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Assessing Genetic Diversity of Five Tanzanian Chicken Ecotypes Using Microsatellite Markers and Mitochondrial DNA D-loop Sequencing

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1.0 Introduction

Tanzania is rich in indigenous farm animal genetic resources of different livestock species including poultry. Traditional poultry farming is dominated by chickens (94.1%), which make an important contribution to the livelihoods of the most vulnerable rural households (Melewas, 1989). In Tanzania, agriculture remains a central source of income, employment and food security especially in rural areas, where about 80% of Tanzanian human population lives. Previous studies revealed genetic and phenotypic variability in Tanzanian indigenous chickens in terms of plumage colour and type, body shape and size as well as productivity (Msoffe, *et al*., 2001, Minga *et al.*, 2004, Msoffe *et al.*, 2004). Several genetic studies suggested multiple origins of African domesticated chickens. From mitochondrial DNA (mtDNA) analysis, Mwacharo *et al.* (2011) reported multiple introductions of chickens into East Africa resulting in five distinct haplogroups of different maternal origins. Muchadeyi *et al.* (2008) found two distinct haplogroups from mtDNA sequence analysis in Zimbabwe village chickens, suggesting an origin of these chickens from southern Asian and the Indian subcontinent, respectively. Mtileni *et al.* (2011) reported that conservation and field chickens in South Africa shared three major haplotypes presumably originating from China, Southeast Asia, and the Indian Subcontinent. The aim of this study was to assess the population structure between Tanzanian chicken populations and to trace their history.

2.0 Materials and Methods

A total of 196 individuals were used in this study which accounted for five ecotypes of Tanzanian local chicken *(Ching'wekwe, Kuchi, Morogoro Medium, Pemba* and *Unguja)* from eight different regions *(Mwanza, Geita, Shinyanga, Tabora, Tanga, Morogoro, Unguja and Pemba)*. Five morphological traits were collected to determine the phenotype of individual birds: (1) *Ulna* bone length, (2) keel length, (3) shank length, (4) shank thickness, and (5) live body weight. Least squares means of phenotypic measurements were calculated and compared with Tukey's HSD procedure by using JMP 9.0.2. Pearson's correlation coefficients between all morphometric traits were estimated, and from the correlation matrix, principal components analysis (PCA) was done. Microsatellite markers were genotyped at 29 loci. Twenty eight of microsatellite markers were taken from the 30 markers suggested for biodiversity studies in chickens by ISAG/FAO advisory group on animal genetic diversity. Allele frequency was computed using Microsatellite-Toolkit.

Wright's fixation indices were estimated using FSTAT 2.9.3.2. Population structure was determined by using a model-based clustering to assign individuals from multilocus genotypes to a population using STRUCTURE 2.3.3. A 708 base pair fragment from the D-loop region of the chicken mitochondrial genome was amplified and sequenced using capillary DNA sequencer (CEQ). DNA sequences were aligned using the AlignIR software and analyzed using NETWORK 4.6.1.0 to construct Median-joining (MJ) networks for determining the evolutionary relationships of haplotypes. In addition to the sequences of the Tanzanian chicken populations, the network analysis included the most frequent haplotypes of nine clades identified by Liu and three clades identified by Oka, which were used as a reference frame in haplotype analysis (Liu *et al.*, 2006; Oka *et al.*, 2007). Oka's haplotypes A3 and A4 from Shamo game birds sampled on Shikoku island of Japan in the Kōchi Prefecture were also used. DNA sequence polymorphism was analyzed using DnaSP 5.10.01.

3.0 Results and Discussion

The first two principal components explained 93.02% of the total variance present in all five phenotypic traits. The distribution of individuals in the plot of the principal components showed *Ching'wekwe* chicken clustering separately from the other four ecotypes due to their unproportional short limbs. *Kuchi* chickens, on the other hand, appeared to be more distributed to the right caused by shank thickness, keel length, and body weight but with greater variation among individuals. The remaining ecotypes *Morogoro, Unguja*, and *Pemba* cluster together in the centre of the plot, overlapping slightly with the *Kuchi* (Fig.1).

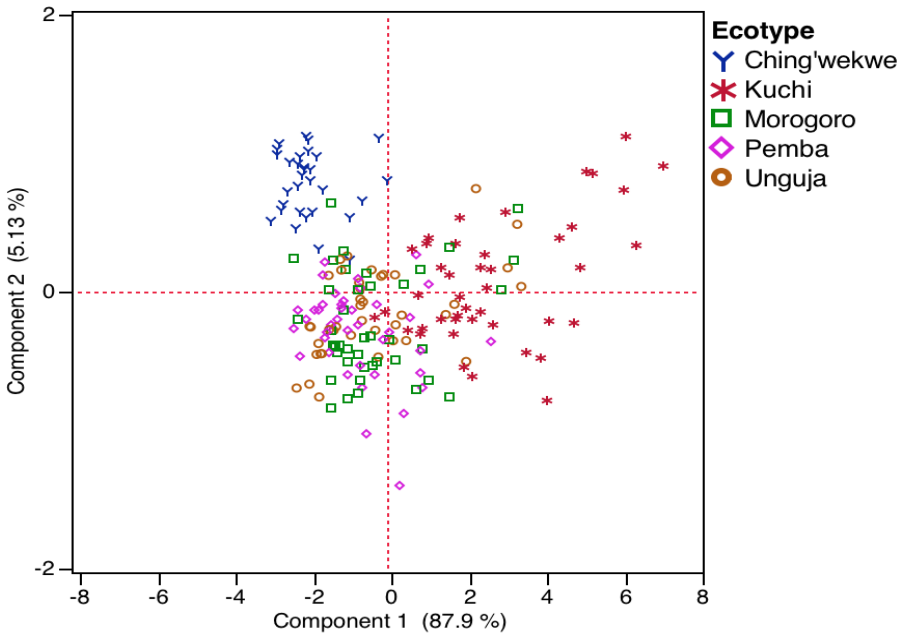

Fig. 1: Principle component plot (PC_1 and PC_2) of five Tanzanian chicken ecotypes based on five morphological traits.

In comparison, Kuchi showed significant ($P \leq 0.05$) longer *ulna* bone lengths, keel lengths, shank sizes, short parrot-like beak, and higher body weight than the other ecotypes under study. These phenotypic characteristics were likewise reported for *Shamo* gamecock by Komiyama *et al.* (2003) when tracing the origin of the Japanese Gamecocks. Results of molecular marker analyses suggested a clear differentiation among Tanzanian chickens. Expected (0.62±0.017) and observed

(0.62±0.028) heterozygosity estimates were higher in Tanzanian indigenous chickens compared to commercial breeds reported earlier (Granevitze *et al.*, 2007, Muchadeyi *et al.*, 2007, Bodzar *et al.*, 2009, Fosta *et al.*, 2011). Furthermore, *Unguja* showed the highest expected heterozygosity (0.67±0.027) while *Kuchi* displayed the lowest values (0.58±0.034) of all five ecotypes. STRUCTURE cluster analysis revealed three groups of Tanzanian chickens. Among these three clusters, *Kuchi* appeared to form an independent cluster immediately at K=2. *Unguja* and *Pemba* ecotypes, which are the Island game birds, split from *Ching'wekwe* and *Morogoro* at K=3 (Fig.2).

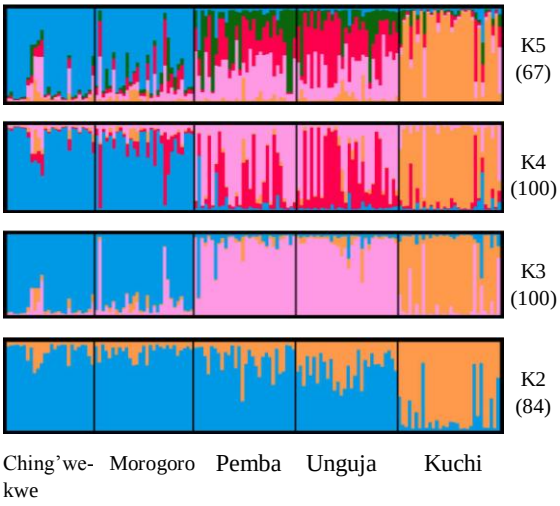

Fig. 2: STRUCTURE clustering of five ecotypes of Tanzanian indigenous chicken ecotypes.

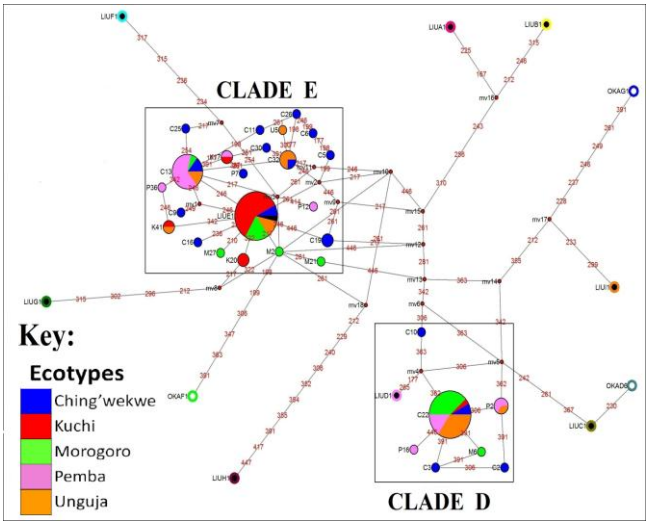

Fig. 3: MJ Network profile of 23 haplotypes observed in Tanzanian chickens merged with the sequences of major haplotypes presented by Liu *et al*. (2006) and Oka *et al*. (2007)

The analysis of mtDNA sequences revealed two maternal lineages in Tanzanian local chicken populations which corresponded to haplogroups D and E described by Liu *et al.* (2006). The skeleton allowed assigning haplotypes observed in this study to clades known from literature. Liu *et al.* (2006) suggested that haplogroups D and E originated from Southeast Asia and Indian subcontinent, respectively. The majority (95.24%) of *Kuchi* were found in haplogroup E (Fig.3), and in particular clustering with Liu's E1 haplotype (76.20%). Latter is identical to haplotype A3 from Oka *et al.* (2007) that was one frequent haplotype in *Shamo* game birds sampled from Shikoku Island of Japan in the Kōchi Prefecture. Among the Tanzanian chicken populations *Kuchi* showed lowest haplotype diversity (0.424 ± 0.131) and nucleotide diversity (0.003), respectively, while *Ching'wekwe* had highest estimates (respective values 0.916±0.038 and 0.012).

From this study we conclude that Tanzanian chickens clustered into three distinct groups with two maternal lineages distributed among the five chicken populations. Haplotype network analysis suggests Tanzanian chickens originated both from Indian Subcontinent and Southeast Asia. The low haplotype diversity in *Kuchi*, phenotypic comparison, and overlap with the haplotype from Shamo in Japan, and the name "Kuchi" which is similar to the prefecture name Kōchi implies that *Kuchi* were recently introduced and might be imported to Tanzania from Japan.

4.0 Literature

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