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MITOCHONDRIAL DNA D-LOOP ANALYSIS OF SOUTHWESTERN NIGERIAN CHICKEN

ANALISIS DE D-LOOP ADN MITOCONDRIAL DE POLLOS DE SW NIGERIA

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ADDITIONAL KEYWORDS

Local poultry breeds. Genetic diversity. Haplotype. Phylogenetic tree.

PALABRAS CLAVE ADICIONALES

Razas aviares locales. Diversidad genética. Haplotipo. Árbol filogenético.

SUMMARY

Mitochondrial DNA (mtDNA) D-loop segment was sequenced for a total of 98 individuals of domestic chicken from South Western Nigeria. Domestic chicken populations were: Anak titan (Israeli breed, n= 1), Frizzle (n= 16), Opipi (n= 5), FrizzleXOpipi (n= 5), Fulani (n= 4), Giriraja (Indian breed, n= 3), Normal (n= 55), Naked neck (n= 8), Yaffa (n= 1). The sequences of the first 397 nucleotides were used for the analysis. Seventeen haplotypes were identified in the samples, 15 for Nigerian indigenous chicken population, 1 for Giriraja and 1 for Anak titan from 23 polymorphic sites. Phylogenetic analysis shows that Nigerian

indigenous and Anak titan chicken were all grouped under clade IV, while the Indian Giriraja was under clade IIIc. Clade IV had 16 haplotypes, while clade IIIc had one haplotype. AMOVA analysis indicates that 97.32% of the total sequence variation between haplotypes was present within population and 2.68% between populations. Our results suggest single multiple maternal origins for the South Western Nigerian domestic chicken.

RESUMEN

Un segmento D-loop de ADN mitocondrial

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(mtADN) fue secuenciado para un total de 98 pollos domésticos de SW Nigeria. Las poblaciones domésticas de pollos fueron: Anak titan (raza israelí, n= 1), Frizzle (n= 16), Opipi (n= 5), FrizzlexOpipi (n= 5), Fulani (n= 4), Giriraja (raza india, n= 3), Normal (n=55), Cuello Desnudo (n=8), Yaffa (n= 1). Las secuencias de los primeros 397 nucleótidos fueron usadas para el análisis. Diecisiete haplotipos de 23 sitios polimórficos, fueron identificados en las muestras: 15 para las poblaciones indígenas nigerianas de pollos, 1 para Giriraja y 1 para Anak Titan. El análisis filogenético, muestra que los pollos indígenas nigerianos pueden agruparse todos dentro del clade IV, mientras que el Giriraja indio, se encuadró en el clade IIIc. El clade IV tiene 16 haplotipos mientras que el clade IIIc tiene sólo un haplotipo. El análisis AMOVA indica que el 97,32% de la variación total de la secuencia entre haplotipos estuvo presente dentro de la población y el 2,68% entre poblaciones. Los resultados sugieren un solo origen maternal múltiple para los pollos domésticos de SW Nigeria.

INTRODUCTION

It is widely established that all populations of domesticated chicken descend from a single ancestor, the red jungle fowl (*Gallus gallus gallus*), which originated in Southeast Asia (Akishinomiya *et al.*, 1994, 1996). Chicken is the most widely distributed of all livestock and poultry species in African countries. It plays a very significant role as a source of income and high quality protein to the rural households.

Knowledge on the distribution of chicken genetic diversity in Africa would be useful in optimizing both conservation and utilization strategies for indigenous chicken genetic resources. The diversity of Nigerian local chickens reported is based mostly on phenotypes including plumage color, feathering pattern, adult body weight, egg weight, reproduction performance and immune responses to various diseases (Omeje and Nwosu, 1983, Nwosu *et al.*, 1985, Ikeobi *et al.*, 1996, Adebambo *et al.*, 1999, Adebambo, 2002) which have limited utility in the study of genetic variation. Mitochondrial DNA (mtDNA) sequences

have successfully been used to determine genetic diversity in Asian chicken (Niu *et al.*, 2002; Liu *et al.*, 2004) and African chicken (Mobegi and Chicken Diversity Consortium, 2005).

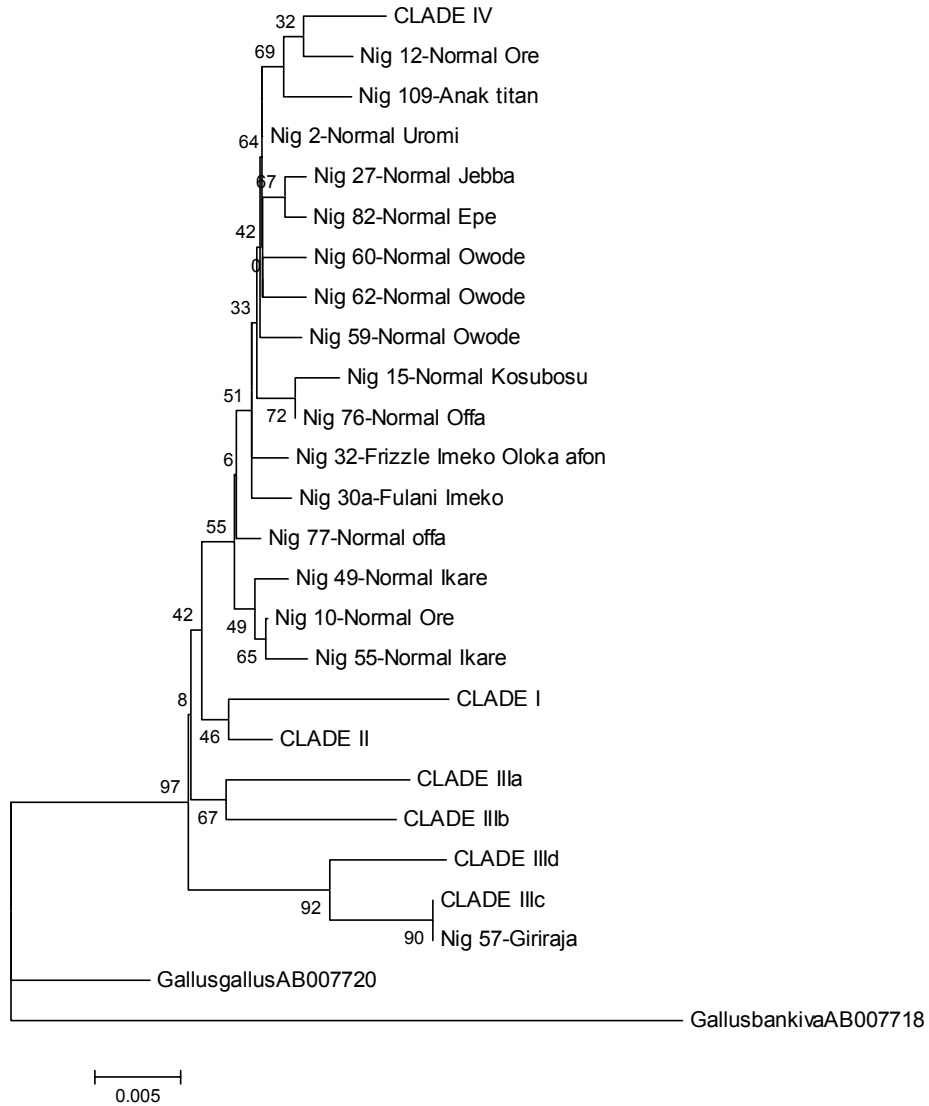
Chicken mtDNA has 16,775 base pairs (Desjardins and Morais, 1990). MtDNA is highly polymorphic compared to nuclear DNA, evolutionary rate being 5 to 10 times faster than the nuclear genome (Brown *et al.*, 1982). Different regions of the mtDNA evolve at different rates (Saccone *et al.*, 1991), making it a marker of choice for studying genetic diversity within as well as between species. The displacement (D)-loop region is non-coding and evolves much faster than other regions of the mtDNA genome. This makes it particularly useful for phylogeographic analysis (Avice, 1994). MtDNA is maternally inherited in most species and does not undergo recombination (Hayashi *et al.*, 1985). These features mean that each molecule as a whole usually has a single genealogical history through maternal lineage.

In the present study, the sequences of the D-loop hypervariable 1 (HV1) segment of the mtDNA were used to study the genetic diversity and relationship of South Western Nigerian domestic chicken.

MATERIALS AND METHODS

Chicken blood samples from a total of 98 individuals belonging to 3 major phenotypes were collected from South-western Nigeria using FTA® classic cards (Whatman BioScience, Maidstone, UK). Geographic location and number of the samples collected were as follow: Lagos (n= 7, Epe), Ogun (n= 20, Imeko, Ipokia, Owode), Oyo (n= 15, Agoare, Oke-iho), Osun (n= 7, Ifewara), Ondo (n= 16, Ikare, Ore), Kwara (n= 24, Jebba, Kosubosu, Offa) Edo (n= 4, Uromi) and University of Agriculture, Abeokuta (n= 5, exotic chicken stocks). Genomic DNA was extracted from air-dried blood spotted on filter paper (FTA® classic cards) following

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Also two haplotypes of the genus *Gallus* retrieved from GenBank; *Gallus gallus gallus* (GenBank accession number AB007720) and *Gallus gallus bankiva* (GenBank accession number AB007718) and seven clade reference haplotypes (Clade I, II, IIIa, IIIb, IIIc, IIId and IV).

The numbers at the nodes represent the percentage bootstrap values for interior branches after 1000 replications.

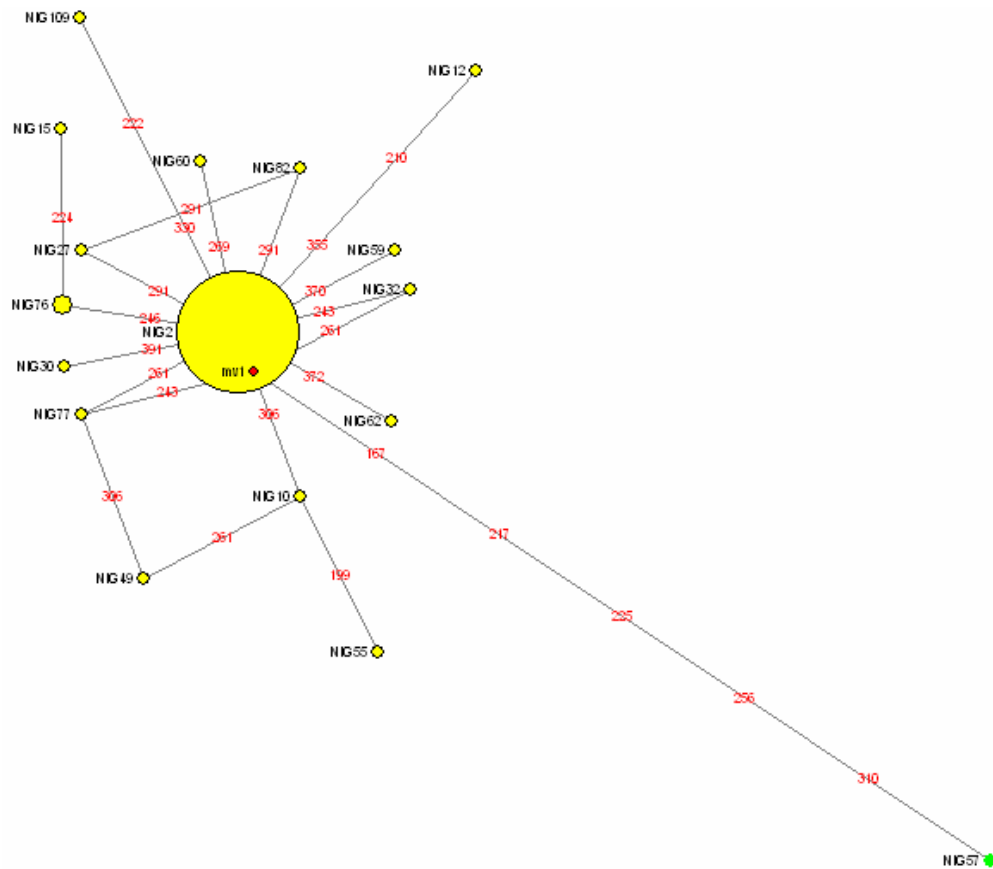
Figure 2. Neighbour-joining tree reconstructed using MEGA 3.1 software from 17 haplotypes identified in 98 sequences of South Western Nigerian chicken. (Árbol neighbour joining reconstruido usando el programa MEGA 3.1 a partir de 17 haplotipos identificados en 98 secuencias de pollos de SW Nigeria).

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indicates that 97.32% of the total sequence variation between haplotypes was present within population and 2.68% between populations. Median joining networks were drawn for the haplotypes identified in 98 sequences of South Western Nigerian domestic chicken. The network illustrates the relationship between 17 haplotypes (fi-

gure 3). There are sixteen haplotypes in clade IV and one in clade IIIc.

Our results indicate that mtDNA (HV1) D-loop region is variable in South Western Nigerian domestic chicken exhibiting 17 haplotypes which indicate multiple maternal origins for the South Western Nigerian domestic chicken.



Area of each circle is proportional to the frequency of the corresponding haplotype. Different classes of haplotypes are distinguished by use of colour codes. (Yellow and bright green circles refer to clades IV and IIIc respectively). The numbers between the haplotype nodes refer to the positions of nucleotide mutations compared to reference sequence (GenBank accession number AB098668).

Figure 3. Median-joining network ($\epsilon=0$) for the 17 haplotypes of South West Nigerian domestic chicken based on the polymorphic sites of the mitochondrial D-loop HV1 region. (Red median-joining ($\epsilon=0$) para los 17 haplotipos del pollo doméstico de SW Nigeria basado en los sitios polimórficos del D-loop HV1 mitocondrial).

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