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Genetic Diversity of mtDNA of Donkeys in Sahel Agro-ecological Zone of Northeastern Nigeria

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Abstract

The molecular characterizations of the donkey in the Sahel agro-ecological zone of the north eastern Nigeria were assessed. A total of 20 adult donkeys were sampled through stratified random sampling in some part of Borno and Yobe States and were used for the molecular characterization. to reduce the genetic relationship among animals and to increase the breed representativeness. Blood samples was collected from the jugular vein and immediately transferred into EDTA bottles. Genomic DNA was extracted following the Accu prep Genomic DNA was extraction and polymerization using donkey MTF primer (Forward primer: 5' TAGCTCCACCATCAACACCC 3') (Reverse: 5'GCATTTTCAGTGCCTTGCT 3'). PCR amplicon was sequenced based on sanger di-deoxy chain termination method at (Inqaba Biotech laboratory in Pretoria south Africa) in West Africa. Bio edits program was used to check and edit ambiguous bases. ClustalW in MEGAX was used for multiple sequence alignments. Numbers of haplotypes, numbers of nucleotide polymorphic sites, haplotype diversity (*h*), and nucleotide diversity (π) were analyzed with DnaSP ver 6.0. A total of 16 sequences from the present study and together with 66 sequences from the gene bank were used for the above analysis. Borno/Yobe donkeys were highly polymorphic compared to donkeys from other locations of the World. The high haplotype diversity and low nucleotide diversity among the donkeys in the study area could be as a result of the differences in the wide geographical spread of the study area, environmental and genotype by environment interaction. It may also be as a result of rates of mutation, founder effect or sudden expansion of the population.

Key words: Genetic diversity**,** Donkey, Mitochondrial DNA, Nucleotide diversity and Haplotype

Introduction

In Africa, donkeys were domesticated in the last 5000 years ago. They were used to satisfy human needs in transport and work and to influence the organization of the first cities and pastoral societies. Their numbers decreased with the advent of motor vehicles (Abdulla *et al.,* 2004: Kimuru *et al.*, 2011: Abdulla *et al.*, 2014). Typical factors such as high temperatures, minimal amount of precipitation and lack of quality nutrients have made the donkeys to develop typical aptitudes, which played a key role to survive in dry areas (Pearson and Ouassat, 2000). The African wild donkey (*Equus africanus*) is the ancestor of the donkey (*Equus asinus*).

Mitochondrial DNA (MtDNA) are maternal markers have been instrumental in identification of wild ancestors, localization of domestication centers and reconstruction of colonization and trading routes (Bruford *et al.*, 2003 and Groeneveld *et al.*, 2010). Mitochondrial DNA (mtDNA) have advantage of maternal inheritance, extremely low paternal leakage, present in large amount in cell, haploid form of DNA (Cummins *et al.*, 1997). It is preferred for genetic diversity, population structure, geography and animal evolution studies (Ankel-Simons and Cummins, 1996 and Merwad *et al.,* 2014).

The molecular characterization of donkeys reveals genetic diversity in the animal population. Genetic diversity study is an excellent field that ensures that genetic variation is maintained in any given species (Ahmad-Syazni *et al*., 2017 and Han *et al*., 2017). Loss of genetic diversity in domestic populations is particularly significant in potentially unsustainable species such as donkeys, causing the simultaneous loss of essential functional traits (Navas *et al*., 2017). The objective of the study was to evaluate the genetic variation of MtDNA of donkeys in the Sahel agro ecological zone in North Eastern Nigeria.

Materials and Methods

Location of the Study

This study was conducted in the Sahel agro-ecological zone of Nigeria. The Sahel agro-ecological zone comprises the following states in North East and some state in the north west of Nigeria: Borno, Yobe, Kano, Katsina and Sokoto. The Sahel ecological zone is characterized by vast grassland and few trees. The temperature ranges from 33 ^{oC} to 40 ^{oC} and humidity ranging from 4 - 12% with annual average rainfall of 400 – 600mm (FAO,1991). The agricultural activities in the area include arable crop farming, livestock rearing, fishing and hunting. This study was carried out specifically in Borno and Yobe State north eastern part of Nigeria. Borno State is located between latitude 10 and 14ºE and Longitude 11 and14º N and an altitude of 354 m above sea level. It covers an area of 61,435 km^2 which is about 12% of the total area of the country. It occupies a greater part of the Chad Basin and shares border with Adamawa State to the south-east, Gombe State to the south west and Yobe State to the north-west. (BOSHIC, 2007).

On the other hand, Yobe state is located within latitude 11º north and longitude 13.5º East with a total land area of 47,153 square kilometers. It shares common boundaries with Borno state to the East and southeast, Jigawa state to the northwest, Bauchi and Gombe states to the southwest. It also shares an international border with the Republic of Niger. This boundary stretches over 323km to the north of the State. Yobe State generally engaged in agrarian activities with more than 80% of the citizens engaged in small scale subsistence farming. Food crops are grown by small-scale farmers to generate household income. The state is also noted to be the largest producer of gum arabic in Nigeria. A significant proportion of the population are pastoralists who pasture commercial livestock including cattle, sheep, goats, donkeys and horses. The State has the largest cattle markets in West Africa. It supplies meat, hides and skin to other parts of the country particularly to the south. Fig 2. present the map of Nigeria showing the agro-ecological zones of the country.

Experimental Animals and DNA isolation

A total of 20 donkeys were used for the study of molecular characterization. The animals were phenotypically selected based on their coat colour either red, grey, white and black. The animals were maintained under the traditional husbandry system of management., with body weight ranging from 174 -184 kg. The animals were selected using the criteria of unrelated individuals, samples from different genetic groups and household in order to reduce the genetic relationship among animals and to increase the breed representativeness. Blood samples was collected through the jugular vein using 5mls syringes, about 2mls blood were collected and immediately transferred into EDTA bottles. All samples were kept at 4 $^{\circ}C$ until the further laboratory process. The DNA extraction, PCR and gel electrophoresis were done in DNA laboratory in Kaduna, Kaduna state of Nigeria.

DNA Extraction

Genomic DNA was extracted following (Accu prep Genomic DNA extraction kit from Bioneer) protocol. The Isolation of DNA from whole blood, buffy coat, and cultured cells was done. About 20 µl of Proteinase K was put into clean 1.5 ml tube and then applied 200 µl of whole blood. About 200 µl of binding buffer was added to the sample and mixed immediately by vortex mixer and then was incubated at 60$^{\circ C}$ for 10 min. About 100 µl of isopropanol was added and mixed well by pipetting. The lysate was carefully transferred into the upper reservoir of the binding column tube (fit in a 2 ml tube) without wetting the rim, then the tube was closed and centrifuged at 8,000 rpm for 1 min. the tube was opened and the binding column tube was transferred to a new 2 ml tube for filtration. About 500 µl of Washing buffer 1 (W1) was used without wetting the rim then the tube was closed and centrifuge at 8,000 rpm for 1min. The tube was opened and the solution from 2ml tube was poured into a disposal bottle. About 500 µl of Washing buffer 2 (W2) was then added carefully without wetting the rim, then the tube was closed and centrifuge at 8,000 rpm for 1 min. The Centrifuge was repeated once more at *ca.* 12,000 rpm for 1 min to completely remove ethanol, and check that there was no droplet clinging to the bottom of Binding column tube. Then the binding column tube was transferred to a new 1.5 ml tube for elution about 200 µl of Elution buffer was added (EL, or nuclease-free water) onto binding column tube and waited for at least 1 min at RT (15~25$^{\circ C}$) until EL was completely absorbed into the glass fiber of Binding column tube. Lastly it was centrifuge at 8,000 rpm for 1 min for elution.

Mitochondrial DNA (MtDNA) marker

The sequence of mitochondrial complete D-loop of *Equus asinus* was accessed from gene bank database (www.ncbi. nlm.gov). Primers was designed by Primer3 software from GenBank: X97337.1 for amplification of specific sequence of mitochondrial D-loop region. The below primers pair was used for amplification of product of 1100 bp .

([Forward primer: 5' TAGCTCCACCATCAACACCC 3']

[Reverse: 5' GGCATTTTCAGTGCCTTGCT 3']).

The optimized PCR of 25 µL recipe for this reaction was used follow DNA 2µL (30-50ng), dNTPs (10mM) 2.5 µL, $Mgcl_2$ 2 µL, buffer 2 µL, primer forward 1 µL (10 pM), primer reverse 1 µL (10 pM), Polymerase (5U) 3µL and water 14.2 µL. Thermo cycler setting adjusted as; 95 ^{oC} for 5 minutes 94 ^{oC} for 30 second, 57 ^{oC} for 30 second, 72 ^{oC} for 1 minute and final extension at 72 ^{oC} was 10 minutes for 25 cycles.

To the results of PCR electrophoresis was run about 1.5 % agarose (3g of agarose gel) was used. The solution was heat in a microwave until agarose was completely dissolved. It was allowed to cool in a water bath set at 50 – 55 ^{oC}. The gel casting was prepared by sealing ends of gel chamber with tape or appropriate casting system. Appropriate number of combs was placed in the gel tray. About 5ul of ethidium bromide was added to cooled gel and pour into gel tray. Then load DNA and standard (Ladder) onto gel, it was then electrophoresing at a given voltage for at least 1hour then visualize DNA bands using UV light box or gel imaging system. (Biorad). The amplicons were sequenced through di-deoxy chain termination method using both orientation of forward and reverse primers and fluorescently labeled products was analyzed on ABI genetic analyzer sequencing (ABI 3100).

Results

Single Nucleotide Polymorphism (SNP) and Haplotypes observed in the study

Table 1: Single Nucleotide Polymorphism (SNP) and Haplotypes observed in the study Regions among the donkey population

S/N	Loci / SNP	Types of mutation	Total number variate
1	54 A > G	Substitution	4
2	55 C > T	Substitution	
3	60 G >A	Substitution	

4	183 G >T	Substitution	
5	124 A > -	Deletion	3
6	254 C > -	Deletion	
7	467 G > -	Deletion	

Populations	Number of haplotype	Haplotypes
Miringa	1	HapWDB 1
Buni – yadi	2	Hap WDY22, Hap WDY21
Gujba	1	Hap WDY23
Dadin kowa	1	HapRDB11
Shikwe	1	HapRDB12
Geidam/Dam	4	HapRDY21, HapRDY22, HapBDY and HapGDB
Geidam	1	HapRDY23
Zuwa	1	HapBDB11
Wula –wula	1	HapBDB12
Bumsa	1	Hap BDB13
Garubula	1	HapBDB
Hausari	1	HapGDY23
Kawuri	1	HapGDY23

Table 1 presents the Single nucleotide polymorphism (SNPs) within the Dloop segments of the donkey mtDNA. The number of single nucleotide polymorphism (SNPs) within the regions of the mtDNA were 4 variants in SNPs with four substitutions at position 54, 55, 60 and 183, respectively. At the other position there existed 3 variants with deletion at position 124, 254 and 467 respectively. A total of 13 haplotypes were discovered within 16 sequences in the study area. The number of included positions was 253, number of site 778 and with haplotype diversity of 0.9500.

Molecular Diversity of Donkeys from the Sahel Agro-ecological Zone of Nigeria based on mtDNA

Table 2: Molecular Diversity of Donkeys from mtDNA in the Sahel A gro-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 presents the molecular genetic variation indices of donkeys in Sahel Agro – ecological zone of Nigeria. The number of sequences were 16, number of polymorphic sites (S) 253, the total number of mutation (Eta) 331, number of haplotype (h) 13, haplotype diversity (Hd) 0.950 and the

nucleotide diversity (Pi) 0.153. The average number of nucleotide difference (k) obtained in the study was 89.31. The selected region was 778 while the total site excluding alignment gaps obtained was 627. The finite site model contained the Theta per site from genetic diversity with the values of π :0.193, Theta per site from polymorphic segregation S: 0.179 and the Theta per site from total number of mutation Eta: 0.211.

Molecular Diversity Indices of mtDNA in Borno and Yobe Donkeys

Table 3: Molecular Diversity Indices from mtDNA in Borno and Yobe States

Indices	Borno	Yobe
Number of sequences	8	8
Number of segregation site (S)	96	63
Number of haplotypes (h)	4	5
Haplotype diversity (Hd)	0.75	0.79
Average number of difference (k)	35.00	28.39
Nucleotide diversity (π)	0.08	0.06
Tajima D	-0.475	-1.113

The molecular diversity indices from the study area are shown in Table 3 Equal number of sequences were used in both locations. Borno population had the higher number of segregating site (S= 96). Yobe population had the higher number of haplotypes (h = 5). Both locations had highest number of Hd of 0.75 and 0.79 for Borno and Yobe population respectively. High nucleotide diversity was obtained in the present study $\pi = 0.08$ and $\pi = 0.06$ for Borno and Yobe, respectively.

Standard and Molecular Diversity Indices from mtDNA of Donkeys in the Study Area and other Regions

Table 4: Standard and Molecular Diversity Indices from mtDNA of Donkeys in the Study Area and Other Regions

Index	Values
Number of sequences	82
Number of haplotypes (h)	25
Haplotypes diversity (Hd)	0.87
Nucleotide diversity (π)	0.05
Number of usable loci	773
Number of segregation site	131
Number of observed transversion	1
Selected region	784
Theta value base on Eta per site	0.1714
Theta value base on Eta	99.75
Theta values base on π	0.1534
Tajima D	-1.1139

Table 4 presents the molecular diversity indices from mtDNA of donkeys in the study area. The number of sequences used for the study were 82 and 25 haplotypes were discovered. High haplotype diversity was observed Hd = 0.87 and medium nucleotide was observed for the nucleotide diversity $\pi = 0.05$. The number of usable loci had 773 and the regions selected was 784. The Theta Value per mutation investigated had high value in the present study 99.75 while the Theta per nucleotide diversity obtained was 0.153.

Discussions

Single Nucleotide Polymorphism (SNPs) Within the Two Hyper Variable Regions of the Dloop Segments of the Donkey mtDNA

The 16 mtDNA – Dloop sequences from Borno and Yobe donkeys were defined by 262 polymorphic sites 65 parsimonious sites and 7 singleton sites that further group into 13 haplotypes. These figures are in contrast with those of Kefena *et al*. (2014) who obtained 29 polymorphic and 22 parsimonious sites in Ethiopian donkeys. Yun and Cho *et al*. (2021) investigated a total indels of 35 variants in the Korea donkeys which is higher than the present study. The variation obtained in the present study may be indicative of the genetic diversity of Nigerian donkeys compared to other locations of the world Different authors recorded lower polymorphic site of 27 and 33 which is lower than the present findings (Mazzatenta *et al*., 2021 and Earnist *et al*., 2021).

Variation in Sequences of Donkey in Sahel Agro –ecological Zone of Nigeria

The present study shows high haplotype diversity and low nucleotide diversity among the studied populations. Similar results were reported by Cozzi *et al*. (2018) who observed high haplotype diversity among Italian donkeys breeds using mt- DNA Dloop and by Xia *et al*. (2019) who reported haplotypes diversities of Hd: 0.910 ± 0.032 and 0.879 ±0.060 among Egyptian and Brazilian donkeys respectively. Similarly, Earnist *et al*. (2021) and Bhardwaj *et al.* (2020) obtained high haplotype diversities of (hd = 0.967±0.037 π = 0.02917 ±0.00307) among (Hd = 0.8152 with low π = 0.1281) among Pakistan and Indian donkey populations respectively. The results of high haplotype diversity may be due to wide difference in geographical areas of the study leading to environmental modifications and hence genotype by environment interactions. It may also be as a result of varying rates of mutation, genetic bottlenecks, founder effect or sudden expansion of the population.

Molecular Diversity Indices in Borno and Yobe

The present study revealed that there was higher number of segregating sites in Borno population as compared to Yobe with corresponding higher average number of difference (k) and nucleotide diversity which may be as a result of high rate of mutation in the location. On the other hand, there was higher number of haplotype diversity in Yobe population as compared to Borno which may be an evidence of purifying selection, geographical modification, high mutation as well as the rate of introduction of donkeys from neighboring countries (Yap *et al.,*2011). The Tajima D had negative values which may be suggestive of population expansion or purifying selection which implies that such population are not at equilibrium but tended to have excess of rare alleles (Tajima, 1996).

Standard and Molecular Diversity Indices of mtDNA of Donkeys in the Study Area and other Regions

The haplotype diversities (hd = 0.87) from this research reveals that Nigerian donkeys were comparatively genetically diverse. Earnist *et al*. (2021) observed moderate-to-high levels of haplotype diversity in Turkish donkey populations (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 in all the Turkish donkey populations. Haplotype and nucleotide diversity values for D-loop were compared with different donkey breeds, Turkish donkey populations were found to be lesser than Ethiopian donkeys (H_D: 0.903 $^{\pm}$ 0.032; π_D: 0.020 $^{\pm}$ 0.003), reported by Kefena *et al*. (2014), Balkan donkeys (H_D: 0.982 $^{\pm}$ 0.002; π_D: 0.017 $^{\pm}$ 0.009) reported by Pérez-Pardal *et al.* (2014;2) and Chinese donkeys (H_D: 0.9055 $^{\pm}$ 0.017–0.9778 $^{\pm}$ 0.0540, π_D: 0.02265 $^{\pm}$ 0.00040–0.0285 $^{\pm}$ 0.0160) reported by (Chen *et al*., 2006). The present finding discovered high diversity of Hd = 0.87 but still lower than Ethiopian donkeys. Results were obtained for Brazilian donkeys with the values of Hd = 0.879 which is slightly similar with the present findings. Negative Tajima D was observed which implies that the populations are experiencing sudden expansion or purifying selection (Tajima, 1996).

Conclusions

At the molecular level, Borno and Yobe donkeys are highly polymorphic compared to donkeys from other locations of the world. The high haplotype diversity and low nucleotide diversity among the donkeys in the study area is traceable to the difference in the wide geographical spread of the study area, environmental and genotype by environment interaction. It may also be as a results of rates of mutation, founder effect or sudden expansion of the population.

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