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Genetic Diversity in Growth Hormone Gene of Three Colour Type Populations of Camels in Yobe State

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Abstract
This research was conducted to investigate the genetic diversity of three camel colour type populations in Yobe State. A total of 100 camels of three distinct colour types (Grey White (GW), JA and Kurri (KR) from Yobe state were sampled for the study. Five samples from each of the populations were sequenced to examine molecular diversity parameters as well as the presence of single nucleotide polymorphism (SNPs). Generally, the result showed transition between the haplo-groups and the reference. Two SNPs (C→T transition) were observed at positions 062 and 232, respectively in the haplotypes. Highest genetic variability was observed in molecular indices among the GW color type. The analysis of molecular variance (AMOVA) revealed higher genetic variability within population than between populations. The neutrality test of population expansion revealed that the GW and KR were expanding while JA population was shrinking. The phylogenetic analysis showed that all the camel colour types shared same origin before divergence, with KR and JA being closer to each other than they were to GW.
Keywords: genetic diversity, growth hormone, gene, camels, colour types

Introduction
Dromedary camels are one of the best adapted animals of the desert and sustainable source of milk, meat, wool and transportation for livelihood in the environment. According to FAO (2019), the total population of dromedary camels in Nigeria is estimated at 289,794. These animals are found in the Northern part (Borno, Yobe, Jigawa, Kano, Katsina, Kebbi, Sokoto and Zamfara States) of (James-Ruga and Jidayi 2004; Mohammed and Hoffman, 2006). With over 80% spreads across Borno, Yobe, Katsina, Sokoto and Kano states, which are desert gateways with important camel trade (Timothy *et al*., 2015).

Growth hormone gene has proven to be the major regulator of postnatal growth and metabolism in mammals including camels and thus affects growth rate, body composition, health, milk production and aging by modulating (regulating) the expression of many genes (Carnicella *et al*., 2003). In livestock, growth hormone genes are of economic importance because they are often associated with faster growth and reduced fat stores (McMahon *et al*., 2001) which extends over 1900 bp.

Genetic diversity is the measure of genetic differences between animals in a population (i.e. genetic variation). Variability among individuals is a basic fact of biological life. The distribution of genetic diversity within and among populations is a function of the rate of gene flow between populations (Hemati *et al*., 2017). Afifi *et al*. (2014) observed that advances in molecular-genetic techniques have made it possible to identify differences between individuals at the DNA level. The aim of this study therefore was to determine population genetic characteristics and SNPs in three color type camels of Yobe State, Nigeria.

Materials and Methods
A total of one hundred camels comprising thirty three each of Kurri (brown black) and JA (dark brown) as well as thirty four Grey white (Fari), from Garin Alkali market in

Northeastern Nigeria were sampled for the study. Hair follicle samples used for molecular analysis were collected from the camel's tail by plucking them directly from the roots and then placed in a small, transparent, labeled container thereafter, for their cells. DNA extraction was carried out with Jena Bioscience Blood-Animal-Plant tissue preparation kit following the manufacturer's instructions.

PCR was conducted in 25µl reaction mixture consisted of 2.5 µl 10x buffer, 0.5 µl dNTPs, 0.2 µl High fidelity Taq polymerase, 1 µl (10 pmol) each of primer, 2 µl DNA template and 17.8 µl sterile nuclease free water. The primer (Table 1) was designed on the basis of DNA sequence of camel GH gene (Ishag *et al*., 2010). The amplification of DNA by PCR consisted of 37 cycles. The first cycle was characterized by pre-PCR denatuaration at 94°C for 2 minutes, annealing at 56°C for 30seconds, extension at 72°C for 40seconds. The next 36 cycles involved denaturation at 94°C for 1 minute, annealing at 56°C for 30 seconds and extension at 72°C for 40 seconds and a final extension at 72°C for 10 minutes.

Sequencing and neutrality test

The purified products (5 samples from each color type) were sequenced using a Big Dye terminator v 1.1 Ready Reaction cycle sequencing kit (Gordon *et al*., 1998) and an ABI PRISM 3100 (Applied Biosystems). The results were analyzed with Chromas Pro and Blast 2.0 software. The sequences were aligned and the positions of the identified SNPs were numbered as per the complete camel reference sequence of accession JX891650. Analysis of molecular variance

Table 1. The primer sequences, location and size of the amplified fragments

Name	Annealing temperature (°C)	Product size (bp)	Sequence (5'-3')	GeneBank Accession No.
KGH1B up	56	508	cagggaccaattccaggat	JX891650
KGH1B low	56	508	ccatccctgaggagcttaca	

(AMOVA), Tajima D, Fu's test and Harpending Raggedness, test of neutrality were determined using Arlequin V3.5. Software (Excoffier and Lischer, 2010). Neighbor-Joining (NJ) tree for camel color types sequences and phylogenetic tree were constructed using Mega Version 7.0 soft ware (Kumar and Tamura, 2016).

Results and Discussion

The alignment of the camel GH gene sequences for various color types and the reference gene JX891650 are shown in Table 2. The results revealed the presence of two SNPs (C→T) at 062 and 232 positions along the 508 bp GH gene. Haplo-group 1 had similar sequence as the reference with 2, 4 and 1 individuals from KR, JA and GW, respectively making a total of seven. The Haplo-group 2 had transition at position 062 with six individuals comprising KR (2), JA (1) and GW (3). However, the Haplo-group 3 showed transition at position 232 only in one individual from GW type. This observation is in line with those of Afifi *et al*. (2014) who reported two SNPs among four Saudi Arabian camels (Saheli, Majaheem, Waddah and Homor) breeds. However, one SNP (C→T) in different breeds had also been reported by some authors (Ishag *et al*., 2010; El-Aziem *et al*., 2015 and Shawki *et al*., 2015). Abdussamad *et al*. (2015) observed 14 polymophisms (13 transitions and one transversion). In this study, two SNPs (transitions) were detected. This is consistent with the report Othman *et al*. (2017) who found two SNPs in six of Egyptian camels. The findings in this study were slightly above

the report of one SNP in Sudanese camels (Ishag *et al*., 2010). While Affifi *et al*. (2014) and Abdussamad *et al*. (2015) found 14 and 13 SNPs, respectively in their works. Similary, Ming *et al*. (2016) also observed 17 SNPs among Bactrian camel breeds of Mongolia, Russia and China.

Table 2. Nucleotide polymorphism observed in camel GH gene

	02 63 22	Colour			N
		1	2	3	
Ref	CC				
Haplo group 1.	..	2	4	1	7
Haplo group 2.	.T	2	1	3	6
Haplo group 3	T.	-	-	1	1

Vertically oriented numbers indicate the variable sites position. Dots (.) indicate identity with the reference (Ref) sequence (GenBank accession number: JX891650), while different base letters denote substitution. 1: Kurri, 2: Ja, and 3: Grey white colour, and N: Number of individuals in each haplotype

The molecular diversity indices showed high genetic variability as presented in Table 3. The results generally showed that GW camels recorded higher values for all the parameters studied compared to the other camel types. The JA (dark brown camels) had the lowest values, while KR was intermediate between the two populations.

Regarding haplotype number, KR and JA had two (2) each while GW had three (3). Haplotypic (H_d) and nucleotide (Л) diversity parameters were highest in GW (0.70±0.22, 0.33±0.31) followed by KR (0.67±o.20, 0.22±0.25) and least among JA (0.40±0.24, 0.13±0.17) color types indicating abundant genetic diversity in GW and KR populations. This finding is in line with observations of Abdussamad *et al*. (2015) in camels of Nigeria-Niger. They observed highest haplotypic and nucleotide diversity in Kurri (H_d=0.897, Л=0.004) followed by GW (H_d=0.700, Л=0.003), Sand brown (H_d=0.857, Л=0.003) and least in JA (H_d=0.476, Л=0.001) populations. According to Ayied *et al*. (2018) high haplotypic and nucleotide (H_d=0.648, Л=0.0017) diversity was observed in Hurra breed in camel of Iraq but zero values (H_d=0.00, Л=0.00) were recorded for Khwar breed; an indication of absence of polymorphic site in Khwar. This was similar to the report in Maghrabi and Baladi breeds of Egypt (Othman *et al*., 2017). The higher theta value based on pairwise difference (θ_π), expected heterozygosity (θ_H), number of alleles (θ_k) and number of segregation sites (θ_s) in the population of GW compared with any of the other colour types (KR and JA) confirms its higher diversity. Similarly, the higher percent of the base thymine (T) and lower cytosine (C) in GW population compared with others could be attributed to its higher haplotypes and transitions.

The results of the analysis of molecular variance between and within color types, showed that genetic variance between and within populations were -4.35% and 104.35%, respectively (Table 4). It implies that the variability was much greater within than between population. By implication there is no gene flow between the populations. This is in line with the report of Abdussamad *et al*. (2015) who observed 99.74% variations among individuals within respective populations. The high within population variability reported in this study may be due to same maternal origin.

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Table 3. Molecular diversity indices of Nigeria camel color types

Parameters	KR	JA	GW	Pooled
Sample size	4	5	5	14
Haplotypes	2	2	3	3
Gene diversity	0.67±0.20	0.40±0.24	0.70±0.22	0.60±0.08
Nucleotide diversity	0.22±0.25	0.13±0.17	0.33±0.31	0.22±0.20
Sum of square frequency	0.50	0.68	0.44	0.44
Observed transitions	1	1	2	2
Observed transversions	0	0	0	0
Number of substitutions	1	1	2	2
Observed indels	0	0	0	0
Number of polymorphic sites	1	1	2	2
Mean number of pairwise differences	0.67±0.63	0.40±0.44	1.00±0.80	0.67±0.55
θ_H	1.52±1.45	0.50±0.49	1.78±1.95	1.15±0.38
θ_k	0.88±0.18	0.69±0.15	2.23±0.53	0.86±0.24
θ_s	0.55±0.55	0.48±0.48	0.96±0.76	0.63±0.47
θ_π	0.67±0.75	0.40±0.51	1.00±0.93	0.67±0.61
C (%)	75.00	90.00	60.00	75.00
T (%)	25.00	10.00	40.00	25.00

Kurri, JA, and Grey White color. *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.*

The mismatch distribution (Table 5.) gives information on the demographic history of a population expansion or contraction. Mismatch observed mean and variance were both higher in GW (1.00, 0.67) and least in JA (0.40, 0.27) while that of KR was intermediate. From the results, GW and KR showed complete divergence (T=1.08), KR (T= 0.98) while JA (T= 0.57) with least divergencestill have the ancestral genes. Harpendings Raggedness index (r), a statistical measure for development of population size which indicates the population is stable shrinking or expanding, was higher in KR (0.56) compared to JA (0.20) and GW (0.11) indicates shrinkage or stationary KR population but expansion of JA and GW populations. This is similar to observation of Burger *et al*. (2013) who reported Harpending Raggedness index in wild (r=0.218) and domestic Bactrian camel (r =0.067), and concluded on theexpansion of the two populations due to their low r values. Similar findings were also observed by Ayied *et al*. (2018). In this study, negative Tajima observed in JA (-0.8165) and Fu's GW (-0.4754) values also confirmed that the two populations were expanding.

Table 4. AMOVA of different camel color types

Source of variation	d. f.	Sum of squares	Variance components	Percentage of variation	F_{ST}
Among populations	2	0.557	-0.0144	- 4.35	- 0.0435
Within Populations	11	3.800	0.3455	104.35	
Total	13	4.357	0.3311		

F_{ST}: Inbreeding coefficient

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This is in agreement with the observation made by Abdussamad *et al*. (2015) who reported a negative Tajima (-01.064) among camel of Nigeria-Niger corridor. Positive Tajima obtained in this study for KR (1.6329) and GW (0.2431) indicates a decrease in population and they are similar to positive Tajima 2.331 and 1.777 reported in domestic and wild Bactrian camels, respectively by Mohandesan *et al*. (2017).

The evolutionary relationship among the population was inferred using the Neighbour-joining method (NJ) as presented in Figure 1. The tree showed that the three populations of camel ecotypes originated from common ancestor before divergence, with KR closely Related to JA but both were far from GW.

Table 5. Mismatch distribution in different camel color types

Parameters	KR	JA	GW	Pooled
Mismatch observed mean	0.67	0.40	1.00	0.67
Mismatch observed variance	0.27	0.27	0.67	0.36
T	0.98	0.57	1.08	0.76
Θ	0.10	0.003	0.31	0.15
M	8191.81	9145.00	4551.66	3452.10
Sum of square deviation	0.09	0.01	0.01	0.04
P (Sim. SSD > = Obs. SSD)	0.00	0.02	0.35	0.01
Harpending's Raggedness index	0.56	0.20	0.11	0.25
P (Sim. Rag. > = Obs. Rag.)	1.00 ns	0.86 ns	0.66 ns	0.21 ns
Tajima's D	1.6329	-0.8165	0.2431	0.1787
P (Sim. D < Obs. D)	0.968 ns	0.305 ns	0.668 ns	0.594 ns
Fu's Fs	0.5400	0.0902	-0.4754	0.0554
P (Sim. Fs < Obs. Fs)	0.499	0.302	0.227	0.412

Brown black (KR), Brown black (JA), and Grey white (GW) color. T: time of expansion; θ: mutation parameters

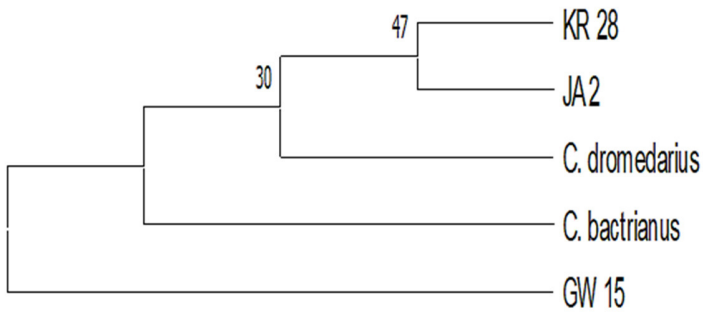

Figure. 1 phylogenetic tree

Conclusion

The sequence analysis revealed a transition in two SNPs in the direction of C→T and within population variability (100%) was observed. Higher genetic variability was observed in GW camels for all molecular parameter indices. The neutrality test of population expansion showed that the GW and KR were expanding while JA was shrinking.

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