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Genetic Characterization of Some Selected Ethiopian Indigenous Chickens using Simple Sequence Repeat Markers

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ABSTRACT: Improving local chicken production in Ethiopia will be a priority toward achieving "zero hunger", food security, and sustainability. Chickens provide an excellent source of protein that can satisfy growing human needs. Indigenous chickens in Ethiopia are not selected and improved, so they have low productive and reproductive performance. The present study aimed to investigate the genetic diversity and population structures of three Ethiopian local chicken ecotypes using 16 SSR markers, and the Koekoek breed was used as a comparison. A total of 97 alleles have been detected, with an average value of 6.062 alleles per locus. Polymorphic information content ranged from (0.54) MCW0183 to (0.85) LEI0166, with an average value of 0.67 per locus. Across all studied populations, the mean observed heterozygosity and expected heterozygosity were 0.026 and 0.60, respectively. The Shannon information index varied from (I = 0.83) MCW 0098 to (I = 1.57) LEI0166. AMOVA showed that genetic variance varied by 3% within individuals, 82% within populations, and 15% between breeds. According to UPGMA, the Horro and Tilili populations were grouped, while the Jarso population was distinct and the Koekoek breed was distinct as expected. The studied population showed high genetic diversity within populations, and the Jarso ecotype showed the highest genetic diversity and a number of unique alleles. The SSR markers used in this finding were polymorphic and useful for determining the genetic variation of Ethiopian local chicken ecotypes. The information obtained will be used for genetic conservation and national breeding program efforts.

Keywords: Indigenous chickens, genetic diversity, simple sequence repeat marker.

INTRODUCTION

In Ethiopia, chickens are the ideal prevalent livestock species, accounting for 57 million and having the largest percentages of indigenous, hybrid, and exotic chickens (78.85%, 12.02%, and 9.111%, respectively) (Sime and Edea 2022). They offer high levels of animal protein in terms of meat and eggs for human diet (Mohammed, 2018; Pius *et al*., 2021). Moreover, they were kept for income and sociocultural roles (Abadula *et al*., 2022; Aleme, 2022).

Local chicken is selected over exotic chickens in many countries due to their flavor, taste, pigmentation, and leanness (Abdulwahid and Zhao 202 2). They tend to adapt to local environmental conditions, can survive well under harsh conditions, and are resistant to some diseases (Perini *et al*., 2020; Tolasa, 2021). To enhance the production of native chickens, selective breeding is preferred over cross-breeding with exotic strains that are unadapted. High levels of inbreeding and genetic dilution can result from random mating or unplanned crossing of local chickens within exotic breeds to improve the low productivity of local chickens (Chebo *et al*., 2022).

It is essential to possess prior knowledge of the prevailing genetic diversity in order to identify unique and valuable genetic resources for breed improvement, assess their genetic potential, and contribute to future strategies for sustainable management (Pius *et al*., 2021). The main strategies being considered for the genetic advancement of local Ethiopian chicken breeds include the introduction of exotic breeds, selection within breeds or lines, and cross-breeding (Shapiro *et al*., 2015; Yonas, 2020).

Morphological characterization helps distinguish animals based on their apparent phenotypes. However, they are exposed to environmental influences, have low polymorphism, and provide no basis for differentiating animals that look similar or have similar expression traits, thereby reducing the accuracy of evaluation or selection. In addition, the evaluation of quantitative traits, or the contribution of each gene to a trait and its location in the genome using morphological markers, has limitations (Tongsiri *et al*., 2019; Marwal and Gaur 2020). Similarly, estimates of genetic variation within and between chicken populations were made using protein polymorphisms and biochemical markers, but they didn't provide enough information.

It is vital to combine simultaneous genetic characterization with phenotypic characterization, which is impacted by the environment, to conserve and

utilize genetic resources (Yadav *et al.,* 2017*;* Simm *et al.*, 2020). Molecular markers indicate the presence of DNA sequence variation that exists at specific locations in the genome and its heritability. Variation identification relies on DNA assays (Reshma and Das 2021). This is important for future monitoring of gene flow, parental definitions, genetic traceability, and effective evidence-based decision-making for successful conservation and selection breeding efforts (Habimana *et al.*, 2020). SSR markers are highly polymorphic and codominant throughout the genome; as a result of their high levels of polymorphism and codominance inheritance, they are abundant and uniformly distributed (Fathi *et al.,* 2017; Okumu *et al.,* 2017; Yacouba *et al.*, 2022). Therefore, the present study aimed to investigate genetic diversity and population structures among three Ethiopian local chicken ecotypes using 16 SSR markers, and the Koekoek breed was used as a comparison.

MATERIALS AND METHODS

A. Study animals, sampling, and DNA extraction

A total of 95 blood samples were collected from unrelated chickens from four populations, including Horro (n = 25), Tilili (n = 25), Jarso (n = 25), and Koekoek (n = 20), with vacutainer tubes containing EDTA as an anticoagulant. Each breed was selected purposefully, and individuals from each population were selected randomly. Genomic DNA was extracted from whole blood using the salting-out method (Nasiri *et al*., 2005) with some modifications. A 300 µl blood sample was added to a 2 ml Eppendorf tube and 800 µl of lysis buffer (0.3M sucrose, 0.01 MTris HCl, pH 7.5,5mM MgCl and 1% triton X 100) was added to each tube and centrifuged at 10,000rpm for 5 min and the supernatant was removed (this step repeated until we get white pellet), then 60µl of 10mM tris-HCl pH 8, was added to the pellets, vigorous vortex and centrifuge for 2 min at 10,000 rpm and the supernatant was removed and 66µl 10mM tris-HCl, 66µl laundry powder solution, glass beads added to the pellets, and vortex for 20 sec, 50µl of 6M NaCl was added and vortex again for 20 sec, then centrifuge for 5 min at 13,000 rpm and the supernatant transferred to fresh tubes and then 150µl of 97% ethanol was added to precipitate the DNA and centrifuge for 3min at 13,000rpm, then the pellet was washed twice with 100µl of 70% ethanol by centrifugation for 2min at 12,000rpm, finally 40µl Elution buffer was added. The concentration of DNA was checked by adding 2µl DNA samples to the Nanodrop machine. Furthermore, the quality of the genomic DNA was tested with 0.8% agarose gel electrophoresis that was run for 45 min at 85 voltages (primarily by loading a 5µl sample of DNA with 2µl loading dye having gel red).

B. Polymerase chain reaction (PCR) and SSR markers

A total of 16 SSR markers were used for genetic characterization (Table 1). The PCR amplification was carried out in a 10µl reaction volume containing 5µl DreamTaq PCR master mix 2X, 10µM forward primer (0.25µl), 10µM reverse primer (0.25µl), 20ng templates DNA (0.5µl), and nuclease-free water (4µl). Touch-down PCR was used in the PCR condition.

Table 1: List of SSR markers used in this study.

Markers	Chr. No.	Repeat Motif	Forward Primer 5'- 3'	Expected (bp)	Annealing Temperature (°C)
MCW0222	3	(GT)8	F: GCAGT TACATTGAAATGATTCC R: TTCTCAAAACACCTAGAAGAC	220–226	59
MCW0078	5	(GT)6 (AT)4	F: CCACACGGAGAGGAGAAGGTCT R:TAGCATATGAGTGTACTGAGCTTC	135–147	59
MCW0081	5	(TG)17	F: GTT GCT GAG AGC CTG GTG CAG R: CCTGTATGTGGAATTACTTCTC	112–135	58
MCW0016	3	(TG)16	F:ATGGCCCAGAAGGACAAGCGATAT R:TGGCTTCTGAAGCAGTTG CTATGG	162–206	58
ADL0268	1	(GT)12	F: CTC CAC CCC TCT CAG AACTA R: CAACTTCCCATCTACCTACT	102-116	58
MCW0067	10	(GT)11	F: GCACTACTGTGTGCTGCAGTTT R: GAGATGTAGTTGCCACATTCCGAC	176-186	58
MCW0165	23	(CA)8	F: CAGACATGCATGCCCAGATGA R: GATCCAGTCCTGCAGGCTGC	114-118	58
MCW0206	2	(AT/GT)15	F: ACATCTAGAATTGACTGTTCAC R: CTTGACAGTGATGCATTAAATG	221-249	60
LEI0166	3	$(CA)_4$(TA) $(CA)_{14}$	F: CTCCTGCCCTTAGCTACGCA R: TATCCCCTGGCTGGGAGTTT	354-370	57
MCW0034	2	(CA)24	F: TGCACGCACTTACATACTTAGAGA R: TGTCCTTCCAATTACATTCATGGG	212-246	58
MCW0098	4	$(TTTTA)_5$ $(TG)_6$ $(TG)_7$	F: GGCTGCTTTGTGCTCTTCTCG R: CGATGGTCGTAATTCTCACGT	261-265	58
ADL0278	8	(TG)18	F: CCAGCAGTCTACCTTCCTAT R: TGTCATCCAAGAACAGTGTG	114-126	55
MCW0183	7	$(CA)_{15}$	F: ATCCCAGTGTCGAGTATCCGA R: TGAGATTTACTGGAGCCTGCC	296-326	58
MCW0104	13	$(TG)_{19}$ $(TG)_{19}$	F: TATTGGCTCTAGGAACTGTC R: GAAATGAAGGTAAGACTAGC	190-234	58
MCW0020	1	(TG)13	F: TCTTCTTTGACATGAATTGGCA R:GCAAGGAAGATTTTGTACAAAATC	179-185	59
MCW0014	6	(CA)18	F: TATTGGCTCTAGGAACTGTC R: GAAATGAAGGTAAGACTAGC	164-182	57

5µl PCR product and 2µl of 6X loading dye (containing gel red) were loaded onto 2% agarose gel and run with 1X TAE buffer for 50 min at 85 constant voltage. A 100-bp base-pair mixed DNA ladder (SIMOBIO, DM2100) was loaded in the peripheral wells to estimate the molecular weight of each amplified product. An amplified product was visualized under UV light using the BioDOC-IT TM Imaging system (Cambridge, UK) to capture gel images for downstream analysis.

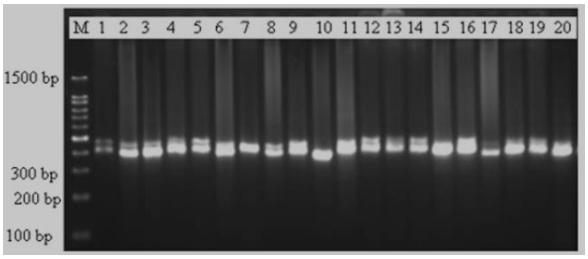

Fig. 1. PCR product of selected chicken populations using SSR marker.

C. Data analysis

PyElph 1.4 software was used to score the genotypes of selected chicken populations using amplified bands that were clearly visible (Pavel *et al*., 2012). Genetic variation was measured by estimating observed (Ho) and expected (He) heterozygosity by Nei (1978). In AMOVA, the fixation indices (F_{IS}, F_{IT}, and F_{ST}) and pairwise F_{ST} values (Weir and Cockerham, 1984) were computed using GenAlex v6.5 software (Peakall and Smouse 2015). The observed heterozygosity was computed using Levene's (1949) algorithm with the POPGEN software package (Yeh *et al*., 1999), version 1.31. Polymorphic information content (PIC), the total number of observed alleles (Na), and the frequency of major alleles were computed using Power Marker 3.25 (Liu and Muse, 2005).

An agglomerative hierarchical clustering technique with unweighted pair groups with an arithmetic mean and Darwin 6.0 were used to illustrate the evolutionary relationships between ecotypes. Using the dendro UPGMA online application (Garcia-Vallve and Puigbo, 2015), trees have been designed and displayed (Page, 1996). A confidence statement about the breeds was established by bootstrapping 1000 replicates to establish cluster dependency.

The rarefied allelic richness and the private rarefied allelic richness were calculated using HP Rare 1.1 software (Kalinowski, 2005). Structure 2.3.4 software (Pritchard *et al*., 2000) was used to estimate the genetic structure of the populations. In this finding, population structures (1–10K) were analyzed using independent alleles and admixture models (burns of 100,000, followed by 100,000 iterations with MCMC). According to the K value computed by the Structure Harvester program, the 95 genotypes were partitioned into three clusters. The number of clusters used to calculate each one's optimal K value was K=3. Kopelman *et al.* (2015) developed the CLUMPAK tool to determine the best alignment. The number of clusters used to calculate each one's optimal K value was K=3. Kopelman *et al.* (2015) developed the CLUMPAK tool to determine the best alignment from the structural data.

RESULTS

A. SSR marker polymorphism

In total, 97 alleles were detected, with a mean of 6.062 alleles per locus, ranging from 4 (MCW0020), (MCW0016), and (MCW0104) to 12 (LEI 0166). PIC values ranged from 0.54 (MCW0183) to 0.85 (LEI0166), with an average of 0.67. Observed heterozygosity (Ho) varied from 0.00 (MCW0067), (ADL0268), (MCW0081), (MCW0183), (MCW0222), (MCW0098), (MCW0165), (MCW0034), (MCW0026), (MCW0206), (ADL0278), (MCW0104), (MCW0078), (MCW0014) to 0.398 (LEI0166), whereas expected heterozygosity (He) varied between 0.44 (MCW0098) and 0.733 (LEI0166), with observed and expected heterozygosity (Ho=0.026).

Major allele frequency varied between 0.22 (LEI0166) and 0.59 (MCW0183), with a mean of 0.4 per locus. MCW0098 and LEI0166 loci had an effective number of alleles of 2.09 and 4.17, respectively, with a mean of 2.14 per locus. The Shannon's information index (I) varied between 0.83 (MCW0098) and 1.57 (LEI0166), with a mean of 0.83.The unbiased expected heterozygosity (uHe = 0.79) and fixation index (F = 0.77) were recorded. The mean of allelic richness (AR) was 4.25, with a range from 3.5 (MCW0016), (MCW0183), and (MCW0222) to 7 (LEI0166), and the private alleles (PA) also ranged from 0.00 (MCW0222), (MCW0020), (MCW0098), (MCW0165), (MCW0104), and ADL0278 to 0.75 (MCW0183), and (LEI0166) with a mean of 0.25 per locus. Out of 97 alleles, 1 (1.03%) of PA was unique to specific breeds with a mean gene diversity of 0.71 across all loci, and the gene diversity (GD) varied from 0.59 (MCW0183) to 0.86 (LEI0166). All of the markers were found to differ considerably (P<0.001) from the proportions predicted by HWE. The 16 SSR markers revealed a highly substantial and significant deviation (P<0.001) from the Hardy-Weinberg equilibrium (Table 2).

Table 2: Genetic diversity indices for 95 genotypes across 16 SSR loci.

SSR Loci	Diversity Indices																		
	Allele in bp	No	Na	MAF	Ne	I	AR	PA	Ho	He	Ht	uHe	NM	Fis	Fit	Fst	PIC	P	P(HWE)
ADL0268	110-140	6	4.25	0.31	3.10	1.22	4.25	0.25	0.0	0.64	0.79	0.662	1.08	1	1	0.19	0.77	0.00	***
MCW0081	110-140	6	4.25	0.32	3.23	1.23	4.25	0.25	0.0	0.67	0.73	0.683	1.79	1	1	0.12	0.73	0.00	***
LEI0166	360-410	12	7	0.22	4.17	1.57	7	0.75	0.39	0.73	0.86	0.749	1.46	0.46	0.5	0.15	0.85	0.00	***
MCW0183	290-325	6	3.5	0.59	2.24	0.91	3.5	0.75	0.0	0.5	0.59	0.508	1.5	1	1	0.14	0.54	0.00	***
MCW0222	218-230	4	3.5	0.47	2.71	1.08	3.5	0	0.0	0.62	0.65	0.639	5.57	1	1	0.04	0.59	0.00	***

MCW0014	166-180	6	4	0.53	2.28	0.98	4	0.25	0.0	0.51	0.64	0.529	0.98	1	1	0.20	0.60	0.00	***
MCW0020	174-186	5	3.75	0.53	2.46	0.98	3.75	0	0.01	0.55	0.65	0.563	1.31	0.98	0.98	0.16	0.61	0.00	***
MCW0098	255-270	5	3.75	0.53	2.09	0.83	3.75	0	0.0	0.44	0.62	0.448	0.59	1	1	0.30	0.57	0.00	***
MCW0067	170-190	6	3.75	0.44	2.31	0.95	3.75	0.5	0.0	0.52	0.63	0.535	1.32	1	1	0.30	0.56	0.00	***
MCW0165	114-126	4	3.75	0.36	2.89	1.13	3.75	0	0.0	0.64	0.71	0.655	2.37	1	1	0.16	0.65	0.00	***
MCW0034	220-245	6	4	0.23	2.65	1.12	4	0.25	0.0	0.62	0.77	0.634	1.02	1	1	0.09	0.73	0.00	***
MCW0016	160-210	5	3.5	0.43	2.56	1.06	3.5	0.5	0.0	0.60	0.65	0.622	3.34	1	1	0.07	0.58	0.00	***
MCW0206	220-250	8	5	0.23	3.15	1.28	5	0.5	0.0	0.66	0.83	0.673	0.97	1	1	0.20	0.80	0.00	***
ADL0278	114-135	8	6.25	0.23	3.97	1.50	6.25	0	0.0	0.70	0.83	0.719	1.38	1	1	0.15	0.81	0.00	***
MCW0104	190-220	4	3.75	0.39	2.81	1.13	3.75	0	0.0	0.63	0.71	0.649	2.18	1	1	0.10	0.66	0.00	***
MCW0078	128-150	6	4	0.48	2.50	1.05	4	0.25	0.0	0.58	0.68	0.589	1.57	1	1	0.14	0.63	0.00	***
Mean		**6.06**		**0.4**	**2.14**	**0.83**	**4.25**	**0.25**	**0.026**	**0.60**	**0.71**	**0.79**	**1.78**	**0.96**	**0.97**	**0.15**	**0.67**	**0.00**	

Key: * P < 0.05, ** P < 0.01, *** P < 0.001,
Allele size range (in bp), allelic richness (AR), private allele (PA), major allele frequency (MAF); number of observed alleles (No); mean number of alleles (Na); number of effective alleles (Ne); Shannon's information index or Shannon's diversity index(I); observed heterozygosity (Ho); expected heterozygosity or gene diversity (He); unbiased expected heterozygosity (uHe); fixation index (F); gene flow (Nm); polymorphic information content (PIC);

B. Genetic diversity among the population's

The gene diversity of the four examined chicken populations was compiled in Table 3 based on their geographical origin. The Horro and Jarso chicken populations showed the highest average number of alleles (Na) (4.75) among the local chicken ecotypes, while the Tilili chicken ecotype showed the lowest (4.69). The population of chickens in Koekoek had an average of 2.81 alleles. Similarly, in the local chicken population, the number of effective alleles (Ne) was highest in the Jarso chicken population and lowest in the Tilili chicken population. The average record was 2.82. The average allelic richness across all populations was 4.25, with higher allelic richness found in Jarso and Horro (4.75) and lower allelic richness found in Koekoek (2.81). Likewise, the Jarso chicken population had the highest number of unique alleles (0.5), followed by Tilili and Horro (0.19).

Shannon's information index was relatively high for the Jarso chicken ecotype (1.32). Similarly, the observed number of heterozygosities (Ho) was higher in the Jarso population (0.035), lowest in the Horro population (0.005), and average overall (0.026). In the Jarso chicken population, both expected heterozygosity (He) gene diversity and unbiased heterozygosity (uHe) were the highest at 0.68 and 0.67, respectively, while the lowest levels were observed in the Tilili population (He = 0.60 and u = 0.616). The Koekoek chicken population had (He = 0.51 and u = 0.52). The mean values of expected heterozygosity (He) and unbiased expected heterozygosity (uHe) in all populations were 0.6 and 0.616, respectively.

C. Analysis of molecular variance and gene flow

The AMOVA analysis showed 15% genetic variation between breeds, 82% within populations, and 3% within individuals. The analysis also confirmed the presence of gene flow (1.474) between populations (Table 4).

Table 3: Summary of genetic diversity parameters among chicken populations using 16 SSR loci.

Breeds	Genetic diversity parameters									
	N	Na	Ne	PA	I	Ho	He	TNA	AR	% PI
Horro	25	4.75	2.96	0.19	1.2	0.005	0.62	76	4.75	100.0%
Tilili	25	4.69	2.74	0.19	1.155	0.028	0.6	75	4.69	100.0%
Jarso	25	4.75	3.45	0.5	1.32	0.035	0.68	76	4.75	100.0%
Koekoek	20	2.81	2.14	0.12	0.833	0.034	0.51	45	2.81	100.0%
Mean		4.25	2.82	0.25	1.13	0.026	0.60	68	4.25	100.0%
SE		0.199	0.122		0.042	0.013	0.017			0.00%

N, number of alleles; Shannon's information index; Ho, observed heterozygosity; He, expected heterozygosity gene diversity; uHe, unbiased expected heterozygosity;%PI, percentage of polymorphic loci. TNA, total number of alleles; NA, mean number of alleles; AR, allelic richness; Ne is the effective number of alleles.

Table 4: Analysis of molecular variance in the studied chicken populations

Source	df	SS	MS	Est. Var	% of variations	F- statistics	P-Value
Among pops	3	154.368	51.456	0.87	15%	**Fst** = 0.145	0.001
Among Indiv	91	918.585	10.094	4.947	82%	**Fis** = 0.961	0.001
Within Indiv	95	19.000	0.200	0.200	3%	**Fit** = 0.967	0.001
Total	189	1091.953		6.020	100%		
NM						1.474	

D. Genetic distance and genetic identity

There was a range of 0.162 to 0.445 pairwise distances between the local chicken populations (Table 5). Jarso chicken ecotypes and Horro chicken populations had the highest genetic distance (0.445), while Horro chicken ecotypes and Tilili chicken populations had the lowest (0.162). A significant genetic distance of (0.676) was observed between the indigenous chicken population of Ethiopia and the Koekoek chicken breed.

Table 5: Gene flow (upper diagonal) and gene differentiation (lower diagonal).

Breeds	Horro	Tilili	Jarso	Koekoek
Horro	0.00	0.85	0.641	0.568
Tilili	0.162	0.00	0.732	0.582
Jarso	0.445	0.312	0.00	0.509
Koekoek	0.565	0.542	0.676	0.00

E. Cluster analysis of the genotypes

In an unweighted neighbor-joining cluster analysis of 95 chicken genotypes (C-I, C-II, and C-III), three major clusters were identified. Each cluster is made up of 21.05 percent C-I, 31.58 percent C-II, and 47.37 percent C-III of the total population. In the first cluster, 20 genotypes were found only in the Koekoek chicken population; in the second cluster, 30 genotypes were found, excluding Koekoek chicken populations; and in the third cluster, 45 genotypes were found in all chicken populations except Koekoek chicken populations.

There are only Koekoek genotypes in cluster one (C-I), Jarso (22%), Tilili (7.37%), and Horro (2%) are the most common genotypes in cluster two (C-II), and the largest cluster was C-III, which contains 47.37 percent genotypes from Horro (24.277%), Tilili (18.82%), and Jarso (4.2%). UPGMA was used to implement clustering to identify the genetic relationship between the four populations (Fig. 2). Three clusters (C-I, C-II, and C-III) have been detected by analysis of the populations of Horro, Tilili, Jarso, and Koekoek. A subgroup of Horro and Tilili formed within the third cluster. Chicken Populations from geographically distant regions were also found to cluster together, despite the pattern of clustering displayed by populations from geographically neighboring areas (Horro vs. Tilili).

F. Principal coordinate analysis (PCoA) and population structure

Principal coordinate analysis was used to ascertain the relationship between the chicken populations and the individual genotypes. Results showed that the first three most informative coordinates explained 25.34 percent of the overall variation. From the total variation, the first, second, and third coordinates are described as 10.03 percent, 8.72 percent, and 6.59 percent, respectively. A clear geographical location clustering of populations and a significant pattern of grouping were seen in the genotype distribution in a two-dimensional plot. PCoA analysis verified the NJ cluster analysis result (Fig. 3). STRUCTURE software was used to infer the 95 samples representing the four populations' admixture model-based population structure. The best number of genetic clusters is three, according to a study of the structure output performed using the Structure HARVESTER tool (Earl and VonHoldt 2012), which used the ΔK method of Evanno *et al*. (2005).

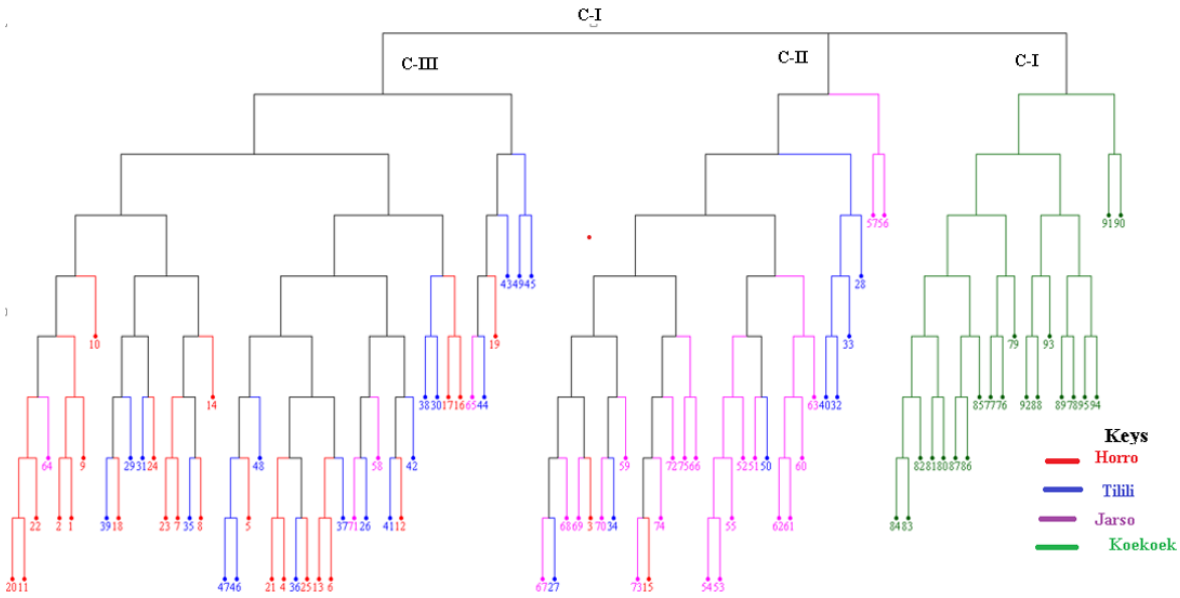

Fig. 2. Hierarchical clustering of selected chicken populations using dendrogram.

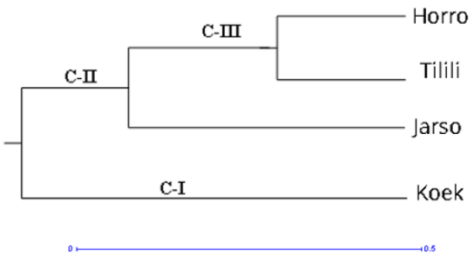

Fig.3. Dendrogram showing the genetic diversity and similarity of three indigenous chicken populations in Ethiopia and the Koekoek chicken population.

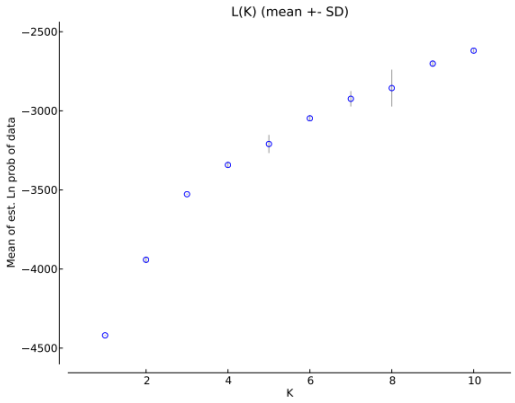

Fig. 1. Evolution of the mean estimate in probability of data with the number of cluster (K) in studied chicken populations.

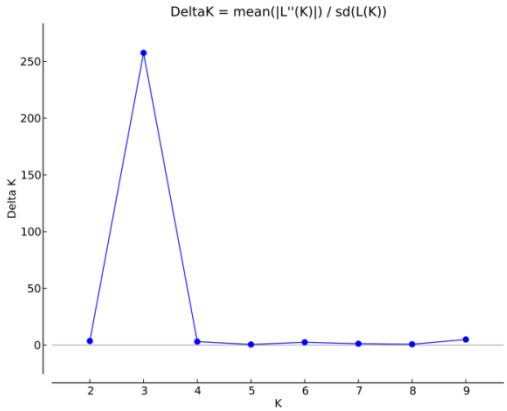

Fig. 2. Results of the STRUCTURE analysis of four chicken populations, highest peak at k =3.

Table 6: Evanno population structure parameters.

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	10	-4419.450000	0.317105	—	—	—
2	10	-3941.670000	17.512221	477.780000	63.130000	3.604911
3	10	-3527.020000	0.892935	414.650000	230.000000	257.577472
4	10	-3342.370000	16.887606	184.650000	51.970000	3.077405
5	10	-3209.690000	54.442905	132.680000	29.460000	0.541117
6	10	-3047.550000	15.404419	162.140000	38.530000	2.501230
7	10	-2923.940000	45.915773	123.610000	55.950000	1.218536
8	10	-2856.280000	113.881135	67.660000	87.110000	0.764920
9	10	-2701.510000	14.693570	154.770000	72.830000	4.956590
10	10	-2619.570000	8.830509	81.940000	—	—

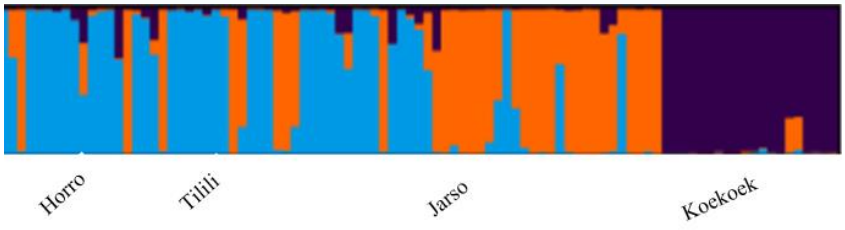

Fig. 3. Population structure of four chicken populations obtained by Structure analysis (K=3) where each color represents a different cluster.

DISCUSSIONS

A. Genetic diversity

The average value of PIC is the best index for assessing allelic polymorphism (Azimu *et al*., 2018). This shows that when PIC > 0.5, more information can be retrieved from the loci, while 0.25 < PIC < 0.5 indicates a moderately informative locus, while PIC < 0.25 indicates a moderately informative locus, and PIC < 0.25 indicates a vaguely informative locus (Habimana *et al*., 2020). Most of the 16 SSR markers used for assessing genetic variation in livestock breeds had a PIC > 0.5, exceeding the FAO recommendation of five alleles per locus (FAO, 2011; Madilindi *et al*., 2019).

The three most effective markers out of 16 were LEI0166, ADL0278, and MCW0206. It was found that MCW00165, MCW0222, and MCW0104 were the least effective markers for maintaining different chicken breeds. Markers with a PIC value between 0.54 and 0.85 were found across populations for MCW0183 and LEI0166. In the present study, the average PIC value (0.67) indicated that the markers were highly informative and that their allelic distribution across the genomes of the populations was significant. The PIC values that we reported in this study are also higher than those reported earlier (Hassen *et al*., 2009; Bekerie *et al*., 2015).

In this study, a total of 97 alleles were found with the use of 16 SSR markers, with an average of 6.062 alleles per locus. The alleles we report here are more than those found by Hassen *et al.* (2009) in seven native chickens from northwest Ethiopia, three South African chicken lines, and two commercial strains, with an average of 6.05 alleles per locus. Four indigenous chicken ecotypes from South and Western Ethiopia were studied for their genetic diversity and population structure by Bekeri *et al.* (2015). A total of 74 alleles with a mean of 4.80 were found across all populations of four indigenous chicken ecotypes from South and South Western Ethiopia across 10 loci, based on genetic diversity and population structure. A lower estimate of (1.726) was produced using eight local Kenyan chickens across eighteen markers (Okumu *et al*., 2017). In this study, more alleles were detected than in previous reports. This might be because of the larger genotypes, the genetic diversity of selected genotypes, and the different markers.

B. Genetic diversity analysis along with populations

Heterozygosity can be considered an estimate of the degree of genetic variation present in a population (Hariyono *et al*., 2019; De Kort *et al.*, 2021). The average level of heterozygosity in a population indicates the level of stability of the population.The population's low heterozygosity is an indication of its high genetic stability. In the current study, He ranged from 0.51 to 0.68 with an overall mean of 0.60, while Ho ranged from 0.005 to 0.034 with an overall mean of 0.026. With the exception of the observed heterozygosity, the mean heterozygosity values found in our study indicate that most intrapopulational genetic diversity and SSR markers are able to resolve heterozygosity and homogeneity (Terefe *et al*., 2023). The number of samples per population and the total population might be the cause of the differences between Ho and He numbers.

The Shannon information index (I), another measure of gene variety, indicates the presence of variation in the studied populations. The values obtained in this study ranged from I = 0.83 to I = 1.57, with a mean value of 0.83. This finding is somewhat in agreement with the findings of Burkina Faso (0.97), obtained from four local chicken ecotypes in Burkina Faso using 20 microsatellite markers (Yacouba *et al*., 2022).

A lower estimate of the number of effective alleles (Ne) was found in this study (2.14), as obtained in Burkina Faso (2.304) using 20 microsatellite markers from four different chicken breeds (Yacouba *et al*., 2022), and in China (4.6), based on genetic diversity and population structure analysis of eight local chicken breeds in Southern Xinjiang across 20 loci. This variation in the average number of alleles per locus and the actual number of alleles may be related to the number or type of markers used, the sample size, and the genetic resources studied.

In Ethiopia, sixteen SSR markers were used to measure genetic variation for local chicken ecotypes, and the results revealed a Fis value (0.97) indicative of inbreeding. Using five microsatellite markers, Hassen *et al*. (2009) reported a Fis value of 0.07, which was lower than the Fis value found in this study. FST levels can represent low (0–0.05), medium (0.05–0.15), high (0.15-0.25), and very high (FST >0.25) genetic variation between populations (Demir and Balcioglu, 2019; Nemera *et al*., 2022).

The F_{ST} value estimated from this study (0.15) was high er than that of the Sinai and Norfa chicken diversity revealed using 20 microsatellite markers (F_{ST} = 0.062) (Soltan *et al*., 2018). It is somewhat consistent with the findings of the assessment of population structure and genetic diversity of 15 Chinese indigenous chicken breeds using 29 microsatellite markers, with a FST of 0.16. Gene flow and animal exchange might be responsible for this FST variation. Populations isolated from each other have a higher FST (Chen *et al*., 2008).

C. Analysis of molecular variance

Molecular variance analysis of this finding revealed a 15% variation in breed, an 82% variation in populations, and a 3% variation in individuals. The results of this finding were inconsistent with population variation analyses in other works (Bekerie *et al*., 2015). The genetic variation within the population was higher than among populations. This might be due to gene flow, inter-population, sexual recombination, and mutation.

The lowest observed genetic distance between local ecotypes was 0.162 (between Tilili and Horro), and the largest was 0.445 (between Horro and Jarso). This might be due to the geographical location and the type of population used. The Koekoek chicken population had a greater genetic distance or less genetic similarity to the Ethiopian chicken ecotypes, and the Koekoek chicken breed was relatively high (0.676). This showed that there is still no dilution of the local Ethiopian chicken population and Koekoek chicken breeds

through agricultural extension programs or the national and regional poultry breeding institutions.

D. Cluster analysis, PCoA, and population structure

In clustering, a dendrogram of cluster analysis based on the NJ algorithm using UPGMA categorized the four chicken ecotypes into three clusters based on geographical location (C-I, C-II, and C-III) with different subgroups.The divergence may be due to the number of markersused, breeds, and sample size. The clustering model showed that there was a relationship between the patterns of genetic diversity and the geographical origins of the sample collection. It was found that the samples collected from Horro and Tilili were strongly related. Horro and Tilili chickens were close to each other, and the existence of gene flow between the neighboring populations seems possible.

Furthermore, this result was reflected in the population structure, which showed a low level of genetic mixing between populations. This indicates the presence of a sub-structure (k = 3) in four chicken populations. Previously, Bekeri *et al*. (2015) also reported a population structure with k = 3, with four local chicken populations representing South and South Western Ethiopia and two exotic chickens. There were genetic admixtures among local chicken populations. This might be due to chicken movement, uncontrolled mating and exchange reproduction, or migration from one area to another. PCoA clustering corresponds to clustering dendrogram-based, which showed consistent results obtained from the UPGMA analysis.

CONCLUSIONS

SSR markers give important insights into the genetic diversity and population structures of local chickens. According to the results of this study, a high level of genetic variation was detected in populations, their geographical origins were sorted, and distinct alleles were observed in specific populations. These findings support the notion that ecotypes are genetically distinct and the reliability of the results. However, within-ecotype variation is found to be very high, which is supported by the high heterozygosity level of microsatellite markers tested in all ecotypes. The evaluation of genetic diversity among indigenous chicken populations studied in the current study was efficient and yielded reliable results. The conservation of diverse native chicken breeds will protect genetic resources from extinction and contribute to the genetic pool of chickens as a whole. Some indigenous (local) chickens possess major genes that allow for survival in unfavorable environments. In the near future, finding indigenous chickens will be more difficult due to unintentional cross-breeding between exotic and indigenous kinds. We are losing agronomically important adaptation traits, so the national breeding program should take quick measures.

Abbreviations. AMOVA: Analysis of molecular variance, AR: Allelic richness, F: fixation index, HWE: Hardy Weinberg equilibrium, Shannon information index, MAF: major allele frequency, NJ: Neighbor joining tree, PA: Private alleles, PCOA: principal coordinate analysis, PIC: polymorphic information content, SSR: simple sequence repeat markers; UPGM: unweighted pairwise group method.

Data availability. The corresponding author will make the datasets generated or analyzed during the current investigation available upon reasonable request.

Ethical Approval. In this study, the authors did not use laboratory animals or experimental animals; instead, they collected blood samples only from the wing veins of selected chicken ecotypes. Additionally, the manuscript does not contain any clinical studies or patient data.

Authors' contributions. SHT, DM, WA, and SHK wrote up and designed the study. BA and GT read and edited the manuscript. Finally, all the authors commented on the early and final responses to the manuscript.

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