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Detection of Homologous Loci in Sheep and Cattle

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ABSTRACT

Background: The study of the homology of loci between different species has allowed the improvement of specific genetic knowledge and the development of several fundamental concepts. Thanks to this method, Wright discovered the correspondence between a gene and an enzyme (Wright, 1917). In ruminants, this correspondence between loci of different species has been demonstrated for a very long time (Lauvergne, 1979). In fact, the objective of this work was, initially, to use microsatellites recommended for sheep (FAO, 2011) for the amplification of bovine DNA to show that these loci are homologous and, then, to study the genetic diversity and population structure of three groups of each of the two species in Tunisia.

Methods: In this study, we used three microsatellite markers (OarFCB304, OarFCB193 and MAF209) to verify whether they can be amplified in both sheep and cattle. For that, we considered 28 samples of sheep species from 3 different populations and 17 samples of cattle belonging to three breeds. The data obtained were also used to study their genetic diversity and population structure.

Result: We have demonstrated the existence of microsatellite regions common to sheep and cattle. Regarding genetic diversity, we observed a heterozygous deficiency in all the studied breeds. Nei's genetic distances were greater between breeds of cattle than between breeds of sheep.

Key words: Cattle, Genetic diversity, Homologous loci, ISSR, Sheep.

INTRODUCTION

In Tunisia, breeding activities mainly concern bovine, ovine and caprine species and in a secondary way, camel and equine species. Studying their history, origin, structure and phylogeny is essential for establishing the management and conservation programs for these animals.

The molecular tools available allow us to trace the evolutionary history of species and to rigorously define the genetic diversity of populations. Microsatellites or SSRs (Simple Sequence Repeats) are highly polymorphic and specific molecular markers. They have been used for animal identification and parentage determination in cattle (Zhao *et al*., 2017), sheep (Rosa *et al*., 2013) and horses (Kang *et al*., 2016) as well as in studies of population genetics (Zhou *et al*., 2015; Sheriff *et al*., 2018) and evolution (Oliveira *et al*., 2006). Although some opinions are controversial, the majority of researches admit that SSR are neutral markers of evolution (Tachida and Lizuka, 1992; Shriver *et al*., 1993; Valdes *et al*., 1993; Di Rienzo *et al*., 1994).

When homologous microsatellites are compared between species, significant differences in mean allele length at a given locus are often noted (Kayser *et al*., 2006). Some studies have assumed that microsatellite sequences become longer during evolution. For example, they are longer in humans compared to chimpanzees (Cooper *et al*., 1998; Webster *et al*., 2002), in Drosophila melanogaster compared to D. simulans (Amos *et al*., 2003). However, several authors have reported that the difference in lengths of SSRs at homologous loci is the result of several phenomena such as genetic linkage, mutations or recombination (Ellegren *et al*., 1995; Amos and Rubinsztein 1996; Jarne and Lagoda, 1996).

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MATERIALS AND METHODS

This work was carried out at the National Gene Bank of Tunisia from March 01 to December 31, 2021.

Collection of samples and DNA extraction

In this study, 45 blood samples were taken from the jugular vein of animals belonging to two species: sheep (n=28) and cattle (n=17).

For sheep, the samples come from three groups: the Sicilo-Sardinian breed (n=14), the Black of Thibar breed (n=5) and a population of crossed animals (n=9) whereas for cattle, we considered three breeds: the Montbéliarde (n=6), the Brown Swiss (n=5) and the local Tunisian (n=6). DNA extraction was performed from blood stored at -20°C using the Iprep INVITROGEN device and its kits. This system uses Magtration® technology (Obata *et al*., 2001). DNA quantity and quality were estimated on 0.8% agarose gel using the Lambda DNA digested with Hind III (Lambda DNA/ Hind III Marker).

PCR conditions

After having tried several primers, we selected, for this study, three microsatellite ones: OarFCB304, MAF209 and OarFCB193. The PCR amplifications were carried out in a reaction medium with a volume of 25 µl containing 15 ng of genomic DNA 0.4 µM of each primer, 100 µM of dNTPs, 2 mM of $MgCl_2$, 0.8 units of Taq DNA polymerase (Invitrogen) and 5 µl of Taq buffer (5X).

In order to detect any contamination, control reactions that were not including genomic DNA were carried out for each amplification. Amplification conditions were: an initial denaturation at 94°C for 5 min, followed by 45 cycles each consisting of a denaturation step of 40 sec at 94°C, annealing step of 40 s and an extension step of 1 min at 72°C. The last cycle was followed by 8 min extension at 72°C. After several optimization runs, the hybridization temperatures used were 63°C for the primers OarFCB304 and OarFCB193 and 65°C for the primer MAF 209. The amplification products were separated by 3% agarose gel electrophoresis containing ethidium bromide in Tris Borate EDTA buffer and visualized in a gel documentation system.

Statistical analysis

The size of the different amplified bands was determined using Total Lab Quant software (Total Lab, UK). Statistical analyzes were carried out using the GENETIX software (Belkhir *et al*., 2004).

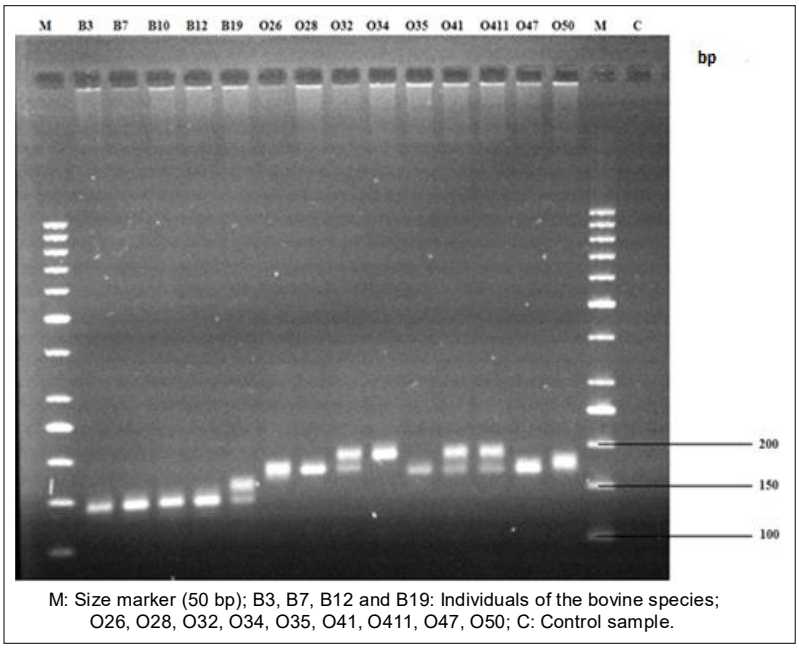
RESULTS AND DISCUSSION

SSR polymorphism

Twenty-four bands were generated in all individuals using the three primers OarFCB304, MAF209 and OarFCB193. Thirteen alleles were amplified at the OarFCB304 locus, six at the MAF209 locus and five at the OarFCB193 locus. The size of the bands varies between 130 and 220 bp for OarFCB304, between 105 and 143 bp for MAF209 and between 108 and 140 bp for the OarFCB193 locus. The number of alleles generated corroborates with the work carried out in sheep in Saudi Arabia by (Mahmoud *et al*., 2020). Indeed, the authors detected an average number of loci equal to 11.8.

Locus OarFCB304

The OarFCB304 marker is located on chromosome 19 in sheep (Forbes *et al*., 1995), while its position is unknown in cattle. Abdelkader *et al*., (2017) used, among others, the marker OarFCB304 to characterize 12 Algerian sheep breeds and reported that the size of the alleles detected for this locus varies between 140 and 192 bp. Buchanan and Crawford, 1993 reported band sizes in the range of 150-188 in sheep for this same marker. In our study, we detected allele sizes varying between 150 and 220 bp in sheep and of the order of 130 and 200 bp in cattle for the OarFCB304 marker. An example of the DNA amplification product by this primer is shown in Fig 1.



M: Size marker (50 bp); B3, B7, B12 and B19: Individuals of the bovine species; O26, O28, O32, O34, O35, O41, O411, O47, O50; C: Control sample.

Fig 1: Example of profiles of DNA fragments amplified by primer OARFCB304.

The sizes of the bands detected in sheep are comparable to the results found by (Forbes *et al*., 1995), the latter reported sizes of the order of 142-192 bp with an average size of 167.6 bp in domestic sheep. Likewise, the band sizes in sheep confirm the data cited by the FAO (FAO, 1997).

Locus MAF209

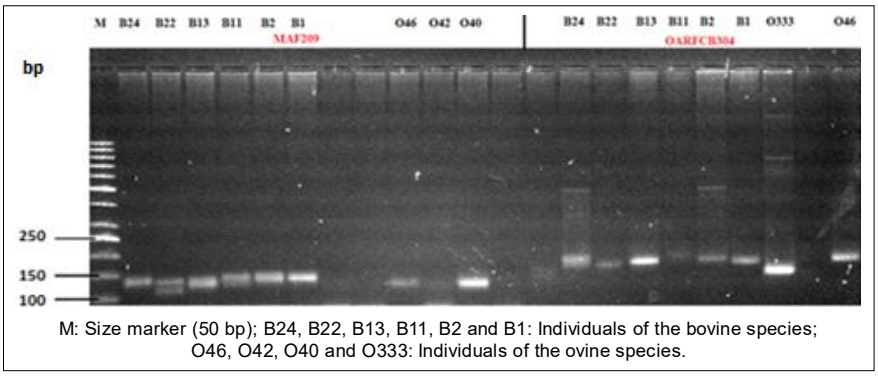
The MAF209 microsatellite marker was originally discovered in sheep by Buchanan and Crawford in 1992 allowing the generation of four alleles. The authors mentioned in their study that the locus could be amplified in alpaca (*Lama pacos*), red deer (*Cervus elaphus*), goats (*Capra hircus*) and cattle (*Bos taurus*). Ellegren *et al*. (1997) used the MAF209 marker to compare homologous loci in sheep and cattle and succeeded to generate eight bands varying in size from 109 to 135 bp. In our case, three bands were detected in sheep and four in cattle with sizes varying between 105 and 133 bp and 115 and 143 bp respectively. Fig 2 illustrates examples of DNA amplified using MAF209 and OARFCB304 primers.

Locus OarFCB193

This microsatellite marker is also of sheep origin; Buchanan and Crawford (1993) used it for the characterization of 50 individuals with band sizes varying between 96 and 136 bp. In our study, the OarFCB193 primer generated three bands in sheep and four bands in cattle bands with sizes between 108 and 140 bp, these results are similar to those found by Buchanan and Crawford (1993). Fig 3 illustrates an example of the product of DNA amplification from few individuals by the primer OarFCB193.

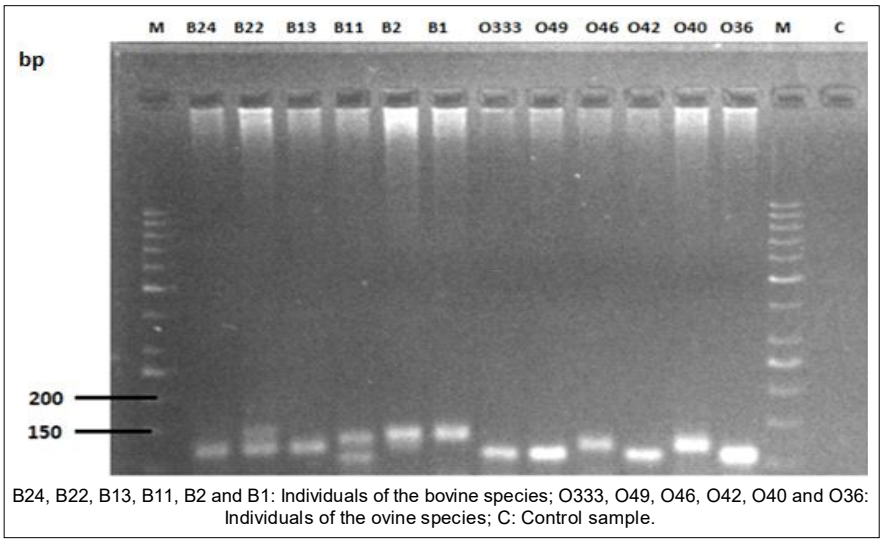
Genetic diversity

The mean heterozygosity over the loci was calculated in both species and in their groups (Table 1, 2 and 3). We note that the observed heterozygosity values are much lower than those of the expected heterozygosity in all populations which shows a heterozygosity deficiency in our sample. It is found that genetic diversity is slightly higher in cattle than in sheep. In addition, the black of Thibar was the breed sheep with the lowest heterozygosity value while the local breed cattle were the ones with the lowest heterozygosity. The study of genetic diversity in three sheep breeds in Tunisia using microsatellite markers also revealed a deficit in heterozygosity (Khaldi *et al*., 2020). Similarly, Ni *et al*. (2018) reported a significant heterozygosity deficit in cattle in Chongqing (Ni *et al*., 2018). The F parameters were



M: Size marker (50 bp); B24, B22, B13, B11, B2 and B1: Individuals of the bovine species; O46, O42, O40 and O333: Individuals of the ovine species.

Fig 2: Example of profiles of DNA fragments amplified by primers MAF209 (left) and OARFCB304 (right).



B24, B22, B13, B11, B2 and B1: Individuals of the bovine species; O333, O49, O46, O42, O40 and O36: Individuals of the ovine species; C: Control sample.

Fig 3: Example of profiles of DNA fragments amplified by primer OARFCB193; M: Size marker (50 bp).

Table 1: Mean heterozygosity at loci in sheep and cattle.

	He	Hnb	Ho	N
		Sheep		
	0.68	0.69	0.13	5
Standard deviation	0.17	0.17	0.05	
		Cattle		
	0.73	0.75	0.14	5.33
Standard deviation	0.1145	0.1179	0.0899	

Table 2: Mean heterozygosity at loci level in sheep populations.

	He	Hnb	Ho	N
		Sicilo-Sardinian		
	0.65	0.67	0.1	4.33
Ecart-type	0.1695	0.1757	0.0825	
		Black of Thibar		
	0.48	0.53	0	2
Ecart-type	0	0	0	
		Crossed animals		
	0.66	0.7	0.26	3.66
Ecart-type	0.11	0.12	0.06	

Table 3: Mean heterozygosity at loci level in cattle populations.

	He	Hnb	Ho	N
		Montbeliarde		
	0.61	0.66	0.17	3.67
Ecart-type	0.21	0.23	0.17	
		Brown swiss		
	0.68	0.76	0.13	3.67
Ecart-type	0.14	0.12	0.12	
		Local breed		
	0.51	0.56	0.11	2.67
Ecart-type	0.18	0.19	0.09	

He: H calculated with bias.

Hnb: H calculated without bias (Nei, 1978).

Ho: H observed.

N: Average number of alleles per locus.

calculated according to the method of Weir and Cockerham (1984) (Table 4). We note that all the F_{IS} values (coefficient of consanguinity) are positive. This again shows a deficiency in heterozygosity in all the populations studied. Likewise, F_{IT} values indicate an overall deficit of heterozygotes in the total population. Ni *et al*. (2018) also reported positive inbreeding coefficients varying between 0.0017 and 0.0367 in five cattle breeds in China (one native and four introduced breeds) and described a significant deficit in heterozygosity in the studied populations. Between two sheep breeds in India (Nellore and Deccani breeds), the FIS values ranged from -0.372 to 0.74, FST values ranged from 0.001 to 0.172 and FIT values ranged from -0.370 to 0.728 (Amareswari *et al*., 2018). The F_{ST} binding index is an index for estimating genetic differentiation between populations. If the F_{ST} is equal to or very close to 0, it means that there is a lot of genetic exchanges between populations (little genetic differentiation, panmictic population). Conversely, if the F_{ST} is close to 1, these results in strong genetic differentiation between populations suggest very little or no flow of genes between populations. According to Wright (1978), an F_{ST} between 0 and 0.05 indicates weak differentiation; an F_{ST} between 0.05 and 0.15 reflects moderate differentiation; an F_{ST} between 0.15 and 0.25 suggests an important differentiation and beyond 0.25, it illustrates a very important differentiation. In our study, the values show a greater differentiation between cattle populations (12%) than between sheep populations (2%). The coefficient of gene differentiation Gst (Nei, 1973) and the genetic distance of Nei (1978) between the two species are respectively 0.12 and 0.957. This important differentiation is expected since they are two well-differentiated species which are also well shown in Fig 4.

The genetic distances between the breeds of each of the two species were calculated (Nei, 1972) (Table 5 and 6). They vary between 0.08 and 0.36 and between 0.5 and 0.6 respectively between the sheep and cattle breeds. Genetic

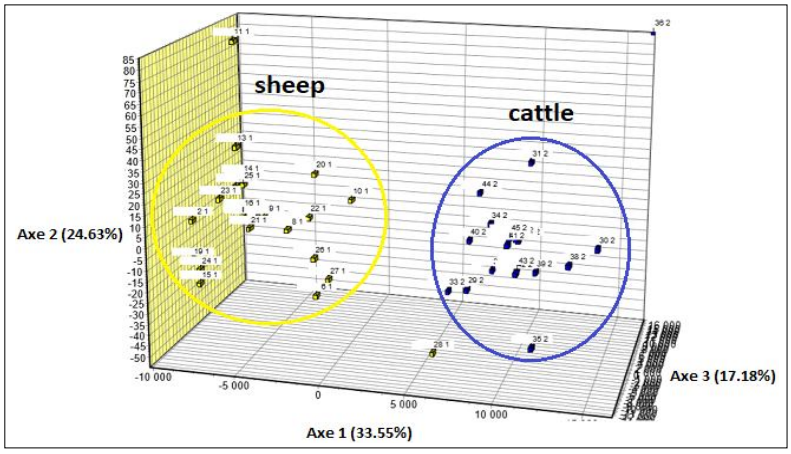


Fig 4: Genetic differentiation between the two species using the method of factorial correspondence analysis, percentage of inertia explained by each axis in parentheses (AFC, Benzécri (1973).

Table 4: Estimation of F parameters (Weir and Cockerham).

	F_{IS}	F_{IT}	F_{ST}
Between species	0.82	0.85	0.18
Between sheep populations	0.81	0.81	0.02
Between cattle populations	0.81	0.83	0.12

Table 5: Genetic distances between populations of the sheep specie.

	Black of Thibar	Crossed animals
Sicilo-Sardinian	0.14	0.08
Black of Thibar		0.36

Table 6: Genetic distances between populations of the bovine specie.

	Brown swiss	Local breed
Montbeliarde	0.5	0.52
Brown swiss		0.6

distances are high within the bovine species because they are two introduced breeds and a local population while the studied sheep breeds are local.

CONCLUSION

The conservation of microsatellite loci in sheep and cattle shows a high applicability of its SSRs for the study of evolutionary links between animal species.

The markers used are highly polymorphic and reproducible and are, therefore, highly recommended for the study of genetic diversity, parentage and phylogeny. On the other hand, they are not effective for marker-assisted selection work since they are neutral.

Studying SSRs can help us understanding many aspects of the organization and function of the genome in animals. It is, for example, interesting to know in the one hand why some repetitions are abundant and why others, in the other hand, are extremely rare. Are the abundance and distribution of these repeats subject to natural selection? Studies on other microsatellite sequences in animal species are needed to understand the evolution, organization and function of their genome.

Conflict of interest: None.

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