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Full Length Research Paper

Study on the genetic diversity of native chickens in northwest Ethiopia using microsatellite markers

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In this study, indigenous chicken populations representing seven different areas of northwest Ethiopia were studied using microsatellite markers to determine genetic diversity and variation. Three local lines of South African chicken and two commercial chicken strains were included for comparison. The Ethiopian chicken population Gassay/Farta had the highest number of alleles per locus (10) for microsatellite marker MCW 158. MCW 154 was the most polymorphic marker across all populations with an average of seven different alleles. A high genetic diversity was observed in overall loci for all populations with heterozygosity (Ho) value of 0.77. The highest heterozygosity (0.93) across all markers was observed in the Mecha chicken population, while the lowest heterozygosity across all loci (0.66) was observed in the White Leghorn breed. The RIR commercial chicken breed showed higher genetic distance (lower genetic similarity) with the Ethiopian chicken populations than the South African fowls. This indicates that the Ethiopian indigenous chicken populations are still not highly diluted by the RIR chicken breed either through the extension program or through the regional poultry breeding and multiplication institutes. Based on the phylogenetic tree result, it is concluded that the clustering of the chicken populations in the present study are in accordance with the origin and marketing systems of these native chickens, which indicates that the microsatellite markers used in this study were suitable for the measurement of the genetic biodiversity and relationship of Ethiopian chicken populations. These results can therefore serve as an initial step to plan the characterization and conservation of native chickens in Amhara region, Ethiopia.

Key words: Ethiopia, genetic variation, native chickens, microsatellites.

INTRODUCTION

Poultry production in Ethiopia is an important livestock sector contributing a high proportion of the protein supply for humans in the form of eggs and meat. About 98.5 and 99.2% of the national egg and poultry meat production is obtained from traditional chicken production systems (AACMC, 1984) with average annual output of 72,300 metric tones of meat and 78,000 metric tones of eggs (ILCA, 1993). It is especially favorable to small holder farmers due to its low capital requirement, high cost efficiency, flexible production systems and low production risk. Indigenous chickens are also kept for income and socio-cultural roles. Indigenous chickens are preferred to exotic chickens because of their pigmentation, taste, flavor and leanness. With regards to these important aspects, their exposure to man-made and natural risks, the indigenous chickens of northwest Ethiopia has been studied for their potential in village-based production system (Halima et al., 2007). The performance traits of these chickens have been also studied under intensive management conditions (Halima et al., 2006) and recommendations made for efficient management, breeding and utilization.

Genetic characterization contribute to breed definition

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especially populations, which are not well defined and provide an indication of the genetic diversity of these lines. It also has potential to identify unique alleles in the breeds or lines studied. Up to date no information is available on the genetic diversity of northwest Ethiopian native chickens, which are becoming important to design effective selection and conservation strategies.

The genome of the domestic chicken has a haploid number of 39 chromosomes, eight pairs of macro-chromosomes, one pair of sex chromosomes (Z and W) and 30 pairs of micro-chromosomes. The size of the chicken genome is estimated to be 1.2 X 10^9 base pairs **(**Groenen et al., 2000). Chickens, like other avian species, differ from mammals in that the female is the heterogametic sex (ZW) and the male is the homogametic sex (ZZ) (Singh, 2000). The chicken is the first bird, as well as the first agricultural animal, to have its genome sequenced and analyzed. As the first livestock species to be fully sequenced**,** the chicken genome sequence is a landmark in both avian biology and agriculture (Burt, 2005) and therefore provides a vast number of microsatellite markers for diversity studies. A number of microsatellite markers based on the degree of polymorphism and genome coverage have been recommended by the Measurement of Domestic Animals Diversity (MoDAD) FAO (2004), for application in diversity studies and detail information on the microsatellite markers are available on FAO website (www.dad-fao.org/en/refer/library/guidelin/marker.pdf).

Microsatellites are highly polymorphic tandem repeat loci with a core motif of 1 to 6 base pairs (bp) repeated several times (Tauzt, 1989). They are the preferred markers for diversity studies and have been used in a number of animal genetic variation and conservation studies, includeing birds (Crooijmans et al., 1996; Ponsuksili et al., 1996; Vanhala et al., 1998; van Marle-Köster and Nel, 2000).These markers are co-dominant and highly reproducible. This study is therefore the first attempt to study the genetic diversity of native chickens of northwest Ethiopia using microsatellite markers.

MATERIALS AND METHODS

Chicken populations

A total of 147 chickens representing seven indigenous chicken populations: Tilili (22), Gelilia (23), Debre-Elias (23), Melo-Hamusit (14), Gassay/Farta (19), Guangua (23) and Mecha (23) were collected from four administrative zones in northwest Ethiopia, for this study **(**Figure 1). Detail on the phenotypic traits and their origin have been reported by Halima et al. (2006, 2007). The Rhode Island Red (RIR) breed was included as control. Further more, blood samples from 95 chickens from four South African chicken strains namely one commercial White Leghorn (WHL) breed and three native chicken lines (Ovambo, Koekoek and Lebowa-Venda) were calculated based on the internal size standards of ROX^{TM} 500 were obtained from Glen poultry state farm, Bloemfontein, South Africa, and tested for comparison Figure 1).

Blood sample collection and DNA extraction

Blood samples from Ethiopian chicken populations were collected in 2 ml tubes containing EDTA in the form of K3E, as anticoagulant and stored at –70^{o}C until DNA extraction, while blood samples from the South African chicken lines were collected from their combs using FTA cards, and stored at room temperature.

The chicken DNA samples were extracted from 50 µl of the whole EDTA blood following the method of Sambrook et al. (1989). The frozen blood was immediately thawed at 37^{o}C for 15 min using a water bath. Seven hundred µl lyses buffer (10 mM Tris–HCl pH 8.0, 100 mM NaCl, 1 mM EDTA, pH 8.0, 0.5 % Sodium Dodecyl Sulphate (SDS)) and 20 µl of 10 mg per ml Proteinase-K were added to the aliquot and incubated overnight at 42^{o}C with gentle shaking. Thirty-three µl phenol, chloroform and isoamyl alcohol mixture at a rate of 25:24:1, respectively was added in each sample, and centrifuged for 5 min at 12000 rpm at 4^{o}C. The supernatant from each sample was collected and added into newly labeled Eppendorf tubes. Isopropanol, stored at -20^{o}C, was added to the supernatant at a rate of 75% per volume, and centrifuged at 12000 rpm for 15 min at 4^{o}C. The liquid phase was then removed by inverting the Eppendorf tubes upside down gently. DNA samples were washed by adding 1 ml of 70% ethanol, and centrifuged at 12000 rpm for 5 min. Ethanol was removed and DNA samples were dried at room temperature. Finally, DNA was diluted by adding 40 µl of 1 x TE buffer, and concentration measured at 260 nm using a spectrophotometer.

DNA extraction from FTA cards was carried out following the method describes by the WHATMAN Company (http://www.whatman.com). FTA cards containing blood samples were dried at room temperature. A piece of the dried blood sample was punched out from FTA filter paper (Whatman Bioscience) using a hole punch and placed in a 1.5 ml micro centrifuge tube. The samples were then washed three times with 200 µl FTA purification reagent. Each time, the samples were stirred manually, vortexed and the liquid was removed with a sterile pipette. The samples were again washed two times with 200 µl TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH = 8.0) in a similar manner and then dried on a heating block at 65°C. The washed and dried FTA disks were used as DNA template for the PCR reaction.

Polymerase chain reaction (PCR) preparation and amplification

A total of twenty-two microsatellite markers were donated by the Department of Animal Breeding and Genetics, Wageningen University, The Netherlands. Markers were optimized and tested for polymorphism and a final panel of seven markers was selected for genotyping of the different chicken lines (Table 1). A PCR reaction mixture with the final volume of 10 µl included 50 ng template genomic DNA, 1µl of Thermophilic DNA poly. 10 X Buffer, 2 µl of 100 mM dNTPs, 0.5 µl of each (10 pmol/µl) forward and reverse primers, 0.2 µl of 5U/µl Taq DNA polymerase, 0.6 µl of 25 mM $MgCl_2$ and double distilled water were prepared
(Table 1). The following program was run for amplification: 1 min denaturation at 95^{o}C followed by 35 cycles of denaturation at 94^{o}C for 30 s, annealing at 55^{o}C for 30 s, 72^{o}C for 30 s and an extension step of 10 min at 72^{o}C using a Thermo-Hybrid PX2 thermal cycler. Thereafter, a mixture of 1.5 µl of PCR products, 0.5 µl of ROX^{TM} 500 internal size standard and 24 µl Formamide was made, heat denatured at 95^{o}C for about 3 min and analyzed on POP-4 polymer using a 36 cm capillary with 55 injection at 15 KV and run for 28 min at 15 KV + 9 µA on ABI 310 genetic analyzer following the Applied Biosystem user manual version 2.1. The fragment sizes using the Gene Mapper ID version 3.2.

Statistical analyses were performed using POPGENE version 1.31 software (Yeh et al., 1999). The following estimations such as observed number of alleles and observed heterozygosity (H_o) were

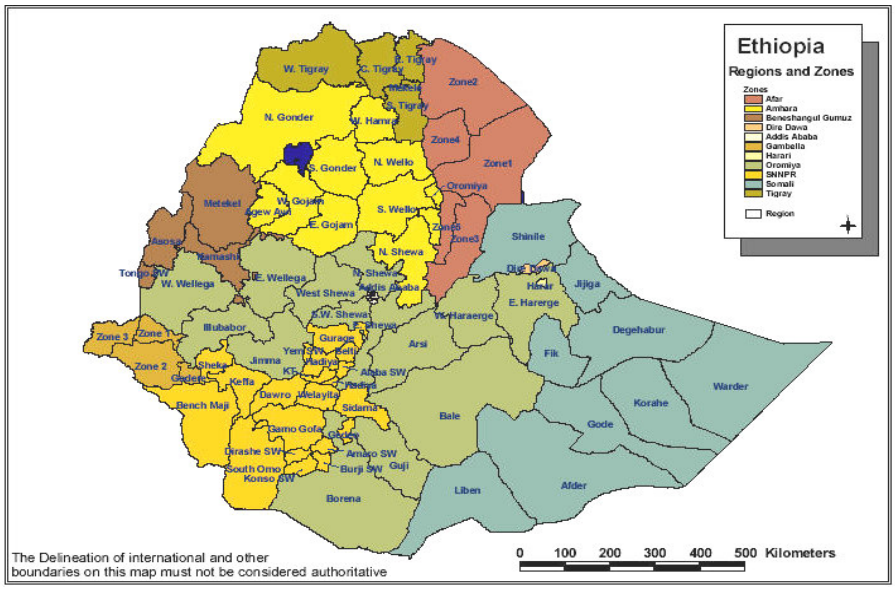

Figure 1. Map of Ethiopia indicating the study zones (South Gondar, Awi, Wes and East Gojam) of the Amhara region.

Table 1. List of microsatellite markers used for genetic variation studies in chickens.

Marker	Chromosomal location	Repeat	Dye	Annealing temperature (°C)	Size range, (bp)	Number of observed alleles
MCW 145	1	$(GTTT)_6$ $(GT)_{20}$	TET	55	150-227	24 (8)*
MCW 154	Z	$(CA)_{11}$	FAM	55	110-236	26 (13)
MCW158	8	$(GT)_{26}$ $(AT)_9$	FAM	55	156-235	27 (13)
MCW 213	13	$(AC)_{25}$	FAM	55	251-314	20 (16)
MCW 214	5	$(CA)_9$	FAM	55	233-300	17 (10)
MCW 228	10	$(GT)_{10}$	TET	55	195-261	23 (14)
MCW 238	5	$(AC)_{21}$	FAM	55	161-232	18 (4)

TET (Green), FAM (Blue), HEX (Yellow).
*-Numbers in the bracket showed the no. of distinct allele observed.

calculated using POPGENE computer program. Estimates of unbiased genetic identity and genetic distances were estimated using Nei (1978) method. The dendrogram was constructed based on Nei (1978) standard genetic distance and the Neighbor Joining (NJ) methods following Unweighted Pair-Group Method (UPGMA), which was modified from NEIGHBOR procedure of PHYLIP version 3.5. Polymorphism information content (PIC) values were calculated using the method described by Botstein et al. (1980).

RESULTS AND DISCUSSION

Genetic variation

All the seven microsatellites used in this study were found to be highly polymorphic. Despite a relatively small sample size 78 distinct alleles were observed across the seven loci tested in 12 chicken lines (Table 1). The highest number of alleles per locus (11) was observed for the Ovambo chicken population using MCW 214 locus, while the lowest number of alleles per locus (2) was recorded for Gassay and Lebowa-Venda chicken populations (MCW 214 and MCW 238). Amongst the Ethiopian chickens, Gassay/Farta chicken population had shown the highest number of alleles per locus (10) for the MCW 158 marker. MCW 154 was the most polymorphic marker across all populations with seven mean numbers of alleles. Amongst the Ethiopian chicken population, D/Elias and Melo-Hamusit chickens have shown the highest mean number of alleles across all loci (6.29) followed by Guangua (6.15). The average number of alleles across all populations in all loci was 6.05 (Table 2). Similar results with regard to the number of alleles were reported by Crooijmans et al. (1993, 1996), Cheng et al. (1995), Olowofeso et al. (2005) and Ponsuksili et al. (1996). The average number of alleles across all populations in all loci was 6.05 (Table 2). Van Marle-Köster and Nel (2000) had reported a mean number of

Table 2. Observed number of alleles and heterozygosity values for twelve chicken populations using seven microsatellite markers.

Population	Trait	Locus							Mean	St. dev
		MCW 145	MCW 154	MCW 158	MCW 213	MCW 214	MCW 228	MCW 238		
RIR	Na*	6	6	3	4	4	6	2	4.43	1.61
	H_o **	0.67	0.75	1.00	1.00	0.33	0.89	0.50	0.73	0.25
Tilili	Na	3	6	6	7	4	6	6	5.43	1.39
	H_o	1.00	0.67	0.80	0.67	1.00	0.60	0.85	0.80	0.17
Gelilia	Na	7	6	3	6	6	3	5	5.15	1.58
	H_o	1.00	0.71	1.00	0.83	0.60	1.00	0.67	0.84	0.17
D/Elias	Na	7	9	6	5	5	8	4	6.29	1.79
	H_o	1.00	1.00	0.50	0.62	0.33	0.89	0.83	0.74	0.26
Melo-Hamusit	Na	9	8	7	6	3	6	5	6.29	1.97
	H_o	0.85	1.00	0.71	0.67	0.33	1.00	0.67	0.74	0.24
Gassay/Farta	Na	4	6	10	5	2	6	5	5.43	2.44
	H_o	1.00	0.80	1.00	0.60	0.20	1.00	0.67	0.75	0.30
Guangua	Na	6	8	6	6	4	8	5	6.15	1.47
	H_o	1.00	0.83	0.80	0.80	0.40	1.00	0.67	0.79	0.20
Mecha	Na	5	4	5	5	4	8	3	4.85	1.58
	H_o	1.00	1.00	0.75	1.00	0.75	1.00	1.00	0.93	0.12
Ovambo	Na	7	7	10	9	11	7	8	8.43	1.61
	H_o	0.75	1.00	0.44	0.89	1.00	0.78	0.89	0.82	0.19
Koekoek	Na	10	9	6	8	7	5	5	7.15	1.96
	H_o	0.75	0.71	0.75	1.00	0.83	0.50	0.50	0.72	0.18
Lebowa Venda	Na	5	6	9	6	6	5	2	5.57	2.07
	H_o	0.83	1.00	0.85	1.00	0.50	0.50	0.00	0.67	0.36
WLH	Na	9	9	6	6	8	6	8	7.43	1.39
	H_o	1.00	0.71	0.33	0.37	0.71	0.50	1.00	0.66	0.27
Mean	Na	6.5	7.0	6.4	6.1	5.3	6.2	4.8	6.05	1.74
	H_o	0.90	0.85	0.74	0.79	0.58	0.81	0.69	0.77	0.23

*Na = Observed number of alleles.
** H_o = Observed heterozygosity.

alleles ranging from 2.3 to 4.3 in five chicken lines representing the “Fowls for Africa” program’ which included the Koekoek, New Hampshire Red, Naked-Neck, Lebowa-Venda and Ovambo. Wimmers et al. (2000) had detected 2 to 11 alleles per locus for the local chickens from Africa, Asia and South America. Similar results were also reported by Osman et al. (2006), Vanhala et al. (1998), Romanov and Weigend (2001) with regard to the polymorphic nature of microsatellite markers tested on indigenous chickens.

Heterozygosity was calculated to determine the genetic variation. The highest observed level of heterozygosity (0.93) was seen in the Mecha chicken population, while the lowest mean heterozygosity across all loci (0.66) was recorded for the White Leghorn breed. The mean hetereozygosity value across all loci for all populations in the present study was 0.77 (Table 2). The present result which is higher than the previous reports by van Marle-Köster and Nel (2000), Wimmers et al. (2000) and Vanhala et al. (1998) who have reported mean heterozygosity values ranging from 0.31 to 0.61, 0.45 to 0.71 and 0.29 to 0.67, respectively. The variation in results may be due to differences in geographical location, chicken types, sample sizes, laboratory as well as the sources of microsatellite markers used (Table 2).

Polymorphic information content (PIC)

The PIC values were estimated in order to assess how informative the markers are. The PIC values observed in this study was similar in trend with the PIC values reported for the various chicken populations by Olowofeso et al. (2005) and Ponsuksili et al. (1996)

In this study the average PIC value for the seven loci tested ranged from 0.59 for MCW 214 to 0.78 for MCW 154 with an average of 0.71 over all the markers. Smaller PIC values were observed for D/Elias, Melo-Hamusit and Gassay/Farta chicken lines on MCW 214 as well as for Lebowa-Venda chicken population on MCW 238 loci

Table 3. Polymorphic information content (PIC) for the twelve chicken populations using seven microsatellite.

Population	Locus							
	MCW 145	MCW 154	MCW 158	MCW 213	MCW 214	MCW 228	MCW 238	Mean
RIR	0.78	0.79	0.55	0.70	0.35	0.75	0.30	0.60
Tilili	0.55	0.72	0.73	0.80	0.70	0.77	0.72	0.71
Gelilia	0.80	0.74	0.51	0.72	0.77	0.55	0.64	0.68
D/Elias	0.75	0.81	0.72	0.68	0.49	0.81	0.62	0.70
Melo Hamusit	0.84	0.85	0.77	0.72	0.27	0.75	0.62	0.69
Gassay/Farta	0.65	0.77	0.87	0.77	0.16	0.79	0.74	0.68
Guangua	0.77	0.85	0.77	0.73	0.54	0.85	0.50	0.72
Mecha	0.75	0.67	0.65	0.71	0.52	0.86	0.50	0.67
Ovambo	0.81	0.77	0.86	0.83	0.87	0.75	0.76	0.81
Koekoek	0.80	0.82	0.80	0.83	0.81	0.62	0.64	0.76
Lebowa Venda	0.62	0.72	0.84	0.79	0.76	0.71	0.30	0.68
WHL	0.87	0.82	0.66	0.73	0.83	0.70	0.85	0.78
Mean	0.75	0.78	0.73	0.75	0.59	0.74	0.60	0.71

Table 4. The genetic Identity (above diagonal) and genetic distances (below diagonal) (Nei, 1978) of twelve chicken lines.

Population	1	2	3	4	5	6	7	8	9	10	11	12
RIR(1)	****	0.436	0.347	0.361	0.138	0.220	0.295	0.438	0.455	0.501	0.473	0.276
Tilili (2)	0.830	****	0.801	0.731	0.512	0.622	0.736	0.730	0.588	0.341	0.501	0.591
Gelilia (3)	1.057	0.221	****	0.791	0.714	0.537	0.829	0.813	0.509	0.376	0.638	0.514
Debre Elias (4)	1.019	0.314	0.234	****	0.741	0.796	0.861	0.929	0.518	0.436	0.646	0.481
Melo-Hamusit(5)	1.984	0.669	0.337	0.299	****	0.831	0.861	0.717	0.345	0.351	0.381	0.277
Gassay/Farta(6)	1.514	0.475	0.621	0.228	0.185	****	0.822	0.728	0.485	0.396	0.449	0.336
Guangua (7)	1.222	0.307	0.187	0.149	0.149	0.196	****	0.891	0.483	0.325	0.475	0.453
Mecha(8)	0.826	0.314	0.207	0.073	0.333	0.318	0.115	****	0.620	0.314	0.650	0.449
Ovambo (9)	0.787	0.531	0.675	0.657	1.064	0.723	0.728	0.478	****	0.478	0.722	0.412
Koekoek(10)	0.691	1.077	0.979	0.831	1.046	0.926	1.122	1.158	0.739	****	0.484	0.439
L. Venda (11)	0.749	0.691	0.450	0.437	0.965	0.800	0.744	0.431	0.325	0.725	****	0.348
WHL (12)	1.286	0.526	0.666	0.732	1.285	1.091	0.791	0.800	0.886	0.823	1.054	****

(Table 3). The mean PIC values over the studied chicken lines ranged from 0.60 (RIR) to 0.81 (Ovambo), while the PIC value for the Ethiopian chicken lines obtained in this study was 0.67 (Mecha) to 0.72 (Guangua) Table 3.

Genetic distance and genetic identity

The genetic distance and genetic identity matrix estimated within and between every pair of populations is presented in Table 4. The smallest genetic distance (0.073) and the highest genetic similarity (0.929) were observed between the D/Elias and Mecha populations. A high genetic distance was indicated between the two commercial breeds, WHL and RIR, of 1.3 which could be due to the fact that they were selected for different production systems (dual-purpose vs egg production). The RIR chicken population has shown higher genetic distance (lower genetic similarity) with the Ethiopian chicken populations than the South African fowls. This indicated that the Ethiopian indigenous chicken populations are still not highly diluted by the RIR chicken breed either through the extension program or through the national and regional poultry breeding institutes. Vanhala et al. (1998) evaluated the genetic variability and genetic distances between eight chicken lines using microsatellite markers and reported the smallest and the largest genetic distances of 0.117 and 1.17, respectively. Similarly, Yang et al. (2003) reported mean genetic distance ranging from 0.194 to 1.758 for Chinese indigenous pig breeds (Table 4).

Phylogenetic tree

The phylogenetic consensus tree (Figure 2) was constructed based on Nei (1978) genetic distance and neighbor-joining method. The tree clearly indicated that

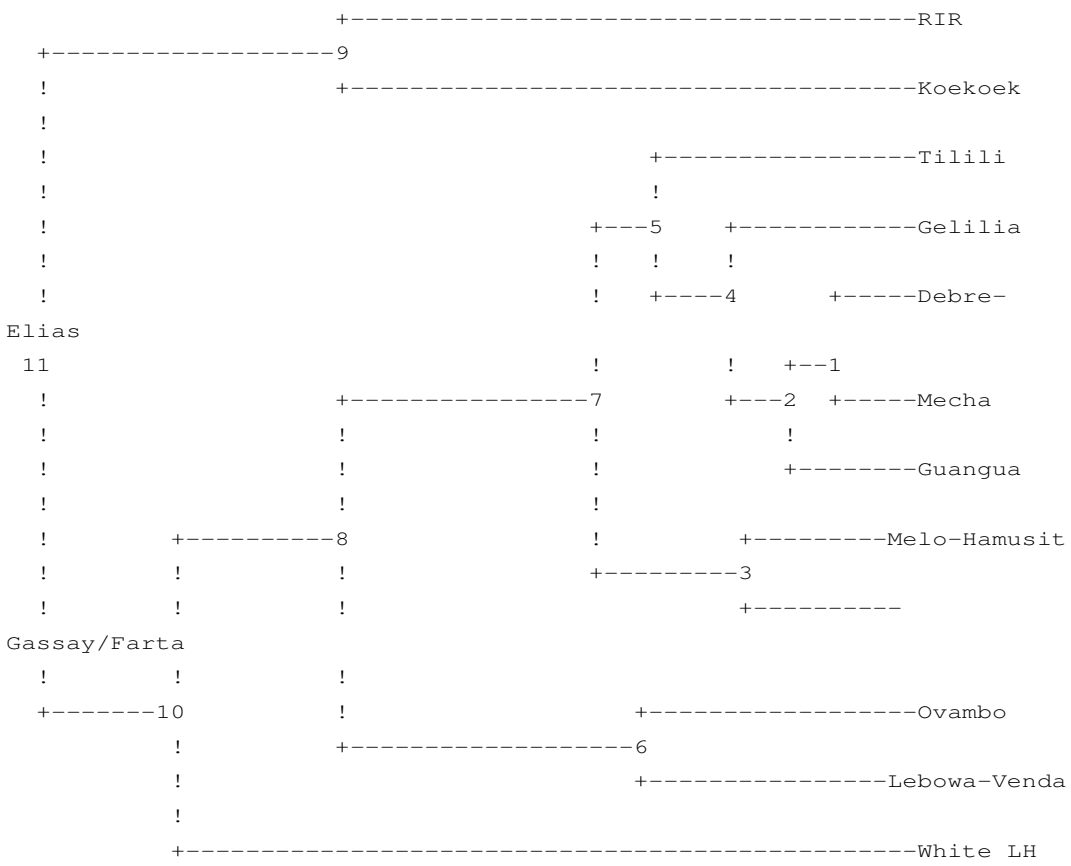

Figure 2. Dendrogram of relationships among 12 chicken lines using Nei's (1978) genetic distance and neighbor-joining methods.

the chicken populations from northwest Ethiopia could be divided into two major categories (Gojam and Gondar) with distributions running generally consistent to their geographical locations, and marketing places. All the populations collected from Gojam regions were grouped under one major cluster. The Tilili and Gelilia populations from this cluster were further divided into different sub clusters. The two populations collected from Gondar region were clustered in the Gondar category, but under separate clusters. The two South African local chickens (Ovambo and Lebowa-Venda) were clustered in the same group, while one of the local South African chicken populations (Koekoek) was grouped with RIR under one major cluster indicating that the two breeds may have intercrossed one another (Figure 2).

In this study, it can be concluded that amongst the Ethiopian chickens, the Gassay/Farta chicken population showed the highest variation with the highest number of alleles per locus (10) using the MCW 158 marker. The mean hetereozygosity value across all loci for all populations (0.77) and the mean wide range of genetic distance (0.073 – 0.669) among the indigenous Ethiopian chickens indicated the presence of substantial amount of genetic diversity. The phylogenic tree clearly indicated that the chicken populations from northwest Ethiopia could be divided into two major categories (Gojam and Gondar) with distributions running generally consistent to their geographical locations, and marketing places. The RIR chicken population has shown higher genetic distance (lower genetic similarity) with the Ethiopian chicken populations, indicating that the Ethiopian indigenous chicken populations have not yet been diluted by the RIR chicken breed either through the extension program and/or through the national and regional poultry breeding and multiplication institutes. The present study confirms the applicability and efficiency of microsatellite markers for assessing genetic variation and relatedness in local chicken populations. The further cataloging and genetic characterization of Ethiopian native chicken populations in each province could be designed as an international and/ or national project to reduce over all investment by one group, and to overcome problems associated with the use of a small number of populations, sample sizes and microsatellites for genotyping. Therefore, it is suggested that more markers and samples should be included for an extension of this type of study as it is clear that genetic diversity exist in the region and should be utilized to improve poultry productivity by designing

proper conservation and breeding strategies.

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