ORIGINAL ARTICLE

Genetic diversity of indigenous chickens from selected areas in Kenya using microsatellite markers

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Abstract   In this study, indigenous chickens were collected from eight different regions in Kenya and kept at InCIP-Egerton University. These were studied using eighteen microsatellite markers to determine genetic variation. Statistics related to genetic variation were estimated using GenA-LEx6. Mean percentage polymorphic loci (PPL) was 96.71% and 4% genetic variance ($p \geq 0.003$) was seen between the eight populations. MCW0123 marker had the highest genetic variance of 13% among populations ($p \geq 0.003$) at 95% CI. Mean He ranged from 0.351 ± 0.031 (SIB) to 0.434 ± 0.022 (BM) with a grand mean He of 0.399 ± 0.011 across the populations using the microsatellite markers. Nei's genetic distance ranged from 0.016 (SIB and WP) to 0.126 (NR and SIB). DARwin6.501 analysis software was used to draw the population dendrogram and two major population clusters were observed, also seen with PCoA. This study found a lot of genetic variation and relatedness within and among populations. Based on the phylogenetic tree result, it is concluded that the clustering of the chicken populations in the present study is not based on geographical proximity. The microsatellite markers used in this study were suitable for the measurement of the genetic biodiversity and relationship of Kenyan chicken populations. These results can therefore serve as an initial step to plan the conservation of indigenous chickens in Kenya.

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1. Introduction

The indigenous chicken is one of the most important animal species worldwide since it provides higher proportion of animal protein in the human diet. Indigenous chickens are also kept for income and sociocultural roles among the Kenyan

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communities. The indigenous chickens are usually preferred over exotic chickens due to their pigmentation, taste, flavor and leanness. The contemporary chicken was most likely developed from its main wild ancestor, the red jungle fowl (*G. gallus*) after its domestication in Southeast Asia in 3200BC. Chicken, over the years, has evolved from the wild form to layers, broilers, bantams, game and fancy breeds as well as the indigenous village chicken we have today. Physically, the diversity within indigenous domestic chicken is extensive and this should provide a breeding base for animals that are adapted to a variety 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. This study was formulated to provide information on genetic diversity of the indigenous chickens in Kenya that would be important in designing effective selection and conservation strategies. The domestic indigenous chicken has a haploid number of 39 chromosomes, 8 pairs of macrochromosomes, one pair of sex chromosomes (Z and W) and 30 pairs of microchromosomes. The size of the chicken genome is estimated to be 1.2×10^9 base pairs [6]. Chickens, like other avian species, differ from mammals in that the female is the heterogamete sex and the male is the homogametic sex [15]. The full genomic sequence of the chicken provides a large number of microsatellites for genetic diversity studies.

Indigenous Poultry farming under free range system is a common practice among the rural small holder farmers in Kenya, but it is still considered a small sector of the poultry industry. Industrial breeding companies in Kenya have just started putting effort into developing stocks that specialize and perform well under this management method e.g. the Kenbro, but at present specialized stocks are not yet available to free range producers. Some free range producers are attempting to develop their own breeding stock e.g. Keleo poultry international in Siaya, but most of them lack the necessary skills and resources.

Genetic variability among chicken population was tested using 18 microsatellite markers. Out of this study, the information on genetic diversity in the selected populations of chickens that can be used to facilitate decision making for conservation and development of breeding stocks of free-range production system is made available.

Microsatellites markers are highly polymorphic loci widely dispersed throughout animal genomes and consist of randomly repeated motifs or simple sequence repeats of mono-, di-, tri-, tetra-, or penta-nucleotide units [16,1]. The variability of microsatellite loci is due to differences in the number of repeat units recognized as a major source of genetic variation [18]. 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 a very rapid rate of evolution making them particularly useful in working out the relationships among very closely related species. Microsatellite markers also provide tools for study of linkages with quantitative trait loci [20,4]. Microsatellites have not been used successfully in reconstructing phylogenies because of some restrictions to divergence caused by range constrains, irregularities and

asymmetries in the mutation process and the degradation of microsatellites over time. They are also inappropriate for the study of deep phylogeny because their high mutation rates lead to a large amount of homoplasy over a relatively short period [3]. Microsatellites exist in both coding and non-coding regions of the genome and are co-dominant and highly reproducible.

2. Materials and methods

2.1. Study area

The research was carried out at Kenya Agricultural and Livestock Research Organization (KALRO) - Biotechnology Research Institute laboratories, Nairobi. For result validation and reliability, twenty samples were picked at random and the same experiments done in replicates.

2.2. Chicken populations

A total of 150 chickens representing eight indigenous chicken populations: Taita Taveta (TT, 20), Siaya Bondo (SIB, 20), Kakamega (KK, 28), Bomet (BM, 12), Narok (NR, 12), West Pokot (WP, 20), Turkana (TK, 18) and Lamu (LM, 20) were selected based on the different phenotypic characteristics (Dwarf, Frizzled, Normal feather, Bantam white, Feathered Shank, Crested head, Bearded Black, Naked neck, Game and Kuchi) and kept at InCIP-Egerton University.

2.3. Blood sample collection and genomic DNA extraction

Whole blood was collected by bleeding from the wing vein of the chickens. This was then transferred into serum tubes containing EDTA or Heparin (anticoagulants) and stored at −40 °C.

Genomic DNA extraction was done using Quick-gDNA MiniPrep kit (Catalog NO: D3025) from ZYMO RESEARCH. 400 μl of genomic lysis buffer was added to 100 μl of whole blood in a microcentrifuge tube. This was mixed completely by vortexing for 6 s and then let to stand for 10 min at room temperature. The mixture was transferred to a Zymo Spin column in a collection tube and then centrifuged at 10000xg for 1 min. The collection tube with the flow through was discarded. The Zymo Spin column was then transferred to a new collection tube and 200 μl of DNA pre-wash buffer added to the Spin column and then centrifuged at 10,000g for 1 min. The Spin column was transferred to a clean collection tube and 500 μl of gDNA wash buffer added to the spin column and centrifuged at 10,000g for 1 min. The spin column was transferred to a clean microcentrifuge tube and 50 μl of DNA elution buffer added to the spin column, incubated (allowed to stand) at room temperature for 5 min, and then centrifuged at top speed for 30 s to elute the genomic DNA. The DNA concentration was measured using a spectrophotometer (NanoDrop 2000c – Thermo Scientific). This was then stored at −20 °C for further molecular based applications.

2.4. Characterization of the 18 microsatellite markers

Eighteen highly polymorphic microsatellite markers, widely distributed over the genome on 13 different chromosomes

(out of the known 39) were studied. Multiple markers on the same chromosomes are well spaced and they are genetically independent [2]. These markers have been used in previous biodiversity studies in chickens [8], (Table 1).

The sequence fragments were amplified by PCR using previously designed primers obtained from the published literature for each of the microsatellite markers [5,8]. The PCR reactions were performed in a total reaction volume of 25 μl containing 19.375 μl of nuclease free water, 2.5 μl of 10× buffer (including 20 mM MgCl$_2$), 1 μl of 2 mM dNTP mix, 0.5 μl (10 pmol/μl) Forward primer, 0.5 μl (10 pmol/μl) Reverse primer, 0.125 μl of Dream Taq polymerase and 1 μl of template DNA. The amplification was carried out in a thermo cycler (GeneAmp™ PCR system 9700) using the cycling conditions shown in Table 2.

The cycling conditions were optimized for each marker. The PCR products were separated by electrophoresis at 75 V through a 1.5% agarose-TBE gel depending on the fragment sizes for 45 min. Ethidium Bromide (1 mg/ml) staining was used for visualization under UV light followed by visual scoring of data.

2.5. 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. DARwin5.0.158. was used to draw dendrogram of relationships among 8 chicken populations using Nei's [14] genetic distance and neighbor-joining methods. The analysis was presented in form of tables and figures.

3. Results and discussion

3.1. Genetic diversity

In this study, substantial amount of genetic diversity using SSRs was found among populations but was narrow. All the

Table 2 PCR cycling conditions.

Cycle no.	Temperature in °C	Time in minutes
1	94	5
	50 (Primer dependent)	2
	72	1
30	94	1
	50 (Primer dependent)	2
	72	1
1	94	1
	50 (Primer dependent)	2
	72	10
Hold	10	Infinity

microsatellites used were polymorphic. A total of 282 alleles were observed over the eighteen loci. The mean number of alleles and the expected heterozygosity are shown in Table 3. The mean number of alleles over the 18 loci for each population ranged from 1.895 ± 0.072 to 2.00 with an average number of alleles across all loci in all the eight populations being 1.961 ± 0.018. Lyimo et al. [9] reported MNA ranging from 5.10 ± 2.08 to 6.28 ± 2.24 in the Tanzanian population. Halima et al. [7] 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 [10] 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. [19] 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. [11] reported a higher mean number of alleles per locus ranging from 3.52 ± 1.09 to 6.62 ± 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. [12] in Zimbabwean, Malawian and Sudanese chicken populations. The lower MNA in the Kenyan populations as compared to these other populations shows the pres-

Table 1 List of microsatellite markers used.

Marker	Chromosomal location	Position CM	Repeat	Annealing temp in °C	Allele size (bp)
MCW0111	1	118	(AC)8	48	96–120
ADL0268	1	288	(GT)12	48	102–116
ADL0185	2	103	(CA)16	53	128–150
MCW0034	2	233	(CA)24	54	223–245
ADL0146	2	403	(TG)17	51	150–166
MCW0004	3	155	(CA)28	60	149–199
MCW0037	3	317	(CA)8	57	154–160
LEI0094	4	153	(AC)16	50	253–285
MCW0029	5	128	(CA)29	58	149–194
ADL0298	5	198	(CA)14	54	120
MCW0014	6	50	(CA)18	52	164–188
MCW0183	7	86	Compound	54	290–311
ADL 278	8	94	(TG)18	47	114–126
MCW0067	10	59	(GT)11	54	178–184
ADL210	11	54	(AC)15	44	124–147
MCW0123	14	45	(CA)10	49	94
MCW0330	17	41	Compound	50	260–290
MCW0069	26	47	(CA)11	60	145–185

Table 3 Mean number of alleles and expected heterozygosity ($\pm$ SE).

Pop	N	Na*	Ne**	I	He***	uHe
BM	12.000	2.000	*1.804 $\pm$ 0.053*	*0.620*	*0.434 $\pm$ 0.022*	*0.453*
KK	27.000	2.000	1.639 $\pm$ 0.072	0.543	0.366 $\pm$ 0.030	0.373
LM	20.000	1.895 $\pm$ 0.105	1.735 $\pm$ 0.066	0.582	0.404 $\pm$ 0.029	0.414
NR	12.000	1.895 $\pm$ 0.072	1.795 $\pm$ 0.074	0.587	0.416 $\pm$ 0.036	0.434
SIB	20.000	2.000	*1.598 $\pm$ 0.068*	*0.524*	*0.351 $\pm$ 0.031*	*0.360*
TK	18.000	1.895 $\pm$ 0.072	1.719 $\pm$ 0.076	0.556	0.389 $\pm$ 0.038	0.400
TT	21.000	2.000	1.787 $\pm$ 0.050	0.619	0.431 $\pm$ 0.020	0.441
WP	20.000	2.000	1.729 $\pm$ 0.062	0.585	0.404 $\pm$ 0.028	0.414
Grand mean over loci and pops						
	N	Na	Ne	I	He	uHe
Total	18.750 $\pm$ 0.375	1.961 $\pm$ 0.018	1.726 $\pm$ 0.023	0.577 $\pm$ 0.013	*0.399 $\pm$ 0.011*	0.411 $\pm$ 0.011

Na* number of alleles.
Ne** number of effective alleles.
He*** expected heterozygosity.

Table 4 Analysis of molecular variance (AMOVA).

Source	Degree of freedom	Sum of Squares	MS	Estimated Variation	Percentage of variation
Among Pops	7	44.017	6.288	0.151	4%
Within Pops	142	494.210	3.480	3.480	96%
Total	149	538.227		3.631	100%
Stat	Value	P(rand $\geq$ data)			
PhiPT	0.042	0.003			

Table 5 AMOVA for each of the 18 markers.

Marker	MCW0111	ADL0268	ADL0185	MCW0034	ADL0146	MCW0004	MCW0037	LEI0094	MCW0029
% AP	0	0	2	0	0	0	0	9	7
% WP	100	100	98	100	100	100	100	91	93
	ADL0298	MCW0014	MCW0183	ADL278	MCW0067	ADL210	MCW0123	MCW0330	MCW0069
% AP	0	5	4	0	6	8	13	8	12
% WP	100	95	96	100	94	92	87	92	88

Table 6 Percentage of polymorphic Loci.

Population	%P
BM	100.00%
KK	100.00%
LM	94.74%
NR	89.47%
SIB	100.00%
TK	89.47%
TT	100.00%
WP	100.00%
Mean	96.71%
SE	1.70%

ence of a relatively limited sample of gene pool, and therefore there is a lower gene flow in the Kenyan populations.

Heterozygosity was calculated to determine genetic variation. The mean expected heterozygosity ranged from 0.351 $\pm$ 0.14 (SIB population) to 0.434 $\pm$ 0.08 (BM population), Table 3. These results concur with other studies of the Ethio-

pian native chickens where Marle-Koster and Nel [10] and Vanhala et al. [17] reported heterozygosity values ranging from 0.31 to 0.61 and 0.29 to 0.67 respectively. Halima et al. [7] and Wimmers et al. [19] reported higher values of 0.66 to 0.93 and 0.45 to 0.71 respectively. Mtileni et al. [11] reported expected heterozygosity of 0.67 $\pm$ 0.02 to 0.69 $\pm$ 0.02 among the South African free range chickens. Lyimo et al. [9] reported expected heterozygosity values of 0.58 $\pm$ 0.034 to 0.67 $\pm$ 0.027 in the Tanzanian populations. These differences in heterozygosity values may be attributed to 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 [3].

The fixation index between Kenyan chicken populations (F_{ST}) is 0.04; that is, the genetic diversity between the eight Kenyan chicken populations constituted 4% of the total genetic variance ($p \geq 0.003$) (Table 4). MCW0123 showed the highest molecular variance of 13% among populations (Table 5). Similar results were also reported by Lyimo et al.

Table 7 Pairwise Population Nei genetic distance.

	BM	KK	LM	NR	SIB	TK	TT	WP
BM	0.000							
KK	0.046	0.000						
LM	0.017	0.051	0.000					
NR	0.034	0.099	0.048	0.000				
SIB	0.082	0.027	0.094	*0.126*	0.000			
TK	0.031	0.059	0.028	0.030	0.096	0.000		
TT	0.021	0.023	0.029	0.055	0.045	0.031	0.000	
WP	0.042	0.017	0.050	0.084	*0.016*	0.060	0.023	0.000
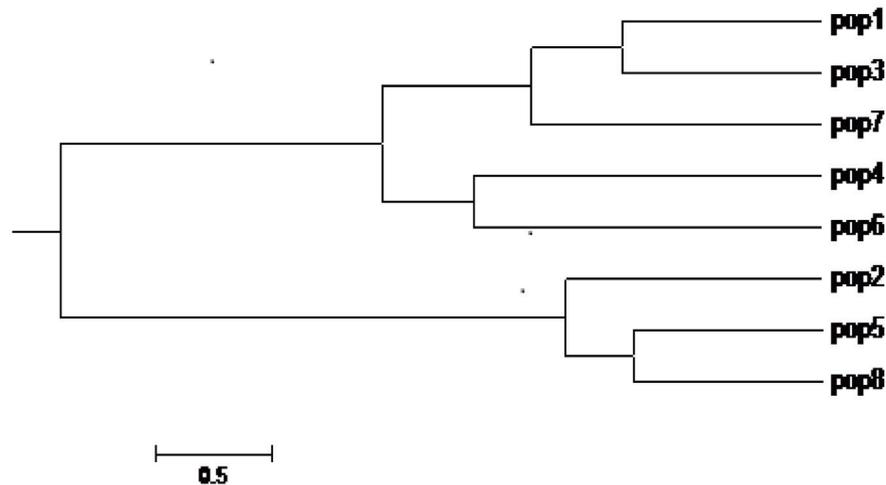
Fig. 1 Dendrogram of relationships. Legend: pop1: Bomet; pop2: Kakamega; pop3:Lamu; pop4:Narok; pop5: Siaya Bondo; pop6: Turkana; pop7: Taita Taveta; pop8:West Pokot.
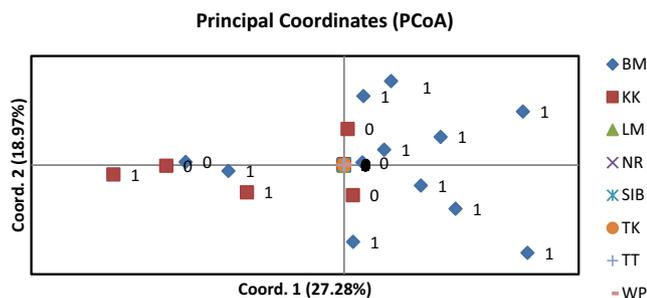

Fig. 2 Principal coordinate analysis (PCoA) (PC$_1$ and PC$_2$).

[9]. Mtileni et al. [11] reported a lower F_{ST} value of 0.008 ± 0.003 among the village chicken populations of South Africa. Similar results were also reported by Muchadeyi et al. [12] who showed that Zimbabwean populations are not genetically differentiated with an average fixation index between breeds (F_{ST}) of 0.008. Mwacharo et al. [13] identified genetic subdivisions between the Kenyan and Ugandan chicken populations and the Ethiopian and Sudanese chicken populations, but within individual country's populations, the F_{ST} values were always < 0.1. This study concurs with the results by Mwacharo et al. [13].

3.2. Percentage of polymorphic loci

Microsatellites are useful in population genetics studies since they showed high percentage polymorphic loci ranging from 89.47% to 100% (Table 6). Halima et al. [7] used polymorphic information content to assess how informative the markers used were and got an average PIC value of 0.71. Both PIC and PPL values in these studies show that the markers used were informative in showing genetic diversity.

3.3. Genetic distance

In the present study, the smallest genetic distance was observed between Siaya Bondo and West Pokot populations while the biggest genetic distance was observed between Narok and Siaya Bondo populations (Table 7). Kenyan indigenous chickens are therefore very closely related to one another. Halima et al. [7] 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. [17] 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. Kenyan indigenous chickens are therefore closely related as compared to their

Table 8 Principal coordinate analysis (PCoA).

Axis no.	1	2	3	4	5	6	7	8
Eigen value	12.362	8.595	7.781	6.199	5.710	2.002	1.889	0.777
%	27.28	18.97	17.17	13.68	12.6	4.42	4.17	1.71
Cum %	27.28	46.25	63.42	77.1	89.7	94.12	98.29	100

Ethiopian counterparts. This could be due to the cultural practices and admixture.

3.4. Phylogenetic tree

The phylogenetic consensus tree in this study showed that the eight chicken populations were divided into two major clusters (Fig. 1). These two clusters were further sub-clustered. Clustering and sub-clustering show possible inbreeding of the populations. This shows that Kenyan indigenous chickens can basically be grouped into two genetic populations. Considering the geographical locations of all these populations, and how randomly a population is linked to another in the current study (for instance Siaya Bondo/West Pokot and Lamu/Bomet), this could be explained by the cultural practices among the people of these communities: traveling with chickens from one location to another. This facilitates inbreeding. Halima et al. [7] clustered the Ethiopian indigenous chickens into two clusters, showing the presence of two major breeds. A cluster shows the level of inbreeding and populations that cluster together could be sharing the same ancestry [7]. Sub-clusters show further differentiation and populations that sub-cluster together are even more related and this shows intense inbreeding.

The distribution patterns of the chickens as revealed by the dendrogram show no distinct grouping with respect to geographical proximity. Two major population clusters seen and sub-clusters reveal social and cultural practices within and among the communities in Kenya.

In this study, the samples were partitioned into two distinct groups using principal coordinates analysis (Fig. 2) with axis 1 showing variation of 27.28% and axis 2 showing variation of 18.97% (Table 8). Lyimo et al. [9] did a plot of the principal component and the Tanzanian ecotypes grouped into three clusters with axis 1 showing variation of 89.9% and axis 2 showing variation of 5.13%.

It can be concluded that the 150 indigenous chickens from the study areas show genetic variation both within and among the eight populations. The distribution patterns of the chickens as revealed by the phylogenetic tree show no distinct grouping with respect to geographical proximity. Two major population clusters seen and sub-clusters reveal social and cultural practices within and among the communities in Kenya, where chickens are transferred from one location to another. The diversity seen at the acquired immunity level (using the 18 microsatellite markers) with P(0.003) value < 0.05 (95% CI) indicates that the Kenyan indigenous chickens are diverse, and need to be conserved.

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