

Original Research Paper

Genetic diversity and origin of Namchi cattle breed inferred by matrilineage analyses

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Abstract:

In animal genetic studies, mitochondrial DNA (mtDNA) analysis offers a way to detect changes in populations over time along with their maternal origin and migration routes. To determine these patterns in Namchi cattle breeds of Cameroon, analyses of 17 sequences of a 336 base pair fragment of the mtDNA D-loop region from two populations (Namchi Poli and Namchi Ngaoundere) were conducted in conjunction with previously published sequences from African, European, Japanese and Chinese subjects. As expected for an African taurine breed, all individuals in the study were found to be members of haplogroup T1. Due to the size of fragment analyzed, only one sub-haplogroup (T1c) could be detected, at a rate of 5.88%. This suggested that The Namchi cattle breed originated most probably from south west Asia and migrated to Cameroon through the Isthmus of Suez to Egypt and through Arabia to Somalia and Ethiopia. A relatively high nucleotide diversity (π) was found in Namchi Poli population (0.03421 ± 0.00582) while in Namchi Ngaoundere a value of 0.00980 ± 0.00185 was obtained, for a total nucleotide diversity of 0.01387 ± 0.00234 . The high genetic diversity observed in Namchi cattle breed reflects not only the presence of sub-haplogroups T1 but also an introgression from hybridization. This study provides new information regarding the sub-haplogroups of the Namchi cattle, their origin and migration routes and their genetic diversity which seems to have been affected by gene flow with zebus like White Fulani or Red Fulani. To facilitate the preservation and the conservation of the Namchi cattle breed in Cameroon, it is therefore important to adopt effective breeding management practices.

Keywords: diversity; haplogroup; mitochondrial DNA; Namchi; Cameroon.

Introduction

Genetic diversity provides the raw material for breed improvement and for the adaptation of livestock populations to changing environments and changing demands (FAO, 2015). During the past decades, analysis of mitochondrial DNA (mtDNA) has become the mainstay of molecular-genetic investigations of animal population diversity and history (Groeneveld et al., 2010). For several livestock species, mtDNA analyses established the ancestral species and contributed to evidence for the localisation of domestication sites (Zeder et al., 2006) and migration routes (Groeneveld et al., 2010).

The mitochondrial DNA (noted mtDNA) was also considered to be the effect genetic marker for the phylogenetic evolution and the genetic diversity among populations and species, because of its maternal inheritance, rapid evolution, less selection pressure, great genetic variance and the absence of recombination (Li et al., 2010). Nowadays, many animal genetic diversity researches are using the mtDNA as molecular tool.

In Cameroon, domestic animal genetic resources were found to present a genetic diversity affected by gene flow between breeds (Keambou et al., 2014; Meutchieye et al., 2014; Ngono Ema et al., 2014). Specifically, among the *Bos taurus* cattle breeds of Cameroon, the Namchi has shown a genetic diversity largely influenced by inbreeding with *Bos indicus* cattle breeds like White fulani or Red Fulani (Ibeagha-Awemu et al., 2006; Ngono Ema et al., 2014).

In this survey, the mtDNA D-loop sequences from two Namchi cattle populations were amplified, sequenced, and analysed to reveal the diversity, origin and evolution of this breed. The conclusion could offer basic material for their origin and genetic differentiations and provided theoretic basis for the reasonable taxon, conservation, development and utilization of the Namchi cattle breed.

Material and Methods

Breed description

The Namchi is a trypanotolerant short horn cattle breed (Achikwi et al., 1997) almost exclusively found in the Soudano-sahelian area of Cameroon, within the Poli Mountains, in the Faro division of the North region. They are mainly used for feast, rituals and draught activities (Ebangui, 2002). This breed is considered as endangered (Thys & Wandji, 1994; Ebangi et al., 2002). Namchi displays a high coat color polymorphism (Fig. 1).

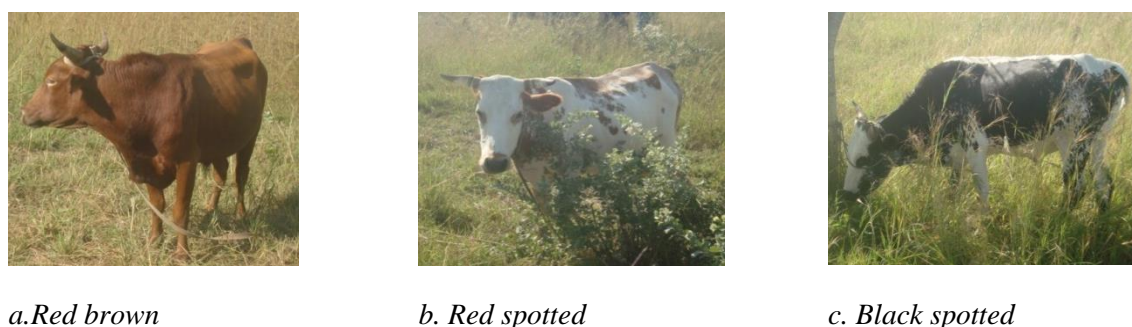


Figure 1. Coat color polymorphism in Cameroon Namchi cattle

Sampling

A total of 17 blood samples were collected on FTA® cards from Namchi cattle breeds in Cameroon. According to their spatial locations Namchi cattle breeds were divided into two populations: Namchi Poli (NP) in the North region of Cameroon and Namchi Ngaoundere (NW) in the Adamaoua region. Individual animals were chosen based on the knowledge of local herdsman, to ensure that they were not closely related. Complete D-loop sequences from 23 DNA Taurine samples from Africa (n=2), Europe (n=10) and Asia (n=11) were retrieved from GenBank under the accession No L27712-L27735, UB7636-UB7640 and AY521077-AY521082.

Amplification and sequencing

Genomic DNA was extracted by a modified protocol of Smith and Bourgoyne (2004). The complete D-loop was amplified by using forward primer L15737:5'-CTGCAGTCTCACCATCAACC-3' (Loftus et al., 1994) and reverse primer H992:5'-GATTATAGAACAGGCTCCTC-3'. These primers derived from the mitochondrial DNA (mtDNA) complete sequence of *Bos taurus* (Anderson et al., 1982). Both primers were used for sequencing. Amplification and PCR products purification were done according to the method described by Ngono Ema et al. (2014). PCR products were directly sequenced by using Big Dye Terminator v3.1 Cycle Sequencing kit (ABI) on an ABI prism 3100 DNA Sequencer according to the manufacturer's manual.

Data analysis

Sequence alignments were performed using the MUSCLE package in MEGA v. 5.03 (Tamura et al. 2011). Variations in the D-loop region were defined by direct comparison with the reference bovine

mtDNA sequence (Accession No.V00654, Anderson et al.(1982)).Gaps in the aligned sequences were excluded from the analyses.

We followed the nomination system of the phylogenetic clades described by Bonfiglio et al. (2012) to analyze *Bos taurus* mtDNA. To demonstrate the phylogenetic clusters, we first constructed an unrooted neighbor-joining tree by using the Kimura 2-parameter model included in MEGA v. 5.03 (Tamura et al. ,2011) and then used the median-joining network (Bandelt et al.,1995) by employing program Network 5.0(<http://www.fluxus-engineering.com/sharenet.htm>) to detect whether the Namchi's clade would undergo possible population expansion we empirically made a judgement by looking at the mismatch distribution and performing the Fs test of Fu (Fu,1997).The mismatch distribution was performed in Network v. 5.0 and the Fs test was estimated by using DnaSP v5 (<http://www.ub.edu/dnasp>). Possible demographic changes in Namchi population was also investigated with Tajima's test with DnaSP v5 (Librado and Rozas, 2009).To estimate the age of the phylogenetic clade generated from Namchi , we computed the nucleotide diversity π by using DnaSP v5,then the divergent rate for partial mtDNA control region,62.81% per site per million years (Bradley et al.,1996), was used to convert it into years (Nei,1987).The number of polymorphic sites, haplodiversity, nucleotide diversity and average number of nucleotide differences were also estimated using Dnasp v5.

Results

Variation in the mtDNA D-loop of Namchi cattle Breed in Cameroon

D-loop analyses in 17 sequences of Cameroonian native Namchi cattle showed a total of 22 variable sites characterizing 15 haplotypes (Figure.1). These polymorphisms included 23 mutations in total by comparison with the bovine reference sequence (Anderson et al., 1982).

	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	5	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6
	9	9	9	9	9	9	9	9	9	9	0	0	0	0	1	1	1	1	1	2	2
	4	4	4	4	5	5	5	6	6	7	1	5	5	8	1	2	2	3	3	3	4
	5	6	8	9	2	4	6	1	9	4	4	0	7	8	3	2	6	1	8	4	7
RS	A	T	A	C	A	G	A	G	C	C	A	C	G	A	T	T	T	T	T	C	C
NP06	T	.	.	T	T	.	G	.	T	G	.	T	.	.	C	.	.	C	C	.	T
NP07	G	.	T	T	T	A	.	C	C
NP10	T	T	.	.	.	T	C
NP11	A	T	T	.	G	C	C
NP12	.	.	C	T	.	.	.	T	.	.	C	C
NW01	T	.	.	C	C	C
NW02	C	C	.	T	.	.	G	G	.	C	.	T	C
NW03	T	T	A	.	C	.	C	.	T	.	C
NW04	T	.	.	C	.	C	.	.	.	C
NW05	T	A	.	C	.	C	.	.	.	C
NW06	T	T	A	.	C	.	C	.	.	.	C
NW07	T	A	.	C	.	C	.	.	.	C
NW08	T	.	.	.	T	A	.	C	.	C	.	.	.	C
NW09	T	.	.	.	T	.	.	C	G	C
NW10	T	T	.	.	.	T	A	.	C	.	C	.	.	.	C
NW11	T	.	.	T	T	.	.	C	.	C	.	.	.	C
NW12	T	C

Figure.1: Variable sites in mtDNA D-loop of haplotypes of Namchi Cattle Breed . The corresponding haplogroups are found in the right column. Mutations are scored relative to the reference sequence (RS) of Anderson et al.1982. Dots (.) denotes identity with the reference sequence.

The three T1 transitions at 16255, 16113 and 16050 from T3 (Troy et al., 2001; Mannen et al., 2004, Bonfiglio et al., 2012) were detected in 14 out of 17 sequences from Namchi cattle breed in Cameroon (figure 1). Three sequences were scored T1 with a 16113C independent back mutation events (NP10, NW02, NW12). Furthermore, according to Bonfiglio et al. (2012), NW01 belonged to the sub-haplogroup T1c. This suggests a Near East original domestication of the Namchi cattle breed followed by an expansion along the Neolithic migration route of human populations (Bonfiglio, 2012).

In order to assess the relationships among the Namchi cattle breed of Cameroon, Ndama cattle breed of West Africa, European, Japanese and Chinese taurines, two types of phylogenetic trees were constructed using individual sequences: a neighbor-joining tree and a Median-joining tree.

The major feature showed by the neighbor-joining tree (Figure 2) is a clear separation between 3 principal haplogroups: African taurines (T1), Japanese taurines (T4) and European and Chinese taurines (T3).

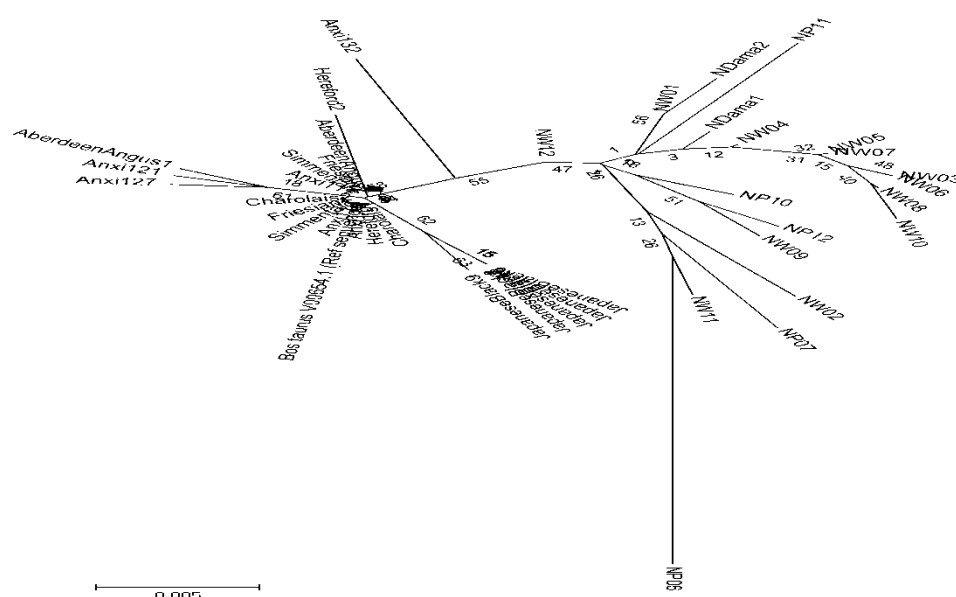


Figure 2: Unrooted neighbor-joining tree constructed from mtDNA belonging to Namchi cattle breed from Cameroon, Ndama cattle breed, European, Japanese and Chinese taurines.

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The median-joining network confirmed the separation between the Namchi cattle breed and the others *Bos taurus* breeds from Europe, Japan and China. Even the Ndama cattle which also belonged to the T1 haplogroup was clearly segregating from the Namchi.

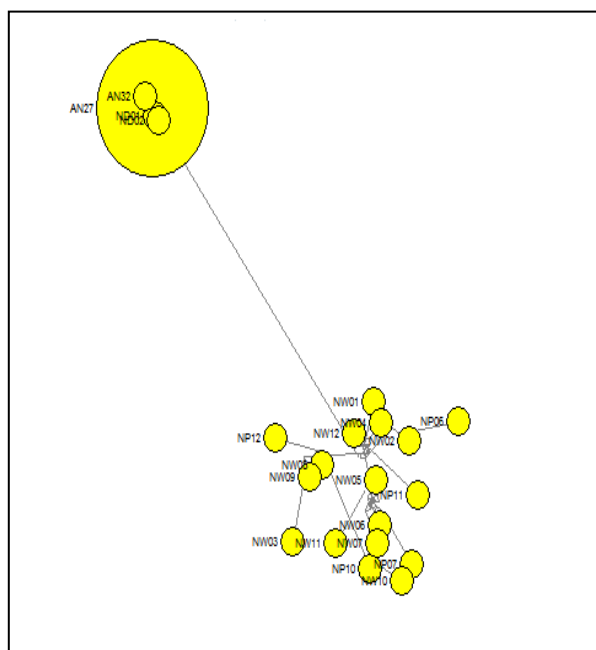


Figure. 3: Median-joining network after star contraction of Namchi cattle breed in Cameroon and other *Bos taurus* mitochondrial DNA sequences from Europe, Japan, China and Africa. *Note: Circles are proportional to the frequency of the haplotypes.*

Population expansion

Mismatch distribution showed a multimodal distribution (Figure.4). This revealed genetic signature consistent with past population expansion in Namchi cattle breed (Rogers et al., 1996). The multimodal shape would be explained by diverged sublineages and highly variation within the T1 haplogroup (Mannen et al., 2004).

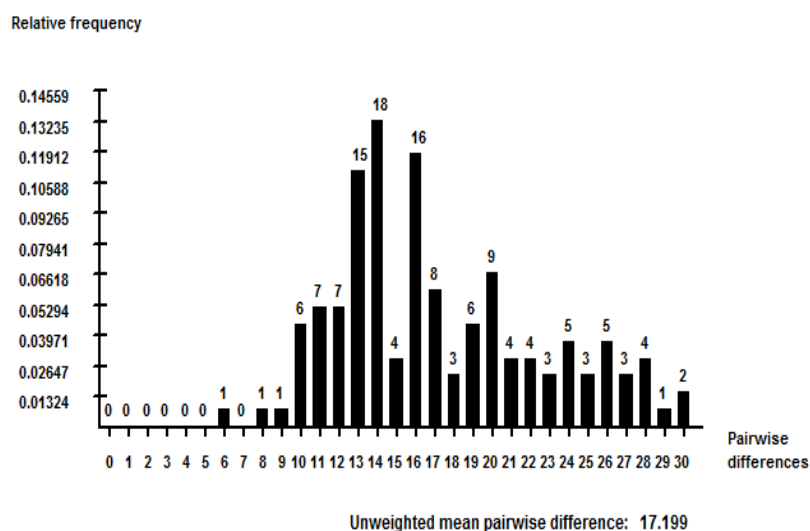


Figure.4: Mismatch distribution within Namchi mtDNA lineage

Genetic diversity of the Namchi cattle Breed

As shown in Table 1, the genetic diversity of the Namchi cattle populations varied a lot. The Namchi Poli presented a very high haplotype diversity ($H_d=1.000$) and a high nucleotide diversity

($\pi=0.03421$). In all the populations, the Tajima's D and the Fs tests appeared to be negative and non-significant ($P>0.10$).

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Table 1. Age estimation and genetic diversity of the Namchi cattle breed in Cameroon

Population	N	h	Hd \pm SD	K	S	$\pi\pm$ SD	Age ^a (10 ³ y)	D	Fs test
NP	05	05	1.000 \pm 0.126	11.700	10	0.03421 \pm 0.00582	54.5 \pm 9.3	-1.36345	-0.134
NW	12	10	0.970 \pm 0.044	3.303	16	0.00980 \pm 0.00185	15.6 \pm 2.9	-0.38780	-5.387
Total	17	15	0.985 \pm 0.025	4.662	26	0.01387 \pm 0.00234	22.1 \pm 3.7	-1.25962	-9.450

Note. All the estimation were based on 336bp fragment (relative to region 15819–16285 in the reference sequence [Accession No. L27733; Loftus et al., 1994]). ^a A divergence rate (62.81% site per million year [Myr])(Bradley et al., 1996) was used to convert the nucleotide diversity into years according to Nei (1987). c According to Fu (1997) and $P < 0.05$ was regarded as statistically significant. N is number of individuals sampled; h is the number of haplotypes; Hd is the haplotype diversity; K is the average number of differences; S is the number of segregating sites (excluding indels); D is Tajima's D statistic ; π is the nucleotide diversity.

Discussion

The Namchi mtDNA sequences were found to belong to the T1 haplogroup. This finding is in good agreement with previous population analyses studies (Troy et al., 2001; Mannen et al., 2004; Achili et al., 2008) who demonstrated the almost exclusive occurrence of haplogroup T1 in Africa. According to Bonfiglio et al.(2012) nomenclature system, in order to determine all the six T1 sub-haplogroups, at least PCR fragments of 1138bp encompassing the control region (nps 15718-517) has to be sequenced. This is not the case in the present study where the analyses were done only in a 336 bp (15819-16285) of the D-loop region. Therefore, only the sub-haplogroup T1c could be detected among the Namchi mtDNA sequences. Hence there is a need to analyze more longer Namchi mtDNA sequences.

As suggested by the multimodal mismatch distribution, the Namchi cattle breed presents many sub-haplogroups in the Haplogroup T1. Six T1 sub-haplogroups have been highlighted by Bonfiglio et al. (2012), from T1a to T1f. Apart from T1e not yet reported in African taurines (Bonfiglio, 2012; Horsburgh, 2013), each of these sub-haplogroups is likely to be found in the Namchi cattle breed according to their geographical location (Bonfiglio et al., 2012; Lengstra et al., 2014). This overrepresentation of the T1 sub-haplogroups in Africa is not yet well understood (Lenstra, 2014).

The Namchi cattle breed originated most probably from south west Asia as suggested by their haplogroup (Bonfiglio et al.,2012;Lengstra et al.,2014;Magee et al.,2014).The presence of a T1c sequence even in a very low frequency (5.9 %) suggest two migratory roads for the Namchi: the first one from the north through the Isthmus of Suez to Egypt and the second one from the south through Arabia to Somalia and Ethiopia (Caramelli, 2006;Bonfiglio et al., 2012; Feliuss et al.,2014).

This inference shows that the nucleotide diversity in the Namchi samples are higher than those found in some other African taurines breeds like in South Africa ($0.000750 \pm .000385$), Egypt (0.00082 ± 0.000464) or Ethiopia (0.00094 ± 0.000524) according to Horsburgh et al. (2013). Genetic diversity can be modified by factors including natural selection, population size, mutation rates or gene flow between populations (Frankham et al., 2001). Furthermore, isolated populations (and it is the case for the Namchi) can present a high genetic diversity if they represent a fusion of clades or have interbred with other species (Zidana et al., 2009; Bay et al., 2011). Therefore, the high genetic diversity observed in Namchi cattle breed reflects not only the presence of sub-haplogroups T1 but also an introgression from hybridization like recently demonstrated by Ngono Ema et al. (2014) in a study on molecular characterisation of Namchi cattle breed using microsatellites.

Tajima's D test (Tajima, 1989) and Fu Fs test (Fu, 1997) have been developed to detect departure of DNA sequence variability from the expectations of the neutral theory of evolution (Kimura,1983.). Both tests showed negative but not significant values ($P>0.10$). This could have implied a presence of rare alleles at high frequencies or a signature of a past population expansion (Simonsen et al.,1995).

Conclusion

This study found that the Namchi cattle breed of Cameroon had high genetic diversity probably due to the presence of sub-haplogroups T1 but also an introgression from hybridization with *Bos indicus* breeds like White Fulani and Red Fulani. The presence of sub-haplogroup T1c was confirmed but whole-mtDNA sequencing may detect additional sub-haplogroups informative for movements of cattle between or within continents and expansion of populations (Bonfiglio, 2012; Horsburgh, 2013). The Namchi cattle breed originated most probably from south west Asia and migrated to Cameroon through two migratory roads: the first one from the north through the Isthmus of Suez to Egypt and the second one from the south through Arabia to Somalia and Ethiopia (Caramelli, 2006; Bonfiglio et al., 2012; Felius et al., 2014). Further research on the genetic diversity and origin of the Namchi cattle breed is required to determine the paternal origin of these breed by using Y-chromosome as molecular basis.

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Author's Contributions

Ngono Ema designed the whole study; Meutchieye and Manjeli contributed to data collection, data analyses and overall write-up of the paper; Keambou contributed to further analyses and paper write-up.

Ethics

Authors declare that there are ethical issues that may arise after the publication of this manuscript.

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