



## Genetic diversity of local chicken ecotypes in selected part of Central Tanzania

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### Abstract

This study was carried out to evaluate the genetic diversity of Central Tanzanian indigenous chicken. Chickens were collected from six different districts of Central part in Tanzania (Dodoma, Kondoa, Singida, Manyoni, Igunga and Tabora). Eighteen microsatellite markers recommended by International Society for Animal Genetics, Food and Agriculture Organization advisory group on animal genetic diversity were used to determine genetic variation. The microsatellite markers used were suitable for the measurement of the genetic diversity and relationship of Tanzanian chicken populations. Statistics related to genetic variation were estimated using GenALEX6. Genetic variation among population was 98% and 2% genetic variance was observed between the six populations. The least number of alleles was seven in MCW0111 locus and highly polymorphic locus was MCW0016 with 23 alleles with a grand mean heterozygosity of  $0.779 \pm 0.018$  across the populations which indicate unlimited gene pool and high gene flow. Nei's genetic distance ranged from 0.203 (lowest) between population of Kondoa and Singida to 0.583 (highest) between population of Tabora and Kondoa suggesting their genetic relatedness and dissimilarity. Principal of Coordinate Analysis (PCoA) results revealed diverse genetic variation within six populations analyzed. The distribution patterns of the chickens showed no distinct group based on the location specific rather than intermixing of the genotypes was observed suggesting random mating of chickens. This study found a lot of genetic variation and relatedness within and among populations. The results from this study can therefore serve as an initial step to plan the conservation of indigenous chickens in Tanzania.

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## Introduction

In Tanzania and Africa in general, indigenous chicken is one of the most important animal species which provides higher proportion of animal protein in the human diet. They produce meat which is usually preferred over exotic chickens due to their pigmentation, taste, flavour and leanness. Tanzania has a good number of local chicken and contributes about 94% of the traditional poultry farming in the country (Swai *et al.*, 2007). Tanzanians local chickens are found in almost every place where there is human settlement, although most of the indigenous chickens are kept in the central corridor regions of Tanzania (FAO, 2007; RDLC, 2010). Most of livestock keepers tend to migrate with their chickens as petty animals when shifting from place to another. Several studies have been conducted on chicken diversity, but most of these studies conducted on local chickens have centred on phenotypic characterization rather than on genetic diversity (Gueye, 1998; Msoffe *et al.*, 2001; Msoffe *et al.*, 2004). Phenotypically, there is an extensive diversity within indigenous domestic chicken and this should be used as a base of breeding for animals that are adapted to a wide range of local environments. However, industrialization and globalization of chicken in the 21st century have adversely affected distribution of chicken genetic resources limiting breed composition to industrial breeds. As a result, many chicken breeds are either extinct or seriously threatened with extinction. Therefore, a need to carry out genetic studies of the indigenous local chicken is of great importance for the purpose of conservation and breeding. Knowing the genotype of the local chicken will be a key point in breeding programs as it reduces the chance of inbreeding. Thus, chicken keepers or producers should not only rely on phenotypic characteristics, rather they should also consider the genotype.

Few studies have reported the genetic diversity of the local chickens in Tanzania (Wimmers *et al.*, 2000; Msoffe *et al.*, 2005; Marle-Koster and Nel, 2013; Lyimo *et al.*, 2013). Several methods of Deoxyribonucleic Acid (DNA) analysis have been employed to study genetic diversity in animals, but

microsatellite DNA genotyping has provided better and reliable results (Marle-Koster and Nel, 2000; Wimmers *et al.*, 2000). DNA based typing methods provide a rapid and reliable method for differentiating individuals in a genetically diverse population (Bidwell, 1994; Parham and Ohta, 1996).

Microsatellites markers are highly polymorphic loci widely dispersed throughout animal genomes and consist of randomly repeated motifs or simple sequence repeats of nucleotide units (Tautz, 1989; Emara *et al.*, 2002). The variability of microsatellite loci is due to differences in the number of repeat units recognized as a major source of genetic variation (Weber and Wong, 1993).

Furthermore, microsatellites are useful in unveiling genetic diversity, individual identification, gene mapping, paternity analysis and the assessment of relatedness, and phylogenetic studies and as a means to measure inbreeding and differences among populations. Microsatellites have an instant rate of evolution making them mostly useful in working out the relationships among very closely related species. In addition, microsatellite markers also provide tools for study of linkages with quantitative trait loci (Zhou and Lamont, 1999).

Accurate determination of the animal genetic variations within animal species is a vital step towards conservation of the animal genetic resources (Oldenbroek, 1999). Thus, this study was carried out to provide information on genetic diversity of the indigenous chicken ecotypes in Central part of Tanzania that would be important in making effective selection and conservation strategies.

## Materials and methods

### Sampling

Blood samples were collected from six districts in the central zone Tanzania. A total of 120 birds were collected from Manyoni (20), Singida (20), Igunga (20), Tabora (20), Kondoa (20) and Dodoma (20). Some of their phenotypic features were recorded including Frizzled, Normal feather, Feathered Shank,

Crested head, Bearded Black, Naked neck and Kuchi. Chickens were randomly sampled from each household, each household distanced by a minimum of 1km from the next household. All chickens sampled were free ranging indigenous chicken. Approximately two millilitres (2ml) of blood was drawn from their wing veins for every bird sampled and stored in serum tubes containing EDTA and were stored at -40°C. Laboratory analysis for the extraction of genomic DNA, its purification and PCR amplification was done at Molecular Biology laboratory, College of Veterinary Medicine and Biomedical Sciences – Sokoine University of Agriculture.

#### *DNA Extraction and Polymerase Chain Reaction*

The genomic DNA was extracted from blood following protocol by Quick-DNA MiniPrep plus Kit (Zymo Research – D4069 Epigenetics CO. Ltd). A total of 18 autosomal microsatellite loci which are among the 30 suggested markers by the International Society for Animal Genetics (ISAG), Food and Agricultural Organisation (FAO) project for surveying chicken biodiversity (FAO, 2012) were used for genotyping. All markers used and their sequences and annealing Temperature are shown in Table 1.

The Polymerase Chain Reaction (PCR) reactions were performed in a total reaction volume of 25µl containing 6.5µl of nuclease free water, 12.5µl of readymade PCR premix (ZymoBIOMICS, Epigenetics CO. Ltd) Primers, 0.5µl (10 pmol/ml) of Forward and Reverse primers and 5µl of template DNA.

The amplification was carried out in a Thermal Cycler (Takara Holdings Inc. Otsu, Shiga Japan). The cycling conditions were; initial temperature for denaturation was set at 95°C for 5 minutes followed by 30 cycles for denaturation at 94°C for 1 minute, annealing temperature was optimized for each marker, extension was 1 minute at 72°C and the final extension was done for 7 minutes at 72°C.

The PCR products were separated by electrophoresis at 100V through a 1.0% agarose-TAE gel for 40 minutes. Cyber safe staining was used for

visualization under UV light followed by scoring of alleles using VisionCapt software (Vilber.de).

#### *Statistical analysis*

The data obtained were processed using GenAlEx6 (Genetic analysis in Excel version 6) which was used to show parameters of genetic diversity within and among populations.

The relationships among 6 chicken populations was estimated by using Nei's genetic distance (Nei, 1987). The analysed results were presented in form of tables, figures and pie chart.

#### *Genetic variability*

Allelic diversity and heterozygosity, Total number of alleles for individual markers (NA), mean number of alleles, number of private alleles by locus and by population, allelic patterns for across populations and genetic diversity were computed using GenAlEx software version 6.502 (Peakall and Smouse, 2012). The analysed results were presented in form of tables and figures.

#### *Genetic distances*

Nei's genetic distance and genetic identity per population were computed using GenAlEx software version 6.502 (Peakall and Smouse, 2012) where a population matrix was used to compare the distances between the different populations and indicate their relationships.

#### *Analysis of Molecular Variance (AMOVA)*

Analysis of molecular variance (AMOVA) was performed to describe variance components within and among populations using GenAlEx software version 6.502 (Peakall and Smouse, 2012) with 1000 permutations.

#### *Principal Coordinate Analysis (PCoA)*

The Principal co-ordinate analysis (PCoA) was carried out using GenAlEx 6.5 based on Nei's genetic estimations. The graph was viewed using the same software to show the distribution of each individual in a sampled population.

## Results and discussion

### Genetic diversity

All the microsatellites used in this study were polymorphic. A total of 197 alleles were observed over the eighteen loci. The number of alleles ranged from  $5.533 \pm 0.322$  to  $8.200 \pm 0.751$ , while the overall mean number of different alleles ( $N_a$ ) was  $6.922 \pm 0.459$ . Effective number of alleles ( $N_e$ ) was  $4.981 \pm 0.354$  and heterozygosity ( $H_e$ ) was  $0.779 \pm 0.018$

(Fig.1 and Table 3). This indicates that there is unlimited sample of gene pool and high gene flow in Tanzanian local chickens. Same study by Lyimo *et al.* (2013) reported mean number of alleles ranging from  $5.10 \pm 2.08$  to  $6.28 \pm 2.24$  in the Tanzanian population of indigenous chickens, which concur with the recent study, this implies that in Tanzanian indigenous chicken there is unrestricted gene pool and higher flow of gene.

**Table 1.** List of microsatellite marker used for genotyping.

Marker	Oligo Seq.	Chromosome location	Annealing Temperature (°C)	Allele range (bp)
ADLo268	CTC CAC CCC TCT CAG AAC TA CAA CTT CCC ATC TAC CTA CT	1	60	102 - 116
MCW0295	ATC ACT ACA GAA CAC CCT CTC TAT GTA TGC ACG CAG ATA TCC	4	60	88 - 106
MCW0081	GTT GCT GAG AGC CTG GTG CAG CCT GTA TGT GGA ATT ACT TCT C	5	60	112 - 135
MCW0014	TAT TGG CTC TAG GAA CTG TC GAA ATG AAG GTA AGA CTA GC	6	55	162 - 182
MCW0183	ATC CCA GTG TCG AGT ATC CGA TGA GAT TTA CTG GAG CCT GCC	7	55	290 - 326
ADLo278	CCA GCA GTC TAC CTT CCT AT TGT CAT CCA AGA ACA GTG TG	8	50	114 - 126
MCW0067	GCA CTA CTG TGT GCT GCA GTT T GAG ATG TAG TTG CCA CAT TCC GAC	10	60	175 - 186
MCW0123	CCA CTA GAA AAG AAC ATC CTC GGC TGA TGT AAG AAG GGA TGA	14	50	75 - 100
MCW0330	TGG ACC TCA TCA GTC TGA CAG AAT GTT CTC ATA GAG TTC CTG C	17	50	256 - 300
MCW0069	GCA CTC GAG AAA ACT TCC TGC G ATT GCT TCA GCA AGC ATG GGA GGA	26	60	158 - 185
MCW0248	GTT GTT CAA AAG AAG ATG CAT G TTG CAT TAA CTG GGC ACT TTC	1	50	205 - 225
MCW0111	GCT CCA TGT GAA GTG GTT TA ATG TCC ACT TGT CAA TGA TG	1	50	96 - 120
MCW0034	TGC ACG CAC TTA CAT ACT TAG AGA TGT CCT TCC AAT TAC ATT CAT GGG	2	60	212 - 248
LEIo234	ATG CAT CAG ATT GGT ATT CAA CGT GGC TGT GAA CAA ATA TG	2	50	216 - 364
MCW0016	ATG GCG CAG AAG GCA AAG CGA TAT TGG CTT CTG AAG CAG TTG CTA TGG	3	60	155 - 206
MCW0037	ACC GGT GCC ATC AAT TAC CTA TTA GAA AGC TCA CAT GAC ACT GCG AAA	3	60	152 - 170
LEIo094	GAT CTC ACC AGT ATG AGC TGC TCT CAC ACT GTA ACA CAG TGC	4	50	247 - 287
MCW0078	CCA CAC GGA GAG GAG AAG GTC T TAG CAT ATG AGT GTA CTG AGC TTC	5	60	132 - 240

In contrast, Okumu *et al.* (2013) reported mean number of alleles over the 18 loci for each population ranged from  $1.895 \pm 0.072$  to 2.00 with an average number of alleles across all loci in all the eight populations being  $1.961 \pm 0.018$ , which indicates limited gene pool and lower gene flow of Kenyan population of local chickens. Halima *et al.*, (2007) reported an average number of alleles across all populations in all loci to be 6.05 in the Ethiopian native chickens. Marle-Koster and Nel (2000) , reported a mean number of alleles ranging from 2.3 to 4.3 in five chicken lines representing the Fowls for Africa program. Wimmers *et al.*, (2000), reported a mean number of alleles ranging from 2 to 11 per locus for the local chickens from Africa, Asia and South

America. Mtileni *et al.*, (2010), reported a higher mean number of alleles per locus ranging from  $3.52 \pm 1.09$  to  $6.62 \pm 3.38$  among the South African chickens. A similar higher mean number of alleles was observed in other free-ranging chickens reported by Muchadeyi *et al.*, (2007), in Zimbabwean, Malawian and Sudanese chicken populations.

These differences in heterozygosity values may be attributed by variation in geographical location, chicken types, sample sizes, laboratory and sources of microsatellites used. Expected heterozygosity value of  $< 0.5$  may also be due to inbreeding and admixture as this occurrence is associated with population constrains and bottlenecks (Fariba, 2011).

**Table 2.** Total allelic patterns by populations.

Population	Manyoni	Tabora	Igunga	Dodoma	Kondoa	Singida
Na	122	83	123	101	96	98
Na Freq. $\geq 5\%$	105.000	83.000	79.000	101.000	96.000	98.000
Ne	32.000	9.000	17.000	5.000	2.000	0.000
No. LComm Alleles ( $\leq 25\%$ )	0.000	0.000	0.000	0.000	0.000	0.000
No. LComm Alleles ( $\leq 50\%$ )	23.000	16.000	31.000	22.000	24.000	27.000

Number of allele with frequency  $\geq 5\%$ , Number of Locally Common alleles (frequency  $\geq 5\%$ ) found in  $\leq 25\%$  and  $\leq 50\%$  of populations.

**Table 3.** Mean and standard errors of the different genetic diversity parameters estimated for six local chickens populations.

Population		Na	Na Freq. $\geq 5\%$	Ne	I	No. Private Alleles	He
Manyoni	Mean	8.133	7.000	5.594	1.836	2.133	0.798
	SE	0.723	0.775	0.555	0.094	0.646	0.018
Tabora	Mean	5.533	5.533	4.463	1.554	0.600	0.748
	SE	0.322	0.322	0.350	0.080	0.190	0.028
Igunga	Mean	8.200	5.267	5.248	1.795	1.133	0.784
	SE	0.751	0.284	0.570	0.092	0.274	0.019
Dodoma	Mean	6.733	6.733	4.888	1.681	0.333	0.778
	SE	0.452	0.452	0.366	0.072	0.159	0.017
Kondoa	Mean	6.400	6.400	4.891	1.691	0.133	0.788
	SE	0.235	0.235	0.226	0.045	0.091	0.011
Singida	Mean	6.533	6.533	4.804	1.683	0.000	0.777
	SE	0.274	0.274	0.317	0.056	0.000	0.017

Total allelic patterns by populations are summarised in Table 2 and Mean and standard errors of the different genetic diversity parameters estimated for six local chickens populations are summarised in Table 3. Briefly, total number of different alleles identified was higher in Igunga (N=123) and lower in

Tabora (N=83). The effective number of alleles was higher in Manyoni (N=32) and lower in Singida (N=0.00) as shown in Table 2.

The number of alleles and allele's frequency identified in each locus are shown in Fig. 2. The least number of

alleles was seven in MCW0111 Locus and highly polymorphic locus was MCW0016 with twenty three alleles. The mean number of information index, private alleles and heterozygosity was higher in Manyoni populations with mean average of  $1.836 \pm$

$0.094$ ,  $2.133 \pm 0.646$  and  $0.798 \pm 0.018$ , respectively (Fig. 1 & Table 3). The summary of private alleles at specific loci and their frequencies in all six populations are shown on table 4.

**Table 4.** Summary of numbers of private alleles at specific loci and their frequencies.

Population	Private alleles (N)	Locus	Alleles	Frequency
Manyoni	32	MCW0295	89	0.125
		MCW0295	92	0.063
		MCW0295	95	0.063
		MCW0081	121	0.050
		MCW0081	126	0.050
		MCW0016	157	0.118
		MCW0016	158	0.059
		MCW0016	166	0.059
		MCW0016	174	0.059
		MCW0016	176	0.059
		MCW0016	183	0.059
		MCW0016	191	0.059
		MCW0016	204	0.059
		MCW0034	218	0.118
		MCW0034	247	0.059
		MCW0034	248	0.059
		MCW0078	132	0.053
		MCW0078	228	0.053
		MCW0069	162	0.045
		MCW0069	164	0.045
		MCW0069	166	0.045
		MCW0069	168	0.045
		MCW0069	169	0.045
		MCW0069	184	0.045
		MCW0183	323	0.100
		MCW0183	325	0.050
		MCW0123	75	0.045
MCW0123	82	0.045		
MCW0123	83	0.095		
MCW0123	84	0.048		
MCW0123	85	0.048		
MCW0248	214	0.095		
Tabora	9	ADL0268	111	0.222
		ADL0268	112	0.222
		MCW0081	128	0.125
		MCW0016	198	0.125
		MCW0034	212	0.111
		MCW0034	242	0.111
		MCW0078	139	0.111
		MCW0069	173	0.091
		MCW0330	297	0.100
Igunga	17	ADL0268	106	0.040
		MCW0081	118	0.043
		MCW0081	119	0.083
		MCW0081	134	0.083
		MCW0016	168	0.040
		MCW0016	185	0.040
		MCW0016	205	0.080
		MCW0034	234	0.038
		MCW0034	245	0.038
		MCW0078	136	0.043
		MCW0078	240	0.043
		MCW0067	175	0.038
		MCW0037	156	0.083
		MCW0037	168	0.043
		MCW0183	312	0.037
MCW0123	98	0.036		
LEI0234	252	0.037		
Dodoma	5	MCW0081	206	0.059
		MCW0034	219	0.063
		MCW0034	232	0.063
		MCW0067	182	0.053
		MCW0037	170	0.056
Kondoa	2	MCW0123	96	0.059
		MCW0248	211	0.176

**Table 5.** Analysis of Molecular Variance (AMOVA) table showing the percentage variations among populations and within populations.

Source	df	SS	MS	Est. Var.	%
Among Pops	5	41.838	8.368	0.109	2%
Within Pops	114	709.739	6.226	6.226	98%
Total	119	751.577		6.334	100%

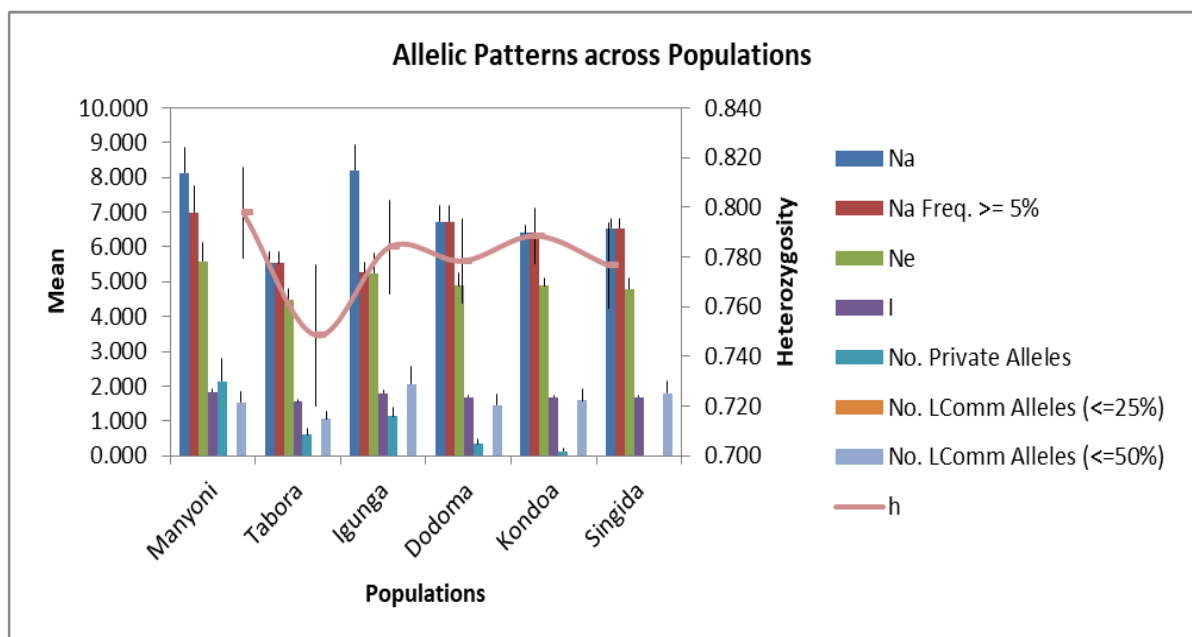
**Table 6.** Pairwise Population Matrix of Nei Genetic Distance.

Manyoni	Tabora	Igunga	Dodoma	Kondoa	Singida	
0.000					Manyoni	
0.359	0.000				Tabora	
0.286	0.308	0.000			Igunga	
0.350	0.451	0.281	0.000		Dodoma	
0.421	0.583	0.462	0.250	0.000	Kondoa	
0.327	0.474	0.378	0.248	0.203	0.000	Singida

*Principal Coordinate Analysis (PCoA)*

PCoA were used to determine the distribution of the populations and the genetic relatedness among the population genotypes (Fig. 3). Majority of the chickens from Igunga and Tabora were clustered together with chickens from Manyoni on the first and second quadrant showing their genetic relatedness. Majority of the chickens from Kondoa were clustered on the right side of axis 1 on the fourth quadrant while the remaining populations from Dodoma and Singida were distributed uniformly in all four

quadrants. Generally, PCoA did not clearly group genotypes based on the location specific. But intermixing of the genotypes across the coordinates was observed. However, some distinct genotypes from Manyoni, Igunga and Kondoa were inclined to stay on the quadrant 1 and IV respectively indicating their genetic identity and dissimilarity. Lyimo *et al.*, (2013) did a plot of the principal component and the ecotypes studied were grouped into three clusters with axis 1 showing more variation than other clusters.



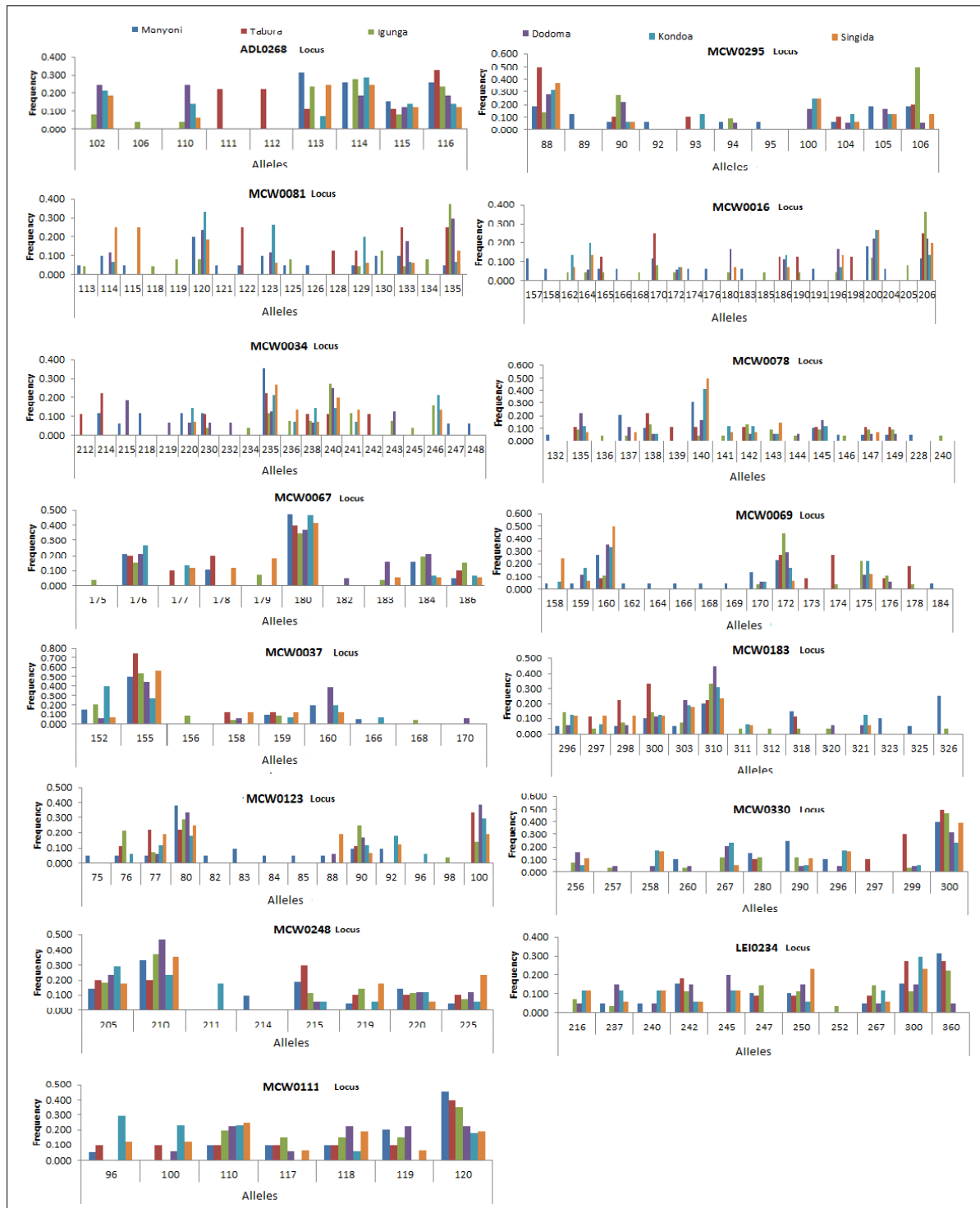
Number of alleles (Na), Number of allele with frequency >=5%, Effective number of alleles, information index (I) and Number of private alleles.

**Fig. 1.** Mean values, Heterozygosity and Allelic patterns across six populations.

*Analysis of Molecular Variance (AMOVA)*

AMOVA was used to examine the partitioning of genetic variation (Table 5). Analysis of the 120 Local chickens from 6 different populations showed that within-group genetic differences accounted for 98% of the total variations indicating panmictic

populations, there are no mating restrictions of individuals within population. Only 2% variations among populations were observed, indicating individuals from different populations are genetically more closely related than within population (Fig. 4).

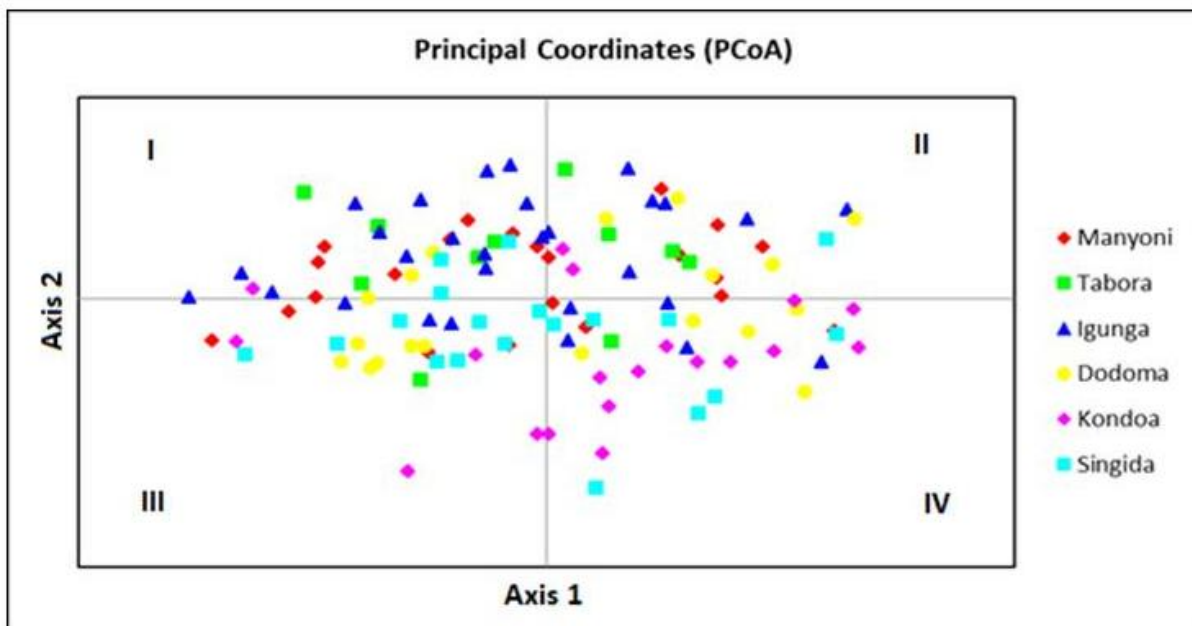


**Fig. 2.** Number of alleles and allele's frequency identified in each locus. Least number of alleles was seven in MCW0111 Locus and highly polymorphic locus was MCW0016 with twenty three alleles.

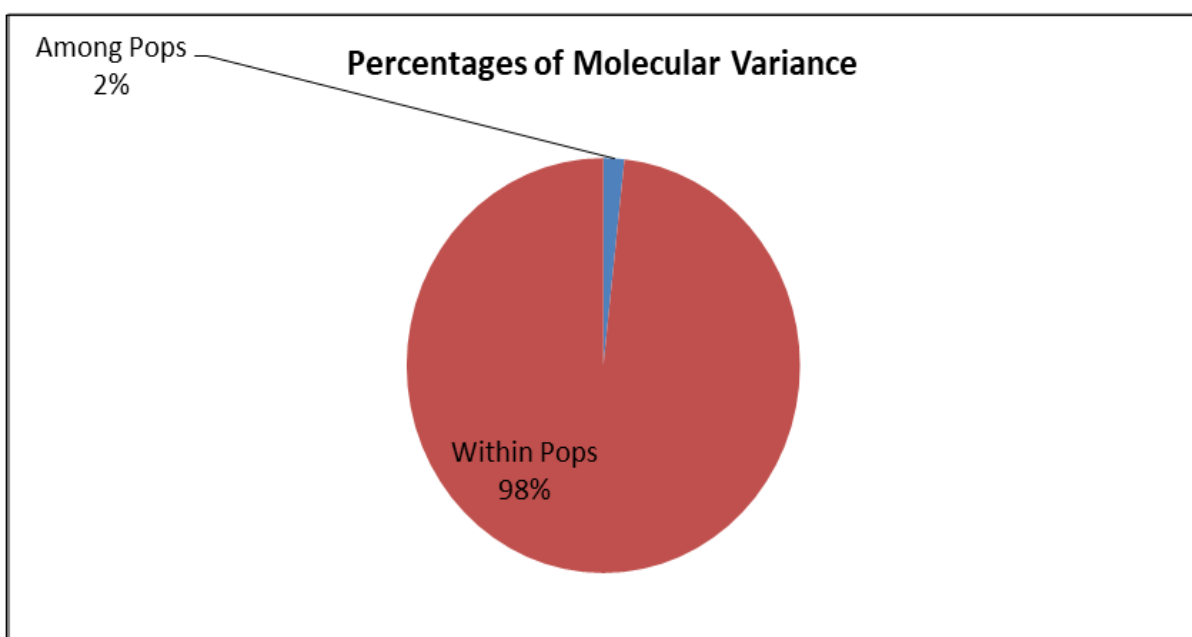


Nei's genetic distance was estimated between six populations (Table 6). The pairwise Nei's genetic distance ranged from 0.203 (lowest) between population of Kondo and Singida to 0.583 (highest) between population of Tabora and Kondo (Table 6) suggesting their genetic relatedness and dissimilarity. Tanzanian indigenous chickens are therefore closely related to one another compare to their counterparts of Ethiopia. This could be due to the cultural practices

and admixture. Halima *et al.*, (2007), studied the genetic variation in the 147 native chickens (seven populations) from northwest Ethiopia, and reported the smallest and largest genetic distance of 0.073 and 1.3, respectively. Vanhala *et al.*, (1998) evaluated the genetic variability and genetic distances between eight chicken lines using microsatellites and reported the smallest and the largest genetic distances of 0.117 and 1.17, respectively.



**Fig. 3.** Scatter diagram showing principal coordinate analysis for 6 local chickens populations collected from Central zone in Tanzania.



**Fig. 4.** Pie chart showing the percentage variations among populations and within populations.

## Conclusion

The results revealed diverse genetic variation within six populations analyzed. The distribution patterns of the chickens show no distinct group based on the location specific. Intermixing of the genotypes is observed suggesting random mating of chickens. However, more extensive sample size required from different sites to draw firm conclusion about genetic diversity of local chickens within populations in central zone. The preliminary findings obtained from this study may provide background for future studies of identification of the genetic uniqueness of Tanzanian native chicken breeds, breeding programmes and development of conservation strategies.

## Competing interests

The authors declare that they have no competing interests

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