Genetic diversity of bovine populations raised in Senegal

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Genetic diversity of bovine populations raised in Senegal

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Abstract

Background: The Gobra zebu and N'dama taurine cattle breeds are important genetic animal resources for Senegal. For several decades, genetic breeding programmes have been devoted to them at the Centre de Recherches Zootechniques de Dahra and Kolda. Since then, these animals have been subjected to mass selection, mainly in closed selection nuclei.

Objective: This study aims to assess the genetic diversity within these selection nuclei in order to orient future selection strategies.

Material and methods: The study was carried out on the Gobra zebu and N'dama taurine populations from selection nuclei of Dahra and Kolda respectively, which were compared to 5 other populations of the main cattle breeds in Senegal. One hundred eighty (180) animals were genotyped with 21 microsatellite markers recommended by the Food and Agriculture Organisation.

Results: All populations were found to be polymorphic with a PIC of over 55%. However, animals from the CRZ-Dahra (indigenous) and CRZ-Kolda stations had the lowest mean heterozygosity (0.643 and 0.591 respectively). The other populations had an average heterozygosity between 0.650 and 0.737.

Conclusion: The cattle populations maintained at the different CRZs show a lower genetic diversity than the other populations described in our study. The main reasons for this are reproductive isolation and selection pressure on these populations.

KEYWORDS
genetic diversity, Gobra, microsatellites, N'dama

1 | INTRODUCTION

In Senegal, livestock farming is the second largest source of incomes for rural populations. It represents more than 28% of the primary sector and contributes to more than 4% of gross domestic product (ANSD, 2014). The economic importance of livestock is also due to the fact that it contributes significantly to the living conditions of the rural population, which represents 55% of the national population (ILRI, 2010; ANSD, 2014). In addition to its irrefutable economic role, livestock farming plays also a very important social role. Indeed, for some ethnic groups in the country, livestock keeping is not only an income-generating activity but also as a sign of wealth, for example, Ladoum breed (Sambe, 2021).

In Senegal, cattle are mainly exploited for milk and meat. In addition, the sale of hides and horns generates direct income, while draught power and dung used in agriculture contribute indirectly to income

FIGURE 1 Gobra zebu (a) and N'dama taurine (b)

generation. In Senegal, local breeds predominate over exotic breeds introduced for their dairy performance. Four indigenous cattle breeds are found in Senegal. These are the Gobra zebu (Figure 1a) cattle in the Sahelian area (in the North and Centre – sylvo-pastoral area), the N'dama taurine (Figure 1b) in the Sudano-Sahelian area (in the South and East), the Djakoré (resulting from the crossbreeding between Gobra zebus and N'dama taurines) in transitional areas and the Moorish zebu in the North of the country (Ndiaye et al., 2015). The most common exotic breed (especially in the sylvo-pastoral area) is the Guzera zebu (Diack et al., 2016).

The importance of the Gobra zebu cattle and the N'dama taurine cattle in the socioeconomic life of farmers is such that it has led to the establishment of genetic breeding programmes dedicated to them. The first programme, dedicated to the mass selection of the Gobra, was initiated in the early 1950s at the Centre de Recherches Zootechniques (CRZ) in Dahra. The second, initiated in the early 1970s at another CRZ in Kolda, is dedicated to the N'dama. Population from CRZ of Dahra enrolled in this selection programme is preserved from crosses with other cattle breeds (Sambe, 2021; Sow et al., 1988; Wane et al., 2017). Indeed, the first population (Gobra) was globally maintained in a closed selection nucleus while the second (N'dama) became an open selection nucleus in the early 1990s (Sambe, 2021; Wane et al., 2017). For the latter, animals from village herds located near the CRZ of Kolda are regularly tested to identify elite animals. Moreover, the study of breeds, using molecular techniques is very important and useful for their characterising (Bordbar et al., 2021). Conservation of genetic diversity in animal species requires the proper performance of conservation superiorities and sustainable handling plans (Mohammadabadi et al., 2021a) that should be based on universal information on population structures, including genetic diversity resources among and between breeds (Mohammadabadi et al., 2017). Genetic diversity is an essential element for genetic improvement, preserving populations, evolution and adapting to variable environmental situations (Mohammadabadi et al., 2021b; Zamani et al., 2015). The objective of this study was to evaluate the impact of this management and isolation on the genetic diversity of this population.

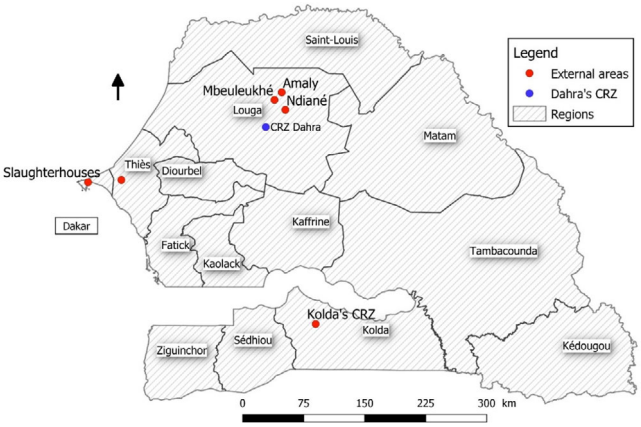

FIGURE 2 Study areas for genotypic characterisation

2 | MATERIALS AND METHODS

2.1 | Study population and blood collection

The study involved 180 animals randomly selected from the seven study areas shown in Figure 2. The study population comprised four breeds: N'dama taurines, Gobra zebus, Guzera zebus and Moorish zebus. Thus, 41 samples of Gobra from the CRZ of Dahra, 68 samples of Gobra from 3 villages located close to CRZ of Dahra (21 in Amaly, 22 in M'beuleukhé and 25 in N'diané); 29 samples of N'dama from the selection nucleus of the CRZ of Kolda; 22 samples of Moorish zebu from the Dakar slaughterhouse; and 20 Guzera samples from a private farm in Thiès (EMAAP). The 41 animals from the CRZ of Dahra were composed of (i) 29 animals from the genetic breeding programme (native) and (ii) 12 animals (from the 33 animals from villages close to CRZ of Dahra) introduced in 2017 to reinforce the nucleus herd. Information about the sex of the animals was entered in Table S1.

Blood collection was carried out between October 2016 and March 2017. On each animal, blood was taken from the jugular vein in ethylene diamine-tetra-acetic acid (EDTA) tubes. The samples thus obtained were centrifuged at 8000 rpm for 10 min to collect the buffy

TABLE 1 Thermal conditions for PCR of microsatellite markers

Steps	Temperature (in°C)	Time	Comments
1	94	15 min	Initial denaturation
2	94	30 s	PCR touch down step 10 cycles – with each cycle decrease the temperature by 0.5°C
3	T° H_{TD}	1 min 30	
4	72	1 min 30	
6	94	30 s	PCR conventional step 20 cycles
7	T° H	1 min 30	
8	72	1 min 30	
9	72	15 min	Final elongation

T° H_{TD}: hybridisation temperature of PCR Touch Down step; T° H: desired hybridisation temperature.

coat. The buffy coat was then placed in 1.5 ml Eppendorf tubes and stored at −20°C until the genomic DNA was extracted.

2.2 | DNA extraction

Genomic DNA was extracted from the buffy coats using the Zymo Research commercial kit (Quick-DNATM Universalt Kit) following the protocol described by the manufacturer. The extracted DNA was stored at −20°C until genotyping.

2.3 | Genotyping of individuals

Individuals were genotyped with 21 microsatellite markers chosen from the list recommended by FAO (2011).

The amplification reactions were performed with Applied Biosystems fluorochrome-labelled forward primers (FAM, VIA, PET and NED) via TouchDown PCRs in simplex or multiplex. Thus, 6 markers could be amplified individually (simplex) while the others were amplified in groups of 2 (duplex), 3 (triplex) or 6 (sextaplex) (Table S1). The amplifications were performed with a total reaction volume of 12 µl containing: 5 µl of mix without primers, 5 µl of primers mix and 2 µl of DNA at the concentration of 10 ng/µl of each sample. The mix without primers consisted of: 3.79 µl distilled water, 0.60 µl 10× Buffer, 0.48 µl $MgCl_2$ (25 mM), 0.05 µl dNTP (25 mM) and 0.08 µl Taq polymerase (5 units/µl). The primer mix consisted of 1 µl of each F-primer, 1 µl of each R-primer and the amount of distilled water needed to obtain a volume of 5 µl (i.e. 4.8, 4.6, 4.4 or 3.6 µl of water for simplex, duplex, triplex or sextaplex amplification respectively). The primers were at a concentration of 20 µM.

These amplifications were performed using a Fisher Scientific Veriti™ 96-Well Fast Thermal Cycler. The thermal conditions for the amplifications are detailed in Table 1.

The amplification products were then subjected to electrophoresis on an Applied Biosystems 3500 Genetic Analyzer automatic capillary sequencer. Five migration groups were formed. For each sample, migration was performed by mixing 0.25 µl of LIZ600, 8.75 µl of formamide and 1 µl of each PCR group constituting the migration group considered. The hybridisation temperatures for each primer as well as the amplification and migration groups are given in the Table S2.

The electrophoretic profiles of each individual were analysed and cleaned using GeneMapper® software, version 5. Thus, for each locus the size of the amplified alleles was determined for each individual.

2.4 | Data analysis

2.4.1 | Genetic variability

Allele identification was done on GenAlEx version 6.5 (Peakall & Smouse, 2012). After identification of the alleles, the genetic variability was estimated according to the loci and populations by calculating: the average numbers of alleles, the allelic richness (Rs: allelic richness per population at all loci; Rt: allelic richness per locus for all populations combined), allelic frequencies, the degree of polymorphism at the 95% and 99% threshold, the Polymorphic Information Content (PIC) and finally the rates of heterozygosity [H_{OBS}: observed heterozygosity; H_{EXP}: Nei's (1987) unbiased expected heterozygosity].

Average allele numbers, allelic richness and allelic frequencies were calculated using Fstat version 2.9.3.2 (Goudet, 2002). Polymorphic Information Content was determined using Cervus software version 3.0.7 (Kalinowski et al., 2007) and heterozygosity was calculated using Arlequin version 3.1 (Excoffier et al., 2006).

2.4.2 | Genetic equilibrium

Hardy–Weinberg equilibrium (per locus and per population) and linkage disequilibrium (between pairs of loci) were evaluated with Genepop version 4.2.2 (Rousset, 2008). Hardy–Weinberg equilibrium was tested by performing 10,000 de-memorisations, 20 batches and 100,000 iterations. The signal of the linkage disequilibrium was evaluated on R with a binomial test as described by De Meeûs (2012). The significance level of the tests performed was set at 5%.

3 | RESULTS AND DISCUSSION

3.1 | Results

After cleaning the data, the HEL5 marker and 5 zebu's individuals (1 Gobra from Amaly, 3 Guzera and 1 Moorish) were eliminated due to poor amplification. The analyses therefore covered 20 microsatellite markers and 175 individuals, representing 3,500 data points or genotypes. Of these 3,500 genotypes, only 74 (approximately 2%) were identified as null alleles (Table 2). On average, in each population and for each locus, a maximum of 2 individuals showed null alleles. For all populations combined, each locus showed on average 3 to 4 individuals with null alleles (Table 2).

TABLE 2 Numbers and percentages of alleles per population

Populations		Nb_{AN}	$Nm_{AN/L}$	Nb_{AI}	Nb_{AP}	P_{AP}
Gobra zebu (CRZ Dahra)	Native	10	0.500	127	6	4.724
	Acquired	1	0.050	124	2	1.613
Gobra zebu (Amaly)		6	0.300	134	5	3.731
Gobra zebu (M'beuleukhé)		3	0.150	128	2	1.563
Gobra zebu (N'diané)		11	0.550	137	4	2.920
Guzera zebu (EMAAP)		8	0.400	121	7	5.785
Moorish zebu (SOGAS Dakar)		29	1.450	140	6	4.286
N'dama taurine (CRZ Kolda)		6	0.300	101	2	1.980
Total		74	3.700	201	35	16.667

Nb_{AN}: total number of null alleles; $Nm_{AN/L}$: average number of null alleles per locus; Nb_{AI}: total number of identified alleles; Nb_{AP}: number of identified private alleles; P_{AP}: percentage of private alleles.

In addition to null alleles, 201 alleles were identified. About 17% of the alleles detected in the studied population were private alleles (Table 2). The proportion of null alleles observed per loci is very low as less than 5% for all loci except for HEL1 and INRA063. These two loci presented a proportion of null alleles equal to about 5.7% (which represents 10 null alleles for 175 targeted genotypes). The populations with the highest proportions of private alleles were the Guzera and the Gobra native to the CRZ of Dahra with about 6%; the Moorish and Gobra of Amaly with about 4% private alleles. The other populations have less than 3% of private alleles (Table 2).

Between 120 and 140 alleles were identified in zebu populations, while 101 were identified in the N'dama (Table 3). Per locus, the number of alleles was on average 10 and varies between 5 (BM1824) and 16 (TGLA122). Considering populations, the zebu populations had on average 6–7 alleles per locus, whereas N'dama had about 5 per locus (Table 3).

When the effects of sample size variability are removed, the populations show allelic richness of about 4–5 alleles per locus/population (Table 4). However, it should be noted that the populations with the lowest allelic richness were the native Gobra of the CRZ of Dahra and the N'dama of the CRZ of Kolda. All populations combined, the loci had an allelic richness between 3 and 6 (Table 4).

No allele had a frequency greater than or equal to 95%, so all loci were polymorphic. Each locus had a PIC greater than 55%. Moreover, except for the BM1824 and ETH152 loci, all the others had a PIC greater than 60% (Table 5). The observed heterozygosities were globally higher than 59%. The N'dama from the CRZ of Kolda and the native Gobra from the CRZ of Dahra had the lowest heterozygosities (0.591 and 0.643, respectively). All other populations had heterozygosity between 0.650 (Gobra from M'beuleukhé) and 0.737 (Gobra from Amaly).

For most of the loci, all populations were in Hardy–Weinberg equilibrium (Table 6). In the zebu populations, five loci (BM2113, CSSM66, HEL9, INRA063 and SPS115) showed recurrent and significant deviations from the Hardy–Weinberg model. They describe a more or less important heterozygosity deficit within these populations (deviations between H_{OBS} and H_{EXP} ranging from 0.086 for HEL9 to 0.725 for SPS115). Five other loci (ETH185, ILSTS005, INRA005, INRA037 and MM12) showed a significant deviation from the Hardy–Weinberg model. These deviations, varied in absolute value between 0.082 for INRA037 and 0.224 for ETH185. For the N'dama population of the CRZ of Kolda, six markers deviated significantly from the Hardy–Weinberg equilibrium model. Four of them (BM1824, BM4440, CSSM66 and ETH152) showed a heterozygosity deficit (differences between H_{OBS} and H_{EXP} between 0.079 for BM1824 and 0.397 for ETH152). The other two (ETH185 and INRA032) showed an excess of heterozygous genotypes.

The multi-loci analysis showed that all populations were significantly in Hardy–Weinberg disequilibrium (Table 6). Overall, this imbalance was in the direction of a slight deficit in heterozygosity in all populations except for the N'dama of the Kolda CRZ, for which a slight excess of heterozygosity was observed. Furthermore, the deviation from the Hardy–Weinberg equilibrium model (differences between observed and expected heterozygosity) is much less important for the N'dama populations of the CRZ of Kolda, Gobra from Amaly and natives of the CRZ of Dahra. Indeed, these populations showed differences varying in absolute value between 0.005 and 0.027, whereas they vary between 0.039 and 0.084 for the other populations studied (Guzera, Moorish, Gobra of M'beuleukhé, N'diané and acquired of the CRZ of Dahra).

Out of 190 loci pairs, only 12 showed a significant *p* value in the linkage disequilibrium test (Figure 3). The linkage disequilibrium signal test gave a non-significant *p* value. This means that the observed linkage disequilibrium for the 12 loci pairs can be explained by chance. Therefore, all loci were used for further analysis as they are considered independent.

3.2 | Discussion

The populations showed significant variability at the studied loci. These loci showed: a high average number of alleles (about 10 alleles per loci),

TABLE 3 Number of alleles identified per locus and per population

Loci	Gobra zebu (CRZ Dahra)		Gobra zebu (Amaly)	Gobra zebu (M'beuleukhé)	Gobra zebu (N'diané)	Guzera zebu (EMAAP)	Moorish zebu (SOGAS Dakar)	N'dama taurine (CRZ Kolda)	Nb_T
	Native	Acquired							
BM1818	7	5	8	9	9	7	8	6	10
BM1824	5	4	4	5	4	4	4	3	5
BM2113	10	9	7	6	6	6	7	8	13
BM4440	6	9	8	9	8	9	8	3	14
CSRM60	5	7	7	5	5	8	7	8	11
CSSM66	10	6	10	6	7	7	10	6	14
ETH10	6	7	7	7	8	4	6	4	8
ETH152	6	4	6	3	4	5	4	3	9
ETH185	7	7	8	5	5	4	10	5	12
HEL1	5	6	6	6	7	7	6	5	10
HEL9	5	8	7	8	9	8	8	8	11
ILSTS005	4	4	4	5	5	5	5	2	5
INRA005	7	5	6	6	5	5	5	4	8
INRA023	7	5	7	7	7	6	6	7	9
INRA032	6	6	7	8	8	4	7	4	8
INRA037	7	7	7	7	7	7	7	5	8
INRA063	4	6	6	5	5	4	7	4	10
MM12	7	8	7	8	9	7	10	6	13
SPS115	4	4	5	5	6	6	6	3	7
TGLA122	9	7	7	8	13	8	9	7	16
Nb_S	127	124	134	128	137	121	140	101	201
Ns	6.350	6.200	6.700	6.400	6.850	6.050	7	5.050	10.050

Nb_T: total number of alleles identified for all populations combined; Nb_S: total number of alleles identified for all loci combined; Ns: average number of alleles identified for each population combined.

allelic richness globally between 3 and 7 alleles per loci/population, a high Polymorphic Information Content (PIC > 50%) and an average heterozygosity globally between 60% and 75%. Animals from the CRZ-Dahra (native) and CRZ-Kolda stations showed the lowest average heterozygosity (0.643 and 0.591, respectively). The other populations had average heterozygosities between 0.650 and 0.737. These results were very similar to those observed in Gobra populations from the groundnut basin, Moorish from the river valley and N'dama from the CRZ of Kolda in Senegal (Ndiaye et al., 2015); taurine breeds or zebu breeds found in Africa (Dayo et al., 2009; Freeman et al., 2004; Foulley & Ollivier, 2006; MacHugh et al., 1997; Ngono Ema et al., 2015); Asian zebu (Chaudhari et al., 2009; Sharma et al., 2009; Shi et al., 2010; Suh et al., 2014; Singh et al., 2015); European cattle breeds (Dayo et al., 2009; Freeman et al., 2004; Foulley & Ollivier, 2006; MacHugh et al., 1997) and Brazilian breeds (da Silva Filho et al., 2014). This indicates a high genetic diversity observed across the loci studied. The low genetic diversity of the populations from the CRZ-Dahra (native) and CRZ-Kolda stations compared to the other populations is attributable to the founder effect (Serre, 2006). Indeed, the reproductive isolation of these populations, combined with the selection pressure exerted on them, can lead to a loss of genetic diversity (Serre, 2006). The PIC of these loci showed that they are suitable for assessing the diversity and genetic structuring of our populations (Botstein et al., 1980; Shi et al., 2010; Singh et al., 2015). Moreover, in view of the results of the linkage disequilibrium test, the information conveyed by these loci is not redundant (De Meeûs, 2012).

Significant deviations from the Hardy–Weinberg model were observed for some loci in the populations studied. These deviations were mainly carried by the loci BM2113, CSSM66, HEL9, INRA063, SPS115, ETH185 and INRA032. In general, they cause a slight heterozygote deficit in zebu populations or a slight heterozygote excess in the N'dama of the CRZ-Kolda. Similar observations were made by Ndiaye et al. (2015) for zebu populations and MacHugh et al. (1997) for several zebu breeds in Africa but also for N'dama samples from Gambia, Guinea and Guinea Bissau.

The heterozygosity deficit observed for the CRZ's acquired Gobra population must be due to the rigorous screening that was done to select these animals according to phenotypic criteria of socioeconomic importance (appearance of the coat, horns and conformation of the animal). Indeed, the distribution of allele frequencies of certain loci can

TABLE 4 Allelic richness by locus and population

Loci	Gobra zebu (CRZ Dahra)		Gobra zebu (Amaly)	Gobra zebu (M'beuleukhé)	Gobra zebu (N'diané)	Guzera zebu (EMAAP)	Moorish zebu (SOGAS Dakar)	N'dama taurine (CRZ Kolda)	Rt
	Native	Acquired							
BM1818	4.427	4.395	5.740	5.777	5.812	4.249	5.689	4.571	5.578
BM1824	3.109	3.504	3.440	3.922	3.302	3.145	3.52	2.066	3.428
BM2113	4.362	5.983	4.475	4.203	4.004	4.355	4.547	4.815	5.095
BM4440	4.291	5.543	5.208	4.628	5.217	5.848	5.059	1.357	5.085
CSRM60	3.173	4.290	3.898	2.862	3.607	4.387	4.46	3.808	4.028
CSSM66	5.972	4.764	5.592	4.555	4.208	5.326	5.224	3.750	5.640
ETH10	3.341	4.950	5.539	4.658	4.841	2.931	4.610	2.626	4.880
ETH152	2.987	3.228	3.234	2.522	3.094	2.867	2.978	2.539	3.282
ETH185	3.892	4.927	4.335	3.790	3.627	2.912	5.555	3.542	4.479
HEL1	3.181	4.422	4.407	4.194	4.942	4.658	4.433	3.171	5.034
HEL9	3.929	5.624	5.181	5.561	6.211	5.655	5.103	4.936	5.816
ILSTS005	3.409	3.389	3.612	3.246	3.357	3.585	4.130	1.997	3.562
INRA005	4.043	3.970	4.097	3.963	3.788	4.238	3.615	3.025	4.001
INRA023	5.020	4.214	4.734	3.600	4.327	4.175	4.081	4.003	4.566
INRA032	3.341	4.545	5.611	5.093	5.079	2.972	4.779	2.630	5.019
INRA037	4.957	5.553	4.676	4.901	4.833	4.941	5.176	2.804	5.098
INRA063	3.433	4.886	4.184	3.663	4.024	2.906	4.744	2.485	3.957
MM12	4.418	5.081	3.710	4.623	5.175	4.429	4.763	3.759	5.059
SPS115	2.238	3.625	2.971	4.159	3.223	4.285	4.294	2.892	3.779
TGLA122	4.824	4.980	4.745	4.729	5.980	5.628	5.044	3.199	5.399
Rs	3.881	4.618	4.420	4.224	4.421	4.166	4.594	3.272	4.627

Rs: allelic richness per population at all loci; Rt: allelic richness per loci for the entire study population.

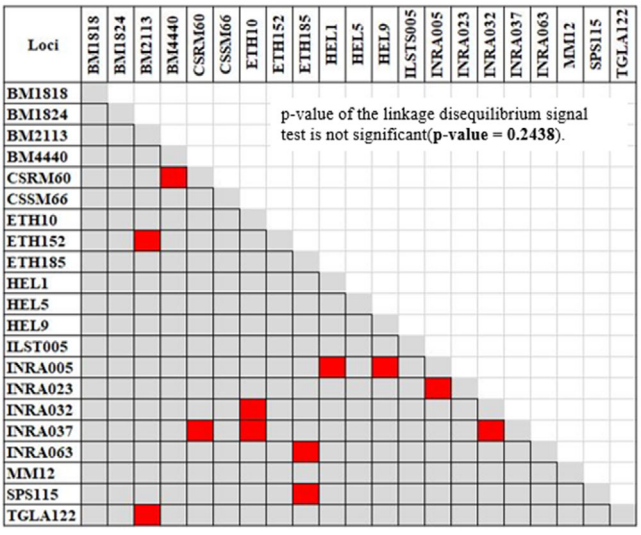

FIGURE 3 Significance matrix of the linkage disequilibrium test (red boxes represent loci pairs with a significant *p* value in the linkage disequilibrium test and grey boxes represent loci pairs without a significant *p* value in the linkage disequilibrium test)

be modified by the proximity of these loci to a portion of DNA coding for a phenotypic trait of interest. This is the hitchhiking phenomenon described by various authors such as Slatkin (1995), Nielsen et al. (2006) and De Meeûs (2012). In addition, Oliveira et al. (2005) showed that in a Brazilian breed (Brangus Ibagé), an association between allele frequencies of the HEL5 loci and some reproductive performance traits such as calving interval. Also, DeAtley et al. (2008) noted an association between ETH10 allele frequencies with growth and carcass traits in Brangus. Brenig and Schütz (2016) noted that in the German Holstein Friesian breed, the allele frequency distributions of the loci ETH3, ETH225, TGLA122, TGLA126 and TGLA227 are most influenced by their selection criteria. They also noted that the reduction in frequency of the 137 bp BM2113 allele over time may indicate the adverse effect of this allele on the performance of these animals.

In addition to the hitchhiking phenomenon, which would apply to all populations for the same reasons explained above, three other phenomena could justify the heterozygosity deficits observed in our study for the other populations. These are: (i) the presence of null alleles; (ii) the nature of the loci involved in Hardy–Weinberg disequilibrium and (iii) the Wahlund effect.

The test for the impact of null alleles performed according to the second Brookfield model shows that, under the assumption of

TABLE 5 Polymorphic information content by locus and population

Loci	Gobra zebu (CRZ Dahra)		Gobra zebu (Amaly)	Gobra zebu (M'beuleukhé)	Gobra zebu (N'diané)	Guzera zebu (EMAAP)	Moorish zebu (SOGAS Dakar)	N'dama taurine (CRZ Kolda)	All populations
	Native	Acquired							
BM1818	0.457	0.614	0.582	0.407	0.581	0.698	0.691	0.593	0.831
BM1824	0.601	0.724	0.679	0.631	0.693	0.417	0.724	0.312	0.581
BM2113	0.652	0.736	0.659	0.633	0.625	0.429	0.794	0.588	0.793
BM4440	0.539	0.746	0.821	0.736	0.716	0.479	0.749	0.451	0.746
CSRM60	0.573	0.759	0.580	0.668	0.660	0.645	0.694	0.749	0.635
CSSM66	0.730	0.686	0.505	0.707	0.762	0.689	0.679	0.642	0.823
ETH10	0.539	0.718	0.824	0.787	0.747	0.479	0.758	0.460	0.775
ETH152	0.708	0.711	0.826	0.834	0.829	0.672	0.818	0.756	0.555
ETH185	0.735	0.738	0.753	0.725	0.801	0.809	0.696	0.450	0.722
HEL1	0.830	0.731	0.789	0.741	0.639	0.791	0.780	0.554	0.791
HEL9	0.455	0.506	0.455	0.417	0.506	0.374	0.446	0.462	0.845
ILSTS005	0.650	0.800	0.718	0.671	0.664	0.687	0.717	0.741	0.640
INRA005	0.639	0.800	0.782	0.810	0.854	0.811	0.762	0.780	0.689
INRA023	0.603	0.620	0.637	0.558	0.626	0.574	0.685	0.367	0.728
INRA032	0.261	0.639	0.481	0.677	0.471	0.713	0.659	0.547	0.789
INRA037	0.576	0.683	0.715	0.702	0.771	0.705	0.703	0.564	0.795
INRA063	0.492	0.547	0.591	0.681	0.614	0.515	0.625	0.232	0.667
MM12	0.708	0.749	0.770	0.682	0.774	0.820	0.734	0.068	0.763
SPS115	0.764	0.786	0.751	0.755	0.754	0.756	0.790	0.406	0.602
TGLA122	0.777	0.703	0.749	0.560	0.679	0.642	0.653	0.623	0.771

panmixia, the null alleles observed should explain all the observed heterozygosity deficits (De Meeûs, 2012). Nevertheless, the proportions of null alleles observed were very low in our study (less than 5% for 18 of the 20 markers used) and would not be sufficient to explain the large heterozygosity gaps noted for several markers such as: BM2113 (in the Guzera samples) and CSSM66 (in the Gobra samples from N'diané). Furthermore, several of these populations, notably those from the Dahra and EMAAP stations, clearly do not have a panmictic breeding system. Indeed, the animals from these stations are subject to selection pressure to keep only those with the characteristics of interest (mainly morphological characteristics such as colour and body weight). Thus, it appears that in our study, null alleles alone cannot explain the deviations from the Hardy–Weinberg model and that other phenomena are at play.

The nature of the loci involved could be another clue to explain at least part of these deviations. Indeed, 6 of the 7 most involved loci (BM2113, CSSM66, HEL9, INRA063, SPS115 and ETH185) are very sensitive to inbreeding and are used to determine the DNA fingerprints of individuals or conduct paternity-test (Curi & Lopez, 2002; Jakhesara et al., 2012; Rodríguez-Ramírez et al., 2011; Rajapaksha et al., 2014; Silva et al., 2014; Brenig and Schütz, 2016). It is therefore possible that part of the heterozygosity deficit observed for certain populations, notably those of the CRZ of Dahra and EMAAP, is due to the inbreeding of individuals that is reflected through these loci. However, this phenomenon would not be sufficient to explain the deficits observed for the Moorish zebu population. The probability that Moorish individuals present such a large deficit in heterozygosity for certain loci (e.g. CSSM66 and INRA063) only because of the relationship between individuals is low. Indeed, these animals are of diverse origin and mostly come from Mali or Mauritania (Mbengue et al., 2007). One explanation could be the fact that Moorish breeders practice rigorous breeding management; unselected males are castrated, which is not often the case with Fulani breeders.

Heterozygosity deficits could also be due to the Wahlund effect, that is, the existence of substructures due to the existence of reproductive barriers between the constituent individuals of these populations or to the divergence of allelic frequencies concomitant with genetic drift (Arora & Bhatia, 2006; De Meeûs, 2012; Nei, 1987). This effect is expected to be most apparent at the stations (Dahra CRZ and EMAAP farm) because of the reproductive patterns exerted on these populations. These patterns would lead, over the generations, to an under-structuring of the said populations. For example, at the CRZ of Dahra, since the 1990s, animals of breeding age have been placed in two isolated herds, each comprising a maximum of 50 females and a breeding bull (Sambe, 2021). If the breeding herds are repeatedly not well homogenised from one breeding season to the next, it is possible that the Gobra population in CRZ of Dahra was subject to the Wahlund effect. For populations sampled outdoors or at the abattoir, although

TABLE 6 Heterozygosity and Hardy–Weinberg equilibrium tests by locus and population

	Gobra zebu (CRZ Dahra)																							
	Native			Acquired			Gobra zebu (Amaly)			Gobra zebu (M'beuleukhé)			Gobra zebu (N'diané)			Guzera zebu (EMAAP)			Moorish zebu (SOGAS Dakar)			N'dama taurine (CRZ Kolda)		
Loci	H_{OBS}	H_{EXP}	HW	H_{OBS}	H_{EXP}	HW	H_{OBS}	H_{EXP}	HW	H_{OBS}	H_{EXP}	HW	H_{OBS}	H_{EXP}	HW	H_{OBS}	H_{EXP}	HW	H_{OBS}	H_{EXP}	HW	H_{OBS}	H_{EXP}	HW
BM1818	0.724	0.759	NS	0.833	0.783	NS	0.900	0.867	NS	0.909	0.872	NS	0.875	0.865	NS	0.706	0.734	NS	0.900	0.859	NS	0.931	0.803	NS
BM1824	0.621	0.547	NS	0.727	0.619	NS	0.833	0.665	NS	0.773	0.744	NS	0.680	0.692	NS	0.765	0.590	NS	0.789	0.698	NS	0.172	0.251	*
BM2113	0.517	0.691	**	0.583	0.855	***	0.700	0.776	NS	0.619	0.727	NS	0.560	0.718	NS	0.588	0.745	*	0.450	0.773	***	0.690	0.782	*
BM4440	0.759	0.761	NS	0.917	0.804	NS	0.800	0.815	NS	0.727	0.728	NS	0.760	0.813	NS	0.941	0.865	NS	0.800	0.778	NS	0.071	0.071	NS
CSRM60	0.429	0.495	NS	0.583	0.678	NS	0.750	0.633	NS	0.409	0.450	NS	0.720	0.636	NS	0.706	0.761	NS	0.667	0.741	NS	0.750	0.644	NS
CSSM66	0.679	0.861	***	0.833	0.797	NS	0.900	0.829	NS	0.714	0.794	NS	0.480	0.686	**	0.588	0.840	**	0.524	0.826	**	0.483	0.591	*
ETH10	0.690	0.604	NS	0.750	0.812	NS	1.000	0.863	NS	0.727	0.786	NS	0.640	0.758	NS	0.588	0.556	NS	0.762	0.801	NS	0.690	0.544	NS
ETH152	0.481	0.512	NS	0.417	0.576	NS	0.450	0.495	NS	0.409	0.498	NS	0.520	0.566	NS	0.471	0.408	NS	0.429	0.496	NS	0.172	0.569	***
ETH185	0.679	0.714	NS	0.667	0.801	NS	0.800	0.714	NS	0.545	0.699	NS	0.542	0.692	NS	0.235	0.480	**	0.762	0.836	NS	0.897	0.652	***
HEL1	0.586	0.657	NS	0.667	0.757	NS	0.750	0.772	NS	0.909	0.762	NS	0.875	0.816	NS	0.824	0.758	NS	0.917	0.775	NS	0.724	0.640	NS
HEL9	0.517	0.690	NS	0.833	0.859	NS	0.750	0.828	NS	0.524	0.851	**	0.800	0.886	*	0.688	0.859	**	0.600	0.809	*	0.821	0.823	NS
ILSTS005	0.714	0.671	NS	0.750	0.714	NS	0.550	0.709	**	0.500	0.634	NS	0.760	0.704	NS	0.706	0.638	NS	0.750	0.746	NS	0.552	0.494	NS
INRA005	0.704	0.693	NS	0.583	0.721	*	0.700	0.728	NS	0.682	0.704	NS	0.792	0.716	NS	0.941	0.772	*	0.632	0.624	NS	0.667	0.618	NS
INRA023	0.821	0.819	NS	0.750	0.779	NS	0.900	0.801	NS	0.545	0.621	NS	0.739	0.730	NS	0.813	0.702	NS	0.611	0.722	NS	0.690	0.669	NS
INRA032	0.690	0.604	NS	0.750	0.790	NS	0.950	0.865	NS	0.727	0.832	NS	0.583	0.789	NS	0.563	0.554	NS	0.667	0.808	NS	0.759	0.559	*
INRA037	0.897	0.805	NS	0.833	0.844	NS	0.700	0.805	NS	0.773	0.801	NS	0.880	0.798	*	0.813	0.810	NS	0.900	0.836	NS	0.552	0.449	NS
INRA063	0.464	0.669	NS	0.500	0.786	*	0.588	0.743	NS	0.455	0.703	*	0.391	0.755	***	0.188	0.466	**	0.556	0.778	NS	0.310	0.336	NS
MM12	0.828	0.781	NS	0.750	0.739	NS	0.550	0.537	NS	0.727	0.756	NS	0.917	0.803	NS	0.733	0.756	NS	0.500	0.722	*	0.724	0.708	NS
SPS115	0.276	0.281	NS	0.000	0.725	***	0.421	0.560	*	0.591	0.730	*	0.400	0.513	*	0.471	0.777	**	0.526	0.710	*	0.643	0.631	NS
TGLA122	0.793	0.777	NS	0.917	0.804	*	0.750	0.805	NS	0.727	0.773	NS	0.875	0.832	NS	0.813	0.857	NS	0.700	0.733	NS	0.517	0.483	NS
Means	0.643	0.670	**	0.682	0.762	*	0.737	0.741	*	0.650	0.723	***	0.689	0.739	***	0.657	0.696	***	0.672	0.754	***	0.591	0.566	***

H_{OBS}: observed heterozygosities; H_{EXP}: expected heterozygosities; HW: significance of Hardy–Weinberg test; NS: non-significant *p* value.
* *p* Value between 0.05 and 0.01.
** *p* Value between 0.01 and 0.001.
*** *p* Value less than 0.001.

theoretically possible, this effect should be negligible as reproductive barriers are less important or non-existent for these populations.

Finally, this study reveals that the animals maintained in the CRZ of Dahra have low genetic diversity compared to the other studied populations. This low genetic diversity could be due to reproductive isolation and selection pressure, which are the basis of the Gobra genetic selection programme in Dahra. It would be interesting for future work to evaluate the impact of this breeding isolation and selection pressure on: (i) inbreeding between individuals in the Gobra selection nucleus population and (ii) the degree of genetic differentiation of this population compared to other Gobra cattle populations maintained in the same area.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

BSS: investigation, formal analysis, funding acquisition, methodology, writing original draft, writing-review & editing; MND: conceptualisation, project administration, investigation, funding acquisition, methodology, supervision, writing original draft, writing-review & editing; IH: formal analysis, methodology, supervision; writing original draft; bn: investigation, formal analysis; MNB: investigation, formal analysis; MD: conceptualisation, methodology, supervision, validation; MS: methodology, supervision, validation. all authors have read and approved the final manuscript.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and that no ethical approval was required for this particular case report.

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REFERENCES

Agence National de la Statistique et de la Démographie (ANSD). (2014). *Recensement général de la population et de l'habitat de l'agriculture et de l'élevage*. Rapport définitif. Ministère de l'Economie, des Finances et du Plan. Rapport technique, Dakar (Sénégal). p. 418.

Arora, R., & Bhatia, S. (2006). Genetic diversity of Magra sheep from India using microsatellite analysis. *Asian Australian Journal of Animal Science*, *19*(7), 938–942.

Bordbar, F., Mohammadabadi, M., Jensen, J., Xu, L., Li, J., & Zhang, L. (2021). Identification of candidate genes regulating carcass depth and hind leg circumference in simmental beef cattle using Illumina Bovine Beadchip and next-generation sequencing analyses. *Animals*, *12*, 1103. https://doi.org/10.3390/ani12091103

Botstein, D., White, R. L., Skolnick, M., & Davies, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*, *32*, 314–331.

Brenig, B., & Schütz, E. (2016). Recent development of allele frequencies and exclusion probabilities of microsatellites used for parentage control in the German Holstein Friesian cattle population. *BioMed Central Genetics*, *17*(18), 9.

Chaudhari, M. V., Parmar, S. N. S., Joshi, C. G., Bhong, C. D., Fatima, S., Thakur, M. S., & Thakur, S. S. (2009). Molecular characterization of Kenkatha and Gaolao (*Bos indicus*) cattle breeds using microsatellite markers. *Animal Biodiversity and Conservation*, *32*(2), 71–76.

Curi, R. A., & Lopes, C. R. (2002). Evaluation of nine microsatellite loci and misidentification paternity frequency in a population of Gyr breed bovines. *Brazilian Journal of veterinary and Research of Animal Science*, *39*(3), 129–135.

da Silva Filho, E., de Azevedo, J. S. N., de Souza Rodrigues, L. F., de Lima Pinheiro, L. M., DeRosia, M. R., Almeida, T. T. G., Monteiro, F. O. B., & Campelo, J. E. G. (2014). Molecular evidences of mutation-drift equilibrium by microsatellite markers in four meat producer bovine breeds in Brazil. *The Journal of Animal & Plant Sciences*, *24*(6), 1691–1694.

Dayo, G. K., Thevenon, S., Berthier, D., Moazami-Goudarzi, K., Denis, C., Cuny, G., Eggen, A., & Gautier, M. (2009). Detection of selection signatures within candidate regions underlying trypanotolerance in outbred cattle populations. *Molecular Ecology*, *18*(8), 1801–1813.

De Meeûs, T. (2012). *Initiation à la génétique des populations naturelles: Applications aux parasites et à leurs vecteurs*. IRD Editions. Marseille (France). p. 338.

DeAtley, K. L., Rincon, G., Farber, C. R., Medrano, J. F., Enns, R. M., Silver, G. A., & Thomas, M. G. (2008). Association of microsatellite ETH10 genotypes with growth and carcass trait levels in Brangus cattle. *Proceedings, Western Section*, American Society of Animal Science, 59, 69–71.

Diack, A., Diop, M., & Sané, I. (2016). Rapport d'étude de la situation de référence du projet de lutte contre la désertification par l'appui au pastoralisme dans les régions de Louga et Matam. Agronomes et Vétérinaires Sans Frontières p. 218.

Excoffier, L., Laval, G., & Schneider, S. (2006). Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, *1*, 47–50.

Food and Agriculture Organization (FAO). (2011). Molecular genetic characterization of animal genetic resources. FAO Animal Production and Health Guidelines. No. 9. Rome. p. 100.

Foulley, J. L., & Ollivier, L. (2006). Diversité Génétique et richesse allélique: Concepts et application à des races bovines. *Rencontre Recherche. Ruminants*, *13*, 227–230.

Freeman, A. R., Meghen, C. M., Machugh, D. E., Loftus, R. T., Achukwi, M. D., Bado, A., Sauveroche, B., & Bradley, D. G. (2004). Admixture and diversity in West African cattle populations. *Molecular Ecology*, *13*(11), 3477–3487.

Goudet, J. (2002). FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3.2). Available from http://www.unil.ch/izea/softwares/fstat.html

International Livestock Research Institute (ILRI). (2010). *Gestion durable du bétail endémique en Afrique de l'Ouest (PROGEBE)*. Etude de référence, Sénégal. p. 77.

Jakhesara, S. J., Rank, D. N., Kansara, J. D., Parikh, R. C., Patel, V. M., Vataliya, P. H., & Solanki, J. V. (2012). *Parentage verification in field progeny*

testing program of Mehsana buffalo. American Society of Animal Science, 90, 4723–4728.

Kalinowski, S. T., Taper, M. L., & Marshall, T. C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, *16*, 1099–1106.

MacHugh, D. E., Shriver, M. D., Loftus, R. T., Cunningham, P., & Bradley, D. G. (1997). Microsatellite DNA variation and the evolution, domestication and phylogeography of taurine and zebu cattle (*Bos taurus* and *Bos indicus*). *Genetics*, *146*(3), 1071–1086.

Mbengue, M., Gueye, A., Faye, O., Toguebaye, B. S., & Konte, M. (2007). Etude séro-épidémiologique de la Cowdriose chez le zébu Maure au Sénégal. *Parasite*, *14*, 169–171.

Mohammadabadi, M., Oleshko, V., Oleshko, O., Heiko, L., & Starostenko, I. (2021a). Using inter simple sequence repeat multi-loci markers for studying genetic diversity in guppy fish. *Turkish Journal of Fisheries and Aquatic Sciences*, *21*(12), 603–613.

Mohammadabadi, M., Bordbar, F., Jensen, J., Du, M., & Guo, W. (2021b). Key genes regulating skeletal muscle development and growth in farm animals. *Animals*, *11*, e835.

Mohammadabadi, M. R., Jafari, A. H. D., & Bordbar, F. (2017). Molecular analysis of CIB4 gene and protein in Kermani sheep. *Brazilian Journal of Medical and Biological Research*, *50*, e6177.

Ndiaye, N. P., Sow, A., Dayo, G. K., Ndiaye, S., Sawadogo, G. J., & Sembène, M. (2015). Genetic diversity and phylogenetic relationships in local cattle breeds of Senegal based on autosomal microsatellite markers. *Veterinary World*, *8*(8), 994–1005.

Nei, M. (1987). *Molecular evolutionary genetics*. Columbia University Press, New York (Etat-Unis). p. 506.

Ngono Ema, P. J., Manjeli, Y., Meutchieyié, F., Keambou, C., Wanjala, B., Desta, A. F., Ommeh, S., Skilton, R., & Djikeng, A. (2015). Genetic diversity of four Cameroonian indigenous cattle using microsatellite markers. *Journal of Livestock Science*, *5*, 9–17.

Nielsen, E. E., Hansen, M. M., & Meldrup, D. (2006). Evidence of microsatellite hitch-hiking selection in Atlantic cod (Gadus morhua L.): Implications for inferring population structure in nonmodel organisms. *Molecular Ecology*, *15*(11), 3219–3229.

Oliveira, J. F. C., Neves, J. P., Almeida, E. A., Steigleder, C. S., Moraes, J. C. F., Gonçalves, P. B. D., & Weimer, T. A. (2005). Association between reproductive traits and four microsatellites in Brangus–Ibagé cattle. *Genetic Molecular Biology*, *28*, 54–59.

Peakall, R., & Smouse, P. E. (2012). GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research – An update. *Bioinformatics*, *28*, 2537–2539.

Rajapaksha, P. I., Lakmali, M. B. D., Dunuwille, S. W. M. B., Janaththani, P., Rajapakse, S., Wijayagunawardane, M. P. B., Kodithuwakku, S. P., & Sooriyapathirana, S. D. S. S. (2014). A DNA fingerprinting scheme to establish the identity of semen samples of cattle breeds in artificial insemination programs of Sri Lanka. *Wayamba Journal of Animal Science*, *30*, 947–954.

Rodríguez-Ramírez, R., Arana, A., Alfonso, L., González-Córdova, A. F., Torrescano, G., Legarreta, G., & Vallejo-Cordoba, B. (2011). Molecular traceability of beef from synthetic Mexican bovine breeds. *Genetics and Molecular Research*, *10*(4), 2358–2365.

Rousset, F. (2008). GENEPOP: A complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources*, *8*, 103–106.

Sambe, B. S. (2021). Amélioration génétique du zébu Gobra au Centre de Recherches Zootechniques de Dahra: Revue du programme; évaluation des performances ; caractérisation génétique des animaux du noyau de sélection et de populations bovines exogène. Thèse en Génétique des Populations, Ecole Doctorale des Sciences de la Vie, de la Santé et de l'Environnement. p. 132.

Serre, J. L. (2006). *Génétique des populations*. Paris: Dunod p. 267.

Sharma, R., Parmar, S. N. S., Joshi, C. G., Thakur, M. S., Bhong, C. D., & Chaudari, M. V. (2009). Molecular characterization of Nimari cattle using fluorescently labelled microsatellite markers. *Indian Journal of Animal Sciences*, *80*(7), 661–665.

Shi, Z., Lee, J. H., Lee, Y. S., Oh, D. Y., & Yeo, J. S. (2010). Analysis of genetic diversity and distances in Asian cattle breeds using microsatellite markers. *Journal of the Korean Data & Information Science Society*, *21*(4), 79–802.

Silva, E. C. D., McManus, C. M., de Paiva Guimarães, M. P. S. L. M., Gouveia, A. M. G., Facó, O., Pimentel, D. M., Caetano, A. R., & Paiva, S. R. (2014). Validation of a microsatellite panel for parentage testing of locally adapted and commercial goats in Brazil. *Genetics and Molecular Biology*, *37*(1), 54–60.

Singh, G., Thakur, Y., Kour, A., Sankhyan, V., & Katoch, S. (2015). Genetic characterization of Gaddi goat breed of Western Himalayas using microsatellite markers. *Veterinary World*, *8*(4), 527–531.

Slatkin, M. (1995). Hitchhiking and associative overdominance at a microsatellite locus. *Molecular Biology and Evolution*, *12*(3), 473–480.

Sow, R. S., Denis, J. P., Trail, J. M. C., Thiongane, P. I., Mbaye, M., & Diallo, I. (1988). Productivité du zebu Gobra au Centre de Recherches Zootechniques de Dahra (Sénégal). *Rapport technique, Institut Sénégalais de Recherches Agricoles*, p. 47.

Suh, S., Kim, Y. S., Cho, C. Y., Byun, M. J., Choi, S. B., Ko, Y. G., Lee, C. W., Jung, K. S., Bae, K. H., & Kim, J. H. (2014). Assessment of genetic diversity, relationships and structure among Korean native cattle breeds using microsatellite markers. *Asian-Australasian Journal of Animal Sciences*, *27*(11), 1548–1553.

Wane, M., Diouf, M. N., Diop, M., & Kamga Waladjo, A. R. (2017). *Revue de programme de sélection bovine: Cas du taurin N'dama au Centre de Recherches Zootechniques de Kolda (Sénégal)*. Institut sénégalais de Recherches agricoles (ISRA). p. 61.

Zamani, P., Akhondi, M., & Mohammadabadi, M. R. (2015). Associations of Inter-Simple Sequence Repeat loci with predicted breeding values of body weight in Sheep. *Small Ruminant Research*, *132*(3), 123–127.

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