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Genetic Diversity and Structure of Local Chicken Populations Raised in Five Agroecological Zones of Togo

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ABSTRACT

Local chickens are the most commonly raised poultry breed in rural areas of Togo, where they help in alleviating poverty and food insecurity in households. The current study aimed to ensure the sustainable management of this genetic resource by evaluating the genetic diversity, phylogenetic relationships, and population structure of local chicken populations from five agroecological zones (Dry Savannah, Atakora, Forest, Wet Savannah, and Littoral) in Togo. Genotyping was carried out using 15 microsatellite markers on 30 unrelated individuals per agroecological zone. Genetic diversity was assessed by estimating the number of alleles per locus, observed heterozygosity, unbiased expected heterozygosity, and the polymorphic information content (PIC). The genetic structure of the populations was analyzed using a Bayesian-based approach. The results revealed a high genetic diversity but weak population structuring among local chickens. Moreover, 98 alleles were detected in all population groups, varying from 3 to 12 per locus, with an average of 6.53 ± 2.67 alleles per locus. The PIC values varied from 0.436 to 0.690, with an average of 0.550 ± 0.087. The mean number of alleles per population across all markers ranged from 4.4 ± 1.4 (Dry Savannah) to 5.4 ± 2.0 (Forest). The unbiased expected heterozygosity was high and varied from 0.58 ± 0.07 (Atakora) to 0.65 ± 0.11 (Forest), while that observed varied between 0.46 ± 0.09 (Dry Savannah) and 0.57 ± 0.14 (Forest). All populations deviated significantly from the Hardy-Weinberg equilibrium. Across populations, F_{IT}, F_{IS}, and F_{ST} fixation indices were 0.150, 0.132, and 0.021, respectively. The genetic distances were low and varied from 0.022 (between Atakora and Dry Savannah) to 0.045 (between Atakora and Forest). These results could be used in potential genetic improvement programs or the preservation of local chickens in Togo.

Keywords: Genetic diversity, Local chickens, Microsatellite markers, Heterozygosity, Togo

INTRODUCTION

Poultry plays a key role in developing countries by providing protein through meat and eggs (Moula et al., 2013). Domesticated chickens (*Gallus gallus domesticus*) are the main poultry genetic resources worldwide. In Sub-Saharan Africa, over 80% of the total chicken populations are local chickens (Ngeno et al., 2015). These local chickens reduce malnutrition and poverty (Osei-Amponsah et al., 2015). In Togo, local chicken is the most common poultry breed raised, particularly in rural areas, so it plays an important role in fighting against food insecurity in households and improves the livelihood of populations. The local chicken is found in all agroecological zones of Togo, where it is known to be well-adapted (Dao et al., 2015). To date, little is known about the genetic diversity of the breed.

One of the fundamental biological characteristics of local chickens is their rusticity since they are disease-resistant and better adapted to survive under harsh

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environmental conditions and poor rearing practices (Ben Larbi et al., 2018). According to Bakare et al. (2021), local chickens are good scavengers, efficient mothers, independent, resilient, and need little care to grow. Additionally, these authors reported that consumers prefer their products because of their taste, leanness, and suitability for special dishes.

However, the generalization of the use of commercial hybrids (resulting from terminal crosses) and uncontrolled crossbreeding to improve the productivity of the local chickens constitute a real threat of loss of their genetic originality in relation to their products' quality and their rusticity (Ben Larbi et al., 2018). Weigend et al. (2004) foresaw that this menace may lead to an unrecognized replacement of local genotypes with commercial hybrids, which have a higher production potential based on high nutrient requirements but are not selected for survival in such a harsh environment. According to Leroy et al. (2012), this situation is due to poor conservation strategies and a lack of incentives for a continued and sustainable use of local chicken populations. For these scientists, a perfect characterization of genetic structure and an assessment of the genetic diversity of local chicken populations are requisite for the development of conservation strategies.

The assessment of genetic diversity is a key step towards identifying and preserving valuable genetic resources to deal with changes in environmental conditions, changes in consumer preferences, and adaptation to different production practices (Suh et al., 2014). Genetic marker polymorphisms are a way of assessing diversities in chickens, and different genetic markers have been used. Microsatellites are markers that have been widely used in genetic diversity studies because of their codominance, availability throughout the genome, and high polymorphic nature (FAO, 2011; Suh et al., 2014). Microsatellite-based studies from Côte d'Ivoire (Loukou et al., 2009), Benin (Youssao et al., 2010), Ghana (Osei-Amponsah et al., 2010), and Burkina Faso (Yacouba et al., 2022) indicated high genetic variation within local chicken populations in these countries. However, studies on the characterization of local chickens in Togo are only phenotypic, including adult body phaneroptic and measurements (Dao et al., 2015). It is, therefore, important to assess the genetic diversity of these agroecologically adapted chicken populations using molecular biomarkers to offer insights into their improvements.

This study aimed to investigate the genetic diversity, phylogenetic relationships, and population structure of local chickens raised in the five agroecological zones of Togo using 15 microsatellite loci.

MATERIALS AND METHODS

Ethical approval

The authors confirm that the sampling procedures and the collection of blood samples for this study were performed in accordance with the guide for the care and use of agricultural animals in research (008/2021/BC-BPA/FDS-UL) edited by the Faculty of Sciences of the University of Lomé (Togo).

Study areas

The study covered the national territory of Togo, divided into five agroecological zones (Dao et al., 2015), including Dry Savannah, Atakora, Wet Savannah, Forest and Littoral (Figure 1).

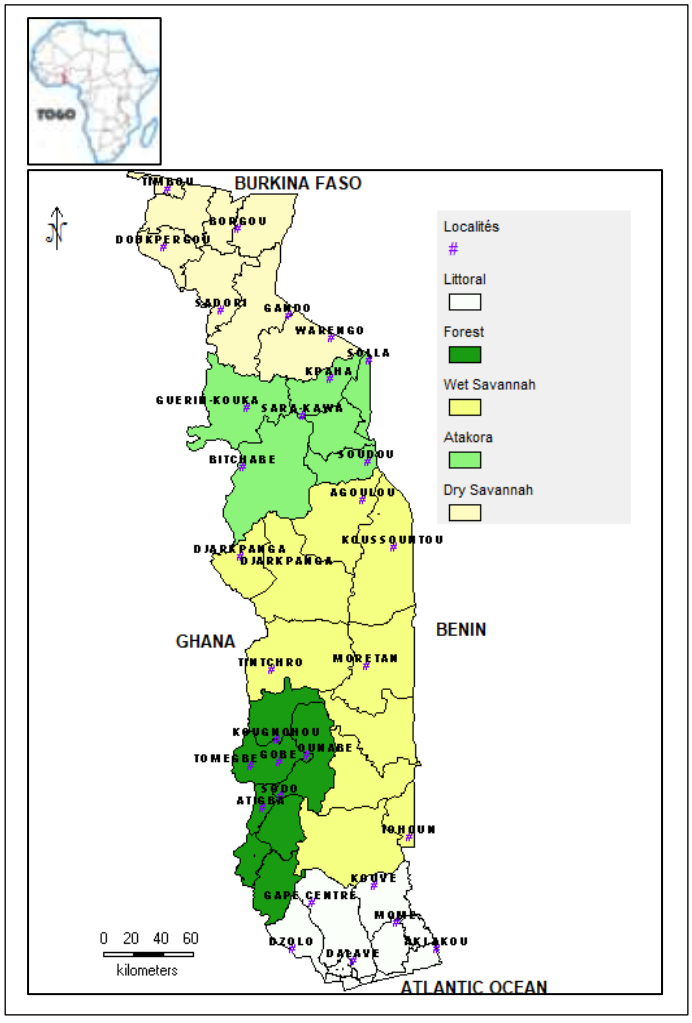


Figure 1. Map of Littoral, Forest, Wet Savannah, Atakora, and Dry Savannah agroecological zones of Togo with localities of sampled local chickens

The Dry Savannah zone is a lowland area in the

extreme north of Togo, whose flora is dominated by the Sudanian Savannah. The climate is Sudanese, with annual rainfall between 1000 and 1100 mm and the annual average temperature of 28.5°C.

The Atakora zone is the northern mountainous area with a studio-Guinean climate. The vegetation is made of a mosaic Savannah, forest with *Isoberlinia doka*, and compact dry forests. The annual rainfall is around 1300 mm, with a maximum in August-September when it rains every other day.

The Wet Savannah is a vast plain in the central and southeast of Togo whose flora consists of Guinean Savannah characterized by numerous fragments of compact forests. The area is characterized by a humid tropical climate with unimodal rainfall. The annual rainfall varies between 1200 and 1500 mm for a number of rainy days, reaching 120 days in the rainy season. The annual temperature variation is between 20 and 32 °C.

The Forest zone corresponds to the southwestern part of the mountains of Togo. The vegetation of the area is made of authentic semi-evergreen forests with a subequatorial climate characterized by bimodal rainfall. The annual average rainfall oscillates between 1500 and 1800 mm. Average annual temperatures are between 22 and 27 °C. The Forest zone is an area of excellence for producing coffee, cocoa, oil palm, plantain, avocado, and citrus fruits.

The Littoral zone corresponds to the coastal plain covered by a mosaic of semi-deciduous forests, Savannah, and grasslands. It is subject to a four-season subequatorial climate (two rainy seasons and two dry seasons) with total annual rainfall varying between 800 and 1200 mm from the south to the north of the area. For temperatures, the absolute maximum is between 32 and 35°C in February and the minimum of 21°C is recorded in the rainy season.

Blood sampling and DNA extraction

Blood samples were collected from 120 hens and 30 cocks of 8 to 10 months of age belonging to five agroecological zones of Togo. In each agroecological zone, 30 individuals of local chickens were sampled from six villages, keeping predominantly local chickens and located at least 20 km apart. Five chickens per village and only one chicken per household were randomly sampled to avoid including genetically related individuals. Approximatively, 2 ml of blood was collected per chicken by a puncture at the wing vein using 19G VENOJECT® needles into Vacutainer EDTA-containing tubes. Prior to DNA extraction, blood samples were stored at -20°C.

DNA was extracted from blood samples using the QIAGEN® kit (QIAGEN, Valencia, CA, USA) at the CIRDES genotyping platform in Bobo-Dioulasso (Burkina Faso). A NanoDrop Spectrophotometer (ThermoFisher Scientific™ Nanodrop 2000, Wilmington, USA) was used to quantify the total DNA extracted, which was stored at +4°C until DNA amplification by polymerase chain reaction (PCR) and genotyping.

Microsatellite genotyping

The DNA polymorphism was assessed using a set of 15 microsatellite loci which were included in previous studies (Loukou et al., 2009; Osei-Amponsah et al., 2010; Youssao et al., 2010; Yacouba et al., 2022) and part of the 30 ISAG-FAO recommended microsatellite markers (FAO, 2011) for chicken genetic diversity assessment. The names of the 15 microsatellite loci, their chromosomal location, and PCR conditions are presented in Table 1.

The PCR was performed in 15 µl reaction volume containing 8.1 µl of sterilized water, 1.6 µl of 10X PCR buffer, 1.6 µl of dNTPs (2.5mM), 0.8 µl of $MgCl_2$ (25mM), 0.2 µl FM13 (Forward) primer (Hillel et al., 2003), 0.3 µl of reverse primer (10 µM), 0.1 µl of Qiagen Taq DNA polymerase (5U/µl); 0.3 µl of fluorochrome dye (Dye 700), and finally 2 µl of DNA samples. The amplifications were performed using an automated thermal cycler (Applied Biosystems Veriti 96 Well, Thermal Cycler) and programmed for 1 cycle of an initial denaturation of DNA and enzyme activation step at 94°C (3 min), followed by 35 cycles consisting of denaturation at 94°C (30 seconds), primer annealing at temperature varying from 58 to 64°C (30 seconds), and extension at 72°C (45 seconds), then a final cycle of extension at 72°C (8 minutes).

The amplified products were then migrated using vertical high voltage electrophoresis (1,500 volts for 1 hour and 30 minutes) in an acrylamide gel on a Li-Cor® automated sequencer (DNA Analyzer Model 4300; LI-COR Biosciences-GmbH, Germany) following the manufacturer's procedures. The electrophoretic profiles were analyzed using SAGA^{GT} Generation 2.0 software to assess DNA polymorphism.

Table 1. Information on the 15 microsatellite loci analyzed in five local chicken populations of Togo

Microsatellite locus	Chromosomal position	Primer sequence (5' > 3') Forward Reverse	Annealing temperature	Alleles sizes (bp)
ADL0268	1	CTCCACCCCTCTCAGAACTA CAACTTCCCATCTACCTACT	60°C	102-116
ADL0278	8	CCAGCAGTCTACCTTCCTAT TGTCATCCAAGAACAGTGTG	60°C	114-126
MCW0034	2	TGCACGCACTTACATACTTAGAGA TGTCCTTCCAATTACATTCATGGG	60°C	212-246
CW0037	3	ACCGGTGCCATCAATTACCTATTA GAAAGCTCACATGACACTGCGAAA	64°C	154-160
MCW0067	10	GCACTACTGTGTGCTGCAGTTT GAGATGTAGTTGCCACATTCCGAC	60°C	176-186
MCW0069	*E60C04W23*	GCACTCGAGAAAACTTCCTGCG ATTGCTTCAGCAAGCATGGGAGGA	60°C	158-176
MCW0078	5	CCACACGGAGAGGAGAAGGTCT TAGCATATGAGTGTACTGAGCTTC	60°C	135-147
MCW0081	5	GTT GCTGAGAGCCTGGTGCAG CCTGTATGTGGAATTACTTCTC	60°C	112-135
MCW0111	1	GCTCCATGTGAAGTGGTTTA ATGTCCACTTGTCAATGATG	60°C	96-120
MCW0183	7	ATCCCAGTGTCGAGTATCCGA TGAGATTTACTGGAGCCTGCC	58°C	296-326
MCW0206	2	ACATCTAGAATTGACTGTTCAC CTTGACAGTGATGCATTAAATG	60°C	221-249
MCW0216	13	GGGTTTTACAGGATGGGACG AGTTTCACTCCCAGGGCTCG	60°C	139-149
MCW0222	3	GCAGTTACATTGAAATGATTCC TTCTCAAAACACCTAGAAGAC	60°C	220-226
MCW0295	4	ATCACTACAGAACACCCTCTC TATGTATGCACGCAGATATCC	60°C	88-106
MCW0330	17	TGGACCTCATCAGTCTGACAG AATGTTCTCATAGAGTTCCTGC	60°C	256-300

All primers of this table are used in the previous studies of Loukou et al. (2009), Osei-Amponsah et al. (2010), and Yacouba et al. (2022).

Genetic diversity estimates

Data generated were analysed using *CERVUS* version 3.0.7 computer program (Kalinowski et al., 2007) and *FSTAT* version 2.9.4 software (Goudet, 2003) to estimate the number of alleles detected, polymorphic information content (PIC, a measure of how a microsatellite locus is informative in relation to expected heterozygosity (Botstein et al., 1980) observed heterozygosity (H_O), unbiased expected heterozygosity (H_E). Moreover, *POPGENE* version 1.32 software (Yeh et al., 1999) was used to estimate F-statistics of Wright's (1978), number of successful migrants per generation (Nm) and significance of deviations from Hardy-Weinberg equilibrium for each of locus across the 5 local chicken populations. Deviations from Hardy-Weinberg equilibrium were assessed using a chi-square goodness-of-fit test. The test compares observed genotype frequencies with expected genotype frequencies calculated from alleles frequencies assuming Hardy-Weinberg equilibrium. Within-population genetic diversity, represented by mean number of alleles, private alleles, observed (H_O), and expected (H_E) heterozygosities and inbreeding coefficient (F_{IS}) for each population across loci was estimated using the *GENETIX* version 4.05 software (Belkhir et al., 2004). The Markov chain Monte Carlo (MCMC) algorithm (100 batches and 5,000 iterations per batch, dememorization step of 10,000) as implemented in *GENEPOP* version 4.7.5 software (Rousset, 2008) was used to test the deviations from Hardy-Weinberg equilibrium for each population across loci.

Genetic relationships and structure

For the estimation of the genetic relationships among the five chicken populations, Nei's D_A distances (Nei et al., 1983) between all pairs of populations were computed based on allele frequencies using the *POPTREE2* computer program package (Takezaki et al., 2010, Kagawa University, Japan). A phylogenetic tree based on D_A distances was constructed using the neighbour-joining method implemented in *MEGA* version 11 software

(Tamura et al., 2021). Furthermore, a bootstrap test (Felsenstein, 1985) with 1,000 resampling of loci was used to evaluate the phylogenetic tree robustness. Moreover, the *GENETIX* version 4.05 software (Belkhir et al., 2004) was used to perform Factorial correspondence analysis (FCA) in order to investigate the differentiation of the individuals within each population. A Bayesian approach, implemented in *STRUCTURE* version 2.3.4 software (Hubisz et al., 2009) was used to reveal probable clustering substructures. The analysis involved 20 independent runs for each number of clusters K (ranging from 2 to 10) with a burn-in period of 50,000 Markov Chain Monte Carlo iterations followed by 120,000 repeat numbers. The most likely number of clusters (K) was determined using the distribution of the ΔK statistic as described by Evanno et al. (2005) and implemented in *STRUCTURE HARVESTER* program (Earl and Vonholdt, 2012).

Statistical analysis

The significant level of deviations from Hardy-Weinberg equilibrium using a chi-square goodness-of-fit test was set as $p < 0.05$. The statistical significance of deviations from Hardy-Weinberg equilibrium based on the Markov chain Monte Carlo (MCMC) algorithm was set at a p value of 5%.

RESULTS

Microsatellite loci polymorphisms

A total of 98 alleles were identified in the 150 chickens assessed at 15 microsatellite loci. The number of alleles per locus across chicken populations varied from 3 (MCW0037) to 12 (MCW0069), with the mean number of alleles 6.53 ± 2.67 in all loci (Table 2). Out of the total alleles identified, 18 were considered private alleles, so they were observed in only one population.

The observed heterozygosity (H_O) mean value of 0.522 was lower than the expected heterozygosity (H_E) means value (0.616). The values of H_O ranged from 0.393 (ADL0278 and MCW0216) to 0.687 (MCW0034), while that of H_E varied from 0.516 (MCW0067 and MCW0078) to 0.733 (MCW0034).

The polymorphic information content (PIC) per locus ranged from 0.436 (MCW0078) to 0.690 (MCW0034), with an average of 0.550. A total of 67% of microsatellite loci had a PIC value above 0.5, indicating that they were highly informative.

The heterozygote deficiency (as determined by F_{IS} index) at the microsatellite locus level, extended between -0.036 (MCW0081) and 0.351 (ADL0278) with a mean of 0.132 for all loci. The global heterozygosity deficit of individuals within the overall populations (F_{IT}) per locus ranged from -0.019 (MCW0081) to 0.388 (ADL0278), and averaged at 0.150. The genetic differentiation among populations (evaluated by F_{ST}) estimates was 0.021 on average and varied from 0.006 (MCW0037 and MCW0330) to 0.056 (ADL0278). The average gene flow between populations, estimated by the number of migrants per generation (Nm) in the overall population and across the fifteen microsatellite loci, was 11.909. The chi-square goodness-of-fit test revealed that about two-thirds of microsatellite loci deviated significantly from Hardy-Weinberg equilibrium ($p < 0.05$).

Genetic diversity within populations

Within the populations, genetic diversity estimates are summarized in Table 3. The mean number of alleles for the overall chicken populations was 4.9 and varied from 4.4 in the Dry Savannah local chicken population to 5.4 in the Forest zone.

The mean H_O ranged from 0.464 (Dry Savannah) to 0.569 (Forest), while H_E ranged from 0.585 (Atakora) to 0.647 (Forest). The inbreeding coefficient (F_{IS}) values varied from 0.111 (Littoral) to 0.212 (Dry Savannah) and were different from 0 ($p < 0.05$), indicating a significant deficit of heterozygotes within the population.

Genetic relationship between populations

The genetic relationships between populations were estimated using the Neighbour-Joining method based on Nei's D_A genetic distances and FACA.

Based on Table 4, the matrix of pairwise genetic distances between populations showed a low genetic distance (0.022) between Atakora and Dry Savannah populations, and between Forest and Wet Savannah (0.025). The highest genetic distance was observed between the Forest and Dry Savannah populations (0.045). The phylogenetic relationship by the Neighbour-Joining tree based on D_A genetic distances showed three main branches (Figure 2). Unlike the other populations, the Littoral chicken population stands alone and constitutes the first main branch. The Forest and Wet Savannah chicken populations were found in the second major branch, while Atakora and Dry Savannah local chicken populations were grouped in the third main branch of the tree.

The FCA was performed using allele frequencies of the 15 microsatellite loci, as an alternative approach to understanding the genetic relationship among chicken populations. Figure 3 shows a weak differentiation

between the five local chicken populations. The three axes of the FCA explained 87.17% of the variability and distinguished three groups. Axis 1 separated two groups, including Group 1 (Atakora and Dry Savannah local chicken populations) and Group 2 of Wet Savannah, Forest and Littoral (costal) local chicken populations. Axis 2 isolated the Littoral local chicken population from the Wet Savannah and Forest local chicken populations.

Genetic structure

The most consistent gain in information was obtained with K: 3 (Figure 4). The STRUCTURE clustering was graphically illustrated in Figure 5, which displays the individual of each population as a vertical line partitioned into three colored segments that represent the individual's estimated membership coefficients in the three assumed clusters. The proportion of membership of each population in each of the three inferred clusters showed that apart from the Forest local chicken population, which individuals clustered fairly in the three clusters, the four other local chicken populations had more than 40% of their individuals clustered in the Cluster 3. The *STRUCTURE* analysis results revealed a low structuring in the local chicken populations studied.

Table 2. Number of alleles, polymorphic information content, observed and unbiased expected heterozygosities, Wright's F-statistics, gene flow and significance of deviation from Hardy-Weinberg equilibrium for each of the 15 microsatellite loci in 5 local chicken populations of Togo

Locus	Na	PIC	H_O	H_E	F_{IS}	F_{IT}	F_{ST}	Nm	HW
ADL268	5	0.597	0.500	0.657	0.224	0.237	0.016	15.07	***
ADL278	5	0.584	0.393	0.645	0.351	0.388	0.056	4.18	***
MCW034	11	0.690	0.687	0.733	0.015	0.060	0.046	5.24	**
MCW037	3	0.466	0.487	0.552	0.109	0.115	0.006	39.14	***
MCW067	5	0.443	0.453	0.516	0.099	0.118	0.021	11.43	ns
MCW069	12	0.562	0.580	0.629	0.064	0.075	0.011	22.13	ns
MCW078	5	0.436	0.487	0.516	0.042	0.054	0.012	19.92	ns
MCW081	6	0.512	0.593	0.584	-0.036	-0.019	0.016	15.72	ns
MCW111	8	0.640	0.513	0.691	0.241	0.255	0.019	12.95	***
MCW183	10	0.680	0.620	0.727	0.135	0.145	0.011	22.09	***
MCW206	7	0.659	0.640	0.711	0.087	0.097	0.011	21.79	***
MCW216	4	0.470	0.393	0.551	0.272	0.283	0.015	16.00	***
MCW222	4	0.515	0.480	0.590	0.171	0.184	0.017	14.93	***
MCW295	6	0.516	0.433	0.574	0.214	0.243	0.037	6.49	***
MCW330	7	0.477	0.567	0.559	-0.023	-0.016	0.006	39.20	ns
Mean	**6.5**	**0.550**	**0.522**	**0.616**	**0.132**	**0.150**	**0.021**	**11.91**	

Na: Number of alleles, PIC: Polymorphic information content, H_O: Observed heterozygosity, H_E: Unbiased expected heterozygosity, F_{IS}: Inbreeding coefficient within populations, F_{IT}: Inbreeding coefficient overall populations, F_{ST}: Inbreeding coefficient of differentiation among populations, Nm: Number of migrants, HW: Significance of deviation from Hardy-Weinberg equilibrium ($p < 0.05$), ns: Not significant, ** $p< 0.01$; *** $p< 0.001$

Table 3. Total, mean, and private number of alleles, observed and expected heterozygosity, and inbreeding coefficient of 5 local chicken populations in Togo

Population	Na	MNa	NPA	H_O	H_E	F_{IS}	HW
Atakora	73	4.87±1.92	1	0.482±0.150	0.585±0.073	0.178	***
Forest	81	5.40±2.03	6	0.569±0.139	0.647±0.105	0.123	**
Littoral	76	5.00±1.73	3	0.547±0.115	0.614±0.109	0.111	***
Wet Savannah	77	5.13±1.81	5	0.547±0.143	0.623±0.089	0.124	**
Dry Savannah	66	4.40±1.40	3	0.464±0.093	0.587±0.083	0.212	***
All	98	6.53±2.67	18	0.522±0.089	0.616±0.075	0.132	

Na: Number of alleles; MNa: Mean number of alleles; NPA: Number of private alleles; H_O: Observed heterozygosity; H_E: Expected heterozygosity; F_{IS}: Inbreeding coefficient within populations; HW: significance of deviation from Hardy-Weinberg equilibrium ($p < 0.05$); ** $p< 0.01$; *** $p< 0.001$

Table 4. Genetic distance between the five local chicken populations in Togo

	Atakora	Forest	Littoral	Wet Savannah	Dry Savannah
Atakora	-				
Forest	0.038	-			
Littoral	0.042	0.039	-		
Wet Savannah	0.032	0.025	0.032	-	
Dry Savannah	0.022	0.045	0.042	0.032	-

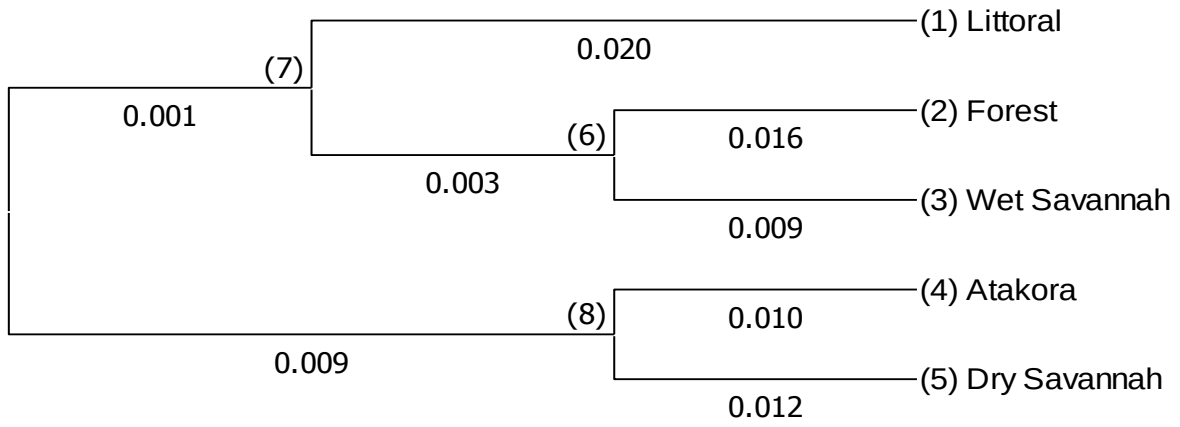


Figure 2. Neighbor-joining tree showing genetic relationships among local chicken populations in Togo based on D_A genetic distance

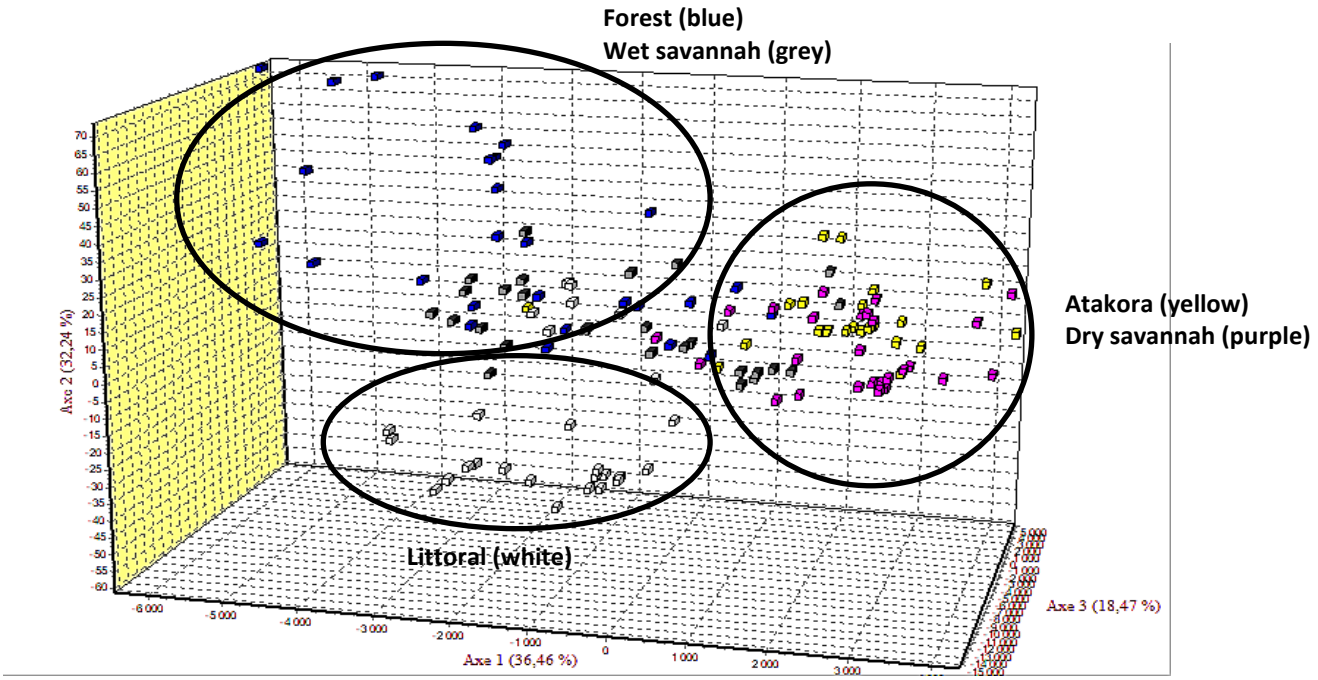


Figure 3. Clustering patterns of all individuals analysed using 15 microsatellite loci as revealed by factorial correspondence analysis (FCA) implemented in GENETIX 4.05. Yellow (Atakora), blue (Forest), white (Littoral), grey (Wet Savannah) and purple (Dry Savannah)

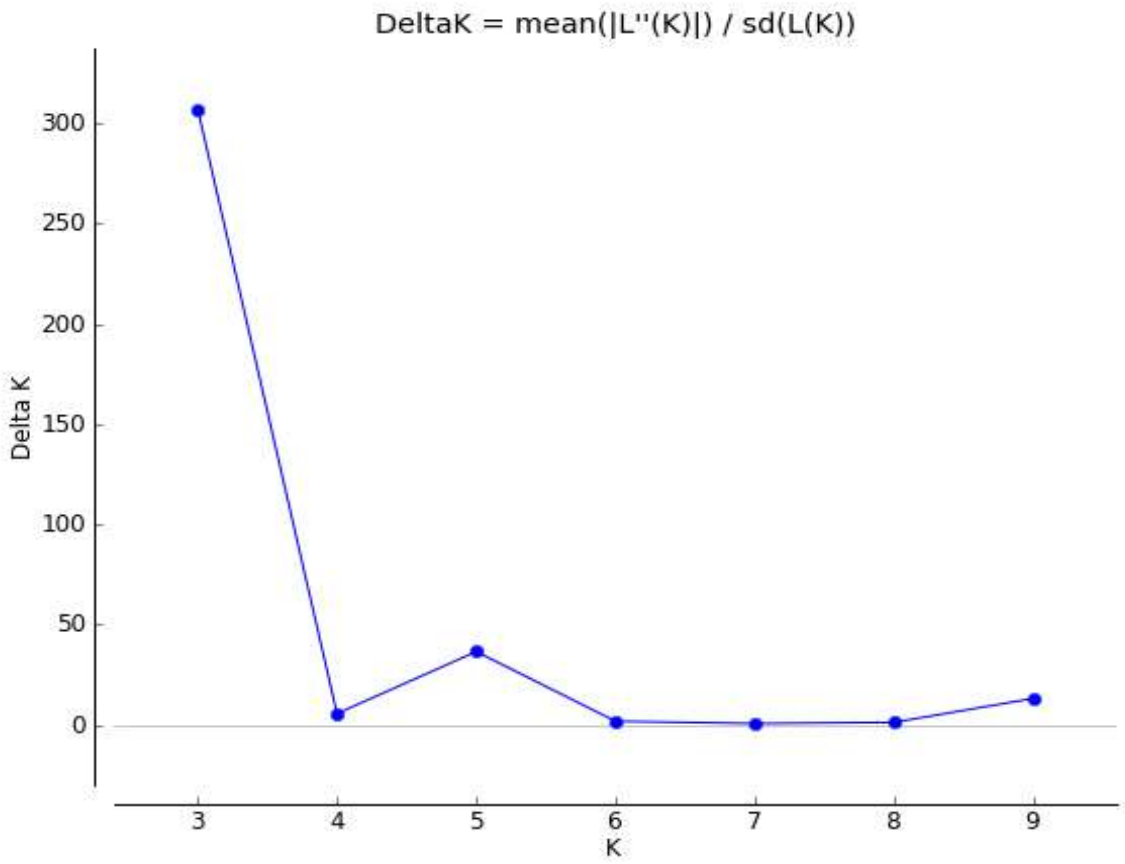
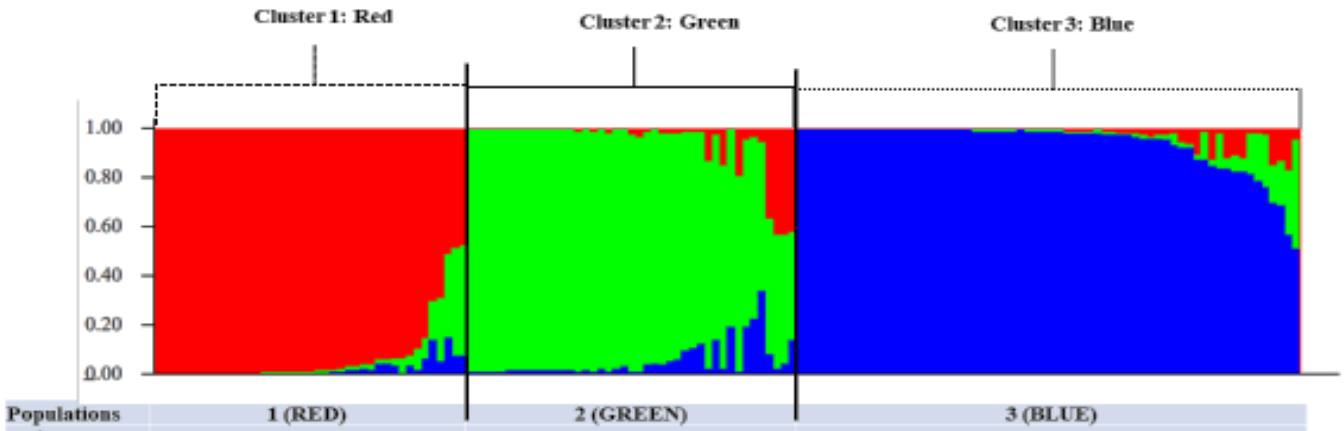


Figure 4 . Delta K values generated by *STRUCTURE HAVESTER* program estimating the most likely number of clusters of the five local chicken populations in Togo



Populations	1 (RED)	2 (GREEN)	3 (BLUE)
Atakora	27%	28%	45%
Forest	31%	32%	36%
Littoral	27%	27%	46%
Wet Savannah	25%	31%	44%
Dry Savannah	30%	24%	47%

Figure 5. Clustering diagram based on structure analysis of the five local chicken populations in Togo. Each of the 150 chickens is represented by a thin vertical line, which is divided into three colored segments that represent the individual's membership coefficients in the three assumed clusters. The percentages represent the proportion of individuals of each population in each cluster.

DISCUSSION

The number of alleles per microsatellite locus across all populations ranged from 3 (MCW037) to 12 (MCW069), with an average of 6.53. This result showed that the 15 loci were polymorphic in the local chicken populations of Togo. The present findings were similar to those found across indigenous chickens from agroecological zones of Côte d'Ivoire (Loukou et al., 2009), Forest and Savannah chicken populations of Ghana (Osei-Amponsah et al., 2010), Cameroon indigenous chickens (Fotsa et al., 2011) and in local chicken ecotypes in Burkina Faso (Yacouba et al., 2022). The allele numbers at loci ADL278 (05 alleles) and MCW183 (10 alleles) observed in Cameroon indigenous chicken ecotypes (Keambou et al., 2014) were similar to those obtained in the current study for the two loci.

The mean number of alleles per locus (MNA) obtained in the current study (6.53) was lower than the previous values reported by Mahammi et al. (2016) in Algeria (10.26), Soltan et al. (2018) in Egypt (9.10) and Habimana et al. (2020) in Rwanda (10.89). However, lower mean numbers of alleles per locus were observed in Benin (5.73-5.91) by Youssao et al. (2010), in Egypt (4.92) by Eltanany et al. (2011), in Sudan (5.3) by Berima et al. (2013) and in Tanzania (5.7) by Lyimo et al. (2013). Ramadan et al. (2012) and Yacouba et al. (2022) reported 6.9 in Egypt and 6.35 in Burkina Faso alleles per locus, respectively, which were closer to the values obtained in the present study. Such differences in the mean number of alleles per marker reported across studies could be attributed to the number of ecotypes/populations of studied chickens, the variation in the sample size, and the number and the loci used for the genotyping.

The polymorphic information content (PIC) is considered by Hubisz et al. (2009) as the best index for estimating the polymorphism of a locus, and based on PIC value. Botstein et al. (1980) classified loci as highly informative (PIC > 0.5), moderately informative (0.25 < PIC < 0.5), and slightly informative (PIC < 0.25). The mean PIC of the microsatellite loci used in the present study was 0.55, indicating their high informativeness and suitability for assessing local chicken populations' genetic diversity in Togo. The mean PIC obtained in the current study was higher as compared to the value reported by Osei-Amponsah et al. (2010) in Forest and Savannah chicken populations of Ghana (PIC = 0.460) but closer to the values reported by Keambou et al. (2014) in five Cameroon chicken populations (PIC = 0.57) and Yacouba et al. (2022) in four Burkina Faso local chicken ecotypes (PIC = 0.541). However, higher mean PIC values were reported by Olowofeso et al. (2016) in three Nigerian chicken populations (0.70) and Rashid et al. (2020) in Bangladeshi native chicken populations (0.7489).

The mean F_{IS} value was 0.132, which was lower than previously reported in Ghana and Egypt (Osei-Amponsah et al., 2010; Ramadan et al., 2012). This result indicated a deficit of heterozygotes. Ten loci out of the 15 used deviated significantly from the Hardy-Weinberg equilibrium. Clementino et al. (2010) and Dorji et al. (2012) reported lower percentages of Hardy-Weinberg equilibrium deviation (44%) and (40%), respectively, in Brazilian chicken ecotypes and native chickens from Bhutan. These differences can be attributed to the farming systems of the local chicken populations in different countries. In the current study zone, mating is random, the proportion of males is lower than that of females, and the roosters remain long in the production systems.

Heterozygosity is one of the basic measures of genetic diversity. The current study indicated that the mean observed heterozygosity (H_O) per chicken population varied from 0.464 to 0.569 with an overall mean value of 0.522, while the unbiased expected heterozygosity (H_E) ranged from 0.585 to 0.647 with an overall average of 0.616, indicating that there is high genetic diversity in the studied local chickens. These results were closer to those reported in local chickens from Cameroon (H_E = 0.65) by Keambou et al. (2014) but lower than the values (H_O: 0.71-0.88 and H_E: 0.47-0.85) reported in Korean native chicken lines by Seo et al. (2013). In contrast, heterozygosity values were higher than those reported by Okumu et al. (2017) in Kenya (H_E = 0.40) with 18 highly polymorphic microsatellite loci and by Yacouba et al. (2022) in Burkina Faso (H_O = 0.391, H_E= 0.539) using 20 polymorphic microsatellite loci. The differences in observed and expected heterozygosity values between studies can be linked to the population structure, characteristics, and the number of microsatellite loci used in the studies. Furthermore, the expected heterozygosity values were higher than those observed in the five local chicken populations, leading to positive F_{IS} values. The positive value of the F_{IS}, indicating heterozygote deficiency in the five local chicken populations, could be due to the population substructure created by clusters of households during the stratified sampling, which could bring the Wahlund effect (Samaraweera et al., 2021). Generally, there are no controls on mating, and very few roosters in village

poultry flocks. This can lead to inbreeding and heterozygote deficiency.

The genetic distances (from 0.022 to 0.045) estimated between studied chicken populations were low, indicating that these populations are not genetically isolated from each other. These estimated genetic distances were much lower as compared to the values obtained in five chicken populations of Bangladesh (0.29 to 0.58) using 16 polymorphic microsatellite loci (Abdur Rashid et al., 2020) and in five Korean native chicken lines (0.08 to 0.17) using 15 microsatellite loci (Seo et al., 2013). Also, the global population differentiation (F_{ST}) was very low (0.021), indicating that only 2.1% of total genetic variation was due to population variation versus 97.9% due to the genetic variation within- populations. These results indicate that within-population variation is the main source of genetic diversity in the local chicken populations of Togo. The results also highlight a high level of gene flow among chicken populations between the different agroecological zones of Togo.

Based on DA genetic distances, the phylogenetic relationship observed on the Neighbour-Joining tree showed three main branches. The clustering patterns of all individuals, as revealed by FCA, confirmed the Neighbour-Joining results within one group, the Atakora and Dry Savannah chicken populations, in the second group, the Wet Savannah and Forest chicken populations, while the Littoral (coastal zone) chicken population clustered in a third group. These groups were so close and revealed little genetic differentiation among local chicken populations studied which can be explained by the large number of common alleles brought out by different individuals due to the important gene flow between them. The little genetic differentiation revealed by FCA was supported by the structure analysis. these results suggest a very mild population sub-structuring among the five populations of chickens examined in this study. This indicates that the chickens from these populations share a highly mixed and admixed genetic background. The low genetic differentiation observed in the present study is certainly due to the uncontrolled migration of chickens from one agroecological zone to another through the live animals or eggs sharing, thus favoring a permanent gene flow. This result is in accordance with Mtileni et al. (2011), who reported that large effective population sizes as well as continuous gene flow may be one of the forces responsible for the lack of population differentiation among local South –African chicken genotypes in their studies. In addition, the traditional rearing system used in the studied zones allows gene flow between the chicken populations. A similar observation was reported in Burkina Faso by Yacouba et al. (2022). These authors did not observe a sub-structuring in four Burkina Faso local chicken ecotypes using 20 microsatellite loci. Berima et al. (2013) also reported an absence of sub-structuring in five Sudanese native chicken breeds using 29 microsatellite loci. This absence or low sub-structuring in the local chicken ecotypes/breeds reported in most African countries could be due to a lack of genetic improvement programs concerning this poultry species. The advantage here is the high genetic variability observed in the local chicken populations, but the disadvantage is that, without a proper breeding program, uncontrolled crossbreeding will continue with a risk of losing some adaptive traits.

CONCLUSION

Fifteen microsatellite loci were used for genotyping local chicken from Dry Savannah, Atakora, Wet Savannah, Forest, and Littoral agroecological zones of Togo. The results indicated high genetic diversity in the studied local chickens. The differences between agroecological chicken populations account for a small fraction (2.1%) of the total genetic variation. The analysis of the local chicken populations' genetic structure showed a low genetic structure among the agroecological chicken populations. Present results suggest that there is no specific and isolated genetic group in the local chicken populations raised in the study area. This study offers crucial details on the genetic makeup of indigenous chickens in Togo that could be applied to preservation or improvement efforts for the species. Prior to preservation or improvement, the growth performances and the reproductive potentialities must be evaluated in a controlled environment with the aim to identify any valuable chickens among local ecotypes.

DECLARATION

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Authors' contribution

Kossoga Kakom Assota contributed to the conceptualization of the protocol, data collection, data analysis, and manuscript drafting. Dayo Guiguigbaza-Kossigan contributed to the conceptualization of the protocol, data analysis, and revising the manuscript.

Bilalissi Abidi N'nanle Ombortime and Oke O. Emmanuel contributed to revising the manuscript. Tete-Benissan Amivi Kafui contributed to the supervision of the data collection and analysis and to the revising of the manuscript. All authors read and approved the final manuscript.

Ethical consideration

The authors have made sure that the manuscript complies with the journal's ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) for submission and publication.

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Availability of data and materials

The corresponding author can provide all the related data of this study upon a reasonable request.

Competing of interests

The authors reported no conflict of interest regarding the publication of this manuscript.

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