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Original Article

Mitochondrial DNA Variation and Genetic Diversity of Donkeys in Sahel Agro – Ecological Zone of Nigeria

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Abstract: *A great field for ensuring that genetic variation is preserved in any particular species is genetic diversity analysis. The study's goals were to assess the molecular genetic diversity and determine the evolutionary history of Nigeria's Sahel agro-ecological donkeys. For the investigation, twenty (20) samples from Borno and Yobe states and sixty-six sequences from the gene bank were employed. The methodology for extracting genomic DNA was followed (Accu prep Genomic DNA extraction kit from Bioneer), and polymerization was carried out at DNA Lab Kaduna utilising donkey MTF primers (forward primer: 5' TAGCTCCACCATCAACACCC 3'; reverse primer: 5' GCATTTTCAGTGCCTTGCT 3'). PCR amplicons were sequenced at the Inqaba Biotech facility in West Africa using the Sanger di-deoxy chain termination method.Uncertain bases were checked and corrected using the Bio Edit programme. MEGAX's ClustalW programme was utilised to align various sequences. DnaSPver 6.0 was used to analyse the numbers of haplotypes, nucleotide polymorphic sites, haplotype diversity (h), and nucleotide diversity (π). Median Joining Network in NETWORK v 10.1 was used to construct the haplotype joining network, and Arlequin software version 3.5 was used to analyse Tajima's test of neutrality (D). The current study's high Hd = 0.87 and negative Tajima D observation suggest that populations are expanding or undergoing purifying selection. When measuring genetic distance, the estimates of (Fst) and Nst were applied, and the results were low for numerous alleles. This indicates that there was little variation in the allele frequency of these genes within the population.The findings reveal that there is no structured population in the population of donkeys (Fst values were not significant at p<0.05).Three clades were found by the evolutionary analysis. All of this category includes the research area. Equine Nubians, Equine Asinus Somalis, and Clade C comprise African wild donkey clade members. These groups' many subclades describe founder effect and expansion from a set of founder mitochondrial DNA haplotypes.*

Keywords: *Mitochondrial, Diversity, Donkeys, Evolution. Sahel.*

I. INTRODUCTION

As draft animals, donkeys (Equusasinus) are a vital part of the livestock and contribute significantly to the agricultural economy. Donkeys, being draught animals, are essential to the economies of developing nations as they provide the primary means of transportation and traction, especially in regions with challenging relief operations (FAO, 2003).Donkeys were first tamed in Africa 5,000 years ago. They influenced the layout of the earliest cities and pastoral cultures, met human requirements for labour and transportation, and permitted the flow of people and products. With the introduction of motor cars, their population declined (Kimuruet al., 2011). Donkeys on the African continent developed typical aptitudes that were essential to their survival in arid regions due to the normal circumstances (high daily temperatures, little precipitation, and poor nutrient quality) (Pearson and Ouassat, 2000).

The need to maintain diverse populations of domesticated birds and mammals is one of the key justifications for protecting genetic diversity in animals used as food sources. Domesticated species enable livestock to adapt to changing environmental conditions and changes in agricultural practices, leading to the creation of over 8,000 recognised breeds (Diamond, 2002 &FAO, 2011). A species' chances of surviving are increased by genetic variety (Neaves et al., 2015).Reconstructing colonisation and trade routes, locating domestication centres, and identifying wild forebears have all benefited from the use of these maternal markers (Brufordet al., 2003; Groeneveldet al., 2010). The hyper variable control region (D-loop) is the focus of most mtDNA studies, however whole mtDNA sequences provide important additional information by revealing the relationship between haplogroups. (Achillie and others, 2008). The current study's goals were to assess the molecular characteristics of the MtDNA in donkeys in Nigeria's Sahel agro-ecological zone.

II. MATERIAL AND METHODS

The Sahel agro-ecological zone of Nigeria served as the site of the tests. The states of Borno, Yobe, Kano, Katsina, and Sokoto in northeastern and western Nigeria are included in the sahel agro-ecological zone. There are not many trees and a lot of grassland in the Sahel ecozone. The temperature ranges from 33 to 40 degrees Celsius, the humidity ranges from 4 to 12 percent, and the annual rainfall is between 400 and 600 millimetres (FAO, 1991).The region's agricultural pursuits include the raising of livestock, fishing, hunting, and arable crop cultivation. Specifically, the northeastern states of Borno and Yobe were the study's location.

A. Animal and DNA isolation

Twenty donkeys in all were utilised in the molecular characterisation analysis. To decrease genetic relationships among animals and maximise breed representativeness, the animals were chosen based on household, unrelated individuals, and samples from various genetic groups. The jugular vein was used to draw blood samples, which were then promptly placed into EDTA vials. All specimens were maintained at 4˚C until subsequent laboratory procedures.

B. DNA Extraction

The methodology used to extract genomic DNA was Accu prep Genomic DNA extraction kit from Bioneer. After isolating DNA from buffy coat, cultured cells, and whole blood, 200 µl of whole blood was added to a clean 1.5 ml tube along with roughly 20 µl of Proteinase K. The sample was combined with about 200 µl of binding buffer using a vortex mixer right away, and it was then incubated at 60°C for 10 minutes. After adding around 100 µl of isopropanol, thoroughly mix with a pipette.

C. Mitochondrial DNA marker (MtDNA)

A total of 16 sequences from the current study database (www.ncbi.nlm.gov) and 66 sequences of Equusasinus's mitochondrial full d-loop were retrieved from the Gene Bank. Primer3 programme created Primers. For the amplification of a particular mitochondrial d-loop region sequence, use GenBank: X97337.1. The amplification of the 1100 bp product was performed using the primer pair listed below.([TAGCTCCACCATCAACACCC 3' Forward primer]) 5' GGCATTTTCAGTGCCTTGCT 3' is the reverse of this. For this reaction, an optimised PCR protocol containing 25 µL of DNA (30–50 ng), 2.5 µL of dNTPs (10 mM), 2 µL of Mgcl2, 2 µL of buffer, 1 µL of primer forward (10 pM), 1 µL of primer reverse (10 pM), 3 µL of polymerase (5U), and 14.2 µL of water were employed. Using both forward and reverse primer orientation, the amplicons were sequenced using the di-deoxy chain termination method, and the fluorescently labelled products were examined on an ABI genetic analyzer sequencing (ABI 3100).DnaSPver 6.0 (Librado and Rozas, 2009) was used to assess the numbers of haplotypes, nucleotide polymorphism sites, haplotype diversity (h), and nucleotide diversity (π). The average difference between random pairs of homologous haplotype sequences in a sample is called haplotype diversity (h), while the average difference between random pairs of homologous nucleotide sites in a sample is called nucleotide diversity (π). Next, utilising the Median Joining Network in NETWORK v 10.1, a haplotype joining network was built.The UPGMA approach was used to infer the evolutionary history (Sneath and Sokal, 1973). Next to the branches were the proportion of duplicate trees where the related taxa clustered together in the bootstrap test (1000 repetitions) (Felsenstein, 1985).

D. Molecular Diversity of Donkeys of mtMNA in the Sahel Agro - Ecological Zone of Nigeria

The molecular genetic variation indices of donkeys in Nigeria's Sahel Agro-ecological zone are shown in Table 1. There were sixteen sequences, 253 polymorphic segregations (S), 331 total mutations, 13 haplotypes (h), 0.950 haplotype diversity (Hd), and 0.153 nucleotide diversity (Pi). In the study, averages of 89.31 nucleotide differences (k) were found. The entire site, excluding alignment gaps, obtained was 627, whereas the targeted region accounted for 778. Theta per site from genetic diversity (π:0.193), Theta per site from polymorphic segregation (S:0.179), and Theta per site from the total number of mutations (Eta:0.211) were all included in the finite site model. Across all Turkish donkey populations, Unnatiet al. (2022) found moderate-to-high levels of haplotype diversity (ranging from 0.533 ± 0.180 to 0.933 ± 0.122) and moderate nucleotide diversity (ranging from 0.01196 ± 0.0026 to 0.02101 ± 0.0041), indicating diversified genetic diversity. When haplotype and nucleotide diversity values for D-loop were compared with different donkey breeds, it was discovered that Turkish donkey populations were smaller than those of Ethiopian donkeys (HD: 0.903 ± 0.032; πD: 0.020 ± 0.003), Balkan donkeys (HD: 0.982 ± 0.002; πD: 0.017 ± 0.009), Chinese donkeys (HD: 0.9055 ± 0.017–0.9778 ± 0.0540, πD: 0.02265 ± 0.00040–0.0285 ± 0.0160) reported by Chen et al. (2006). The current research found that although the diversity was higher than that of Ethiopian donkeys, it was still Hd = 0.87. Brazilian donkeys produced results with Hd = 0.879, which is marginally comparable to the current findings. Tajima (1996) observed a negative Tajima D, indicating that populations are either expanding or undergoing purifying selection.

E. The Gene Flow Estimation

The gene flow from estimation utilising the information from the haplotype data is shown in Table 2. The investigation yielded a genetic coefficient of difference (Gst) of 0.12221 for the gene, and an effective population number (Nm) of 1.80 for the number of migrants. Using sequencing data, the estimation of gene flow yielded the following results: Nm = 0.18, Gamma St = 0.58661, and Delta St = 0.02911. The population sub- division Fst: 0.58010 and the nucleotide sub- division Nst: 0.58010 and Nm: 0.18 are measured. Both the sequencing and the haplotype data were captured by the gene flow calculation. Gene flow is inversely proportional to the genetic coefficient of differentiation (Gst), an estimate that quantifies genetic differentiation. Gst values that were comparable were found using Agaviezoret al. (2017), Gst = 0.014. The Gst, as predicted, spans from 0.0 to 1.0, where 0.0 denotes no variation in allele frequency between two populations and 1.0, which denotes that the two populations are fixed for a different allele. According to certain authors, there is a reported gene flow among the donkey population in the research area (Hedrick, 2005: Meirmans and Hedrick, 2011 & Gupta et al., 2018). Genetic distance was measured using the nucleotide sub-division Nst and the population sub-division Fst, which were generalised for multiple alleles. Both values obtained were low. This indicates that there was little variation in the allele frequency of these genes within the population. According to the results of (Anilaet al., 2014 & Agaviezoret al. 2017), the current study concurred. According to Wolf and Soltis (1991), Nm represents the average number of individuals each generation that migrate between populations if those populations are equal in size. Wright (1969) developed the Island model, which postulated that distinct alleles at a locus in two populations would not become fixed by genetic drift if more than one person migrates between populations every other generation (Nm> 0.5). When Nm < 0.5, gene drift may cause significant genetic differentiation, and when Nm > 0.05, it plays a crucial role in determining population structures (Wolf and Soltis, 1991).

F. The Analysis of Molecular Variance

The molecular variance analysis is shown in Table 3. The findings showed that maternal genetic differences in the study area accounted for 46.67% of the variation. The sample country population exhibited a variance of just 27.79%, whereas the research area's among-group variation was found to be 8.73%. The study's Fst result, Fst = 0.132, was not statistically significant.According to the current research on Borno and Yobe donkeys, they were not in a state of neutral equilibrium, which is consistent with the findings of the following studies: Jobling et al., 2004; Harrison, 1992; Rogers, 1995; Schneider and Excoffier 1999. These populations were not in neutral equilibrium because they fit an assumed sudden demographic model. The parameters of mutation (because t >0 and σ1 > Θ0). The Yobe/Borno donkeys are currently in a state of abrupt population transition. The non-significant Fst value found in this study suggests that the donkey population was not organised, which is consistent with the findings of (Tor et al., 2021).

Table 1: Molecular diversity of donkeys from mtMNA in the Sahel Agro Ecological zone of Nigeria

Analysis of Pair Wise Comparisons	Values
Number of sequences	16
Number of polymorphic segregation (S)	253
Total number of mutation (Eta)	331
Number of haplotypes (h)	13
Haplotype gene diversity (Hd)	0.950
Nucleotide diversity (π)	0.15345
Average number of nucleotide differences (K)	89.31
Selected region	778
Total site	627
Theta (per site) from genetic diversity (Pi)	0.1929
Theta (per site) from segregation (S)	0.17930
Theta (per site) from mutation (Eta)	0.21161

Table 2: Gene Flow Estimation

Haplotype Data information	
Gst: 0.12221 Nm: 1.80	
Sequence Data information	
Delta St: 0.02911	Gammer St: 0.58661 Nm:0.18
Nst: 0.59542	Nm: 0.17

Fst: 0.58010	Nm:0.18

Where: Gst = genetic coefficient of differentiation, Nm= number of migrant per population, Fst = estimation of population sub division and Nst= nucleotide sub – division.

Table 3: Analysis of Molecular Variance within and Among Donkey Populations Using MTDNA Sequences in the Study Area

Sources of Variation	Variance component	Percent Variance	Fst	Probability value
Among group	74.63	8.73	0.132	1.000
Among population	680.167	46.67		
Within group	222.33	27.79		
Total variance	977.12	65.73		

Fst = estimation of population sub division differentiation and Fst values not significant P< 0.05

G. Maternal Distribution Evolution Origin of Borno/Yobedonkeys.

The genesis of evolution in the maternal distribution of Borno and Yobe donkeys. It is commonly recognised that the ancestors of modern donkeys were from Somalian and Nubian tribes (Kimura et al., 2018). To enhance comprehension of this research, D-loop was utilised to conduct a genetic diversity analysis on 16 sequences and 66 published sequences, totaling 82 samples from diverse regions such as India, Cameroon, Pakistan, Turkey, Africa, and Europe (Nei and Kumar, 2000). The participants in the current study comprised Clade A, of which Borno White was the sole member; the other participants were from other geographical areas. The donkeys in Clade C are Red Borno, White Yobe, Grey Yobe, Black Borno, and White Yobe; the donkeys in Clade B are Black Borno, Black Yobe, Grey Borno, and Red, Rugged Yobe donkeys. The results of Earnistet al. (2021) who identified two distinct Nubian and Somali lineages verifying the African maternal origin of donkeys are consistent with the findings of this study. The current study concurs with Kimura et al. (2011), who found that, aside from the two main clades, the likely recognised extinct wild ancestors in the domestication of donkeys event. This group has several subclades that describe founder effect and expansion from a set of founder mitochondrial DNA haplotypes. Three clades were found in the current investigation. All of this category includes the research area. The black Borno, black Yobe, grey Borno, and red/rust donkey Yobe belong to Equine asinus Somalis, and clade C is made up of a lineage of African wild donkeys. The white Borno donkey originated in Clade A of the Equine Nubians. This group has several subclades that describe founder effect and expansion from a set of founder mitochondrial DNA haplotypes. According to the current results, Clade B donkeys diverged first, followed by Clade C and Clade A donkeys, in that order. This could suggest that Borno and Yobe donkeys once shared maternal ancestors. This work suggests a little more ancient divergence in the time range of 0.910–0.303 Ma, which is consistent with the estimate of divergence time made by Pereira et al. (2004) for the two donkey maternal clades. The coalescence times between the several E. asinus lineages are examined by Wang et al. (2021). The split of the two domestic donkey populations occurred at 0.715 Ma (95% CI: 1.169–0.305 Ma), which is the divergence time between Somali wild ass and domestic donkey.

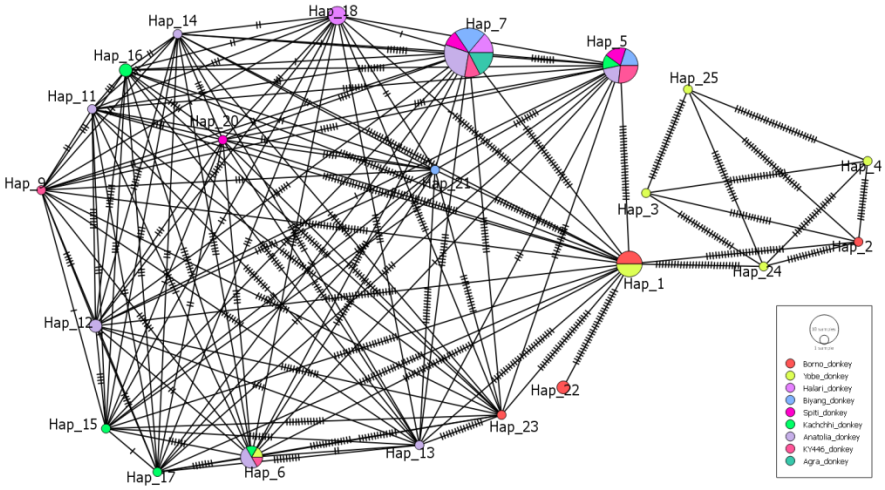

Figure 1: Used Mega Version 10 Software to Link the phylogenetic

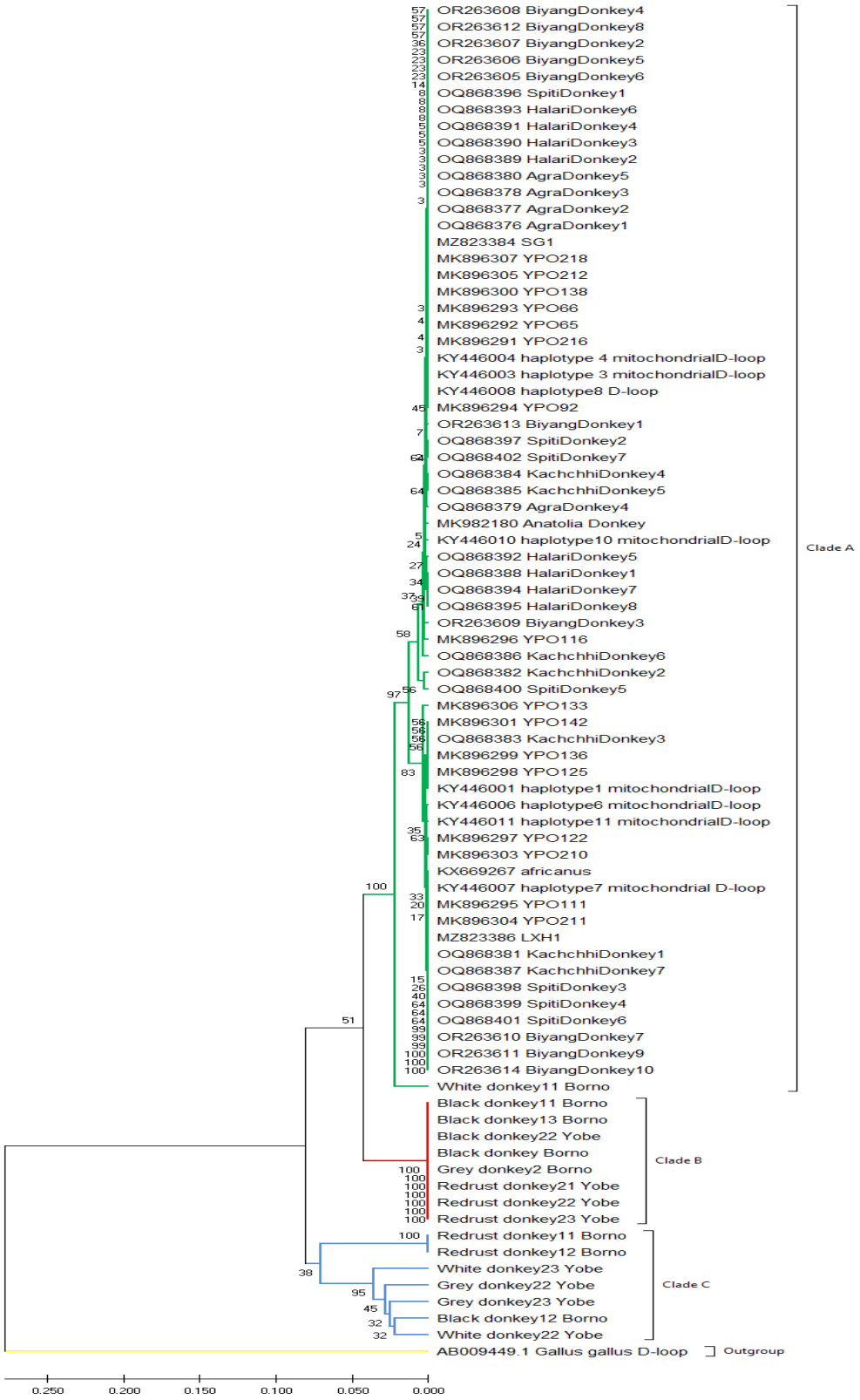

Figure 2: The Network Results

Fig 1. Neighbour – used MEGA version 10 software to link the phylogenetic tree that was generated for donkeys in the sahel agro-ecological zone of Nigeria with populations from other locations based on consensus sequences. After 1000 replications, the % bootstrap values for inner branches are represented by the number at the nodes. With a sum of branches length of 0.93113977, the ideal tree.

Fig 2. Median – The network result for the association between the world's population and the donkeys of Borno and Yobe. Colour codes were used to identify different populations, according to Tor et al. (2021) (Purple= India, light blue= Cameroon, pusher pink= India, light green= India, light purple= Turkey, red= Pakistan donkey, yellow= Yobe, red= Borno, green= India) Each circle's area corresponds to the matching haplotype's frequency (s).

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