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Genetic Diversity and Population Structure of Local Chicken in Burkina Faso

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ABSTRACT

Poultry farming plays a vital role in rural livelihoods and is a primary source of animal protein in Burkina Faso. However, the increasing introduction of fast-growing exotic breeds threatens the genetic integrity of local chicken populations. This study aimed to assess the genetic diversity and structure of local chickens using 27 microsatellite markers, of which 23 were selected and 19 polymorphic markers were retained for analysis. A total of 116 individuals from local populations were genotyped, along with 20 individuals of the exotic broiler breed Cobb 500 for comparison. Genetic diversity, Hardy-Weinberg equilibrium, genetic distances, and population structure were evaluated using Bayesian and multivariate approaches. Results showed that 19 loci were polymorphic, revealing high allelic diversity with 117 alleles and a mean of 6.16 alleles per locus. The overall heterozygosity deficit (FIS) was low (0.04). Genetic differentiation among all populations was moderate (F_{ST} = 0.10), with higher differentiation between local populations and the exotic breed (F_{ST} = 0.19) and very low differentiation among local subpopulations (maximum F_{ST} = 0.04). Phylogenetic and Bayesian analyses indicated high admixture among local chickens, suggesting a largely homogeneous genetic structure, although the Sahelian population appeared slightly distinct. Evidence of introgression from exotic breeds was also observed, particularly in the Sudanian and Sudano-Sahelian zones. In conclusion, the local chickens of Burkina Faso form a single, weakly structured genetic pool characterized by high allelic diversity and a low heterozygosity deficit.

Keywords: diversity; structure; microsatellite; chickens; Burkina Faso

INTRODUCTION

Indigenous chickens play a major socio-economic role in low- and middle-income countries by contributing to household income, food security, and nutrition, particularly in rural communities. In sub-Saharan Africa, village chicken production systems remain essential because they require low inputs and are well adapted to local environmental conditions, including high temperatures, disease pressure, and feed scarcity (Mogano *et al.*, 2024). In Burkina Faso, poultry production represents one of the most accessible livestock activities for rural households and contributes substantially to animal protein supply and poverty alleviation. Recent national statistics estimated the poultry population at more than 44 million birds, of which approximately 90% belong to traditional village production systems dominated by indigenous chickens (MRAH/DGESS, 2023). Despite their adaptive advantages, local chicken populations in Burkina Faso face increasing genetic pressure due to the rapid dissemination of exotic commercial breeds and uncontrolled crossbreeding practices. Improved breeds are increasingly introduced to enhance meat and egg production; however, continuous introgression may progressively erode the unique genetic resources

of indigenous populations. Similar concerns have been reported in several African countries, where genomic studies have revealed substantial gene flow and introgression between indigenous and exotic poultry populations, potentially affecting the conservation of locally adapted genetic resources (Okumu *et al*., 2017; Lawal *et al*., 2018; Lawal *et al*., 2021). Indigenous chickens possess valuable adaptive traits, including tolerance to harsh tropical environments, scavenging ability, and resistance to endemic diseases, making their conservation strategically important for sustainable livestock production under climate change conditions (Gheyas *et al.,* 2021). The characterization of animal genetic resources is therefore considered a priority for the development of sustainable breeding and conservation strategies. Leroy *et al*. (2018) reported that the diversity of animal genetic resources underpins a broad range of ecosystem services, underlining the need to characterize and conserve indigenous livestock populations. Advances in molecular genetics have considerably improved the assessment of genetic diversity and population structure in poultry populations. Although single nucleotide polymorphism (SNP) technologies and whole-genome sequencing are increasingly used in population genomics studies (Zhi *et al*., 2023), microsatellite markers remain highly informative and cost-effective tools for evaluating genetic diversity, population differentiation, and introgression patterns, particularly in developing-country contexts (Samaraweera *et al*., 2021; Zhuang *et al*., 2023). Several studies have investigated the genetic diversity of indigenous chickens in Africa and Asia using microsatellite and genomic approaches, revealing substantial within-population diversity but generally weak population structuring associated with extensive gene flow and traditional free-ranging management systems (Fathi *et al*., 2017; Mwambene *et al*., 2019; Nxumalo *et al*., 2020; Samaraweera *et al*., 2021; Xu *et al*., 2023). In Burkina Faso, previous investigations mainly focused on phenotypic and morphobiometric characterization of indigenous chickens (Pindé *et al*., 2020), while molecular characterization remains relatively limited. Previous molecular studies on indigenous chickens in Burkina Faso mainly focused on selected ecotypes and revealed substantial genetic diversity with weak population structuring (Yacouba *et al*., 2022). That study focused on specific ecotypes from selected regions; however, comprehensive assessments integrating all major agro-ecological zones of Burkina Faso and explicitly evaluating potential introgression from commercial breeds remain limited. The present study differs from former studies in several key aspects: (i) it encompasses populations from three distinct agro-ecological zones using a broader geographic sampling framework covering six regions and 57 villages; (ii) it explicitly includes a commercial exotic breed (Cobb 500) as a reference population to quantify introgression levels; and (iii) it applies additional multivariate approaches to resolve population structure. Moreover, beyond cost considerations, microsatellite markers are highly informative, technically accessible with standard PCR equipment, require no specialized bioinformatics pipelines, and are endorsed by the FAO for genetic characterization of livestock in low-resource settings (FAO, 2007). Understanding the genetic structure and diversity of indigenous chickens is essential for designing breeding programs, conserving adaptive genetic resources, and limiting uncontrolled genetic erosion. Therefore, the present study aimed to evaluate the genetic diversity and population structure of local chicken populations from different agro-ecological zones of Burkina Faso using microsatellite markers and to assess their phylogenetic relationships and potential introgression with exotic commercial chickens.

MATERIALS AND METHODS

Sampling Sites

The present study was conducted in Burkina Faso between July 2023 and October 2025. It encompassed six regions: Centre, Centre-East, Centre-North, Central Plateau, Hauts-Bassins, and Sahel. In each region, one province was selected. Across the six provinces, surveys were carried out in 11 municipalities covering a total of 57 villages.

Biological Material

The survey conducted in the present study focused on the subspecies *Gallus gallus domesticus*, primarily on the local chicken population of Burkina Faso and on the exotic broiler breed "Cobb 500". The selection of the subspecies *Gallus gallus domesticus* was based on its predominance in rural poultry production systems, whereas Cobb 500 is mainly used in peri-urban poultry farms. Regarding the local chicken population of Burkina Faso, the different breeds or strains it may comprise are insufficiently described and inventoried within the country. This species therefore constitutes an appropriate model for a genetic characterization study. The biological material consisted of local breed chickens with a minimum estimated reproductive age of six months and the exotic "Cobb500" breed, with an estimated age of 35 days. Age estimation of the chickens was provided by the farmers themselves, based on their knowledge of their flocks.

Sample Collections

The study focused on traditional poultry production systems. Breeders were selected based on two criteria: a minimum flock size of three reproductive-age birds and sufficient geographic distance between farms (at least 1 km) to limit animal exchanges. All birds within each flock, including hens, pullets, roosters, and cockerels, were considered. According to these conditions, 10 to 15 breeders per municipality were chosen by the heads of the Livestock Technical Support Units (ZATE). At the time of the survey, each breeder owned at least three adult birds and was interviewed using a structured questionnaire.

Blood samples were collected from 325 unrelated local individuals and 20 exotic Cobb 500 birds using 3

mL syringes and 5 mL tubes containing EDTA. Due to financial constraints limiting laboratory genotyping costs, a subset of 116 individuals (96 local chickens distributed proportionally across the three agro-ecological zones and 20 exotic Cobb 500 birds) was selected for genotyping. Between 2 and 3 ml of blood per bird was obtained via brachial vein puncture. To avoid related sampling and ensure genetic diversity, a maximum of three local hens per flock were sampled. Additionally, 20 hens of the exotic Cobb 500 breed were collected from one farm. Samples were stored at +4 °C until DNA extraction.

DNA Extraction

Genomic DNA was extracted from whole blood using the MasterPure DNA Purification Kit (Biozym Illumina Inc., USA), following the manufacturer's instructions. Extracted DNA was stored at + 4 °C until polymerase chain reaction (PCR) amplification.

PCR Amplification and Genotyping

A conventional PCR was performed using 23 microsatellite markers selected from 27 initially screened. Of these, four were excluded due to monomorphism or amplification failure, and 19 polymorphic markers were retained for analysis. The microsatellites were labeled with one of three fluorescent dyes (FAM, HEX, or ATTO550). PCR conditions consisted of an initial denaturation at 95 °C for 15 minutes, followed by 40 cycles of denaturation at 95 °C for 50 seconds, primer annealing for 50 seconds at 53, 55, 57, 58, or 60 °C (depending on the optimal annealing temperature of each marker), and extension at 72 °C for 1 minute, with a final extension step of 10 minutes at 72 °C.

PCR products were subjected to electrophoresis following multiplexing on an automated DNA analyzer (SeqStudio, Applied Biosystems, Thermo Fisher Scientific), using LIZ600 (Applied Biosystems, Thermo Fisher Scientific) as the internal size standard. The 23 microsatellite loci were multiplexed into five panels for genotyping, as indicated in Table 1. Genotypes were subsequently scored using GeneMapper v4.1 software (Applied Biosystems, USA).

Data Analysis

The software Micro-Checker version 2.2.3 (Oosterhout *et al*., 2004) was used to detect and account for null alleles. Genetic diversity indices, including the mean number of observed alleles (Na), observed heterozygosity (Ho), expected heterozygosity (He), and Wright's F-statistics, were calculated using MICROSATELLITE ANALYZER (MSA) version 3.15 (Dieringer & Schlötterer, 2003). Exact tests for heterozygote excess and deficit relative to Hardy-Weinberg equilibrium were performed using GENEPOP version 1.2 (Raymond & Rousset, 1995). Polymorphism Information Content (PIC) was estimated using CERVUS (Kalinowski *et al*., 2007). The Estimated Heterozygosity deficit (FIS) values per locus across all populations were determined using the jackknife method over loci, and confidence intervals were estimated by bootstrapping over loci. The significance of FIS values was inferred based on 95% confidence intervals. The FIS of Weir and Cockerham (1984) was calculated using FSTAT version 2.9.3.2 (Goudet, 2002). The Fixation Index (F_{ST}) values were estimated per locus across all populations using FSTAT version 2.9.3.2 (Goudet, 2002), applying the jackknife procedure over loci and bootstrapping to generate 95% confidence intervals. Statistical significance was inferred from the 95% confidence intervals. The Overall genetic variation (FIT) values were also calculated per locus across all populations using FSTAT version 2.9.3.2 (Goudet, 2002), with jackknife and bootstrap procedures over loci, and significance was assessed using 95% confidence intervals. The analysis of molecular variance (AMOVA) was performed using Arlequin v.3.5 (Excoffier & Lischer, 2010). The significance of the variance components was assessed using 10,000 permutations (α = 0.05). The proportions of shared alleles among individuals and among populations, calculated using Microsatellite Analyzer (MSA) version 3.15 (Dieringer & Schlötterer, 2003), were used to construct phylogenetic trees of individuals and populations based on the Neighbor-Joining (NJ) method implemented in PHYLIP version 3.5 (Felsenstein, 1993). The robustness of tree nodes was assessed by bootstrap analysis with 1,000 resampling iterations. Dendrograms were visualized using MEGA version X (Kumar *et al*., 2018). To investigate the occurrence of independent genetic clusters (K) and to assign each individual to the population in which its genotype had the highest probability of occurrence, Structure version 2.3.4 (Pritchard *et al*., 2000) was used. This program applies a Bayesian Markov Chain Monte Carlo (MCMC) approach based on a clustering model to infer population genetic structure and to assess individual assignment to populations according to a membership probability (Q), representing the proportion of ancestry from inferred clusters. Results generated by STRUCTURE were analyzed using Structure Harvester (Earl & VonHoldt, 2012) to determine the most likely number of genetic clusters (K) based on the log-likelihood method L(K) and the Evanno method through the modal distribution of ΔK values (Evanno *et al*., 2005).

RESULTS

Biodiversity and Inbreeding Estimates

Of the 23 microsatellite markers initially screened, 19 were polymorphic and retained for analysis, generating 2,204 genotypes across 116 individuals. A total of 117 alleles were detected (Table 2). The Sudano-Sahelian subpopulation (SDS) showed the highest allelic count (83), followed by the Sudanian (77) and Sahelian (75) groups, while the exotic population (EXO) had the lowest (70). Across loci, allele numbers (Na) ranged from 2 (MCW0098) to 13 (MCW0034 and LEI0192), with a mean of 6.16 per locus. Mean number of alleles per population varied slightly among zones (SDS: 4.37;

Table 1. List and characteristics of the markers used to genotype the local chicken (*Gallus gallus domesticus*) populations of Burkina Faso and the exotic Cobb 500 breed

Panel no	Locus	Primer sequences	Fluorescent dye	Allele size range	Annealing temperature
1	ADL0112-F	GGCTTAAGCTGACCCATTAT	HEX	120-134	58 °C
1	ADL0112-R	ATCTCAAATGTAATGCGTGC			
1	MCW0016-F	ATGGCGCAGAAGGCAAAGCGATAT	ATTO550	120-180	60 °C
1	MCW0016-R	TGGCTTCTGAAGCAGTTGCTATGG			
1	MCW0034-F	TGCACGCACTTACATACTTAGAGA	FAM	211-247	60 °C
1	MCW0034-R	TGTCCTTCCAATTACATTCATGGG			
1	MCW0295-F	ATCACTACAGAACACCCTCTC	FAM	81-109	62 °C
1	MCW0295-R	TATGTATGCACGCAGATATCC			
1	MCW0330-F	TGGACCTCATCAGTCTGACAG	HEX	246-300	64 °C
1	MCW0330-R	AATGTTCTCATAGAGTTCCTGC			
2	LEI0094-F	GATCTCACCAGTATGAGCTGC	HEX	247-287	62 °C
2	LEI0094-R	TCTCACACTGTAACACAGTGC			
2	MCW0014-F	TATTGGCTCTAGGAACTGTC	ATTO550	165-189	62 °C
2	MCW0014-R	GAAATGAAGGTAAGACTAGC			
2	MCW0078-F	CCACACGGAGAGGAGAAGGTCT	HEX	135-147	62 °C
2	MCW0078-R	TAGCATATGAGTGTACTGAGCTTC			
2	MCW0111-F	GCTCCATGTGAAGTGGTTTA	FAM	96-120	60 °C
2	MCW0111-R	ATGTCCACTTGTCAATGATG			
3	ADL0278-F	CCAGCAGTCTACCTTCCTAT	FAM	99-127	58 °C
3	ADL0278-R	TGTCATCCAAGAACAGTGTG			
3	MCW0067-F	GCACTACTGTGTGCTGCAGTTT	ATTO550	174-186	60 °C
3	MCW0067-R	GAGATGTAGTTGCCACATTCCGAC			
3	MCW0098-F	GGCTGCTTTGTGCTCTTCTCG	HEX	255-265	60 °C
3	MCW0098-R	CGATGGTCGTAATTCTCACGT			
3	MCW0206-F	CTTGACAGTGATGCATTAAATG	FAM	221-249	58 °C
3	MCW0206-R	ACATCTAGAATTGACTGTTCAC			
3	MCW0216-F	GGGTTTTACAGGATGGGACG	HEX	136-150	60 °C
3	MCW0216-R	AGTTTCACTCCCAGGGCTCG			
4	ADL0268-F	CTCCACCCCTCTCAGAACTA	FAM	102-116	60 °C
4	ADL0268-R	CAACTTCCCATCTACCTACT			
4	MCW0037-F	ACCGGTGCCATCAATTACCTATTA	HEX	152-160	64 °C
4	MCW0037-R	GAAAGCTCACATGACACTGCGAAA			
4	MCW0103-F	AACTGCGTTGAGAGTGAATGC	HEX	264-276	62 °C
4	MCW0103-R	TTTCCTAACTGGATGCTTCTG			
4	MCW0104-F	TAGCACAACTCAAGCTGTGAG	ATTO550	190-234	64 °C
4	MCW0104-R	AGACTTGCACAGCTGTGTACC			
5	LEI0192-F	TGCCAGAGCTTCAGTCTGT	FAM	244-370	62 °C
5	LEI0192-R	GTCATTACTGTTATGTTTATTGC			
5	MCW0069-F	GCACTCGAGAAAACTTCCTGCG	HEX	155-177	62 °C
5	MCW0069-R	ATTGCTTCAGCAAGCATGGGAGGA			
5	MCW0081-F	GTTGCTGAGAGCCTGGTGCAG	FAM	107-135	60 °C
5	MCW0081-R	CCTGTATGTGGAATTACTTCTC			
5	MCW0183-F	ATCCCAGTGTCGAGTATCCGA	HEX	292-326	58 °C
5	MCW0183-R	TGAGATTTACTGGAGCCTGCC			

SD: 4.05; SHL: 3.95; EXO: 3.68). Observed heterozygosity (Ho) ranged from 0.192 (MCW0248) to 0.686 (ADL0278) with a mean of 0.484, while expected heterozygosity (He) varied from 0.180 (MCW0248) to 0.702 (MCW0111) with a mean of 0.545. The mean PIC was 0.486, with 42.1% of loci below 0.50. Allelic richness (Rt) ranged from 1.52 (MCW0248) to 3.18 (LEI0192), averaging 2.51 alleles per locus.

Among the 76 locus x population combinations analyzed, 36.84% showed significant deviation ($p<0.05$) from Hardy–Weinberg equilibrium due to heterozygote deficiency, while 5.26% deviated due to heterozygote excess. No significant deficiency-related deviation was observed in the SHL subpopulation. In contrast, four (04) loci showed heterozygote deficiency in the SDS subpopulation (MCW0034, MCW0330, MCW0206, MCW0081) and four (04) in the SD subpopulation (MCW0295, MCW0330, MCW0111, MCW0069), while one (01) locus (MCW0081) was affected in the EXO population. Only one (01) significant case of heterozygote excess was detected at locus MCW0067 in the EXO population (Table 3).

Table 2. Genetic variability parameters of the different chicken populations studied per locus

LOCI	Variability parameters				Na per population				
	Ho	He	PIC	Rt	Na	SDS	SHL	SD	EXO
MCW0034	0.589	0.630	0.605	3.094	13	9	6	8	7
MCW0295	0.564	0.637	0.583	2.876	9	5	6	6	4
MCW0330	0.277	0.455	0.427	2.356	7	5	4	4	3
LEI0094	0.478	0.486	0.450	2.426	10	4	5	5	5
MCW0078	0.539	0.571	0.514	2.566	4	4	4	4	4
MCW0111	0.632	0.702	0.643	3.052	5	5	4	4	3
ADL0278	0.686	0.639	0.559	2.673	5	3	2	4	4
MCW0067	0.396	0.442	0.399	2.193	3	3	3	3	3
MCW0098	0.382	0.499	0.373	1.967	2	2	2	2	2
MCW0206	0.609	0.648	0.583	2.837	6	5	4	4	4
MCW0216	0.441	0.503	0.385	2.018	3	2	2	3	2
ADL0268	0.518	0.619	0.583	2.930	5	4	5	5	5
MCW0037	0.504	0.533	0.439	2.235	3	3	2	3	3
MCW0103	0.292	0.302	0.264	1.760	3	3	2	2	2
LEI0192	0.651	0.700	0.648	3.181	13	8	7	5	5
MCW0069	0.485	0.587	0.500	2.483	6	4	3	5	3
MCW0081	0.417	0.592	0.504	2.472	6	5	2	3	2
MCW0183	0.542	0.621	0.597	3.076	11	6	9	4	6
MCW0248	0.192	0.180	0.171	1.519	3	3	3	3	3
Total					117	83	75	77	70
Mean	0.484	0.545	0.486	2.511	6.158	4.368	3.947	4.053	3.684

Note = Ho: observed heterozygosity; He: Expected heterozygosity; PIC: Polymorphism Information content; Rt: Allelic richness; Na: Mean number of observed alleles; SD: Local chickens from the Sudanian zone; EXO: Exotic chickens; SHL: Local chickens from the Sahelian zone; SDS: Local chickens from the Sudano-Sahelian zone.

Table 3. Results of the Hardy-Weinberg equilibrium test by locus and chicken population

LOCI	Heterozygote deficiency				Heterozygote excess			
	SDS	SHL	SD	EXO	SDS	SHL	SD	EXO
MCW0034	0.00	0.96	0.88	0.10	1.00	0.08	0.33	0.92
MCW0295	0.22	0.68	0.00	0.08	0.79	0.42	1.00	0.92
MCW0330	0.01	0.17	0.02	0.14	0.99	0.97	0.98	0.86
LEI0094	0.87	0.74	0.47	0.57	0.33	0.49	0.62	0.64
MCW0078	0.59	0.61	0.14	1.00	0.42	0.43	0.88	0.28
MCW0111	0.08	0.77	0.04	1.00	0.92	0.23	0.96	0.18
ADL0278	0.77	1.00	0.06	0.88	0.24	0.80	0.95	0.22
MCW0067	1.00	0.20	0.68	0.99	0.66	0.86	0.58	0.03
MCW0098	0.72	0.15	0.51	1.00	0.57	0.96	0.81	0.97
MCW0206	0.01	0.09	0.71	0.92	1.00	0.93	0.39	0.19
MCW0216	0.62	0.66	0.17	0.82	0.67	0.62	0.93	0.64
ADL0268	0.21	0.12	0.29	0.86	0.88	0.89	0.74	0.24
MCW0037	0.27	0.47	0.75	0.15	0.83	0.77	0.27	0.86
MCW0103	0.06	0.50	0.44	1.00	0.98	0.90	0.95	0.18
LEI0192	0.29	0.14	0.60	0.96	0.75	0.89	0.52	0.14
MCW0069	0.56	0.11	0.00	1.00	0.59	0.91	1.00	0.18
MCW0081	0.03	0.77	0.70	0.03	0.97	0.49	0.41	1.00
MCW0183	0.22	0.58	0.58	0.85	0.84	0.60	0.58	0.21
MCW0248	1.00	1.00	1.00	1.00	0.88	0.89	0.96	0.63

Note: Loci deviating significantly from HWE equilibrium (p<0.5) are marked in bold. SD: Local chickens from the Sudanian zone; EXO: Exotic chickens; SHL: Local chickens from the Sahelian zone; SDS: Local chickens from the Sudano-Sahelian zone.

Genetic Relationships and Population Structure

Across all populations, F_{IS} values ranged from - 0.16 (ADL0278) to 0.36 (MCW0330), with an overall mean of 0.04. About 26.31% of loci showed heterozygote excess ($F_{IS} < 0$). The overall F_{IT} indicated a 13% heterozygote deficiency (Table 4), with values between - 0.07 (MCW0248) and 0.41 (MCW0330). Considering all loci, the F_{ST} value was moderate (0.10), suggesting that 10% of the total genetic variation was due to differences among subpopulations.

Pairwise F_{ST} values among subpopulations and

between each subpopulation and the total population showed varying levels of genetic differentiation. F_{ST} ranged from 0.03, observed between SDS-SHL and SD-SHL, to 0.19, recorded between each local subpopulation and the EXO population. The highest genetic differentiation occurred between local chickens and the exotic population, accounting for 19% of total genetic variation (Table 5). Among local subpopulations, SDS-SHL and SD-SHL showed the lowest pairwise F_{ST} values (0.03 each), indicating equally close genetic relationships. The greatest differentiation among local groups was between SDS and SD (F_{ST} = 0.04). Nei's genetic distances followed a similar trend, being high (≥ 0.18) between local and exotic populations and low (0.05) between SHL and SDS (Table 5). These results show, therefore, stronger genetic divergence between local and exotic chickens than within local subpopulations.

Table 4. The values of some F-statistics by locus in the chicken populations studied

LOCUS	FIT	FST	FIS
MCW0034	0.07	0.03	0.04
MCW0295	0.13	0.06	0.08
MCW0330	0.41	0.08	0.36
LEI0094	0.04	0.09	-0.05
MCW0078	0.07	0.06	0.01
MCW0111	0.12	0.07	0.05
ADL0278	-0.04	0.11	-0.16
MCW0067	0.16	0.22	-0.08
MCW0098	0.27	0.19	0.10
MCW0206	0.06	0.00	0.06
MCW0216	0.15	0.12	0.04
ADL0268	0.18	0.10	0.10
MCW0037	0.07	0.07	0.01
MCW0103	0.04	0.02	0.02
LEI0192	0.09	0.07	0.02
MCW0069	0.19	0.07	0.13
MCW0081	0.34	0.24	0.14
MCW0183	0.17	0.18	-0.01
MCW0248	-0.07	0.00	-0.07
Mean	0.13	0.10	0.04

Note : F_{IS} = Estimated Heterozygosity deficit; F_{IT}= Overall genetic variation; F_{ST}= Fixation Index among Subpopulations

Table 5. Pairwise FST values (lower triangle) and Nei's genetic distances (upper triangle) among the chicken populations studied

Type of chicken	SDS	SHL	SD	EXO
SDS	-	0.05	0.06	0.18
SHL	0.03	-	0.07	0.20
SD	0.04	0.03	-	0.20
EXO	0.19	0.19	0.19	-

Note: SD: Local chickens from the Sudanian zone; EXO: Exotic chickens; SHL: Local chickens from the Sahelian zone; SDS: Local chickens from the Sudano-Sahelian zone.

The AMOVA results are presented in Table 6. Without hierarchical grouping, analyses showed that 9.63% of the total genetic variation was distributed among populations, whereas 90.37% was distributed within populations, with a significant overall differentiation ($p<0.001$). Under Grouping I (EXO vs. SD, SDS, and SHL), 16.54% of the total variation was attributed to differences among groups, 2.92% to variation among populations within groups, and 80.53% to variation within populations, with all variance components being significant ($p<0.001$). Under Grouping III (SD vs. SDS and SHL), the variance component among groups was negative (-1.34%) and non-significant ($p = 0.792$), whereas 4.59% of the variation occurred among populations within groups and 96.74% within populations, both showing significant differentiation ($p<0.001$).

The phylogenetic tree based on shared allele proportions revealed that Burkina Faso local chicken subpopulations and the exotic population were grouped into three distinct clades with strong bootstrap support (Figure 1). The first clade separated the SHL subpopulation from the others. The second clade clearly distinguished the SD subpopulation from SDS and EXO, with very high support (100%). The third clade, supported by a bootstrap value of 57%, differentiated the SDS subpopulation from the EXO population.

The phylogenetic tree based on shared allele proportions among individuals showed partial clustering patterns across subpopulations. SHL individuals tended to group together, although their cluster overlapped with many SD individuals. SD individuals displayed a less defined structure, with branching that sometimes included SDS individuals. Overall, the tree revealed a heterogeneous arrangement, mixing individuals from different subpopulations, with only partial clustering observed for EXO and SDS individuals (Figure 2).

Table 6. Analysis of molecular variance among Burkina Faso chicken populations

Group	Source of variation	Sum of squares	Variance components	Percentage of variation	P-Value
No Grouping	Among population	84.64	0.4777	9.62	0.000
	Within populations	894.346	4.48501	90.37	0.000
Grouping I (Group I = EXO; Group II = SD, SDS, SHL)	Among groups	55.242	0.92128	16.54	0.000
	Among population	29.405	0.16278	2.92	0.000
	Within populations	894.346	4.48501	80.53	0.000
Grouping III (Group I = SD; Group II = SDS; SHL)	Among groups	13.139	-0.06079	-1.33	0.792
	Among population	16.265	0.20899	4.59	0.000
	Within populations	760.228	4.40229	96.74	0.000

Note = SD : Local chickens from the Sudanian zone ; EXO : Exotic chickens ; SHL: Local chickens from the Sahelian zone ; SDS : Local chickens from the Sudano-Sahelian zone.

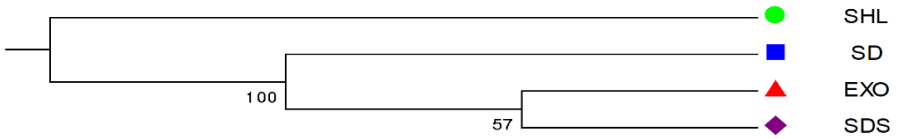

Figure 1. Phylogenetic tree of Neighbor Joining based on distances between subpopulations (SD: Local chickens from the Sudanian zone; EXO: Exotic chickens; SHL: Local chickens from the Sahelian zone; SDS: Local chickens from the Sudano-Sahelian zone.

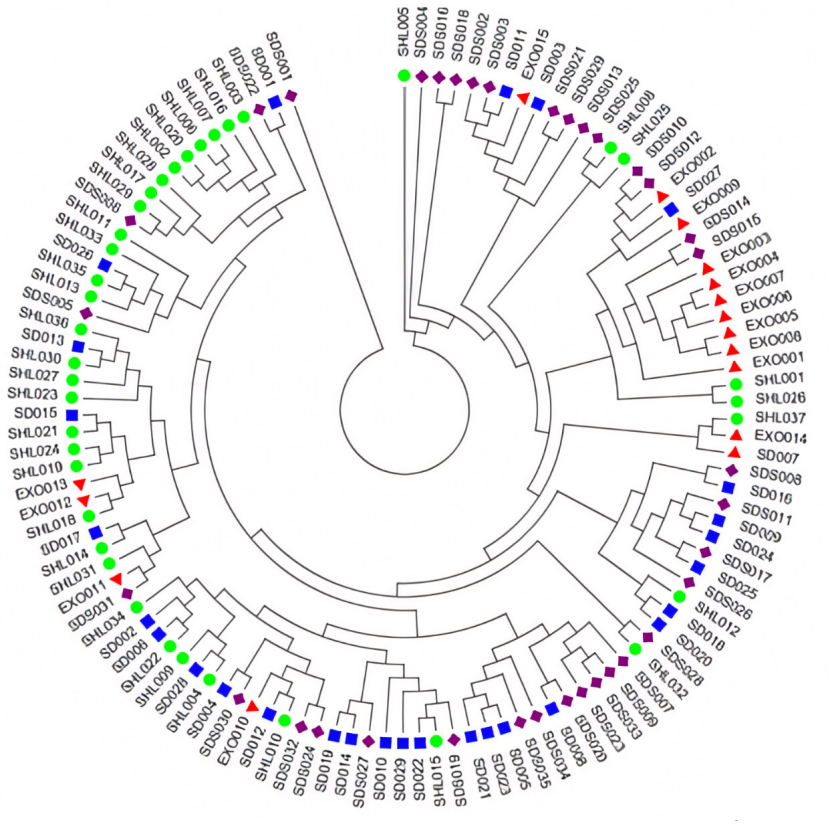

Figure 2. Phylogenetic tree of Neighbor Joining based on inter-individual distances in the 3 local chicken subpopulations and the EXO chicken population (SD: Local chickens from the Sudanian zone; EXO: Exotic chickens; SHL: Local chickens from the Sahelian zone; SDS: Local chickens from the Sudano-Sahelian zone). Note : ● SHL, ■ SD, ▲ EXD, ◆ SDS

The principal components derived from F_{ST} values enabled the graphical representation of the different populations across the dimensions of the scatterplot (Figure 3). The configuration of the plot displayed a three-dimensional triangle, with a basal tripod formed by the clustering of the local chicken subpopulations (SD, SDS, and SHL), and the apex represented by the EXO population.

To infer the cryptic genetic structure of local chicken populations in Burkina Faso, individuals from the different populations studied were clustered using a Bayesian approach. The results indicated that K = 4 best described the structure of the local chicken population sampled across the three agro-ecological zones of Burkina Faso in the present study (Figure 4).

The genotypic structure of Burkina Faso's local chicken population revealed different patterns depending on the assumed number of ancestral populations (K) (Figure 5). At K = 1, the population appeared as a single homogeneous group. With K = 2, two distinct clusters emerged, clearly separating the EXO population from local subpopulations, although the latter still shared part of their genetic background with EXO. At K = 3, all local subpopulations (SDS, SD, and SHL) were distinguished from the EXO population, but showed mutual introgression, with only limited genetic contribution from EXO. Among them, SHL appeared less introgressed than SDS and SD. At the most informative level (K = 4), SDS and SD displayed strong introgression with other subpopulations. SHL also showed introgression, but some individuals retained a relatively conserved and homogeneous genetic background. Similarly, most EXO individuals exhibited a highly homogeneous genotypic structure.

DISCUSSION

Biodiversity and Inbreeding Estimates

The microsatellite markers selected in this study have previously been used to characterize local chicken populations in Burkina Faso (Yacouba *et al.*, 2022),

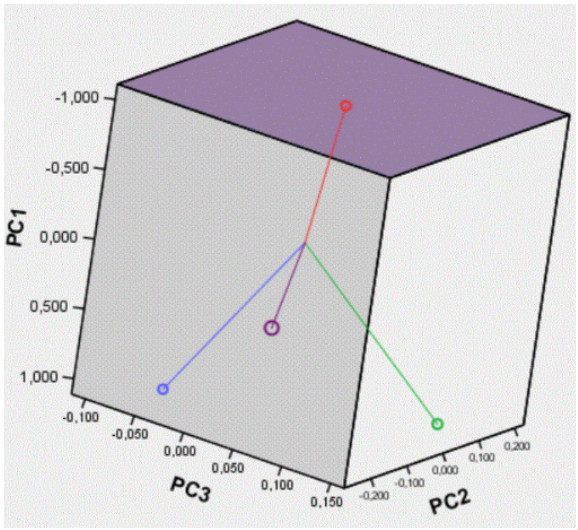

Figure 3. Scatter plot showing the distribution of chicken populations according to the different principal components based on F_{ST} values (SD: Local chickens from the Sudanian zone; EXO: Exotic chickens; SHL: Local chickens from the Sahelian zone; SDS: Local chickens from the Sudano-Sahelian zone). Note : ○ EXO, ○ SD, ○ SDS, ○ SHL

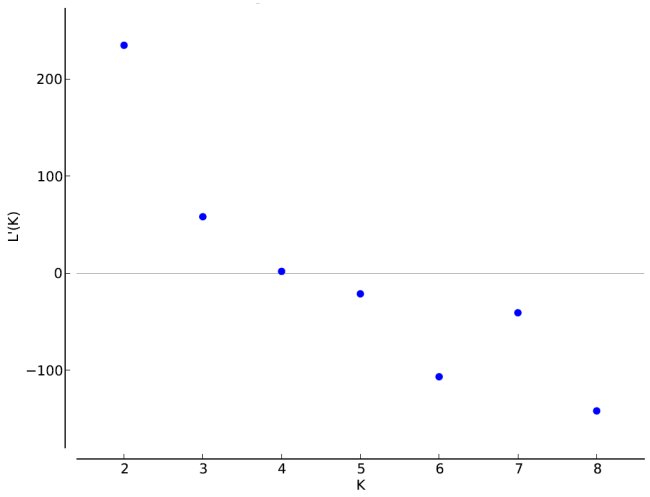

Figure 4. Rate of change of the log-likelihood, L'(K), as a function of the number of clusters (K), used to determine the optimal K for the chicken populations studied following the Evanno method.

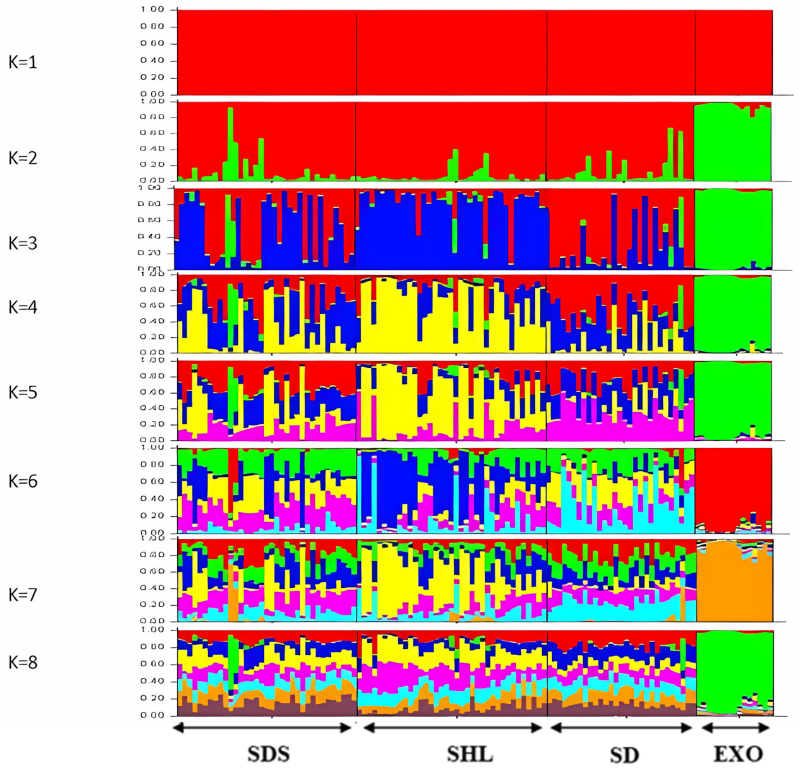

Figure 5. Genetic structure of the local sub-populations and the EXO chicken population (SD: Local chickens from the Sudanian zone; EXO: Exotic chickens; SHL: Local chickens from the Sahelian zone; SDS: Local chickens from the Sudano-Sahelian zone).

Nigeria (Manjula *et al.*, 2021), and Kenya (Okumu *et al.*, 2017), as well as indigenous chicken populations across Asia (Rashid *et al.*, 2020; Roh *et al.*, 2020; Ren *et al.*, 2022). The number of alleles detected per locus indicated a sufficient level of polymorphism, since loci carrying several alleles allow allele-frequency differences among populations to be detected more readily and thus yield better-resolved estimates of genetic differentiation (Sunde *et al.*, 2020). Accordingly, the 19 microsatellite markers used in the present study can be considered appropriate for assessing genetic diversity in the local chicken population of Burkina Faso, consistent with Long *et al.* (2017), who likewise found 19 microsatellite loci to be highly polymorphic

in nine local chicken breeds. All markers proved to be informative, with a mean polymorphism information content (PIC) of 0.486. According to Botstein *et al*. (1980), PIC values are classified into three categories : highly informative markers (PIC > 0.5), reasonably informative markers (0.25 ≤ PIC ≤ 0.5), and slightly informative markers (PIC < 0.25). In the present study, 52.63% of the microsatellite markers were highly informative, 42.11% were reasonably informative, and 5.26% were slightly informative. These proportions differ from those reported by Okumu *et al*. (2017), who found 100 % highly informative markers. These results reflect the moderate marker informativeness also reported for Ethiopian indigenous chicken ecotypes (Hailu *et al*., 2020). The overall mean number of alleles per locus across all populations was 6.158. When comparing population-level allelic diversity, local chickens showed higher means (SDS: 4.37; SD: 4.05; SHL: 3.95) compared with the EXO population (3.684), a difference attributable to the open mating system prevailing in Burkina Faso local chickens, which favors the maintenance of a greater number of alleles. The lower allelic diversity observed in the EXO population is consistent with its origin from genetically selected individuals, in agreement with the reduced allelic diversity reported for commercial breeds relative to indigenous Asian populations (Roh *et al*., 2020). The mean allelic diversity per population observed here is lower than values reported by Samaraweera *et al*. (2021) for local chickens in Sri Lanka (ranging from 6.50 to 7.30). These differences may reflect the smaller sample size in this study, the different number of markers analyzed, and potentially higher historical admixture in Sri Lankan populations associated with their geographic position along ancient trade routes (Samaraweera *et al*., 2021). Substantial intra-population allelic variability was detected. Expected heterozygosity (He) exceeded observed heterozygosity (Ho) in all populations (local and EXO), except at loci ADL0278 (Ho = 0.686; He = 0.639) and MCW0248 (Ho = 0.192; He = 0.18), suggesting potential deviations from Hardy-Weinberg equilibrium (HWE). Results of HWE tests, together with positive FIS values in the local sub-populations, indicate a heterozygote deficit consistent with a low level of inbreeding (approximately 4%). According to Wright (1978), FIS values below 0.05 are generally considered indicative of low inbreeding. Similar findings have been reported in African village chicken populations (Traoré *et al*., 2018), where poultry are typically raised under traditional systems with small flock sizes per household. No deliberate selection was practiced by farmers during the present study. Uncontrolled mating within small flocks likely contributed to the observed heterozygote deficit. The presence of null alleles and a potential Wahlund effect may also have influenced these results. However, the EXO population exhibited a negative FIS value, indicating heterozygote excess. A similar observation was reported by Samaraweera *et al*. (2021), who attributed this pattern to controlled mating schemes in commercial populations, typically involving a limited number of selected males and females bred for either egg or meat production. The mean observed and expected heterozygosity values in the Burkina Faso local chicken population (Ho = 0.484; He = 0.545) indicate a moderate level of genetic diversity, consistent with the substantial phenotypic variability observed. The expected heterozygosity falls within the range reported for African and Asian local chicken populations (Manjula *et al*., 2021; Yacouba *et al*., 2022). The relatively high diversity observed in the Burkinabè populations is consistent with their random mating system. The absence of organized selection for specific production traits and the occurrence of uncontrolled crossbreeding likely promote continuous gene flow among local sub-populations. Comparable patterns have been reported in other African local chicken populations (Okumu *et al*., 2017; Manjula *et al*., 2021).

Genetic Relationships and Population Structure

The overall F_{ST} value obtained in the present study indicates moderate genetic differentiation among the sub-populations. Indeed, F_{ST} values ranging from 0.05 to 0.15 are generally interpreted as reflecting moderate genetic differentiation (Hall, 2022; Nematbakhsh *et al*., 2024). Despite the geographic distances separating the sub-populations (52-650 km), inter-population genetic differentiation remained low. This limited differentiation may be explained by the reproductive mode and extensive management system of local chickens, as well as bird movements associated with livestock mobility and commercial exchanges, which can generate substantial gene flow between regions (Okumu *et al*., 2017; Traoré *et al*., 2018; Samaraweera *et al*., 2021; Tapsoba *et al*., 2024). The low among-population component of variation is consistent with Mwambene *et al*. (2019), who found that only 2% of the total genetic variation in Tanzanian local chicken ecotypes was attributable to between-ecotype differences and with Ajibike *et al*. (2022), who reported greater within- than among-population variation in Nigerian indigenous chickens. The highest pairwise F_{ST} values involved the EXO population (0.19). This likely reflects the distinct origin of exotic breeds and their more limited admixture with local populations compared to gene flow among local sub-populations. Moreover, exotic breeds are subject to directional selection and specialization, which may further accentuate genetic differentiation (Xu *et al*., 2023; Nematbakhsh *et al*., 2024; Wen *et al*., 2025). The relatively lower F_{ST} observed in Burkina Faso may also result from crossbreeding practices, whereby farmers mate local hens with improved cocks to enhance zootechnical performance (Okumu *et al*., 2017; Zhuang *et al*., 2023). The lowest pairwise F_{ST} values were observed between SDS-SHL and SD-SHL (both 0.03), followed by SDS-SD (0.04), indicating very small genetic distances. It is noteworthy that the NJ phylogenetic tree-based on shared allele proportions may yield a different topology from F_{ST} rankings when pairwise distances are similarly low; the tree topology should therefore be interpreted in conjunction with the F_{ST} matrix. These results suggest substantial gene flow between the Sudano-Sahelian agro-ecological zone (SDS) and the other local sub-populations (SD and

SHL). Consequently, SDS, SD, and SHL are genetically close, sharing a high proportion of common alleles. Strong demand for local chicken meat in major urban centers such as Ouagadougou and Bobo-Dioulasso may also contribute to sustained commercial flows of birds toward these cities. Overall, a moderate level of genetic diversity is maintained both within and among local chicken sub-populations in Burkina Faso.

The AMOVA results obtained in the present study revealed that most of the genetic variation was distributed within populations rather than among populations, which is consistent with the general pattern commonly observed in indigenous chicken populations. Without hierarchical grouping, 90.37% of the total genetic variation was found within populations, whereas 9.63% was attributable to differences among populations, indicating moderate genetic differentiation among the studied populations. Under Grouping I, where EXO was separated from SD, SDS, and SHL, the proportion of variation among groups increased to 16.54%, while only 2.92% of the variation was observed among populations within groups. In contrast, Grouping III, which included SD, SDS, and SHL as separate groups, showed a non-significant variance component among groups, indicating weak genetic structuring among these populations. Overall, these results suggest that the main genetic differentiation in the dataset is driven by the divergence of EXO, whereas SD, SDS, and SHL are genetically closer to each other. These findings are broadly consistent with those reported by Yacouba *et al*. (2022) in indigenous chicken populations from Burkina Faso, where AMOVA also revealed that the majority of genetic variation was distributed within populations, with limited differentiation among ecotypes. Their study further identified a relatively distinct genetic structure for the Konde ecotype, which originates from the Sudan-Sahelian (SDS) zone. In the present study, however, no significant structuring was detected among SD, SDS, and SHL when analyzed separately, suggesting a lower level of differentiation among populations from this zone. This discrepancy may reflect differences in sampling design, population composition, or levels of gene flow and admixture across production systems. Nevertheless, both studies consistently highlight that indigenous chicken populations in Burkina Faso maintain high within-population genetic diversity and generally low to moderate population structure.

Neighbor-Joining phylogenetic analysis based on shared alleles revealed substantial overlap among individuals from different sub-populations, indicating weak genetic structuring at the population level relative to within-subpopulation variation. The SHL sub-population and the EXO population appeared relatively distinct from the others. Similar observations were reported by Okumu *et al.* (2017) in Kenyan local chickens and Samaraweera *et al.* (2021) in local chickens of Sri Lanka. In the case of the SHL sub-population, this relative distinctiveness may be associated with the limited introduction of improved breeding males in that region.

In the present study, allelic frequencies were used to assign genotypes based on probabilistic and Bayesian approaches. Individuals from the EXO population were correctly assigned at 100%, whereas individuals from the SDS, SHL, and SD ken population of Burkina Faso. A similar pattern of high admixture has been reported in other African local chicken populations, including those from Kenya (Okumu *et al*., 2017), Tanzania (Mwambene *et al*., 2019), and Nigeria (Manjula *et al*., 2021). According to Samaraweera *et al.* (2021), such structuring reflects low differentiation and substantial gene flow. This contrasts with commercial reference populations, which typically display clearer genetic separation.

Clustering of local sub-populations according to agro-ecological zones was not supported, owing to low inter-population genetic differentiation. This absence of geographic structuring can be attributed to several interacting factors: (i) the traditional extensive management system promotes continuous gene flow across boundaries; (ii) commercial trade networks connecting rural areas to urban markets facilitate bird displacement across agro-ecological zones; and (iii) the absence of organized selective breeding prevents artificial divergence. The SHL sub-population appeared relatively distinct and showed the lowest level of introgression, possibly reflecting limited access by exotic breeds in the more remote Sahelian zone. Similar findings were reported by Okumu *et al*. (2017) in Kenya, Yacouba *et al*. (2022) in Burkina Faso, Habimana *et al.* (2020) in Rwanda and Samaraweera *et al.* (2021) in Sri Lanka. In contrast, Xu *et al*. (2023) found that the structure of certain Chinese indigenous breeds strongly reflects their geographic distribution and distinct breeding histories.

CONCLUSION

The present study provides a comprehensive assessment of the genetic diversity and population structure of local chickens across the major agroecological zones of Burkina Faso. Using microsatellite markers, we observed a relatively high level of genetic diversity, marked by substantial intra-population variability, high allelic richness, and heterozygosity. Despite the geographic and agroecological distribution of populations, no clear genetic structuring by agroecological origin was detected. Instead, the populations formed a single broad genetic complex shaped by continuous gene flow, with only limited introgression from commercial breeds. This high within-population diversity, combined with weak differentiation, indicates strong potential for genetic improvement and underscores the need for sustainable management and conservation of these local genetic resources.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other

people or organizations related to the material discussed in the manuscript.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, a generative AI tool was used to assist with the translation and summarization of certain sections of the manuscript. After using this tool, we carefully reviewed, edited, and validated the content as needed and took full responsibility for the accuracy, integrity, and final content of the publication.

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