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Absence of population substructuring in Zimbabwe chicken ecotypes inferred using microsatellite analysis

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Summary

The objective of this study was to investigate the population structure of village chickens found in the five agro-ecological zones of Zimbabwe. Twenty-nine microsatellites were genotyped for chickens randomly selected from 13 populations, including the five eco-zones of Zimbabwe ($n = 238$), Malawi ($n = 60$), Sudan ($n = 48$) and six purebred lines ($n = 180$). A total of 280 alleles were observed in the 13 populations. Forty-eight of these alleles were unique to the Zimbabwe chicken ecotypes. The average number (±SD) of alleles/locus was 9.7 ± 5.10. The overall heterozygote deficiency in the Zimbabwe chickens (F_{IT} ± SE) was 0.08 ± 0.01, over 90% of which was due to within-ecotype deficit (F_{IS}). Small Nei's standard genetic distances ranging from 0.02 to 0.05 were observed between Zimbabwe ecotypes compared with an average of 0.6 between purebred lines. The STRUCTURE software program was used to cluster individuals to $2 \leq K \leq 7$ assumed clusters. The most probable clustering was found at $K = 6$. Ninety-seven of 100 STRUCTURE runs were identical, in which Malawi, Sudan and purebred lines split out as independent clusters and the five Zimbabwe ecotypes clustered into one population. The within-ecotype marker-estimated kinships (mean = 0.13) differed only slightly from the between-ecotype estimates. Results from this study lead to a rejection of the hypothesis that village chickens are substructured across agro-ecological zones but indicated high genetic diversity within the Zimbabwe chicken population.

Keywords chicken ecotypes, genetic diversity, microsatellites, population structure.

Introduction

Indigenous chickens are an important contribution to the livelihoods of smallholder families in Africa (Anderson 2003). In spite of their advantages to households, the existence of local chickens is threatened by a number of factors. In Zimbabwe, for example, commercial chicken production contributes 55% of the total chicken population and makes use of exotic genetic resources (Faranisi 1995; Mhlanga *et al.* 1999). The dependency on imported breeds sidelines the village chickens to communal small-scale subsistence farming. The lack of inventory data, particularly for the indigenous chicken populations, is a sign of negligence and poses a threat to poultry genetic resources (Weigend & Romanov 2002).

The use of ecotypes to describe village chicken populations is common in most village chicken production systems (Msoffe *et al.* 2001) and have been used as a sampling framework in previous diversity studies (Wimmers *et al.* 2000). The local chickens in Zimbabwe and other developing countries consist of different phenotypic strains (Mhlanga *et al.* 1999; Msoffe *et al.* 2001; Tadelle *et al.* 2003; McAinsh *et al.* 2004) raised by communal farmers across distinct agro-ecological zones. Within eco-zones, subpopulations can be formed through selective breeding of

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distinct phenotypes. In addition, geographical isolation of the populations could lead to substructuring through drift, mutation and different natural selection forces. However, it is not known whether these ecotypes represent genetically distinct populations. Characterization of genetic structure and variation of local populations is an important step towards identifying unique and valuable genetic resources.

Genetic marker polymorphisms are a reliable way of assessing the differences within and among chicken populations. Within-population diversity is an important component of species variation, particularly in domesticated species (Caballero & Toro 2002). Between-population diversity is usually assessed using genetic distance measures (Nei 1972; Reynolds *et al.* 1983). Alternatively, mean kinships between populations (Eding & Meuwissen 2001) provide a statistic that relates directly to quantitative genetic variation. Clustering individuals into populations based on their genotypic data (Pritchard *et al.* 2000) allows one to interpret group relations without *a priori* definitions of breeds and lines.

The aim of this study was to characterize the genetic differentiation within- and between-Zimbabwe chicken populations sampled from different eco-zones and to relate the extent of differentiation to other African and purebred populations. Data on microsatellite genotypes in Zimbabwe chicken populations were compared with two other African chicken population and purebred lines. A number of alternative methods were used to investigate differentiation among indigenous Zimbabwe chicken ecotypes.

Materials and methods

Zimbabwe ecotypes

Five local chicken ecotypes were obtained from Zimbabwe. Zimbabwe has an area of 390 757 km^2 and extends from latitude 15°47′S to 22°24′S and from longitude 25°14′E to 33°04′E. It is landlocked, and altitude ranges from 197 to 2592 m above sea level. The five agro-ecological zones (I–V) vary in rainfall distribution (>1000 mm per annum in eco-zone I and <450 mm per annum in eco-zone V) and temperatures (mean temperature = 15 °C in eco-zone I and >35 °C in eco-zone V). Five districts (Risitu, Hurungwe, Gutu, Gokwe-South and Beitbridge) in agro-ecological zones I through V (ECO-I to ECO-V respectively) were used for this study. Fifty chickens were sampled in eco-zones I, III and IV while 51 and 37 chickens were sampled for eco-zones II and V respectively. For each eco-zone, one chicken was sampled per household and 2–5 villages were selected for each district. Ten households were selected in each village. These chickens have not been formally selected for any commercial production traits and are raised by communal farmers under a scavenging system of production. They are characterized by high morphological variation.

Reference populations

Six populations were selected from the AVIANDIV[1] project, a European collaborative project on chicken biodiversity. These consisted of broiler dam (BRD) and 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 the white egg layer line A (WL_A) were commercial lines. The other white egg layer (LS_S) was the experimental White Leghorn line_Rs maintained at the Institute for Animal Breeding as a conservation flock (Hartmann 1997). The purebred lines are managed as closed populations with known pedigree and breed histories. These characteristics made them well suited to be used as reference populations in comparison with extensively raised chickens from Zimbabwe.

Sixty scavenging chickens that were sampled from a 50-km radius in Malawi (MAL) and 48 Sudanese (SUD) chickens from a similar extensive system of production were also used. Similar to Zimbabwe chicken ecotypes, Malawi and Sudanese chickens have not been selected for any particular production traits and show high levels of phenotypic heterogeneity. The geographical coordinates of Malawi are 13°30′S and 34°00′E while Sudan is located at 15°00′N and 30°00′E. The large geographic distances, the mountains and rivers separating the countries and, more importantly, the official border, restrict the exchange of genetic material among the African countries.

Collection of blood samples and DNA isolation for the Zimbabwe ecotypes

A drop of blood was sampled from the wing vein of each bird onto Whatman FTA® filter cards (Whatman International Ltd), dried and stored in an aluminium foil envelope at room temperature. DNA isolation was carried out using the phenol-chloroform method (Sambrook & Russell 2001).

DNA polymorphisms

A set of 29 microsatellite markers (Table 1) were used to examine genetic variability. Twenty-eight of these were part of the 30 microsatellites recommended by the FAO (http://dad.fao.org/en/refer/library/guidelin/marker.pdf) MoDAD project for assessing chicken genetic diversity. *MCW80* was not included in the FAO list but had been previously used together with some of the FAO markers in the multiplex reactions for the AVIANDIV populations.

Multiplex PCR was carried out according to FAO recommendations (http://dad.fao.org/en/refer/library/guidelin/

[1]AVIANDIV EC Contract No. BIO4-CT98-0342 (1998–2000); S. Weigend (Coordinator), M.A.M. Groenen, M. Tixier-Boichard, A. Vignal, J. Hillel, K. Wimmers, T. Burke and A. Mäki-Tanila (http://w3.tzv.fal.de/aviandiv).

Table 1 Observed allele size ranges and number of alleles in all populations and the number and frequency of alleles unique to the Zimbabwe ecotypes.

	All 13 populations		Zimbabwe population	
Locus	Allele range (bp)	No. alleles ($N = 526$)	No. alleles ($N = 238$)	Unique alleles
ADL112	122–134	7	6	
ADL268	104–116	7	6	
MCW330	256–290	9	6	
MCW295	88–108	9	9	108 (0.42)[1]
MCW248	207–223	6	3	
MCW222	220–226	4	4	
MCW216	137–149	7	7	137 (0.84)
MCW206	221–249	14	11	233 (0.42); 249 (0.84)
MCW183	296–326	15	12	297 (4.20); 309 (0.42); 326 (0.42)
MCW165	114–118	3	3	
MCW123	76–94	10	9	76 (2.10); 84 (0.84); 94 (5.46)
MCW111	98–114	7	6	114 (0.42)
MCW104	190–228	17	17	198 (0.42); 212 (1.26); 216 (0.42); 228 (0.84)
MCW103	262–274	4	4	262 (0.42); 274 (0.42)
MCW98	261-265	3	2	
MCW081	112–145	11	10	141 (0.42); 131 (0.42); 133 (0.84); 145 (0.42)
MCW080	266–282	14	11	272 (3.36); 273 (0.42); 282 (1.26)
MCW078	135–145	6	5	
MCW069	158–176	9	9	
MCW067	176–190	8	7	182 (0.42) 188 (1.68)
MCW037	154–160	7	6	157 (10.01); 159 (3.78)
MCW034	214–246	15	13	214 (2.95); 244 (0.84)
MCW020	179–185	4	5	
MCW016	170–204	11	11	176 (0.84); 184 (1.68); 186 (2.10); 198 (0.84); 204 (0.84)
MCW014	160–182	12	8	
LEI234	216–368	24	22	256 (1.26); 260 (1.26) 311 (1.68); 368 (0.42) 356 (2.10)
LEI166	350–366	7	5	354 (2.94)
LEI094	245–289	20	18	245 (0.42); 253 (6.72); 273 (4.20); 277 (0.42)
ADL278	114–123	10	5	115 (8.40); 117 (0.84); 121 (0.84)
Total		280	240	48

[1]Value in brackets indicate the absolute frequency (%) of the unique alleles found in the Zimbabwe chicken gene pool ($N = 238$).

marker.pdf). Electrophoregram processing and allele-size scoring were performed with the RFLPSCAN software package (Scanalytics). The reference populations were already typed in previous projects. The genotyping of the Zimbabwe ecotypes was performed in the same laboratory as the reference populations, and standard alleles were used to adjust the allele scores appropriately.

Marker polymorphisms and within-population diversity

Total number of alleles, allele frequencies, average number of alleles per locus, observed heterozygosity, expected heterozygosity and inbreeding coefficients (F_{IS}) per population were determined using the FSTAT version 2.9.3 (http://www.unil.ch/izea/softwares/fstat.html) software package. The Weir & Cockerham's (1984) estimations of Wright's (1951) fixation indices (F_{IT}, F_{ST} and F_{IS}) were calculated in order to quantify the partitioning of variance between and within populations. Standard errors for the fixation indices were generated using jacknifing over loci and populations using the FSTAT software.

Among-population diversity

Pairwise F_{ST} (proportion of genetic variability due to population substructuring) values were computed for all pairs of the 13 populations using the FSTAT software package. Nei's standard genetic distances (Nei 1972) were estimated among pairs of populations using the PHYLIP software (http://evolution.genetics.washington.edu/phylip.html). Mean genetic distances among the groups (Zimbabwe, other African and purebreds) were estimated using JMP version 5.1 (JMP 2003; SAS Institute Inc.).

Assignment of individuals to populations

The algorithm implemented in STRUCTURE 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 (burn-in phase) and then 50 000 iterations for $2 \leq K \leq 8$ with 100 runs for each K value. K was the number of assumed clusters to be examined. A pairwise comparison of the 100 solutions was carried out using SIMCOEFF software (Rosenberg *et al.* 2002). Solutions with over 95% similarity were considered identical. The most frequent solution was considered to be the most probable, and the clustering pattern was visualized using DISTRUCT software (Rosenberg 2004).

Marker-estimated kinships

Similarity indices between and within populations were calculated from allele frequencies using Malecot's definition of similarity (Eding & Meuwissen 2001):

$$S_{ij} = \sum \left(p_{i,x} p_{j,x}\right),$$

where $p_{i,x}$ was the xth allele frequency in population i and $p_{j,x}$ was the xth allele frequency in population j. These similarity indices were subsequently used to calculate marker-estimated kinships (MEK) among populations using a weighted log-linear model (Eding & Meuwissen 2003):

$$\log\left(1 - S_{ij,L}\right) = \log(1 - f_{ij}) + \log(1 - s_L),$$

where $S_{ij,L}$ was the average similarity between population i and j for L loci, f_{ij} was the kinship coefficient between population i and j and s_L was the probability of alleles identical-in-state. In this model, observations on allele frequency similarities per locus and pairs of populations were weighted with the expected error variance of the similarity indices to account for variation in the informativeness of different loci. In order to construct a phylogenetic tree, the MEK values were converted to kinship distances using the formula:

$$D(i,j) = \hat{f}_{ii} + \hat{f}_{jj} - 2\hat{f}_{ij},$$

where $\hat{f}_{ii}$ and $\hat{f}_{jj}$ were kinship estimates within population i and j respectively. $\hat{f}_{ij}$ was the kinship estimate between population i and population j (Mateus *et al.* 2004). A phylogenetic tree was constructed using the Neighbour-Joining method (Saitou & Nei 1987), with the broiler sire line (BRS) as the out-group, using the PHYLIP software package (http://evolution.genetics.washington.edu/phylip.html).

Results

Marker polymorphisms and within- and among-population diversity

All microsatellite loci typed were polymorphic. The number of alleles per locus for the 13 populations, and for the five Zimbabwe ecotypes alone, are given in Table 1. A total of 280 alleles were observed. The average number of alleles (±SD) was 9.7 ± 5.10 per locus. Expected heterozygosity (±SD) was 0.7 ± 0.02 while the observed heterozygosity (±SD) was 0.5 ± 0.04. The five ecotypes of Zimbabwe yielded 240 alleles with an average (±SD) of 8.4 ± 4.72 alleles/locus. Forty-eight of the observed alleles were unique to the Zimbabwe chicken ecotypes. Twenty-eight of these unique alleles occurred at a frequency of <1% while the allele frequency of the remaining 20 ranged from 1.3% to 10.0%.

The average number of alleles per locus, the expected and observed heterozygosity values and the F_{IS} value for each of the 13 populations are given in Table 2. The average number of alleles/locus (±SD) ranged from 2.8 ± 1.3 in the purebred line (WL_A) to 6.7 ± 3.8 in the Zimbabwe

Table 2 Mean number of alleles per locus, number of unique alleles, expected (H_E) and observed (H_O) heterozygosity and inbreeding coefficient (F_{IS}) per population.

Population	*N*	Alleles/locus (±SD)	Unique alleles	H_E ± SD	H_O ± SD	F_{IS}
Eco-I	50	6.7 ± 3.8	8 (2.0–8.0)[1]	0.642 ± 0.026	0.590 ± 0.013	0.083*
Eco-II	51	6.1 ± 2.9	5 (2.0)	0.650 ± 0.026	0.605 ± 0.013	0.070*
Eco-III	50	6.2 ± 3.2	5 (2.0–6.0)	0.647 ± 0.026	0.594 ± 0.013	0.083*
Eco-IV	50	6.4 ± 3.5	4 (2.0–4.0)	0.656 ± 0.024	0.598 ± 0.013	0.090*
Eco-V	37	6.2 ± 3.3	1 (2.7)	0.661 ± 0.023	0.625 ± 0.015	0.055*
MAL	60	5.9 ± 3.0	12 (1.7 –11.7)	0.607 ± 0.029	0.554 ± 0.012	0.088*
SUD	48	5.6 ± 2.5	4 (2.1–8. 3)	0.561 ± 0.025	0.517 ± 0.013	0.081*
LS_S	30	2.9 ± 1.1	1 (3.3)	0.355 ± 0.038	0.332 ± 0.016	0.067*
WL_A	30	2.8 ± 1.3	2 (2.3)	0.338 ± 0.039	0.309 ± 0.016	0.086*
BL_C	30	2.9 ± 1.1	0	0.393 ± 0.038	0.399 ± 0.017	–0.015
BL_A	30	2.9 ± 1.2	0	0.418 ± 0.039	0.391 ± 0.017	0.065*
BRD	30	4.8 ± 1.9	6 (3.3–20.0)	0.626 ± 0.023	0.614 ± 0.017	0.019
BRS	30	3.8 ± 1.5	0	0.547 ± 0.035	0.526 ± 0.017	0.039*

*Significantly different from zero at $P < 0.05$.

[1]Minimum and maximum allele frequency (%) for the unique alleles in each population.

Table 3 Overall-population (F_{IT}), between-population (F_{ST}) and within-population (F_{IS}) inbreeding coefficients of the Zimbabwe, African (Malawi, Sudan and Zimbabwe) and purebred populations[1].

Population	F_{IT} ± SE	F_{ST} ± SE	F_{IS} ± SE
Zimbabwe	0.084 ± 0.012	0.008 ± 0.012	0.077 ± 0.012
African	0.115 ± 0.013	0.039 ± 0.004	0.079 ± 0.011
Purebred	0.383 ± 0.024	0.357 ± 0.020	0.041 ± 0.001
Overall	0.218 ± 0.014	0.159 ± 0.010	0.070 ± 0.009

*$P < 0.05$.

[1]The *F*-statistics were calculated according to Weir & Cockerham (1984) estimations.

chicken Ecotype I. Greater expected and observed heterozygosity estimates were found in the Zimbabwe ecotypes compared with the purebred lines.

The mean F_{IT}, F_{IS} and F_{ST} estimates per population of the five Zimbabwe ecotypes, the three African populations and the six purebred lines, are given in Table 3. The overall population heterozygote deficiency [F_{IT} (±SE)] was 0.218 ± 0.014. A hierarchical analysis of F_{IT} showed that the heterozygote deficiency was greatest in the purebred lines [F_{IT} (±SE) $= 0.383 \pm 0.024$] followed by the African (Zimbabwe, Malawi and Sudanese) and least in the Zimbabwe [F_{IT} (±SE) $= 0.084 \pm 0.012$] population. A contrast in the distribution of within- and between-population variation (F_{ST} vs. F_{IS}) was observed between African populations, in particular between Zimbabwe ecotypes and the purebred lines. For the purebred lines, high F_{ST} and low F_{IS} were found. In contrast, almost all of the F_{IT} was accounted for by the within-ecotype heterozygote deficiency (F_{IS}) in the Zimbabwe population, with corresponding low F_{ST} estimates.

Pairwise genetic distances

Low (0.01 ± 0.01) mean (±SD) pairwise F_{ST} values were observed between pairs of the Zimbabwe ecotypes compared with a mean (±SD) of 0.36 ± 0.09 between purebred lines (Table 4). Nei's standard genetic distance estimates among the Zimbabwe chicken ecotypes, brown egg layers, white egg layers and broiler dam and BRSs are also given in Table 4. Small genetic distances ranging from 0.03 to 0.05 were observed between pairs of the Zimbabwe ecotypes. The genetic distances were larger [mean (±SD) $= 0.12 \pm 0.037$] between the other African populations (Malawi and Sudan) and Zimbabwe ecotypes, and largest [mean (±SD) $= 0.61 \pm 0.183$] between pairs of purebred lines.

Cluster analysis

The results of the STRUCTURE clustering are displayed in Fig. 1. At a lower number of assumed clusters ($K = 2$ and 3), the Zimbabwe ecotypes clustered together with the Malawi, Sudanese and the two broiler lines. At $K = 2$, two solutions with approximately equal frequencies were observed. Both placed the white egg layers into one group and the two broiler lines, and African populations in the second cluster. At $K = 3$, the most frequent ($N = 71$) solution showed the white and brown egg layers split to form two distinct gene pools, while the broiler lines clustered with the African chickens. The solutions with the highest similarity coefficient (94 identical runs) were observed at $K = 4$ and at $K = 6$. At $K = 4$, the purebred lines clustered into three distinct clusters (white egg layers, brown egg layers and broiler lines) separate from the African gene pool. At $K = 6$, the Malawi, Sudanese and purebred lines clustered as independent clusters and the five Zimbabwe ecotypes gave one cluster. Above $K = 6$, the similarity coefficient dropped dramatically. The reference populations remained as distinct clusters, while individuals in the Zimbabwe ecotypes were randomly assigned to any of the added K clusters without showing any substructuring between ecotypes.

Marker-estimated kinships

Marker-estimated kinships within and between the populations are given in Table 4. The within-population MEK for the Zimbabwe ecotypes did not differ very much from the between-ecotype MEK estimates. The mean MEK (±SD) value within ecotypes was 0.130 ± 040, while the mean between-ecotype estimate was 0.110 ± 0.005. The latter estimate was slightly elevated in comparison with MEK

Table 4 Mean Nei's standard genetic distances, pairwise F_{ST} and marker estimated kinships (MEK) within and between the Zimbabwe five ecotypes, Malawi and Sudanese chickens and purebred lines.

Population category	Nei's standard genetic distance	Pairwise F_{ST}	MEK
Within Zimbabwe ecotypes	–	–	0.13 ± 0.04
Within Malawi and Sudanese	–	–	0.22 ± 0.06
Within purebreds	–	–	0.58 ± 0.04
Between Zimbabwe ecotypes	0.04 ± 0.01	0.01 ± 0.01	0.11 ± 0.01
Between Malawi and Sudan	0.24	0.13	0.11
Between purebreds	0.61 ± 0.18	0.36 ± 0.09	0.12 ± 0.02
Between Zimbabwe and Malawi and Sudan	0.12 ± 0.04	0.05 ± 0.03	0.11 ± 0.03
Between Zimbabwe and purebreds	0.35 ± 0.09	0.19 ± 0.07	0.08 ± 0.02

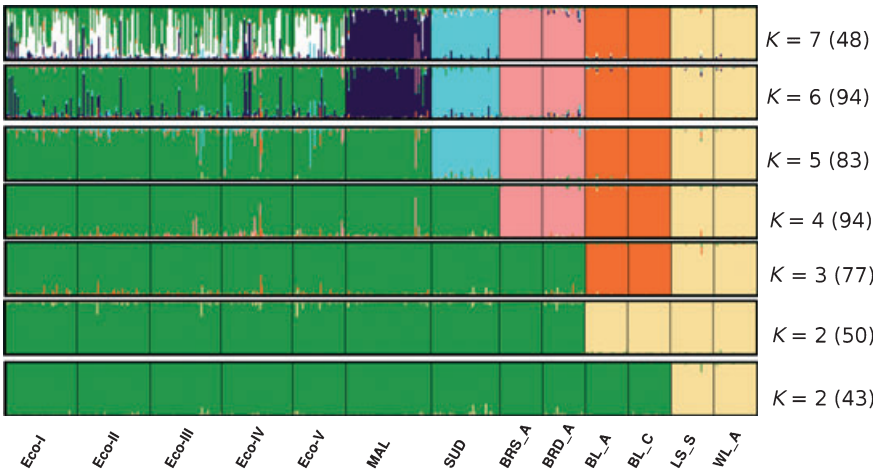

Figure 1 STRUCTURE clustering of Zimbabwe chicken ecotypes in reference to the extensively raised Malawi and Sudanese chickens and purebred broiler, white and brown egg layers. Numbers in parenthesis indicate the number of identical solutions at 95% threshold. Eco-I to Eco-V are the five Zimbabwe ecotypes; MAL = Malawi; SUD = Sudan; BRS_A = broiler sire line A; BRD_A = broiler dam line A; BL_A = brown egg layer line A; BL_C = brown egg layer line C; LS_S = white egg layer experimental line; WL_A = white egg layer line A.

estimates between ecotypes and other populations. High between-population kinship estimates were observed between pairs of purebred lines, particularly between the four egg layers.

A phylogenetic tree derived from the MEK estimates is illustrated in Fig. 2. The clustering indicates separation of the broiler lines from the layer lines, with the African populations clustered in between. Note the short branch lengths of the Zimbabwe ecotypes.

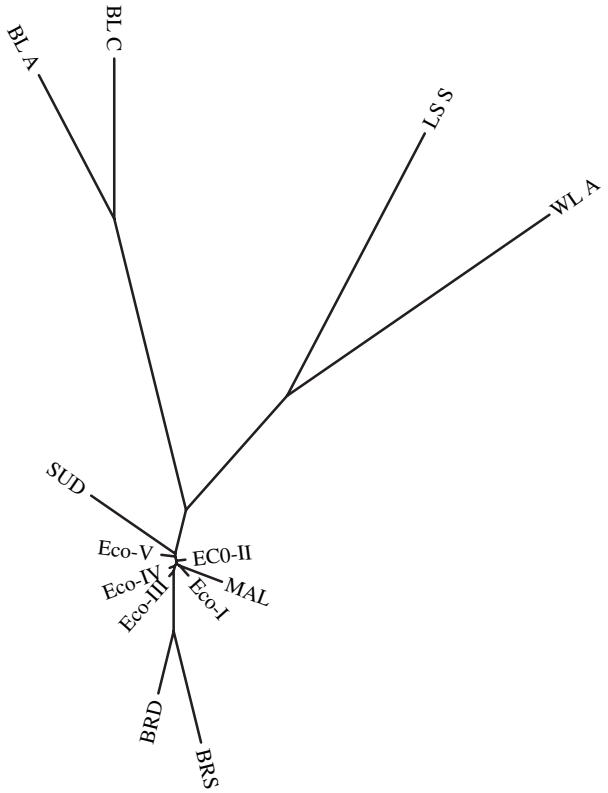

Figure 2 Neighbour-Joining tree derived from marker estimated kinships. Eco-I to Eco-V are the five Zimbabwe ecotypes; MAL = Malawi; SUD = Sudan; BRS_A = broiler sire line A; BRD_A = broiler dam line A; BL_A = brown egg layer line A; BL_C = brown egg layer line C; LS_S = white egg layer experimental line; WL_A = white egg layer line A.

Discussion

Compared with the other eight populations used in this study, the Zimbabwe ecotypes contributed more unique alleles and are thus a source of genetic diversity (Petit *et al.* 1998). However, some of these alleles have low frequencies, contributing little to genetic variation (Falconer & MacKay 1996). In addition to the new alleles, the overall number of alleles/locus was higher in the Zimbabwe ecotypes than in the purebred lines.

Both expected and observed heterozygosity estimates were high for the Zimbabwe ecotypes, together with the Malawi and Sudanese chickens (Table 2). Whereas purebred lines were founded on a limited number of breeds (Crawford 1990) and selected for specific production traits, the Zimbabwe chicken ecotypes have not been bred for any particular trait and roam freely during scavenging. The latter fact might result in migration of birds from one flock to a neighbouring one, causing a continuous gene flow between flocks, conserving a high number of alleles and heterozygosity in ecotype populations.

Zimbabwe ecotypes raised under scavenging systems of production are highly polymorphic compared with the purebred lines (Tables 1 and 2). This agrees with other studies (Wimmers *et al.* 2000; Hillel *et al.* 2003; De Marchi *et al.* 2006) in which wild and extensively raised chickens were found to be genetically diverse. Relatively high

observed heterozygosity and allelic diversity have also been found in Tanzanian ecotypes (Wimmers *et al.* 2000) and free-ranging village chickens from Mozambique and Botswana (Marle-Köster & Nel 2000).

Contrary to what is implied by the large geographical distances between ecotypes (300–800 km), the low between-ecotype (F_{ST}) variation (Table 3) indicated absence of clear substructuring of the Zimbabwe populations along agro-ecological zones. In fact, the observed total inbreeding (F_{IT}) was almost fully explained by within-population inbreeding (F_{IS}, Table 3). Each Zimbabwe ecotype population seemed to represent the full range of genetic diversity present in Zimbabwe indigenous chickens. Although null alleles could lead to elevated F_{IS} values, there was no indication of the presence of null alleles in our analysis.

The relatively high F_{ST} estimates for commercial breeds indicates that each population represents a limited sample of the total gene pool. This high level of population divergence in purebred lines was expected because they are based on different founder breeds, raised as closed flocks and selected for different production traits (Delany 2003).

STRUCTURE-based clustering further supports the low among ecotype differentiation of the Zimbabwe chickens (Fig. 1). The lack of observed substructuring among Zimbabwe ecotypes at values of $K \geq 6$ suggest that Zimbabwe indigenous chickens essentially form one population. This finding agrees with observed Wright's (1951) fixation indices (Table 3). Substructuring according to geographic location (ecotype) could not be observed. Furthermore, clustering of the Zimbabwe chickens was not related to phenotypic classes (data not shown).

The separation of the purebred lines at $K \leq 4$ followed by the Sudanese and lastly Malawi populations emphasizes the distinctiveness of the Zimbabwe population. The splitting of the Sudanese populations from the Zimbabwe populations at a lower K value ($K = 5$) than from the Malawian ($K = 6$) shows a geographical trend.

In Zimbabwe populations, the mean within-population kinships were only slightly higher than the mean between-population kinships (Table 4). This observation could be due to either a very large effective population size or relatively strong and continuous gene flow between populations. Gene flow among populations would result in equal allele frequencies across all five ecotypes and give no cause of the inferred substructures. In addition to the lack of population substructuring, the MEK estimates showed low within-population kinships in the Zimbabwe chicken ecotypes compared with the purebred lines, in particular the white egg layers. The closer association of the Zimbabwe chicken ecotypes with chickens from Malawi and Sudan (Fig. 2) suggests that indiscriminate hybridization with exotic commercial lines (Wollny 2003; Hall 2004) does not have a strong impact.

In conclusion, results from this study gave no indication that village chickens are substructured across agro-ecological zones. There is no evidence that the Zimbabwe chicken ecotypes are locally adapted and restricted to their respective agro-ecological zones. The results did show high genetic variation within the Zimbabwe village chicken population.

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