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Lack of Phylogeographic Structure in Nigerian Village Chickens Revealed by Mitochondrial DNA D-loop Sequence Analysis

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Abstract: Genetic diversity studies that utilize phenotypic and genetic information are informative when formulating breeding and conservation plans. The present study utilizes sequences of mitochondrial DNA (mtDNA) D-loop region of 232 village chickens from Southern and Northern Nigeria to determine the origin and diversity of Nigerian local chickens. Thirty-six polymorphic sites which generate 35 haplotypes are identified. Phylogenetic analyses group Nigerian local chickens to a single clade and 97.8% of the total maternal variation occurs within populations. Reference sequences representing the major chicken mtDNA lineages from Asia indicate the Indian subcontinent to be the likely main center of origin of Nigerian village chicken. Lack of phylogeographic structure among Nigerian village chickens suggest extensive genetic intermixing within the country.

Key words: mtDNA, D-loop, genetic relationship, indigenous village chicken, Nigeria

INTRODUCTION

Genetic evidence confirms that chickens are derived from multiple maternal origins in Asia (Liu *et al*., 2006). Chickens are the most widely distributed of all livestock species in Nigeria with a population of 166 million birds (FAOSTAT, 2007). Chicken play very significant socio-cultural and economic roles in most African societies. Quantifying the structure of genetic diversity in different African chicken populations is of significance in optimizing conservation and utilization strategies. The description of Nigerian local chickens is based on phenotypic traits (Nwosu *et al*., 1985; Adebambo *et al*., 1999). Such information if complemented with findings obtained using molecular markers could be useful in formulating long term inference/plans for genetic improvement programs. The D-loop region of mitochondrial DNA (mtDNA) has been widely used to assess the diversity and phylogeographic structure of various chicken populations (Niu *et al*., 2002; Mobegi *et al*., 2006; Muchadeyi *et al*., 2008; Razafindraibe *et al*., 2008). So far, however, no such data has been reported on Nigerian local chicken. In the present study, the hypervariable segment 1 (HV1) region of the D-loop was sequenced to investigate the phylogeographic structure, diversity and origin of Nigerian local chicken.

MATERIALS AND METHODS

Genomic DNA was extracted from 232 local unrelated chickens from four geographic regions in Nigeria; 58 samples from the Northwest region (NW), 46 from the Northeast region (NE), 96 from the Southwest region (SW) and 32 from the Southeast region (SE). Five hundred and ninety two base pairs of the mtDNA D-loop region were amplified (Mobegi *et al*., 2005) using L16750 (5'-AGGACTACGGCTTGAAAAGC-3', accession number NC_001323, Desjardins and Morais 1990) as the forward primer and H547 (5'-ATGTGCCTGACCGAGGAACCAG-3', accession number AB098668, Komiyama *et al*., 2003) as the reverse primer. Direct sequencing of PCR products was done with the BigDye® Terminator Cycle Sequencing Kit version 3.1 (Applied Biosystems, USA) using two internal primers CR-for (5'-TCTATATTCCACATTTCTC-3') and CR-rev (5'-GCGAGCATAACCAAATGG-3'). PCR products were sequenced on ABI 3730 XL capillary sequencer (Applied Biosystems, USA).

A 397 bp long fragment, including the HV1 region was subsequently used for analysis. Following 1000 bootstrap replicates, a Neighbour-Joining (NJ) tree was generated using MEGA 3.0 software (Kumar *et al*., 2004). Two D-loop sequences of wild junglefowl; *Gallus gallus gallus* and *G. g. bankiva* (GenBank accession numbers AB007720 and AB007718, respectively), were included as out-groups. Seven reference sequences that correspond to the different major clades found in Asian domestic chickens and spanning the geographic range of wild red jungle fowls (Bjørnstad *et al*., in preparation) were also included in the analysis. The

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sequences used were those of the most common haplotype observed in the respective clades. A Median Joining (MJ) network constructed using the program NETWORK 4.1.0.8 (Bandelt *et al*., 1999) was also generated. Genetic differentiation based on the Analysis of Molecular Variance (AMOVA) and population expansion inferred from mismatch distributions, Tajima's D (Tajima, 1989) and Fu's Fs (Fu, 1997), were calculated using Arlequin version 3.0 (Excoffier *et al*., 2005).

RESULTS

The 232 sequences from local Nigerian chicken populations generated 35 haplotypes from 36 polymorphic sites. All haplotypes have been deposited with the Genbank under accession numbers FJ851656-FJ851686 and GU951751-GU951758 and are shown in Fig. 1. Phylogenetic analysis (Fig. 2) grouped Nigerian local chickens into a single clade (Clade IV) out of the seven clades (Clade I, II, IIIa, IIIb, IIIc, IIId and IV) identified in Asian domestic chicken (Bjørnstad *et al*., in preparation).

	1112222222222222222333333333333333333	
	2691112224456689999001134445555677 99	
	6790272483661911267680500242459702 16	N
Reference	CTTCATATCTTTCCAAATATCCTCTAACTTCTTACT	
NE51	....GC...CCCT....C...TC..........C	6
NE62	....GC...CCCT....C...TC........C....	5
NE73	....GC...CCCT....C...TC...C.......C	1
NE74	....GC...CCCT....C...TC...C.....	1
NE79	....GC...CCCT....C...TC......C.....	1
NE81	....GC...CCCT....C...TC.....C.....	1
NE84	....GC...CCCT....C...TC.....	177
NE96	....GC...CCCT.G..C...TC.....	1
NW7	....G....CCCT....C...TC.....	1
NW9	....GC...CCCT...GC...TC........C....	1
NW18	....GC...CCCT....C...TC.G.....	1
NW25	....GC...CCCT....C....C.....	1
NW35	....GC...CCCT....CT..TC.....	1
NW36	....GC..TCCCT....C...TC.....	2
NW59	....GC...CCCT....C...TC..G.....	1
SE2	....GC...CCCT....C..GTC.....	1
SE5	T...GC...C.CT....C...TC.....	1
SE8	....CCG..CCCT....C...TCT.....	1
SE10	....GC...C.CT....C...TC.......T.....	1
SE18	....GC...CCCT....C...TC....T.....	1
SE32	.C..GC...CCCT....C...TC.....	1
SW10	....GC...CCCT....C.C.TC.....	2
SW12	...TGC...CCCT....C...TC......C.....	1
SW15	....GC.C.C.CT....C...TC.....	1
SW20	....GC...C.CT....C...TC.....	7
SW27	....GC...CCCT..G.C...TC.....	1
SW30	....GC...CCCT....C...TC.....T.	1
SW32	....GC....CCT....C...TC.....	3
SW49	....GC...CCC.....C.C.TC.....	2
SW55	..C.GC...CCCT....C.C.TC.....	1
SW59	....GC...CCCT....C...TC.........C...	2
SW60	....GC...CCCTT...C...TC.....	1
SW62	....GC...CCCT....C...TC.........G..	1
SW77	....GC...CCC.....C...TC.....	1
SW82	....GC...CCCT..T.C...TC.....	1

Fig. 1: Nucleotide polymorphisms observed in D-loop HV1 domain of 232 chicken sequences and their frequencies (N). Vertically oriented numbers indicate the site position and the sequences shown are only the variable sites. Dots (.) indicate identity with the reference sequence (GenBank accession number AB098668) (Komiyama *et al*., 2003). The abbreviations denoting the haplotype names are as follows: NE (North East), NW (North West), (NE), SE (South East) and SW (South West)

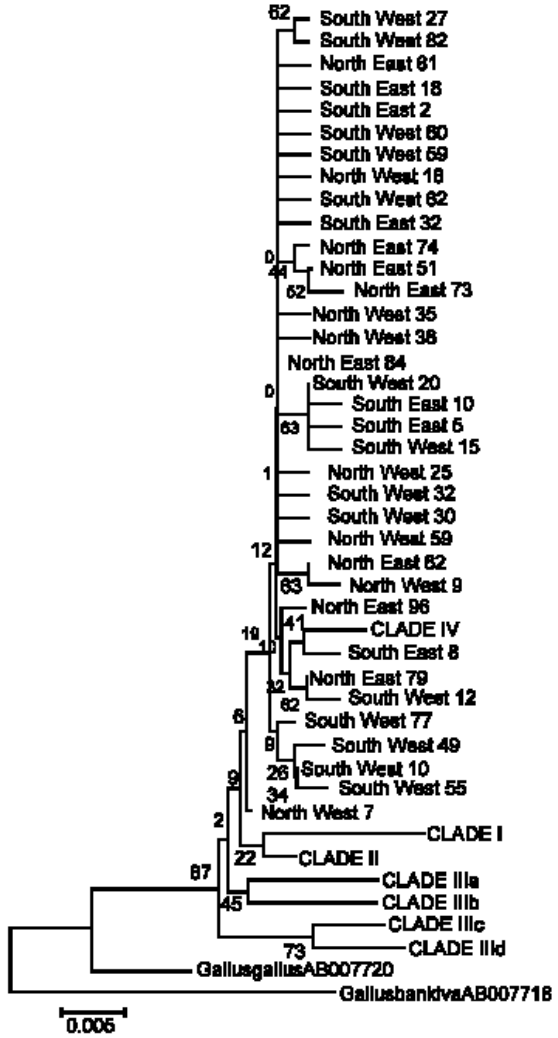

Fig. 2: Neighbour-joining tree reconstructed using MEGA 3.1 software from 35 haplotypes identified in 232 sequences of Nigerian chicken. Two haplotypes of the genus *Gallus* retrieved from GenBank; *Gallus gallus gallus* (GenBank accession number AB007720) and *Gallus gallus bankiva* (GenBank accession number AB007718) and seven haplotypes representing the major chicken mitochondrial DNA clades (Clade I, II, IIIa, IIIb, IIIc, IIId and IV) are also included in the tree for reference purposes. The numbers at the nodes represent the percentage bootstrap values for interior branches after 1000 replications

Analysis of molecular variance shows that 97.8% of the maternal genetic variation occurs within populations; the remaining variation is found among populations. Figure 3 shows the MJ networks of the sampled Nigerian local chicken populations. As in the phylogenetic tree, the 35 haplotypes clustered together in Clade IV. Figure 4

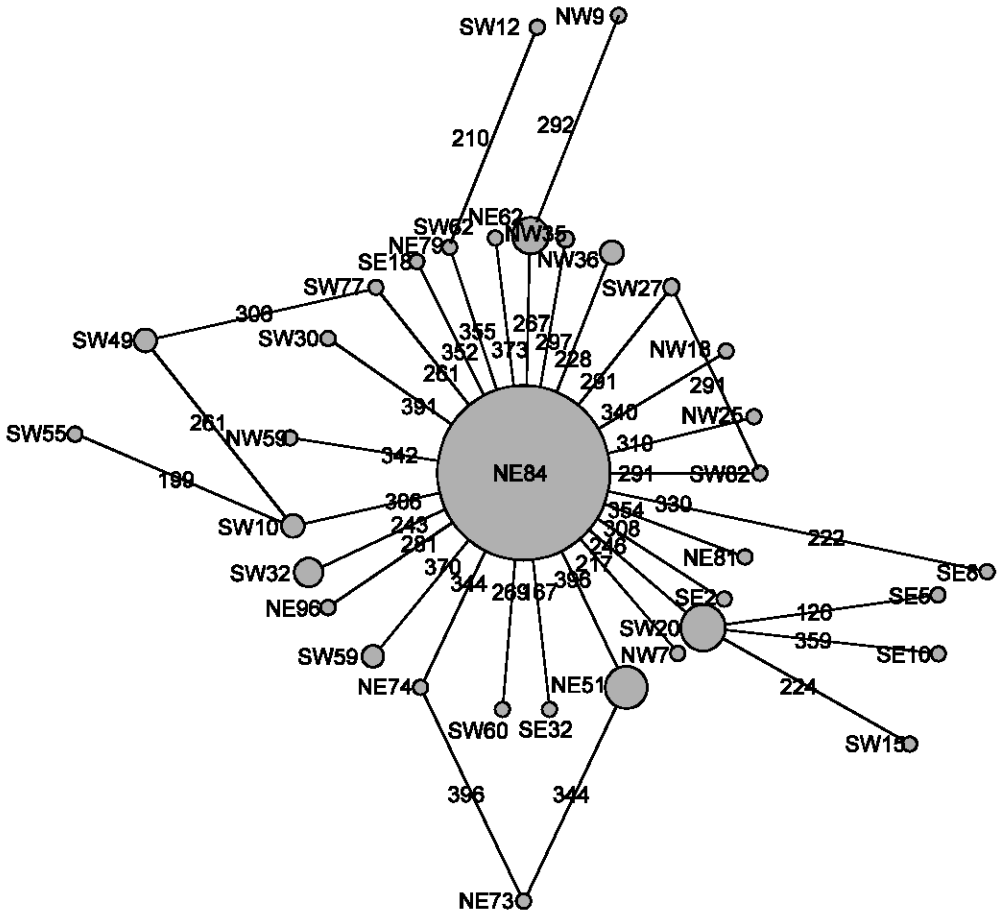

Fig. 3: Median-joining network (ε = 0) for the 35 haplotypes of Nigerian indigenous chicken based on the polymorphic sites of the mitochondrial D-loop HV1 region. Area of each circle is proportional to the frequency of the corresponding haplotype. The numbers between the haplotype nodes refer to the positions of nucleotide mutations compared to reference sequence (GenBank accession number AB098668)

shows the geographic distribution of haplotypes in Nigeria. Haplotypes NE84 and SW20 are common to all the populations, while NE84 is the most common one. It shows a Southwest to Northeast cline in its frequency of occurrence. Haplotype SW32 is common to Northwest Southwest and Southeast populations. Southwest and Northwest populations share haplotypes SW59 and SW10, Northwest and Northeast populations share haplotypes NE51 and NW36 while Southeast and Southwest populations only share haplotype SW49. The mismatch distribution pattern (Fig. 5) shows a half bell-shaped curve indicative of signatures of population expansion. Low haplotype (0.4217±0.0419) and nucleotide diversities (0.001578±0.01376) were observed in the populations studied. Estimates of Tajima's D (-2.548) and Fu's F_S (-13.3522) were both significant ($p<0.05$) and negative indicating departure from neutrality, therefore population expansion. The values of SSD (0.00015) and Harpending's Raggedness index r (0.125) were not significant, further supporting the theory of population expansion among Nigerian local chickens. Positive values shown by SSD and Harpendings's Raggedness index *r* also indicate increasing contribution of new and low-frequency mutations to population variation typical in expanding populations.

DISCUSSION

Results from the current study indicate that mtDNA (HV1) D-loop region is highly variable in Nigerian local chickens revealing a total of 35 haplotypes. All the mtDNA D-loop sequences observed in Nigerian village chickens radiate from a single sequence (NE84) belonging to Clade IV, which likely originates from the Indian subcontinent (Bjørnstad *et al*., in preparation). Mobegi *et al*. (2006) also observed that majority of haplotypes found in West African village chicken populations cluster in clade IV, while Muchadeyi *et al*. (2008) and Razanfindraibe *et al*. (2008) observed two distinct major clades amongst both Zimbabwean and

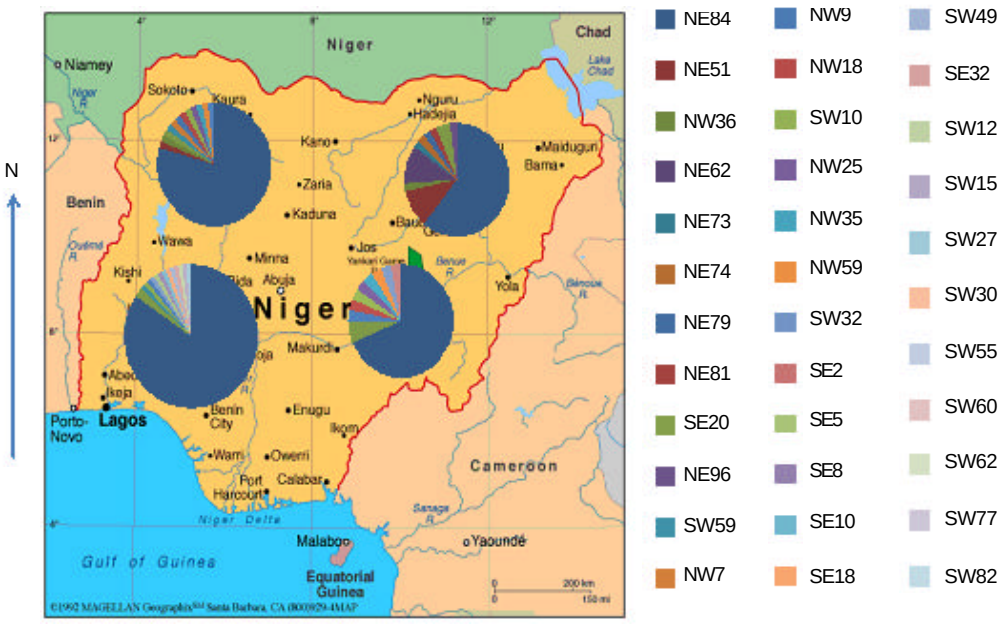

Fig. 4: Geographic distribution of the 35 haplotypes observed in the D-loop HV1segment of the 232 Nigerian chicken sequences

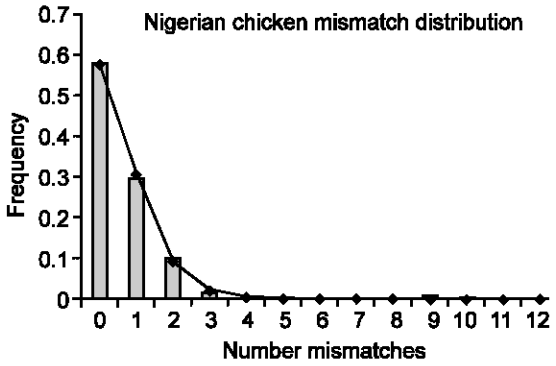

Fig. 5: Mismatch distribution of Nigerian indigenous chicken. Observed and model frequencies are indicated

Malagasy chickens, indicating a different history of domestic chicken between the West African and the Southern African regions. Fig. 3 and Fig. 4 indicate no clear geographic sub-structuring in Nigerian chicken population, likely a consequence of the relative recent arrival of domestic chicken in the region and the lack of phylogeographic resolution of the marker used in this study. Alternatively, it could equally be the result of intensive genetic intermixing between populations following human migrations and trading. Haplotype NE84 shows the widest geographic distribution, but with an increasing frequency in the Southwest direction. This possibly infers a North-South dispersal of this haplotype. The D-loop region in Nigerian chicken shows a departure from equilibrium (significant negative values for Tajima's *D* and Fu's *F*s) suggesting population expansion. These results are consistent with the observed mismatch distribution patterns and non-significant value for Harpending's raggedness index. Therefore, a population expansion of Nigerian local chicken likely followed their arrival in the country. The lack of phylogeographic structure observed among the different populations sampled from different geographic regions suggests population intermixing across long distances within the country.

The ancestors of the today's Nigerian chicken could have reached the country from the North of the African continent through trans-Saharan trading and/or could have originated from East Africa through migrations along the Sahelian belt. Alternatively, they could have also reached Nigeria from the coast, e.g. following early European exploration. Our data do not allow us to distinguish between these hypotheses but the relative simple network expansion observed, characterized by an expansion from a single ancestral haplotype (Fig. 3) suggests a single geographic origin. Whatever the case, it appears that the Asian maternal ancestor of Nigerian local chicken would have originated initially from the Indian subcontinent as all haplotypes identified in local Nigerian chicken belong to clade IV which most likely originates from the Indian subcontinent (Bjørnstad *et al*., in preparation).

Conclusion: Overall, Nigerian local chicken populations would have likely been introduced first into the Northern parts of the country. Thereafter the chicken populations

dispersed towards the southernmost region following human movements and commercial activities. The lack of substructure in the chicken populations is evidence of a single maternal origin and extensive genetic intermixing in the past and present times.

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