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Full Length Research Paper

Allelic and genotypic frequencies of *ASIP* and *MC1R* genes in four West African sheep populations

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In West Africa, consumers pay a major attention on the coat colour of the sheep due to religious and cultural reasons. White coated individuals reach selling prices up to three-fold higher than black coated sheep. The aim of this study was to ascertain the genotypic and allelic frequencies of *MC1R* and *ASIP* genes in order to assess possible implementation of breeding programmes focusing on the increase of the white coated sheep frequencies. A total of 113 individuals belonging to three Burkina Faso sheep breeds (Burkina-Sahel, Djallonké and Mossi) and one Niger sheep breed (Touareg) were genotyped for the *MC1R* and *ASIP* genes. The wild allele of the *ASIP* gene (A^{wt}; 54.30%) was the most frequent in the four West African sheep, particularly in Burkina-Sahel (85%) and Touareg breeds (80%). The dominant black E^D allele was not identified in Burkina-Sahel and Touareg. Most of the analysed individuals were homozygous for wild *MC1R* allele (E^+/E^+) with 100, 73.5, 59 and 100% frequency in Burkina-Sahel, Djallonké, Mossi and Touareg, respectively. The A^{wt}/A^{wt} was the most frequent genotype on the *ASIP* gene in the four West African breeds (80.53%). No individuals were homozygous for the deletion (allele A^{del}). Although, no routine methods for detection of the genetic basis of the recessive black coat colour patterns can be easily implemented, the current results suggest the feasibility of a selection programme aiming at decreasing the frequencies of the dominant black E^D allele in Burkina Faso sheep breeds.

Key words: Sheep, coat colour, *MC1R* gene, *ASIP* gene, Burkina Faso, Niger.

INTRODUCTION

In West Africa, sheep are important genetic resources that play a major role in the sustenance of the impoverished families in rural areas, feeding and cultural rites. In Burkina Faso, they represent 29% of the total grazing domestic animals (ENEC II, 2004).

Coat colour is an inherited feature allowing the fitness of animals to different climatic zones (Odubote, 1994; Ebozoje and Ikeobi, 1998) and is one of the most important traits used to distinguish between farm animal breeds (Fontanesi et al., 2011). It has a considerable influence on the price of animals in Africa. Therefore, the proportion of black lambs born in a flock therefore determines substantial losses in the wool sheep industry (Fleet, 2006). In West Africa, due to religious and cultural reasons, consumers pay a major attention on the coat colour of the sheep as it is used for Tabaski (Muslim celebration day) also called Aid el Kebir or "Fête du mouton". White coated sheep has selling prices up to three-fold higher than black coated sheep. Consequently, the identification of causes of recessive pigmentation in wool is a matter of economic importance.

Coat colour is influenced by a large number of genes that are involved in determining the presence, the distribution and the biochemical activities of the melanocytes (Bennett and Lamoreux, 2003; Fontanesi et al., 2011). In

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mammals, coat colour depends basically on the amount of two pigments: eumelanin, which is always black or brown and pheomelanin which appears yellow or red (Lauvergne, 1992). The two pigments are controlled by both the *Extension* (E) and the *Agouti* (A) loci (Searle, 1968), encoded, respectively by the melanocortin-1 receptor (*MC1R*) and the Agouti-signalling peptide (*ASIP*) (Bultman et al., 1992). In sheep, black coat colour may be partially determined by a dominant *Extension* allele (Våge et al., 1999) or a recessive *ASIP* allele (Royo et al., 2008).

The determination of white coat colour in sheep is complex and does not only depend on the absence of black; it is usually accepted that the white-coated phenol-type has pheomelanin background from the dominant white/tan (A^{wt}) agouti allele, and that the most recessive ovine allele, non-agouti (A^{a}), results in a eumelanic (black/brown) coat pattern (Sponenberg, 1997). Norris and Whan (2008) have identified the genetic basis of the dominant white colour in sheep.

The aim of this study was to ascertain the genotypic and allelic frequencies of MC1R and ASIP genes in order to assess possible implementation of breeding programmes focusing on the increase of the frequency of white coated sheep.

MATERIALS AND METHODS

Sheep breeds and their environment

Sampling was performed in the three main environmental areas of Burkina Faso according to climate conditions and types of vegetation (Ouadba, 1997): (a) the Sahel area; (b) the Sudan-Sahel area and (c) the Sudan area. The Sahel domain is an arid area covering the northern part of Burkina Faso with an annual rainfall less than 600 mm, high temperatures varying from 15 to 47°C, and grassy, bushy, shrubby and thicket steppe vegetation.

The Sudan-Sahel domain is a transitional zone with regards to rainfall and temperature, covering the central part of the country with a short rainy season and temperature ranging from 20 to 42°C with better hydric conditions.

The Sudan domain covers the area of Southern Burkina Faso that shares with the Sudan-Sahel area a similar rainy season with annual rainfall >900 mm and a predominance of woodlands and Sudan and Guinean-type savannahs; temperatures are relatively low, varying from 17 to 35°C.

The three environmental areas described above are assumed to be the habitat of three different sheep breeds (Traoré et al., 2008) (Figure 1): (a) the Djallonké dwarf breed, which inhabits the Sudan area, is a hairy-thin tailed sheep belonging to the West African Dwarf sheep family; (b) the Burkina-Sahel breed, which inhabits semi-arid and arid areas of Northern Burkina Faso and maintained by the Peul (Fulani) agro-pastoral communities; and (c) the Mossi breed, which inhabits the Sudan-Sahel area of Burkina Faso, and considered as a transition breed probably nearer to the Djallonké breed.

The Touareg breed have been introduced from Niger and maintained in the experimental farm of 'Centre de Multiplication des Animaux Performants (CMAP)'. The most common coat colour pattern in the three Burkinabé sheep breed is spotted in black (Traoré et al., 2008), whereas the Touareg breed consists of white coated individuals only.

Sampling and DNA extraction

Blood samples were obtained from a total of 113 individuals belonging to four West African sheep breeds (Table 1). Sampling was carried out on individuals in their respective environmental area: Burkina-sahel in sahelian area, Mossi in Soudan-sahel area and Djallonké in Sudan area. Within flock sampling was limited to three individuals (the youngest reproductive male and the older reproductive females, if available) to avoid relatedness between sampled individuals, thus maximizing genetic variability. About 10 to 13 farms were sampled in each environmental area. Touareg breed was introduced from Niger in an experimental farm and all the individuals were sampled at this location. Within breed, individuals were selected in accordance with the standards determined in previous works (Traoré et al., 2008). Total DNA was extracted following standard phenol-chloroform procedures (Sambrook et al., 1989).

Polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism (RFLP) amplification and genotyping

All the individuals were genotyped for the presence of both the A^{del} allele on the *ASIP* gene, partially responsible for the recessive black coat colour pattern (Royo et al., 2008), and the black dominant extension allele (E^{D}) on the *MC1R* gene.

Amplification of E^{D} allele of the *MC1R* gene was performed, as previously described by Våge et al. (2003): i) a Met-73-Lys RFLP using the primers named E3–E4 by these authors followed by a NlaIII digestion; and ii) an Asp-121-Asn RFLP using the primers named as E3–E8 followed by digestion with MseI. Full description of the laboratorial methods used is as shown in the work of Väge et al. (1999).

For the ASIP gene, a PCR protocol was performed following Royo et al. (2008) using primers flanking the deletion (5'-GCACCTGAGGAAAAGCCCAGAGATG-3' and 5'-CTTGATTCCT-CCAGAATTGTTCTG-3'). *ASIP* alleles were determined based on their size: 242 bp for the A^{+} allele and 237 bp for the A^{del} allele.

Genotyping was performed from the PCR product on an ALFexpressII automated sequencer (Amersham Biosciences, Barcelona) using the Thermo Sequenase Cy5 Dye Terminator Cycle Sequencing Kit (Amersham Biosciences, Barcelona).

RESULTS AND DISCUSSION

Genotypic and allelic frequencies on the *MC1R* and *ASIP* genes for the sampled populations are given in Table 1.

The A^{wt} allele was the most frequent in the four West African sheep breed sampled in this study (54.30%), particularly in Burkina-Sahel (85%) and Touareg breeds (80%). However, the E^{D} allele was not found in these two breeds and consequently the genotype E^{D}/E^{D} was also absent.

The A^{del} allele was found in the four breeds analysed at various frequencies, ranging from 1.47% in Djallonké to 20% in Touareg. The E^{+} allele is virtually fixed in both the Burkina-Sahel and the Touareg breeds.

Most sheep sampled in the four breeds were homozygous for the E^{+} allele with frequencies of 100, 73.5, 59 and 100% of the individuals showing the E^{+}/E^{+} in, respectively the Burkina-Sahel, Djallonké, Mossi and Touareg breeds. The A^{wt}/A^{wt} genotype was the most frequent in the four West African breed (80.53%) and no individuals in this study were homozygous for the deletion

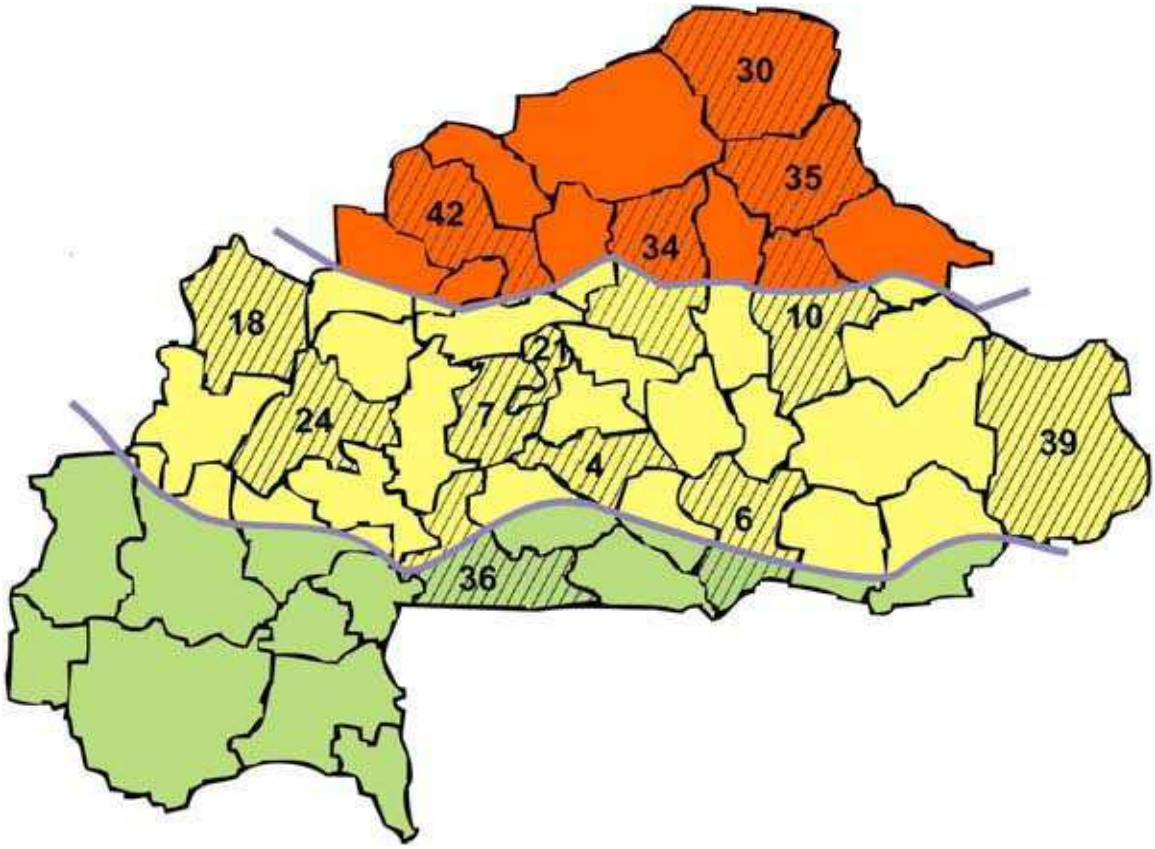

Figure 1: Map of the three environmental areas adapted from Ouabda (1997) (In red: Sahelian area; in yellow: Sudan-sahel area; in green: Sudan area)

Table 1. Genotypic and allelic frequencies for the *MC1R* and *ASIP* genes in four West African sheep populations.

Parameter	Population			
	Burkina-Sahel	**Djallonké**	**Mossi**	**Touareg**
Samples size	30	34	39	10
Alleles				
E^D	0 (0)	13 (19.12)	19 (24.36)	0 (0)
E^+	60 (100)	55 (80.88)	59 (75.64)	20 (100)
A^{wt}	51 (85)	66 (97.06)	71 (91.03)	16 (80)
A^{del}	9 (15)	2 (2.94)	7 (8.97)	4 (20)
Genotypes				
Extension				
E^D/E^D	0 (0)	4 (11.8)	3 (7.7)	0 (0)
E^D/E^+	0 (0)	5 (14.8)	13 (33.3)	0 (0)
E^+/E^+	30 (100)	25 (73.5)	23 (59)	10 (100)
Agouti				
A^{wt}/A^{wt}	21 (70)	32 (94.1)	32 (82)	6 (60)
A^{wt}/A^{del}	9 (30)	2 (5.9)	7 (18)	4 (40)
A^{del}/A^{del}	0 (0)	0 (0)	0 (0)	0 (0)

Frequencies are given in absolute values and as percentages (in brackets).

(A^{del}/A^{del}).

A previous study describing the morphological variation in Burkina Faso sheep (Traoré et al., 2008) reported that white coat colour had a limited frequency (14.38%, ranging from 9.64% in Burkina-Sahel sheep to 21.86% in Djallonké sheep). Most Burkinabé sheep (82.70%) were spotted in black or brown, while the black coat colour pattern was in a low overall frequency (1.60%). The presence of spotted patterns may be partially related to the presence of the E^D in the Djallonké and Mossi sheep breeds. Therefore, the possibilities of homogenisation of the colour in Burkinabé sheep are high since the detection of the E^D allele is feasible. The decrease of the frequency of the recessive black coat colour pattern is more difficult due to the low frequency of the A^{del} allele in the analysed breeds and to the absence of the A^{del}/A^{del} genotype in the samples analysed. The recessive black in sheep has a complex genetic determination which not only involves variation in the coding sequence (homozygous presence of the recessive allele A^{del}) but also differences in expression of the *ASIP* gene (Royo et al., 2008).

Black pigmentation in sheep is determined by a dominant *Extension* allele (Våge et al., 1999) or a recessive *ASIP* allele (Royo et al., 2008). However, genetic determination of white coat colour in sheep is more complex. The *MC1R* gene controls whether or not the agouti gene will partake in determining whether the sheep is white, grey or coloured in other patterns (Fontanesi et al., 2011). Agouti alleles would normally be phenotypically expressed only in the presence of the wild type allele (E^+) at the *Extension* locus. Most sheep of both primitive and modern breeds carry the recessive pair E^+/E^+. This allows the full expression of agouti. Therefore, the major genotype in breeds selected for white coat colour will be: A^{wt}/A^{wt} and E+/E+.

According to our results, although no routine methods for detection of the genetic basis of the recessive black coat colour patterns can be easily implemented, the feasibility of decreasing the frequencies of the dominant black E^D allele in Burkina Faso sheep breeds exists. Indeed, other strategies, such as the increase of the dominant white alleles, may be implemented to increase the frequency of white coat colour in sheep. Dominant white colour is caused by deregulated expression of the agouti protein, resulting from a 190-kbp genomic duplication that places a functional ASIP-coding sequence under the control of a duplicated promoter from the neighbouring itchy homolog E3 ubiquitin protein ligase (ITCH) locus as described in detail by Norris and Whan (2008). The implementation of routine methods to identify this genetic variant in a wide field population is not straight forward and may not be advised.

Conclusion

This study was carried out to determine the allelic and genotypic frequencies of the *MC1R* and *ASIP* genes in Burkina Faso sheep breeds in order to assess possible implementation of breeding programmes focusing on the increase of the frequency of white coated sheep. The results show the allelic and genotypic frequencies at these two genes and suggest that there are possibilities of decrease in the frequencies of the dominant black E^D allele in Burkina Faso sheep breeds by selection.

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