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Genetic Diversity and Demographic Evolution Using *SRY* Gene Polymorphism in Four Lineages of Ladoum Sheep in Senegal

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Abstract: Knowledge of spatial and temporal distribution of genetic diversity within and between populations is a crucial step for establishing strategies management of Animal genetic resources. This study aims to contribute to a better understanding of the genetic structure of local sheep breeds. Specifically, it will characterize the genetic diversity, differentiation and evolution of the *SRY* gene in four main lineages of Ladoum sheep in Senegal. After alignments and corrections, a total of 42 sequences were used for analysis of *SRY* genetic diversity and demographic evolution. The standard indices of genetic diversity, genetic differenciation, analyses of hierarchical molecular variance (AMOVA) and demographic tests were compute. Analysis of genetic variability parameters in the overall population revealed a low level of polymorphism in the *SRY* gene. Haplotypic diversity is much higher than the average nucleotide divergence between all pairs of haplotypes. The genetic distances, as well as the values of the genetic differentiation factor beetween lineages, reveal two genetic groups; one made up of the individuals of “Birahim” and the other, grouping the three lineages individuals. Differences in frequencies of essential proteins for a multitude of functions in the body are observed between the "Birahim" lineage and the others. The comparative trends in genetic diversity indices, the shape of the mismatch distribution curves, demographic indices and demogenetic tests show a rapid and recent expansion population from an ancestral small size. The Z-test analysis of selection revealed that *SRY* gene is under selection. Unlike “Batling” who is under negative selection, "Birahim" and "Gorgui" are under positive selection. This study has made a significant contribution to understanding the genetic structure of the Ladoum raised in Senegal.

Keywords: Genetic, Diversity, Evolution, SRY, Polymorphism, Scheep, Ladoum

1. Introduction

Local breeds represent an original and unique heritage due to the processes that have shaped them in relation to the history of the region. Deeply embedded in the local production systems, through their breeding methods and uses, they have thus developed particularly useful zootechnical aptitudes in terms of production performance and quality of adaptation. Among them, sheep have played an important role for human throughout their evolution. They were among the first domesticated animals and have proved useful for both food, providing meat and milk [1]. In addition to the wealth that these sheep represent for the most vulnerable sectors of the rural world, they also provide several services within the ecosystems of which they are part. In 2010, the Senegalese herd included 10 million small ruminants [2], and a wide variety of sheep breeds reared in different agro-ecological zones according to different management methods. Indeed, depending on the area, the most adapted breeds are favoured by breeders.

In the Sudanese regions of the country, infested by tsetse flies, the Djallonké sheep breed is mainly characterized by its

trypanotolerance which allows it to evolve in such environment [3]. However, in the northern and central part of the country, the so-called Sahelian, Peul-Peul, Touabire, Bali-Bali and Waralé breeds coexist, resulting from the crossing between Peul-Peul and Touabire [4]. On the outskirts and inside the big cities, intensive breeding is practiced [3, 5] mainly on the Ladoum sheep [2, 6]. Other imported breeds such as the Bali-bali or the Balami commonly called Azawak are also raised as hut sheep. With the advent of urban breeding and the craze for the Ladoum sheep, several associations have been created to promote and develop this breed. The process of creating the Ladoum breed, which in its early days was driven by passion and prestige, is now of great importance on the national economic level (improvement of the local herd and self-sufficiency in sheep meat), but also on the scientific level (standardization and certification of the breed, genetic study). In the breeders' jargon, there are several lineages within this breed, each headed by a male whose reproductive performance has been confirmed by his phenotypic criteria, which are appreciated and measured during the annual competitions organised by the breeders' associations (mainly SALADAM and SALADEL), but also by the performance of his offspring. This work, which today has resulted in this beautiful and great breed of sheep that is the Ladoum, was done empirically and did not follow the conventional process of creating a breed. There remains today a scientific void as to its origin and the different breeds involved in this process. During annual trade fairs and shows such as the Salon de l'Alliance pour le Développement et L'Amélioration des races (SALADAM), the most reputable "breed breeders" exhibit the most prized specimens, especially of the Ladoum breed. In order to participate in these events, the breeders practice a harsh selection within their herd for a continuous improvement of the breed to be able to present choice animals. Thus, animals reaching weight performances and morphometric measurements never reported in sheep are presented and are the subject of competitions under the wise eye of veterinarians and specialists in the field selected on the shutter. The presented Ladoum sheep can reach exceptional measurements and weights. Despite this performance, speculation about the origin of the Ladoum sheep remains persistent [7]. Indeed, some authors [8-10] describe it as a subpopulation of Touabire obtained after a long selection on the latter. Other results reported by Sadio [11] have led to the hypothesis that the Ladoum sheep is a variety of the Touabire breed, which, according to the breeders association of Thiès (REIT), is the result of a selection carried out by a breeder since 1975 on his herd. The use of this breed, which is unstable and recent from a geological point of view, in the improvement of the local herd, requires a better knowledge of its genetic structure and evolution. In Senegal, for example, the Ladoum, Peul-peul, and Djallonké races, among others, are only recognized on the basis of phenotypic characteristics. This is why molecular analysis, based on the neutralist theory of evolution and which postulates that the evolutionary time separating the different taxa is reflected by the divergence of DNA sequences, is an essential tool for elucidating the evolution of intermediate structures. The present study is part of this context and aims to contribute to a better understanding of the genetic structure of local sheep breeds. Specifically, it will characterize the genetic diversity, differentiation and evolution of the *SRY* gene of the four main lineages of Ladoum sheep raised in Senegal. The *SRY* gene is a valuable tool in the search for paternal relationships, but also for intra and inter species evolutionary divergences morphologically or ecologically structured in populations.

2. Material and Methods

2.1. Study Site and Choice of Animals

The study was conducted in the Dakar region, where all the Ladoum "lineages" converge during the fairs. Located on the Cape Verde peninsula, Dakar region covers an area of 550 km², or 0.28% of the national territory. It lies between 17° 10 and 17° 32 West longitude and 14° 53 and 14° 35 North latitude. Sheep farming in this area is intensive and mainly involves Ladoum sheep [2, 6, 12]. Animals are raised in enclosures in front of houses or indoors on stall terraces. The choice of flocks was guided by the collaboration of the owners. A total of 47 Ladoum sheep were selected, of which 22 were from the "Batling" lineage, 12 from the "Birahim" lineage, 5 from the "Gorgui" lineage and 8 from the "Tyson" lineage. Table 1 summarises the sampling parameters.

2.2. Collection and Storage

Samples were collected from January to April 2022. The biological material for this study consisted of whole blood obtained from the jugular vein of the animal and collected in EDTA (ethylene diamine tetra acetic acid) tubes of 4 ml capacity, which allow a better long-term conservation of the nucleic acids in the blood. After collection, the blood samples were directly sent to the genomics laboratory of the Department of Animal Biology of the Faculty of Science and Technology of Cheikh Anta Diop University in Dakar. The blood samples obtained were then stored at -18°C until DNA extraction.

Table 1. *Sampling parameters of the individuals studied.*

N°	Name	Codes	Farms	Lineage	Collection date
1	Recteur	REC	Bergerie KAS	«Batling»	09/01/2022
2	Ma Alassane	ALA	Bergerie KAS	«Batling»	09/01/2022
3	Sembène	SEM	Bergerie KAS	«Batling»	09/01/2022
4	Dunya	DUN	Bergerie KAS	«Batling»	09/01/2022
5	Prof de Suspens	BEN1	Bergerie KAS	«Batling»	09/01/2022

N°	Name	Codes	Farms	Lineage	Collection date
6	Douma Yène	DO.Y	TEKROUR	«Batling»	06/02/2022
7	Aïdara	Aid	Wakeur S. M. Khabane	«Batling»	06/02/2022
8	Nicéphore	NIC	Mame Faty Niang	«Batling»	06/02/2022
9	Diégo	DIEG1	Modou Gueye	«Batling»	16/02/2022
10	Nothern Dancer	NOTH	Ngayène Family	«Batling»	16/02/2022
11	Kamal	KAM	Cheikh Mbaye	«Batling»	27/02/2022
12	Mbarodi	MBA	Cheikh Mbaye	«Batling»	27/02/2022
13	Boy Lamine	B.LA	Modou Sarr	«Batling»	10/04/2022
14	Bour	Bour	Baye Dieng	«Batling»	10/04/2022
15	Légende	LEG	Cheikh Mbaye	«Batling»	27/02/2022
16	Frère Hamza	Fr.H	Bergerie KAS	«Batling»	10/04/2022
17	Fils Tête Cornée	TE.C	Bergerie KAS	«Batling»	10/04/2022
18	Fils Robe Noire	RO.N	Bergerie KAS	«Batling»	10/04/2022
19	Fils Assistante	F.ASSI	Bergerie KAS	«Batling»	10/04/2022
20	Fils Surprise	F.SURP	Bergerie KAS	«Batling»	10/04/2022
21	Fils Suspense	F.SUSP	Bergerie KAS	«Batling»	10/04/2022
22	Galactique Junior	GF	Diobass	«Batling»	10/04/2022
23	Amorce	AMO	Bergerie KAS	«Birahim»	09/01/2022
24	Prof Hamady	Pr.H	Wakeur S. M. Khabane	«Birahim»	06/02/2022
25	Mame goor	MG	Mame Faty Niang	«Birahim»	06/02/2022
26	Chéikh Gadiaga	CH.G	Touba Elevage Taïsir	«Birahim»	09/02/2022
27	Kemtane	KEM	Diobass	«Birahim»	11/02/2022
28	Khewlou	KHE	Bay Dieng	«Birahim»	11/02/2022
29	Lamine Ndiaye	L.N	Modou Sarr	«Birahim»	11/02/2022
30	Modou Sarr	M.S	Modou Sarr	«Birahim»	11/02/2022
31	Républicain	REP	Ngayène Family	«Birahim»	16/02/2022
32	Benzéma	BEN	Mansour Gueye	«Birahim»	16/02/2022
33	Diégo	DIEG	Mansour Gueye	«Birahim»	16/02/2022
34	Fils Jackpot	F. Jac	Lamine Ndiaye	«Birahim»	31/04/2022
35	Rimka	RIM	Chez Paco	«Gorgui»	10/04/2022
36	Paco Marron	PAC.M	Chez Paco	«Gorgui»	10/04/2022
37	Paco Noir	PAC.N	Chez Paco	«Gorgui»	10/04/2022
38	Fils Rimka	F.RIM	Bergerie KAS	«Gorgui»	10/04/2022
39	Ado1	Ado1	Lamine Ndiaye	«Gorgui»	21/04/2022
40	Modou Gassama	MO.G	Wakeur S. M. Khabane	««Tyson»»	06/02/2022
41	GEP	GEP	TEKROUR	««Tyson»»	09/02/2022
42	Jonson	JON	Yandé Djiké	««Tyson»»	11/02/2022
43	Alfa	ALF	Cheikh Mbaye	««Tyson»»	27/02/2022
44	Tidjane	TIJ	Cheikh Mbaye	««Tyson»»	27/02/2022
45	Waraba	WAR	Tonton Gueye	««Tyson»»	28/02/2022
46	Tijane	Tij	Baye Dieng	««Tyson»»	21/04/2022
47	Boy Walo	B.Wa	Lamine Ndiaye	?	21/04/2022

2.3. DNA Extraction, Amplification and Sequencing SRY Gene

Total DNA from the blood was extracted using the standard protocol of the Zymo research kit. After extraction, Fragments of *SRY* gene was amplified in a reaction volume of 25 µl with two primers: F 5'CAACTTTCAAGTTTGCCTTATGG-3' and R 5'ACAGCCCAATCCTGTTATATA-3'. The PCR was performed in an Eppendorf thermal cycler with the initial denaturation performed at 94°C for five minutes, followed by a repeat of 35 cycles with denaturation at 94°C for 30 seconds, hybridization of the primers at 48°C for 40 seconds, elongation of the complementary DNA strands at 72°C for 40 seconds, and terminated by a final elongation at 72°C for 10 minutes. Each target gene was amplified by performing an electrophoretic migration on a 2% agarose gel. The Sanger method was used to identify the nucleotide sequence of *SRY* gene.

2.4. Sequence Cleaning and Alignment

The resulting sequences were corrected, manually cleaned and aligned with BioEdit 7.0.8.0 software [13] using the Clustal-W algorithm [14]. The nucleotide sequences were checked and corrected thoroughly with reference to the electrophoregram. They were subjected to a BLAST (Basic Local Alignment Search Tool) to check the similarity with the reference sequence in GenBank (https://blast.ncbi.nlm.nih.gov).

2.5. Genetic and Statistical Analyses

2.5.1. Analysis of Genetic Variability and Diversity

The nucleotide composition of the sequences was calculated with the editor BioEdit. The standard indices of genetic variations (number of polymorphic sites, number of informative sites, number of total haplotypes and by lines) are explained with the MEGA 7 software [15]. The ratio

between transitions and transversions and the frequency of nucleotides were also calculated using this same software by the Pattern substitution test. For sequence polymorphism analysis, haplotypic diversity (Hd) and nucleotide diversity (π) [16, 17] were calculated using DNAsp version 5.10.01 [18]. All these analyses were performed in the study population and for each lineage.

2.5.2. Analysis of Differentiation and Genetic Distance

The genetic differentiation factor (F*ST*) within and between lineages was determined by ARLEQUIN V3.1 software [19]. According to Wright, the closer F*ST* is to 1, the more genetically structured populations are to each other. On the other hand, populations do not show allelic differences if the F*ST is* zero. For each value of F*ST*, the value of P allows to accept or reject it according to whether it is significant or not.

Genetic distance, which is a measure of the genetic relationships of population samples, was calculated within and between lineages using MEGA7 software [15]. Several distance measures have been proposed, however the Nei's [20] standard genetic distance (dS) widely used in natural population genetics studies has been retained. The use of Nei's distance and genetic diversity is justified by their biological significance [20]. Nei's genetic distance is intended to measure the average number of substitutions that occur after the divergence of two populations, and is expected to increase linearly with time [21]. dS assumes that the rate of gene substitution per locus is uniform across sequences and lineages.

2.5.3. Analysis of Molecular Variance (AMOVA)

Analysis of molecular variance (AMOVA) is used to understand the structure of the study population. This procedure seeks to estimate indices of genetic structure using information on the allelic content of haplotypes and their frequencies [22]. The significance of the covariance components associated with the different hierarchical levels (based on a priori groupings of the total population into subpopulations) is tested using the 5040 permutation procedure with ARLEQUIN version 3.1 software [19].

2.5.4. Detecting Natural Selection in Populations

Using models that account for demographic processes is essential for identifying regions of the genome under selection. The Ka/Ks or dN/dS test [23, 24] was used. This test compares the ratio of the rate of non-synonymous substitutions (dN) to the rate of synonymous substitutions (dS) per polymorphic site between populations [23, 25, 26]. At equilibrium (in the absence of selection), dN is (approximately) equal to dS. In contrast, if dN is greater than dS, selection is divergent. On the contrary, a dN lower than dS indicates a purifying selection. The Z-test is performed assuming that the gene is under positive selection. This hypothesis states that non-synonymous mutations are superior to synonymous mutations (dN > dS). This test was performed through MEGA 7 software [15] using the Nei-Gojobori model and the pairwise deletion method. A p value < 0.05 was considered significant.

2.5.5. Demographic Evolution

The inference of demographic change (in the broad sense) in populations was tested using 3 approaches: (i) comparison of haplotypic and nucleotide diversities [27, 28]: diversity indices (hd) and (π) have been used to infer demographic change in a population; the advantage is that the result is independent of fragment length and sample size [17, 29]; (ii) Demogenetic tests based on the allele frequency spectrum have been done. These tests determine whether the frequency spectrum of mutations conforms to the expectations of the standard model of neutrality. Tajima's D [30, 31] is the difference between the total number of observed polymorphic sites (S) and the average number of observed differences between pairs of sequences (K); Fu's FS [32] which compares the average number of observed differences between pairs of sequences (K) with the number of haplotypes (H) in a population; R2 [33] which is a complementary statistic based on the differences between the number of singleton mutations (SS) and the average number of observed pairwise sequence differences (K). The significance of the D, FS and R2 statistics was tested by generating random samples under the assumption of selective neutrality and population equilibrium, using a coalescent simulation algorithm adapted from Hudson [34] with the software ARLEQUIN (D and FS statistics) and DNAsp (R2 statistics); (iii) Analysis of the Mismatch distribution and demographic indices which is the qualitative graphical representation of the distribution of genetic distances between individuals in a population taken in pairs, was carried out. Two models were defined to infer the demographic evolution of a population from the Mismatch distribution graph: constant size population and growing - declining population. Following the graphs deduced from these two models, two distributions can be obtained: amultimodal distribution which indicates the signature of a stable (constant size) population and a unimodal distribution which indicates the signature of an expanding population. Mismatch analysis combines two indices that test the goodness of fit of the distribution. These indices, the SSD (sum of squares of deviations) and the Rag (Harpending's Raggedness index), which quantifies the smoothness of the distribution of observed pairwise differences, have been estimated in Arlequin version 3.1 [19].

2.5.6. Amino Acid Variability

The frequency distribution of the 20 amino acids that can be encoded by the *SRY* gene of the different ladoum lineages sampled was studied. *SRY* is a coding gene. The transformation of *SRY* nucleotide sequences into amino acid sequences is performed using MEGA7 software [15] by choosing the universal genetic code and the best reading frame (the one with the least number of stop codons). To see if there is a difference in the frequency distribution of each amino acid between the Birahim lineage and the other lineages, the database was run through R software version 1.1.463 [35]. The Shapiro-Wilk normality test was performed to see if the data were from a normally distributed

population. In the case of a normal distribution, the Student's t-test is performed for comparison of means; otherwise, the Wilcoxon test is used. A significance level of 5% was used.

3. Results

3.1. Variability and Genetic Diversity of the SRY Gene in Ladoum Population

3.1.1. Genetic Variability

After amplification of the DNA extracts, the sequences of 42 amplicons were analyzed. The sequences of individuals coded DIEG1, KAM, F. Jac and Tij are not included in the analyzes due to their poor quality. The sequence of the coded individual B.Wa is not used in the analysis because of a doubt of its belonging to a given lineage. Although there is some intra- and inter-lineage genetic variability, observation of the different electropherograms reveals an insertion of CAAA, which essentially differentiates the "Birahim" lineage from the others (figure 1).

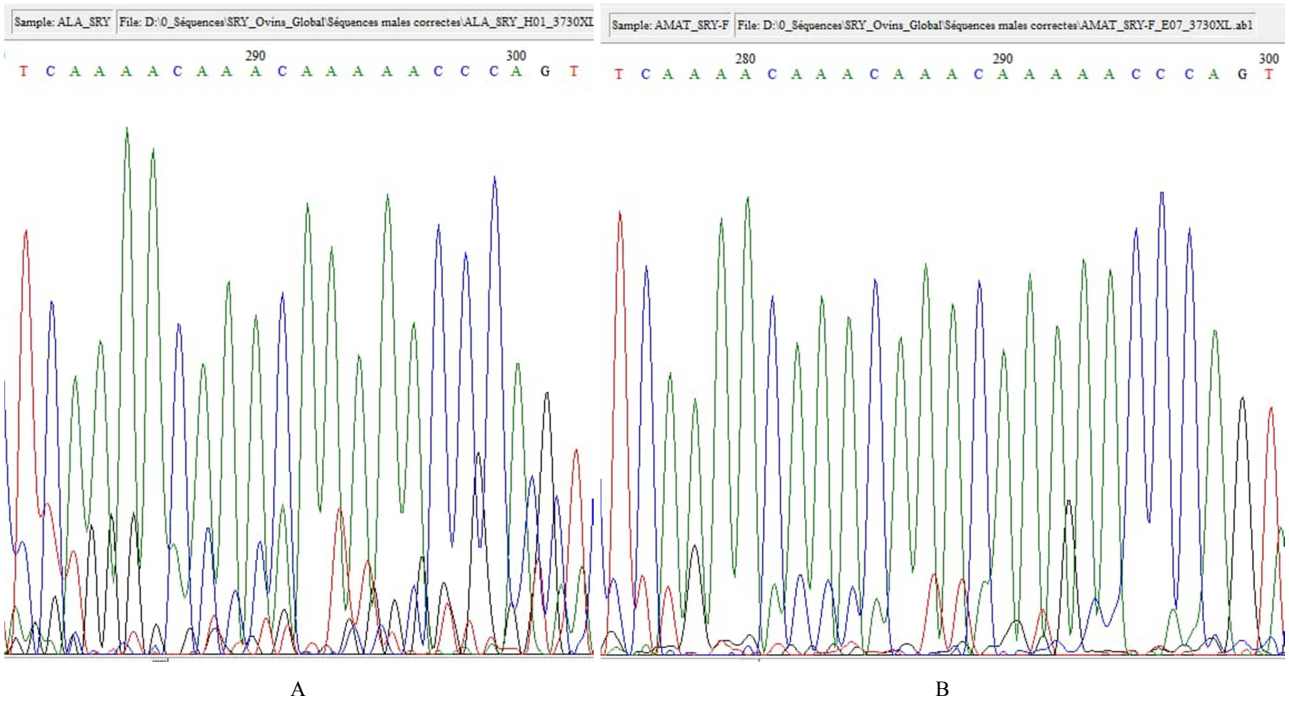


Figure 1. *Chromatogram: A. «Batling», «Gorgui» and «Tyson» lineages; B. «Birahim» lineage.*

The data set obtained after alignment and cleaning of the sequences revealed 558 sites of which 96.77% (540/558) of them are monomorphic and 03.22% (18/558) are variable, of which 01.61% (09/558) of singleton sites and 01.61% (09/558) of informative sites in parsimony (table 2). Transversions (51.18%) appear to be more numerous than transitions (48.83%). The 42 sequences are divided into 13 haplotypes. The mutation rate is 0.83; the average number of nucleotide differences is estimated at 2.907 (Table 2).

Table 2. *Genetic variability parameters of SRY gene sequences.*

Parameters		Variabilities
Number of sites 558		558
Monomorphic sites		540
	Polymorphic sites	18
Variable sites	Non-informative (singleton)	09
	Informative (parsimony)	09
Total number of Eta mutations		18
Number of haplotypes (h)		13
Average number of nucleotide differences K		2.907
Transition rate (%)		48.83
Transversion rate (%)		51.18
Mutation rate R		0.83

The nucleotide composition of all lineages shows that the percentage of A+T is generally higher than C+G, both within and between haplotypes (Table 3).

Table 3. *Nucleotide frequency of the SRY gene in each population and in the overall population.*

	A (%)	T (%)	C (%)	G (%)	A +T (%)	C +G (%)
«Batling»	29.49	32.66	20.26	17.59	62.15	37.85
«Birahim»	30.07	32.61	20.43	16.89	62.68	37.32
«Gorgui»	29.46	32.69	20.25	17.60	62.15	37.85
«Tyson»	29.57	32.62	20.25	17.56	62.19	37.64
Overall population	29.66	32.64	20.31	17.39	62.3	37.7

A = Adenine; T = Thymine; C = Cytosine and G = Guanine.

3.1.2. Haplotypic and Nucleotide Diversity

The *SRY* gene shows, both in the overall ladoum population and by lineage, a high haplotypic diversity and a low nucleotide diversity except for the "Tyson" population where haplotypic and nucleotide diversities are zero. In the "Gorgui" lineage, haplotypic diversity is maximal and nucleotide diversity is very low (Table 4).

Table 4. *Haplotypic and nucleotide diversity parameters estimated within each population and at the total population.*

Genetic Diversity Parameters		Haplotypic diversity (Hd)	nucleotide diversity (Pi)
By population	«Batling»	0.643	0.00236
	«Birahim»	0.800	0.00339
	«Gorgui»	1.000	0.00269
	«Tyson»	0.000	0.00000
Overall population		0.774	0.00522

3.2. Differentiation and Genetic Distances

F*ST* values are statistically significant both between lineage pairs (P-value between 0.0013 and 0.0072) and in the total population ($P = 0.01241 < 0.05$) showing a hierarchical structuring of the study population (Table 5). The F*ST* values between the "Birahim" lineage and the others are much higher in absolute value than those observed between the other lineages. The lowest value (0.013) is obtained between "Gorgui" and "Tyson". There is also a low value of F*ST* (0.074) between "Gorgui" and "Batling" although statistically significant (Table 5).

Table 5. *Overall and between-pair population FST values. The p-values are in brackets.*

	F*ST* between population pair			Global F*ST*
Lineage	«Batling»	«Birahim»	«Gorgui»	
«Birahim»	0.577 (0.0072)			
«Gorgui»	0.074 (0.0045)	0.861 (0.0021)		0.543 (0.012)
«Tyson»	0.376 (0.0068)	0.710 (0.0034)	0.031 (0.0013)	

Even if all the genetic distances taken two by two are significant, those between the "Birahim" lineage and the others are in absolute values higher. The largest one is observed between the "Birahim" lineage and the "Gorgui" lineage (D=0.011) comparable to the one obtained between the "Birahim" lineage and the rest of the study population (D=0.009). The genetic distances within the "Batling" and "Tyson" lineages are very small and statistically equal to zero. The "Tyson" lineage is genetically homogeneous (Table 6).

Table 6. *Genetic distances (inter and intra populations).*

Race	Genetic distances inter-population			Genetic distances intra-population
	«Batling»	«Birahim»	«Gorgui»	
«Batling»	-			0.001 (0.001)
«Birahim»	0.008 (0.005)	-		0.002 (0.004)
«Gorgui»	0.005 (0.004)	0.011 (0.006)	-	0.008 (0.008)
«Tyson»	0.000 (0.000)	0.008 (0.005)	0.004 (0.003)	0.000 (0.000)
«Birahim»	0.009 (0.005)			0.007 (0.004)
Overall population				0.002 (0.001)

3.3. Molecular Variance (AMOVA)

Analysis of the overall population by AMOVA indicates that the source of molecular variance between lineages is 41.89% and is significant ($P = 0.00289$). 58.11% of molecular variance is explained by intra-lineage variation (Table 7).

Table 7. *Results of the analysis of molecular variance.*

AMOVA test			
Source of variation	**Variance components**	**Percentage of Variation**	**P-value**
Between lineages	0.01957 Va	41.89	0.00289
Within lineages	1.01655 Vb	58.11	0.00100
Overall population	1.03612	100	

3.4. Detection of Natural Selection Signatures Within Populations

Synonymous substitutions are statistically more important than non-synonymous ones in the study population as observed in the "Birahim" and "Gorgui" lineages and contrary to what is obtained in the "Batling" lineage. No selective constraints on the nature of the amino acids of the protein were observed in the "Tyson" lineage (Table 8). The results of the Z-test reveal that "Birahim", "Gorgui"and "Tyson" lineages are under positive selection; only the lineage "Batling" is under negative selection (Table 8).

Table 8. *Types of substitutions and selection test (Z-test).*

	«Batling»	**«Birahim»**	**«Gorgui»**	**«Tyson»**	**Total population**
dS	0.001	0.007	0.008	0.000	0.006
P-value	(0.001)	(0.004)	(0.007)	(0.000)	(0.003)
dN	0.003	0.003	0.002	0.000	0.005
P-value	(0.001)	(0.001)	(0.002)	(0.000)	(0.002)
Z-test	(1.292)	(-0.765)	(-0.367)	(0.000)	(-0.086)
P-value	0.099	1	1	1	1

dS = synonymous substitutions; dN = non-synonymous substitutions.

3.5. Demogenetic and Demographic Evolution

3.5.1. Demogenetic Indices

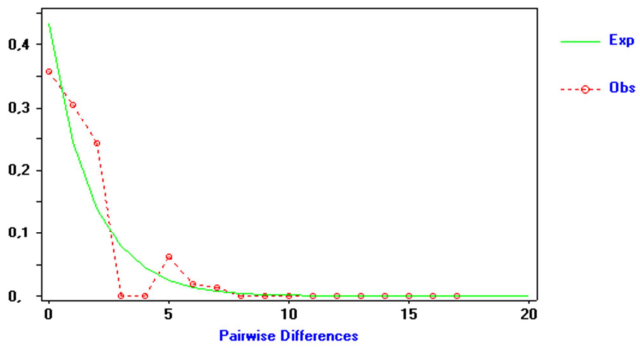
Negative D and Fs values with p-values well above 0.05 are obtained from the sequence analysis of the total population as well as those of the 'Birahim' and 'Gorgui' lineages. The "Tyson" lineage has a Tajima D of zero. Only the "Batling" lineage shows positive Tajima D and Fu Fs values. Apart from the "Tyson" lineage for which the determination of the value is impossible, all values of Ramos R2 are positive (table 9).

Table 9. *Estimation of Tajima's D, Fu's Fs and Ramos' R2 and Rg indices by population and total population.*

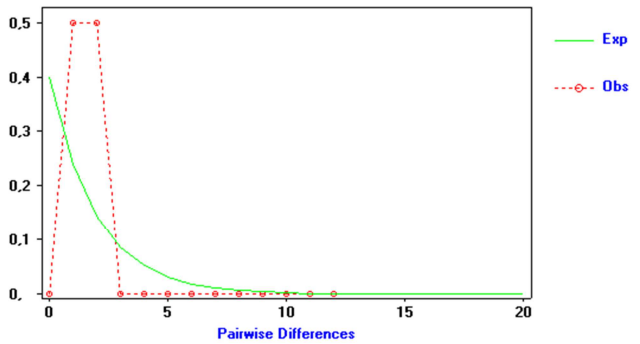
	«Batling»	**«Birahim»**	**«Gorgui»**	**«Tyson»**	**Total population**
Tajima's *D*	0.645	-0.476	-0.314	0.000	-0.03633
P-value	(0.770)	(0.341)	(0.548)	(1.00)	
Fu's *Fs*	1.217	-1.419	-1.157	-	-0.33972
P-value	(0.756)	(0.134)	(0.091)		
Ramos' *R2*	0.1388	0.1571	0.1443	-	0.0838

3.5.2. Analysis of the Mismatch Distribution

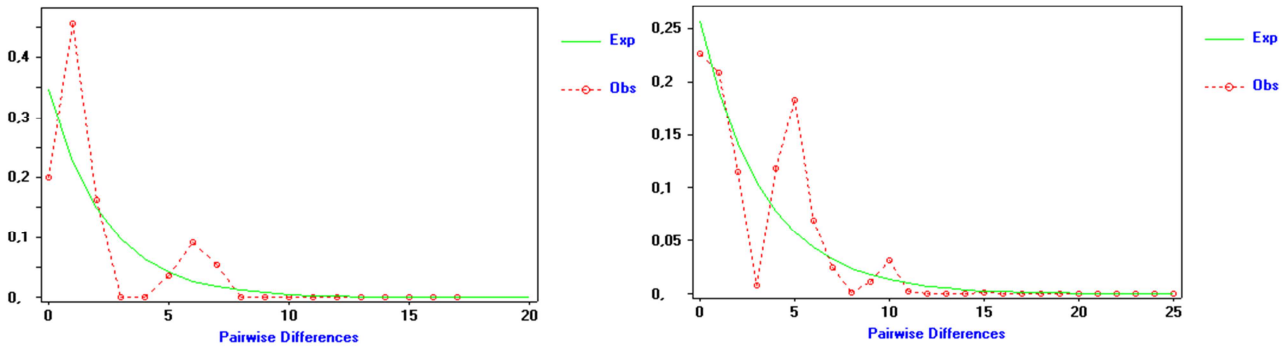
The mismatch plots show a different pattern of the studied population with a population growth signal for the "Batling" and "Gorgui" lineages (unimodal distribution) (figure 2). The "Birahim" lineage shows a stable growth (multimodal distribution).



A: «Batling» mismatch distribution



B: «Gorgui» mismatch distribution



C: «Birahim» mismatch distribution

D: Global mismatch distribution

Figure 2. *Mismatch distribution curves of the 3 lineages and the overall population.*

These graphs are supported by the values of the SSD and Rag indices, which are not significant indicating that there is no discrepancy between the observed and expected values. The disparity distribution was not calculated for the "Tyson" lineage of zero genetic distance between individuals of this lineage (Table 10).

Table 10. *Demographic distribution indices SSD (Sum of Squared Deviation) and Rg (Raggedness) of each population and the total population.*

	«Batling»	«Birahim»	«Gorgui»	«Tyson»	Total population
SSD	0.051	0.027	0.079	0.000	0.0395
P-value	(0.350)	(0.360)	(0.480)	(0.00)	(0.297)
Rg	0.122	0.092	0.222	0.000	0.109
P-value	(0.530)	(0.540)	(0.810)	(0.00)	(0.470)

3.6. Amino Acid Frequency

The amino acid frequency distribution shows similar proportions in absolute values between the “Batling”, “Gorgui” and “Tyson” lineage compared to the “Birahim” lineage.

Table 11. *Amino acid frequency of the SRY gene in the 'Birahim'population compared to the rest of the population.*

	«Birahim»	Population	P-value
Ala	1.737	3.484	0.0754
Cys	2.248	2.857	0.1120
Asp	1.686	1.769	0.4722
Glu	1.788	3.429	0.0513
Phe	7.307	10.287	0.0091
Gly	3.832	3.429	0.7604
His	2.248	2.286	0.678
Ile	8.380	4.572	0.0231
Lys	2.620	2.839	0.2012
Leu	12.927	11.449	0.0499
Met	0.000	2.286	0.0003
Asn	6.796	6.286	0.1140
Pro	6.745	6.858	0.4762
Gln	4.496	3.982	0.5420
Arg	3.781	3.263	0.7455
Ser	10.168	13.882	0.0039
Thr	6.745	5.088	0.0985
Val	7.307	5.660	0.0719
Trp	1.686	1.143	0.1087
Tyr	4.496	5.143	0.0929

A statistically significant difference in the frequency of some differentially expressed amino acids was noted between the 'Birahim' lineage and the others combined (Table 11). Phenylalanine and serine are less important in the "Birahim" lineage; on the other hand, leucine and isoleucine are more expressed in the "Birahim" lineage. Methionine is not expressed in the Birahim lineage (Table 11).

4. Discussion

Each individual presents the characteristics of its species with variations that are specific to it, resulting from the expression of its genetic program and environmental influence. The hereditary characteristics, specific to the species, are transmitted by the parents, from generation to generation. These hereditary characteristics are determined by a set of information contained in the chromosomes, the support of the genetic program (genome) constituted by the deoxyribonucleic acid (DNA) molecule. The *SRY (Sex-determining Region of Y chromosome)* gene is a portion of this DNA molecule, located on the Y chromosome responsible for male gonadal differentiation and conserved throughout evolution [36]. According to Meadows *et al* (2006), autosomal microsatellites and genetic variation especially in the 5' promoter region of the *SRY* gene are studied in discrimination research and origins of domestication in sheep [37]. In this study, the promoter region and a coding portion of the *SRY* gene were targeted to highlight genetic diversity, evolutionary mode as well as phylogenetic relationships between four lineages of Ladoum, a local sheep breed from Senegal. Any interpretation of the results will have to take into account the disproportion between the samples, explained by the fact that some breeders are reluctant to let their animals be sampled because of a strong suspicion of mysticism that reigns in this environment.

Analysis of genetic variability parameters in the overall population revealed a low level of polymorphism in the *SRY*

gene and a combination of polymorphic nucleotides throughout the sequences. This low genetic variability of the *SRY* gene may be due to its specific function as a male determinant, which is transmissible from generation to generation [38, 39]. In the work of Fu *et al* [40] on marsh buffalo, it was described that in the 5' region, the promoter sequences of transcriptional regulation were highly conserved confirming the observations made in our study population. Furthermore, it has been shown that mutations in the Y chromosome are very rare and that the *SRY* gene sequence is very stable within the same species, a group of potentially or actually interbreeding natural populations that are productively isolated from other similar groups [39]. Haplotypic diversity, which indicates the probability that two haplotypes drawn at random from the entire population are similar, is much higher than the average nucleotide divergence between all pairs of haplotypes. Although Ndiaye [41] had shown that the Ladoum breed is the most stable compared to other sheep breeds in Senegal, the comparative trends in genetic diversity indices show a signal of rapid population growth from a small ancestral population that has been effective for a sufficient time for recovery of haplotypic diversity through mutation, but too short for the accumulation of strong sequence differences. This finding is supported by the shape of the mismatch distribution curves and validated by demographic indices (SSD and Rg) and demogenetic tests (Tajima's D, Fu's FS and Ramos & Rosas' R2). It is therefore an event of recent expansion from an ancestral population of low effective size for a sufficient time for a recovery of haplotypic diversity through an accumulation of mutations. This richness in variants would imply that this gene is subject to high selection pressure, probably due to a high rate of exchange between different Ladoum lineages, but also to the introduction of exotic males to improve certain reference criteria. The genetic processes responsible for the diversity of the populations are selective or random and lead to the appearance of domestic phenotypes. These selective processes, which are artificial selection (a controlled process), natural selection and the relaxation of natural selection (both partially controlled processes), are subject to uncontrolled random evolutionary forces consisting of mutation, reproductive isolation, inbreeding and genetic drift [42, 43]. According to the same authors, these processes are increasingly used in the Ladoum breed to produce offspring with good quality characteristics, which may be the reason for the observed population expansion. Our results are in agreement with those of Sy *et al* [7] where high Hd and low Pi were obtained in Ladoum and Touabire populations. The exception to this process is the 'Tyson' lineage, which shows a severe and prolonged signal of a recent bottleneck with zero Hd and Pi values. In contrast to the 'Batling' population which expresses negative selection with an amino acid change and the 'Tyson' population where no constraint was observed, the Z-test analysis of selection revealed statistically significant positive or bidirectional selection for both the 'Birahim' and 'Gorgui' lineages and the study population. Although the vast majority of mutations are neutral (i.e. have no phenotypic effect) and advantageous mutations are very rare, the results of the selection test reveal that the derived state of a genetic variant has a selective advantage over the ancestral state in our study population. Usually, only parts of a protein are under positive selection while others are under strong purifying selection to maintain the basic structure and function of a protein. Thus, if a gene is expressed, codon usage, nucleotide bias and other factors (protein toxicity) will generate purifying selection. Moreover, generally positive selection only works for a certain evolutionary time. This recent population explosion could therefore be due to the massive and intensive exploitation of the Ladoum breed as described in the work of Fall *et al* [3]. The "Batling" population is undergoing a loss of highly deleterious alleles through so-called purifying negative selection. It is described as stabilising because it contributes to the reduction of genetic diversity [44]. The "Tyson" population is not under selection and evolution according to Kimura's neutralist theory. It should be noted that congruence is not found when one attempts to reconstruct the demogenetic history of the lineages leading to consider the population as a whole as forming a homogeneous group, distinguishing it from other sheep breeds.

The observed amino acids frequencies are globally low. According to Robelin & Theriez [45], this low frequency may be due to the fact that the protein content of the empty body changes little in absolute value after birth. For these authors, variations between individuals as well as variations between lineages are closely linked to variations in lipid and water content. In any case, proteins are essential for a multitude of functions in the animal organism and yet statistically significant differences between the "Birahim" lineage and the rest of the study population are obtained for phenylalanine, leucine, isoleucine and serine. The higher phenylalanine frequencies in the "Batling", "Gorgui" and "Tyson" populations suggest a difference in phenotype of the group compared to the "Birahim" population. A high frequency of phenylalanine may be due to its function as a precursor of tyrosine, which has the capacity to synthesize dopamine, itself a precursor of catecholamines (adrenaline and noradrenaline), thyroid hormones and melanin (pigment responsible for the coloring of hair, eyes and skin) As for serine, it is involved in the biosynthesis of cysteine and brain phospholipids, which are necessary for cognitive functions. The higher frequencies of leucine and isoleucine in the "Birahim" population indicate a major signal of protein synthesis and thus of muscle tissue renewal in this lineage. Both leucine and isoleucine are branched chain amino acids. Isoleucine is an essential amino acid that is mainly concentrated in muscle tissue in the human body. It is thought to be involved in the uptake of glucose into the cells and breaking it down into energy. This specific function of isoleucine is the reason why it can help to improve sports and resistance performance [46], characteristics observed in the "Birahim" lineage (personal communication). As for methionine, it is known for its benefits for the beauty of hair and nails. Indeed, it has the particularity of containing sulfur,

an essential element for the elaboration of sulfur proteins such as keratin. As a reminder, keratin is the main constituent of the phanera (horns, nails, hair and hair). Its absence in the "Birahim" lineage could explain the fact that the horns of the individuals of this lineage would be globally less developed compared to those of the other lineages. The estimation of the genetic distance between Ladoum lineages reveals that there is a small distance between lineages, highlighting however the genetic distance of the "Birahim" lineage compared to the others. The values of genetic distances between Ladoum lineages are comparable to those obtained between West and Central African breeds of zebus and bulls [47], between Mozambican and Cameroonian cattle breeds [48, 49]. This trend is supported by the values of the *FST* which measures, according to Hartl & Clark [50], the deficit of heterozygotes relative to that expected under Hardy-Weinberg equilibrium. According to Balloux & Lugon-Moulin [51], it has been suggested that *STF* values between 0 and 0.05 indicate weak genetic differentiation; between 0.05 and 0.15, moderate differentiation; between 0.15 and 0.25, strong genetic differentiation; and above 0.25, very strong genetic differentiation. The average *STF* (0.546), which seems very high, nevertheless reveals moderate genetic differentiation between the lineages studied. Although such an interpretation may be accurate, the actual genetic differentiation of a population may not be representative due to loss of heterozygosity when analyses are done on sequence data of diploid species. Indeed, it is recalled that the expected *STF* under full differentiation will not always be maximal because the effect of polymorphism (due to mutations) can drastically increase the expected values of the *STF* [51].

The genetic distances, as well as the values of the genetic differentiation factor, support a structuring of the study population because, even if there are no reproductive barriers between the four population groups, the singularity of the gene studied (gene with quasi-paternal transmission) and the control of the matings by the owners of the subjects could lead to a radiative, purifying evolution of the different lineages. Furthermore, most of the molecular variance has been observed within lineages that essentially value individual characteristics within them and favour selection within a lineage by some breeders [3], sometimes going as far as controlling reproduction by injecting hormones. According to Ndiaye *et al.* [4], breeders rigorously select sires according to coat, animal conformation and sociological considerations, with a view to genetic improvement of the herd. This variability between individuals can also be attributed to the origin [52] and regional distribution of the different populations [53] involved in the selection of the Ladoum breed. Indeed, the Ladoum breed is increasingly bred mainly on the outskirts and inside large cities [41] and this breeding is considered by some as a prestige activity, becoming attractive to certain socio-professional categories [9].

5. Conclusion

Such a study certainly includes several shortcomings in the analytical approach, as a great variability is noted in the conception, by ecologists and geneticists, of a population, ranging from a lack of knowledge of its limits and composition to a detailed study of individuals and their interactions in both space and time. This variability also reflects a continuity of viewpoints, from a mathematical conception to an ecological and ethological consideration of the population. It is possible, however, to draw up a more or less complete inventory of different types of populations considered from a genetic point of view by referring to the genetic diversity in natural populations. Taking into account one of the limitations of this study, which is the disproportion in the number of individuals between lineages, it should be noted that: (i) The *SRY* gene is an excellent molecular marker; (ii) The *SRY* gene is under selection. The previously defined lineages "Birahim" and "Gorgui" are under positive selection; only the lineage "Batling" is under negative selection; the lineage "Tyson" is not under selection; (iii) There is a genetic structuring within the Ladoum breed in Senegal; (iv) In Senegal, there are only two genetically identified groups within the Ladoum breed: the one formed by individuals of the "Birahim" lineage and another grouping the other lineages.

This study makes a significant contribution to the management of animal genetic resources which constitute a considerable part for food and agriculture. They are an essential part of the biological basis of global food security, and they provide livelihoods for more than a billion people. A diverse resource base is essential for human survival and well-being and to contribute to the eradication of hunger. The results provide decision-makers with new elements for the management of this new breed of sheep, the adaptation and evolution of which must be mastered in socio-economic and environmental conditions in constant change due to climate change.

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