Assessing the genetic diversity of five Tanzanian chicken ecotypes using molecular tools

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Abstract

The study aimed to evaluate the genetic diversity of Tanzanian chicken populations through phylogenetic relationship, and to trace the history of Tanzanian indigenous chickens. Five ecotypes of Tanzanian local chickens (*Ching'wekwe*, *Kuchi*, *Morogoro-medium*, *Pemba* and *Unguja*) from eight regions were studied. Diversity was assessed based on morphological measurements and 29 microsatellite markers recommended by ISAG/FAO advisory group on animal genetic diversity. A principal component analysis (PCA) of morphological measures distinguished individuals most by body sizes and body weight. *Morogoro Medium*, *Pemba* and *Unguja* were grouped together, while *Ching'wekwe* stood out because of their disproportionate short shanks and *ulna* bones. *Kuchi* formed an independent group owing to their comparably long body sizes. Microsatellite analysis revealed three clusters of Tanzanian chicken populations. These clusters encompassed i) *Morogoro-medium* and *Ching'wekwe* from Eastern and Central Zones ii) *Unguja* and *Pemba* from Zanzibar Islands and iii) *Kuchi* from Lake Zone regions, which formed an independent cluster. Sequence polymorphism of D-loop region was analysed to disclose the likely maternal origin of Tanzanian chickens. According to reference mtDNA haplotypes, the Tanzanian chickens that were sampled encompass two haplogroups of different genealogical origin. From haplotype network analysis, Tanzanian chickens probably originated on the Indian subcontinent and in Southeast Asia. The majority of *Kuchi* chickens clustered in a single haplogroup, which was previously found in *Shamo* game birds sampled from Shikoku Island of Japan in the Kōchi Prefecture. Analysis of phenotypic and molecular data, as well as the linguistic similarity of the breed names, suggests a recent introduction of the *Kuchi* breed to Tanzania.

Keywords: Tanzanian indigenous chickens, genetic diversity, microsatellites, mitochondrial DNA
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Introduction

Tanzania is rich in indigenous farm-animal genetic resources of livestock species, including poultry. Traditional poultry farming is dominated (94.1%) by chickens (Swai *et al*., 2007), which make a substantial contribution to the livelihoods of the most vulnerable rural households, which account for 80% of the Tanzanian human population (Swai *et al*., 2007; Lwelamira *et al*., 2008). The scavenging local chickens have been reared by the local community of Tanzania since time immemorial (Kabatange & Katule, 1989; Mutayoba *et al*., 2012). Local chickens in Tanzania can be found in almost every place with human settlement, although most of the indigenous chickens are kept in the central corridor regions of Tanzania (FAO, 2007; RLDC, 2010).

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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; Msoffe *et al*., 2006). In these reports, Tanzanian indigenous chickens were characterized based on their phenotypic traits and geographical origin in Tanzania (Msoffe *et al*., 2005). Assessment of genetic differentiation between Tanzanian chicken breeds was based on a few microsatellite markers, with only one of the 20 microsatellite markers being in the recommended list of the markers proposed for chicken biodiversity studies by FAO (2011).

Several genetic studies have 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 origin. 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. Mtileni *et al*. (2011b) reported that conserved 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 examine the existing diversity of five chicken ecotypes of Tanzania to obtain a more comprehensive picture of these genetic resources and their phylogenetic relationships, and to examine the historical background of Tanzanian local chickens by analysing the degree of shared mtDNA haplotypes with those of known origin to disclose probable maternal lineages of Tanzanian chickens.

Materials and Methods

A total of 196 individuals were used in this study, which represent five ecotypes of Tanzanian local chicken (*Ching'wekwe, Kuchi, Morogoro-medium*, *Pemba* and *Unguja*) from eight regions of Eastern Zone, Central Zone, Lake Zone and Zanzibar islands (Table 1). *Kuchi*, *Pemba* and *Unguja* ecotypes are characterized by upright posture, resembling game birds, while *Morogoro-medium* and *Ching'wekwe* ecotypes are of Bankiva type with very short shanks in the *Ching'wekwe* ecotype (Msoffe *et al*., 2001; 2004). Forty-eight villages were randomly selected in 21 districts of these regions, which were chosen according to the predominant ecotype of indigenous chickens kept with less introgression from exotic populations. To avoid collecting closely related individuals, four chickens were sampled in each village and only one bird per household. The number of hens was higher than cocks, as farmers keep more breeding females than males.

These morphological traits were collected to assess the phenotype of individual birds: 1) forearm length of the *ulna*, measured along the surface from the elbow *(olecranon)* to the wrist *(carpus)*; 2) shank length *(tarso-metatarsus)* taken from the hock joint to the foot pad; 3) shank thickness measured from the top of an outstretched shank at the point right above the spur; 4) keel length, taken from the tip of the *chondral* across the keel/bone towards the sternum where the bones of the clavicle *(clavicula)* form a triangle; and 5) live body weight, assessed with a top-hanging weighing scale of 10 kg capacity with 10% margin of error and tolerance of 50 g (0.05 kg).

Blood samples were taken from the *ulna* vein of each bird and stored on Whatman filter paper (Whatman Biosciences, Brentford, UK). From the filter paper, approximately one cent coin was collected from the field. A half cent coin was extracted in the laboratory, which then provides an average of 25 µg in a concentration of 250 ng/µ. Genomic DNA was isolated using the phenol-chloroform extraction method (Sambrook & Russell, 2001). Individuals were genotyped at 29 microsatellite loci, 28 of them taken from the 30 that have been suggested for biodiversity studies in chickens (FAO, 2011). LEI 0192 and MCW0284 was not analysed, but microsatellite locus MCW0080 was added. PCR products were generated using primers labelled with fluorescent dyes (IRD700 and IRD800), and PCR products were visualized on 8% polyacrylamide gel using a LI-COR DNA analyser (LI-COR Inc. Nebraska, USA). Electropherogram and allele-size scoring were performed with RFLPscan plus software (Scanalytics, Division of CSP, Billerica, USA). Internal allele ladders and five DNA standard samples with known genotype were loaded on all gels and used to adjust the allele scoring between runs.

The mtDNA was amplified and sequenced as described by Muchadeyi *et al*. (2008). DNA sequences were aligned using the AlignIR software (LI-COR Inc.). Extra nucleotide sequences that were outside the nucleotide sequences from 167 to 521 bp of the D-loop region were excluded from analysis.

Least square means of phenotypic measurements of *ulna* length, shank length, shank thickness, keel length, and body weight for all ecotypes under study were compared with Tukey's HSD procedure using the

JMP 9.0.2 statistical package. Pearson's correlation coefficients between all morphometric traits were estimated, and from the correlation matrix, principal component factor analysis (PCA) was done. The first two principal components (PC) were used to identify population clusters, and a variance maximization method (Varimax) was used for factor rotation (SAS/STAT, 2009).

Table 1 List of Tanzanian indigenous chickens ecotypes used for genotyping

Ecotype	Number of birds		Region(s)	Districts
	Female	Male		
Ching'wekwe	20	6	Morogoro and Tanga	Gairo, Kilindi and Mvomero
Kuchi	20	10	Mwanza, Shinyanga, Tabora and Geita	Misungwi, Magu, Shinyanga Rural, Kahama, Ushirombo/Bukombe, Geita, Sengerema, Nyegezi and Nzega
Morogoro-medium	20	9	Morogoro	Kilosa, Gairo, Morogoro Rural and Mvomero
Pemba	20	10	Pemba Island	Chakechake, Wete and Mkoani
Unguja	20	10	Unguja Island	Magharibi, Kaskazini mashariki and Kaskazini

Allele frequency, mean number of alleles (MNA), polymorphic information content (PIC), expected (H_E) and observed (H_O) heterozygosity of the populations were estimated using Microsatellite-Toolkit (Park 2001). Wright's fixation indices were calculated using FSTAT 2.9.3.2 software (Goudet, 2002) to quantify within and between sub-population partitioning variances. Variance estimates were obtained by jack-knifing over loci and populations using the FSTAT software. The level of genetic differentiation was determined using Weir & Cockerham's (1984) estimation of Wright's (1951) fixation index. Analysis of molecular variance (amova) was done with the algorithms suggested by Excoffier *et al*. (1992), implemented in Arlequin software version 3.5.1.3.

Table 2 Liu and Oka's haplotypes names and their GenBank accession number

Haplotype name	Accession number	References
Liu_A_1	AB114069	Liu *et al*. (2006)
Liu_B_1	AB007744	Liu *et al*. (2006)
Liu_C_1	AB114070	Liu *et al*. (2006)
Liu_D_1	AY588636	Liu *et al*. (2006)
Liu_E_1	AB114076	Liu *et al*. (2006)
Liu_F_1	AF512285	Liu *et al*. (2006)
Liu_G_1	AF515588	Liu *et al*. (2006)
Liu_H_1	D82904	Liu *et al*. (2006)
Liu_I_1	AB009434	Liu *et al*. (2006)
Oka_D_6	AB268535	Oka *et al.* (2007)
Oka_G_1	AB268545	Oka *et al.* (2007)
Oka_F_1	AB268543	Oka *et al.* (2007)
Oka_A_3	AB268508	Oka *et al.* (2007)
Oka_A_4	AB268509	Oka *et al.* (2007)

Reynolds' genetic distance among Tanzanian chickens was estimated (Reynolds *et al*., 1983), and 1000 bootstrapping replicates over loci were performed to test the robustness of the tree topology, using the PHYLIP software package (Felsenstein, 2005). The obtained tree was depicted using SplitsTree4 software version 4.12.3 (Hudson & Bryant, 2006).

Population structure was determined by using a model-based clustering for assigning individuals from multilocus genotypes to a population with STRUCTURE 2.3.3 software (Pritchard *et al*., 2000; Falush *et al*., 2007; Hubisz *et al.*, 2009). The analysis involved an admixture model with correlated allele frequencies. Some 50 000 iterations in the burn-in phase were applied, followed by 100 000 iterations. The user-defined number of clusters ranged from $2 \leq K \leq 5$. Individuals were grouped into the predefined number of clusters with 100 independent Structure runs repeated for each K value. A pair-wise comparison of the 100 solutions using simCoeff (Rosenberg *et al*., 2002) was carried out, and the solutions with over 95% similarities were considered identical. The most frequent solution was considered the most probable clustering and was visualized using Distruct 1.1 software (Rosenberg, 2004). In addition, the approach developed by Evanno *et al*. (2005) was applied from K = 1 to K = 5 to determine the optimal number of clusters.

Median-joining networks were constructed to determine the evolutionary relationships of haplotypes following the algorithms of Bandelt *et al*. (1995), using Network 4.6.1.0 software (http://www.fluxus-engineering.com/sharenet.htm). Besides the sequences of the Tanzanian chicken populations, the network analysis included the most frequent haplotypes of nine clades from Liu's network and of three clades from Oka's, which were used as a reference frame in haplotype analysis (Liu *et al*., 2006; Oka *et al.*, 2007). The list of haplotypes and their GenBank accession numbers are given in Table 2. Haplotype diversity and Tajima's *D* value were analysed using DnaSP 5.10.01 software (Librado & Rozas, 2009).

Results

Ulna length, shank length, shank thickness, keel length and body weight were of larger size in male birds than in females in all ecotypes (Table 3). Highest mean values of all traits ($P \leq 0.05$) were found in *Kuchi* ecotype, which is a game-type chicken, while *Ching'wekwe* ecotype had the lowest values. *Unguja*, *Morogoro* and *Pemba* ecotypes revealed no significant differences in all traits.

Table 3 Least square means (± SE) of phenotypic measurements in five ecotypes of Tanzanian local chickens

Phenotypic traits	Sex	Ecotypes				
		Chingw'ekwe	*Morogoro*	*Pemba*	*Unguja*	*Kuchi*
Ulna bone length	M	$7.53^{c} \pm 0.31$	$9.92^{b} \pm 0.24$	$9.47^{b} \pm 0.23$	$9.78^{b} \pm 0.24$	$11.5^{a} \pm 0.21$
(cm)	F	$7.01^{c} \pm 0.12$	$8.28^{b} \pm 0.11$	$8.15^{b} \pm 0.11$	$8.26^{b} \pm 0.11$	$10.1^{a} \pm 0.11$
Shank length	M	$7.18^{c} \pm 0.48$	$11.0^{b} \pm 0.34$	$10.3^{b} \pm 0.32$	$11.3^{b} \pm 0.34$	$13.8^{a} \pm 0.30$
(cm)	F	$6.22^{c} \pm 0.15$	$8.64^{b} \pm 0.14$	$8.59^{b} \pm 0.14$	$8.61^{b} \pm 0.14$	$11.0^{a} \pm 0.14$
Shank thickness	M	$10.1^{b} \pm 0.66$	$11.9^{b} \pm 0.51$	$11.4^{b} \pm 0.49$	$12.5^{b} \pm 0.51$	$15.6^{a} \pm 0.45$
(mm)	F	$9.92^{b} \pm 0.16$	$9.34^{c} \pm 0.14$	$9.65^{bc} \pm 0.14$	$9.59^{bc} \pm 0.14$	$12.3^{a} \pm 0.14$
Keel length	M	$14.5^{b} \pm 0.76$	$16.8^{b} \pm 0.58$	$15.7^{b} \pm 0.56$	$16.8^{b} \pm 0.59$	$19.9^{a} \pm 0.51$
(cm)	F	$12.9^{c} \pm 0.24$	$14.2^{b} \pm 0.21$	$13.9^{b} \pm 0.22$	$13.7^{bc} \pm 0.22$	$16.4^{a} \pm 0.22$
Body weight	M	$1.65^{c} \pm 0.17$	$2.42^{b} \pm 0.14$	$1.59^{c} \pm 0.14$	$2.36^{b} \pm 0.14$	$3.29^{a} \pm 0.13$
(kg)	F	$1.34^{c} \pm 0.06$	$1.52^{b} \pm 0.05$	$1.25^{c} \pm 0.05$	$1.53^{b} \pm 0.05$	$2.57^{a} \pm 0.06$

NB: Means within a row with same letter are not significantly different at $P \geq 0.05$; M: male; F: female.

Loading of shank length (0.857) and *ulna* length (0.851) were highest for the first PC, which explained 87.9% of the total variation present in all five phenotypic traits, while the second PC explained 5.13% of the total variance. Shank thickness (0.867), body weight (0.774), and keel length (0.697) contributed heavily to the second PC. The score plot of the first two PCs (Figure 1) showed *Ching'wekwe* chicken clustering separately from the other four ecotypes mainly owing to their disproportionately short

legs. *Kuchi* chickens, on the other hand, were distributed more to the upper right because of greater shank thickness, longer keel length and higher body weight, with a greater variation among individuals. The remaining ecotypes, *Morogoro*, *Unguja* and *Pemba*, cluster together in the centre of the plot, overlapping partly with *Kuchi*.

The overall means of expected and observed heterozygosity estimates were 0.62 and 0.62, respectively (Table 4). The expected heterozygosity was highest in *Unguja* ecotype (0.67) and lowest in *Kuchi* ecotype (0.58). None of the F_{IS}-estimates differed significantly from zero (P >0.05) indicating that the observed frequencies of heterozygotes were close to what is expected if populations were in Hardy-Weinberg equilibrium. The fixation index between Tanzanian chicken breeds (F_{ST}) is 0.048, that is, the genetic diversity between the five ecotypes of Tanzanian chicken populations constituted 4.8% of the total genetic variance (Table 5).

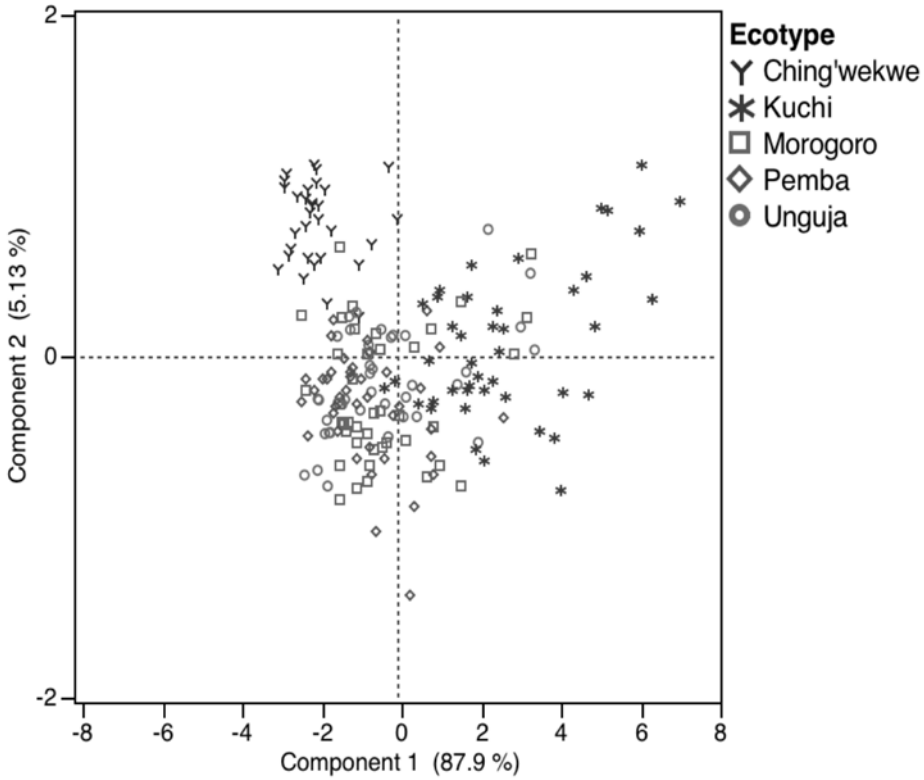

Figure 1 Principle component plot (PC_1 and PC_2) of five Tanzanian chicken ecotypes based on five morphological traits.

Table 4 Genetic diversity within chicken population in Tanzania

Population	No. of birds	No. of loci	MNA ± SE	HE ± SE	HO ± SE	F_{IS}
Ching'wekwe	26	29	5.41 ± 2.29	0.62 ± 0.027	0.65 ± 0.017	–0.061
Kuchi	30	29	5.10 ± 2.08	0.58 ± 0.034	0.56 ± 0.017	0.028
Morogoro-medium	29	29	5.69 ± 2.63	0.60 ± 0.026	0.58 ± 0.017	0.038
Pemba	30	29	6.00 ± 2.80	0.65 ± 0.028	0.67 ± 0.016	–0.029
Unguja	30	29	6.28 ± 2.24	0.67 ± 0.027	0.63 ± 0.016	0.065
Overall mean	29	29	5.70 ± 2.61	0.62 ± 0.028	0.62 ± 0.017	0.01

MNA: mean number of alleles; H_E: expected heterozygosity; H_O: observed heterozygosity; SE: standard error of the mean; F_{IS}: average inbreeding coefficient within subpopulation.
Different F_{IS} estimates were not significantly different from zero at $P \geq 0.05$.

Table 5 Analysis of molecular variance (AMOVA) within and between five ecotypes of Tanzanian chicken population

Source of variation	Sum of squares	Variance component	Percentage of variation
Between populations	142.075	0.45848	4.81333
Within population	2575.917	9.06672	95.18667
Total	2717.992	9.52520	

Genetic clustering based on STRUCTURE analysis of the five Tanzanian indigenous chicken ecotypes is shown in Figure 2. The most likely clustering appeared at K = 3 as indicated by applying Evanno method (Evanno *et al*., 2005). The maximum number of 100 identical runs were observed at K = 3 and at K = 4, respectively. Clustering populations into more than three clusters did not change overall structure: *Ching'wekwe* clustered with *Morogoro-medium*, and *Unguja* clustered together with *Pemba* while *Kuchi* ecotype formed an independent cluster immediately at K = 2. *Unguja* and *Pemba* ecotypes which are the Island game birds split from *Ching'wekwe* and *Morogoro-medium* ecotypes at K = 3.

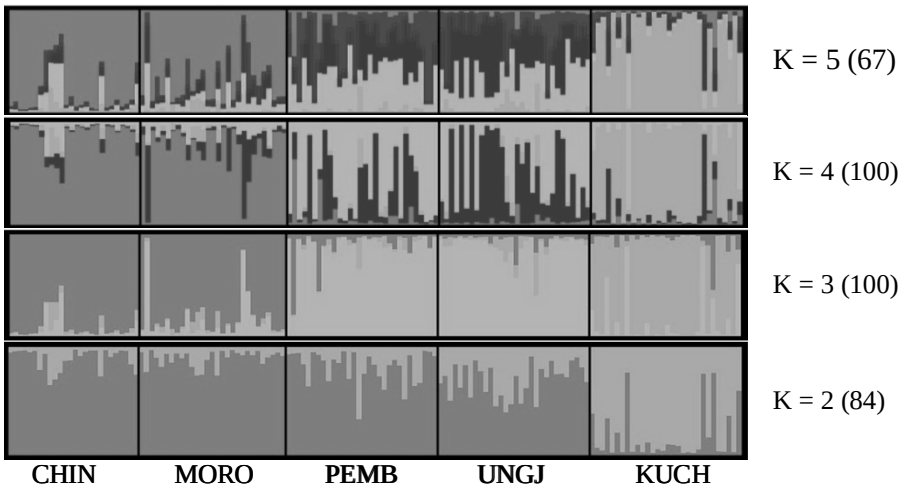

Figure 2 Clustering of five ecotypes of Tanzanian indigenous chickens: *Ching'wekwe* (CHIN); *Morogoro-medium* (MORO); *Pemba* (PEMB); *Unguja* (UNGJ); and *Kuchi* (KUCH) ecotypes. The numbers in brackets indicate the number of identical solutions at 95% threshold.

Genetic distance estimates between Tanzanian indigenous chicken populations was used to form a neighbour net illustrating the relationship between breeds (Figure 3). The largest genetic distance was observed between *Kuch*i and *Ching'wekwe* ecotypes. *Unguja* and *Pemba* ecotypes exhibited the closest phylogenetic relationship, followed by *Morogoro-medium* and *Ching'wekwe* ecotypes.

The median-joining (MJ) network analysis of the mtDNA D-loop haplotypes observed in Tanzanian local chickens, together with most frequently observed haplotypes from Liu *et al*. (2006) and Oka *et al*. (2007) as a skeletal frame reference, is shown in Figure 4. Twenty-three haplotypes were observed in Tanzanian chickens and were found to cluster with haplogroups D and E identified by Liu *et al*. (2006). *Kuchi* (95.2%) and *Ching'wekwe* (75.0%) clustered in clade E, while *Morogoro*, *Unguja* and *Pemba* were distributed within clades E and D. Oka's haplotypes A3 and A4 clustered in clade E. Most of the *Kuchi* chickens (76.2%) clustered in haplotype Liu E1 in clade E. Analysis of sequence polymorphism revealed an overall haplotype diversity of 0.831, nucleotide diversity of 0.012, and Tajima's *D* value of 0.67475

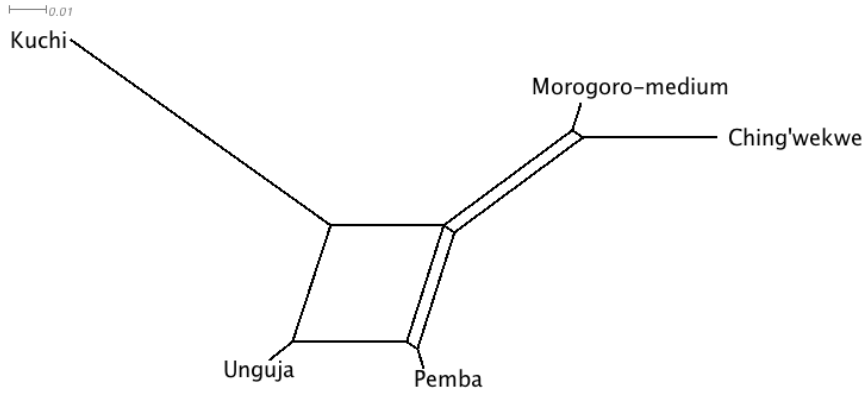

Figure 3 Neighbour net of five ecotypes of Tanzanian indigenous chickens.

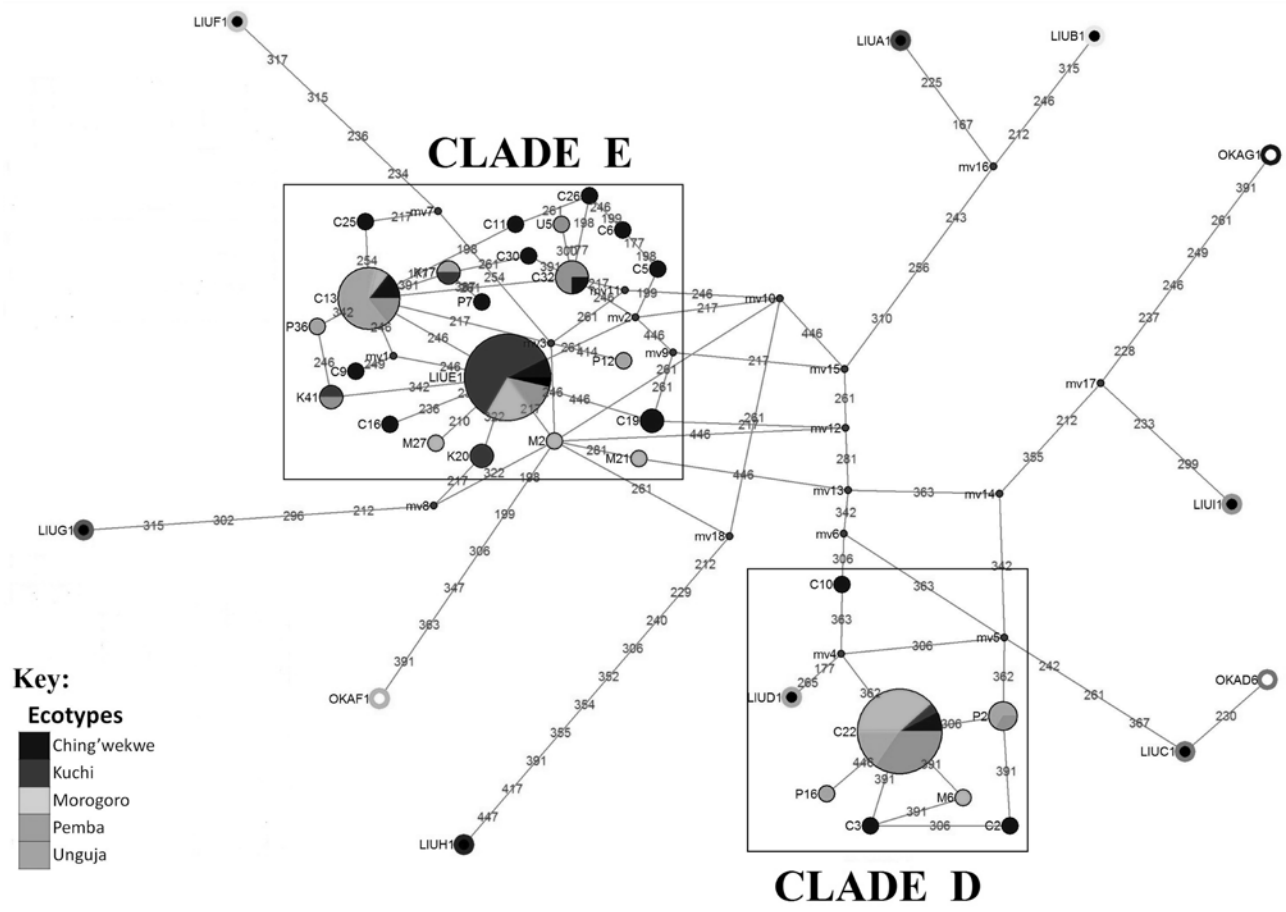

Figure 4 Median-joining network profile of 23 haplotypes observed in Tanzanian indigenous chicken merged with the sequences of major haplotypes presented by Liu *et al.* (2006) and Oka *et al.* (2007). Note that the circle size corresponds to haplotype frequency.

(*P* >0.10) in Tanzanian chicken populations (Table 6). Among the Tanzanian chicken populations, *Kuchi* showed lowest haplotype diversity (0.424) and nucleotide diversity (0.003), respectively, while *Ching'wekwe* had highest estimates (respective values 0.916 and 0.012). Estimate of Tajima's *D* values were neutral in *Ching'wekwe*, *Morogoro* and *Pemba* chicken populations. *Unguja* tested a significant positive

value, while *Kuchi* recorded a significant negative value (P <0.05). *Kuchi* (95.2%) and *Ching'wekwe* (75.0%) clustered in clade E, while *Morogoro*, *Unguja* and *Pemba* were distributed within clades E and D. Oka's haplotypes A3 and A4 clustered in clade E. Most of the *Kuchi* chickens (76.2%) clustered in haplotype Liu E1 in clade E. Analysis of sequence polymorphism revealed an overall haplotype diversity of 0.831, nucleotide diversity of 0.012, and Tajima's D value of 0.67475 (P >0.10) in Tanzanian chicken populations (Table 6). Among the Tanzanian chicken populations, *Kuchi* showed the lowest haplotype diversity (0.424) and nucleotide diversity (0.003), respectively, while *Ching'wekwe* had highest estimates (respective values 0.916 and 0.012). Estimate of Tajima's D values were neutral in *Ching'wekwe*, *Morogoro* and *Pemba* chicken populations. *Unguja* tested a significant positive value, while *Kuchi* recorded a significant negative value (P <0.05).

Table 6 Number of haplotypes, haplotype diversity, number of nucleotide diversity and Tajima's D test in mitochondrial DNA sequences of Tanzanian chickens

Population	Sample size	Number of Haplotypes (h)	Haplotype Diversity (Hd) ± S.E.	Nucleotide Diversity (π)	Tajima's D
Ching'wekwe	20	11	0.916 ± 0.038	0.01152	1.13118
Morogoro-medium	20	7	0.711 ± 0.089	0.01131	1.04689
Pemba	20	8	0.795 ± 0.071	0.01225	1.34791
Unguja	20	7	0.763 ± 0.079	0.01286	2.15115*
Kuchi	21	5	0.424 ± 0.131	0.00317	–2.05611*
Total	101	23	0.831 ± 0.023	0.01147	0.67475

* P <0.05 significant, indicating the rejection of the hypothesis neutral expansion.

Discussion

Molecular genetic information and morphological variation were used to achieve deeper insight into genetic diversity within and the relationship between five ecotypes of Tanzanian chickens. From molecular genetic marker analyses, the expected and observed heterozygosity estimates were higher in Tanzanian indigenous chickens compared with commercial breeds reported earlier (Granevitze *et al.*, 2007; Muchadeyi *et al.*, 2007; Bodzar *et al*., 2009; Fosta *et al*., 2011). Furthermore, the differentiation between Tanzanian chicken ecotypes (F_{ST} 0.048) was found to be smaller than between commercial chicken lines. This is in agreement with several molecular studies, which revealed higher heterozygosity and lower F_{ST} values between African local chickens than between commercial lines (Muchadeyi *et al*., 2007; Eltanany *et al*., 2011; Fosta *et al*., 2011; Goraga, *et al*., 2011; Mtileni *et al*., 2011a). In contrast to commercial lines, which have been managed as distant breeding populations for many generations, following a strict selection scheme, a higher genetic diversity in Tanzania chickens can be expected, as they are managed in a free-range system with random breeding and no selection for performance traits.

Two maternal lineages in Tanzanian local chicken populations were revealed in the analysis of mtDNA sequences, which corresponded to haplogroups D and E described by Liu *et al*. (2006), who identified Southeast Asia and the Indian subcontinent as places of origin, respectively. Liu's clades D and E appear to be the common haplotypes in Eastern Africa. Muchadeyi *et al*. (2008) found the existence of two distinct maternal lineages of Liu's haplogroups D and E, which were evenly distributed among the five Zimbabwean chicken ecotypes. Mwacharo *et al*. (2011) reported the presence of haplotype E in chicken populations in Sudan and Ethiopia, and the presence of haplotype D in Kenya, Uganda, Sudan, and Ethiopia without frequent exchange of genetic materials.

Analyses of microsatellite and phenotypic data revealed population stratification among Tanzanian chicken populations. The results of the cluster analysis using the STRUCTURE software suggest that *Kuchi* might have originated from a different ancestral population than *Ching'wekwe* and *Morogoro*. *Unguja* and *Pemba*, *Morogoro* and *Ching'wekwe*, which clustered together, were distributed in a closer geographical distance without a permanent boundary on the Tanzanian mainland. Although Unguja and Pemba are

islands, 80 km apart, *Unguja* and *Pemba* chicken populations showed a higher degree of admixture among each other than with any of the other three Tanzanian chicken populations under study. This could be the result of a higher exchange of genetic materials between these islands, which form a sovereign state. Social and agriculture interrelationships between Unguja and Pemba were recorded in a Greco-Roman text from the first century AD, when these islands were used as a base for voyages between the Middle East, India, and other parts of Africa (Chami, 2005; Walsh, 2006). In the PC plot based on phenotypic traits, *Unguja* and *Pemba* chickens, which were characterized as island game birds, clustered with *Morogoro-medium* ecotype. Furthermore, mtDNA analysis results indicated that *Unguja*, *Pemba* and *Morogoro* chickens shared a rather equal distribution of haplotypes D and E. These results obtained from mtDNA and microsatellite analysis suggest that effects of genetic drift were stronger within these populations than gene flow between island and mainland populations (Johnson *et al.*, 2003). This is further supported by positive Tajima's *D*-value in *Unguja,* which might indicate a decrease in population size (Johnson *et al*., 2007).

Kuchi sampled from Lake Zone region of Tanzania not only clustered differently owing to its significantly larger body size, but had a pronounced parrot-like beak, which was not found in the other chicken types. These characteristic features were also reported in *Shamo* gamecock by Komiyama *et al.* (2003) when tracing the origin of Japanese gamecocks. The phenotypic similarity of *Kuchi* and *Shamo* birds might be owing to a common ancestry. Mitochondrial DNA sequence analyses revealed that the *Kuchi* haplotype is the same as that found in *Shamo* fighting birds sampled from Shikoku island of Japan in the Kōchi Prefecture Livestock Experiment Station (Oka *et al*., 2007). Although it is not known how *Kuchi* were eventually brought to Tanzania, our genetic data, together with the striking similarity in the names of the chickens, suggest that the *Kuchi* population in Tanzania might have been originated from Kōchi Prefecture in Japan. Furthermore, *Kuchi* showed the lowest genetic diversity among the Tanzanian chicken populations investigated. This finding might be a result of recent isolation of this population from an ancestral population (Crow, 1986; Manthey, 2011; Peters *et al.*, 2012). A low genomic evolutionary rate and elevated inbreeding frequency may have contributed to the low genetic variation observed in this population. Demographic analyses (Tajima's *D*) using mtDNA sequence polymorphism showed a signal of population expansion in the *Kuchi* population characterized by an excess of rare variants consistent with population growth (Tajima, 1989; Aris-Brosou & Excoffier, 1996; Schmidt & Pool, 2002; Johnson *et al*., 2007).

Unguja and *Pemba* game birds were both distributed in Liu's clade D and clade E. Oka *et al.* (2007) and Gongora *et al.* (2008) found Indian fighting birds in haplogroups that have been associated with Liu's clade D and fighting birds from Western Asia and Japan in Liu's clade E (Liu *et al.*, 2006). Cockfighting was among the traditional sports in the Tanzanian islands of Unguja and Pemba, introduced by Austronesians in 945 - 946 AD, as reported by Walsh (2006; 2010). During the great maritime trade in the Indian Ocean between the tenth and eleventh centuries, Zanzibar was the main centre for trading with the mainland Swahili coast (Arsenat *et al*., 2006; Vernet, 2009). The traders carried large amounts of ivory, slaves and animals to Zanzibar (Royer, 2000; UNESCO, 2012). This may possibly be another way in which chickens were introduced to the Zanzibar islands from the East African mainland.

Conclusion

Based on microsatellite information, Tanzanian chickens are clustered into three distinct groups which are related mainly to geographical distribution. *Unguja* and *Pemba* island game birds are clustered together, as well as *Ching'wekwe* and *Morogoro* ecotypes from the East and Central Zones of Tanzania mainland, while *Kuchi* from the Lake Zone forms an independent group. Based on body measurements *Ching'wekwe* ecotype formed an isolated group owing to their short legs and *ulna* bone length, while *Kuchi* ecotype with significant higher in body size formed another group, which overlapped partly with *Morogoro, Unguja* and *Pemba* ecotypes. Two maternal lineages were distributed among the five populations, although *Kuchi* ecotype was found to dominate in one haplotype. In all these analyses, *Kuchi* ecotype tended to remain in a distinct group. *Ching'wekwe*, *Morogoro-medium*, *Unguja* and *Pemba* might have been distributed to Tanzania with the two early main waves of introduction of chickens to Africa in which chickens were introduced along the African East Coast from the Indian Ocean or through Egypt from the Mediterranean before being spread inland through overland routes (MacDonald, 1992; Van Marle-Köster *et al*., 2008; Gifford-Gonzales & Hanotte, 2011). In contrast, *Kuchi* seems to have been introduced recently and is highly associated with *Shamo* gamebirds from Japan.

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