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GENETIC DIVERSITY OF DJELLI ZEBU OF NIGER USING MICROSATELLITE DNA MARKERS

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ABSTRACT

The present study was carried out to evaluate the genetic diversity in of the Djelli zebu of Niger sampled in three departments (Kollo, Say, Tera) of the Tillaberi region and in the peri-urban area of Niamey. A total of 20 microsatellite markers were used to genotype 114 animals. The results showed a high variability in the subpopulations studies. The number of alleles ranged from 5 (BM1824 and HAUT27) to 12 alleles (MM12) and while the allelic richness calculated using the rarefaction correction varied from 0.427 (SPS115) to 0.802 (HEL9). The highest genetic diversity was observed in the department of Kollo and the lowest in the subpopulation of Niamey. The highest genetic distance values were observed between the Say and Niamey subpopulations while the lowest were between Kollo and Tera. The Bayesian clustering method showed that the most consistent gain in information was obtained with K = 2 and from K = 3 to K = 6, some individuals displayed less than 1% of their genome in other clusters.

KEYWORDS: zebu Djelli, diversity genetic, microsatellite markers, Niger.

INTRODUCTION

Livestock sector is an important asset for economic growth, food safety and poverty reduction in Niger as it provides income and numerous services for households. Livestock is mainly dominated by cattle particularly zebus or indicine cattle with a cervicothoracic hump (*Bos indicus*). According to the statistics (Niger, 2014), the country has about 37,835,272 heads of animals with 11 377 313 heads of cattle representing about 30% of the livestock. Djelli zebu is one of the five cattle breeds of this country. Its breeding is very popular because of its zootechnical skills. Comparatively to the other zebu breeds of Niger (Azawak, Bororo and Gudali zebus), Djelli zebu is less known.

A recent study on phenotypic characteristics of Djelli zebu (Zakari et al., 2019) showed that most of the characteristics of the Fulani zebu of Niger were influenced by the factor "breeding locality" and the « sex » of the animal. Djelli breed is characterized by a straight profile of the head, variable horn shapes but mostly cup-shape and crescent-shape and lyre-shape. The coat pattern of body hair of Djelli zebu is mostly spotted and the color is either red-pied or black-pied.

The sustainable management of this zebu breed of Niger requires other knowledge on the genetic diversity using molecular markers studies in order to complete the information generated by morphobiometric characterization (Zakari et al. 2019). Thus, the present study was carried out to evaluate the genetic diversity in of the Djelli zebu of Niger sampled in three departments (Kollo, Say, Tera) of the Tillaberi region and in the peri-urban area of Niamey.

MATERIALS AND METHODS

Study area and animal material

The study was conducted in three departments of Tillabery region (Kollo, Say and Téra) and the peri-urban zone of Niamey, located along the Niger River which is the natural extension area of Djelli zebu in Niger. The study area is shown in Figure 1.

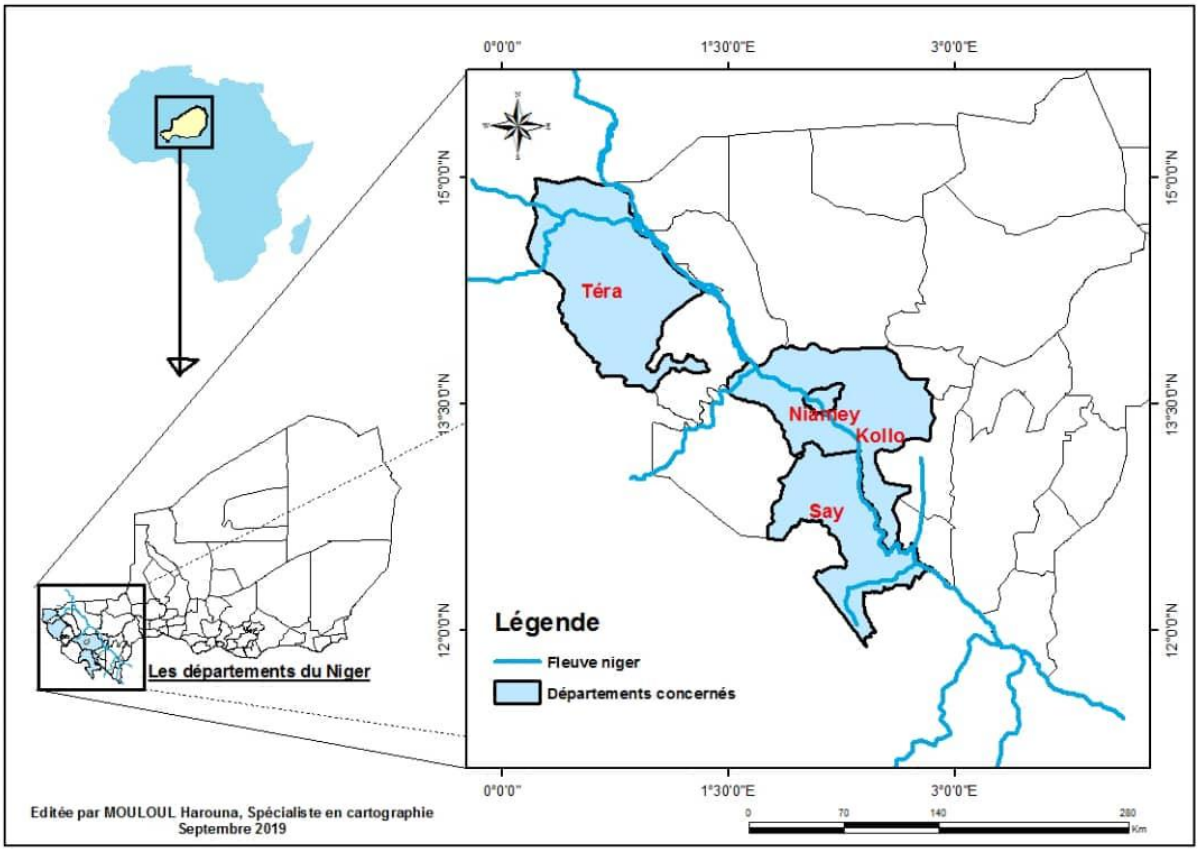

Figure 1: Study area.

Animal sampling and genotyping method

The genotyping data were collected from the blood samples of 114 individuals belonging to the Fulani zebu (Djelli) race from Niger from the study area mentioned above. The numbers of animals sampled for each department were: Kollo (64), Say (29), Téra (13) and the peri-urban area of Niamey (8). The blood samples were taken from the jugular vein of the animals in EDTA vacutainer tubes and then stored on ice before they were transferred to the laboratory for DNA extraction.

Genomic DNA was extracted from whole blood samples using the Promega Wizard Kit and stored at -20°C until genotyping.

Twenty-two microsatellite markers were genotyped among which 21 were chosen in the Food and Agriculture Organization panel recommended for livestock biodiversity studies and one (BM4440) was detected as under selection and is located within the confidence interval of a previously described QTL underlying a trypanotolerance-related trait (Dayo et al., 2009; Hanotte et al., 2003) (table 1).

The forward primer for each locus was labelled with one of the four fluorescent dyes FAM, VIC, NED and PET (Applied Biosystems, USA). Multiplexed Polymerase chain reaction was performed in a total reaction volume of 12 µl containing 5 µl of mix primers of multiplex, 5 µl of mix of other reagents (Buffer, $MgCl_2$, Taq polymerase) and 2 µl of DNA. The following thermal conditions, 94˚C for 15 minutes, followed by 30 cycles of 94˚C for 30 seconds, specific annealing temperature (58°C and 60°C according to the multiplex) for 1 minute 30 seconds and 72˚C for 1 minute 30 seconds and a final extension at 72˚C for 15 minutes was used for sample amplification by PCR. DNA fragments analysis of microsatellite markers was carried out using Applied Biosystems 3500 automated DNA sequencer along with LIZ600 (Applied Biosystems, USA) as an internal lane control. The allele size data for each sample was extracted using Genemapper software, version 5.

Table 1: Characteristics of the microsatellite markers used.

Markers	SEQUENCE (5' - 3')	Dye	Expected size	Annealing Temperature (°C)	Multiplex
CSRM60F	AAGATGTGATCCAAGAGAGAGGCA	6-FAM	(79 - 115)	60	1
CSRM60R	AGGACCAGATCGTGAAAGGCATAG		(79 - 115)		1
HEL9F	CCCATTCAGTCTTCAGAGGT	6-FAM	(141 - 173)		1
HEL9R	CACATCCATGTTCTCACCAC		(141 - 173)		1
HEL1F	CAACAGCTATTTAACAAGGA	NED	(99 - 119)		1
HEL1R	AGGCTACAGTCCATGGGATT		(99 - 119)		1
INRA63F	ATTTGCACAAGCTAAATCTAACC	NED	(167 - 189)		1
INRA63R	AAACCACAGAAATGCTTGGAAG		(167 - 189)		1
INRA 023F	GAGTAGAGCTACAAGATAAACTTC	VIC	(195 - 225)		1
INRA 023R	TAACTACAGGGTGTTAGATGAACTC		(195 - 225)		1
BM4440F	CCCTGGCATTCAACAAGTGT	PET	(100-121)		1
BM4440R	TAGATGCAAAACACACACACA		(100-121)		1
BM1824F	GAGCAAGGTGTTTTTCCAATC	PET	(176 - 197)		1
BM1824R	CATTCTCCAACTGCTTCCTTG		(176 - 197)		1
BM1818F	AGCTGGGAATATAACCAAAGG	NED	(248 - 278)	57	2
BM1818R	AGTGCTTTCAAGGTCCATGC		(248 - 278)		2
MM12F	CAAGACAGGTGTTTCAATCT	VIC	(99 - 145)		2
MM12R	ATCGACTCTGGGGATGATGT		(99 - 145)		2
HAUT27F	AACTGCTGAAATCTCCATCTTA	NED	(120 -158)		2
HAUT27R	TTTTATGTTCATTTTTTGACTGG		(120 -158)		2
ILST005F	GGAAGCAATGAAATCTATAGCC	VIC	(176 - 194)		2
ILST005R	TGTTCTGTGAGTTTGTAAGC		(176 - 194)		2
TGLA122F	CCCTCCTCCAGGTAAATCAGC	PET	(136 - 184)		2
TGLA122R	AATCACATGGCAAATAAGTACATAC		(136 - 184)		2
INRA035F	TTGTGCTTTATGACACTATCCG	PET	(100 - 124)		2
INRA035R	ATCCTTTGCAGCCTCCACATTG		(100 - 124)		2
HEL5F	GCAGGATCACTTGTTAGGGA	6-FAM	(145 - 171)		2
HEL5R	AGACGTTAGTGTACATTAAC		(145 - 171)		2
ETH10F	GTTCAGGACTGGCCCTGCTAACA	6-FAM	(207 - 231)		2
ETH10R	CCTCCAGCCCACTTTCTCTTCTC		(207 - 231)		2
TGLA53F	GCTTTCAGAAATAGTTTGCATTCA	NED	(143 - 191)	60	3
TGLA53R	ATCTTCACATGATATTACAGCAGA		(143 - 191)		3
ETH152F	TACTCGTAGGGCAGGCTGCCTG	VIC	(181 - 211)		3
ETH152R	GAGACCTCAGGGTTGGTGATCAG		(181 - 211)		3
BM2113F	GCTGCCTTCTACCAAATACCC	PET	(122 - 156)		3
BM2113R	CTTCCTGAGAGAAGCAACACC		(122 - 156)		3
INRA0005F	CAATCTGCATGAAGTATAAATAT	6-FAM	(135 - 149)		3
INRA0005R	CTTCAGGCATACCCTACACC		(135 - 149)		3
CSSM66F	ACACAAATCCTTTCTGCCAGCTGA	6-FAM	(171-209)		3
CSSM66R	AATTTAATGCACTGAGGAGCTTGG		(171-209)		3
SPS115F	AAAGTGACACAACAGCTTCTCCAG	NED	(234 - 258)		3
SPS115R	AACGAGTGTCCTAGTTTGGCTGTG		(234 - 258)		3
TGLA227F	CGAATTCCAAATCTGTTAATTTGCT	VIC	(75 - 105)		3
TGLA227R	ACAGACAGAAACTCAATGAAAGCA		(75 - 105)		3

Data analysis

Allele numbers, allelic richness, the unbiased estimator of Wright's inbreeding coefficient F_{IS} was calculated according to Weir et Cockerham (1984) for each locus using FSTAT software version 2.9.4 (Goudet, 2003).

Additionally, observed and unbiased expected heterozygosities per locus (Nei 1987), genetic distances (Nei, 1978) were estimated using GENETIX 4.03 (http://www.genetix.univ-montp2.fr) as well as the Factorial Correspondence Analysis.

Departures from Hardy–Weinberg equilibrium over all loci were evaluated using Fisher's method implemented in Genepop v. 4.7.2 (Rousset, 2007). The same software was used to perform the score test for Hardy-Weinberg

equilibrium (Rousset and Raymond, 1995) per locus using a Markov chain algorithm with 10,000 dememorizations, 200 batches and 5000 iterations per batch.

The Hardy-Weinberg equilibrium test measures the difference between the observed numbers of population genotypes and the theoretical genotypic numbers obtained with the Hardy-Weinberg relationship.

The detection of null alleles, the excess of homozygotes at this locus, the detection of short alleles (large allele dropout or short allele dominance), cases of stuttering were estimated using the software Microchecker (Van Oosterhout et al. 2004).

The effective number of alleles (Ae) and the polymorphic information content (PIC) for each locus were analyzed by using Molkin v. 3.0 software (Gutierrez et al., 2005).

To assign individuals to K populations and estimate the posterior distribution of each individual's admixture coefficient, we used STRUCTURE software 2.3.4 (Pritchard et al., 2000) in which a model-based Bayesian clustering analysis is implemented. Because genotyping information for the putative parental populations was not available, we hypothesized k parental unknown populations (k varying from 1 to 8 with 10 replicated runs for each K). Analysis was performed with a burn in length of 50,000 followed by 100,000 Markov chain Monte Carlo iterations for each of K using independent allele frequencies between the parental populations and an no admixture model.

The optimal 'K' was identified based on ΔK, the second order rate of change in LnP(D) following the likelihood procedure of Evanno et al. (2005) using Structure Harvester (available at http://taylor0.biology.ucla.edu/structureHarvester/). Structure Harvester is a web-based program for collating results generated by the STRUCTURE program to identify the best value of K. The program provides a fast way to assess and visualize likelihood values across multiple values of K and to detection of the number of genetic groups that best fit the data.

RESULTS

Genetic diversity

Of the 22 microsatellite markers used, two (INRA063 and INRA005) presented an amplification problem and were eliminated from the analysis. Indeed, either no individual could be genotyped with these markers in at least one population or else they had a high rate of genotyping failure in populations generating a lot of missing data). In addition, three pairs of loci showed a linkage imbalance ($p < 0.05$): BM4440 X HEL1, MM12 X TGLA53 and BM2113 X TGLA53. HEL1 and TGLA53 were then removed for further analysis.

Table 2: Number of alleles (Na), allelic richness (RA), expected (He) and observed (Ho) heterozygosities per loci in subpopulations of Djelli zebu.

Loci		Kollo	Say	Tera	Niamey	Overall sampling locations (department)	Mean ± SD
BM1824	Na	4	4	5	4	5	**4.25 ± 0.5**
	RA	3.5	3.7	4.5	3.7	3.8	**3.88 ± 0.45**
	He	0.72	0.73	0.80	0.74		**0.75 ± 0.04**
	Ho	0.81	0.86	0.80	0.43		**0.72 ± 0.2**
BM4440	Na	8	8	5	5	10	**6.5 ± 1.73**
	RA	4.6	4.8	4.5	4.2	4.7	**4.52 ± 0.22**
	He	0.77	0.81	0.81	0.78		**0.79 ± 0.02**
	Ho	0.76	0.78	0.92	0.88		**0.83 ± 0.08**
CSRM60	Na	8	8	7	4	10	**6.75 ± 1.89**
	RA	4.2	4.0	4.7	3.2	4.2	**4.03 ± 0.6**
	He	0.65	0.61	0.70	0.58		**0.63 ± 0.05**
	Ho	0.58	0.59	0.85	0.75		**0.69 ± 0.13**
HEL9	Na	9	8	7	5	10	**7.25 ± 1.71**
	RA	5.5	5.2	5.4	4.4	5.3	**5.09 ± 0.5**
	He	0.85	0.82	0.84	0.76		**0.82 ± 0.04**
	Ho	0.78	0.64	1.00	1.00		**0.86 ± 0.18**
INRA023	Na	7	5	3	3	8	**4.5 ± 1.91**
	RA	4.0	3.2	3.0	2.8	3.4	**3.27 ± 0.51**
	He	0.69	0.59	0.64	0.59		**0.63 ± 0.05**
	Ho	0.50	0.50	0.80	0.50		**0.58 ± 0.15**
BM1818	Na	9	7	6	6	9	**7.00 ± 1.41**
	RA	5.1	5.1	4.6	5.1	5.1	**4.96 ± 0.26**
	He	0.81	0.83	0.78	0.82		**0.81 ± 0.02**
	Ho	0.82	0.81	0.77	0.88		**0.82 ± 0.04**

ETH10	Na	7	9	6	5	9	**6.75 ± 1.71**
	RA	4.8	4.6	3.9	4.3	4.6	**4.41 ± 0.4**
	He	0.80	0.79	0.64	0.73		**0.74 ± 0.08**
	Ho	0.87	0.67	0.69	0.88		**0.78 ± 0.11**
HAUT27	Na	4	4	4	3	5	**3.75 ± 0.5**
	RA	3.1	3.2	3.8	3.0	3.3	**3.27 ± 0.37**
	He	0.67	0.65	0.76	0.68		**0.69 ± 0.05**
	Ho	0.47	0.63	0.82	1.00		**0.72 ± 0.23**
HEL5	Na	5	6	3	3	6	**4.25 ± 1.5**
	RA	3.1	4.1	2.5	3.0	3.3	**3.17 ± 0.65**
	He	0.46	0.66	0.50	0.64		**0.57 ± 0.1**
	Ho	0.24	0.35	0.22	0.40		**0.3 ± 0.09**
ILST005	Na	5	5	5	4	5	**4.75 ± 0.50**
	RA	4.0	4.1	4.4	3.2	4.0	**3.92 ± 0.49**
	He	0.76	0.74	0.80	0.58		**0.72 ± 0.1**
	Ho	0.77	0.70	0.77	0.50		**0.68 ± 0.13**
INRA35	Na	6	4	4	2	6	**4.00 ± 1.63**
	RA	3.1	2.7	3.5	1.6	2.9	**2.72 ± 0.81**
	He	0.50	0.42	0.68	0.13		**0.43 ± 0.23**
	Ho	0.41	0.42	0.46	0.13		**0.35 ± 0.15**
MM12	Na	10	11	8	7	12	**9.00 ± 1.83**
	RA	5.2	5.4	5.4	5.7	5.3	**5.41 ± 0.22**
	He	0.81	0.80	0.80	0.87		**0.82 ± 0.03**
	Ho	0.63	0.81	0.85	1.00		**0.82 ± 0.15**
TGLA122	Na	10	8	6	7	11	**7.75 ± 1.71**
	RA	4.3	4.8	4.8	5.3	4.7	**4.79 ± 0.44**
	He	0.66	0.77	0.78	0.79		**0.75 ± 0.06**
	Ho	0.61	0.78	0.69	1.00		**0.77 ± 0.17**
BM2113	Na	7	6	7	4	7	**6.00 ± 1.41**
	RA	4.9	5.0	5.2	3.6	4.9	**4.68 ± 0.7**
	He	0.81	0.82	0.82	0.69		**0.78 ± 0.06**
	Ho	0.75	0.94	0.77	0.43		**0.72 ± 0.21**
CSSM66	Na	10	6	6	6	11	**7.00 ± 2.00**
	RA	5.3	4.9	4.4	5.3	5.1	**4.96 ± 0.43**
	He	0.84	0.81	0.76	0.84		**0.81 ± 0.04**
	Ho	0.66	0.88	0.85	0.71		**0.78 ± 0.1**
ETH152	Na	6	4	3	3	6	**4.00 ± 1.41**
	RA	3.2	2.9	2.4	2.7	2.9	**2.8 ± 0.34**
	He	0.55	0.47	0.46	0.47		**0.49 ± 0.04**
	Ho	0.57	0.50	0.38	0.29		**0.44 ± 0.13**
SPS115	Na	6	4	4	4	7	**4.50 ± 1.00**
	RA	3.2	2.9	2.4	3.4	3.1	**2.98 ± 0.43**
	He	0.48	0.50	0.29	0.49		**0.44 ± 0.1**
	Ho	0.46	0.53	0.31	0.57		**0.47 ± 0.12**
TGLA227	Na	9	6	6	3	10	**6.00 ± 2.45**
	RA	4.2	4.3	3.9	2.7	4.1	**3.76 ± 0.75**
	He	0.70	0.77	0.66	0.32		**0.61 ± 0.2**
	Ho	0.47	0.77	0.75	0.33		**0.58 ± 0.21**
	mean Na ± SD (all loci)	**7.2 ± 2.0**	**6.3 ± 2.1**	**5.3 ± 1.5**	**4.3 ± 1.5**	**8.2 ± 2.3**	
	mean RA ± SD (all loci)	**4.2 ± 0.8**	**4.2 ± 0.9**	**4.1 ± 1.0**	**3.7 ± 1.1**	**4.1 ± 0.8**	
	mean He ± SD	**0.696 ±**	**0.699 ±**	**0.695 ±**	**0.638 ±**		

	(all loci)	**0.126**	**0.132**	**0.147**	**0.192**		
	mean Ho ± SD (all loci)	**0.620 ± 0.172**	**0.676 ± 0.168**	**0.705 ± 0.216**	**0.648 ± 0.285**		

SD: Standard deviation

Information on the number of alleles/locus and number of alleles/locus/population, expected heterozygosities (He) and observed heterozygosities (Ho) are summarized in Table 2. All loci used were polymorphic with a large number of alleles per locus (Na), ranging from 5 (BM1824 and HAUT27) to 12 alleles (MM12) while the allelic richness ranged from 0.427 (SPS115) to 0.802 (HEL9) and the same trend was observed for the effective allele number (from 1.81 for SPS115 to 5.66 for HEL9). The highest genetic diversity was observed in the Department of Kollo has the highest values of the genetic diversity parameters (He, PIC, Ae and k) while the lowest values were obtained in the subpopulation of Niamey (table 3).

Within the subpopulation, the mean Ho varied from 0.62 (Kollo department) to 0.71 (Tera department), while the mean He varied from 0.64 (Niamey department) to 0.70 (Kollo, Say and Tera departments).

Table 3: Average values of Polymorphic Information Content (PIC), effective number of Allele (Ae), average number of allele per locus (k) and the average number of allele per locus using the rarefaction method (K (10)) using molkin 3.0.

Subpopulations	Ho	He	PIC	Ae	K	K (10)
Kollo	0.626	0.684	0.5405	3.64	7.22	4.17
Say	0.681	0.682	0.5254	3.59	6.28	4.18
Tera	0.698	0.664	0.5119	3.37	5.28	4.07
Niamey	0.663	0.593	0.44.44	2.88	4.33	3.74
Total	**0.658**	**0.688**	**0.6538**	**3.68**	**8.17**	**4.15**

Exact tests of deviation from Hardy–Weinberg expectations (HWE) showed a significant deviation from HWE ($P < 0.01$) of some markers in Kollo and Say subpopulations, suggesting that null alleles might be segregating in these populations (Table 4).

Table 4: F_{IS} per locus and per subpopulation.

Loci	Kollo	Say	Tera	Niamey
BM1824	-0.128	-0.186	0.000	0.438
BM4440	0.016	0.025	-0.142	-0.126
CSRM60	0.113*	0.026	-0.222	-0.333
HEL9	0.082	0.226*	-0.210	-0.355
INRA023	0.286	0.165	-0.280	0.167
BM1818	-0.013	0.017	0.016	-0.077
ETH10	-0.083	0.161	-0.091	-0.225
HAUT27	0.302*	0.042	-0.078	-0.538
HEL5	0.480**	0.473***	0.573	0.407
ILST005	-0.008	0.053	0.036	0.138
INRA35	0.187	-0.011	0.330	0.000
MM12	0.222*	-0.015	-0.065	-0.167
TGLA122	0.090	-0.006	0.111	-0.287
BM2113	0.083	-0.145	0.063	0.400
CSSM66	0.219*	-0.085	-0.123	0.155
ETH152	-0.048	-0.076	0.178	0.415
SPS115	0.041*	-0.004	-0.079	-0.171
TGLA22	0.333**	0.000	-0.145	-0.053
Over all loci	**0.113***	**0.037**	**-0.016**	**-0.016**

*** p<0.001, **p<0.05

The overall value for F-statistics F_{IT}, F_{ST} and F_{IS} in the studied population of Djelli zebu were 0.068, 0.004 and 0.064 respectively (table 5).

Table 5: Polymorphic Information Content (PIC), effective number of Allele (Ae), F-Statistics (F_{IS}, F_{IT} *and* F_{ST}) according Weir and Cockerham (1984) per locus in the Djelli population studied.

Locus	PIC	Ae	F_{IT}	F_{ST}	F_{IS}
BM1824	0.675	3.61	-0.084	-0.008	-0.075
BM4440	0.754	4.66	-0.009	0.010	-0.019
CSRM60	0.612	2.72	-0.006	-0.004	-0.003
HEL9	0.802	5.66	0.054	-0.002	0.056
INRA023	0.571	2.76	0.168	0.000	0.168
BM1818	0.779	5.1	-0.007	-0.003	-0.005
ETH10	0.740	4.37	0.000	0.015	-0.015
HAUT27	0.611	3.08	0.094	0.002	0.093
HEL5	0.509	2.18	0.483	0.003	0.482
ILST005	0.703	3.93	0.043	0.014	0.029
INRA35	0.439	1.9	0.181	0.024	0.162
MM12	0.782	5.08	0.063	-0.006	0.069
TGLA122	0.703	3.64	0.030	0.006	0.024
BM2113	0.771	4.95	0.054	-0.000	0.054
CSSM66	0.798	5.61	0.104	0.017	0.088
ETH152	0.453	1.98	0.007	-0.021	0.027
SPS115	0.427	1.81	-0.003	0.002	-0.005
TGLA227	0.639	3.11	0.170	0.028	0.146
All loci	**0.654**	**3.68**	**0.068**	**0.004**	**0.064**

Genetic structure of the population

Genetic distances and pairwise F_{ST} of subpopulation

The overall differentiation level of the subpopulations was very low (F_{ST} = 0,004). Among the four subpopulations, the highest values of genetic distance and F_{ST} were observed between Say and Niamey subpopulations while the lowest were between Kollo and Tera for F_{ST} and between Kollo and Say subpopulations for distance of Cavalli-Sforza & Edwards (Table 6).

Table 6: Pairwise F_{ST} (above of the matrix) and pairwise genetic distance of Cavalli-Sforza & Edwards (below of the matrix).

	Kollo	Say	Tera	Niamey
Kollo	**0**	0.0036	**-0.0025**	0.0132
Say	**0.031**	**0**	0.0028*	**0.0192**
Tera	0.044	0.057	**0**	0.0054
Niamey	0.072	**0.079**	0.069	**0**

Genetic structuring of populations by factorial analysis of correspondences

The clustering obtained by the factorial correspondence analysis (FCA 3D and FCA 2D) is shown in figure 2 (A and B). On the FCA 2D, there is no clear delimitation between the four subpopulations. Although the pairwise values of the F_{ST} were very low, the FCA 3D allowed to represent the different subpopulations. The factorial axis 1 (40.56%) separates all individuals of Niamey, Tera, a large part of Kollo and a small part of Say subpopulations in one side while the large part of Say and a small part of Kollo were clustered in the other side. The factorial axis 2 (32.91%) groups together Kollo, Tera and a small proportion of Say subpopulations in one hand and the Niamey and the large part of Say subpopulations.

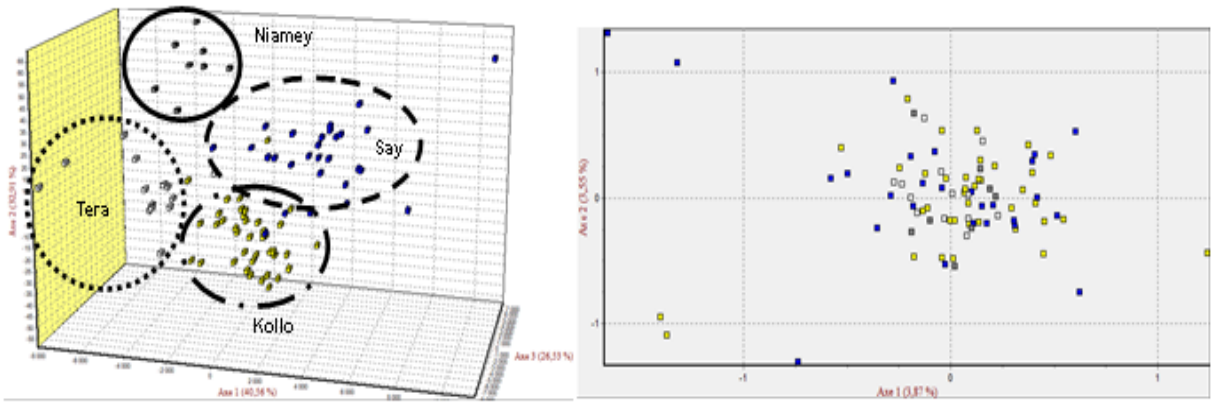

Figure 2: Factorial correspondence analysis (A: 3D and B: 2D).

Genetic structuring of populations by the Bayesian inference method

The number of probable genetic groups from sub-populations (departments) determined by the Evanno method amounts to K = 2 (figure 3).

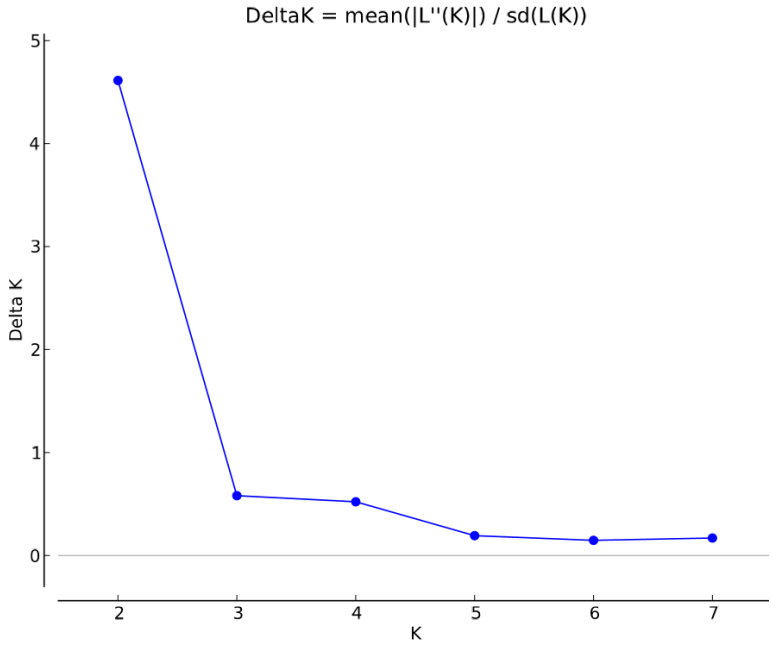

Figure 3: Graphical method allowing detection of the magnitude of ΔK as a function of K.

Table 7: Information on Evanno test results (number of clusters k).

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	10	-4130.6800	0.0919	-	-	-
2	**10**	**-4366.3200**	**53.1785**	**-235.640000**	**245.380000**	**4.614274**
3	10	-4356.5800	24.8996	9.740000	14.460000	0.580732
4	10	-4361.3000	18.8798	-4.720000	9.850000	0.521722
5	10	-4356.1700	33.5782	5.130000	6.460000	0.192387
6	10	-4357.5000	28.4545	-1.330000	4.180000	0.146901
7	10	-4354.6500	29.5495	2.850000	5.010000	0.169546
8	10	-4346.7900	15.1544	7.860000	-	-

The results show that the value of k which better explains the structuring of the population of the djelli zebu is k = 2 (table 23, figure 18). With k = 2, individuals were predominantly grouped in group group 1 with more than 80% (in the Kollo, Tera and Niamey subpopulations) and 75% in the department of Say while individuals in group 2 did not represented only 16% (Kollo sub-population) to 25% (Say sub-population) (table 8).

Table 8: Proportion of membership of each pre-defined population in 2 clusters (K = 2).

Sous-populations	Clusters	
	1	**2**
Kollo	0,842	0,158
Say	0,748	0,252
Tera	0,829	0,171
Niamey	0,814	0,186

From K=3 to K=6, some individuals displayed less than 1% of their genome in other clusters. From K=6 to K=8, increasing in the prior number of clusters does not change significantly the genetic structure of Djelli population. Say department seemed to be the administrative department where introgression of Djelli zebu was the highest among the four subpopulations studied. Individuals from Tera and Niamey departments were the most homogenous and seemed to have the same origin as showed by the neighbour-joining tree obtained using Cavalli-Sforza & Edwards genetic distances (figure 5).

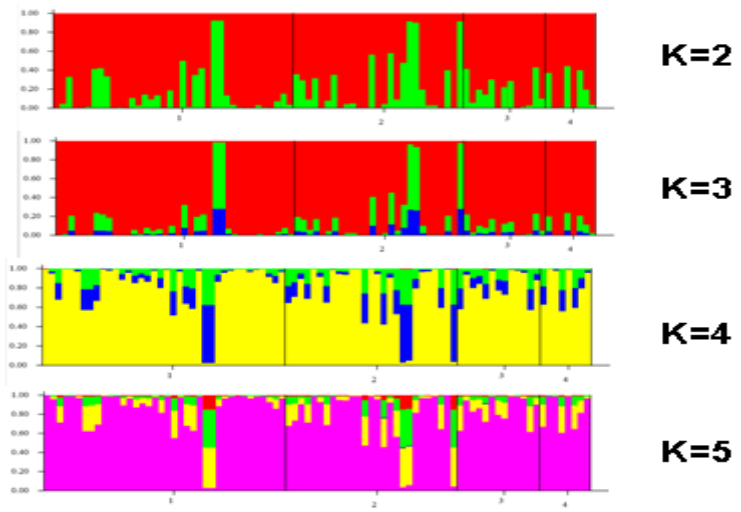

Figure 4: Population structure assessed by Structure. Each individual is represented by a vertical bar, often partitioned into coloured segments with the length of each segment representing the proportion of the individual's genome from K = 2, 3, 4 and 5 ancestral populations. Subpopulations are separated by black lines.

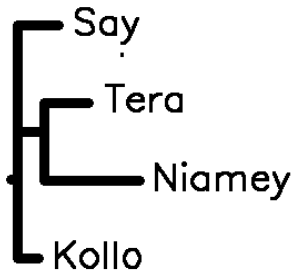

Figure 5: Neighbour-joining tree obtained with Cavalli-Sforza & Edwards genetic distances

DISCUSSION

The results presented in this study are the first for in the Fulani zebu population of Niger named Djelli zebu. Similar studies were conducted in other cattle breeds of Niger: Kouri taurine breed (Grema *et al.* 2017) and Bororo zebu breed or Wodaabe from Niger (Maaouia *et al.* 2019). The four administrative departments included in this study were the most important breeding areas of the Djelli zebu in Niger. Our results can be considered as reflecting a good overview of the genetic diversity of the Djelli zebu in Niger.

Genetic diversity of Djelli zebu of Niger

A high genetic diversity was observed in the four subpopulations (departments) of Djelli zebus of Niger. Indeed, the set of 22 loci used in the study showed an average value of the Polymorphic Information Content (PIC) of 0.654. The similar mean value of PIC was reported in a characterization study of the Bororo zebu in Niger with 27 microsatellite markers (Maaouia *et al.* 2019) (0.65). High PIC average (0.75) have been reported in four cattle breeds (Zebu Gobra, Zebu Maure, Zebu Djakore and taurin N'dama) in Senegal (Ndiaye *et al.* 2015) and in Cameroon (Ngono Ema *et al.* 2014) for microsatellite loci. Botstein *et al.* (1980) recommended to consider microsatellite markers as informative when the PIC value is above 0.5. In a general way, the microsatellite loci used are sufficiently informative to describe the genetic diversity of the Djelli zebu population studied. The average number of alleles per locus and the heterozygosities obtained were higher than those previously reported by Freeman *et al.* (2004) in Fulani zebu from Burkina Faso, Goudali zebu from Sokoto and white Fulani zebu from Nigeria. However, the expected and observed heterozygosities, the average number of alleles per locus (Na = 8.2) and the effective number of alleles (Ae = 3.68) approximated those obtained by Traore *et al.* (2015) in the Fulani zebu in Burkina Faso. Furthermore, the average number of alleles per locus (Na) is higher than those reported in the Bororo zebu (Wodaabe) populations of Niger (Na = 5.22) (Maaouia *et al.* 2019) and in the Kouri breed from Niger (Na = 6.74) (Grema *et al.* 2017) and in zebu breed of Benin (Na = 7.9) (Moazami-Goudarzi *et al.* 2001). However, this number remains lower than those observed in the cattle populations of Senegal (Na = 10.45) (Ndiaye *et al.* 2015) and Cameroon (Ngono Ema *et al.* 2014. The high genetic diversity is a characteristic of West African zebu (*Bos indicus*) and crossbreeds (*Bos indicus* X *Bos taurus*) compared to the West African taurine cattle and European cattle generally subject to selection and artificial insemination (*Bos taurus*) (Traore *et al.* 2014; Dayo *et al.* 2009, Freeman *et al.* 2004).

In our study, the expected heterozygosity was greater and close to that estimated in the cattle populations in Turkey and in the northern part of the Arabian Peninsula (Freeman *et al.* 2004), and in the mixed cattle populations of Cameroon and Nigeria (Ibeagha-Awemu *et al.* 2004).

The high genetic diversity of West African zebus and crossbreeds (*Bos indicus X Bos taurus*) is linked to their history. Indeed, West African zebus contain a certain

proportion of taurine genes because there would have been a continuous introgression between zebus *B. indicus* and taurins *B. taurus* (MacHugh *et al.* 1997, Hanotte *et al.* 2002, Freeman *et al.* 2004). In the crossbreeds (*Bos indicus X Bos taurus*), stabilized (Borgou, Djakore) or not, the strong genetic diversity is explained by the effect of combining alleles of two genetic types of cattle (Dayo et al., 2009, Freeman et al., 2004) and by the combination of a relatively larger effective population size associated with a higher migration rate, linked to a transhumant breeding system.

A slight difference in panmixia (significant average F_{IS}) exists in Kollo subpopulation while for other subpopulations, although F_{IS} is significantly high at some locus levels, it is not significant at the subpopulation level. The significant F_{IS} may be due to technical problems (presence of null alleles on certain markers, dominance of short alleles), the presence of a few related individuals (inbreeding) or even a hidden structure in the population also called the Wahlund effect.

With regard to locus BM4440, it was shown in the population of the zebu Djelli a higher variability as reported by Dayo *et al.,* (2009) in the zebu populations. Indeed, Dayo *et al.* (2009) compared the distribution of allelic frequencies of the BM4440 locus in the trypanotolerant West African Taurine populations and trypanosusceptible West-African zebus. The authors reported a loss of variability at this locus in West African Taurine marked by the reduction in the number of alleles to two and the almost fixation of the most frequent allele (allelic frequency greater than 0.95) except in Lagune taurine which have presented two almost equi-frequent alleles – (0.45 and 0.55).

Genetic structure of the population

Across all subpopulations, the overall value for F_{ST} was 0.004. This value is lower than the 0.03 to 0.06 previously reported by Dayo *et al.* (2009) and Freeman *et al.* (2004) in the West African zebu populations. The level of differentiation (F_{ST}) between the Djelli zebu subpopulations is also lower than those obtained in other cattle populations of Niger as in Bororo zebu (Maaouia *et al.* 2019) with a F_{ST} of 0.036 and in the Kouri with a F_{ST} value of 0.026 (Grema *et al.* 2017). It is important to note that the Kouri cattle is classified as taurine because having a *Bos taurus* submetacentric Y chromosome (Zafindrajaona *et al.* 1999) and being) humpless, but all molecular studies up to now showed in the Kuri breed, genetic characteristics close to the crossbreeds *Bos taurus X Bos indicus* (Souvenir Zafindrajaona *et al.* 1999, Freeman *et al.* 2004, Dayo *et al.* 2009). The values of F_{ST} obtained between the subpopulations of the Djelli zebu are also lower than those obtained in other cattle populations on the African continent, in particular on Ethiopian cattle breeds (F_{ST} = 0.013, Dadi *et al.* 2008), between the Sudanese Fulani zebu subpopulations (F_{ST} = 0.084, Hussein *et al.* 2015), between the Cameroonian indigenous zebus (F_{ST} = 0.061, Ngono Ema *et al.* 2014).

Latch *et al.* (2006) reported that using factorial correspondence analysis (FCA) and a Bayesian approach for detecting population structure (STRUCTURE; Pritchard et al., 2000) performs well at low levels of population differentiation. Results from both Cavalli-Sforza & Edwards distance-based analysis and Bayesian analysis were concordant and tally well with the hypothesis that the administrative division into "department" has very little influence on the genetic structure of the Djelli zebu population in Niger. These results show that in the Tillabery region, the Djelli zebu population is almost homogeneous and that the current levels of differentiation observed between the subpopulations would be due to the various farming systems practiced (place, duration and degree of influence of transhumance) by breeders in the different administrative departments. The differences in the breeding systems led in a different way to various degrees of introgression of the zebu Djelli especially in the departments of Kollo and Say while in Niamey where the animals were bred in the sedentary breeding system, it was noted a low level of introgression. The results of the Bayesian analysis for the detection of genetic structure (Figure 19) suggested that most of the animals in Niamey would come from the Tera region. The same trend was observed on the Neighbour-joining tree (figure 5). A gradient of admixture among the subpopulations studied was observed: from the less to the most level of introgression, we have Niamey department subpopulation followed by Tera, Kollo and Say subpopulations respectively.

The molecular results are in consistence with those obtained in phenotypic characterization of the Djelli zebu (Zakari et al. 2019). Indeed, Zakari et al. (2019) showed that the differences observed in the breeding conditions between the for administrative department influenced significantly the phenotypic characteristics of Djelli zebu in the study area. The promotion of this breed will require the improvement of its breeding conditions and the promotion of initiatives for its sustainable use through for example the creation of a breed or Djelli zebu breeders association, the creation of a value chain around the Djelli zebu.

CONCLUSION

A high genetic diversity was observed in Djelli zebu population of Niger. A weak genetic structuration exists in Djelli zebu population characterized by a low level of differentiation among the four subpopulations included in this study. The farming system of the breed based essentially on pastoralism might influence the level of introgression of the Djelli zebu observed. The identification of the origin of this introgression requires another study including all the cattle breeds of Niger (Bororo, Azawak, Gudali, and Kuri). The high genetic diversity observed in Djelli zebu population is very important to initiate a program of genetic improvement and sustainable management of this breed in Niger with a high involvement of the local breeder's communities.

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