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Abstract

The evolutionary history of African ungulates has been largely explained in the light of Pleistocene climatic oscillations and the way these influenced the distribution of vegetation types, leading to range expansions and/or isolation in refugia. In contrast, comparatively fewer studies have addressed the continent’s environmental heterogeneity and the role played by its geomorphological barriers. In this study, we performed a range-wide analysis of complete mitogenomes of sable antelope (*Hippotragus niger*) to explore how these different factors may have contributed as drivers of evolution in South-Central Africa. Our results supported two sympatric and deeply divergent mitochondrial lineages in west Tanzanian sables, which can be explained as the result of introgressive hybridization of a mitochondrial ghost lineage from an archaic, as-yet-undefined, congener. Phylogeographic subdivisions into three main lineages suggest that sable diversification may not have been solely driven by climatic events affecting populations differently across a continental scale. Often in interplay with climate, geomorphological features have also clearly shaped the species’ patterns of vicariance, where the East Africa Rift System and the Eastern Arc Mountains acted as geological barriers. Subsequent splits among southern populations may be linked to rearrangements in the Zambezi system, possibly framing the most recent time when the river attained its current drainage profile. This work underscores how the use of comprehensive mitogenomic datasets on a model species with a wide geographic distribution can contribute to a much-enhanced understanding of environmental, geomorphological, and evolutionary patterns in Africa throughout the Quaternary.

**KEYWORDS:** *Hippotragus niger*, mitogenome, Pleistocene, phylogeography, ghost mtDNA capture, Africa
1. INTRODUCTION

The African savannah biome, including most tropical grasslands, woodlands, and scrublands of southcentral and eastern Africa, harbors the richest diversity of ungulate species on earth (DuToit & Cumming, 1999). The evolutionary patterns of such diversity have been largely explained by habitat fluctuations associated with Pleistocene climate changes (deMenocal, 2004; Lorenzen et al., 2012). However, though the geomorphology of the African continent is very heterogenous and dynamic, having experienced drastic landmass configuration changes in recent geological times (Plio-Pleistocene) (Doucouré & de Wit, 2003; Goudie, 2005; Moore et al., 2009), its role has received far less attention in the literature (but see Cotterill, 2003a,b). The relative roles of climatically determined habitat fluctuations and geological features on species evolutionary patterns have been historically difficult to assess, though their complex interplay has been recognized in tropical Africa (Couvreur et al., 2021; Stankiewicz & de Wit, 2006).

African climates shifted towards cooler temperatures and greater aridity around 2.8 million years ago (deMenocal, 2004). This climatic event enabled the expansion of the savannah biome that is thought to have triggered a speciation pulse within the Bovidae, a family harboring approximately 80% of all extant African ungulates (DuToit & Cumming, 1999; Vrba, 1995). Throughout the Pleistocene, marked oscillations between cold-dry and moist-warm periods led to the successive expansion and contraction of savannahs and woodlands (Dupont, 2011). Broadly, during dry periods savannahs expanded and displaced tropical rainforests, allowing for savannah-species to expand their range accordingly. During moist periods the scenario reversed (Dupont, 2011; Trauth et al., 2009), leading to habitat fragmentation and isolation of formerly contiguous populations in refugia (Hewitt, 2000, 2004). Such dynamics are thought to be currently reflected in the patterns of vicariance and diversity of many ungulate species (Arctander et al., 1999; Lorenzen et al., 2012). Nevertheless, global climatic trends must have differently affected sympatric taxa and may have had regionally distinct impacts on species widely distributed across Africa’s heterogeneous landscapes.

The regions of southcentral and eastern Africa remained tectonically active throughout the Pleistocene, being affected by uplifting forces, rifting and volcanism that repeatedly modified landscapes, fragmenting and reconnecting habitats and populations (Chorowicz, 2005; Cotterill, 2003a; Goudie, 2005; Grubb et al., 1999; Moore et al., 2009). One of the most striking geological features of the African continent is the East African Rift System (EARS) which, associated with uplands such as the Eastern Arc Mountains (EAM), strongly influenced local climates and the
distribution of different habitats in the region (Chorowicz, 2005; Faulkes et al., 2010; Grubb et al., 1999). For example, an arid corridor has been proposed to connect the arid Horn of Africa with the Namib Desert in the southwest, along the EAM and crisscrossing the EARS and the Zambezi basin to the south (Coe & Skinner, 1993; Grubb et al., 1999). Drainage systems in central and southern Africa have also undergone major reorganizations (Flügel et al., 2015; Goudie, 2005; Moore & Larkin, 2001). In particular, the most important east flowing drainage in southcentral Africa, the Zambezi, reflects a turbulent history of geomorphological events, including a series of river captures and sharp course changes, some of which occurred during the Pleistocene (Moore et al., 2009, 2012; Moore & Larkin, 2001). Additionally, new river basins that developed across Africa also played a significant role in shaping local climates (Stankiewicz & de Wit, 2006).

Ultimately, as the result of complex and dynamic topographic heterogeneity and environmental sensitivity, African geomorphological features have long since acted as barriers and/or corridors to dispersal, driving speciation events and biodiversity hotspots (Chorowicz, 2005; Cotterill, 2003a; Faulkes et al., 2010; Goodier et al., 2011; Grubb et al., 1999; Scholz et al., 2007; Taylor & Maree, 2009; Trauth et al., 2010).

The role of such complex interplay between climate change and geomorphological features on the evolutionary history of African savannah fauna is, however, still poorly understood (Taylor & Maree, 2009). This concerns not only the understanding of diversity and divergence of species but also to the appreciation of potential phenomena driven by the contact between different biological entities, like introgressive hybridization and the existence of ghost diversity (from as-yet-undefined or extinct taxa) persisting in extant taxa (Ottenburghs, 2020). Comparative phylogeographic studies have also been limited by the inconsistent use of different molecular markers, the model assumptions employed, and the lack of comprehensive datasets covering species’ full geographic ranges (e.g., Lorenzen et al., 2012). This has led to incongruent results in estimating divergence times and establishing general phylogeographic patterns across species. Ever-growing advances in high-throughput DNA sequencing make it feasible to obtain whole mitochondrial genomes from hundreds of individuals for phylogeographic studies (Du et al., 2019; Fabre et al., 2016; Lv et al., 2015). While there are biases inherent to the sole use of a maternally inherited single-locus marker, the mass application of whole mitogenome sequencing still allows solving complex within-species relationships, divergence patterns and histories of geographic populations in a cost-effective way (Stiller et al., 2009; Foote et al., 2011; Shamblin et al., 2012; Zinner et al., 2013; Gibb et al., 2015; Du et al., 2019). Importantly, mitochondrial DNA may
constitute the only accessible or reliable source of genetic material in highly degraded samples, given its high copy number in the cell, thus allowing for larger sample sizes in species that are not amenable to high-quality large-scale sampling (Van der Valk et al., 2017). Taken together, these features make mitogenomes amenable to the resolution of complex phylogeographic patterns (Du et al., 2019). However, only a handful of studies have made use of fully sequenced mitogenomes to provide insights on the evolutionary history of non-primate mammals across central, southern and eastern Africa (Gonçalves et al. 2021; Harley et al., 2016; Heller et al., 2012).

The expansion of open habitats during the late Neogene has been linked to the origin and diversification of many ungulate clades, such as the tribe Hippotragini (Hassanin et al., 2012). Within this tribe, the African endemic genus *Hippotragus* was more speciose and ecologically diverse during the Pliocene, as inferred from the fossil record, before experiencing progressive decline throughout the Pleistocene (Estes, 2013), with the sable antelope, *Hippotragus niger*, standing today as one of only two surviving members. Two combined aspects make the sable antelope a great model to investigate the relative signatures of climate shifts and the continent’s geomorphological dynamics on species evolutionary history. First, the species is one of Africa’s most habitat-dependent antelopes, being widely scattered throughout the mesic savannahs of southcentral and eastern Africa strongly associated to miombo woodlands (East, 1999; Estes, 2013). Secondly, its’ wide distribution ranges across some of Africa’s most relevant geomorphological features, such as the EARS, EAM and the southcentral African plateau centered on the Zambezi drainage system (Ansell, 1971; Estes, 2013; Groves, 1983). Geographical variation in morphological features allowed the description of four to five subspecies (Ansell, 1971; Estes, 2013; Groves, 1983), although the intraspecific taxonomy of sable remains unresolved. Geological barriers like the EAM, Lake Malawi, Muchinga Escarpment or the Zambezi River have been suggested to define different sable populations (Ansell, 1971; Estes, 2013; Groves, 1983; Groves & Grubb, 2011), but the proposed boundaries were often inconsistent. The most isolated and morphologically distinct subspecies is the critically endangered and iconic giant sable (*H. n. variani*), restricted to the Cuanza River basin in northern Angola (Estes, 2013). As in other ungulate assessments, the lack of geographically representative samples, adding to the low phylogenetic resolution resulting from analyzing only one or few mtDNA fragments (Matthee & Robinson, 1999; Pitra et al., 2002, 2006; vanVuuren et al., 2010), has limited the ability to fully understand the evolutionary history of the species.

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In this study we generated hundreds of complete mitochondrial genomes covering the species’ whole geographic range, combining contemporary and historic samples to disentangle the evolutionary patterns and timings of vicariance and dispersal of sable populations, interpreted within the context of both Pleistocene climate and geomorphological features. Ultimately, and by using the sable as a model species, we highlight the main drivers that may have shaped the evolutionary histories of other taxa in central, southern and eastern Africa.

2. MATERIALS AND METHODS

2.1 Sample collection, DNA extraction and library preparation

A total of 262 *H. niger* samples (231 contemporary and 31 historic) were collected from 22 populations covering the whole geographic range of the species in Africa. Additionally, four samples of *Hippotragus equinus* (2 contemporary and 2 historic) were included to outgroup *H. niger* in data analysis (Tables S1 and S2). For contemporary samples, DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit following manufacturer’s instructions. DNA extractions of historic (museum) samples were performed in a dedicated laboratory for ancient DNA following Dabney et al. (2013). Double stranded DNA library preparation followed Meyer & Kircher (2010), with the modifications described in Kircher et al. (2012). Four blank controls were carried through all steps. Libraries were amplified and purified as described in Dabney & Meyer (2012) (see Supporting Information, Text S1).

2.2 MtDNA capture, sequencing and assembly of mitogenomes

Four overlapping biotinylated PCR products encircling the *H. niger* whole mitochondrial genome were produced and used as probes for capture, using the Expand Long Range dNTPack kit (Roche) (details in Supporting Information Text S2). For contemporary samples, multiplexed capture of mtDNA sequences was performed as in Maricic et al. (2010). For historic samples, mtDNA capture was performed using a protocol updated from Fu et al. (2013; Text S3 in Supporting Information). DNA enriched libraries were sequenced on the MiSeq platform in a single lane with 76+7 cycles, following manufacturer’s instructions for Illumina Multiplex sequencing, and using double-indexed paired-end sequencing (Kircher et al., 2012). Base calling was performed with Bustard (Illumina Inc.). Reads longer than 35bp, after adapter trimming and
merging, were mapped against the *H. niger* complete mitochondrial genome (Genbank JN632648.1) using the mapper BWA v.0.5.10 (Li & Durbin, 2009). A customized mapping workflow was used for mapping to a circular reference (Text S4 in Supporting Information) and the consensus mitochondrial sequence was subsequently called for each sample (Renaud et al., 2015; Text S5 in Supporting Information). Reads of outgroup samples were mapped against the *H. equinus* mitogenome (Genbank NC_020712.1).

2.3 Data analysis

The full set of 262 *H. niger* and 4 *H. equinus* complete mitochondrial genomes were aligned using MUSCLE v3.8.31 (Edgar, 2004) and Mafft v.7.017b (Katoh & Standley, 2013) after considering the impact of deamination-induced substitutions during consensus calling (Figure S1). Alignments were tested for the maximum likelihood-tree and for the most parsimonious tree using Phangorn R-package (Schliep, 2011) to select the best multiple sequence alignment. Fifty *H. niger* and three *H. equinus* mitogenome sequences with masked bases (N) or poor quality were removed from downstream analyses (see Text S6 in Supporting Information). The final dataset comprising 212 *H. niger* sequences was translated to proteins using DnaSP v.5.10 (Librado & Rozas, 2009) to ensure no stop codons were found in unexpected positions of the mitochondrial genome. To resolve phylogenetic relationships and identify potential phylogeographic groupings among *H. niger* mitogenomes we constructed a Neighbor-Net network (Bryant & Moulton, 2004) based on uncorrected patristic distances and bootstrap analysis with 1000 replicates in SplitsTree v.4.13.1 (Huson & Bryant, 2006), using *H. equinus* as outgroup. Then we performed Bayesian phylogenetic analyses and divergence time estimation with BEAST v1.10.4 (Drummond & Rambaut, 2007; Drummond et al., 2012) using our dataset and additional antelope sequences downloaded from GenBank (Text S7 in Supporting Information). The control region was excluded from these analyses due to alignment ambiguities. Best-fit substitution models were estimated with JMODELTEST v.2.1.5 (Darriba et al., 2012). Molecular divergence age estimates for members of the tribes Hippotragini and Alcelaphini (Bibi, 2013) were used as priors to estimate divergence times for *H. niger* (Text S7 in Supporting Information). Next, we explored a dataset made exclusively of *H. niger* complete mitochondrial sequences. First, we estimated DNA polymorphism, number of haplotypes and genetic diversity parameters for the whole dataset, and for each lineage, haplogroup and sub-haplogroup identified in our phylogenetic analysis using DnaSP v.5.10. Second, we constructed a median-joining network for each haplogroup using
PopART v.1 software (http://popart.otago.ac.nz/links.shtml) to assess the relationships between the different haplotypes at a population level. Finally, to investigate past population dynamics in *H. niger* we performed demographic reconstructions for phylogeographic clades using three models of population size change (constant size, Bayesian Skyline and Bayesian Skyride) as implemented in BEAST v1.10.4 (Text S8 in Supporting Information).

3. RESULTS

3.1 Mitogenome Diversity and Divergence

We found 77 haplotypes among 212 complete mitochondrial sequences (Table 1). Bayesian phylogenetic analyses and the Neighbor-Net network retrieved the same intraspecific relationships, revealing the existence of four main mitochondrial lineages in *H. niger* (Figure 1; Figure S2). The most divergent lineage, here designated as relic (R), harbored a group of 27 haplotypes found in western Tanzania and highly differentiated from other west Tanzanian sable haplotypes (Figures 1 and 2; Figure S2). The remaining three lineages exhibited strong geographic structure, comprising an eastern lineage (E; 5 haplotypes) found in eastern Africa, a southern lineage (S; 37 haplotypes) present across the Zambian plateau and southern regions, and a central lineage (C; 8 haplotypes) found in western Tanzania, Malawi and northern Angola. These sable lineages can be further divided into six haplogroups (E1, E2 for eastern; C1, C2 for central; and S1, S2 for southern), and further subdivided into six sub-haplogroups (C1a, C1b for haplogroup C1; and S1a and S1b, S2a and S2b for haplogroups S1 and S2, respectively) with high support (Figure 1; Figure S2). Bayesian phylogenetic analyses further confirmed the phylogenetic placement of the extinct bluebuck (*H. leucophaeus*) as sister species of sable antelope (*H. niger*), then outgrouped by the roan antelope (*H. equinus*) (Espregueira Themudo & Campos, 2018).

The average number of nucleotide substitutions (Dxy) and number of fixed differences between relic and remaining sable lineages supports a strong and significant genetic divergence between them (Dxy = 0.03436 ± 0.00140; 406 fixed differences for the whole mitogenome; Table S3, Figure S2). In contrast, the levels of genetic distance among any of the remaining sable lineages were on average five times lower (Dxy = 0.00729 ± 0.00253; 86 fixed differences for the whole mitogenome; Table S3).

Remarkably, the 46 relic (R) mitogenomes exhibited similar haplotype diversity (H) to the remaining 166 sable mitogenomes altogether (E, C, S), though presenting about 30% lower nucleotide diversity (π) (Tables 1 and S4). We observed a decrease in haplotype diversity from
southern (S) to eastern (E) and from eastern to central (C) lineages, but similar nucleotide diversity between southern and eastern lineages, and lower nucleotide diversity in the central lineage. The lowest overall genetic diversity was found in haplogroups E1 and C2, restricted to Kenya and Angola respectively, the former exhibiting a complete lack of diversity among six mitogenomes, and the latter exhibiting only three haplotypes among 36 mitogenomes and a very low nucleotide diversity (Table 1).

3.2 Geographical partition of diversity

The distribution and frequency of each lineage, haplogroup and sub-haplogroup was depicted to understand the geographic context of the sable evolutionary history (Figure 2). Median-joining networks were also generated per haplogroup and for the relic lineage to uncover population partition of genetic diversity (Figure 3). The relic lineage was found exclusively in western Tanzania though locally widespread and consistently exhibited predominant frequency over the sub-haplogroup C1a, which is also confined to this region (Figure 2). The 27 haplotypes found for the relic lineage are sparsely connected in the median-joining network, having similar frequencies and no apparent geographical structure among seven locations in western Tanzania (Figure 3).

For sable haplogroups, we observed that E1 and C2 show very strong geographic specificity, the former restricted to one population in Kenya (Shimba Hills) and the latter to Angola (Figure 2). Haplogroups S1 and S2 are widespread among southern populations without an apparent geographic structure (Figure 2). However, this is in sharp contrast with the pattern found within each of these southern haplogroups, as we found a strong geographical segregation relative to the Zambezi River: sub-haplogroups S1a and S2a were only present north of the Zambezi River in Zambia and Congo, whereas S1b and S2b were only observed south of the river, namely in Zimbabwe, southern Mozambique, Namibia and Botswana (Figures 2 and 3). In general, eastern and central haplogroups exhibited very low number of haplotypes (between one and five) and no haplotype sharing among populations, while southern haplogroups show high number of haplotypes highly dispersed along the networks and a few shared haplotypes among sites (Figure 3).

3.3 Divergence Time Analyses
Our estimate of divergence time for the sister species of sable antelope, the extinct bluebuck, was 3.8 million years ago (mya) with a 95% highest posterior density interval (HPDI) between 3.0 and 4.6 mya, while the outgroup to this clade, the roan antelope, diverged around 5.9 [95% HPDI = 5.0–6.7] mya (Table S5). The divergence between relic (R) and other sable lineages (E, C, S) was estimated to have occurred around 1.5 mya with HPDI between 1.1 and 2.0 mya, tracing back to the early Pleistocene (Figure 1; Table S5). The second oldest split in sable antelope occurred between eastern and southcentral populations in the mid-Pleistocene, with an estimated median time of around 374.8 [288.4–471.3] thousand years ago (kya), followed by the split leading to central and southern lineages around 203.4 [156.5–256.9] kya. We found the split into haplogroups within Eastern and Southern lineages to have occurred contemporaneously, estimated at around 154.0 [104.7–211.2] kya for E1 and E2, and 148.3 [110.7–190.4] kya for S1 and S2, respectively. Later, also the Central lineage split into C1 and C2, which occurred around 114.2 [80.6–153.4] kya (Figure 1; Table S5).

Each of the southern haplogroups (S1 and S2) further subdivided into sub-haplogroups in the Late Pleistocene, with an extensive overlap in HPDIs of the timing of both splits estimated at 97.0 [67.3–128.8] and 66.1 [44.4–91.7] kya, respectively (Figure 1; Table S5). The emergence of these sub-haplogroups was contemporary to the split of the central haplogroup C1 into C1a, present in western Tanzanian sables, and C1b found in Malawian sables, which happened around 93.2 [61.7–128.1] kya (Figure 1; Table S5).

### 3.4 Demographic History

Bayes factor analyses for different demographic models favored the Bayesian skyline model over constant population size and Bayesian skyride for southern haplogroups (S1, S2) and sub-haplogroups (S1a, S2a, S2b) (Table S6). Bayesian skyline plot (BSP) analysis for these clades revealed that changes in effective population size through time seem to have occurred within the last 10 kya, at the beginning of the Holocene (Figure S3). Specifically, BSP analysis showed that haplogroups S1 and S2, and their respective sub-haplogroups, experienced a decrease in effective population size from 10 kya. However, the effective population size of these phylogeographic clades was steadier within the 95% HPDI of time of divergence from their respective sister clade during the Pleistocene, except for S2b, which experienced an increase in effective population size from 60 to 30 kya. Bayes factors did not favor BSP over constant effective population size model
for eastern (E1, E2) and central (C1, C2) haplogroups and for sub-haplogroup S1b, for which BSP analysis also revealed constant effective population sizes through time (Table S6; Figure S3).

4. DISCUSSION

Owing to our extensive dataset we were able to draw the most complete picture of the maternal phylogeographic history of sable antelope to date. While acknowledging that a complete understanding of sable evolutionary history requires a subsequent exploration of genome-wide data, the use of complete mitochondrial genomes allowed us to produce consistent inferences of divergence times for the mtDNA lineages, haplogroups and sub-haplogroups recorded with much higher resolution than previous studies based on small mtDNA fragments (Matthee & Robinson, 1999; Pitra et al., 2002, 2006; vanVuuren et al., 2010). Such findings can both contribute to the debate of possible driving forces explaining patterns of vicariance and diversity observed for African organisms, such as the importance of geomorphology and climate change, but also help on further efforts trying to date specific events in Africa, particularly those associated with drainage evolution.

Overall, the phylogeographic clades (lineages, haplogroups or sub-haplogroups) uncovered in this study broadly fit the geographical subdivision of sable antelope in four subspecies proposed by Ansell (1971). However, we were not able to consistently match phylogeographic clades and subspecies. While the eastern lineage (E) is congruent with the subspecies classification of *H. n. roosevelti* and the central sub-haplogroup C2 is carried by sables ascribed to the giant sable of Angola, *H. n. variani*, sub-haplogroups S1b and S2b can both be found in south Zambezi sables classified as *H. n. niger*. Divergent lineages from the central and southern haplogroups, namely the relic (R) lineage and sub-haplogroups C1a, C1b, S1a and S2a, seem to be carried by sables ascribed to the fourth subspecies of sable antelope, *H. n. kirkii*. Future genome-wide analyses and additional large-scale geographic sampling efforts, particularly in Eastern and central Africa, are needed to ascertain whether the current intraspecific taxonomy and associated geographic boundaries needs to be revised.

4.1 Ghost Introgression in Western Tanzanian Sable
Our results confirmed the existence of two divergent mtDNA clades confined to western Tanzania, Relic (R) and C1a, one of which (R) harboring similar levels of haplotype diversity to those of all other sable lineages combined. Pitra et al. (2002) explained the co-existence of these two lineages as the result of a long-distance colonization event from southern Africa, followed by intraspecific hybridization and outbreeding among two extant sable subspecies. Later, it was suggested that the invading population may have shared a common source with Angolan sables (Pitra et al., 2006), while Groves & Grubb (2011), based on morphological characters of a few specimens and earlier genetic findings, suggested that the current west Tanzanian population could be a hybrid swarm between eastern and Angolan sables. However, these hypotheses fail to recognize the high levels of divergence found between relic (R) and eastern (E), central (C) and southern (S) mtDNA lineages, which are five times higher than those found only among E, C and S lineages, and yet are not reflected in ostensible differences in morphological traits, nor at the nuclear level (Vaz Pinto, 2018).

The early Pleistocene 1.5 [1.1–2.0] mya split time between R and remaining sable mitochondrial lineages concurs with a speciation pulse affecting tribes Hippotragini and Alcelaphini and other savannah bovids in eastern Africa (Bibi, 2013; Lorenzen et al., 2010, 2012), and predates all other sable diversification events during mid-to-late Pleistocene that led to ostensible morphological variability within sable (Ansell, 1971). As such, we hypothesize that the co-existence of two divergent mitochondrial lineages in apparently morphologically undifferentiated western Tanzanian sables comprising a single population (carrying R and C1a mtDNA) with shallow nuclear divergence from other sable populations (Vaz Pinto, 2018), could result from past mtDNA introgression with an older as-yet-undefined resident congener. Specifically, we propose that an archaic and now presumably extinct west Tanzanian sable population (carrying R mtDNA) evolved geographically isolated (between the EARS and the EAM; Figure 4) from other (hereafter modern) sables for more than one million years. Subsequently, central African sables carrying the C1 haplogroup came into secondary contact with this archaic population after moving across the EARS into western Tanzania, from which they captured the relic mitochondria via introgression. Notably, the node age for extant relic haplotypes is of approximately 79.1 [53.6–110.2] kya, matching the estimated divergence time between C1a and C1b sub-haplogroups (93.2 [61.7–128.1] kya), which have likely diverged after crossing the EARS. Such spatiotemporal overlap could support secondary contact after central African sables (C1) reached western Tanzania.
Our hypothesis is consistent with the current understanding that colonization processes can result in massive introgression of genes from local populations into the genome of a colonizing population (Currat et al., 2008), and that in species with male-biased dispersal, such as the sable antelope (Estes, 2013), female inherited markers are expected to experience higher levels of introgression than biparental markers (Petit & Excoffier, 2009). Furthermore, there has been increasing evidence that introgression of ghost lineages among extant lineages is frequent in mammals (Achilli et al., 2008; Durvasula & Sankararaman, 2020; Hailer et al., 2012; Kuhlwilm et al., 2019; Ottenburghs, 2020; Roca et al., 2005; Sacks et al., 2021; Seixas et al., 2018; Toews & Brelsford, 2012; Zhang et al., 2019) and would be possible in *Hippotragus* spp. A case of early stages of introgressive hybridization between the giant sable of Angola and the roan antelope (*H. equinus*) lends itself to the argument of introgression events among *Hippotragus* lineages (Vaz Pinto et al., 2016).

Alternatively, the R lineage could be a case of mitochondrial incomplete lineage sorting (ILS). Though disentangling introgression from ILS can be challenging, under a scenario of ILS we would expect to find the R lineage in other remaining sable populations irrespective of geographic region. Instead we find it strictly present in west Tanzanian sables, confined between the EAM and the EARS, locally widespread within the region and in high frequency, as theoretically expected in cases of mtDNA introgression of male-biased dispersing species (Currat et al., 2008; Petit & Excoffier, 2009). Such strong geographic context in western Tanzania favors an introgression scenario over ILS (Funk & Omland, 2003; Toews & Brelsford, 2012).

4.2 Geomorphological features shaping patterns of vicariance in eastern Africa

Sable mitochondrial diversification appears to have been strongly influenced by the EARS in eastern Africa, consistent with the role attributed to local biogeographical barriers as drivers of population fragmentation and speciation (Arctander et al., 1999; Colangelo et al., 2013; Flagstad et al., 2001; Trauth et al., 2007). Separating the Tanzanian plateau from southern Africa, the geologically complex middle section of the western branch of the EARS is credited in promoting discontinuities in faunal distribution (Grubb et al., 1999). Some of the modern topography in the EARS was generated throughout the last two million years (Chorowicz, 2005; Delvaux et al., 2012; Denys et al., 1986) and may have contributed to the isolation of the relic (R) lineage in central Tanzania.
Eastern African biogeography is also shaped by the rain shadow effect that resulted in the long-standing arid corridor present along the western edge of the EAM (Coe & Skinner, 1993; Grubb et al., 1999). The large contiguous lakes linked to mountain chains and the long-standing arid corridor in eastern Africa form a formidable barrier to the dispersal of many species, particularly mesic savannah species that, like the sable, avoid arid habitats, open grasslands, and highlands (Estes, 2013). It is thus not surprising that the deepest split found in our work for modern sable lineages, around 374.8 [288.4–471.3] kya, led to the diversification of the eastern (E) lineage, reflecting a clear geographical signature, where the EAM, the EARS and Lake Malawi seem to define the dividing boundary (Figure 4A). Notably, the roan antelope, sable’s closest living relative, largely overlaps in range with sable in southern Africa, yet it is conspicuously absent to the east of the EAM and Lake Malawi (East, 1999). Furthermore, the sub-haplogroup C1b may have evolved to the west of Lake Malawi and been constrained to the east by the southern extension of the same arid corridor present along the Luangwa valley and Muchinga escarpment in eastern Zambia (Grubb et al., 1999), but additional sampling would be needed to test this hypothesis.

4.3 Diversification modeled by climate change

In sub-Saharan Africa, marked oscillations between warm-wet and cold-dry periods throughout the mid-to-late Pleistocene led to expansion and contraction of savannah habitats and tropical rainforests in tandem (Dupont, 2011; Maley, 1996). While tropical forest taxa persisted in forest refugia that maintained habitat stability during dry periods (e.g. Barrat et al., 2021), the presence of savannah habitat refugia during moist periods is thought to have enabled the persistence of savannah-adapted species, resulting in species divergence and strong genetic structure among populations (Lorenzen et al., 2010, 2012). This may, however, be an oversimplification, as the savannah biome may have shifted in latitude in response to glacial/interglacial cycles rather than becoming fragmented (Couvreur et al., 2021). In addition, and on a finer scale, the savannah biome includes, for example, edaphic grasslands, semi-arid savannahs, mesic woodlands and deciduous forests (White, 1983), which may respond differently to climatic changes and affect species adapted to different habitats accordingly. Miombo woodlands, for example, the habitat in which sable are specialists, are strongly associated with relatively high rainfall and have been found to contract in drier areas during glacial periods while expanding into wetter regions, and to colonize higher ground during warmer interglacials.
We found no strict correlation between divergence events and either glacial or interglacial periods, although ascribing splitting episodes to specific climatic periods is often hampered by dating estimates with wide credible intervals (Figure 5). Specifically, the median estimates for the divergence between the eastern (E) and remaining lineages, the earliest within modern sables, and subsequent contemporaneous splits within eastern and southern (S) lineages, seem to have occurred during glacial periods, while the remaining splits corresponded to interglacials.

Though the median node age for the split between eastern and remaining sable lineages dates back to the Elster glaciation or MIS (Marine Isotopic Stage) 10, the associated 95% HPDI largely overlapped with both glacial and interglacial periods (Figure 5). As such, we were not able to associate this diversification event with a specific climatic event. On the other hand, the following split between the southern and central (C) lineages likely took place during a warm interglacial period, around 203.4 [156.5–256.9] kya, a time concordant with a turnover of speciation in African ungulates (Flagstad et al., 2001; Lorenzen et al., 2010). Notably, the Lichtenstein’s hartebeest (*Alcephalus buseaphus lichtensteinii*), another miombo woodland specialist whose current distribution matches that of the sable, was estimated to have diverged from the red hartebeest (*A. b. caama*) at around 212 kya (Flagstad et al., 2001). It is thus possible for miombo woodlands to have retreated in moister regions during that period pushing miombo specialist species into refugia in southcentral Africa, with the timing and mechanism that led to the differentiation of Lichtenstein’s and red hartebeest coinciding with sable’s central and southern lineages, respectively.

Divergence within both eastern and southern lineages, establishing haplogroups E1 and E2 and S1 and S2, respectively, was estimated to be concurrent at around 150 kya with 95% HPDIs overlapping between 110 and 190 kya. This contemporaneous fragmentation in two geographically distant regions is best explained by the extreme dry-cold period during the MIS 6, characterized by extensive fragmentation of African forests (Maley, 1996). MIS 6 also marks the onset of episodes of climatic and ecological volatility that persisted throughout the late Pleistocene (Stewart & Jones, 2016), and are thought to have triggered profound changes in the local composition of ungulate species by opening and closing dispersal corridors (Faith et al., 2016). We therefore propose that these two intra-lineage divergence events occurred during the penultimate glacial maximum period (PGM) at MIS 6 and could have been the result of a regional contraction of miombo woodlands in drier regions (Dupont, 2011). During the PGM most of the
eastern African coastal plain possibly became too dry for sable, which may have found refugia in moister woodland patches in valleys along the EAM or closer to the Indian Ocean and thus promoted the diversification found in eastern haplogroups (Figure 4B). Such a scenario would be in accordance with the expansion of arid habitats in eastern Africa, and the persistence of moister refugia along the eastern coastline during glacial periods (Hamilton & Taylor, 1991), and with the more recent expansion of woodlands, as the rainfall increased since the last glacial maximum (Ivory et al., 2012). Similarly, southern sables may have found distinct refugia in southcentral Africa (Figure 4B). Both the eastern and the southern haplogroups reconnected following range expansions when conditions improved (Figure 4C).

Consistent with previous findings (Pitra et al., 2006), the central lineage (C) revealed a link between sable present in Angola, Malawi and western Tanzania, but the geographic origin and dynamics of these populations are puzzling. Angolan sables are currently separated from those found in western Tanzania and Malawi by extensive lowland rainforests and the Congo and Zambezi drainage systems. Yet, none of them shares a recent ancestry with sables currently found across the Zambian plateau carrying southern haplogroups S1 and S2, thus making the existence of a corridor across the Zambezi basin unlikely. We hypothesize that the central lineage may have been confined to the central Congo basin during the PGM, when the same dry cold conditions that contracted eastern woodlands may have greatly expanded central African mesic woodlands at the expense of Congo rainforests (Figure 4B). The subsequent warming period that characterized the last interglacial period (LIG) and began at around 128 kya, was very pronounced and led to the re-establishment of rainforests (Maley, 1996; Piñeiro et al., 2017). Such a sequence of events could have fragmented the range occupied by the central lineage and originated the haplogroups found in west Tanzania and Malawi (C1) and Angola (C2) around 114.2 [80.6–153.4] kya, even though the HPDI for this split also partially overlapped with the PGM (Figure 5). We hypothesize that, forced by climate change, sables may have disappeared from the central Congo basin to colonize more suitable areas. By dispersing eastwards, sables could have reached the western branch of the EARS (Figure 4C). However, defining a cross-rift dispersal corridor is challenging, as the most direct route is currently blocked by the upper Congo River, a region that experienced dramatic drainage rearrangements in the past and has remained tectonically active to date (Flügel et al., 2015). The subsequent split of the C1 haplogroup into C1a and C1b at approximately 93.2 [61.7–128.1] kya fully overlaps with the LIG and marks the period when sables were able to move across the EARS into western Tanzania, while the remaining sable evolved in Malawi (Figure 4C). The
cross-rift dispersal that resulted in the differentiation of sable populations on either side of the
EARS may have been enabled by the opening of a temporary corridor between lakes Rukwa and
Malawi, which suffered pronounced water reductions owing to sudden megadroughts between 85
and 110 kya (Scholz et al. 2007; Trauth et al. 2007), and even disappearance of arboreal species
that characterize miombo (*Brachystegia*, *Julbernardia*, *Uapaca*) at 95 kya (Beuning et al., 2011).
Although primarily pushed by climatic shifts, it is likely that the divergence of the
haplogroup currently found in central Angola (C2) was also shaped by physiographic features
(Figure 4C). Draining from the Angolan plateau and south-north oriented over 1,100 km, the
Kwango River is the largest Congo tributary within the Kasai sub-basin and could have easily
constrained sable dispersal to the east. Furthermore, it has been suggested that Pleistocene
tectonics caused various river capture and reorientation events between the upper Kwango and
Cuanza drainages (Monteiro Marques, 1992), and these may help explain how sables carrying the
haplogroup C2 ended up confined within the upper Cuanza basin, with the current giant sable
population in Angola representing the last remnant of this haplogroup.

### 4.4 Zambezi drainage evolution promoting diversification

Although the combined role of Pleistocene refugia and rivers in structuring species distribution
ranges and genetic diversity has been recognized for African forest mammals (e.g., Anthony,
2007; Mitchell et al., 2015; Nicolas et al., 2011), only a handful of studies have assessed the
specific impact of river barriers on the patterns of vicariance and diversity of savannah species
(Cotterill, 2003a,b; Gonçalves et al., 2021; Van Daele et al., 2007). The Zambezi River system is
the most important drainage feature in southern Africa, having been radically modified and
disrupted by river capture events as a result of recent tectonic activity (Moore & Larkin, 2001). In
this work we found two pairs of southern sub-haplogroups geographically separated on either side
of the Zambezi (S1a, S2a in the north versus S1b, S2b in the south; Figure 4D), suggesting that the
river could have acted as a dynamic biogeographic barrier to dispersal. The upper Zambezi used to
be part of an endorheic system that fed Paleolake Makgadigadi, before being captured by the mid-
Zambezi in the mid- to late-Pleistocene (Burrough et al., 2009; Moore & Larkin, 2001; Thomas &
Shaw, 1992). Geological evidence also suggests this connection has subsequently been broken and
reestablished several times (Burrough et al., 2009; Cotterill, 2003b; Moore et al., 2009, 2012;
Moore & Larkin, 2001). Some of the most compelling evidence for river capture events are based
on phylogeographic patterns found in the ichthyofauna of the upper and mid-Zambezi and

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adjacent drainages (Goodier et al., 2011). Also, Pleistocene speciation in antelopes of the genus *Kobus* and *Damaliscus* appears to be tightly linked to key events in the recent evolution of the Zambezi River (Cotterill, 2003a,b, 2005), even though the precise timing and sequence of those episodes await better resolution (Cotterill, 2003b).

We consider that divergence of S1 and S2 haplogroups on either side of the Zambezi is best explained by a single episode associated with the Zambezi River. This is supported by the extensive overlap in the 95% HPDI of these estimates, which may frame this event as having occurred between 91.7 and 67.3 kya. This chronology overlaps, to some extent, with the proposed existence of Palaeolake Makgadigadi within the approximate period of 112 to 89 kya, possibly driven by regional tectonic shifts and diversion of the Zambezi flow into the Kalahari (Burrough et al., 2009). Thus, the split in southern sub-haplogroups likely resulted from a reconfiguration event of the Zambezi River. During the period when Palaeolake Makgadigadi was flooded, the mid-Zambezi would not constitute a barrier for dispersal, allowing sables to move between the Zambian plateau and the remaining regions of southern Africa, but the subsequent reestablishment of the Zambezi link could have led to the differentiation observed. Five additional mega-lake phases were proposed for the last 64,000 years, but these are thought to be better explained by palaeoclimatic shifts rather than by drainage shifts (Burrough et al., 2009) and are unlikely to have re-opened corridors for further sable dispersal across the Zambezi. We therefore suggest that the patterns of vicariance here uncovered may frame the most recent time when the Zambezi River attained its current drainage profile.

### 4.5 General diversity patterns across Africa

Among modern sable mtDNA clades, the southern lineage and its haplogroups consistently exhibit the highest levels of haplotype diversity, despite plausible range changes across the Zambezi explained by the presence of two divergent southern clades to the north (S1a+S2a) and south (S1b+S2b) of the river. This is particularly evident in the structure of the median-joining networks for southern sables, with long separate branches and many unique haplotypes. Consistent with this, our demographic reconstructions supported that the region centered in the Zambezi basin has held large, long-standing sable populations with stable effective population sizes throughout most of the Pleistocene. Notably, changes in effective population size for three out of four southern sub-haplogroups occurred only within the past 10 kya, not within the HPDI of divergence from their sister clades. In contrast, we found very low mitochondrial haplotype and nucleotide...
diversity in the critically endangered giant sable of Angola (C2 haplogroup). This is consistent
with the subspecies’ low nuclear diversity and was already expected for this lineage, which
suffered a severe bottleneck within the last century, after decades of war in Angola (Vaz Pinto et
al., 2015, 2016). Though eastern haplogroup E1 comprises a single haplotype carried by sables
inhabiting the coastal forests in the northern limit of the sable’s East African range, our sampling
size is comparatively much smaller and holds large geographical gaps in this region, which could
have prevented the detection of additional genetic diversity. Currently, the Kenyan population,
where haplogroup E1 likely originated, also holds in sympatry the haplogroup E2, additionally
found in sables from northern Mozambique. Haplogroup E2 thus seems to have evolved more to
the south of the East African sable range and have dispersed north, enriching the genetic diversity
of Kenyan sables. In similarity to southern haplogroups, and despite much lower genetic diversity
and/or sampling size, central (C1, C2) and eastern (E1, E2) haplogroups also exhibited
demographic signatures of constant population sizes throughout the mid-to-late Pleistocene. This
could suggest that changes in sable distribution range driven by changes in African climate and
geo morphology were rarely accompanied by changes in effective population size (Excoffier et al.,
2009). However, it is important to appreciate the limitations of demographic inference obtainable
from mitochondrial sequences, as they represent only one nonrecombining haplotype sequence
that reflects only the maternal lineage.

Ultimately, the geographic distribution of sable mitochondrial diversity leads us to
hypothesize that the fringes of the species’ northern range, in the Angolan plateau and along the
EARS, possibly represent patches of refugial populations that have differentiated and are thus
responsible for an increase in the intraspecific diversity of sable. Similar patterns of diversity have
been found for a few mammal populations occupying the southern and central regions defined in
this work (Castiglia et al., 2012; Lorenzen et al., 2012) and may be indicative of a common and
broader phylogeographic scenario.

4.6 Conclusions

To the best of our knowledge this study is among the first to provide a comprehensive
population analysis using fully sequenced mitochondrial genomes of a non-primate species in
Africa. By analyzing sequence divergence and diversity in sable antelope over the Pleistocene, we
were able to observe how various lineages may have responded differently when exposed to
severe climatic oscillations in interplay with the highly complex and dynamic geomorphological

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history of Africa. This allowed us to provide explanations for long-standing questions about the species’ evolutionary history. First, we were able to provide a credible explanation for the origin of a deeply divergent lineage from western Tanzanian sables. We were additionally able to explain the parapatric pattern of differentiation in southern African lineages, as the result of isolation, reconnection, and later fragmentation by the Zambezi River, and by doing so, we refined dates for when the river may have last attained its current profile. Finally, we were able to formulate a hypothesis for the origin of the critically endangered giant sable antelope of Angola. Our results enforce the principle that a sound understanding of African faunal evolution can only be achieved when both climatic history and landscape dynamics are considered. Although intraspecific patterns are ideal to assess the signs of the impact of climate and geomorphology in African species, it is important to look beyond isolated or single-process responses. We expect this study to represent a milestone in understanding key events in southcentral and eastern Africa that could ultimately clarify the evolutionary history of other species in these regions. Future large-scale comparative efforts may very much benefit from the insights here provided.

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**DATA ACCESSIBILITY STATEMENT**

Unique mitogenome haplotypes for *Hippotragus niger* are available on NCBI Nucleotide Database (OM617391 to OM617467).

**AUTHOR CONTRIBUTIONS**

P.V.P, N.F. and R.G designed the study. J.R. carried out experiments and analyzed data. M.M. provided supervision on museum samples mitogenomics. H.R.S. and B.J.vV. provided most of samples and contributed to analyses and the interpretation of phylogeographic patterns. L.V.

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contributed with the execution of figures of the manuscript. J.R., P.V.P. and R.G. wrote the manuscript with inputs from all authors.
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<th>Hd</th>
<th>π</th>
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<td>144</td>
<td>18</td>
<td>0.881 ± 0.028</td>
<td>0.00247 ± 0.00121</td>
<td>40.704 ± 17.960</td>
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N, number of samples; S, number of polymorphic (segregating) sites; h, number of haplotypes; Hd, Haplotype diversity (and respective standard deviation); π, nucleotide diversity (and respective standard deviation); MPD, mean pairwise distance (and respective standard deviation). All parameters were estimated accounting for insertion/deletions and allowing 5% of missing data. Haplogroups and sub-haplogroups are labeled as in the text.
Figure Captions

FIGURE 1 Bayesian phylogenetic tree of 212 *Hippotragus niger* mitochondrial genomes (excluding control region). Modern sable lineages, haplogroups and sub-haplogroups were labeled accordingly to the respective African region (see Figure 2). Posterior estimates of the time to the most recent common ancestor and 95% HPD intervals are indicated for nodes of interest in million years ago (mya) and thousand years ago (kya) (see Table S5 and Text S7 in Supporting Information).

FIGURE 2 Geographic distribution of mitochondrial haplogroups and sub-haplogroups across sable range (in grey). Haplogroups and sub-haplogroups within lineages are labeled according to the respective African region (see text). E1 and E2: Eastern 1 and 2; C1 and C2: Central 1 (with sub-haplogroups a and b) and 2; S1 and S2: Southern 1 and 2 (with respective sub-haplogroups a and b). The highly divergent mtDNA lineage from Western Tanzania is labeled R (relic). Abbreviations for countries’ names are as follows: KEN=Kenya, MWI=Malawi ZMB=Zambia, TZA=Tanzania, AGO=Angola, ZWE=Zimbabwe, BWA=Botswana, NAM=Namibia, MOZ=Mozambique and COD=Democratic Republic of the Congo.

FIGURE 3 Median-joining networks based on complete mitogenomes for sable haplogroups and the relic lineage. Networks were generated using PopART. Number of mutations separating haplotypes are presented as hatch marks on top of the branches. Haplotypes labeled Zambia correspond to museum specimens with unknown exact origin. Abbreviations for countries’ names are indicated in Figure 2.

FIGURE 4 Evolutionary history of sable antelope lineages in relation to major river basins and geomorphological features. Location of populations and river courses are schematic representations to help interpretation. A: representation of geographical location after splitting of
the four lineages in the mid-Pleistocene and prior to the Penultimate Glacial Maximum (PGM), namely eastern (E), central (C), southern (S) and relic (R); B: split of eastern and southern lineages into haplogroups E1-E2 and S1-S2), respectively, and retreat of the central lineage into the Congo basin as a result of the PGM; C: representation of possible evolutionary dynamics and suggested routes at the beginning of the late Pleistocene following the last interglacial period, with the split of the central lineage into haplogroups C1 and C2, subsequent bridging of the C1 haplogroup across the EARS, and secondary contact within southern and eastern haplogroups; D: representation of sable lineages, haplogroups and sub-haplogroups, attained in the Holocene, showing the split of southern haplogroups into sub-haplogroups a and b (S1a-S1b, S2a-S2b) caused by the reconnection of the Zambezi River, and the split within a central haplogroup into C1a-C1b as some of these sables moved across the rift leading to the ghost introgression event (C1a+R).

**FIGURE 5.** Splitting events (median divergence time and 95% HPDI) within sable lineages, haplogroups and sub-haplogroups. Representation over the past half million years overlaying glacial cycles inferred from sea surface temperatures (SST; adapted from Hughes & Gibbard, 2018).
Populations
1 - Shimba Hills (KEN)
2 - Liwonde NP (MWI)
3 - Masebe-Mkushi (ZMB)
4 - Lusaka-Kafue (ZMB)
5 - Mufumbwe (ZMB)
6 - Mulobezi (ZMB)
7 - Kafue NP (ZMB)
8 - Kizigo (TZA)
9 - Kigosi (TZA)
10 - Ugalla (TZA)
11 - Niensi (TZA)
12 - Mlele (TZA)
13 - Wembere (TZA)
14 - Rungwa (TZA)
15 - Cangandala NP (AGO)
16 - Luando (AGO)
17 - Matesi (ZWE)
18 - Triangle (ZWE)
19 - Chobe (BWA)
20 - Mahango NP (NAM)
21 - Niassa (MOZ)
22 - Katanga* (COD)
23 - Gorongosa NP* (MOZ)
24 - Sofala-Manica* (MOZ)
* Sample type: Museum

Sample Size
1 - 2
3 - 7
8 - 10
11 - 19
20 - 27