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Research Article

The Major Histocompatibility Complex Region and Diversity of the Local Chicken Populations in Niger

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ABSTRACT

Introduction: The major histocompatibility complex (MHC) of chicken is highly polymorphic and is linked to several disease resistance or susceptibility traits. Therefore, the current study aimed to analyze the genetic diversity in the MHC region of Nigerien local chickens (Dourgou, Goggori, Kolonto, Tchagara, Gouzou-gouzou, and Popular) using a high polymorphic microsatellite marker named LEI0258 to determine the diversity of chickens kept at the four agroecological zones in Niger.
Materials and methods: A total of 601 chickens from six local Nigerien chicken breeds were sampled. By capillary electrophoresis, using the EI0258 marker, 403 samples with different fragment sizes were randomly chosen and sequenced.
Results: The findings indicated 80 different alleles ranging in size from 181 to 474 bp. A total of 22 new alleles and 39 private alleles (that existed in only one breed) were detected. The alleles 309, 295, and 193 were the most predominant in the Nigerien local chicken population. Nine polymorphisms were observed along the LEI0258 sequence, including three in the upstream (one indel and two Single Nucleotide Polymorphism [SNP]), one in the repeat region at the last R12 (SNP), and five in the downstream (two indels and three SNPs).
Conclusion: The chickens are not clustering according to their agroecological zone of origin. They are randomly distributed across the four investigated agroecological zones. The information found in this study is invaluable in breeding and conservation programs associated with several disease resistance or susceptibility traits.

1. Introduction

Genetic markers, including single nucleotide polymorphisms[1,2], microsatellites[3-5], and mitochondrial sequences[6], have been used to characterize chicken populations. However, in recent years, researchers have shifted their focus to a region of significant importance, that is the major histocompatibility complex (MHC) of chickens. This complex plays an important role in immune response[7,8,9] and has been associated with disease resistance[10-15] and productivity[16,17].

The tandem repeat LEI0258 was first identified[18] and subsequently described[19]. The microsatellite LEI0258 represents a sequence of repetitions of four nucleotides located in the region that codes for MHC, precisely between the BG and BF regions of chromosome 16[20]. The marker LEI0258 presents two flanking regions (upstream and downstream) surrounding one repeat region[19]. The upstream region starts from position -78 to -1, and the downstream region begins from position 1 to 88. The repeat region consists of two independent repeat elements, a 13-bp repeat of "ATGTCTTCTTTCT" and a 12-bp repeat of "TTCCTTCTTTCT"[21].

The marker LEI0258 has been successfully used in many chicken diversity studies[7,13,21-24] and has also been employed in studies investigating associations with

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diseases[11,12,16,25,26].

Family poultry is a small-scale poultry farming practiced in households mainly found in developing countries[27]. In Niger, family poultry requires low inputs. It contributes to rural food security through its protein intake. This activity helps the rural population fight against poverty through the income it provides to producers, and it also plays an important socio-cultural role[28,29].

In 2006, the avian influenza epidemic reduced poultry production in Niger[30]. Concurrently, the population of Niger has been steadily increasing. These factors have collectively led to increased demand for poultry, which currently accounts for 95% of the poultry supply in Niger[30]. To resolve that problem, the government started to import poultry meat. However, the challenge with importation lies in the uncertainty surrounding the preservation quality. Importation, while addressing the concern of food security, falls short in tackling broader issues, such as poverty and the multifaceted roles family poultry plays in the rural community. Thus, the sub-sector of family poultry, which includes most rural households, is expected to increase production to reduce poverty and malnutrition. Local chickens represent 55% of poultry breeds in Niger[31]. Therefore, it is normal to focus on the local chicken to increase poultry production. This motivates the authors of the current study to focus on the molecular characterization of the local chicken population of Niger. Therefore, the current study aimed to genetically characterize the chickens to provide a database for the improvement of local chickens in Niger.

This study was a cross-sectional survey to determine the diversity of chickens (six local breeds) kept at the four agroecological zones in Niger. It could be, therefore, an opportunity to establish the genetic diversity targeting the LEI0258 microsatellite marker in the MHC region.

2. Materials and Methods

2.1. Ethical approval

The National Committee of Ethics on Health Research authorized the use of this blood sample data (authorization No.010/2017/CNERS).

All the producers were first informed about the main purpose of the study, and their participation was voluntary and anonymous. A verbal agreement was obtained from each producer at the beginning of the blood sample collection from their chicken.

2.2. Sampling

This cross-sectional survey was conducted in the four agroecological zones of Niger from south to north of the country, including the Sahelo-Sudanese zone, Sahelian zone, Sahelo-Saharian zone and Sahara zone. The distribution of localities by zone was made by considering the most productive areas of the local chicken. In the current study, the samples were collected from 6, 12, 4, and 2 localities in the Sahelo-Sudanese, Sahelian, Sahelo-Saharian, and Saharian zones, respectively (Figure 1).

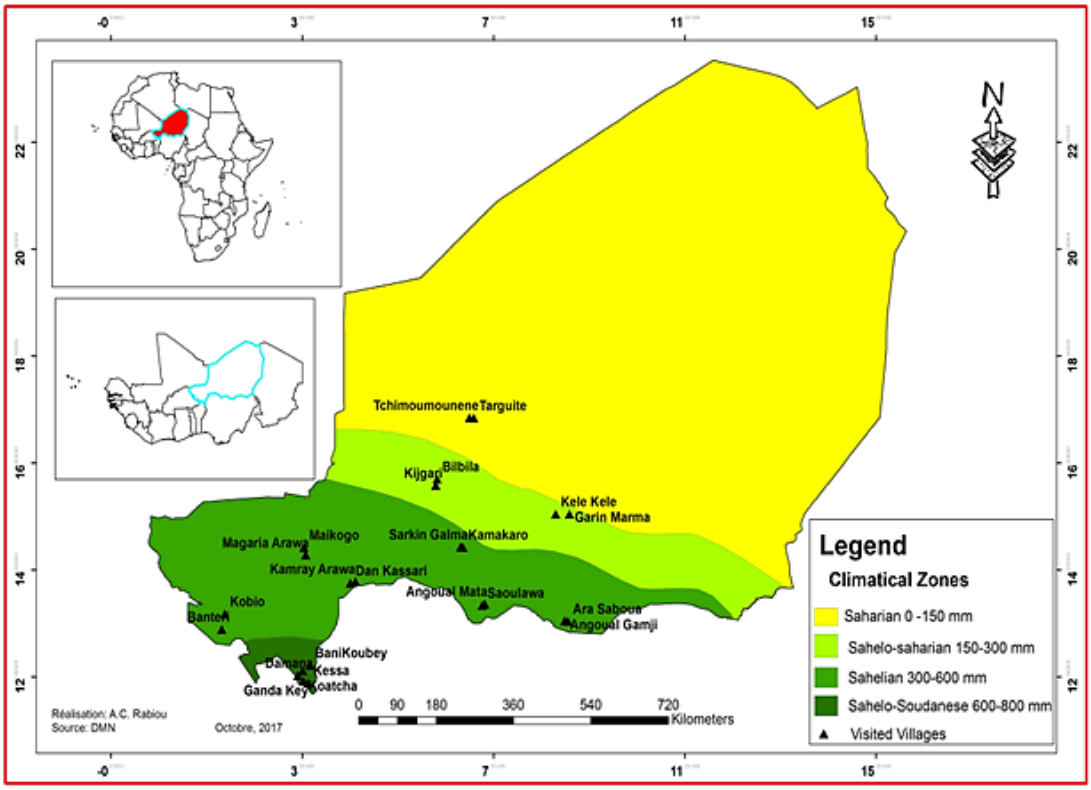
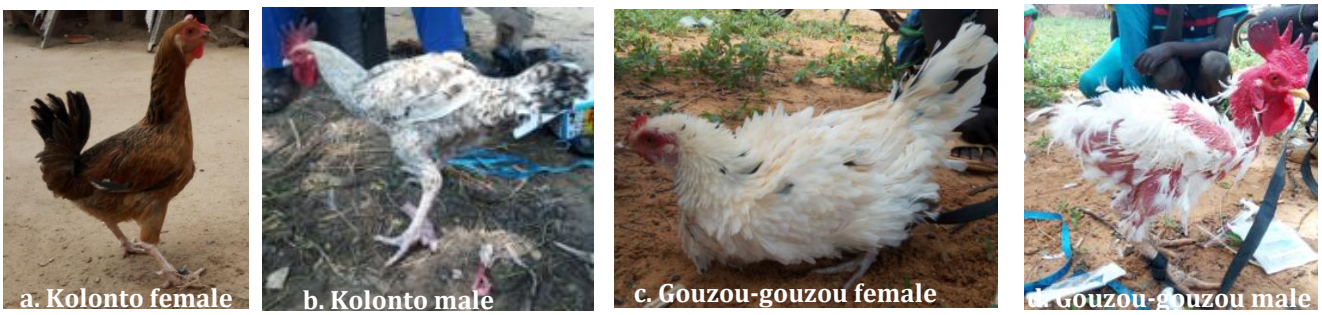


Figure 1. Distribution and number of areas sampled by agroecological zones.



Kolonto chicken has large size and fast growth. It is found in sahelo-soudanese and Sahelian zone.

Gouzou-gouzou is a frizzle chicken which is rare in Niger. They are found in the Sahelian and Sahelo-Sudanese zone.

Goggori chicken is characterized by the absence of tail. Especially encountered in the south of Sahelian zone.

Tchagara chicken is characterized by its small size and large laying capacity. It is more met in the Sudanese zone.

Popular chicken is an ecotype with medium size found in all the agroecological zones of Niger.

Dourgou chicken is a dwarf chicken very rarely encountered in the local chicken population of Niger.

Figure 2. Some phenotypic attributes of indigenous chicken ecotypes encountered across the four agroecological zones of Niger (Hassan et al.)[32]

The Saharian zone, encompassing the Sahara Desert, holds significance in the agricultural landscape Niger. The southern region of the country emerges as agriculturally pivotal, hosting a substantial number of chicken breeders. Recognizing the importance of this area, the study incorporated a larger number of sampling sites from the southern Saharian zone. The decision to select localities was a collaborative effort with the livestock ministry, aligning with specific criteria. Key considerations included seniority of the keeping of the local chicken of the village, the accessibility of the village, and a minimum distance of 15 km between villages.

Blood samples were collected from 601 local chickens in the 12 localities selected in the whole country of Niger. Due to the importance of the practice of keeping local chickens in Niger keeping, the sample size across the agroecological zone was different. Thus, the ratio per village was 29 chickens in the Sahelian zones, 25 in the Sahelo-Sudanese zone, 19 in the Sahelo-Saharian zone, and 10 in the Saharian zone. Figure 2 presents some phenotypic attributes of indigenous chicken ecotypes encountered across the four agroecological zones of Niger[32].

Approximately, 2 ml of blood was obtained by venipuncture of the brachial vein from local chicken and preserved on Whatman FTA paper (GE Healthcare Life Sciences, Buckinghamshire, UK), dried under a shade, and then stored in envelopes at room temperature until DNA extraction.

2.3. PCR amplification

2.3.1. Amplification of LEI0258 and capillary electrophoresis

The LEI0258 microsatellite marker was used to amplify the DNA. The PCR primers for LEI0258 were 5'-NED-CACGCAGCAGAACTTGGTAAGG-3' (forward) and 5'-AGCTGTGCTCAGTCCTCAGTGC-3' (reverse)[18,19]. The reaction volume for PCR was 10 µl and was composed of 15 ng/µl genomic DNA, 0.1 µM each primer, 2× MasterMix. Thermocycling was set at 94°C for 3 minutes, 30 cycles at 94°C for 30 seconds, 63°C for 45 seconds, and 72°C for 45 seconds, and a final extension at 72°C for 10 minutes. Then, 1 µl PCR product was mixed with a HIDI formamide (Applied Biosystems, USA) and GeneScan 500 LIZ Size Standard (Applied Biosystems, USA) cocktail premixed at a 9:1 ratio, respectively. The mixture was then denatured at 95°C for 3 minutes, followed by immediate snap-chilling on wet ice for 5 minutes, and analyzed by capillary electrophoresis on a Genetic Analyzer 3730 (Applied Biosystems, USA). The fragments were sized based on the internal size standards of the electropherogram using the GeneMapper version 4.1 (Applied Biosystems, USA)[33,34].

2.3.2. Amplification of LEI0258 and Sanger Sequencing

Of 601 samples, 403 samples with distinct sizes of the LEI0258 fragment were selected for Sanger sequencing. PCR was set up in a total volume of 50 µl using the concentrations of 15 ng/µl genomic DNA, 10 µM of each unlabeled primer (same primers used for capillary electrophoresis without the fluorescent label NED), 2X of Master Mix, and 19 µl Milli-Q water. PCR run conditions were the same as for the genotyping profile.

For homozygous samples, amplicons were purified using QIAquick PCR Purification Kit (Qiagen, Germany). For heterozygous samples, the amplicons were separated by electrophoresis on a 2% agarose gel and purified using the QIAquick Gel Extraction Kit according to the manufacturer's protocol. The purified PCR products were then Sanger sequenced at the Macrogen sequencing unit in the Netherlands[35].

2.4. Data analysis

GeneMapper v4.1 (Applied Biosystems, USA) was used for allele scoring. The sequences with poor quality bases were trimmed, and contigs assembled using CLC Main Work-bench 7.9.1. Consensus sequences were aligned using the Clustal W program in Mega 7 software. The phylogenetic tree was built with the Mega 7 software. The GenAlEx 6.41 software was used to calculate expected and observed heterozygosity values, the allele number, and the establishment of the genetic variations between and within populations by analyzing molecular variance (AMOVA)[36]. The evaluation of the heterozygote excess or deficiency by overall sample and per ecotype and the test for conformity to Hardy–Weinberg equilibrium (HWE) were performed by GenAlEx 6.41 software[37]. The assessment based on allele frequencies of the standard genetic distances (DA) between ecotypes was conducted by the GenAlEx 6.41 software[38]. PowerMarker software V.3.25 was used to assess the polymorphic information content (PIC)[39].

The R software was utilized to perform the discriminant analysis of principal component (DAPC) based on the allele frequencies to assess genetic relationships among chickens found in the four agroecological zones of Niger.

3. Results

3.1. LEI0258 Polymorphism

The two levels of the marker LEI0258 polymorphism were the flanking region (upstream and downstream) and the repeat region (Table 1). The upstream region had 57 bp, and the downstream region had 52 bp (without the primer sequence). The polymorphism at the upstream region revealed two deletions at position -32 bp and -31 bp (TT/Δ) and two Single Nucleotide Polymorphisms (SNPs) at position -30 bp (Guanine [G]/Adenine [A]) and -13 bp (G/A). In the downstream region, three Single NPs (Thymine [T]/Cytosine [C]) at position 1 bp, A/T at position 25 bp, T/A at position 32 bp), one deletion at position starting from 9 bp to 16 bp (ATTTTGAG/Δ) and one insertion at position 19 bp (Δ/A) were identified. The repeat region contained two repeat fragments, a 13 bp repeat of ATGTCTTCTTTCT (R13) and a 12 bp repeat of TTCCTTCTTTCT (R12). These repeats were 1-22 times for R13 and 2-20 times for R12. Most of the sequences had one repeat of R13 and more repeats of R12 (Table 1).

3.2. Allelic variability

From 403 alleles sequenced from 601 individual samples of 6 Nigerien local chickens, 80 different alleles were found in the local chicken population. The size of LEI0258 alleles ranged from 181 to 474 bp. All 80 alleles were described by their number of repetitions, total size, the combination of the indels, and the SNPs in the flanking regions (Table 1). The findings indicated 22 new alleles and 39 private alleles that exist in only one breed. The popular type of Nigerien local chicken had the highest number of private alleles, followed by the Tchagara type. The alleles 309, 295 , and 193 were the most frequent in the Nigerien local chicken population (Table 2). They were followed by alleles 357, 249, and 307. The allele 205 was only found in the popular and Tchagara chicken type. The alleles 307, 309, and 295 were found in the majority of the types of local chicken except in the gouzou-gouzou type.

Table 1. Polymorphisms and origin of the 80 LEI0258 alleles of the local chickens in Niger

Allele name	Size (bp)	Cluster	Upstream				Repeats				Downstream				N	Populations	Genbank accession number
			Position	-32_-31 TT/Δ	-30 G/A	-13 G/A	R13	R12	Last R12 C/T	1 T/C	9_16 ATTTTGAG/Δ	19 Δ/A	25 A/T	32 T/A			
181A	181	A					1	2			Δ				2	Pop*	MH376179
193A	193	A					1	3			Δ				8	Tch, Kol,Pop,	MH375953
193B	193	A					1	3	T		Δ				26	Kol,Pop,Tch, Gog	MH376110
193C	193[1]	A				A	1	3			Δ				1	Tch*	MH376127
201A	201[1]	A					1	3							1	Tch*	MH375954
205A	205	A					1	4			Δ				12	Pop, Tch,	MH375947
205B	205	A					1	4	T		Δ				1	Pop*	MH376154
206A	206	A					1	4			Δ	A			2	Pop, Kol	MH376048
213A	213[1]	A					1	4							1	Kol*	MH376095
217A	217	A					1	5			Δ				10	Pop,kol,Gog,	MH375899
217B	217	A					1	5	T		Δ				1	Pop*	MH375945
237A	237	C					1	6						A	10	Kol,Tch,Pop	MH376092
241A	241	A					1	7			Δ				1	Pop*	MH375942
249A	249	A					1	7							4	Kol,Pop	MH376040
249B	249	C					1	7						A	13	Pop,Kol,Tch,	MH376037
249C	249	A					1	7					T		6	Pop, Kol, Tch	MH376150
259A	259	C		Δ			1	8						A	2	Tch,Kol	MH376028
259B	259	B		Δ			1	8		C				A	14	Tch,Pop,Kol,Gog	MH375891
261A	261	A					1	8							2	Pop*	MH376038
261B	261	C					1	8						A	2	Kol,Tch	MH376126
261C	261	A					1	8					T		8	Tch,Pop,Kol,	MH375897
271A	271[1]	B		Δ			1	9		C				A	6	Tch,Pop	MH376117
273A	273	C					1	9						A	1	Dou*	MH376030
283A	283	A		Δ			1	10							3	Gou,Gog,Pop	MH375903
283B	283	C		Δ			1	10						A	2	Pop*	MH376247
283C	283	A		Δ	A		1	10							1	Tch*	MH376014
285A	285	A					1	10							1	Pop*	MH376049
285B	285	C					1	10						A	3	Pop*	MH376149
285C	285	A					1	10					T		1	Pop*	MH375952
295A	295	A		Δ			1	11							39	Kol,Tch,Pop,Gog,	MH375921
295B	295	C		Δ			1	11						A	1	Pop*	MH375966
297A	297[1]	A					1	11							7	Kol,Tch,Pop,	MH375986
297B	297	C					1	11						A	1	Tch*	MH376068
297C	297	A					1	11					T		2	Pop*	MH375962
307A	307	A		Δ			1	12							8	Pop,Gog,Tch	MH376277
307B	307	C		Δ			1	12						A	4	Kol,Pop	MH376161
307C	307	A		Δ	A		1	12							7	Kol,Tch,Pop	MH376015
309A	309	A					1	12							16	Tch,Pop,Kol	MH375934
309B	309	C					1	12						A	6	Pop,Kol	MH376174
309C	309	A					1	12					T		19	Tch,Pop,Gog,Kol,Dou	MH375884
309D	309	A					1	12					T	A	1	Tch*	MH376026
319A	319[1]	A		Δ			1	13							1	Gog*	MH376063
319B	319	C		Δ			1	13						A	1	Pop*	MH375965
319C	319	A		Δ	A		1	13							11	Tch,Pop,	MH375931
321A	321[1]	A					1	13							5	Gog,Kol,Pop	MH376055
321B	321	C					1	13						A	6	Kol,Pop	MH376070
321C	321	A					1	13					T		5	Pop*	MH376132
331A	331[1]	A		Δ			1	14							3	Pop,Dou	MH376016
331B	331[1]	B		Δ			1	14		C					1	Pop*	MH376236
331C	331[1]	B		Δ			1	14		C				A	2	Tch, Pop	MH376145
331D	331	A		Δ	A		1	14							1	Tch*	MH376111
333A	333[1]	A					1	14							2	Pop*	MH376025
333B	333	C					1	14						A	4	Tch,Pop	MH376113
343A	343[1]	A		Δ			1	15							1	Pop*	MH375967
343B	343[1]	B		Δ			1	15		C				A	1	Pop*	MH376120
343C	343	A		Δ	A		1	15							1	Pop*	MH375999
345A	345	A					1	15							4	Kol,Gog,Pop	MH376197
345B	345	C					1	15						A	1	Pop*	MH375993
345C	345	A					1	15					T		12	Kol,Pop,Gog,Tch,	MH375983
355A	355[1]	C		Δ			1	16						A	1	Pop*	MH375930
357A	357	A					1	16							9	Pop,Kol,Gou	MH376102
357B	357	C					1	16						A	6	Kol,Tch,Pop,	MH376207
357C	357	A					1	16					T		15	Tch,Pop,Kol,Gou,	MH376079
367A	367	A		Δ	A		1	17							1	Pop*	MH375933
369A	369	C					1	17						A	1	Pop*	MH376192
369B	369	A					1	17					T		6	Pop,Kol	MH376261
379A	379[1]	C		Δ			1	18						A	1	Pop*	MH375929
379B	379	A		Δ	A		1	18							1	Pop*	MH376213
381A	381[1]	A					1	18							1	Pop*	MH376067
381B	381	A					1	18					T		4	Pop,Tch	MH376112
393A	393[1]	A					1	19							1	Pop*	MH375987
393B	393	A					1	19					T		8	Tch,Kol,Pop	MH375981
394A	394[1]	D					14	5							1	Gog*	MH375972
405A	405[1]	A					1	20							5	Gou,Pop	MH376156
405B	405	A					1	20					T		12	Dou,Pop,Tch,Kol	MH375961
420A	420	D					16	5							3	Kol,Tch	MH376274
420B	420[1]	D					16	5						A	1	Tch*	MH376109
443A	443	D					15	8							6	Pop, Gog, Tch	MH376275
444A	444[1]	D					16	7							1	Pop*	MH376270
474A	474	D					22	3							1	Tch*	MH376269

[1]New allele detected in Nigerien local chicken; N: Number of animals, *Private allele found in only in one population; Pop: popular, Gou: Gouzou-gouzou, Tch: Tchagara, Gog: Goggori, Kol: Kolonto, Dou: Dourgou; A: Adenine, G: Guanine, T: Thymine, C: Cytosine

Table 2. Frequencies of LEI0258 alleles in 6 local chickens' strain of Niger

Allele	Strains						Overall frequency	Population
	Dourgou	Goggori	Gouzou-Gouzou	Kolonto	Popular	Tchagara		
181	0.000	0.000	0.000	0.000	0.006	0.000	0.004	1
193	0.000	0.000	0.000	0.092	0.076	0.118	0.081	3
201	0.000	0.000	0.000	0.000	0.000	0.013	0.002	1
205	0.000	0.000	0.000	0.000	0.046	0.013	0.032	2
206	0.000	0.000	0.000	0.000	0.006	0.000	0.004	1
213	0.000	0.000	0.000	0.000	0.003	0.000	0.002	1
217	0.000	0.056	0.000	0.013	0.034	0.000	0.026	3
237	0.000	0.000	0.000	0.066	0.012	0.013	0.020	3
241	0.000	0.000	0.000	0.000	0.003	0.000	0.002	1
249	0.000	0.000	0.000	0.092	0.049	0.066	0.055	3
259	0.000	0.167	0.000	0.039	0.024	0.066	0.037	4
261	0.000	0.000	0.000	0.026	0.027	0.053	0.030	3
271	0.000	0.000	0.000	0.000	0.012	0.026	0.012	2
273	0.167	0.000	0.000	0.000	0.000	0.000	0.002	1
283	0.000	0.056	0.250	0.000	0.009	0.026	0.014	4
285	0.000	0.000	0.000	0.000	0.018	0.000	0.012	1
295	0.000	0.056	0.000	0.132	0.119	0.079	0.110	4
297	0.000	0.000	0.000	0.039	0.030	0.053	0.033	3
307	0.000	0.056	0.000	0.053	0.055	0.039	0.051	4
309	0.500	0.222	0.000	0.118	0.104	0.145	0.120	5
319	0.000	0.056	0.000	0.013	0.030	0.039	0.030	4
321	0.000	0.111	0.000	0.053	0.037	0.013	0.037	4
331	0.167	0.000	0.000	0.000	0.021	0.013	0.018	3
333	0.000	0.000	0.000	0.000	0.018	0.013	0.014	2
343	0.000	0.000	0.250	0.000	0.009	0.000	0.008	2
345	0.000	0.111	0.000	0.092	0.027	0.039	0.041	4
355	0.000	0.000	0.000	0.000	0.003	0.000	0.002	1
357	0.000	0.000	0.250	0.092	0.061	0.079	0.067	4
367	0.000	0.000	0.000	0.000	0.003	0.000	0.002	1
369	0.000	0.000	0.000	0.026	0.021	0.000	0.018	2
379	0.000	0.000	0.000	0.000	0.006	0.000	0.004	1
381	0.000	0.000	0.000	0.000	0.021	0.000	0.014	1
393	0.000	0.000	0.000	0.013	0.034	0.000	0.024	2
394	0.000	0.056	0.000	0.000	0.000	0.000	0.002	1
405	0.167	0.000	0.250	0.013	0.055	0.013	0.043	5
420	0.000	0.000	0.000	0.026	0.003	0.039	0.012	3
443	0.000	0.056	0.000	0.000	0.012	0.026	0.014	3
444	0.000	0.000	0.000	0.000	0.003	0.000	0.002	1
474	0.000	0.000	0.000	0.000	0.000	0.013	0.002	1
Na	4	11	4	18	35	23		

Gouzou-gouzou had the highest frequency of the allele 357.

3.3. Genetic diversity

The result showed that the overall observed heterozygosity (0.656 ± 0.07) was lower than the expected (0.848 ± 0.046). The PIC at this marker was high (0.981). The observed heterozygosity of the Gouzou-gouzou chicken ecotype showed the highest value (1.000), while the Goggori chicken ecotype indicated the lowest (0.556). The expected heterozygosity of the Dourgou chicken ecotype revealed the lowest value (0.667), while the Popular chicken ecotype had the highest (0.945). The average of the fixation index was 0.226, and the overall one was 0.199.

The fixation index values of the Popular, Tchagara, and Kolonto chicken ecotypes were significantly different from zero, which were 0.413, 0.377, and 0.370, respectively (Table 3).

Table 3. Number of alleles, observed and expected heterozygosity, effective number of alleles, and Hardy–Weinberg equilibrium (HWE) status of the local chicken population of Niger

Sub-pop	N	N_{all}	Allele range	Ne	Ho	He	F	HWE deviation
Dourgou	3	4	273 to 405	3.00	0.667	0.667	0.000	b^{ns}
Goggori	9	11	217 to 443	8.10	0.556	0.877	0.366	b^{ns}
Gouzou-Gouzou	2	4	283 to 405	4.00	1.000	0.750	-0.333	b^{ns}
Kolonto	38	18	193 to 420	12.34	0.579	0.919	0.370	b***
Popular	164	35	181 to 444	18.09	0.555	0.945	0.413	b***
Tchagara	38	23	193 to 474	14.02	0.579	0.929	0.377	b***
Mean		15.83±4.9		9.93±2.42	0.656±0.07	0.848±0.046	0.199±0.123	
Entier population		39	181 to 474		0.656	0.848	0.226	

N: Number of animals, N_{all}: Number of alleles, Ne: Number of effective alleles, He: Expected heterozygosity, Ho: Observed Heterozygosity; F: Fixation index; b***: Populations not in HWE ($P < 0.001$); b^{ns}: Populations with non-significant deviation from Hardy Weinberg Equilibrium (HWE)

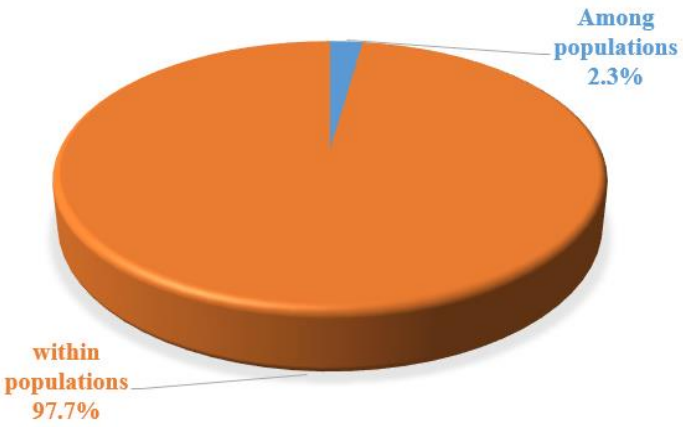


Figure 3. Analysis of molecular variance of the local chicken population of Niger

Based on the chicken ecotype, the number of alleles was different. The popular type, which was the widespread chicken in Niger, had the highest number of alleles. Popular ecotype was followed by Tchagara and Kolonto. Goggori, Dourgou, and Gouzou-gousou chicken types had the lowest number of alleles.

Overall, 12 private alleles were detected across the agroecological zones of Niger, namely six alleles (181, 273, 355, 369, 379, and 394) in the Sahelian zone, four alleles (213, 241, 367, and 444) in the Sahelo-Saharian zone, and two alleles (201 and 474) in the Sahelo-Sudanese zone. The frequency of each of these private alleles was less than 5%. On the other hand, six alleles were the most frequent in the local chicken population of Niger. These are alleles 309, 295, 193, 357, 249, and 307 with the respective frequencies of 12%, 11%, 8.1%, 6.7%, 5.5% and 5.1%. Four alleles 295, 297, 307, and 309, were encountered in the four agroecological zones of Niger.

3.4. Distribution of total genetic variation

Results from AMOVA indicated that 97.7% of the total genetic variation of studied chicken across the agroecological zones was due to variations within populations (individual variations). Only 2.3% of the total genetic variation was due to variations among populations (Figure 3).

3.5. Genetic distance and relationships

Table 5 summarizes pairwise standard genetic distances and gene differentiation (F_{ST}) indices among chickens found in the four agroecological zones in Niger. The genetic distances ranged from 1.919 for the Sahelo-Sahara and Sahara zones to 0.112 for the Sahelian and Sahelo-Sudanese zones. The gene differentiation (F_{ST}) indices ranged from 0.166 for the chicken found in the Sahara zone and Sahelian zone, for the Sahara zone and Sahelo-Saharan zone, to 0.001 for the chicken found in the Sahelian zone and Sahelo-Sudanese zone.

The population structure analysis of local chicken in Niger is represented in Figure 4. As can be seen, the local chicken population in Niger can be divided into three colors which linked to the number of sub-groups estimated. The individuals found in the Sahel zone had a probability higher than 0.5 to be assigned to a sub-group represented by pink color. This is the same case for the chickens found in the Sahelo-Saharan zone and those found in the Sahelo-Sudanese zone. However, the chickens found in the Sahara zone had a probability higher than 0.5 to be assigned to a sub-group represented by green color.

The DAPC showed two sub-groups, as can be seen in Figure 5. This DAPC also demonstrated that the chickens found in the Sahara zone show a significant difference compared to the chickens found in other agroecological zones.

3.6. Phylogenetic analysis

The phylogeny tree built from the all-alleles sequences found in Niger's local chicken using the microsatellite LEI0258 discriminated two main clusters with six subclusters (Figure 6). Local chickens from the four agroecological zones were found in all clusters except clusters 3 and 4, which did not have chickens from the Sahara zone.

Figure 7 presents the repartition of the local chicken ecotype within the six subclusters. Popular local chicken

Table 5. Pairwise Nei unbiased genetic distances between chicken found in four agroecological zones (D_A below diagonal) and fixation indices (F_{ST} above diagonal) between local chicken population of Niger

Agroecological zones	1	2	3	4
1. Sahara zone	--	0.166^{1}	0.166^{1}	0.122^{1}
2. Sahelian zone	0.980	--	0.003^{ns}	0.001^{ns}
3. Sahelo-saharian zone	1.919	0.276	--	0.014^{ns}
4. Sahelo-Sudanese zone	1.062	0.112	0.239	--

Note: $^{ns}F_{ST}$ values not significant, $^{1}F_{ST}$ values are significantly different from zero ($p \leq 0.01$).

Table 4. Allelic variability based on the agroecological zone of the local chicken population of Niger

Zones	N	N_{all}	Ne	Ho	He	F	HWE Deviation
Sahara	10	5	2.50	0.300	0.600	0.500	b^{ns}
sahelian	143	33	15.91	0.573	0.937	0.388	b^{***}
Sahelo_saharian	42	24	13.83	0.595	0.928	0.358	b^{***}
Sahelo_soudanese	59	25	13.76	0.576	0.927	0.379	b^{***}
Mean		21.75±5.9	11.50±3.04	0.51±0.07	0.85±0.08	0.41±0.03	

N: Number of animals, N_{all}: Number of alleles, Ne: Number of effective alleles, He: Expected heterozygosity, Ho: Observed Heterozygosity; F: Fixation index; b^{***}: populations not in HWE ($P < 0.001$); b^{ns}: Populations with non-significant deviation from Hardy Weinberg Equilibrium (HWE)

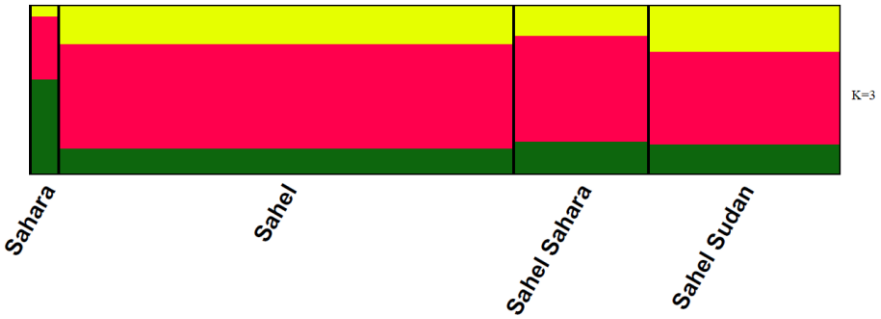


Figure 4. Population structure of the local chicken in Niger, K: Group number considered. The colors designate the number of subgroups in the population.

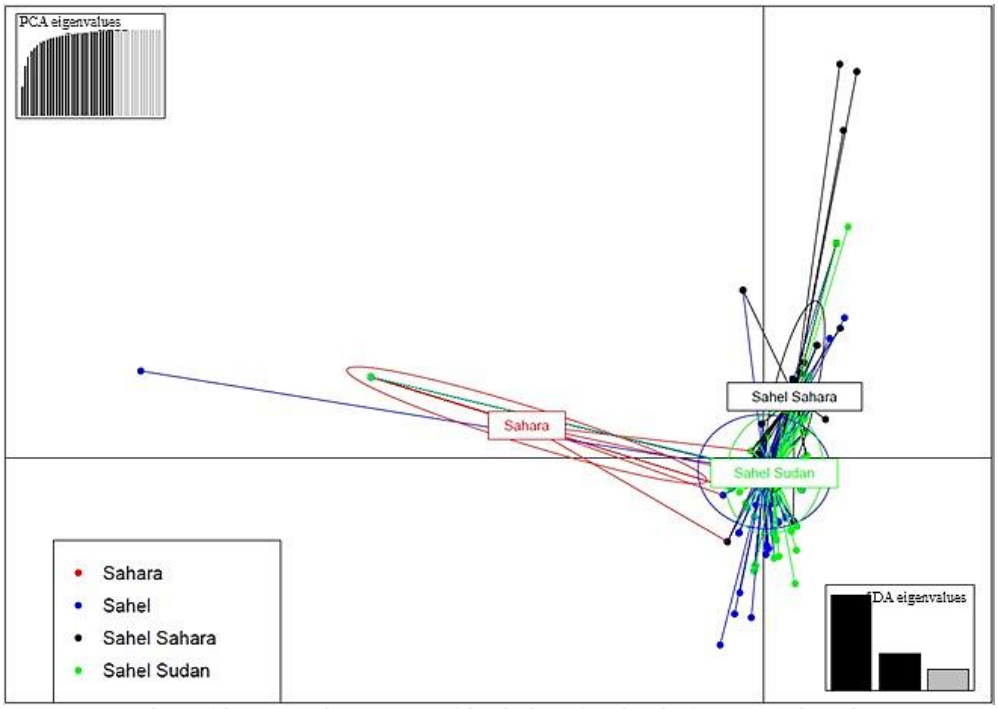


Figure 5. Discriminant analysis of the principal component of the chickens found in the four agroecological zones in Niger

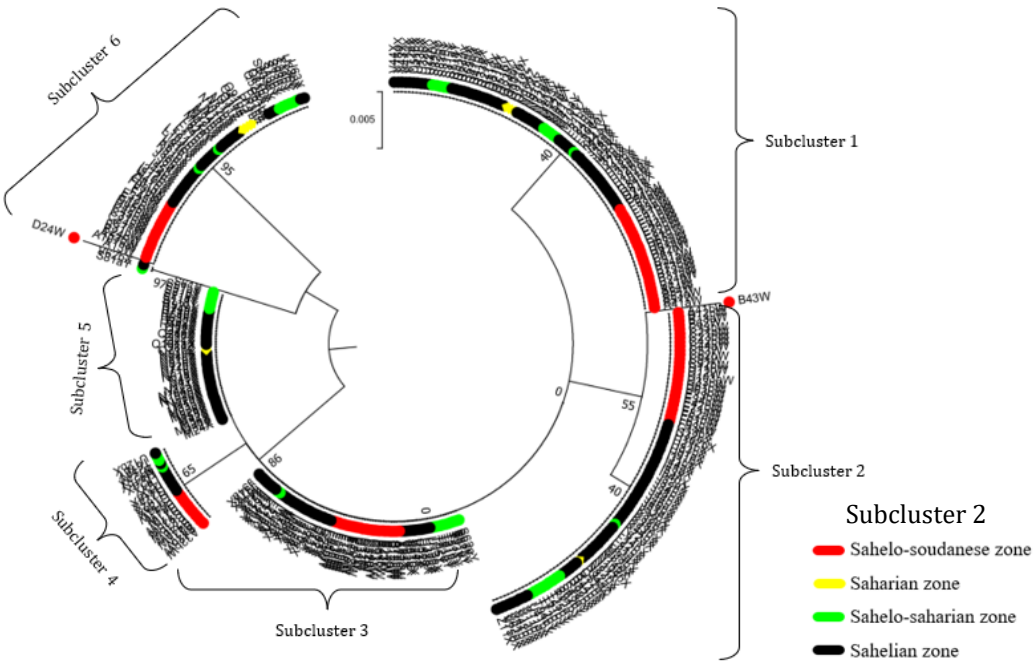


Figure 6. Neighbor-joining tree of the alleles defined for the VNTR LEI0258 of the local chicken population in Niger in 2021

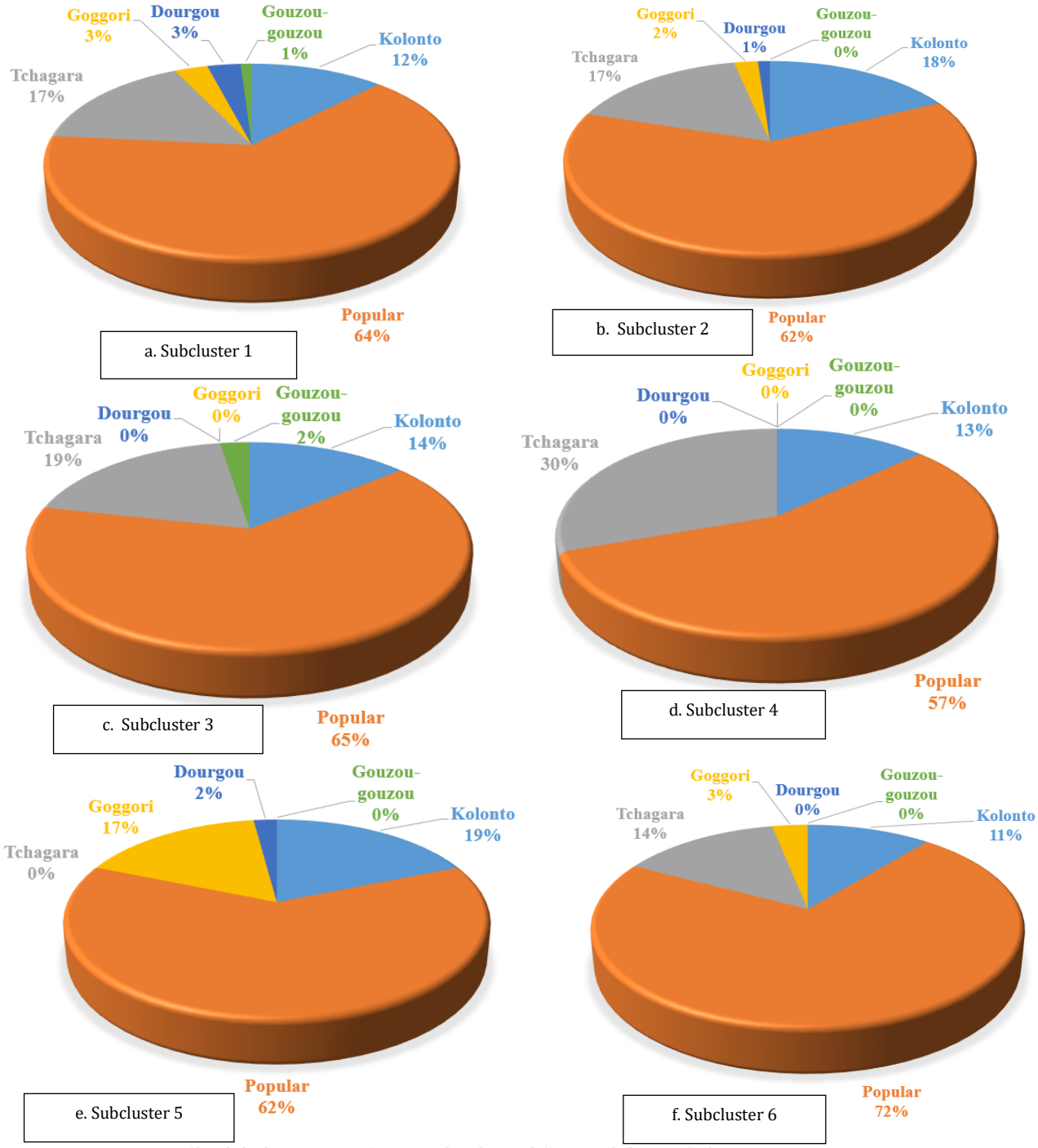


Figure 7. Repartition of local chicken ecotype of Niger within the six different subclusters (a-f)

ecotype exceeded the 50% rate in all subclusters. Gouzou-gouzou was only found in sub-clusters 1 and 3, while Dourgou was found in sub-clusters 1, 2, and 5. Goggori was mostly found in subcluster 5. Kolonto was in all subclusters. Subcluster 5 was the only one in which no Tchagara local chicken ecotype was found.

4. Discussion

4.1. LEI0258 allele

This study described 80 different alleles indicating the polymorphism of the LEI0258. In two Tanzanian chicken ecotypes, 22 and 23 alleles were found[16], 15 alleles were identified in Brazilian chicken, blue-egg caipira[40], 25 alleles in 3 Iranian indigenous chicken populations[41], 25 alleles were observed in 2 chicken breeds in Vietnam[42], 26 LEI0258 alleles, were detected in North American and European layer-type chickens[19], and 69 alleles found in 1617 individuals of 33 Chinese indigenous chicken strains[22].

Overall, 39 allele sizes were observed, ranging from 181 to 474bp. This finding is consistent with the previous report indicating the size of the allele ranged from 182 to 552 bp[19], or another study, which reported the size of the allele ranged from 181 to 552 bp[21].

This study revealed 22 new alleles and 39 private alleles. The alleles 206, 237, 259, 283, 331, 343, and 379 might be specific to Chinese indigenous chickens[22]. However, these alleles were found in the Nigerien chicken population. This means these alleles are not specific to Chinese indigenous chicken.

Nine polymorphisms were observed along the LEI0258 sequence, including three in the upstream, one in the repeat region (last R12), and five in the downstream. Moreover, 10 were observed in polymorphisms, 4 in the upstream and 6 in the downstream[21]. Compared to the result of this study, the difference in the upstream region is at position -2. The prevailing nucleotide at this position is typically C although it has the potential for substitution with nucleotide G. However, the findings of this study revealed that at this specific position, the nucleotide was consistently C.

Fulton et al.[19] also did not observe this polymorphism. Chazara et al.[21] did not detect polymorphism in the repeat region. The current study identified that the SNP Charaza reported at position '+3' in downstream was a SNP within the last R12. Thus, the result of this study is the same as Chazara et al.[21] and Mpenda et al.[12], who have also observed nine SNPs and Indels. Three SNPs and two Indels were detected downstream, while three SNPs and an Indel were detected upstream.

The number of repeats (R13 and R12) highly varied, as reported by Mpenda et al.[12], Mwambene et al.[24], and Chazara et al.[21] R13 appeared 1 to 22 times, whereas R12 appeared 2 to 20 times. This number of repeat regions is higher than what Mpenda et al.[12] have found but likely the same as that of Chazara et al.[21] and Mwambene et al.[24].

4.2. Association of LEI0258 microsatellite alleles with some traits

The microsatellite marker LEI0258 is physically located within the MHC, between the BG and BF regions on chromosome 16[20]. The association between different LEI0258 alleles and the performance was evaluated in previous studies. Studies have reported that the 307 allele was positively correlated with body weight traits[23,43]. This allele has been found in four types of local Nigerien chickens, namely Goggori, Kolonto, Tchagara, and Popular. Naturally, Kolonto and Goggori have high body weights compared to the other local Nigerien chickens. The fact that scavenging is the principal breeding system in Niger, the allele 307 has been shared with the other small body type of Nigerien local chicken such as Tchagara or Popular. The major MHC haplotypes are associated with variations in disease susceptibility and resistance[10,13,44,45]. The allele 357, which is strongly associated with Marek's disease resistance[19,46,47], is found in Gouzou-gouzou, Kolonto, Tchagara, and a popular type of Nigerian local chickens. This allele is found in Tanzanian and Chinese chickens[22,24] but was absent in chicken populations found in Brazil and Vietnam[40,42]. The allele 205 was correlated positively with antibody response to the vaccination of the Newcastle[16]. The allele 307 was negatively correlated with antibody response to the Newcastle vaccination. The allele 205 is mostly found in the Saharian zone of Niger and only found in the Tchagara and Popular chicken ecotypes of Niger.

4.3. Genetic diversity of major histocompatibility complex region

This study described the MHC region of six different types of Nigerien local chicken using microsatellite LEI0258. The number of alleles and observed and expected heterozygosity were considered to estimate genetic diversity. The Nigerian local chicken types (Popular, Tchagara, and Kolonto) were of high genetic variability. However, in the case of Gouzou-gouzou, Goggori, and Dourgou, the result was insignificant. This could be due to their sample size. Of note, these local chicken ecotypes are rare in the local chicken population in Niger. Local chickens are characterized by their high genetic diversity[23]. Therefore, the local chickens are adapted to harsh conditions. The exposure to numerous pathogens can explain their MHC high diversity. The heterozygosity of the MHC region may be linked to the environment in which the populations were bred and their selection history[23]. The polymorphism in local chickens' MHC locus is high compared to commercial breeds[7].

4.4. Distinctiveness and relationships of the chickens among regions

This study revealed that 97.7% of the observed genetic differentiation can be attributed to variations within the population. This implies that the overall genetic diversity across the four agroecological zones of Niger is primarily a result of individual heterogeneity rather than differences among populations. The study indicates a 2.7% level of genetic differentiation among populations. In comparison, Lyimo et al.[48] reported a 4.8% level of genetic differentiation among ecotypes in Tanzanian local chicken populations. It is important to note that the Tanzanian study employed 29 neutral microsatellite loci, whereas this study utilized the LEI0258 marker in Niger.

The phylogenetic tree defined two clusters with six non-homogeneous subclusters in which the different local chicken ecotypes were distributed. With the DAPC, the two clusters were confirmed. The chickens were not subclustering according to their agroecological zone of origin; they were randomly distributed across the four agroecological zones studied. This is due to the production system, indicating the prevalence of scavenging[49]. The popular ecotype is widespread and found throughout the country, contributing to its significant representation in the subclusters.

The genetic differentiation between the different types of Nigerien chicken population was low due to the small values of pairwise F_{ST}. Low genetic differentiation between chickens found in the agroecological zone can be due to the scavenging production system of the local chicken population in Niger[49].

Low genetic differentiation between chickens found in the agroecological zone can be attributed to high gene

flows, admixture, interbreeding in the study area, and sharing a common ancestry among individual chickens.

5. Conclusion

This study has evaluated genetic polymorphism in the Nigerien local chicken population by detecting the MHC region using LEI0258 microsatellite marker. It revealed high polymorphism and genetic diversity within the Nigerien local chicken MHC region. All the types of Nigerian local chicken have been described. A set of alleles is now available to identify and classify MHC genotypes and should be used for further studies on local chicken of Niger. The alleles 205, 307, and 357 should be considered in the cross-breeding plan. Unique alleles in this study should be considered in the improvement and conservation program. The function of many alleles that have been found is not well recognized. Finally, there is a need for further investigation to reveal the specific function of the dominant alleles (193, 295, and 309).

Declarations

Competing interest

The authors declare that they have no competing interests.

Authors' contribution

Roger Pelle supervised this study. He has played a pivotal role from the conceptualization of the project up to the manuscript redaction. Moussa Hassan Ousseini has collected data and worked in the laboratory with Machuka Eunice and Keambou Tiambo Chistian. In terms of methodology, all authors have contributed. Martina Kyallo and Jean-Baka Domelevo Entfellner played a significant role in data analysis. Regarding the draft preparation and subsequent revision, the authorship team worked together to ensure the quality and coherence of the manuscript. All authors checked and approved the final version of this manuscript.

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Availability of data and materials

The manuscript contains all datasets generated and/or analyzed in the current study, which are available from the corresponding author upon reasonable request.

Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by all the authors.

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