have been identified in sheep (Meadows et al., 2006; Meadows and Kijas, 2009).

SNP markers are based on elementary alterations in the DNA molecule, that is, mutations in single nucleotides (adenine, cytosine, thymine, or guanine), and are bi-allelic, that is, only two variants are generally found in a single species (such as an allele corresponding to the base pair A/T and the other to G/C). SNPs are extremely abundant in the genomes of non-endogamic species and can occur in coding regions or with regulatory functions – although, in most cases, they are found in intergenic spacer regions. Until recently, the standard techniques of prospecting for SNP markers were based on Sanger's sequencing method, although second-generation sequencing technologies (Roche 454 - Margulies et al., 2005; Solexa-Illumina – Bennett, 2004; and ABI Solid - Valouev et al., 2008) are now able to produce large data sets (on the order of millions of sequenced bases).

As such, a number of molecular markers can be used to analyze genetic diversity, and while microsatellites have been widely employed (McManus et al., 2010; Paiva et al., 2011b; Souza et al., 2012), SNPs have been

found to be very useful in these studies (Kijas et al., 2012).

Microsatellites

Microsatellites are the most frequently used markers to explore genetic diversity and the population structures of domestic animals (Baumung et al., 2004). Relatively large genome abundance and high levels of polymorphism and co-dominance in this kind of marker make it an important tool for genomic analyses (Crispim et al., 2012).

Evaluations of eight microsatellite loci polymorphism in five unrelated sheep breeds (Romney, Border Leicester, Suffolk, Awassi, and Australia and New Zealand Merino) showed highly significant differences in allelic frequencies among individuals, indicating that microsatellite markers can be valuable tools in evaluating evolutionary relationships between breeds (Buchanan et al., 1994). Arranz et al. (1998) and Stahlberger-Saitbekova (2001) used microsatellites for genotypic characterization and assessment in Spanish and Swiss sheep breeds, respectively, and these markers were found to be efficient for evaluating genetic diversity and calculating the genetic distances between the animals involved.

Almeida (2007) determined the variability of 20 microsatellites in 717 animals of 14 Portuguese sheep breeds, which allowed this author to evaluate the degree of structuring in these Portuguese sheep populations and estimate genetic diversity parameters for each breed. Several studies have used microsatellite markers to investigate local Brazilian breeds. Paiva et al. (2005a) used microsatellite markers for 18 loci to evaluate genetic diversity in both naturalized and exotic sheep (Santa Ines, Bergamacia, Rabo Largo, Morada Nova, and Somalis) and the results were efficient in characterizing the breeds, although they showed low genetic variability.

Microsatellites have thus been shown to be excellent marker for the characterization of naturalized breeds (Paiva et al., 2003) and in population studies (Paiva et al., 2005b; El Nahas et al., 2008). Table 2 lists the microsatellite markers data recommended for paternity testing and genetic diversity and variations in sheep by the Food and Agriculture Organization of the United Nations (FAO, 2011).

Mitochondrial DNA markers

Mitochondria have their own circular DNA (mtDNA) and replication capacities. Mitochondrial inheritance is also known as maternal inheritance as the molecular markers in mitochondrial DNA are only passed through the female (distinct from the biparental inheritance of most nuclear molecular markers) (Olson et al., 2009). The unique genetic and structural characteristics of mitochondrial markers have been explored by a number of researchers.

mtDNA sequencing can be used as a tool for determining evolutionary origins and population dynamics, and is useful in domestication studies because mtDNA has high mutation rates, lacks recombinants, and is maternally inherited – thus allowing the evaluation of divergence between wild and domestic populations over the relatively short time scales of human domestication (Bruford et al., 2003; Toro et al., 2009). mtDNA has been used to investigate the origin of bovines (Loftus et al., 1994), equines (Vila et al., 2001), swine (Giuffra et al., 2000; Larson et al., 2005), goats (Joshi et al., 2004; Sardina et al., 2006), and sheep (Wood and Phua, 1996; Hiendleder et al., 1998; Guo et al., 2005; Pedrosa et al., 2005; Pereira et al., 2006; Tapio et al., 2006; Meadows et al., 2006).

Bruford et al. (2003) demonstrated that the majority of mtDNA sheep lineages are derived from a probable site of initial domestication in the “Fertile Crescent” region (which now comprises Israel, West Bank, and some regions of Lebanon, as well as Jordan, Syria, Iraq, Egypt, southeastern Turkey and southwestern Iran). According to Olson et al. (2009), many mitochondrial genes are highly conserved, so these markers can be used to examine very long-term phylogenetic and taxonomic relationships. Indiscriminate crossbreeding between different sheep breeds makes their characterization by nuclear molecular markers more difficult, but mtDNA markers may help solve problems related to the origins of several naturalized breeds throughout the world.

A study of genetic diversity undertaken by Meadows et al. (2005) that examined 17 sheep breeds of European and Asian origin found 57 single haplotypes among the 121 animals sequenced. The distributions of the haplotypes indicated that most of the animals were confined to a single breed (51 from 57), while six haplotypes were present in more than one breed. The distributions of these differences showed five distinct peaks, which indicated the presence of divergent haplotype groups. The majority of the haplotypes formed one large group that contained sheep lineage B (of European origin), while the remaining haplotypes formed a separate group (probably corresponding to lineage A of Asian origin).

Paiva et al. (2005a) examined six breeds of naturalized sheep in Brazil and observed an overwhelming presence of the European haplogroup, except for two Dorper animals (of African origin), which had mtDNA typical of the Asian haplogroup. These results support the hypothesis that Brazilian sheep originated largely from the European continent.

Gonçalves et al. (2010) examined the mitochondrial sequences of the ND5 gene (of subunit 5 of NADH dehydrogenase) in 225 animals of two native breeds (Frontier and Mountain) from southern Brazil (Santa Catarina and Rio Grande do Sul states) and observed significant differences between them. Bayesian phylo-

Table 2. Microsatellite markers panel of the International Society for Animal Genetics (ISAG) and FAO by locus, chromosome, primer sequences, annealing temperature, genbank accession number, and allele size in base pairs.

Locus	Chromosome	Primer sequence (5' > 3') forward and reverse	Annealing temperature (°C)	Genebank access number	Allele sizes (bp)
OarFCB128	OAR2	ATTAAAGCATCTTCTCTTTATTTCCTCGC CAGCTGAGCAACTAAGACATACATGCG	55	L01532	96-130
OarCP34	OAR 3	GCTGAACAATGTGATATGTTCAGG GGGACAATACTGTCTTAGATGCTGC	50	U15699	112-130
OarCP38	OAR 10	CAACTTTGGTGCATATTCAAGGTTGC GCAGTCGCAGCAGGCTGAAGAGG	52	U15700	117-129
OarHH47	OAR 18	TTTATTGACAAACTCTCTTCCTAACTCCACC GTAGTTATTTAAAAAAATATCATACCTCTTAAGG	58	L12557	130-152
OarVH72	OAR 25	GGCCTCTCAAGGGGCAAGAGCAGG CTCTAGAGGATCTGGAATGCAAAGCTC	57	L12548	121-145
OarAE129	OAR 5	AATCCAGTGTGTGAAAGACTAATCCAG GTAGATCAAGATATAGAATATTTTTCAACACC	54	L11051	133-159
BM1329	OAR 6	TTGTTTAGGCAAGTCCAAAGTC AACACCGCAGCTTCATCC	50	G18422	160-182
BM8125	OAR 17	CTCTATCTGTGGAAAAGGTGGG GGGGGTTAGACTTCAACATACG	50	G18475	110-130
HUJ616	OAR 13	TTCAAACTACACATTGACAGGG GGACCTTTGGCAATGGAAGG	54	M88250	114-160
DYMS1	OAR 20	AACAACATCAAACAGTAAGAG CATAGTAACAGATCTTCCTACA	59	...	159-211
SRCRSP9	CHI12	AGAGGATCTGGAAATGGAATC GCACTCTTTTCAGCCCTAATG	55	L22201	99-135
OarCB226	OAR 2	CTATATGTTGCCTTTCCCTTCCTGC GTGAGTCCCATAGAGCATAAGCTC	60	L20006	119-153
ILSTS5	OAR 7	GGAAGCAATGAAATCTATAGCC TGTTCTGTGAGTTTGTAAGC	55	L23481	174-218
ILSTS11	OAR 9	GCTTGCTACATGGAAAGTGC CTAAAATGCAGAGCCCTACC	55	L23485	256-294
ILSTS28	OAR 3	TCCAGATTTTGTACCAGACC GTCATGTCATACCTTTGAGC	53	L37211	105-177
SRCRSP5	OAR 18	GGACTCTACCAACTGAGCTACAAG GTTTCTTTGAAATGAAGCTAAAGCAATGC	56	L22197	126-158
MAF214	OAR 16	GGGTGATCTTAGGGAGGTTTTGGAGG AATGCAGGAGATCTGAGGCAGGGACG	58	M88160	174-282
SRCRSP1	CHI13	TGCAAGAAGTTTTTCCAGAGC ACCCTGGTTTCACAAAAGG	54	L22192	116-148

Table 2. Continued.

MAF33	OAR 9	GATCTTTGTTTCAATCTATTCCAATTTC GATCATCTGAGTGTGAGTATATACAG	60	M77200	121-141
MCM140	OAR 6	GTTCGTACTTCTGGGTACTGGTCTC GTCCATGGATTTGCAGAGTCAG	60	L38979	167-193
OarFCB20	OAR 2	AAATGTGTTTAAGATTCCATACAGTG GGAAAACCCCCATATATACCTATAC	56	L20004	95-120
OarFCB193	OAR 11	TTCATCTCAGACTGGGATTCAGAAAGGC GCTTGGAAATAACCCTCCTGCATCCC	54	L01533	96-136
OarFCB304	OAR 19	CCCTAGGAGCTTTCAATAAAGAATCGG CGCTGCTGTCAACTGGGTCAGGG	56	L01535	150-188
OarJMP29	OAR 24	GTATACACGTGGACACCGCTTTGTAC GAAGTGGCAAGATTCAGAGGGGAAG	56	U30893	96-150
OarJMP58	OAR 26	GAAGTCATTGAGGGGTCGCTAACC CTTCATGTTCACAGGACTTTCTCTG	58	U35058	145-169
MAF65	OAR 15	AAAGGCCAGAGTATGCAATTAGGAG CCACTCCTCCTGAGAATATAACATG	60	M67437	123-127
MAF70	OAR 4	CACGGAGTCACAAAGAGTCAGACC GCAGGACTCTACGGGGCCTTTGC	60	M77199	124-166
MAF209	OAR 17	GATCACAAAAAGTTGGATACAACCGTGG TCATGCACTTAAGTATGTAGGATGCTG	63	...	...
BM1824	OAR 1	GAGCAAGGTGTTTTTCCAATC CATTCTCCAACTGCTTCCTTG	58	...	...
INRA063	OAR 14	ATTTGCACAAGCTAAATCTAACC AAACCACAGAAATGCTTGGAAG	58	...	...

genetic analysis based on ND5 18 haplotypes pointed to the following geographic structures: the frontier haplotype was clustered in a monophyletic clade, while the mountain haplotype showed two paraphyletic clusters. The studies indicated the occurrence of geographic isolation associated with differences in the ways the herds were distributed (which could have caused gene flow reductions)–thus reinforcing the idea that they are two evolutionary lineages.

Paiva et al. (2011a) analyzed the Somali sheep breed in northeastern Brazil using molecular and pedigree data, microsatellites, and 404 bp of mtDNA control region and obtained an average of 5.32 alleles of herd diversity, with an expected heterozygosity of 0.5896 and an observed heterozygosity of 0.6451 for the microsatellite loci. Sixteen mtDNA haplotypes were identified, and network analysis made it possible to observe the relationships between all of the haplotypes identified. The mitochondrial genetic variability in this population indicated at least two major haplotypes groups. The maintenance of several similar haplotypes is not desirable in herd genetic conservation, and it would therefore be better to maintain isolated populations of individuals with distinct haplotypes.

When the control regions of mitochondrial DNA (mtDNA) were sequenced to identify phylogenetic relationship between naturalized and commercial Brazilian sheep breeds, the nucleotide diversity value was found to be 0.005 (Silvério et al., 2006). These results indicated that mtDNA AMOVA values were greater than those of nuclear markers. Naturalized Brazilian breeds showed deviations from selective neutrality, as tested by Fu's Fs ($p<0.01$), suggesting that these populations are undergoing demographic expansion.

Single nucleotide polymorphism (SNP)

SNP markers used in association studies, genetic mapping, diagnostic paternity assays, individual identification

(traceability), and to detect genetic diseases and/or polymorphisms associated with production traits have long been limited by technological constraints. However, there have been significant advances in the sequencing of mammalian genomes and in the development of bioinformatics tools during the last decade that have improved SNP massal genotyping.

Until recently, the standard method of prospecting for SNP markers was based on Sanger's sequencing method, but there are now high-density SNP panels, that have high-coverage genome-wide SNP with markers and there is the probability that these SNP are close to genes of interest and account for some of the population genetic variation. Kijas et al. (2009) developed a panel of 1,536 SNP from 23 domestic and two wild breeds of sheep to analyze the nuclear genome, generating clusters of large groups based on the animals' geographic origins and could excessively identify the population substructures within individual breeds. The high-density genotyping platform available for this specie currently belongs to Illumina (San Diego, CA). The Ovine SNP50 BeadChip was developed in collaboration with the International Genomics Consortium Sheep and contains more than 54,000 SNP, providing uniform genome coverage. This chip was validated in 75 economically important sheep breeds, including Brazilian breeds such as Santa Ines, Morada Nova, and Crioula Lanada. The applications of such high-density chips include association studies of genomic selection, paternity testing, and pedigree mismatching, as well as more accurate analyses of the diversity and compositions of animal breeds (Illumina, 2012).

A genome-wide association study (GWAS) of 486 typed animals of the Wild Soay breed (*Ovis aries*) was undertaken by Jhonston et al. (2011) with the Ovine SNP50 BeadChip and identified (using ~36,000 SNP) by an autosomal gene candidate for horns (RXFP2, *Relaxin-like receptor 2*). It also appears that there is an additional SNP in the gene which is supported by a new model of horn inheritance in this breed. Thus, the SNP50 BeadChip could be used to determine if the same gene group could explain horn polymorphisms in different breeds or species.

Kijas et al. (2012) conducted a study with the goal of assembling a global diversity chip of sheep breeds and typed 2,819 animals from 74 sheep breeds from Asia, Africa, Southwest Asia (Middle East), Caribbean, North America, South America (Santa Ines and Morada Nova), Europe and Australia using the Ovine SNP50 BeadChip (~49,034 SNP). Among the reported results, they were able to show that sheep breeds have maintained high levels of genetic diversity (in contrast to other domestic species such as dogs). These authors also identified specific genomic regions that contained strong evidence of the rapid changes of artificial selection in sheep evolution.

In order to understand genetic structure, it is essential to reach the genetic improvement by genome-wide association studies, genomic selection, and dissection of quantitative characteristics (Kijas et al., 2009). Thus, the information provided by dense SNP chips can aid in our understanding of genetic structure and the recent evolution of domestic species.

Paiva et al. (2012) conducted a research project using 17 markers in 467 individuals of six sheep breeds (Crioula n=300 Bergamacia n=24; Corriedale n=28; Pantaneira n=50; Rabo Largo n=20; Santa Ines n=45). Only two SNP markers did not produce consistent results and were excluded. The selected markers were then used in allocation tests using the structure of five repetitions with a total of 250k permutations each, which was efficient in distinguishing between the breeds. The only exceptions were Pantaneira and Crioula, which were grouped together, suggesting that they were closely related and probably should be classified as two ecotypes of the same breed. Thus, even a reduced panel could serve as a useful tool for animal breed-certification and the identification of their sub-products.

PERSPECTIVES

Analyses of patterns of molecular genetic variation are fundamental to reconstructing the evolutionary history of species and breeds, as well as for evaluating their genetic diversity, population structures, and their taxonomic definitions – which can help us to conserve and reproduce these breeds and minimize loss of genetic variability. Microsatellite and mitochondrial molecular markers are very useful in conservation studies, although SNP markers are rapidly becoming the markers of choice in genetic studies due to their genomic abundance and low costs for large-scale genotyping. The implementation of sequencing projects in domestic species will allow breeders and scientists to objectively evaluate genetic resources, and the genetic variability linked to productive traits and resistance or tolerance to diseases in naturalized breeds represent an obvious target.

REFERENCES

Almeida PAR (2007). Diversidade genética e diferenciação das raças portuguesas de ovinos com base em marcadores de DNA – microssatélites: uma perspectiva de conservação. Doutorado em Ciência Animal. Universidade Trás-os-Montes e Alto Douro, Vila Real.

ARCO (2011). Assistência ao Rebanho de Criadores de Ovinos. Associação Brasileira de Criadores de Ovinos. http://www.arcoovinos.com.br. Accessed 10 de February 2012.

Aro DT, Polizer KA, Pena SB (2007). O Agronegócio na Ovinocultura de Corte no Brasil. Rev. Cien. Eletro. Med. Vet. 9(5):1-6.

Arranz JJ, Bayón Y, Primitivo FS (1998). Genetic relationships among Spanish sheep using microsatellites. Anim. Genet. 29(6):435-440.

Arranz JJ, Bayón Y, San Primitivo F (2001). Genetic variation at microsatellite loci in Spanish sheep. Small Rumin. Res. 39(1):3-10.

Baumung R, Simianer H, Hoffmann I (2004). Genetic diversity studies in farm animals – a survey. J. Anim. Breed. Genet. 121:361-373.

Bennett S (2004). Solexa Ltd. Pharmacogenomics 5(4):433-438.

Bruford MW, Bradley DG, Luikart G (2003). DNA markers reveal the complexity of livestock domestication. Nat. Rev. Genet. 4(11):900-910.

Buchanan FC, Adams LJ, Littlejohn RP, Maddox JF, Crawford AM (1994). Determination of evolutionary relationships among sheep breeds using microsatellites. Genomics 22:397-403.

Cañon J, Checa ML, Carleos C, Vega-Pla JL, Vallejo M, Dunner S (2000). The genetic structure of Spanish celtic horse breeds inferred from microsatellite data. Anim. Genet. 31(1):39-48.

Crispim BA, Silva DBS, Banari AC, Seno LO, Grisolia AB (2012). Discriminação Alélica em Ovinos Naturalizados do Pantanal Sul-Matogrossense por Meio de Marcadores de Microssatélites. J. Selva Andina Res. Soc. 3(1):3-13.

El Nahas SM, Hassan AA, Mossallam AAA, Mahfouz ER, Bibars MA, Oraby HAS e Hondt HA (2008). Analysis of genetic variation in different sheep breeds using microsatellites. Afr. J. Biotechnol. 7(8):1060-1068.

Elmaci C, Oner Y, Ozis S, Tuncel E (2007). RAPD Analysis of DNA Polymorphism in Turkish Sheep Breeds. Biochem. Genet. 45:691-696.

FAO (2011). The State of the World's Animal Genetic Resources for Food and Agriculture, edited by Barbara Rischkowsky & Dafydd Pilling. Rome.

Ferreira ME, Grattapaglia D (1996). Introdução ao uso de marcadores moleculares em análise genética. 2ª Ed. Embrapa/Cenargen, Brasilia-DF, Brazil.

Giuffra E, Kijas JMH, Amarger V, Carlborg Ö, Jeon JT, Anderson L (2000). The origin of the domestic pig: independent domestication and subsequent introgression. Genetics 154:1785-1791.

Gonçalves GL, Moreira GR, Freitas TR, Hepp D, Passos Weimer TADT (2010). Mitochondrial and nuclear DNA analyses reveal population differentiation in Brazilian Creole sheep. Anim. Genet. 41(3):308-310.

Grisolia AB, Moreno-Cotulio VR (2012). Molecular Markers and Genetic Diversity in Neotropical Felids, Analysis of Genetic Variation in Animals, Mahmut Caliskan (Ed.), ISBN: 978-953-51-0093-5, InTech, Available from: http://www.intechopen.com/books/analysis-of-genetic-variation-in-animals/molecular-markers-and-genetic-diversity-in-neotropical-felids.

Grisolia AB, Moreno VR, Campagnari F, Milazzotto M, Adania CH, Garcia JF, Souza EB (2007). Genetic diversity of microsatellite loci in *Leopardus pardalis*, *Leopardus wiedii* and *Leopardus tigrinus*. Genet. Mol. Res. 6(2):382-389.

Guo J, Du LX, Ma YH, Guan WJ, Li HB, Zhao QJ, Li X, Rao SQ (2005). A novel maternal lineage revealed in sheep (*Ovis aries*). Anim. Genet. 36:331-336.

Hanotte O, Tawah CL, Bradley DG, Okomo M, Verjee Y, Ochieng J, Rege JE (2000). Geographic distribution and frequency of a taurine *Bos taurus* and an indicine *Bos indicus* Y specific allele amongst sub-Saharan Africa cattle breeds. Mol. Ecol. 9(4):387-396.

Hiendleder S, Lewalski H, Wassmuth R, Janke A (1998). The Complete Mitochondrial DNA Sequence of the Domestic Sheep (*Ovis aries*) and Comparison with the Other Major Ovine Haplotype. J. Mol. Evol. 47(4):441-448.

Illumina (2012). Ovine SNP50 Genotyping BeadChip. http://www.illumina.com/documents/%5Cproducts%5Cdatasheets%5Cdatasheet_ovinesnp50.pdf. Accessed 10 de February 2012.

Johnston SE, McEwan JC, Pickering NK, Kijas JW, Beraldi D, Pilkington JG, Pemberton JM, Slate J (2011). Genome-wide association mapping identifies the genetic basis of discrete and quantitative variation in sexual weaponry in a wild sheep population. Mol. Ecol. 20(12):2555-2566.

Joshi MB, Rout PK, Mandal AK, Tyler-Smith C, Singh L, Thangaraj K (2004). Phylogeography and origin of Indian domestic goats. Mol. Biol. Evol. 21(1):454-462.

Kantanen J, Olsaker I, Adalsteinsson S, Sandberg K, Eythorsdottir E, Pirhonen K, Holm LE (1999). Temporal changes in genetic variation of North European cattle breeds. Anim. Genet. 30(1):6-28.

Kijas JW, Lenstra JA, Hayes B, Boitard S, Porto Neto LR, San Cristobal M, Servin B, McCulloch R, Whan V, Gietzen K, Paiva S (2012). Genome-Wide Analysis of the World's Sheep Breeds Reveals High Levels of Historic Mixture and Strong Recent Selection. PLoS. Biol. 10(2):e1001258. doi:10.1371/journal.pbio.1001258.

Kijas JW, Townley D, Dalrymple BP, Heaton MP, Maddox JF, McGrath A, Wilson P, Ingersoll RG, McCulloch R, McWilliam S, Tang D, McEwan J, Cockett N, Oddy VH, Nicholas FW, Raadsma H (2009). A genome wide survey of SNP variation reveals the genetic structure of sheep breeds. PLoS ONE 4(3):4668.

Kumar KG, Kumar P, Bhattacharya TK, Bhushan B, Patel AK, Choudhary V, Sharma A (2003). Genetic Relationship Among Four Indian Breeds of Sheep Using RAPD-PCR. J. Appl. Anim. Res. 24(2):177-183.

Larson G, Dobney K, Albarella U, Fang M, Matisoo-Smith E, Robins J, Lowden S, Rowley-Conwy P, Andersson L, Cooper A (2005). Worldwide phylogeography of wild boar reveals multiple centers of pig domestication. Science 307(5715):1618-1621.

Ligda CH, Altarayrah J, Georgoudis A (2009). Genetic analysis of Greek sheep breeds using microsatellite markers for setting conservation priorities. Small Rumin. Res. 83(1):42-48.

Loftus RT, Machugh DE, Bradley DG, Sharp PM, Cunningham EP (1994). Evidence for two independent domestications in cattle. Proc. Natl Acad. Sci. 91:2757-2761.

Lopes MS, Lopes MTG, Figueira A, Camargo LEA, Fungaro MHP, Carneiro MS, Vieira MLC (2002). Marcadores moleculares dominantes (RAPD e AFLP). Biotecnol. Cienc. Desenvolv. 29(5):56-60.

Margulies M, Egholm M, Altman WE (2005). Genome sequencing in microfabricated high-density picolitre reactors. Nature 437(7057):376-380.

Mariante AS, Albuquerque MSN, Egito AA, McManus C (1999). Advances in the Brazilian animal genetic resources conservation programme. Anim. Genet. Res. Inform. 25(1):107-121.

Mariante AS, McManus C, Mendonça JF (2003). Country report on the state of animal genetic resources: Ministério da Agricultura Pecuária e Abastecimento. Brazil.

McManus C, Paiva SR, Araújo RO (2010). Genetics and breeding of sheep in Brazil. R. Bras. Zootec. 39:236-246.

Meadows JRS, Li K, Kantanen J, Tapio M, Sipos W (2005). Mitochondrial sequence reveals high levels of gene flow between breeds of domestic sheep from Asia and Europe. J. Hered. 96(5):494-501.

Meadows JRS, Hanotte O, Drögemüller C, Calvo J, Godfrey R, Coltman D, Maddox JF, Marzanov N, Kantanen J, Kijas JW (2006). Globally dispersed Y chromosomal haplotypes in wild and domestic sheep. Anim. Genet. 37(5):444-453.

Meadows JRS, Kijas JW (2009). Re-sequencing regions of the ovine Y chromosome and wild sheep reveals novel paternal haplotypes. Anim. Genet. 40(1):119-123.

Melo DC, Oliveira DAA, Seerig A, Carvalho DC (2008). Aplicações práticas de marcadores microssatélites na caracterização genética e identificação de plantéis de tilápia. Rev. Bras. Reprod. Anim. 32(4):220-224.

Morais ORO (2001). O melhoramento genético dos ovinos no Brasil. In: Melhoramento genético aplicado à produção animal. Belo Horizonte, FEPMUZ Editora, Brazil.

Notter DR (1999). The importance of genetic diversity in livestock populations of the future. J. Anim. Sci. 77:61-69.

Oklahoma State University (2007). Animal Science. http://www.ansi.okastate.edu/breeds/sheep Acessed 10 December 2007.

Oliveira ACB, Sediyama MAN, Sediyama T, Finger FL, Cruz CD (2002). Variabilidade genética em batata-doce com base em marcadores isoenzimáticos. Hortic. Bras. 20(4):576-582.

Olson ZH, Whittaker DG, Rhodes OE (2009). The use of molecular markers in wild sheep Research in North America: a review. Proceeding of the Northern Wild Sheep and Goat Council Biennial Symposium 16:251-269.

Paiva SR, Facó O, Faria DA, Lacerda T, Barretto GB, Carneiro PL, Lobo RN, McManus C (2011a) Molecular and pedigree analysis applied to conservation of animal genetic resources: the case of Brazilian Somali hair sheep. Trop. Anim. Health Prod. 43(7):1449-1457.

Paiva SR, Mariante Ada S, Blackburn HD (2011b). Combining US and Brazilian microsatellite data for a meta-analysis of sheep (*Ovis aries*) breed diversity: facilitating the FAO Global Plan of Action for

Conserving Animal Genetic Resources. J. Hered. 102(6):697-704.
Paiva SR, Silvério VC, Egito AA, MacManus C, Faria DA, Mariante AS, Castro SR, Albuquerque MSM, Dergam J (2003). Caracterização Genética da Raça Santa Inês. In: 2ND International Symposium on Sheep and Goat Production, João Pessoa, Anais do Segundo Sincorte 1:487-499.
Paiva SR, Silvério VC, Egito AA, McManus CM, Faria DA, Mariante AS, Castro STR, Albuquerque MSM, Dergam JA (2005b). Genetic variability of the Brazilian hair sheep breeds. Pesq. Agrop. Bras. 40(9):887-893.
Paiva SR, Silvério VC, Paiva DAF, Mcmanus CM, Egito AA, Faria DA, Mariante AS, Castro STR, Albuquerque MSM, Dergam JA (2005a). Origin Of The Main Locally Adapted Sheep Breeds Of Brazil: A RFLP-PCR Molecular Analysis. Arch. Zootec. 54:395-399.
Paiva SR, Vieira FD, Yamagishi MEB, Lacerda T, Vasconcelos C, Mcmanus C, Tanno P, Higa R, Carneiro PLS, Azevedo HC, Faco O, Souza CJH, Araujo AM, Martins VM, Caetano AR (2012). Validation of a low density SNP panel for breed certification testing in Brazilian sheep (*Ovis aries*) breeds as a tool for flock genetic management. In: International Plant and Animal genome Conference, San Diego.
Pariset L, Joost S, Gargani M, Valentini A (2012). Landscape Genomics in Livestock. In: Analysis of Genetic Variation in Animals. INTECH, Rijeka, Croatia.
Pedrosa S, Uzun M, Arranz JJ, Gutiérrez-Gil B, Primitivo FS, Bayón Y (2005). Evidence of three maternal lineages in near eastern sheep supporting multiple domestication events. Proc. R. Soc. B. 272(1):2211-2217.
Pereira F, Davis SJM, Pereira L, Mcevoy B, Bradley DG, Amorim A (2006). Genetic signatures of a Mediterranean influence in Iberian Peninsula sheep husbandry. Mol. Biol. Evol. 23:1420-1426.
Pérez-Pardal L, Royo LJ, Beja-Pereira A, Curik I, Traoré A, Fernández I, Casais R, Goyache F (2009). Y-specific microsatellites reveal an African subfamily in taurine (Bos taurus) cattle. Anim. Genet, doi: 10.1111/j.1365-2052.2009.01982.x.
Pidancier N, Jordan S, Luikart G, Taberlet P (2006). Evolutionary history of the genus Capra (Mammalia, Artiodactyla): discordance between mitochondrial DNA and Y-chromosome phylogenies. Mol. Phylogenet. Evol. 40:739-749.
Qasim M, Ahmad H, Ghafoor S, Afridi SG, Muhammad I, Imtia AK (2011). Estimation of Genetic Diversity in Sheep (*Ovis aries*) using Randomly Amplified Polymorphic DNA. Int. J. Anim. Vet. Adv. 3(1):6-9.
Ramey II RR, Luikart G, Singer FJ (2000). Genetic Bottlenecks Resulting from Restoration Efforts: The Case of Bighorn Sheep in Badlands National Park. Rest. Ecol. 8(4):85-90.
Rosa AJM, Paiva SR (2009). Marcadores moleculares e suas aplicações em estudos populacionais de espécies de interesse zootécnico. Planaltina, DF: Embrapa Cerrados, Brazil.
Sardina MT, Ballester M, Marmi J, Finocchiaro R, Van Kaam JBCHM, Portolano B, Folch JM (2006). Phylogenetic analysis of Sicilian goats reveals a new mtDNA lineage. Anim. Genet. 37(4):376-378.
Sechi T, Miari S, Piras D, Crasta L, Mulas G, Carta A (2009). Genetic variation of goat Y chromosome in the Sardian population. Ital. J. Anim. Sci. 8(2):159-161.
Silvério VC, Paiva SR, Faria DA, McManus CM, Dergam JA, Oliveira AA, Lôbo RNB, Sousa WH, Mariante AS, Egito AA (2006). Phylogenetic study on the main Brazilian naturalized sheep breeds. In: 8th World Congress on Genetics Appl. Livestock Production, Proceedings of 8th World Congress on Genetics Appl. Livestock Production. Belo Horizonte: Instituto Prociência. Brazil.
Souza CA, Paiva SR, McManus CM, Azevedo HC, Mariante AS, Grattapaglia D (2012). Genetic diversity and assessment of 23 microsatellite markers for parentage testing of Santa Inês hair sheep in Brazil. Genet. Mol. Res. 11(2):1217-1229.
Stahlberger-Saitbekova N, Schläpfer J, Dolf G, Gaillard C (2001). Genetic relationships in Swiss sheep breeds based on microsatellite analysis. J. Anim. Breed. Genet. 118(6):379-387.
Tapio M, Marzanov N, Ozerov M, Inkulov M, Gonzarenko G, Kiselyova T, Murawski M, Viinalass H, Kantanen J (2006). Sheep mitochondrial DNA variation in European, Caucasian, and Central Asian areas. Mol. Biol. Evol. 23(9):1776-1783.
Toro MA, Fernández J, Caballero A (2009). Molecular characterization of breeds and its use in conservation. Livest. Sci. 120(3):174-195.
Valouev A, Ichikawa J, Tonthat T (2008). A high-resolution, nucleosome position map of *C. elegans* reveals a lack of universal sequence-dictated positioning. Gen. Res. 18(7):1051-1063.
Vila C, Leonard JA, Götherström A, Marklund S, Sandberg K, Liden K, Wayne R, Ellegren H (2001). Widespread origins of domestic horse lineages. Science 291(5503):474-477.
Wood NJ, Phua SH (1996). Variation in the control region sequence of the sheep mitochondrial genome. Anim. Genet. 27(1):25-33.
Xiao F, Yong F, Ting S, Jin-Fu W (2009). Six Local Sheep Breeds AFLP Analysis of Genetic Diversity in Xinjiang. China Animal Husbandry & Veterinary Medicine. China.