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Research Article

Genetic Diversity and Maternal Ancestry of Liberian Country Chickens Indicated at the Hypervariable-1 Section of the Mitochondrial DNA D-loop

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ABSTRACT

The vast majority of animal production in Africa, Liberia inclusive is in the hands of traditional operators based in the rural or countryside, upon which there is a general dearth of genetic information, especially at the molecular level regarding their origins and characterization. The study sought to evaluate the molecular genetic diversity and trace the evolutionary origins of the Liberian country chickens using the maternally inherited mitochondrial DNA as a profiling marker. The D-loop regions of the mtDNA of 34 Liberian country chickens sampled from four different locations were analysed following standard DNA isolation protocol (www.whatman.com) and polymerisation using AV1F2: 5′-AGGACTACGGCTTGAAAAGC-3′ and 5′-TGCTTAAGGTTAATTACTGCTG-3′ , as forward and reverse primers respectively. PCR amplicons were sequenced based on Sanger's di-deoxy chain termination method at Stabvida genetic laboratories, Portugal and thereafter edited with BioEdit 7.0 and truncated to 490bp covering the hypervariable-1 segment, using MEGA 7. Genetic diversity indices and molecular variance were analysed using DnaSP V.5.10 and Arlequin 3.5.1.3 softwares respectively. The evolutionary relationships of the Liberian chickens with 268 global chicken mtDNA sequences from Mainland Asia, Pacific Asia, Mediterranean region and Africa extracted from Genbank depository (leading to a total sample size of 302), and their maternal origins were inferred following reconstruction of Neighbour-joining phylogenetic and Median-joining network analyses using MUSCLE software and NETWORK 4.6. Results from the study show no population structure (F_{ST} values not significant at $P<0.05$) and low genetic diversity (Haplotype diversity = 0.06±0.05, Nucleotide diversity = 0.002±0.006) among the Liberian chickens probably due to purifying selection and founder effect, possibly occasioned by genetic bottlenecks in the recent history of the country, like the prolonged civil war. One polymorphic site and two Liberian chicken haplotypes (LibE1 and LibE2) belonging to Clade E were detected. This evidence along with others overwhelmingly pointed to India as the ancestral root of the matriarch of the current populations of the Liberian country chickens. Findings from this study hold the potential for the genetic improvement of the Liberian country chickens and further understanding of the possible impact of human conflicts on animal genetic resources in Africa.

Key words: Chicken, Liberia,Genetic diversity,Haplotypes, Mitochondrial DNA, D-Loop

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INTRODUCTION

According to DAGRIS (2007), the domestic chicken is found throughout the African continent where it scavenges freely as village chicken in all the agro-ecological zones, ranging from the humid and sub-humid tropical rainforest of West and Central Africa to the temperate highlands of East Africa, to the arid and semi-arid regions of the Sahel and Kalahari Desert. Most African households keep indigenous birds essentially for eggs and meat production (Guèye, 1998). The initial motivation for the adoption of domestic chickens by African communities, as it has been assumed for the initial domestication of the chicken in Asia, could have been for socio-cultural and or recreation (e.g. cock fighting) rather than as a new source of food (Hassaballah, *et al.,* 2015). Of all the major farm animal species, the domestic chicken is the only one whose origin has not been traced to the Middle East (Liu, *et al.,* 2006). The Indus River Valley, particularly the Mohenjo-Doro site (Zeuner, 1963) has been associated with the domestication of the chicken (Akishinonomiya, *et al.,* 1996) as far back as 3250BC (Moiseyeva, 1998). Given their close association with humans, chickens were dispersed from these original geographic domestication regions to the rest of the world, Africa inclusive, entirely by humans (Mwacharo, *et al*., 2013). There is currently scarcity if not a total lack of molecular evidence on the introduction of the freely roaming country chicken into Liberia. Also, like much of the rest of Africa, only little is known of their genetic diversity (FAO, 2011b). It is a widely held notion that Egypt is the primary gateway of introduction of chicken into Africa (Mwacharo, *et al*., 2013, Osman *et al.,* 2016). Liberia however, has a long history of trans-Atlantic slavery, having been established since 1821 as a settlement of freed American slaves, making it the oldest republic in Africa, and the only state in sub-saharan Africa never to be colonized (Jones, *et al.,* 2020).This along with its prime location on the south west coast of West Africa, along the Atlantic trade routes, could have possibly facilitated the introduction of some of the chickens by returning African slaves from the U.S and the West Indies or by European sailors.The FAO (2011a), has recommended the mitochondrial DNA as a marker of choice for the study of maternal evolutionary ancestry of farm animals. This study was therefore carried out to investigate the genetic characterization and evolutionary origin of the country chicken of Liberia using the analysis of the D-loop region of the maternal mtDNA. The D-loop with a total length of 1232bp is thought to contain two hypervariable segments, making it very informative for evolutionary studies (FAO, 2011a) this study was based on the analysis of a 490 base pair segment of the D-loop, which contains the hypervariable-1 section.

MATERIALS AND METHODS

0.2mls of blood was collected from the wing vein of each chicken and dropped onto dry FTA papers, from a total of 34 chickens from different locations spread across two counties located in the west (Monteserrado) and extreme eastside (Maryland) (Figure 1). 268 additional sequences of chicken mtDNA from across the world especially Asia, Africa and Europe were harvested from Genbank (Table 1) and incorporated into the study, bringing the total sample size to 302. DNA from the dry blood was extracted in accordance with recommendations of the manufacturer (www.whatman.com). Purity and concentration of the DNA was carried out using Nanodrop spectrometer according to the protocol of Desjardins and Conklin (2010). The mtDNA D-loop region was amplified by PCR using a primer pair of AV1F2: 5′-AGGACTACGGCTTGAAAAGC-3′ and 5′-TGCTTAAGGTTAATTAC TGCTG-3′ (Nishibori,*et al.,* 2001). The products of the PCR were sequenced based on Sanger's di-deoxy chain termination method while trimming to 490bp was done with MEGA 7 (Kumar, *et al.,* 2016). The Polymerase Chain Reaction (PCR) to amplify the mtDNA D-loop and subsequent Sanger sequencing were done at Stab Vida Genetics Laboratory in Monte de Caparica, Portugal. Editing of the sequences was done with BioEdit 7.0 (Hall, 1999). Multiple alignments of all sequences to detect nucleotide variations were conducted with MUSCLE software hosted in MEGA 7. Molecular diversity indices were evaluated using DnaSP V.5.10 (Librado and Rozas, 2009) and Arlequin 3.5.1.3 (Excoffier*et al*., 2005) softwares. In order to investigate the maternal ancestry of the Liberian country chickens, phylogenetic trees were reconstructed for the Liberian chickens and other African and global chicken mtDNA sequences. The study adopted the haplotypic nomenclature of Liu, *et al*. (2006) and Miao, *et al*. (2013). To further infer the evolutionary relationships among the West African chicken populations and other global chicken populations and referenced clades, median-joining networks (Bandelt *et al.,* 1999) were constructed using NETWORK 4.6 software (http://www.fluxus-engineering.com/sharenet.htm).

RESULTS

Two Liberian chicken haplotypes (LibE1 and LibE2) and one polymorphic site were detected. Result for the single nucleotide polymorphisms are shown on Table 2. A single nucleotide polymorphism was observed at position 231 at which one mutant (from Johnsonville, Monteserrado County) (LibE2) had a Thymine nucleotide (T)as asubstitute nucleotide for the wild Cytosine (C), which occurred in the remaining 33 studied individuals (97.1%) (LibE1). Similarly, percentage haplotype distributions are shown on Figure 2.

Results for the standard and molecular diversity indices (including nucleotide and haplotype diversities) are shown on Table 3. Similarly, results for the mismatch indices and distribution are contained on Table 4 and Figure 3 respectively. The mismatch shows a half bell shaped or uni-modal distribution. The distribution shows strong presence of rare alleles especially of low frequencies and a lack of average or mid frequency alleles.

Results showing the phylogenetic relationship between Liberian country chickens and other African chicken populations, and chickens from major centers of chicken domestication in Asia, based on consensus sequences are shown in Figure 4. The result grouped Liberian chickens, along with other African chickens, into

Table 1: Geographic and identity details of sequences obtained from Genbank.

Source	No. of Sequences	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): Thailand (10), Vietnam (1)	11	AB009441-AB009444	Miyake *et al.* (1997)
		KC817527-KC817533	Pramual *et al.* (2013)
Mediterranean Italy (3), Spain (4), Malta (1)	8	LK391757-LK391764	Ceccobelli *et al.* (2015)
Island South-East Asia (ISEA): Indonesia (54), Philippines (38), Fiji Island (5)	97	KX642436-KX643040	Herrera *et al.* (2017)
	Total = 268.		

Table 2: Nucleotide Polymorphisms observed in the sampled populations

	231	n
Ref	C	
H_1(LibE1)	.	33
H_2(LibE2)	T	1
Total		34

The first column signifies identification number of the generated chicken haplotypes. Vertically oriented numbers indicate the variable sites position. Dots (.) indicate identity with the reference (Ref) sequence (GenBank accession number AB829474) (Osman *et al.*, 2016) sequences shown are only the variable sites.

Table 3: Standard and molecular diversity indices from mt DNA of sampled Liberian country chickens

Index	value
Number of Sequences	34
Number of Haplotypes	2
Haplotypes diversity (Hd)	0.06±0.05
Nucleotide diversity	0.002±0.006
Mean number of pairwise differences	0.06±0.13
Sum of square frequency	0.74
Number of observed transitions	1
Number of observed transversions	0
Number of substitutions	1
Number of observed indels	0
Number of polymorphic sites	1
θ_H	0.05±0.05
θ_k	0.27±0.06
θ_s	0.24±0.24
θ_π	0.06±0.15
C (%)	37.38
T (%)	29.29
A (%)	25.00
G (%)	8.33

θ_H: Theta value based on expected homozygosity; θ_k: Theta value based on number of alleles; θ_s: Theta value based on number of segregating sites; θ_π: Theta value based on the average number of pairwise differences; C: Cytosine; T: Thymine ; A: Adenine; G: Guanine

one haplogroup with India, Pakistan and Egypt. Similarly, the median-joining network results (Fig. 5) shows Liberian chickens (red) clustering with other African chickens along with chickens from Egypt and India, into Clade E defined by Liu *et al.* (2006). The analysis of molecular variance result (Table 5) indicates that the overwhelming (96.26%) maternal genetic differentiation was within the sampled country populations rather than between them. On the other hand all results for the pairwise genetic distances (Φ_{FT}) (Table 6) were not significant (P<0.05).

Table 4: Mismatch distribution indices of the mtDNA of sampled Liberian country chickens

Index	Value
Mismatch observed mean	0.06
Mismatch observed variance	0.06
T	3.00
θ_0	0.00
θ_1	0.07
Sum of square deviation	0.00
P (*Sim. SSD* ≥ *Obs. SSD*)	0.17
Harpending's Raggedness index	0.782
P (*Sim. Rag.* ≥ *Obs. Rag*.)	0.880
Tajima's *D*	-1.138
P (Sim. D < Obs. D)	0.027
Fu's *Fs*	-1.32
P (Sim. Fs < Obs. Fs)	0.05

T: time of expansion; θ_0 and θ_1: mutation parameters

Table 5: Analysis of molecular variance within and among Liberian country chicken populations using mt DNA sequence.

Source of variation	Variance components	Percentage of variation	F_{ST}	P-value
Among population	0.00111	3.74	0.03737	0.41838
Within population	0.02857	96.26		

Note: F_{ST} values not significant at P<0.05

Table 6: Pairwise Φ_{FT}(below the diagonal) and P values (above the diagonal)

	LB	LJ	LP	LM
LB	-	0.44144	0.99099	0.99099
LJ	0.02041	-	0.99099	0.30640
LP	0.00000	0.00000	-	0.99099
LM	0.00000	0.08197	0.00000	-

Note: all pairwise Φ_{FT} values not significant at P<0.05

DISCUSSION

Results from all the genetic diversity indices used in this study (number of haplotypes, haplotype diversity, number of polymorphic sites, nucleotide diversity etc) indicate that the Liberian country chickens are genetically less diverse, compared to other African (Mtileni, *et al.*, 2011, Wani, *et al.*, 2014, Adebambo, *et al.*, 2010 and 31; Ajibike, *et al.*, 2017,Hassaballah, *et al.*, 2015), Mwacharo, *et al.*, 2011,Osman, *et al.*, 2016) and global chicken populations (Cuc, *et al.*, 2011, Hoque, *et al.*, 2013, Kawabe, *et al.*, 2014). Consequently, results of the fixation index and pairwise distance imply lack of genetic structure among the populations. The low diversity observed could

Fig. 1: An adapted map of the study area showing the various sampling locations (originally sourced from Britannica.com).

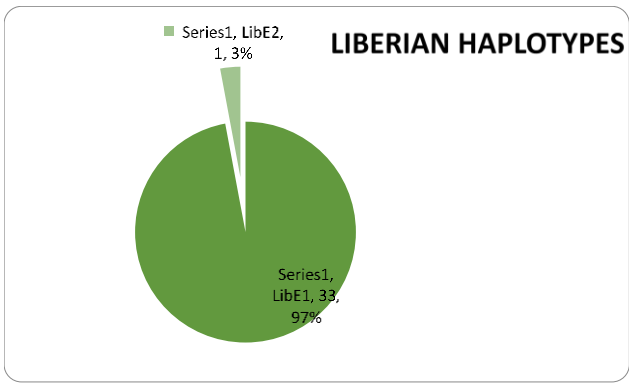

Fig. 2: Percentage occurrence of the two observed haplotypes from the mtDNA of the Liberian country chicken.

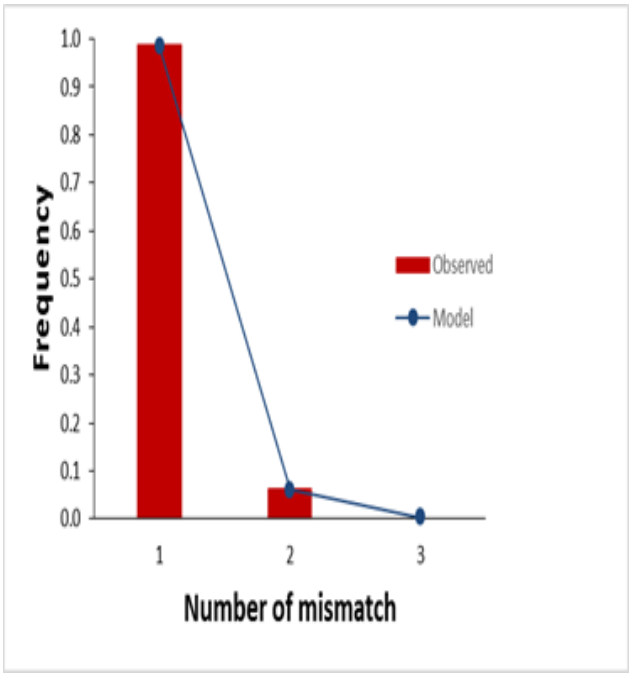

Fig. 3: Mismatch distributions of Liberian country chickens

be an indication that the Liberian chickens were evolutionarily younger, as Savolainen, *et al.* (2002) had observed that derived or recent populations were genetically less diverse than ancient ones. Furthermore, the

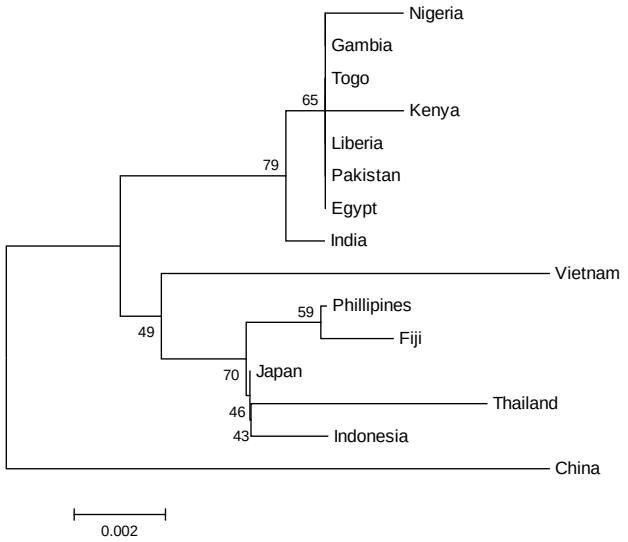

Fig. 4: Neighbour-joining tree reconstructed for Liberian country chickens, and other West African chicken populations and major Asiatic centers of chicken domestication based on consensus sequences using MEGA 7.0 software. The numbers at the nodes represent the percentage bootstrap values for interior branches after 1000 replications.

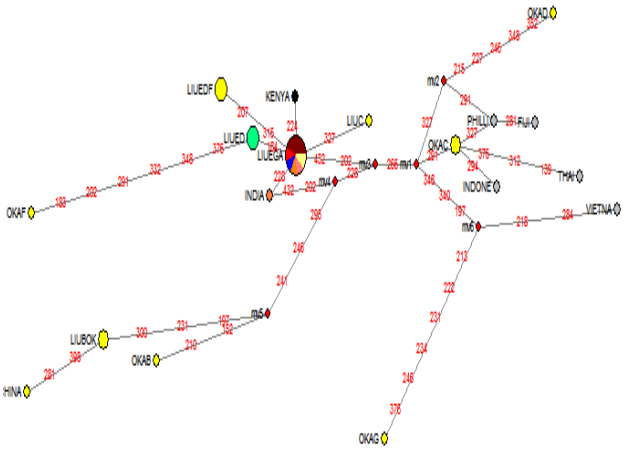

Fig. 5: Median-joining network result for the relationship between the Liberian country chickens, other West African chicken populations, seven international chicken haplotypes defined by Liu*et al.* (2006) (deep yellow) and Oka*et al.* (2007) (deep yellow), and some leading centres of chicken domestication based on 490bp consensus sequences of polymorphic sites of the mtDNA D-loop. Different populations are distinguished by use of colour codes (blue = Gambia, light yellow = Togo, red = Liberia, green = Nigeria, orange = India, black = Kenya, dark brown = Egypt, gray = Indonesia, Philippines, Fiji, Thailand and Vietnam). Area of each circle is proportional to the frequency of the corresponding haplotype(s).

informativeness or otherwise of a marker (FAO, 2011a), sample size and breeding histories of the chickens (Joshi, *et al.*, 2013), as well as natural selection (Yap, *et al.*, 2011) have great influence on diversity. However, It is more likely that the low genetic diversity in the Liberian chickens seen in this study, is due to the effect of purifying selection (Yap, *et al.*, 2011). This thinking is boosted by the negative Tajima's D result suggestive of

population expansion or purifying selection (Tajima, 1996), indicating that such populations are not at equilibrium nor experiencing random (neutral) selection. Hahn, *et al.* (2002) observed that such populations tended to have excess of rare alleles, which is in sync with the mismatch distribution result, with excess high and low frequency alleles, a further indication of departure from equilibrium occasioned by recent or past population expansion activities (Tajima, 1996). Similarly, results of sum of square deviations (*SSD*), Harpending's raggedness index (*r*) and Fu's *Fs* are suggestive of conformity with sudden population expansion model (Rogers and Harpending, 1992; Rogers, 1995; Schneider and Excoffier, 1999; Jobling, *et al.,* 2004), implying that the country chickens of Liberia were not at neutral equilibrium (Jobling, *et al.,* 2004; Okello, *et al*., 2005; Joshi, *et al*., 2013). Similarly, results of analysis of molecular variance, FST and Pairwise reveal a lack of population substructuring an indication of high homozygosity and low diversity.

Since it has been widely observed that dispersal of the chicken is closely associated with human activities (Osman, *et al.,* 2016; Eltanany and Hemeda, 2016*;* Mwacharo, *et al.,* 2013; 2011), it is therefore logical that the prolonged Liberian civil war (TheGuardian, 2003) followed by the outbreak of the deadly Ebola disease (WHO, 2019), could have not only drastically reduced the human population but also that of the chicken and discourage human immigration and trade, consequently obstructing possibilities for introduction of foreign chicken alleles. It can therefore be inferred that the current country chicken populations might likely be the progeny of a few founder progenitors, resulting to the observed low heterozygosity and diversity, an often characteristic of founder effect (Chen *et al.,* 2017).

Evidence from phylogenetic tree reconstruction from this study strongly suggests a south Asian or Indian sub-continental origin for the Liberian country chickens. The high bootstrap value at the node connecting Indian and Liberian, along with other African chickens, is a sign of the strength of the relationship at that topology. Median-joining network results also supports India as the matrilineal origin of the Liberian chicken, as it was grouped along with India in haplogroup E, which has earlier been predefined and reported to have its evolutionary roots in India, from where it has spread ubiquitously around the world (Liu *et al.,* 2006). It may be worthy of note that even if the returning African-American slaves had introduced *gallus gallus* on their arrival, it is most likely that such chickens would have been of the E haplogroup, as the haplogroup has been reported not only to be dominant in India, Middle East and Africa, but much of the Western world (Liu, *et al*., 2006, Miao *et al.,* 2013, Wood *et al.,* 2016) and the Americas (Gongora *et al.,* 2008), a further support for the observed low haplotype diversity.

Conclusions

Our study provides compelling evidence that the Liberian country chickens were of low genetic diversity likely due to being the descendants of a few founder parents that survived genetic bottleneck inducing events in the country's past, like the long civil war, leading to sudden expansion demographic hypothesis. The results also demonstrate that the Liberian chickens are of Indian descent. Findings from this study could be adopted for the genetic improvement of the Liberian chickens and for furtherance of understanding on the historic movement and introduction of chickens (and possibly other commensals) into Liberia. It may also be used as a template or motivation for studying the impact of natural and man-made catastrophes on farm animal genetic resources especially in Africa.

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