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Population structure and genetic diversity of Sudanese native chickens

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The objectives of this study were to analyze genetic diversity and population structure of Sudanese native chicken breeds involved in a conservation program. Five Sudanese native chicken breeds were compared with populations studied previously, which included six purebred lines, six African populations and one Sudanese chicken population. Twenty-nine (29) microsatellite markers were genotyped individually in these five populations. Expected and observed heterozygosity, mean number of alleles per locus and inbreeding coefficient were calculated. A model based cluster analysis was carried out and a Neighbor net was constructed based on marker estimated kinships. Two hundred and one alleles were detected in all populations, with a mean number of 6.93 ± 3.52 alleles per locus. The mean observed and expected heterozygosity across 29 loci was 0.524 and 0.552, respectively. Total inbreeding coefficient (F_{IT}) was 0.069±0.112, while differentiation of subpopulations (F_{ST} 0.026±0.049) was low indicating the absence of clear sub-structuring of the Sudanese native chicken populations. The inbreeding coefficient (F_{IS}) was 0.036±0.076. STRUCTURE software was used to cluster individuals to 2 ≤ k ≤ 7 assumed clusters. Solutions with the highest similarity coefficient were found at K=5 and K=6, in which Malawian, Zimbabwean, and purebred lines split from Sudanese gene pool. The six Sudanese native chicken populations formed one heterogeneous cluster. We concluded that Sudanese native chickens are highly diverse, and are genetically separated from Malawian, Zimbabwean chickens and six purebred lines. Our study reveals the absence of population sub-structuring of the Sudanese indigenous chicken populations.

Key words: Genetic diversity, microsatellites, population structure, Sudanese native chickens.

INTRODUCTION

In recent years, animal biodiversity management has become an important issue in the international community because of changes in large-scale production systems (FAO, 2007). In the absence of comprehensive breed characterization data and documentation of the origin of breeding populations, molecular marker informa-

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Abbreviations: LBZ, Large beladi from Zalingei; **LBDa,** large beladi Dammzein; **BAL,** large beladi Khartoum; **BNAb,** bare neck Abu Naama; **BNOb,** bare neck Obeid; **BT,** Betwil are six Sudanese populations; ZA, ZB, ZC, ZD, ZE , five Zimbabwe eco-types; **MA,** Malawi; **BRS_A,** broiler sire line A; **BRD_A,** broiler A; **BL_A,** brown egg layer line A; **BL_C,** brown egg layer line C; **WL_A,** white egg layer line A; **PCR,** polymerase chain reaction; **BRD,** broiler dam.

Table 1. The geographical description of the study area and sample size.

Agro-ecological zone	Rainfall (mm)	Temperature (°C)	Geographical region	Chicken population	Sample size
I-Damzein	600-800	28 - 30	High rainfall Savannah	LBDa	17
I-Abu Naama	400-600	28- 30	High rainfall Savannah	BNAb	18
II-Abassia					
II-Rashad					
II-Tajmala	600-800	26 - 28	High rainfall mountain Savannah	BTNm	36
II- Dalinj					
II- El farshi					
II-El Obeid	200-400	26 - 28	Low rainfall Savannah	BNOb	12
III-Zalingei	400-600	24 - 26	High rainfall avannah	LBZ	16

Sources: IPCC and CRU; SIM (Sudan Interagency Mapping); vmaplv0, NIMA; UN Cartographic Section. LBZ, Large beladi from Zalingei; LBDa, large beladi Dammzein; BAL, large beladi Khartoum; BNAb, bare neck Abu Naama; BNOb, bare neck Obeid; BT, Betwil are six Sudanese populations.

tion may provide reliable estimates of genetic diversity within and between a given set of populations (Zanetti et al., 2010; Granevitze et al., 2007). Molecular marker information has been used to monitor genetic diversity of populations (DeMarchi et al., 2006), and to valorize genetic resources using genetic traceability systems (Dalvit et al., 2007).

Among molecular markers, microsatellites have been intensively used over the last two decades as they are well dispersed in the genome and highly polymorphic (Cheng et al., 1995). They have been used in many countries to study the genetic relationships among local chicken breeds (Muchadeyi et al., 2007; Dalvit et al., 2009). Microsatellite markers have also been used to assess population structure and diversity of a number of native chickens in Africa (Mtileni et al., 2011; Mwacharo et al., 2011). Several molecular studies of local chicken populations in Africa have been done separately for different countries (Muchadeyi et al., 2007; Mtileni et al., 2011).

More than 1.3 billion chickens are found in Africa today, producing approximately 1.7 and 2.1 million metric tons of eggs and meat, respectively, of which 80% are from indigenous stocks (FAO, 2006). In Sudan, the traditional sector comprises 70% of the total chicken's annual production of 20.1 million birds and 900 million eggs (Sulieman, 1996). The Sudanese fowls with various types, which collectively are called Beladi (means native), were characterized by Desai (1962). These birds are commonly classified as Large Beladi (LB), Bare-Neck (BN) and Betwil (BT) ecotypes (Desai, 1962). Indigenous fowl is less productive compared to exotic breeds, but play an integral role in the smallholders farming systems. The aim of the present study was to evaluate the genetic variability within and between Sudanese native chickens, and study the level of population differentiation between Sudanese native chicken and other village chicken populations from similar extensive systems of production in Africa and pure bred populations with known breed history.

MATERIALS AND METHODS

Geographical description of the study area

Different areas were selected for samples collection, namely Damazein, Abu Naama, Abassia, Nuba Mountains El Obeid and Zalingei (Table 1). These areas are located between 10° N and 15° N latitude, 23° E and 35° E longitude, and 453 and 1350 m above sea level. The rainfall ranges from 200 to 800 mm and the average temperatures are between 24 and 30°C.

Sampling of household

Five Sudanese native chicken populations including two large Beladi chicken populations from Zalingei (LBZ =16; 6 ♀ + 10 ♂) and Damazein region (LBDa = 17; 10 ♀ + 7 ♂), two populations of Bare Neck chicken from Abu Naama (BNAb =18; 8 ♀ + 10 ♂) and EL-Obeid (BNOb = 12; 7 ♀ + 5 ♂), and one population of Betwil from Nuba Mountains (BTNm = 36; 19 ♀ + 17 ♂) were collected based on the phenotypic characteristics of each local breed (Desai, 1962). DNA samples of the sixth Sudanese population (BAL) used in this study were originally collected by Muchadeyi et al. (2007) from Khartoum state.

Reference populations

Microsatellite data of six populations were selected from AVIANDIV project. These consisted of one broiler dam (BRD) and one broiler sire (BRS) lines, two brown egg layers (BL-A and BL-C) and two white egg layers (LS-S and WL-A) with 30 individuals per population. The broiler dam and sire lines, brown egg layers and white egg layer line A (WL-A) were commercial lines, whereas the other white egg layer (LS-S) was an experimental White Leghorn line-Rs maintained at the Institute of Farm Animal Genetics as a conservation flock (Hartmann, 1987).

The pure lines were managed as closed populations with known pedigree and breed history. These characteristics made them well suited to be used as reference populations for comparison with the Sudanese chickens studied.

In addition, data of seven populations were collected from previous studies conducted by Muchadeyi et al. (2007), including five local chicken populations from Zimbabwe eco-zone ZA, ZB, ZC, ZD and ZE with sample sizes of 50, 51, 50, 50 and 37, respectively, and 60 birds from a scavenging chickens population, sampled in Malawi (MA).

Table 2. Nei's estimation of expected and observed heterozygosity, mean number of alleles per population and locus and F-statistics over all loci.

Population	H_E	H_O	No of alleles per locus	F_{IT} ± SE	F_{ST} ± SE	F_{IS} ± SE
Sudanese breeds	0.552	0.524	5.3	0.069±0.112	0.026±0.049	0.036±0.076
African breeds	0.646	0.595	6.28	0.074±0.121	0.013±0.024	0.059±0.104
Commercial breeds	0.439	0.424	3.18	0.336±0.428	0.317±0.403	0.007±0.066
Over all mean	0.546	0.514	-	0.187±0.237	0.137±0.177	0.050±0.083

$H_{E,}$ Expected heterozygosity; $H_{O,}$ observed heterozygosity; F_{IT}, total of inbreeding coefficient; F_{ST}, inbreeding of subpopulation relative to the total population; inbreeding coefficient.

Collection of blood samples and DNA extraction

From Sudanese chickens, a drop of blood was sampled from the wing vein (brachial vein) onto Whatman FTA® classic filter cards (Whatman International Ltd). Blood samples were air dried and stored in original packaging box at room temperature. Genomic DNA was extracted at the International Central Lab, Ministry of Science and Technology (Souba-Sudan) using phenol-chloroform method as described by Sambrook and Russel (2001).

DNA polymorphism

A set of 29 microsatellite markers were used to examine the genetic variability, twenty-eight of which are part of the 30 microsatellites recommended by FAO-ISAG (2004) in measurement of Domestic Animal Diversity (MoDAD) Project for assessing chickens genetic diversity. MCW80 is not included in FAO list but had been previously used together with some of FAO markers in multiplex reaction for the AVIANDIV populations. Polymerase chain reaction (PCR) was used to amplify the specific DNA fragments containing microsatellites as described elsewhere (Muchadeyi et al., 2007). The DNA fragments produced by amplification were visualized on 8% polyacrylamide gel, which was performed with a LI-COR semi-automated DNA analyser (LI-COR Biotechnology, Division, Lincoln, NE68504). Electrophoregram processing and allele-size scoring was done using the RFLPscan software package (Scanalytics, Division of CSP, Billerica, USA).

Statistical analyses

Marker polymorphism and population diversity

Total number of alleles, allele frequencies, average number of alleles per locus, observed heterozygosity, expected heterozygosity and inbreeding coefficients (F_{IS}) for each population across loci were determined using Microsatellite-Toolkit for Excel (Park, 2001). The Weir and Cockerham (1984) estimate of Wright's fixation indices (F_{IT}, F_{ST} and F_{IS}) was calculated in order to quantify the partitioning of variance between and within populations using FSTAT Software (Goudet, 2001). Standard errors for fixation indices were generated using bootstrapping over loci and population. Between populations pairwise F_{ST} estimates and Nei's standard genetic distances (Nei, 1972) were calculated.

Assignment of individuals to populations

The algorithm implemented in STRUCTURE software was used to cluster individuals based on multilocus genotypes (Pritchard et al., 2000). The analysis involved an admixture model with correlated allele frequencies. The model was tested using 20 000 iterations of burn-in phase and 50 000 iterations for each $2 \leq K \leq 7$ assumed cluster with 100 runs for each *K* value. A pairswise comparison between runs for each number of clusters defined a priory was done by SIMCOEFF software (Rosenberg et al., 2002). The solutions with over 95% similarity were considered identical. The most frequent solution for each K-value was considered to be the probable clustering for the given number of assumed groups and visualized by DISTRUCT software (Rosenberg et al., 2004). Clustering was done in two data sets, the full set of all populations under study, and the Sudanese chicken populations only.

Estimation of the optimal number of cluster in structure

To determine the optimum number of clusters for each K-value, a method described by Evanno et al. (2005) was applied to determine the number of clusters that fits best to underlying structure of these populations. In the present study, of the 100 runs for a given value of K, some outliers were detected in the distribution of log likelihoods. Runs of which the log likelihood deviated from the mean with more than three standard deviations, were removed from analyses. All removed runs showed a log likelihood that deviated downwards from the mean, indicating that these runs got stuck in a local optimum.

Marker estimate kinships

Similarity indices between and within populations were calculated from allele frequencies using Malecot's definition of similarity (Eding and Meuwissen, 2001). A network tree was constructed from the MEK using SPLITSTREE-4 software packages (Hudson and Bryant, 2006).

RESULTS

Genetic diversity within and between populations

The total number of alleles among the six Sudanese native chicken populations across all loci was 201. All microsatellite loci were polymorphic. The number of alleles PER locus ranged from three (MCW103, MCW098, MCW248, MCW1650 to 17 (LEI234). The mean number of alleles per locus for the Sudanese chicken populations was higher than that of the reference populations, and lower than that of the other African breeds (Table 2). In addition, the other African chicken breeds showed higher estimates of both expected and observed heterozygosity followed by Sudanese breeds

Table 3. Mean number of alleles per locus, expected ($H_{E.}$), observed (H_O) and F_{IS} for Sudanese chicken populations.

Population	Sample size	No of alleles ± SD	F_{IS}	H_E ± SD	H_O ± SD
LBDa	17	4.76 ± 2.0	0.054	0.560 ± 0.024	0.531 ± 0.023
LBZ	16	4.10 ± 1.4	0.093	0.507 ± 0.031	0.461 ± 0.023
BT	36	5.00 ± 2.0	0.040	0.562 ± 0.028	0.540 ± 0.015
BNAb	18	4.62 ± 2.1	0.032	0.535 ± 0.031	0.518 ± 0.022
BNOb	12	4.00 ± 1.6	0.005	0.581 ± 0.026	0.578 ± 0.027
BAL	48	5.62 ± 2.5	0.081	0.561 ± 0.025	0.517 ± 0.013

Table 4. Marker estimated kinship (above the diagonal) and pair wise F_{ST} (below the diagonal) within and between Sudanese native chicken populations.

Pop	BAL	LBDa	LBZ	BT	BNAb	BNOb
BAL	0.063	0.043	0.000	0.028	0.053	0.043
LBDa	0.008	0.069	0.005	0.039	0.049	0.048
LBZ	0.105	0.098	0.159	0.021	0.042	0.028
BT	0.030	0.011	0.076	0.061	0.036	0.046
BNAb	0.008	0.006	0.114	0.026	0.091	0.045
BNOb	0.013	0.007	0.080	0.007	0.020	0.094

BAL, Large Beladi from Khartoum; LBDa, large Beladi Damazein; LBZ, large Beladi Zalingei; BT, Betwil form Nuba Mountains; BNAb, bare neck Abu Naama; BNOb, bare neck El-Obied.

and commercial lines. The observed and expected heterozygosity estimates and inbreeding coefficient (F_{IS}) of each of the six Sudanese native chicken populations are shown in Table 3. The average observed and expected heterozygosity across 29 loci was 0.524, and 0.552, respectively. The inbreeding coefficient (F_{IS}) within populations ranged from (0.005 to 0.093). The mean inbreeding coefficient F_{IS} for the Sudanese native chicken populations was lower than that of African populations but it was greater than that of purebred lines. The mean fixation coefficient of Sudanese sub-population F_{ST} was slightly higher than that of other of African populations, but both of them were much lower than the value found for purebred lines.

Genetic distances

Kinship coefficient estimated within and between the Sudanese chicken populations (above the diagonal) as well as pairwise F_{ST}-value below the diagonal) are shown in Table 4. The highest kinship was calculated between individuals within LBZ (0.159), while lowest kinship was estimated between BAL and LBZ (o.ooo). Pairwise-F_{ST}-value of Sudanese chicken populations revealed the lowest genetic differentiation (0.006) between LBDa and BNAb populations while it was highest between LBZ and BNAb (0.114) populations.

Cluster analysis

Results STRUCTURE clustering are displayed in Figure 1. At *K* = 2, the six Sudanese native chicken populations clustered together with the five Zimbabwean populations, the Malawian chicken population and the two broiler lines, whereas the two white egg layer lines and the two brown egg layer lines formed a separate group. At *K* = 3, for the most likely solution (N = 29) the six Sudanese native chicken populations split from African chickens while the broiler lines clustered with African chickens from Zimbabwe and Malawi. At *K* = 4, the white egg layers and the brown egg layers split from each other. The most stable solutions with the highest similarity coefficient between runs (97 and 99 identical runs, respectively) were observed at *K* = 5 and *K* = 6, respectively.

At *K* = 5, the purebred lines (the white egg layers, brown egg layers and broilers) and the six Sudanese native chicken populations clustered into four distinct clusters and were separated from the other African populations. At *K* = 6, the Sudanese chicken, Malawian chicken, and purebred chicken lines made up independent clusters whereas the five Zimbabwean eco-types grouped together.

At *K*=7 (and above *K*=8, 9 data not shown) similarity coefficients decreased considerably. The purebred lines and Malawi chickens remained as homogenous and distinct clusters, while the five Zimbabwe eco-types appeared as a heterogeneous group. At these *K* values, the six Sudanese chicken populations did not show any sub-structuring. In the same manner, analysis was done for the Sudanese populations only and results supported the non-existence of a population sub-structure (data not shown).

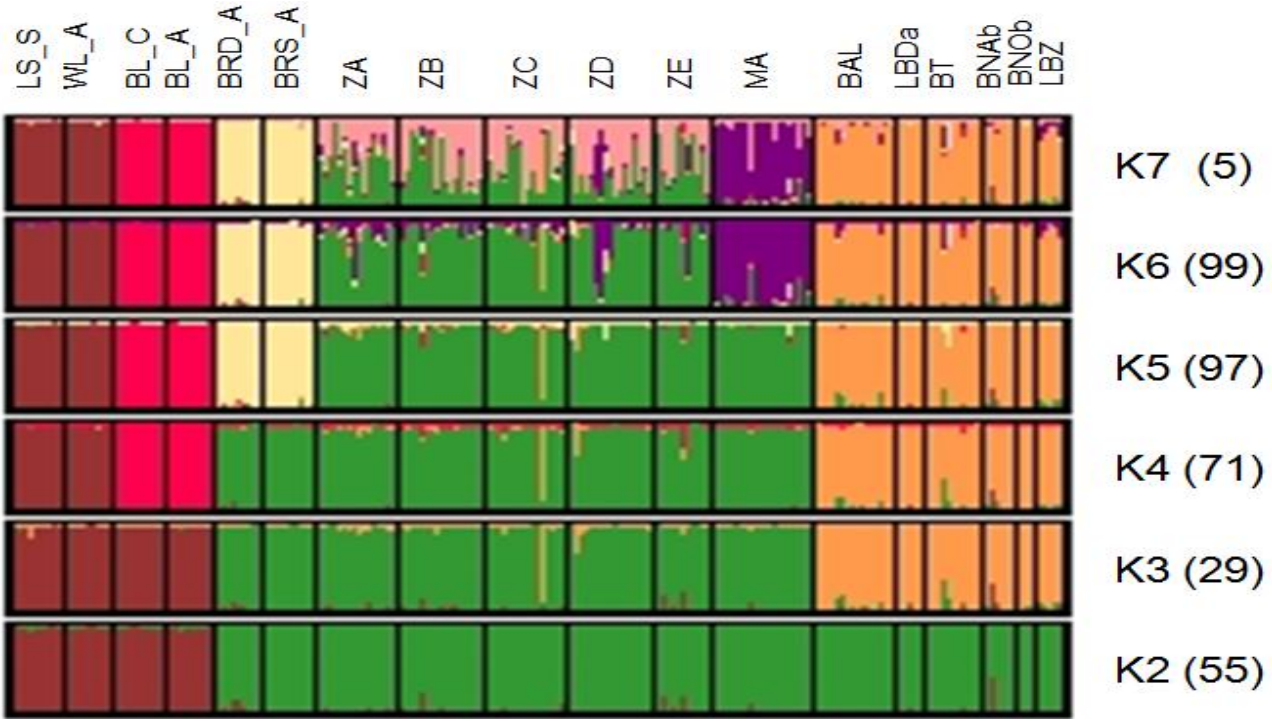

Figure 1. STRUCTURE clustering of Sudanese native chickens in reference to the extensively raised chickens (Zimbabwian and Malawian) and purebred lines (broilers, white and brown egg layers). LBZ, Large beladi from Zalingei; LBDa , large beladi Dammzein; BAL, large beladi Khartoum; BNAb, bare neck Abu Naama; BNOb, bare neck Obeid; BT, Betwil are six Sudanese populations; ZA, ZB, ZC, ZD, ZE , five Zimbabwe eco-types; MA, Malawi; BRS_A , broiler sire line A; BRD_A , broiler A; BL_A , brown egg layer line A; BL_C brown egg layer line C; white egg Layer experimental line and WL_A , white egg layer line A.

Network tree

Network tree based on marker estimated kinships is shown in Figure 2. The clustering shows the separation of broiler lines from the layer lines, with the African populations being clustered in between. Sudanese chicken populations from their own separate cluster, shows LBZ being the most distinct population.

DISCUSSION

Sudanese native chickens were highly polymorphic compared to purebred lines. The average number of alleles per locus for the Sudanese native chicken populations was lower than that for Zimbabwean chicken ecotypes and other African chickens as reported earlier using the same 29 microsatellite markers (Muchadeyi et al., 2007; Granevitze et al., 2007), and for Italian local chicken breeds (5.6 ±2.1) (Zanetti et al., 2010; Bianchi et al., 2011), whereas it was higher than that estimated for the Japanese-native chickens (Nagoya breed) which ranged from 2.35 to 2.85 (Tadano et al., 2012). Variation in number of alleles per locus could be due to sample size such that sampling strategies of each study should be taken into consideration when comparing results from different studies. In addition, African chicken breeds showed higher Nei's estimation of expected and observed heterozygosity followed by Sudanese breeds and commercial breeds. The average observed and expected heterozygosity across 29 loci for Sudanese native chicken populations was higher than the Japanese-native chickens (Nagoya breed), the average observed and expected heterozygosity was 0.438 and 0.433, respectively (Tadano et al., 2012). Granevitze et al. (2007) studied 65 populations using 29 markers and found the expected heterozygosity was 0.52 which was lower than that of Sudanese chicken breeds. On the other hand, the high heterozygosity levels, ranging from 0.51 to 0.67, were reported for chickens across Cameroon, Benin, Ghana, Cote d'Ivoire, and Morocco, corresponding to the values usually found in scavenging populations worldwide (Leroy et al., 2012). Mtileni et al. (2011) reported that village chicken populations were more diverse than conservation flocks. Sudanese indigenous chickens in different rural districts are raised under a typical extensive production system with feed scarcity, disease prevalence and absence of extension services

The F_{IS} values indicate a reduction of the observed heterozygosity compared to what is expected under random mating and serves as an indication of inbreeding

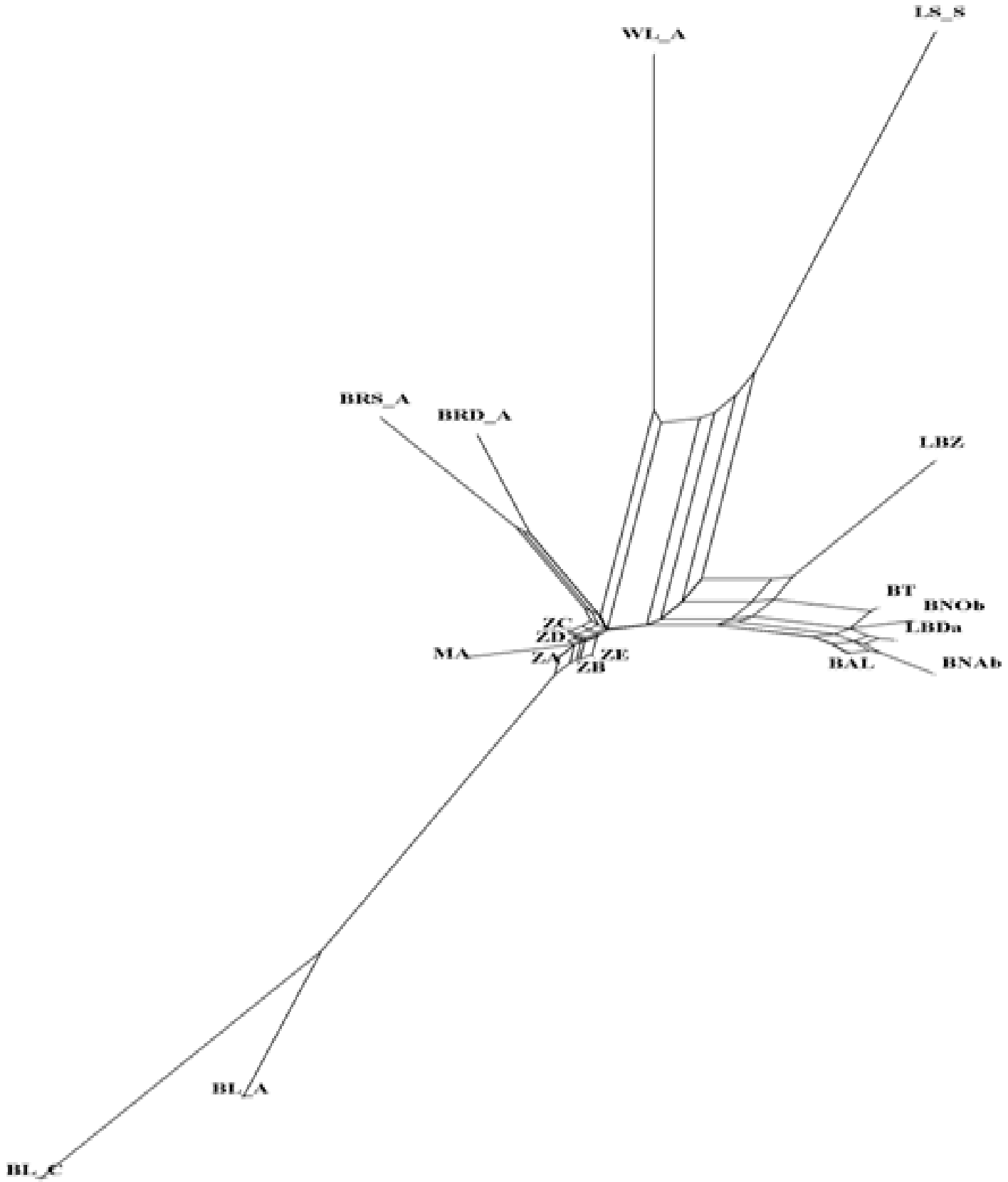

Figure 2. Network tree derived from marker estimated kinships. LBZ, Large beladi from Zalingei; LBDa , large beladi Dammzein; BAL, large beladi Khartoum; BNAb, bare neck Abu Naama; BNOb, bare neck Obeid; BT, Betwil are six Sudanese populations; ZA, ZB, ZC, ZD, ZE , five Zimbabwe eco-types; MA, Malawi; BRS_A , broiler sire line A; BRD_A , broiler A; BL_A , brown egg layer line A; BL_C brown egg layer line C; white egg Layer experimental line and WL_A , white egg layer line A.

within the population (Hartl, 1998). The mean F_{IS} for the Sudanese native chicken populations was lower than that of other African populations but was greater than that of the purebred lines. Pure lines are managed as closed populations but nevertheless Fis estimates are lower than in Sudanese chickens, this might be due to sub-strucuture within the populations avoiding non-random mating (Wahlund effect). The exotic Wyandotte chicken breed was introduced into Sudan earlier in 1926 by a British Veterinarian to improve poultry production. Some other exotic breeds such as White Leghorn, Rhode Island Red, New Hampshire and Sussex were imported shortly after the establishment of Kuku Research Centre in 1962. Recently, several large scale integrated poultry projects have been established using a number of modern commercial chicken strains. In the present study, the marked difference in F_{IS} between the Sudanese chicken populations and the reference pure bred lines implies the fact that these groups were selected from areas that have not been subjected to governmental programs of upgrading indigenous chickens with exotic strains.

On the other hand the subdivision of the lines (F_{ST}), as an indication of genetic differentiation among the lines, revealed a moderate to high differentiation among these groups. Population differentiation as determined from pair-wise F_{ST} values between all combinations of six Sudanese native chicken populations was low but it is slightly greater than that obtained for other African populations. The smallest and largest genetic distances were obtained for LBDa vs BNAb and LBZ vs BNAb, respectively. These patterns of distances may relate to differences among the agro-ecological zones. The LBDa and BNAb populations were collected from almost similar zone with short distance apart where nomadic herders move freely with their herds and carrying with them their animal companions including birds, therefore admixture of the flocks are possible. On the other hand, BLZ population was obtained from a different agro-ecological zone of the Western part of Sudan. This area is surrounded by the Marrah mountain which considered as the highest mountainous region in Sudan (10000 Feet above the sea level), thus representing a natural geographical barrier and minimizing the possibility for any flocks exchange. Eltanany et al. (2011) assessed the genetic diversity of three Egyptian local chicken strains (Fayoumi, Dandarawi and Sinai) and six synthetic breeds derived from Fayoumi and Sinai, and showed that the global inbreeding (F_{IT}) was 0.11, among-population differentiation (F_{ST}) was 0.07, and within-population differentiation (F_{IS}) was 0.04.

Applying the method of Evanno et al. (2005) suggested the most stable clustering solutions at K = 5 and K = 6. Mtileni et al. (2011) found the most probable clustering at K = 5 (95% identical runs) in South African indigenous chicken populations. Structure based clustering further supports the low level of differentiation among the Sudanese native chicken. The lack of observed sub-structuring among Sudanese native chicken populations at K value = 6 suggests that the Sudanese native chickens do not separate into different sub-population. The early separation of the Sudanese chickens at $K \leq 3$ from the African gene pool and purebred lines in STRUCTURE based clustering suggest the differentiation of the Sudanese native chickens from populations located in the southern part of the African continent. Leroy et al. (2012) indicated that from K = 2, most African chicken populations appeared clearly differentiated from commercial lines and the Moroccan population, with Cameroon chicken populations showing intermediate results but indicated that, these results could not be generalized for African chicken populations at the individual level, and there was a relatively high heterogeneity of membership coefficients within populations, particularly in comparison with commercial lines (Leroy et al., 2012). Sudanese chickens make up a gene pool that is separated from other African chickens as well as pure bred lines.

Conclusions

We concluded that Sudanese native chickens are less diverse, and are genetically separated from Malawian, Zimbabwean and six purebred lines. Microsatellite marker revealed the absence of population sub-structuring in Sudanese native chickens.

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