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Indian and Chinese Origins of West African Village Chickens as Revealed by Mitochondrial DNA Partial D-Loop Regions

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ABSTRACT: The study assessed the maternal genetic origins of West African village chickens using a 920-bp mtDNA D-loop region fragment of 127 samples from four populations (Gambia, Togo, Liberia and Nigeria). The mtDNA D-loop region was amplified following standard PCR protocols, using AV1F2: 5′-AGGACTACGGCTTGAAAAGC-3′ and 5′-TGCTTAAGGTTAATTACTGCTG-3′. as the forward and reverse primers respectively. Sequencing was done using Sanger's dideoxy chain termination method. In addition, 268 sequence samples harvested from Genbank representing Gallus gallus from Africa, Mediterranean and different regions of Asia, were included in the analysis, bringing the total sample size to 395. 24 polymorphic sites and 13 West African chicken haplotypes (WAC1-13) were detected. The evolutionary relationships amongst studied populations and other African and global chicken populations were investigated using MEGA 7 and NETWORK 4.6 softwares. The results grouped 11 of the haplotypes including the dominant one (WAC1) with India, Pakistan and Egypt into haplogroup E, indicating an Indian sub-continental origin and Egypt as an entry point, while the other haplotypes (WAC8 and 10) clustered with haplogroup B widely believed to be of Chinese matrilineage and reported for the first time in West Africa by this study. Haplogroup B is thought to be a heritage of recent introgression of exotic alleles from commercial lines. The study shows that India and China were most likely geographic origins of the matriarchs of the West African village chickens.

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KEYWORDS: Chicken, West Africa, origin, haplogroup, mitochondrial DNA.

INTRODUCTION

There is no evidence based on ancient or modern DNA suggesting the existence of any wild *Gallus* species on the African continent, hence the entire African village chickens cannot be said to be natives of Africa. Furthermore, It has been observed that there is an absence of genetic information standards across the continent, occasioned by low cross-border research activities for greater global significance (FAO, 2011). With the exception of a few (like Mwacharo *et al.*, 2011), much of the other results on evolutionary origins of African chickens are restricted within individual countries. This study therefore seeks to investigate the origins of the West African village chicken populations from four countries using the mitochondrial DNA D-loop region as a molecular marker. The haplogroup nomenclature of this study follows that of Miao *et al*. (2013) and Liu *et al.* (2006).

MATERIALS AND METHODS

A total of 127 blood samples from four countries (Gambia, 25; Togo, 14; Liberia, 34 and Nigeria, 54) were collected to represent the village chicken populations of West Africa (Figure 1).

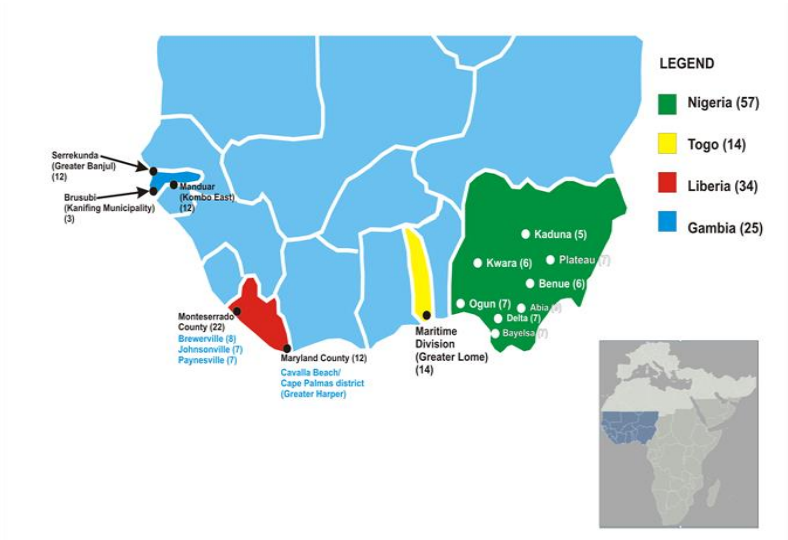

Figure 1: **Map of sampling locations.**

In addition to the 127 West African samples, 268 chicken mtDNA sequences were included from Genbank, representing major Asian centres of chicken domestication, Europe and Africa, bringing the total number of samples for study to 395 chickens (Table 1).

Table 1: Details of the samples downloaded from Genbank.

Source	Number of Sequences	Genbank Accession number	Reference
Egypt	18	AB829473-AB829490	Osman *et al.* (2016)
Kenya	30	EU095192-EU095163	Mwacharo *et al.* (2011)
China	23	AB098666-AB098664	Komiyama *et al.* (2003)
		KY3008169-KY308150	Gao *et al.* (2017)
South Korea	20	HQ836363-HQ836343	Cho *et al.* (2010)
Pakistan	40	MH094617-MH094656	Nisar*et al.* (2018)
India	21	KF411029-KF411009	Ghosh *et al.* (2013)
Mainland South-East Asia (MSEA):	11	AB009441-AB009444	Miyake *et al.* (1997)
Thailand (10), Vietnam (1)		KC817527-KC817533	Pramual *et al.* (2013)
Mediterranean	8	LK391757-LK391764	Ceccobelli *et al.* (2015)
Italy (3), Spain (4), Malta (1)			
Island South-East Asia (ISEA):	97	KX642436-KX643040	Herrera *et al.* (2017)
Indonesia (54),			
Philippines (38), Fiji Island (5)			
Number of samples downloaded from Genbank = 268; Total samples used for this study = **395**			

Blood samples were collected using FTA classic cards, genomic DNA was isolated following the manufacturer's protocol (www.whatman.com). The mtDNA D-loop region was amplified by PCR using the primer pair of AV1F2: 5′-AGGACTACGGCTTGAAAAGC-3′ and 5′-TGCTTAAGGTTAATTACTGCTG-3′ (Nishibori *et al.*, 2001) followed by Sanger sequencing. All 127 raw sequences were edited using BioEdit 7.0 and truncated to a uniform sizes of 920bp and 491bp respectively, using MEGA 7. Multiple alignments and evolutionary relationships of the West African village chicken haplotypes were established by the reconstruction of neighbor joining (NJ) trees following a 1000 bootstrap replicate for all samples from West Africa and the reference sequence (GenBank accession number AB829474), species of the genus *Gallus*, and sub-species of *Gallus gallus*, using the MEGA application. Similarly Neighbour joining trees were constructed for West African samples and the 13 and 9 major Asian clades of Miao *et al.* (2013) and Liu *et al.* (2006) respectively. Phylogenetic relationships between the four West African populations and consensus sequences drawn from eleven countries representing major regions in Asia, Africa and Europe, were reconstructed.

The median-joining networks were constructed using NETWORK 4.6 software. Futhermore, 268 mtDNA sequences downloaded from the GenBank, representing domestic chickens from different regions of Asia, Africa and Europe were used to construct a median-joining network, along with the 127 from this study, in order to confirm the results from phylogenetic analysis. The number of haplotypes were evaluated with DnaSP 5.10.

RESULTS AND DISCUSSION

Haplotype analysis produced 13West African haplotypes (WAC1-13) from 24 polymorphic sites. WAC1 did not only exhibit the widest geographic spread, it also had the highest frequency of occurrence (59.8%) in the populations, while WAC8 and 10 existed only in Nigeria. Futhermore, evolutionary relationship among the thirteen West African chicken haplotypes (Figure 2), showed a star-like distribution of the rest of the haplotypes about haplotype 1 (WAC1), with WAC 8 and10 more remotely distributed.

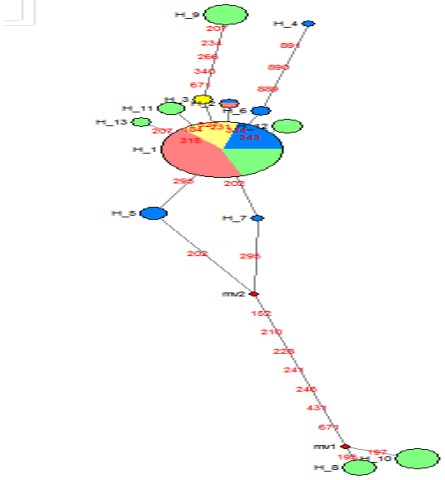

Figure 2: Median-joining network of relationship among West African chicken haplotypes based on 920bp region of polymorphic sites of the mtDNA D-loop. The red colour between the haplotype nodes refer to the positions of median vectors. Different classes of haplotypes are distinguished by use of colour codes (blue = Gambia, yellow = Togo, red = Liberia, green = Nigeria). Area of each circle is proportional to the frequency of the corresponding haplotype. The red colour between the haplotype nodes refer to the positions of median vectors.

Results from phylogenetic relationship (Figure 3 and 4) showed all African chicken mtDNA clustering with samples from the Indian sub-continent namely Pakistan and India, separated from all other Asian entries. Similarly, Indian and all the African samples clustered into one clade, *E* (Figure 4).

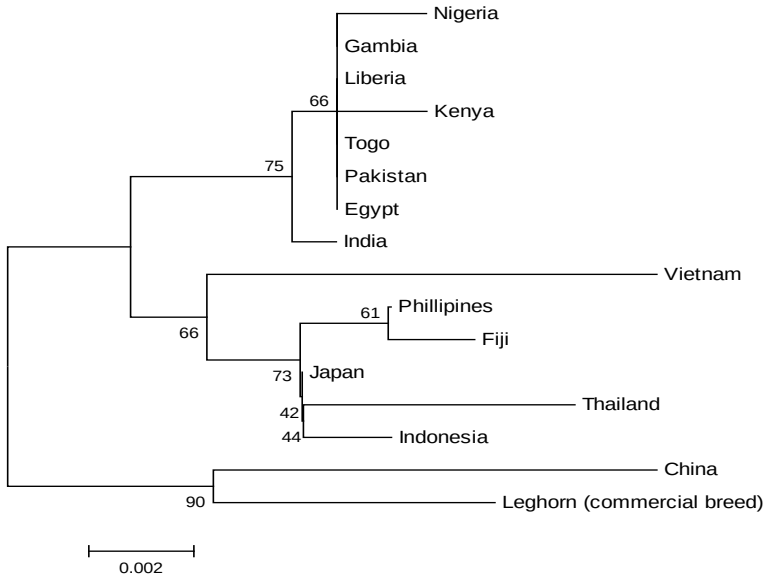

Figure 3: Neighbour-joining tree reconstructed for four West African chicken populations, major Asiatic centers of chicken domestication and a commercial breed (Leghorn) based on consensus sequences using MEGA 7.0 software. The numbers at the nodes represent the percentage bootstrap values for interior branches after 1000 replications.

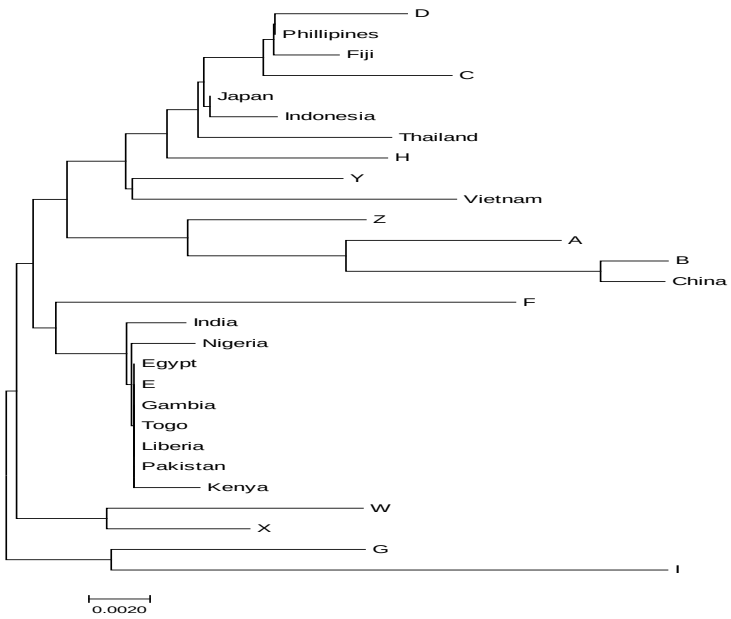

Figure 4: Neighbour-joining tree showing the geographic relationship of the 13 major chicken clades of Miao *et al.* (2013) with African and Asian chicken populations based on a 495bp segment of the D-loop, using MEGA 7.0 software. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree (Tamura and Nei, 1993). The sum of branch length = 0.15955349.

The results of the network analyses were in complete consonance with positions of the phylogenetic analyses. The matrix (Figure 5), show the West African haplotypes in cluster with haplotype E (LiuE1, LiuE2 and LiuE6), defined by Liu *et al.*, 2006 to be of Indian origin, while two of the haplotypes clustered with haplotype B (LiuB1 and OkaB01), defined by Liu *et al.* (2006) and Oka *et al.* (2007) to be of Chinese roots.

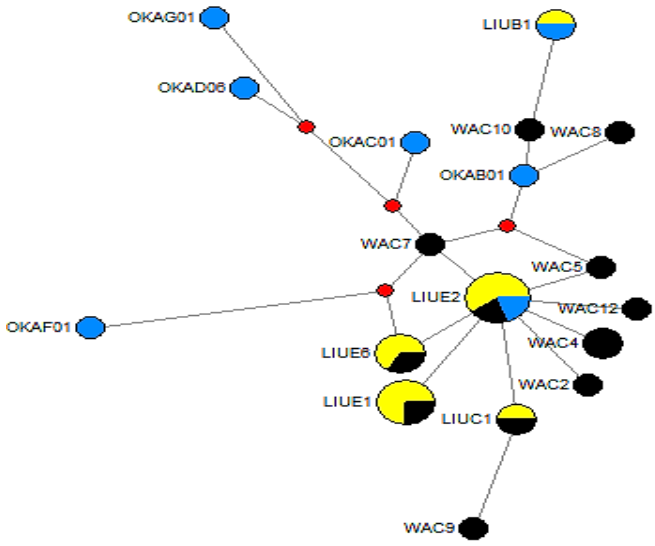

Figure 5: Median-joining network result for the relationship between the West African chicken haplotypes (black) and seven international chicken haplotypes defined by Liu *et al.* (2006) (yellow) and Oka *et al.* (2007) (blue), based on 920bp region of polymorphic sites of the mtDNA D-loop.Area of each circle is proportional to the frequency of the corresponding haplotype(s).The red colour between the haplotype nodes refer to the positions of median vectors.

The network analysis based on the hypervariable-1 region (491bp) (Figure 6), also showed a similar pattern in which most of the 13 West African haplotypes clustered with themselves and haplotypes from the Indian sub-continent. Two of the haplotypes from Nigeria (coloured green), can however clearly be seen branched off to form a smaller separate cluster with Chinese haplotypes.

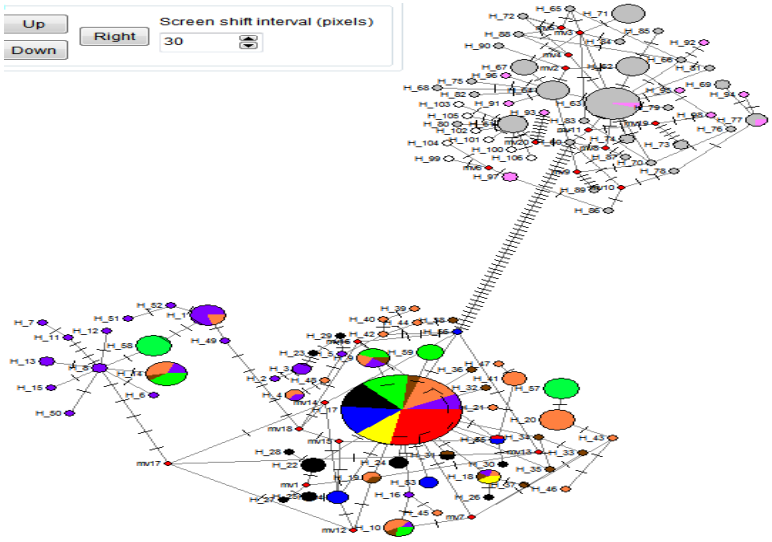

Figure 6: Median-joining network result for the relationship between the West African and global chicken populations from 395 individuals based on 491bp (HV1) segment of polymorphic sites of the mtDNA D-loop. Different populations are distinguished by use of colour codes (blue = Gambia, yellow = Togo, red = Liberia, green = Nigeria, dark brown = Egypt, gray = Kenya, orange = Indian Sub-continent, purple = East Asia, pink = Mainland South East Asia, gray = Island South East Asia and white = Mediterranean). Area of each circle is proportional to the frequency of the corresponding haplotype. The red colours between the haplotype nodes refer to the positions of median vectors.

Origin	Region	Colour	Origin	Region	Colour	Origin	Region	Colour	Origin	Region	Colour
Nigeria	Africa (176)	55	India	Indian Sub-continent (61)	21	China	East-Asia (43)	23	Thailand	MSEA (11)	10
Liberia		34							Vietnam		1
Gambia		25									
Togo		14	Pakistan		40	South Korea		20	Philippines	ISEA (97)	38
Egypt		18							Indonesia		54
Kenya		30							Fiji		5
Spain	Medite-rranean (8)	4									
Italy		3									
Malta		1									

Total = 395 MSEA = Mainland South East Asia ISEA = Island South East Asia

DISCUSSION

The observed number of haplotypes and polymorphic sites were in tandem with some reports elsewhere on the continent (Wani *et al.*, 2014), thus implying that the West African chicken populations are highly polymorphic at the mtDNA hypervariable region.

The results pointed to WAC1 as the most likely first arrival or at least predominant haplotype introduced into West Africa, from whence the other haplotypes evolved. Besides the distribution of WAC 8 and 10 only in Nigeria depicts the phylogeographic structure of that population. Similarly, the more remote distribution of WAC 8 and 10 from WAC1, is a reflection of its evolutionary remoteness or time of divergence, suggestive of a more likely different maternal ancestry.

It could be inferred (Figure 4) that the maternal origins of the West African village chicken was most traceable to clade *E* and *B* in Asia. According to Liu *et al.*, 2006; Oka *et al.*, 2007 and Miao *et al.*, 2013, haplogroup *E* was predominantly found in South Asia region (also known as Indian sub-continent), with India alone having a prevalence rate of 55.56, while *A* and *B* were mostly found in China. The Yellow River in China and the Indus River Valley in India, have been associated with the domestication of the chicken (Akishinonomiya *et al.*, 1996). Thus, the findings from this study pointed to India as the most likely ancestral home of the matriarch of majority of village chickens in West Africa. It is probable that from India, the chicken could have been moved by humans further west through Pakistan to Mesopotamia, Egypt and further on to Europe by means of trade (Osman *et al.*, 2016). From Egypt it would have spread further south to the rest of Africa including West Africa following human interactions. The finding from this study that Haplogroup *E* was the most dominant in West Africa corroborates earlier reports across Africa (Miao *et al.*, 2013), North Africa (Osman *et al.*, 2016), Middle East and Europe (Liu *et al.*, 2006 and Miao *et al.*, 2013). This finding suggests that the matrilineal

progenitor of most West African chickens was from India, agreeing with the few other findings from the region (Adebambo *et al.* 2010).

That Chinese matrilineage of WAC8 and 10 (constituting 13.4% of the sampled populations) was evident in their clustering with haplogroup *B*, and with the consensus sequence from China, which agrees with Liu *et al.* (2006) that clades B might have originated in Yunnan and/or surrounding areas in China. These might have been introduced to West Africa through introgression of exotic alleles from commercial stock rather than direct pre-colonial trade with China. Presence of *B* have been verified in commercial layers of both brown and white stock (Dana *et al.*, 2010). There have been reports in West Africa (Leroy *et al.*, 2012) and elsewhere on the continent (Mwacharo *et al.*, 2011) of introgression from commercial lines through breeding programs established by governments or in conjunction with development partners targeted at improving village poultry production, often involving the distribution or encouragement of the free-range rearing of exotic cockerels. Hence, Mwacharo *et al.* (2011) opined that haplogroup *B* might have reached Africa following recent introductions of improved commercial chickens. Leroy *et al.* (2012) further suggested that phenotypic observations might also be used to confirm this assertion since some traits, like yellow shank colour, were typical of commercial layer lines and some broiler lines, while the wild type was grey-blue shank colour. It is on record that the yellow shank is a common phenotype among African village chickens (Daikwo *et al.*, 2011). Thus, both molecular and phenotypic evidences corroborated the phylogenetic result in which a commercial layer (White Leghorn) sequence clustered with the consensus sequence from China.

All network analyses showed that West African chickens and all the other sampled African chickens in this study were of the matrilineage of Indian sub-continent, while two haplotypes (WAC8 and 10) had consistently alluded to a likely Chinese origin. In addition, the networks indicated that, chickens of Indian, Middle East and African extraction were together very genetically distinct from those of Island South East Asia (Pacific Asia), as could be seen in the highly resolved distance between the two clusters, indicated by the multiple mutation points.

CONCLUSION

Molecular evidence from this study showed that the matrilineage of West African chickens was traceable to the Red Jungle Fowl but supports multiple introductions. While the Indian sub-continent was demonstrated to be the original home of the matriarch of most West African village chickens, there were evidences of a sub-structuring of the population, with haplotypes WAC8 and 10 geographically isolated to Nigeria. The ancestry of this isolated haplogroup (B) was shown to be of Chinese origin, and thought to have arrived West Africa through introgression from commercial lines. The Chineses haplogroup B was reported for the first time in West Africa by this research. The study provides useful insights for further understanding of the global dispersal of the chicken out of Asia from an African perspective, which could be exploited for local and international breed improvements.

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