Mitochondrial diversity of Yoruba and Fulani chickens: A biodiversity reservoir in Nigeria

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ABSTRACT Poultry are the most widely distributed type of livestock in Nigeria. Indigenous chickens are extremely common throughout the country. Indeed, approximately 83 million chickens are raised in extensive systems and 60 million in semi-intensive systems. To provide the first comprehensive overview of the maternal lineages in Southwest Nigeria, we analyzed 96 mitochondrial DNA control region sequences from 2 indigenous chicken ecotypes: Fulani and Yoruba. All samples belonged to the most frequent haplogroup (E) in Africa and Europe and showed noticeably low haplotype diversity. Although only 11 different haplotypes were detected, with 2 of them never found before in Nigeria, the presence of unique sequences among our indigenous samples testified to their status as an important genetic resource to be preserved. Furthermore, a total of 7,868 published sequences were included in the comparative analysis, which revealed an east-west geographic pattern of haplogroup distribution and led to the conclusion that the gene flow from Southeastern Asia mainly involved one mitochondrial clade. Moreover, owing to the extensive genetic intermixing among Nigerian chickens, conservation efforts are required to safeguard the extant mitochondrial variability in these indigenous ecotypes and establish future improvement and selection programs.

Key words: uniparental marker, Africa, haplogroup, conservation, indigenous breed

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INTRODUCTION

Indigenous poultry breeds often represent a valuable genetic resource incomparable to commercial strains (Padhi, 2016). Over the centuries, many populations have been selected for their phenotypes as much as for their adaptation to the environment and resistance to the prevailing diseases (Mpenda et al., 2018); this is shown by a wide range of chicken breeds and ecotypes found across the world (Di Lorenzo et al., 2015). The term "ecotype" refers to a population within a breed that is genetically adapted to a specific habitat (FAO, 2013). Henderson's Dictionary of biological terms defines "ecotype" as a subspecific form within a true species resulting from selection and genetic adaptation to a particular environment but which can interbred with other individuals of the species (Lawrence, 1989). Owing to the high human population growth in Africa and the growing income, the demand for eggs and poultry meat has significantly increased in recent years across large parts of the continent (Mottet and Tempio, 2017). One African country where this trend is clearly appreciable is Nigeria (Heise et al., 2015). In fact, with more than 200 million inhabitants, Nigeria is the most populous nation in Africa and the seventh in the world. The poultry industry is one of the most important sectors in the Nigerian economy, contributing substantially to the nation's gross domestic product (**GDP**) (Ambali et al., 2003). Poultry are the most widely distributed livestock species in Nigeria, with a population of 180

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million chickens, of which only 21% are intensively reared (FAO, 2019). Native chickens play an important role as a household food supply in the rural areas of Nigeria. They serve as a rapid means of bridging protein deficiency and providing an additional income to the generally resource-poor farmers, thereby helping to alleviate poverty (Kyarisiima et al., 2004).

Fulani and Yoruba are 2 of the most widespread indigenous chicken ecotypes in Nigeria and are most commonly found in the southwestern area of the country. The Fulani ecotype is native to the harsh parts of the country, and its purity has been preserved by the isolated family group lifestyle of the Fulani keepers, which hinders its interbreeding with other native chickens (Fayeye et al., 2005).

Yoruba is considered another indigenous ecotype of Nigeria and has the potential to be selected as an egg-type chicken because of its morphology (Osaiyuwu et al., 2009). Ige (2013) described high genetic variation in this chicken, which sometimes reflects the ability to adapt to environmental changes and stress. Indeed, the Yoruba ecotype is found in the backyards of poultry keepers in villages, towns, and cities and is well adapted to live in stressful and harsh conditions (Sola-Ojo et al., 2013). Although native chickens represent a fundamental animal genetic resource in Nigeria, they have remained largely genetically uncharacterized and unimproved (Oluyemi and Roberts, 2000). The Fulani and Yoruba ecotypes have been studied for morphological and morphometrical surveys (Osaiyuwu et al., 2009) and for characterization based on breeding systems (Momoh et al., 2010; Sola-Ojo et al., 2012, 2013; Ige and Salako, 2014). The available genetic studies concern only the characterization of adaptive genes (Ojo, 2002; Fayeye et al., 2006; Fayeye and Oketoyin, 2006) and not the identification of genetic diversity among populations. In a recent study, Ajibike et al. (2017) assessed the first phylogenetic evaluation based on mitochondrial DNA (**mtDNA**) variation in indigenous chickens from different areas of Nigeria, but that study lacked information on specific breeds and types.

Mitochondrial DNA control region sequences have been frequently used to assess the diversity and phylogeographic structure of various chicken populations (Niu et al., 2002; Mobegi et al., 2006; Ceccobelli et al., 2013; 2015). Recently, these kinds of studies have been able to take advantage of complete chicken mtDNA sequencing (Xu et al., 2018).

The aim of this study was to investigate the extant genetic diversity of 2 Nigerian native chicken ecotypes by analyzing the hypervariable segment 1 (**HVS-1**) of the control region (D-loop) to obtain a more comprehensive picture of these important genetic resources and their phylogenetic relationships. The evaluation of the historical background of African local chickens and the degree of shared mitochondrial haplotypes will allow the disclosure of the probable maternal lineages of Nigerian chickens to be used in conservation programs and prevent a dramatic and unrecoverable loss of biodiversity. Furthermore, the scarce number of published African sequences and the low mtDNA variability recorded for this continent led to the analysis of 2 indigenous chicken ecotypes typical of Southwest Nigeria and a comparison with all the other African samples available in GenBank.

MATERIALS AND METHODS

Blood Sampling, DNA Extraction, and mtDNA Control Region Amplification and Sequencing

A total of 96 whole-blood samples from the wing vein (2 mL for each animal, collected with Vacutainer tubes containing EDTA as an anticoagulant and stored at −20°C until DNA extraction) of Fulani (n = 48) and Yoruba (n = 48) chickens were collected from flocks in different geographic areas of Southwest Nigeria (Supplementary Figure 1). Blood sample collection was conducted as part of a routine health screen by qualified veterinarians following guidelines established by the Institutional Animal Care and Use Committee (**IACUC**). Because collecting blood samples from the animals was part of the veterinarians' routine work, no ethics committee approval was required.

Total DNA was extracted using an automated extraction system using the MagCore Automated Nucleic Acid Extractor (RBC Bioscience, New Taipei City, Taiwan), following the provided protocol (Diatech Lab Line Srl).

The D-loop region between sites 16,387 and 721 was amplified using 2 primers specifically designed from the GenBank-published chicken mitochondrial sequence (accession number NC_007235.1) (Nishibori et al., 2005). PCR amplification was performed in a total volume of 25 μL containing 30 ng of DNA, 2.5 μmol of each dNTP, 0.3 μmol of each primer, 5 μL 5X Reaction Buffer GoTaq (Promega Corporation, Madison, WI), and 0.03 unit/μL of GoTaq DNA Polymerase (Promega Corporation). The thermal reaction was carried out in a Swift MaxPro thermocycler (Esco Technologies, Inc., Horsham, PA), and the amplification was performed with the protocol described in Ceccobelli et al. (2013).

The PCR fragment of 1,842 bp, encompassing part of the entire mtDNA control region (nps 1-1,232), was first purified using exonuclease I and alkaline phosphatase (ExoSAP-IT enzymatic system-USB Corporation, Cleveland, OH) and then sent to BMR-Genomics Srl (www.bmrgenomics.com) for Sanger sequencing with the forward primer 5′- CCACACGTTCCCCTTAAATA-3'.

Mitochondrial DNA Sequence Analyses

Sequences were assembled and aligned to the chicken reference sequence using Sequencher 5.10 (Gene Code Corporation, Ann Arbor, MI). All the obtained sequences were subjected to chromatogram quality control: only sequences with a minimum nucleotide quality were used.

To avoid ambiguities, subsequent analyses were restricted to a total of 534 bp of the D-loop. The mtDNA sequences have been deposited in GenBank with accession numbers MN010418 - MN010513. All sequences

Table 1. Genetic diversity parameters within the Nigerian chickens.

Ecotype	N	nh	π	Hd	Hd variance	N sites	S
Fulani	48	6	0.00054	0.270	0.00693	534	5
Yoruba	48	8	0.00070	0.308	0.00757	534	8
Fulani + Yoruba	96	11	0.00062	0.288	0.00372	534	10
All Nigeria[1]	149	41	0.00381	0.673	0.00204	291	38

N, number of analysed samples; nh, number of unique haplotypes; π, nucleotide diversity; Hd, haplotype diversity; S, number of polymorphic sites.
[1]range was restricted to the shared fragment from np 107 to np 397.

were compared with the reference sequence and classified in haplotypes and haplogroups, following the nomenclature previously published by Liu et al. (2006).

Mitochondrial DNA sequence variation parameters were estimated by using DnaSP, version 5.1, software (Librado and Rozas, 2009). Analysis of molecular variance (**AMOVA**) and pairwise F_{ST} calculations were performed using the Arlequin, version 3.5, software package (Excoffier et al., 2005). The statistical significance of the values was estimated by permutation analysis using 100 replications. Intrapopulation and interpopulation comparisons were performed based on the number of pairwise differences between sequences and calculated using an Arlequin integrated R script (http://www.rproject.org/).

The evolutionary relationships among the haplotypes were visualized through the construction of a median-joining network using Network 4.6 (Fluxus Technology Ltd., Colchester, England), in which redundant parallel mutations at nucleotide positions 217, 243, 261, 269, and 310 were downweighted to reduce excess reticulations. The network included both Yoruba and Fulani ecotypes and all available sequences from Nigerian chickens retrieved from GenBank, including the commercial line, which was used as an outgroup (Ajibike et al., 2017).

To ascertain the genetic affinities of the native Nigerian populations to other African and worldwide chicken populations, the survey was extended to all available chicken mtDNA control region sequences.

Principal component analyses (**PCA**) were performed using Excel software implemented by XLSTAT (Addinsoft, Paris, France), as described in the study by Cardinali et al., 2016. This method considers each chicken mtDNA haplogroup as a discrete variable and allows a summary of the initial data set into principal components (**PC**) to graphically display (and summarize) the relationships among all Nigerian extant sequences. The analysis was carried out by grouping the available samples in specific geographic macroareas identified in the 5 continents, highlighting only the African countries.

RESULTS AND DISCUSSION

mtDNA Sequence Variation

A total of 96 mtDNA control region sequences spanning from nucleotide position (**np**) 107 to np 640, including 534 bp of the mtDNA control region, and belonging to 2 Nigerian chicken ecotypes, were analyzed.

The overall sequence alignment revealed the presence of 10 polymorphic sites (S), represented by 6 singletons and 4 parsimony informative sites (Table 1). The average number of nucleotide differences between ecotypes was 0.329 (data not shown). Nucleotide diversity (π) across all individuals was estimated at 0.00062, and the haplotype diversity (**Hd**) was determined at 0.288. These values are markedly low, in accordance with those reported for other indigenous Nigerian populations, especially from the southwestern part of the country, where the lowest haplotype diversity was detected (Hd = 0.25) (Ajibike et al., 2017). The entire Nigerian population reached a mean haplotype diversity value of 0.673. Low levels of molecular diversity were also reported in other populations across the continent, such as Abu Naama in Sudan, 3 populations of Uganda (Teso, Langi and Nganda) (Mwacharo et al., 2011), and Dokki-4 Egyptian chickens (Osman et al., 2016).

A total of 11 distinct mtDNA haplotypes were detected (Table 2): 3 for the Fulani ecotype, 5 for the Yoruba ecotype, and 3 haplotypes shared among the 2 (HT01,

Table 2. Control region haplotypes and haplogroup classification of the 96 mtDNA sequences from Fulani (n = 48) and Yoruba (n = 48) Nigerian chicken ecotypes.

HT ID	HT	Ecotype	N
HT01	212,217,243 246 256 261 310 315 446		**81**
		Fulani	41
		Yoruba	40
HT02	212,217,243 246 256 261 265 310 315 446		**1**
		Fulani	1
HT03	212,217,243 246 256 306 310 315 446		**1**
		Yoruba	1
HT04	212,217,243 246 256 261 310 315 355 446		**1**
		Yoruba	1
HT05	212,217,243 246 256 261 310 315 342 446		**4**
		Fulani	3
		Yoruba	1
HT06	212,217,243 256 261 310 315 446		**2**
		Fulani	1
		Yoruba	1
HT07	212,217,243 246 256 261 310 315 396 446		**1**
		Fulani	1
HT08	212,217,243 246 256 261 306 310 315 446		**1**
		Fulani	1
HT09	212,217,243 246 256 261 310 315 446 447		**2**
		Yoruba	2
HT10	212,217,237 243 246 256 261 310 315 446		**1**
		Yoruba	1
HT11	212,217,243 246 256 261 310 315 344 446		**1**
		Yoruba	1
Total			**96**

Bold values represent the total amount of animals (Fulani + Yoruba) for each haplotype.
Abbreviation: mtDNA, mitochondrial DNA.

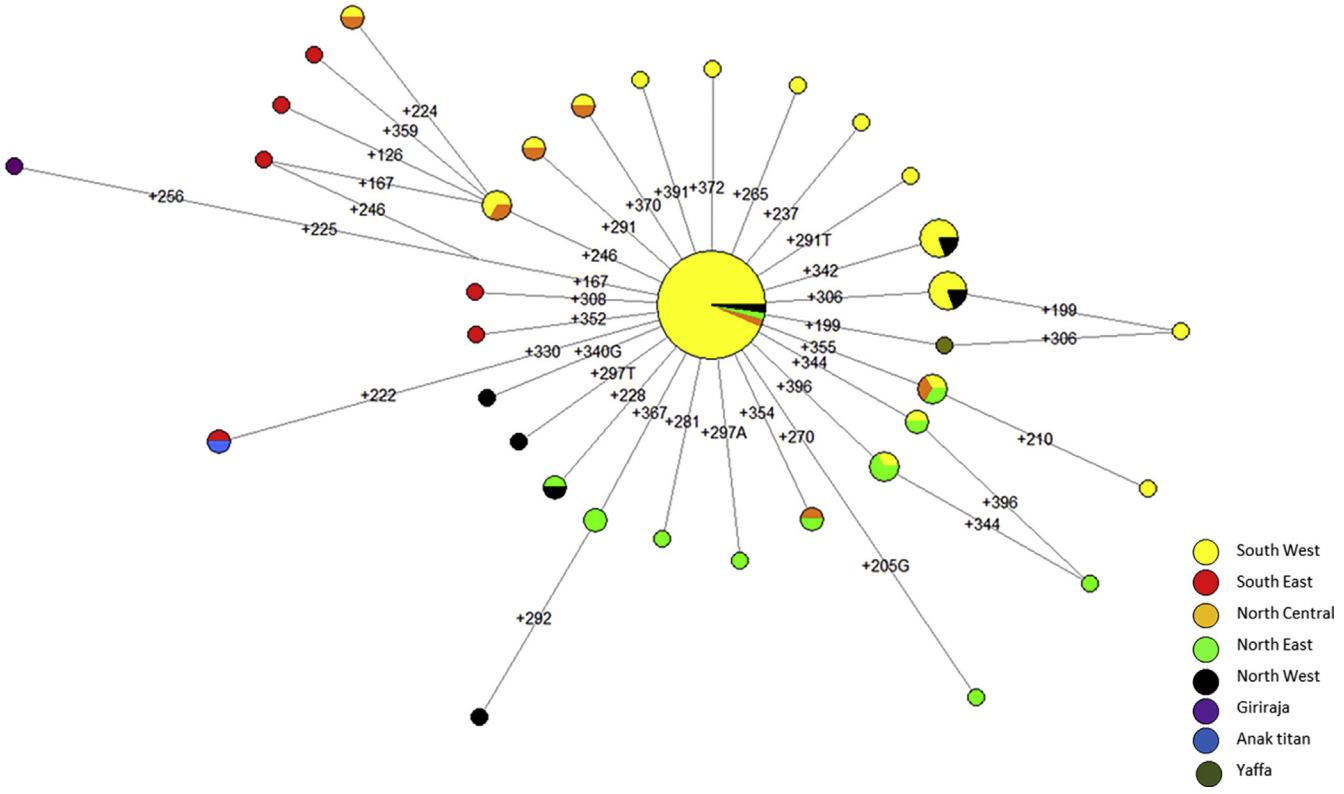

Figure 1. Median-joining network based on control region sequences of 96 studied samples and all published haplotypes from Nigerian chickens. Node size is proportional to the number of samples carrying the same haplotype; the smallest circle represents the unit. Colors reflected the geographical distribution of different indigenous chicken populations in Nigeria and commercial egg line strains; Fulani and Yoruba ecotypes were included in the South West group.

HT05, and HT06). HT05 was found in 3 Fulani samples and one Yoruba, while HT06 was detected in one sample of each ecotype. The most common haplotype (HT01) was found in 81 samples (41 Fulani and 40 Yoruba) out of 96 (84.4%) and was identical to the sequence AB114076, classified as haplotype LIUE1 of clade E by Liu et al. (2006) and called haplogroup D in other studies (Mwacharo et al., 2011; Hassaballah et al., 2015). It is reported to be the most frequent throughout the world (29%) in both local and commercial breeds. In particular, in Europe, it reaches the highest frequencies in Swedish (73%) (Englund et al., 2015), Hungarian (Revay et al., 2010), and Dutch (Dana et al., 2011) breeds. Although at a lower frequency, it was also identified in Polish chickens (Siwek et al., 2013) and recurred in Asia (Silva et al., 2009; Miao et al., 2013) and South America (Gongora et al., 2008).

Phylogenetic Analyses of African Chickens

To estimate the phylogenetic relationships within and among the populations, all the mtDNA D-loop sequences investigated here were processed together with the Nigerian chicken sequences available from GenBank (Supplementary Table 1). Additional nucleotide bases that exceeded np 397 were excluded from the comparison, and a total of 149 sequences were considered. The resulting shorter range included the hypervariable region 1 and spanned from nps 167 to 397.

By performing a network analysis, we obtained a median-joining tree showing a starlike conformation centered on the predominant haplotype (212,217,243 246 256 261 310 315) and confirming the close relationship among all haplotypes that radiate from a single sequence belonging to haplogroup E (Figure 1). This

Table 3. Hierarchical analysis of molecular variance (AMOVA).

Source of variation	df	Sum of square	Variance component	Variance (%)	Fixation index[1]	*P*-value
Among groups (Nigerian ecotypes vs other Nigerian geographic areas)	1	1.428	0.00442	0.94	Φ_{CT} = 0.00944	0.32258
Among populations within groups	5	4.743	0.02963	6.33	Φ_{SC} = 0.06394	0.21505
Within populations	139	60.295	0.43377	92.72	Φ_{ST} = 0.07278	0.000*
Among African areas	8	155.978	0.38386	19.86	Φ_{CT} = 0.19855	0.000*
Among populations within groups	6	13.085	0.01853	0.96	Φ_{SC} = 0.01196	0.01271
Within populations	506	774.634	1.53090	79.19	Φ_{ST} = 0.20814	0.000*

*$P \leq 0.001$.

[1]Φ_{CT} = variation among groups divided by total variation; Φ_{SC} = variation among sub-groups divided by the sum of variation among sub-groups within groups and variation within sub-groups; Φ_{ST} = the sum of variation groups divided by total variation.

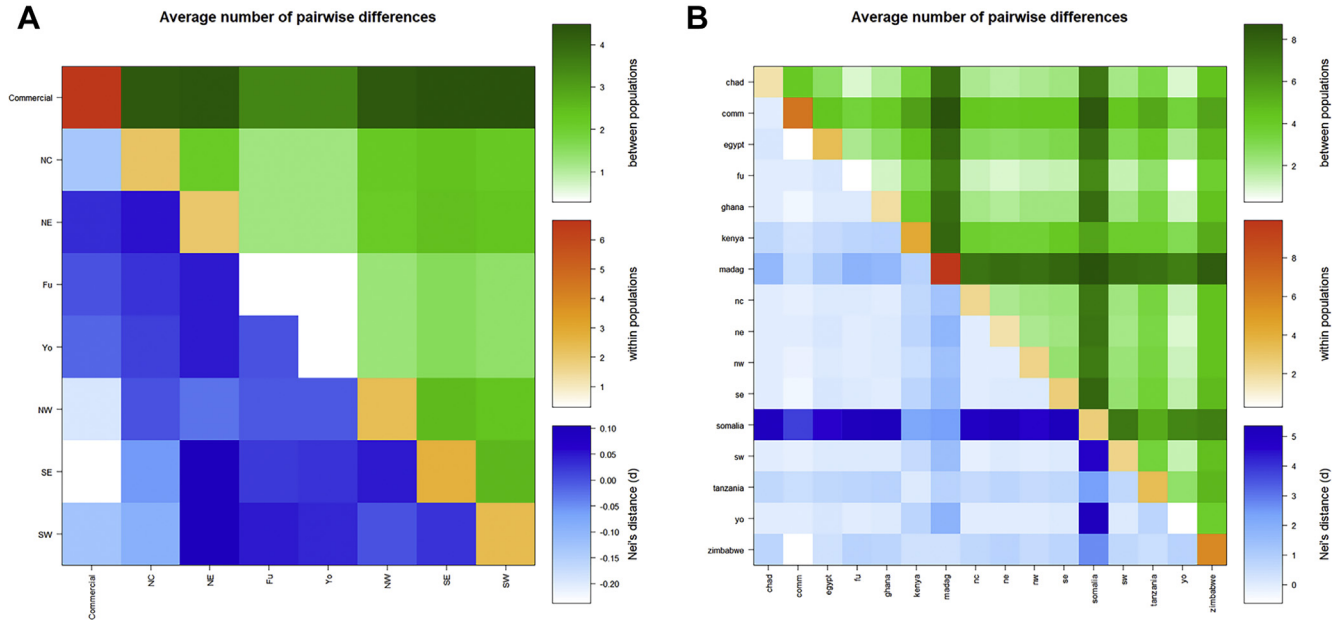

Figure 2. The average number of pairwise differences within and between populations. The graph shows scales of the average number of pairwise differences between populations sampled in Nigeria (A) and Africa continent (B) in 3 different colors. Abbreviations: Comm, commercial; NC, north-central; NE, north-east; Fu, Fulani; Yo, Yoruba; NW, north-west; SE, south-east; SW, south-west.

haplogroup seems to originate from the Indian subcontinent with a single expansion event (Liu et al., 2006), suggesting a relatively recent population expansion of Nigerian ecotype chickens that likely followed their arrival in the country. Except for HT01, distributed all over Nigeria with 57.72% frequency distribution, and the second most frequent haplotype (HT05 = 3.36%), which was detected in both Fulani and Yoruba as well as GU951752, very few haplotypes were shared between different Nigerian geographical areas. Only one haplotype (HT04) was shared between southwestern (Yoruba), north-central, and northeastern regions; by using the Basic Local Alignment Search Tool (BLAST, https://blast.ncbi.nlm.nih.gov/Blast), we found that this haplotype was identical to only one sequence (KP307129) belonging to the Russian breed Orloff.

The other southwestern samples shared 4 haplotypes with north-central Nigeria, 2 haplotypes with the northeastern region, and 2 other haplotypes (including both Fulani and Yoruba) with the northwestern area. Conversely, the commercial egg line strains (Anak titan, Giriraja, and Yaffa by Ajibike et al., 2017) occurred separately.

Finally, HT03 and HT08 (from the Yoruba and Fulani ecotypes, respectively) were previously reported by Adebambo et al. (2010), while HT02, detected only in a Fulani sample, and HT10, observed only in Yoruba, were never found before in Nigeria. Through BLAST analysis, we verified that HT02 was unique among all published chicken mitochondrial data, while there was only one sequence (AB829485) identical to HT10, found in a Fayoumi sample in Egypt (Osman et al., 2016). This is considered an ancient pure native breed that has been raised along the River Nile for centuries; then, during the 19th century, it was imported from Egypt to the United States and the United Kingdom. The sharing of HT10 between our Yoruba sample and the published Fayoumi sequence could indicate that Yoruba likely arrived into West Africa from Egypt after a terrestrial migration from the Middle East.

The genetic peculiarity of the Yoruba and Fulani ecotypes was also quantified by hierarchical AMOVA (Table 3): 0.94% of the observed variance is due to differences between the Nigerian ecotypes belonging to this study and other ecotypes from Nigerian geographic areas; this value clearly represents the homogeneity of the Nigerian chickens studied so far. The same results were graphically revealed by the plot of pairwise population genetic distances shown in Figure 2A, in which the Fulani

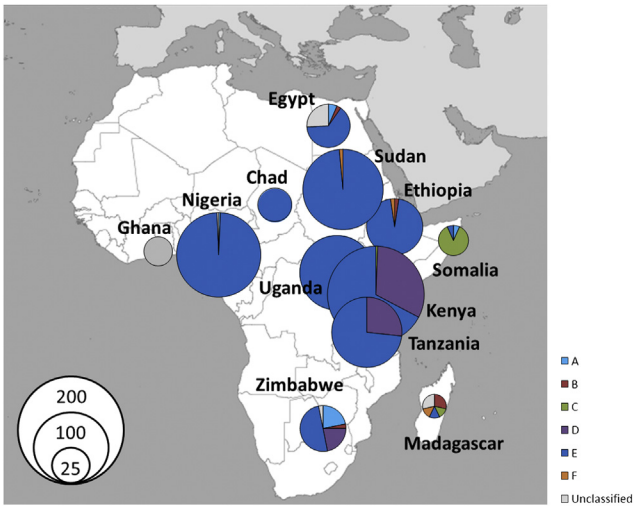

Figure 3. Frequency distribution of mtDNA haplogroups in Africa (Table 4), by including all sequences recorded in GenBank. Abbreviations: mtDNA, mitochondrial DNA.

Table 4. List of African chicken mtDNA data recorded in GenBank.

Country	A	B	C	D	E	F	G	Unclassified	Total
Chad					20				20
Indigenous village chickens					20				20
Egypt	2	1			20			8	31
Dandarawi	1				2			1	4
Fayoumi					2			3	5
Sinai chicken								3	3
Unspecified	1	1			16			1	19
Ethiopia		1			40	1			42
Unspecified		1			40	1			42
Ghana					10				10
Unspecified					10				10
Kenya			1	51	107				159
Homa Bay					14				14
Kakamega				1	13				14
Kilifi				2	8				10
Kisii					15				15
Kitui			1	8	5				14
Marsabit				10	5				15
Meru				9	5				14
Muranga				10	5				15
Naivasha				2	13				15
Nandi					14				14
Taita				4	2				6
Unspecified				5	8				13
Madagascar		2	2	2	1	4		5	16
Red jungle fowl			1	2		3		3	9
Unspecified		2	1		1	1		2	7
Nigeria	1				147			1	149
Commercial egg line strain	1				2				3
Fulani					48				48
Indigenous Nigerian chicken	1				49			1	50
Yoruba					48				48
Somalia	1		13		1				15
Unspecified	1		13		1				15
Sudan					133	2			135
Unspecified					133	2			135
Tanzania				27	74				101
Unspecified				27	74				101
Uganda					123				123
Unspecified					123				123
Zimbabwe	7	1		7	16			1	32
Zimbabwe village chicken	7	1		7	16			1	32
Total	11	5	16	87	682	7		25	833

and Yoruba ecotypes showed low variability between populations compared with all other Nigerian ecotypes, except for commercial. In this graph, Fulani and Yoruba showed the lowest variability within the populations, confirming the lack of phylogeographic structure in Nigerian indigenous chickens, previously described by Adebambo et al. (2010).

To evaluate the African mtDNA variability and allow a comparison between Nigerian and the other African populations, we analyzed a total of 833 data derived from 11 African countries (Supplementary Table 1). Map of frequency distribution in Figure 3 (see Table 4 for details) showed a clear division between Central–West Africa, where almost all the published sequences belonged to haplogroup E, and the East–South regions, including Kenya, Tanzania, and Zimbabwe, where a notable frequency of haplogroup D was also detected (Muchadeyi et al., 2008; Mwacharo et al., 2011; Lyimo et al., 2013). The highest mtDNA variability in Africa, with representatives of haplogroups B, C, E, and F, was detected in Madagascar, even though it is an island, and hence a geographically isolated context, and in Zimbabwe (with haplogroups A, B, D, E).

A further analysis of pairwise genetic distances was performed by including all the populations from the 11 African countries (Figure 2B). For most samples, Fulani and Yoruba showed the highest population genetic distances from chickens from Madagascar, Somalia, and Zimbabwe and the lowest diversity within populations.

Then, by grouping the populations depending on their geographic areas, the hierarchical AMOVA pointed out a total of 19.9% of genetic variance linked to different African areas, even if the majority of the observed variance was attributable to differences among samples within populations (79.2%) (Table 3).

To graphically display (and summarize) the mitochondrial relationships among the analyzed chicken populations, we performed a PCA.

After variable reduction to PC (haplogroup frequencies based on different haplotypes), the coordinates of the observations for the populations were reported in a two-dimensional plot representing the genetic landscape of chicken across the whole African continent in a wider context that included worldwide populations (Figure 4). The Central and West African (Chad, Ghana, and Nigeria) sequences fell into the third quarter, which was

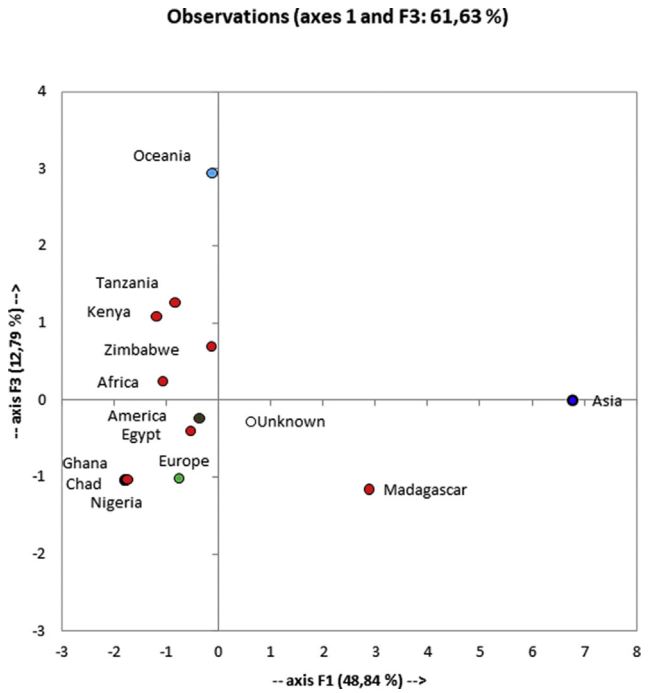

Figure 4. A two-dimensional region-based PCA plot obtained by including studied samples and all available chicken mtDNA data (Supplementary Tables 1 and 2). Colors reflect different continents. The African chickens are reported as red blots. The upper box represents the plot of the contribution of each haplogroup to the first and second principal component. Abbreviations: PCA, principal component analysis.

entirely determined by the component of haplogroup E, close to those from Europe (Greece, Russia, and Turkey). The expected outlier position of the Asian populations was confirmed, along the first PC, partially flanked only by the Madagascan populations, while the second PC clearly split the different African populations, resulting in Nigerian chickens falling closer to Ghana and Chad than to the Southeastern populations.

To determine whether the overall haplogroup frequencies in our Nigerian samples were different from those in other worldwide populations, we performed a second PCA by grouping all available samples from the 5 continents and highlighting only the African countries (Figure 4 and Supplementary Tables 1 and 2). The overall plot highlighted the outlier position of Central-Western African chickens (Chad, Ghana, and Nigeria) from those of the other African countries because of the high frequency of haplogroup E. Furthermore, considering the worldwide distribution of all the chicken haplogroups, Figure 5 shows that except for Asia and Oceania, where the typical haplogroups were H and W, respectively; clade E was the most represented in Africa, America, and Europe.

Moreover, in accordance with previous surveys, the plots highlighted an overall east-west geographic pattern from Asia into the African continent. At least 3 distinct maternal lineages, from which current domestic populations derived, were recently identified by Muchadeyi et al. (2008), explaining the lack of a Zimbabwean population substructure as the result of an ancestral maternal influence from Southeast Asia.

Recently, Herrera et al. (2017) described a phylogenetic link between haplogroups of chickens from East Africa and South Asia, and Ajibike et al. (2017) described the lack of phylogeographic structuring in Nigerian village chickens without mentioning specific ecotypes. Here, we report, for the first time, the same conclusion for the Fulani and Yoruba ecotypes. Our findings suggested extensive genetic intermixing both among the indigenous Southwestern Nigerian chickens and the whole country, following human migrations and trading. Interestingly, the gene flow seems to have involved only one of the main mitochondrial clades (haplogroup E), and the recent introgression of commercial haplotypes into the gene pool of village chickens might explain the rare presence of haplogroups A and B (Mwacharo et al., 2011).

As previously reported by Adebambo et al. (2010), different origins were indicated for East and West African chickens. Indeed, chickens seem to have reached East Africa and Madagascar through the Pacific route (Herrera et al., 2017), but different discussions need to be carried out for West Africa, as the ancestors of these chickens might derive from the Indian zone and have reached Africa through 2 different paths. In the first, they followed terrestrial routes across the Middle East to Egypt and then diffused into Central and West Africa along the Nile River (Osman et al., 2016); in the second, the ancestors of chickens arrived from the west coast of Africa, for example, following early European exploration (Adebambo et al., 2010).

CONCLUSIONS

This study represents the first comprehensive overview of the maternal lineages of 2 indigenous Nigerian chickens through the analysis of the mtDNA control regions of 96 samples belonging to the Nigerian ecotypes Fulani and Yoruba. However, we observed a noticeable presence of unique haplotypes among our native samples, which make these ecotypes a genetic resource that should be preserved.

A comparative analysis with the other African countries and the worldwide haplogroup distribution pointed out an east-west gradient with a higher mtDNA variation in Asia than in Africa and Europe, both characterized by a frequency of 82% for haplogroup E. This lineage likely originated from the Indian subcontinent and later reached Africa and Europe; our results showed a probable dilution of mitochondrial variability after the expansion events and the existence of unusual genetic mtDNAs among Fulani and Yoruba ecotypes highlighted the need to prevent loss of biodiversity in indigenous chicken populations.

Furthermore, we observed a close relationship among the west-central African populations (in particular, chickens from Chad, Ghana, and Nigeria) and the detection of HT10 in Yoruba, shared with only one Egyptian Fayoumi sequence, could reveal an arrival in Nigeria from Egypt after a terrestrial diffusion from the Middle East.

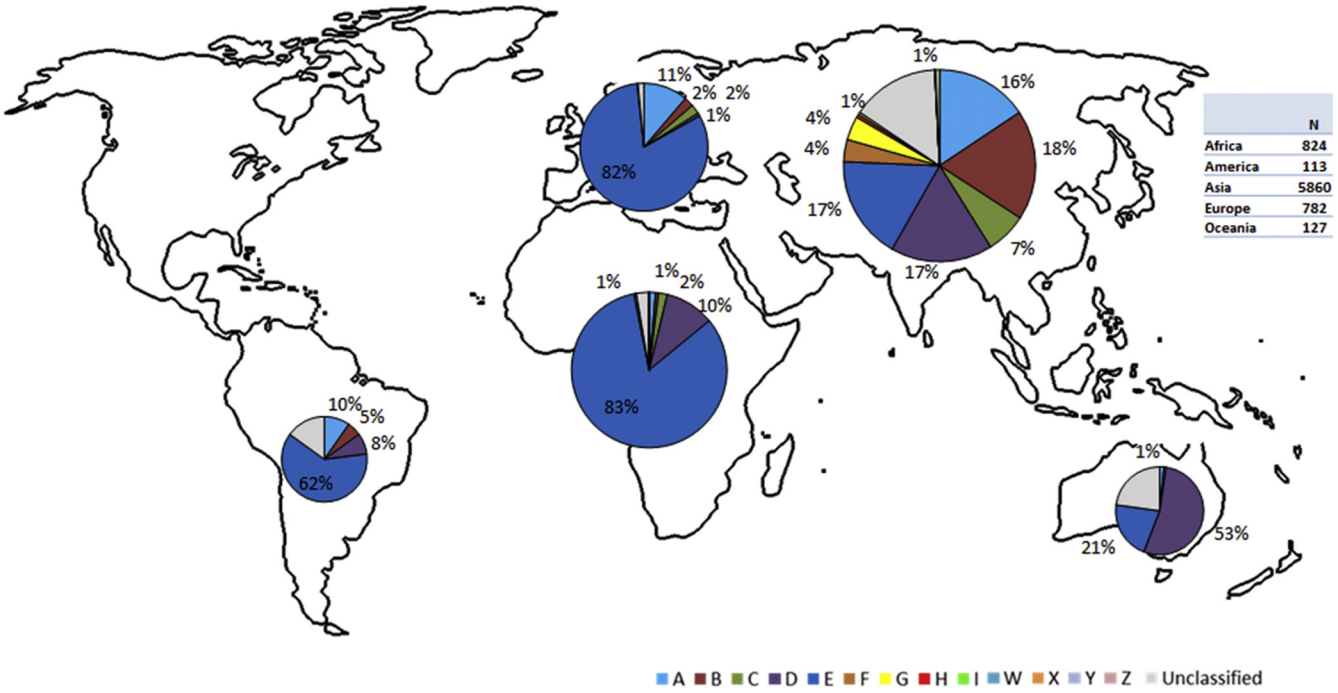

Figure 5. Frequency distribution of mtDNA haplogroups all over the world (for details see Supplementary Table 2). Abbreviation: mtDNA, mitochondrial DNA.

The present research unveils the maternal inheritance of Fulani and Yoruba, pinpointing them as important genetic resources, but other molecular markers, including nuclear loci, should be further investigated to complete the characterization of Nigerian indigenous chickens.

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Conflict of Interest Statement: The authors declare that there is no conflict of interest.

SUPPLEMENTARY DATA

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