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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 GenALEx6. 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.

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 10,000*g* 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 10,000*g* 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