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Use of microsatellites and mitochondrial DNA to assess genetic diversity within and between Zimbabwe chicken eco-types

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Abstract

The objective of the study was to determine genetic diversity of free ranging Zimbabwe chicken eco-types in comparison to pure-bred lines and other extensively raised indigenous chickens. Twenty-nine microsatellite markers were genotyped for 526 chickens randomly selected from five Zimbabwe eco-zones (n = 238), Malawi (n = 60), Sudanese (n = 48) and six purebred lines (n = 180). The overall population variability (F_{IT}) amounted to 0.218 (± 0.014). Over 90% of genetic differentiation in Zimbabwe chickens was due to within eco-zone variation. The most probable STRUCTURE solution at $K = 6$ gave 97 % identical solutions in which Malawi, Sudanese and the pure bred lines split out from Zimbabwe chickens. The within eco-zone marker estimated kinships (MEK) (mean = 0.130) differed slightly from the between eco-zone MEK (mean = 0.110). Mitochondrial DNA sequence analysis yielded twenty–six variables sites on the 326bp mtDNA region resulting in 16 haplotypes. Four haplotypes were unique to the Zimbabwe chicken eco-types. The 133 individual chickens clustered into 3 clades consisting of Zimbabwe and Malawi chickens, pure bred lines and a mixture of pure bred lines and African chickens. Results showed a highly diverse Zimbabwe chicken population that is not genetically sub-structured into eco-types. In contrast to the high microsatellite genetic diversity the Zimbabwe eco-types seem to have been derived from few maternal lineages.

Key words: chicken eco-types, population structure, genetic variability, microsatellites, mtDNA.

Introduction

The local chickens in Zimbabwe and other African countries consist of different phenotypic strains raised by communal farmers across distinct agro-ecological zones (AEZ). It is not certain whether these eco-types represent distinct populations. The aim of this study was to characterize the genetic differentiation within and between Zimbabwean chicken eco-types, and to relate the extent of differentiation to other African chickens, commercial and experimental lines.

Materials and methods

Zimbabwe eco-types: Zimbabwe is located within latitudes 15° 47` S to 22° 24` S and longitude 25° 14`E to 33° 04' E. There are five agro-ecological zones (I-V) that vary in rainfall distribution (> 1000mm per annum in eco-zone I and <450mm per annum in eco-zone V) and temperatures (mean temperature = 15 °C in eco-zone I and > 35°C in eco-zone V). Altitude ranges from 197m to 2592m above sea level. Fifty chickens/eco-zone were sampled in eco-zones I, III and IV (ECO-I; ECO-III and ECO-IV) while fifty-one and thirty-seven chickens were sampled for eco-zones II (ECO-II) and V (ECO-V) respectively.

Reference populations: Eight populations were selected from the AVIANDIV project. These consisted of the broiler dam (BRD_A) and sire (BRS_A) line, two brown egg layers (BL_A and BL_C) and two white egg layers (LS_S and WL_A) with 30 individuals per population. Sixty scavenging chickens sampled from a 50km radius in Malawi and 48 Sudanese chickens from a similar extensive system of production were also used.

DNA polymorphism: The DNA polymorphism was assessed using a set of 29 microsatellite markers recommended by FAO for assessing chicken genetic diversity. The reference populations were already typed in the AVIANDIV project and allele scoring were adjusted using standard alleles. In addition, 326bp of the mtDNA control region were sequenced for 133 individual chickens from the 13 populations.

Statistical analyses: The degree of population subdivision was assessed using the Wright (1951)'s fixation indices. STRUCTURE analysis (Pritchard *et al.,* 2000) was used to cluster individuals to $2 \leq K \leq 7$ assumed clusters with 100 runs for each K value. The 100 runs were compared to each other using SIMCOEFF (Rosenberg *et al.,* 2002). Solutions with over 95% similarity were considered identical and the most frequent of these was visualised using DISTRUCT (Rosenberg, 2004). Marker Estimated Kinships (MEK; Eding and Meuwissen, 2001) between populations were estimated using a log-linear model. For visualisation purposes the MEK matrix was converted to a kinship contour plot. Genetic distances between pairs of the 133 sequences were estimated using the Kimura-2-parameter model (Kimura, 1980), and based on these estimates a Neighbor-joining tree of the haplotypes was constructed.

Results and discussion

The low between (F_{ST}) and high within eco-type variation observed (Table 2) indicates absence of population sub-structuring in the Zimbabwean eco-types.

Table 2: Per population F statistics

Population	F_{IT}	F_{ST}	F_{IS}	HWE*
Zimbabwe	0.084 (0.012[1])	0.008 (0.012)	0.077 (0.012)	*
African	0.115 (0.013)	0.039 (0.004)	0.079 (0.011)	*
Pure bred lines	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]Standard errors generated using jacknifing over loci with the *FSTAT* program

The results of the STRUCTURE analysis are indicated in Figure 1.

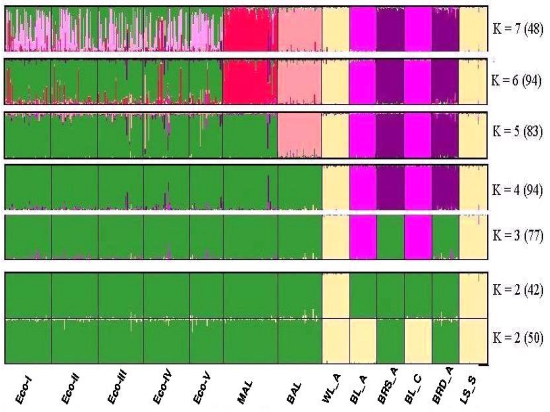

Figure 1: Clustering of Zimbabwe chicken eco-types in reference to the broiler, white and brown egg layers, and extensively raised Malawi and Sudanese chickens. (Number in parenthesis is the number of identical solutions at 95% threshold).

The separation of the white egg layers at both K = 2 solutions indicate high level of distinctiveness of this population. However, the separation of all the pure bred lines at $K \leq 4$ shows clear the distinction of the Zimbabwe population from all these pure bred lines but still clustered together with the Malawi and Sudanese chickens. The results imply that indiscriminate crossbreeding of indigenous lines to exotic commercial lines suggested by Hall (2004) might not be a major threat to these chickens. The lack of observed sub-structuring among Zimbabwe eco-types at values of $K \geq 6$ indicate that Zimbabwe indigenous chickens essentially form one big population separated from the Malawi and Sudanese chickens.

The high genetic variability and distinctiveness of the Zimbabwe chicken eco-types is further corroborated by the MEK estimates (Figure 2).

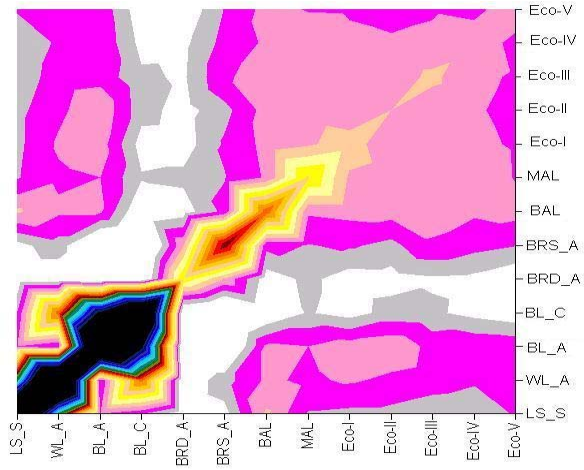

Figure 2: Contour plot of the Marker Estimated Kinships (MEK) within and between populations. (Darker shades indicate higher kinship estimates).

There is a clear distinction between the cluster of African populations and the clusters of pure bred populations. The relatively high MEK within and between the four egg layers agrees with the splitting of these pure bred lines into distinct populations at K ≤ 4 (Figure 1) The within (diagonal) and between eco-zone MEK estimates did not differ much indicating like STRUCTURE little sub-structuring of the Zimbabwean population. However, the elevated MEK between the white egg layers (LS_S and WL_A) and African chickens contrasted with their early split from this gene pool at K = 2 in Figure 1.

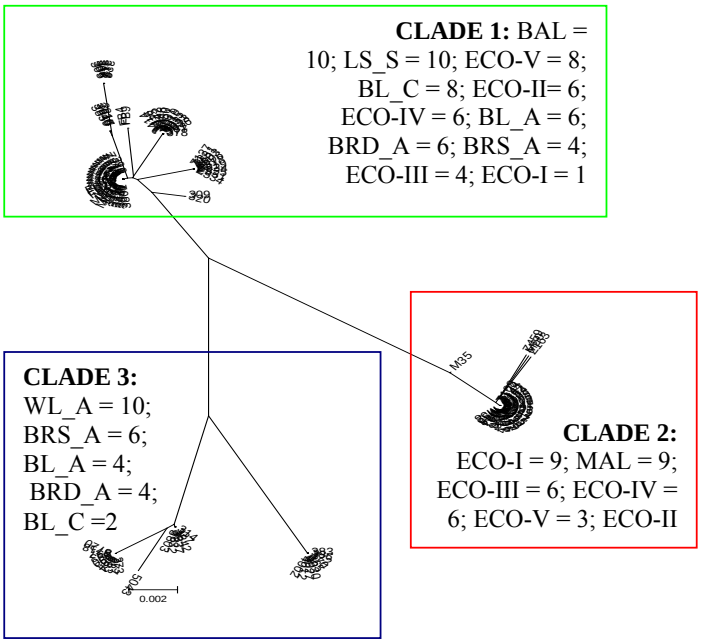

Figure 3: Neighbor joining tree of individual haplotypes

The 133 individual haplotypes clustered into three clades as shown in Figure 3. Clade 1 had a mixture of Sudanese Baladi chickens, ECO II – V, brown and white egg layers while Clade 2 was made up of the Zimbabwe chicken eco-types and the Malawi individuals. Clade 3 consisted of the pure bred lines. Among clade diversity accounted for 86.41% of the total variation while within clade diversity accounted for 13.6%. Unlike in microsatellite analysis the Zimbabwe chicken eco-types shared haplotypes and clustered into a common clade with some of the pure bred lines.

Conclusion

Results from this study reject the hypothesis that village chickens are sub-structured across contrasting agro-ecological zones. The study indicated high genetic variation within the village chicken eco-types. Unlike the free ranging African gene pool, the commercial lines form distinct clusters in both STRUCTURE and MEK estimates.

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