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Nucleotide Variation and Selective Pressure in the Mitochondrial Genome of African Elephants

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Abstract: ***Among the large mammals of Africa, elephants are probably the worst affected by human activities. Although they have been listed as endangered and protected since 1989, illegal poaching and habitat destruction continue to diminish and isolate remaining populations that are dispersed widely over 37 sub-Saharan African countries. Their populations currently exist in small isolated habitat, and this threatens elephant genetic diversity. Although literature is available on the taxonomy and phylogeny of African elephant, few studies have focused on codon usage on mitochondrial genomes. To analyze nucleotide diversity, selective pressure and demographic history of African elephants, we used the portion of mitochondrial sequences of 102 individuals available in the genome database. Our data indicated a low codon bias index (CBI) and a relatively high effective number of codons (ENC) value in the mitochondrial genome, suggesting that African elephants are less biased in their codon usage preference. The data also support a strong purifying selection in the mitochondrial genome of African elephants. However, few sites are under positive selection in the mitochondrial genome of African elephants, with Loxodonta africana presenting more sites under positive selection compared to L. cyclotis. The present work supports the idea that different evolutionary rate among nucleotide sites in L. africana and L. cyclotis, attributable to differences in the frequency of positive selection and probably different environmental conditions, are the driving forces for the codon usage bias in African elephants. Further studies are needed to investigate the contribution of different subpopulation in the genetic structure and diversity of African elephants.***

Keywords: African elephant, *Loxodonta africana*, *Loxodonta cyclotis*, codon usage biais, selective pressure, mitochondrial DNA

1. Introduction

Africa is home to at least 400,000 elephants (Blanc *et al*., 2003). They occur in a wide variety of habitat, from tropical swamp forests to deserts (Blanc *et al*., 2007). A number of genetic studies have suggested that the previously recognized subspecies of African elephant, namely the savannah elephant *Loxodonta africana africana* and the forest elephant *Loxodonta africana cyclotis*, may, in fact, constitute two separate species, namely *Loxodonta africana* (Blumenbach 1797) and *Loxodonta cyclotis* (Matschie 1900) respectively (Comstock *et al*., 2002 ; Roca and O'Brien, 2005). In addition, the existence of a third species, a West African elephant inhabiting both forests and savannah in that region, has also been postulated (Eggert *et al*., 2002).
There is still no consensus in the scientific community as to the number of species of elephant currently extent in Africa (Debruyne *et al*., 2003; Debruyne, 2005).

Among the large mammals of Africa, elephants are probably the worst affected by human activities. Although African elephants have been listed as endangered and protected since 1989, illegal poaching and habitat destruction continue to diminish and isolate remaining populations that are dispersed widely over 37 sub-Saharan African countries (Said *et al*., 1995; Barnes *et al*., 1999). Their populations currently exist in small isolated pockets of habitat, and this threatens elephant genetic diversity (Georgiadis *et al*., 1994).

Because they live in matriarchal societies, the DNA (mtDNA) genealogies in both African (Roca *et al*., 2005; Lei *et al*., 2008) and Asian elephants (Vidya *et al*., 2009) exhibit deeper divergence and/or different phylogeographic patterns than the nuclear genome. Mitochondrial DNA has been the most widely used tool for reconstructing population and species histories. Its mutation rate is high on average and, additionally, appears variable between animal lineages, as indicated by evolutionary analyses (Martin and Palumbi, 1993; Rand, 1994; Mindell *et al*., 1996). Several studies have been conducted to determine the nucleotide differences between forest and savanna elephants used mitochondrial sequence (Brandt *et al*., 2012), nuclear sequences (Ishida *et al*., 2011) or microsatellite data (Roca *et al*., 2001). Although literature is available on the taxonomy and phylogeny of African elephant, few studies have focused study of codon usage on mitochondrial genomes. Finch *et al*. (2014) studied the adaptive evolution of the mitochondrial genome by combining phylogenetic and protein prediction methods to better understand the structural biology of the OXPHOS pathway in the African elephant.

Here we present data on the nucleotide diversity, selective pressure and demographic history of African elephants using the portion mitochondrial genome sequences of 102 individuals available in the genome database using codon usage analysis and mismatch distribution. Understanding the genetic diversity and affinities of this population, and determining the effects selective pressure, can contribute to scientifically sound conservation practices to ensure their long-term persistence.

2. Methods

2.1 Sequence data collection

We extracted from National Center for Biotechnology Information/GenBank the African elephant mitochondrial

sequences. Accession number of the sequences is available as a supplementary material (Annex A). The mitochondrial sequences of African forest elephant (*Loxodonta cyclotis*) and savanna elephant (*Loxodonta africana*) include NADH dehydrogenase subunit 5 (ND5) gene, partial cds; NADH dehydrogenase subunit 6 (ND6) gene, complete cds; tRNA-Glu gene, cytochrome b (CYTB) gene, tRNA-Thr and tRNA-Pro genes, and D-loop sequence. The final set included the 102 sequences originating from 12 African countries: Central African Republic, Zimbabwé, Republic of the Congo, Namibia, Gabon, Botswana, Botswana, South Africa, Tanzania, Kenya, Cameroon, Sierra Leone and Côte d'Ivoire.

The sequences were first aligned using the ClustalX program (Thompson *et al*., 1997). All sites with deletions and insertions were then excluded in order to preserve the reading frames of the genes. The final alignment was 4257 bp long and is presented as supplementary information.

2.2 Nucleotide diversity

DnaSP v.5 software (Librado and Rozas, 2009) was used for quantifying genetic polymorphism by calculating the number of haplotypes (n), haplotype diversity (h), and nucleotide diversity (π). Pairwise comparisons were performed to estimate interspecific divergence (Dxy; average number of nucleotide substitutions per site between species) and intraspecific nucleotide diversity (Pi) using DnaSP v.5 program.

2.3 Codon usage analyses

The Relative Synonymous Codon Usage (RSCU) values were calculated for the dataset. The RSCU statistics is calculated by dividing the observed usage of a codon by that expected if all codons were used equally frequently. If RSCU value of a codon >1, that codon is frequently used than expected whereas RSCU value <1, means that the codon is less frequently used than expected. If RSCU equals 1, it means that the codon is used randomly and equally with other synonymous codons (Sharp *et al*., 1986). If the RSCU value is <0.6, the codon is under-represented and if the RSCU value of a codon is >1.6, the codon is over-represented (Gupta et *al*., 2001). RSCU analysis was conducted using Mega 6 software (Tamura *et al*., 2013).

Parameters related to codon usage bias, such as the codon bias index (Morton, 1993), the effective number of codons (Wright, 1990), and G + C content at second and third positions as well as overall were estimated for savannah elephant and forest elephant mitochondrial protein coding genes using DnaSP v.5 (Librado and Rozas, 2009). Furthermore, to determine whether the compositional changes of the nucleotide content in the mitochondrial protein-coding genes are caused by directional mutation pressure or a result of positive selection, we performed a correlation analysis. The correlation analysis of GC content at the second codon position (nonsynonymous mutations related, GC2) and GC content at third position (synonymous mutations related, GCs3) with CG content of all protein codon genes (GCc) were implemented using SPSS 17.0 (SPSS, 2009).

2.4 Demographic history and population expansion

To test for recent demographic expansion in each elephant species, Tajima's D (Tajima, 1989) and Fu's FS (Fu, 1997) were calculated in DnaSP v.5 (Librado and Rozas 2009) with 16,000 permutations. Negative results of these tests are indicative of a population that has undergone a recent expansion, as rare alleles are more common than expected. These metrics are expected to have significantly negative values if the populations are not in equilibrium between mutation and drift, e.g., because of demographic expansion. These negative values can arise under selective effects, but they can also be indicative of population expansion or bottlenecks (Tajima, 1993; 1996). To further investigate demographic history, we analysed the distribution of pairwise differences (mismatch distribution) for all sequences using the same program. Mismatch distribution analysis (Schneider and Excoffier, 1999) was also used to analyse if expansion occurred in the analysed populations. In addition, Harpendings reggedness index (Harpending, 1994) was calculated for any deviation from population expansion model. Mismatch distributions showing the pattern of nucleotide (or restriction) site differences between pairs of individuals in a sample (Rogers *et al*., 1996) were tested against a model of sudden population expansion with a bootstrap resampling procedure (Schneider and Excoffier, 1999). A population that is at equilibrium is expected to have a multimodal mismatch distribution, whereas populations that have experienced recent growth should have a unimodal mismatch distribution (Slatkin and Hudson, 1991: Rogers and Harpending, 1992).

2.5 Analysis of selective pressures

The Non-synonymous/synonymous substitution ratios (ω = dN/dS, also called Ka/Ks) was used for quantifying the impact of natural selection on molecular evolution (Ohta, 1992). The ratio of the number of nonsynonymous substitutions per nonsynonymous sites (dN) to the number of synonymous substitutions per synonymous sites (dS) indicates the level of selection against nonsynonymous substitutions relative to synonymous ones. dN/dS distributions in pairwise comparisons between DNA sequences were calculated (Nei and Gojobori, 1986) using DnaSP. dN/dS > 1 indicates positive selection, dN/dS < 1 negative (purifying) selection, and dN/dS = 1 neutrality. HyPhy software (Pond *et al*., 2005) was used to generate simulated data under a neutral model with trees generated from the original alignments. The same sequence alignments used as input in the initial analysis were used and one hundred simulated datasets were generated for each alignment. Each simulated dataset was then analyzed using the Dual Model. The minimum value of mean dS across all sliding windows of three adjacent codons, in all of the one hundred simulated datasets, was used as a conservative threshold to identify windows of reduced dS in the observed data. This stringent threshold and a less stringent one that included 95% of the values inferred

from the simulated data are shown in the sliding window plots.

3. Results

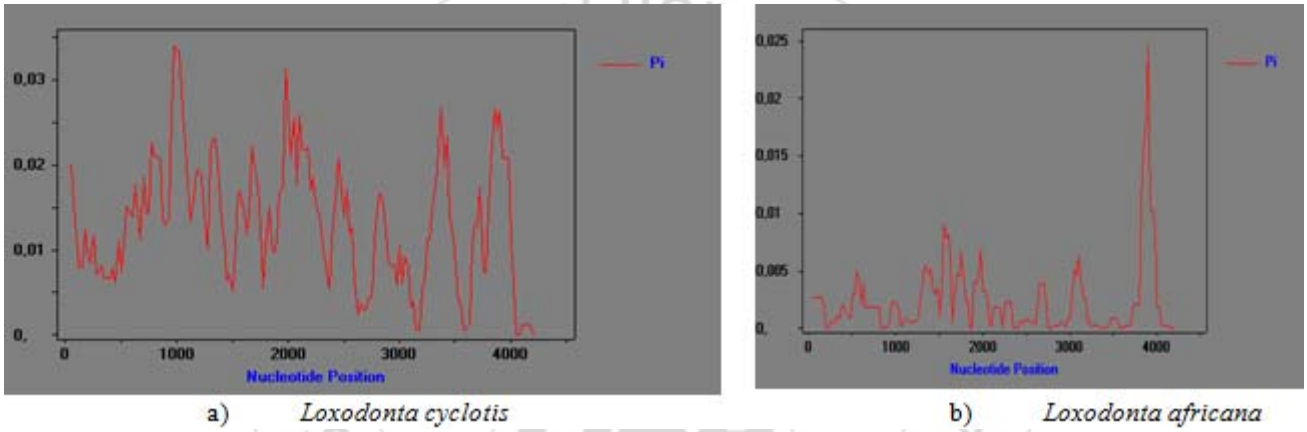
3.1. Patterns of nucleotide change and genetic diversity

The nucleotide composition of the mitochondrial sequences analysed was similar in *Loxodonta africana* and in *Loxodonta cyclotis*: T(U): 27.7%, C: 27.5%, A: 33.2%, G: 11.5% for *L. africana* and T(U): 27.8%, C: 27.6%, A: 33.5%, G: 11.2% for *L. cyclotis*.

We measured nucleotide diversity for each species using p (Nei and Li, 1979; Tajima, 1983), a standard estimator of the population mutation rate (Table 1). The nucleotide diversity observed in *L. cyclotis* (1.19%) is five time higher than the nucleotide diversity in *L. africana* (0.22%). Both the taxa presented similar haplotype diversity: 0.99 and 0.97, respectively for *L. africana* and *L. cyclotis*. The gene region having the highest π value was found between nucleotides 500 to 2,300 in *L. cyclotis* (Figure 1).

Table 1: Results of neutrality tests (Fu's FS and Tajima's D) by region and the total data set

Species	N	Haplotype	Haplotype diversity	Pi (π)	D	Fu Fs	Raggedness index (p)
Loxodonta africana	68	40	0.987	0,0022	- 1.2150	-17.18	0.0346
Loxodonta cyclotis	27	17	0.972	0,0119	0.746	5.105	0.0203



a) *Loxodonta cyclotis* b) *Loxodonta africana*

Figure 1: Sliding window analyses of nucleotide diversity (π) along the portion of mitochondrial sequence of (a) *Loxodonta cyclotis* and (b) *Loxodonta africana*.

The red line shows the value of nucleotide diversity Pi (π) in a sliding window analysis of window size 3 00 bp with step size 10, and the value is inserted at its mid-point.

3.2. Codon usage

Twenty seven codons occurred more frequently versus 31 that occurred less frequently (overall RSCU value of frequently and less frequently used codons, Supplementary Materials) than expected in *Loxodonta africana*. For *L. cyclotis*, 29 codons occurred more frequently versus 30 occurred less frequently. Of the codons that occurred more frequently 25 are shared by both the species (86% for *L. africana* vs 93% for *L. cyclotis*) (Figure 2). For the codons that occurred less frequently 26 are shared by both the species (84% for *L. africana* vs 87% for *L. cyclotis*) (Figure 3). No significant difference was found between the frequencies of the shared codons (t test $p > 0.05$) between the two species.

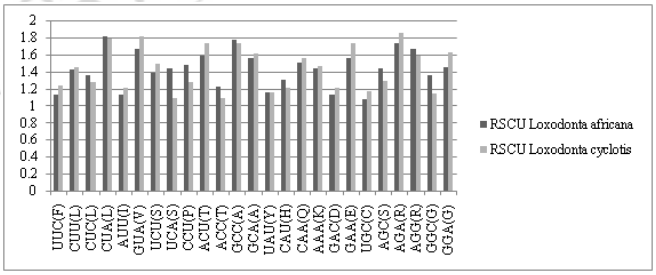


Figure 2: More frequently used codons than expected (RSCU>1) for the portion of mitochondrial DNA of African elephant

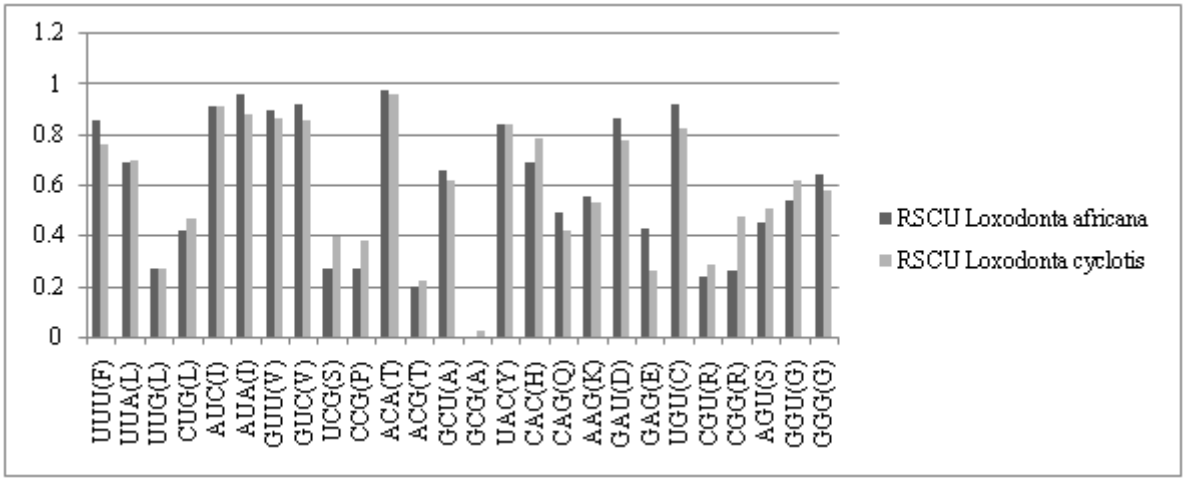


Figure 3: Less frequently used codons than expected (RSCU<1) for the portion of mitochondrial DNA of African elephant

The Codon Bias Index (CBI) is a measure of the deviation from the equal use of synonymous codons with values ranging from 0 (uniform use of synonymous codons) to 1 (maximum codon bias). CBI values were near low, respectively 0.227 and 0.238 for *L. africana* and *L. cyclotis* (Table 2). The Effective Number of Codons (ENC), which may range from 20 (only one codon is used for each amino acid; i.e., the codon bias is maximum) to 61 (all synonymous codons for each amino acid are equally used; i.e., no codon bias), were similar for the both species and approach 61: respectively 52.514 and 52.133 for *L. africana* and *L. cyclotis*. This indicated that the codon usage bias is not remarkable for mitochondrial sequence in these species. GC content at the second position (GC_2) is similar for both the species 39.80%, GC content at third codon position (GCs3) is also similar in both the species 40%. For *L. africana*, there was a highly significant correlation between the GC3s and GCc ($r = 0.436$, $P < 0.001$). However, the correlation between GC2 and GCc was not significant ($r = 0.191$, $P = 0.11$). For *L. cyclotis*, the correlation between the GC3s and GCc was not significant ($r = -0.036$, $P = 0.85$). However, the correlation between GC2 and GCc was significant ($r = 0.191$, $P < 0.001$). The correlation analyses indicate that the nucleotide bias affects synonymous sites in *L. africana* whereas nonsynonymous sites are affected in *L. cyclotis*.

The overall GC content range from 31.8% in tRNA-Thr to 41.2% in tRNA-Pro (Table 3). Within the 6 genes analysed, no significant difference were observed between *L. africana* and *L. cyclotis*.

Table 2: Summary of codon usage index of portion of mitochondrial genome of African elephants

Species	ENC	CBI	GC_2	GC_{3S}	GC_C	G+C:
Loxodonta africana	52,514	0,227	0,398	0,393	0,401	0,390
Loxodonta cyclotis	52,133	0,238	0,398	0,403	0,398	0,388

Table 3: Average GC content in the mitochondrial genome of African elephants

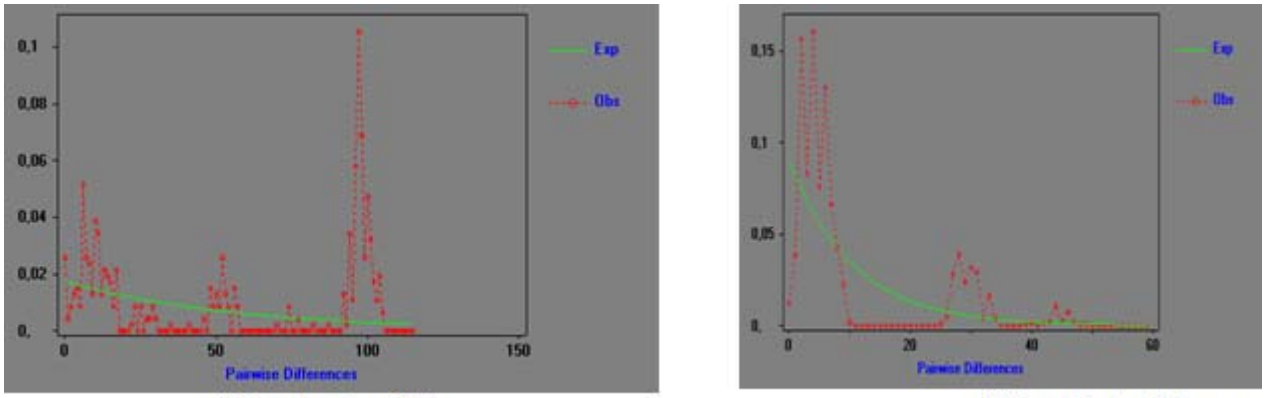
Gene	Size (bp)	GC content (%) *Loxodonta africana*	GC content (%) *Loxodonta cyclotis*
ND5	1798	38.4	38.0
ND6	527	38.3	37.5
tRNA-Glu	64	35.3	33.8
CYTB	1136	40.7	41.0
tRNA-Thr	65	33.3	31.8
tRNA-Pro	67	41.2	41.2
D-loop	589	39.6	39.2

3.3 Demographic history and population expansion

Both standard tests of neutrality were negative and not significant for *Loxodonta africana* : Tajima's $D = -1.22$, $p > 0.10$; Fu's $Fs = -17.18$, $p > 0.10$; a result indicating demographic change. For *L. cyclotis*, the neutrality tests were positives, in contrary to *L. africana*; however, they were also not significant: Tajima's $D = 0.746$, $p > 0.10$; Fu's $Fs = 5.105$, $p > 0.10$.

Multimodal mismatch distribution graphs were observed for either the species (Figure 4), which indicates stable population or a population in equilibrium.

However, non significant raggedness index did not supported population stability for both the African savannah elephant ($r = 0.035$, $p > 0.05$) and African forest elephant populations ($r = 0.020$, $p > 0.05$) (Table 1).



a) *Loxodonta cyclotis* b) *Loxodonta africana*

Figure 4: Mismatch distribution analysis for African elephants. Graphs of the mismatch distributions of (a) *Loxodonta cyclotis*; (b) *Loxodonta Africana*. The X axis shows the observed distribution of pairwise nucleotide differences, and the Y axis shows the frequencies. The dotted lines with circles represent the observed frequency of pairwise differences, and the solid lines show the expected values under the sudden population expansion model.

3.4 Analysis of selection pressure

The dN/dS ratio of the portion of mtDNA was less than 1 in both the species: 0.6 and 0.62, respectively for *Loxodonta africana* and *L. cyclotis*. A low dN/dS ratio indicates that, the sequences did not undergo adequate immune pressure to lead to changes in amino acids. The dN/dS ratio of *L. africana* showed no significant difference ($P < 0.022$) compared to *L. cyclotis*. The synonymous substitution rate was always significantly higher ($P = 0.001$, Student's t) than the nonsynonymous substitution rate.

These results indicate that these mitochondrial genome regions analyzed are subject to very strong purifying selection. The location of the midpoints of the window showing negative selection is given in Figures 5 and 6. Very few sites were under positive selection, with more sites in *L. africana* (4%) (Figure 5) compared to *L. cyclotis* (1%) (Figure 6).

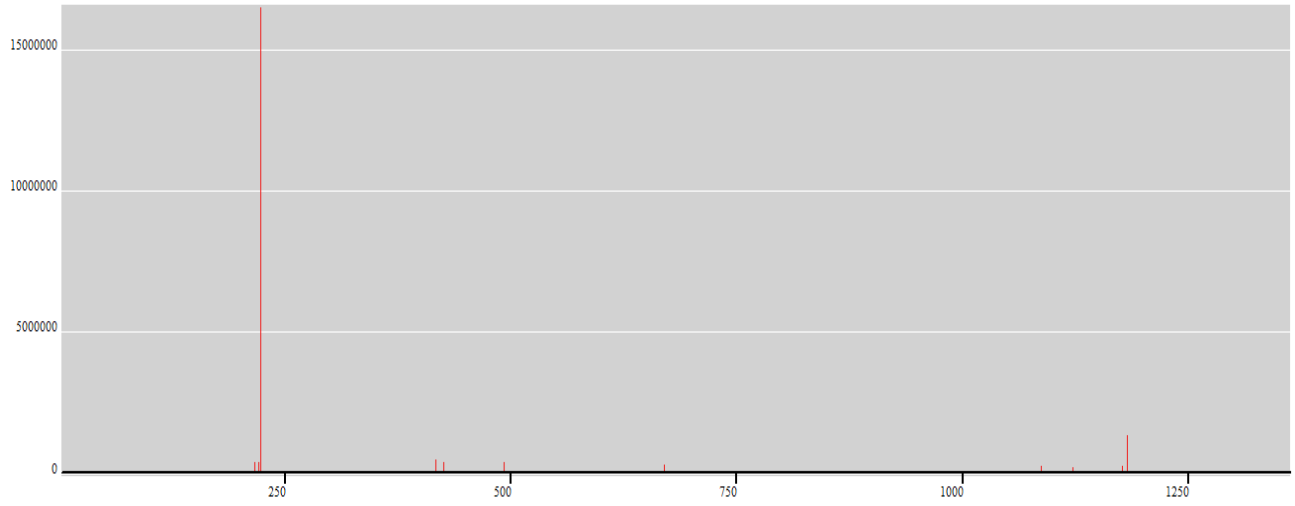


Figure 5: Sliding-window analysis of the cumulative dN/dS across Bayes factor for the event of positive selection at a site along the portion of mitochondrial DNA of *Loxodonta cyclotis*

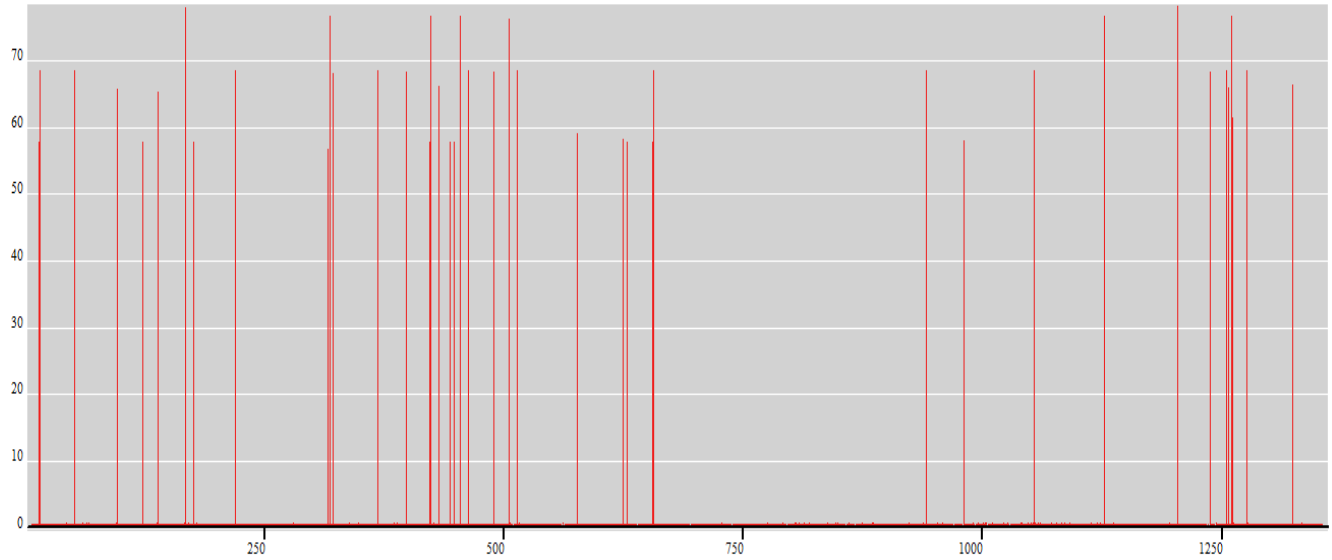


Figure 6: Sliding-window analysis of the cumulative dN/dS across Bayes factor for the event of positive selection at a site along the portion of mitochondrial DNA of *Loxodonta africana*.

4. Discussion

The nucleotide diversity observed in *Loxodonta cyclotis* (1.19%) is five time higher than the nucleotide diversity in *L. africana* (0.22%). This adds further evidence supporting species level distinction for the two African groups (Comstock *et al*., 2002). Using microsatellite data, Comstock *et al*. (2002) found that genetic divergence distances computed between forest and savannah elephant populations are almost great as the difference between Asian and African genera (forest vs savannah Fst = 0.243 microsatellite data, Comstock *et al*., 2002). This suggests forest elephant populations may be completely or almost completely isolated from gene flow from savanna elephant populations (Rohland *et al*., 2010). High genetic diversity between the two species may reflect the biogeographical history of the subspecies. However, regards the actual threat affecting African elephants, the loss of this diversity is ineluctable. With their small size, African elephant populations are genetically isolated and are at risk losing genetic variation (Blanc *et al*., 2003). Loss of genetic diversity can reduce the ability of populations to adapt to environmental change, as well as reducing reproductive fitness and disease resistance (Reed and Frankham, 2003). Therefore, the maintenance of genetic diversity of these populations should be a priority for their long-term conservation.

Our data indicated a low codon bias index (CBI) and a relatively high effective number of codons (ENC) value in African elephant's mitochondrial genome. The relatively high ENC value suggests that African elephants are less biased in their codon usage preference. The low G+C content and GC3s reveal that mutational pressure is the mild factor that contributes to the less biased synonymous codon usage pattern in African elephants.

The results suggested that compositional constraint was one of the major factors in shaping codon usage in the genomes. The correlation analyses indicate that the nucleotide bias affects synonymous sites in *L. africana* whereas nonsynonymous sites are affected in *L. cyclotis*.

In general, there are two traditional paradigms that account for the phenomenon of synonymous codon usage bias: mutational bias and translational selection (Duret, 2002). Shackelton *et al*. (2006) indicated that the genome of mammal tends to exhibit the pattern of synonymous codon usage that is shaped by mutational pressure. Geographic factors are also involved in determining some synonymous codon usage pattern (Liu *et al*., 2011). Previous studies have shown that variation in the evolutionary rate among nucleotide sites may be attributed to differences in the frequency of positive selection (Yang *et al*., 2000) or in the magnitude of selective constraints (Li, 1997; Rausher *et al*., 2008). For a number of different organisms, it was suggested that codon usage was best explained by selection for tRNA abundance, gene expression levels, and translational optimization (Duret, 2000). In other cases, the codon usage was explained by mutation rate, mutation preference (Powell and Moriyama, 1997), environmental conditions (Goodarzi *et al*., 2008), generation time (Subramanian, 2008), and recombination rates (Meunier and Duret, 2004).

Our results support the idea that different evolutionary rate among nucleotide sites in *Loxodonta africana* and *L. cyclotis*, attributable to differences in the frequency of positive selection and probably different environmental conditions are the driving forces for the codon usage bias in African elephants.

Our data indicates a multimodal mismatch distribution for both the savannah and forest elephant populations, supporting the hypothesis of stable population or a population in equilibrium.

With small migration rates between subpopulations, the mean of the mismatch distribution increases and thus the mismatch distribution can become multimodal (Hartl,

2004). Therefore, geographical subdivision might explain the mismatch distributions that were observed for the populations considered in this study. However, these results should be interpreted with caution given the limited reliability of these types of tests (Yang *et al*., 2012).

The analyses indicate that the mitochondrial sequences of African elephants are subject to purifying selection overall and that the derived proteins are not subject to positive selection favoring diversity at the amino acid level but actually tend to be conserved evolutionarily. A similar result was first found during analysis of the mitochondrial genomes of humans, in primates (Zaho *et al*., 2012) chimpanzees, and gorillas (Hasegawa *et al*., 1998) and also reported in additional studies on fruit flies, humans and Atlantic cod (Ballard, 2000 ; Elson *et al*., 2004; Marshall *et al*., 2009). The role of purifying selection, necessary for maintaining mitochondrial gene functions, has been clarified in many papers based on in silico analysis, and also by in vivo experiments. Rand and Kann (Rand and Kann, 1996; Kann and Rand, 1998) used the neutrality index (Rand and Kann, 1996) to determine the influence of selection on protein coding genes in fruit fly and some mammalian species (*Mus musculus*, *Homo sapiens*). Indeed, it is well known that strong negative (purifying) selection plays a central role in the evolution of mitochondrial DNA to keep its important functions in energy metabolism (Stewart *et al*., 2008; Shen *et al*., 2010; Sun *et al*., 2011).

Our hyphy analysis indicates evidence for positive selection limited to only a few sites. Hence positive selection at these sites, are masked by the continuous negative selection that occurs on most sites in the mitochondrial sequence as indicated by several authors (Zhang *et al*., 2005; da Fonseca *et al*., 2008; Shen *et al*., 2009).

5. Conclusion

Our study based on the analysis of partial mitochondrial DNA sequences from African savannah and a forest elephant indicates a higher nucleotide diversity in forest elephant (*Loxodonta cyclotis*) compared to savannah elephant (*Loxodonta Africana*).

A low codon bias index (CBI) and a relatively high ENC value were observed in the mitochondrial genome of both the species, suggesting that these mammals are less biased in their codon usage preference. The data also support a strong purifying selection in the mitochondrial genome of African elephants. However, few sites are under positive selection in the mitochondrial genome of African elephants, with *L. africana* presenting more sites under positive selection compared to *L. cyclotis*.

The present work supports the idea that different evolutionary rate among nucleotide sites in *Loxodonta africana* and *L. cyclotis* that are attributable to differences in the frequency of positive selection and probably different environmental conditions, are the driving forces for the codon usage bias in African elephants. Further studies should be performed to investigate the contribution of different subpopulation in the genetic structure and diversity of African elephants.

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Annex A: List of African elephants mitochondrial sequences used in the present study. Their region of isolation, size and GenBank accession numbers are indicated

Geographical origin	*Source*	*Size (bp)*	*GenBank*
Loxodonta cyclotis			
Central African Republic:	Ishida *et al.*,	4,258	JQ438305
Central African Republic:	Ishida *et al.*,	4,258	JQ438302
Central African Republic:	Ishida *et al.*,	4,258	JQ438273
Central African Republic:	Ishida *et al.*,	4,258	JQ438269
Zimbabwe: Hwange	Ishida *et al.*,	4,258	JQ438373
Republic of the Congo:	Ishida *et al.*,	4,258	JQ438621
Namibia: Northern	Ishida *et al.*,	4,258	JQ438588
Gabon: Lope	Ishida *et al.*,	4,258	JQ438510
Central African Republic:	Ishida *et al.*,	4,258	JQ438266
Central African Republic:	Ishida *et al.*,	4,258	JQ438265
Botswana: Chobe	Ishida *et al.*,	4,258	JQ438244
Central African Republic:	Ishida *et al.*,	4,258	JQ438308
Central African Republic:	Ishida *et al.*,	4,258	JQ438301
Central African Republic:	Ishida *et al.*,	4,258	JQ438267
Central African Republic:	Ishida *et al.*,	4,258	JQ438264
Central African Republic:	Ishida *et al.*,	4,258	JQ438261
Central African Republic:	Ishida *et al.*,	4,258	JQ438263
Central African Republic:	Ishida *et al.*,	4,258	JQ438260
Gabon: Lope	Ishida *et al.*,	4,258	JQ438512
Central African Republic:	Ishida *et al.*,	4,258	JQ438268
Central African Republic:	Ishida *et al.*,	4,258	JQ438262
Gabon: Lope	Ishida *et al.*,	4,258	JQ438513
Gabon: Lope	Ishida *et al.*,	4,258	JQ438503
Gabon: Lope	Ishida *et al.*,	4,258	JQ438502
Central African Republic:	Ishida *et al.*,	4,258	JQ438310
Gabon: Lope	Ishida *et al.*,	4,258	JQ438507
Gabon: Lope	Ishida *et al.*,	4,258	JQ438504
Loxodonta africana			
South Africa: Kruger	Ishida *et al.*,	4257	JQ438464

Geographical origin	*Source*	*Size (bp)*	*GenBank*
South Africa: Kruger	Ishida *et al*.,	4257	JQ438451
Kenya: Aberdares	Ishida *et al*.,	4257	JQ438135
Botswana: Savuti	Ishida *et al*.,	4257	JQ438629
			JQ438719
Tanzania: Tarangire	Ishida *et al*.,	4257	JQ438686
Tanzania: Tarangire	Ishida *et al*.,	4257	JQ438685
Zimbabwe: Sengwa	Ishida *et al*.,	4257	JQ438677
Tanzania : Serengeti	Ishida *et al*.,	4257	JQ438664
Tanzania: Ngorongoro	Ishida *et al*.,	4257	JQ438613
Namibia: Northern	Ishida *et al*.,	4257	JQ438575
Namibia: Northern	Ishida *et al*.,	4257	JQ438573
Namibia: Northern	Ishida *et al*.,	4257	JQ438564
Namibia: Northern	Ishida *et al*.,	4257	JQ438562
Namibia: Northern	Ishida *et al*.,	4257	JQ438554
Namibia: Northern	Ishida *et al*.,	4257	JQ438551
Namibia: Northern	Ishida *et al*.,	4257	JQ438550
Namibia: Northern	Ishida *et al*.,	4257	JQ438547
Botswana: Mashatu	Ishida *et al*.,	4257	JQ438533
Botswana: Mashatu	Ishida *et al*.,	4257	JQ438532
South Africa: Kruger	Ishida *et al*.,	4257	JQ438467
South Africa: Kruger	Ishida *et al*.,	4257	JQ438466
South Africa: Kruger	Ishida *et al*.,	4257	JQ438463
South Africa: Kruger	Ishida *et al*.,	4257	JQ438462
Kenya: Central	Ishida *et al*.,	4257	JQ438402
Zimbabwe: Hwange	Ishida *et al*.,	4257	JQ438341
Botswana: Chobe	Ishida *et al*.,	4257	JQ438221
Botswana: Chobe	Ishida *et al*.,	4257	JQ438220
Kenya: Amboseli	Ishida *et al*.,	4257	JQ438172
Namibia: Northern	Ishida *et al*.,	4257	JQ438577
Zimbabwe: Zambezi	Ishida *et al*.,	4257	JQ438758
Namibia: Northern	Ishida *et al*.,	4257	JQ438594
Namibia: Northern	Ishida *et al*.,	4257	JQ438545
Namibia: Northern	Ishida *et al*.,	4257	JQ438544
Kenya: Aberdares	Ishida *et al*.,	4257	JQ438126
Kenya: Aberdares	Ishida *et al*.,	4257	JQ438125
Zimbabwe: Zambezi	Ishida *et al*.,	4257	JQ438767
Zimbabwe: Sengwa	Ishida *et al*.,	4257	JQ438681
Zimbabwe: Sengwa	Ishida *et al*.,	4257	JQ438676
Kenya: Central	Ishida *et al*.,	4257	JQ438391
Botswana: Chobe	Ishida *et al*.,	4257	JQ438245
Kenya: Amboseli	Ishida *et al*.,	4257	JQ438142
Kenya: Amboseli	Ishida *et al*.,	4257	JQ438140
Kenya: Amboseli	Ishida *et al*.,	4257	JQ438138
Kenya: Amboseli	Ishida *et al*.,	4257	JQ438137
Kenya: Amboseli	Ishida *et al*.,	4257	JQ438136
Kenya: Aberdares	Ishida *et al*.,	4257	JQ438129
Kenya: Central	Ishida *et al*.,	4257	JQ438406
Cameroon: Benoue	Ishida *et al*.,	4257	JQ438211
Kenya: Amboseli	Ishida *et al*.,	4257	JQ438174
Kenya: Central	Ishida *et al*.,	4257	JQ438443
Kenya: Central	Ishida *et al*.,	4257	JQ438410
Kenya: Central	Ishida *et al*.,	4257	JQ438407
Kenya: Central	Ishida *et al*.,	4257	JQ438393
Kenya: Central	Ishida *et al*.,	4257	JQ438388
Kenya: Amboseli	Ishida *et al*.,	4257	JQ438152
Kenya: Aberdares	Ishida *et al*.,	4257	JQ438120
Kenya: Aberdares	Ishida *et al*.,	4257	JQ438119
Kenya: Central	Ishida *et al*.,	4257	JQ438411
Kenya: Amboseli	Ishida *et al*.,	4257	JQ438155
Kenya: Amboseli	Ishida *et al*.,	4257	JQ438151
Cameroon: Waza	Ishida *et al*.,	4257	JQ438734
Cameroon: Waza	Ishida *et al*.,	4257	JQ438730
Kenya: Aberdares	Ishida *et al*.,	4257	JQ438134
Kenya: Aberdares	Ishida *et al*.,	4257	JQ438131
Democratic Republic of	Ishida *et al*.,	4257	JQ438322
Cameroon: Waza	Ishida *et al*.,	4257	JQ438742

Geographical origin	*Source*	*Size (bp)*	*GenBank*
Cameroon: Waza	Ishida *et al*.,	4257	JQ438737
Unknown	Murata *et al*.,	16945	AB443879
Unknown	Hauf *et al*.,	16866	AJ224821
Unknown	Rogoev *et al*.,	16913	DQ316069
Sierra Leone	Brandt *et al*.,	16109	JN673264
Cote d'Ivoire: Tai NP	Finch et *al*.,	16030	KJ557424
Central African Republic:	Brandt *et al*.,	161028	JN673263