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Forest elephant mitochondrial genomes reveal that elephantid diversification in Africa tracked climate transitions

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Abstract

Among elephants, the phylogeographic patterns of mitochondrial (mt) and nuclear markers are often incongruent. One hypothesis attributes this to sex differences in dispersal and in the variance of reproductive success. We tested this hypothesis by examining the coalescent dates of genetic markers within elephantid lineages, predicting that lower dispersal and lower variance in reproductive success among females would have increased mtDNA relative to nuclear coalescent dates. We sequenced the mitochondrial genomes of two forest elephants, aligning them to mitogenomes of African savanna and Asian elephants, and of woolly mammoths, including the most divergent mitogenomes within each lineage. Using fossil calibrations, the divergence between African elephant F and S clade mitochondrial genomes (originating in forest and savanna elephant lineages, respectively) was estimated as 5.5 million years ago (Mya). We estimated that the (African) ancestor of the mammoth and Asian elephant lineages diverged 6.0 Mya, indicating that four elephantid lineages had differentiated in Africa by the Miocene-Pliocene transition, concurrent with drier climates. The coalescent date for forest elephant mtDNAs was ca. 2.4 Mya, suggesting that the decrease in tropical forest cover during the Pleistocene isolated distinct African forest elephant lineages. For all elephantid lineages, the ratio of mtDNA to nuclear coalescent dates was much greater than 0.25. This is consistent with the expectation that sex differences in dispersal and in variance of reproductive success would have increased the effective population size of mtDNA relative to nuclear markers in elephantids, contributing to the persistence of incongruent mtDNA phylogeographic patterns.

Introduction

A number of studies have indicated that the African savanna or bush elephant (*Loxodonta africana*) is a distinct species from the African forest elephant (*L. cyclotis*) (Groves & Grubb 2000; Grubb *et al.* 2000; Roca *et al.* 2001; Comstock *et al.* 2002; Roca *et al.* 2005; Rohland *et al.* 2010). Analyses of DNA sequences have demonstrated that little or no nuclear gene flow occurs between forest elephant and savanna elephant populations, providing evidence that the two groups would satisfy the definition, under the biological species concept, that "species are groups of interbreeding natural populations that are reproductively isolated from other such groups" (Mayr 1963; Meier & Wheeler 2000; Roca *et al.* 2001; Roca *et al.* 2005; Roca *et al.* 2007; Petit & Excoffier 2009). Corroborating this evidence are reports that forest and savanna elephants can be completely distinguished morphologically using a discriminant function (Groves & Grubb 2000), that between forest and savanna elephants F_{st} values greater than 0.90 have been estimated for nuclear sequences and microsatellites (Roca *et al.* 2001; Comstock *et al.* 2002), that phylogenies inferred using nuclear markers place forest and savanna elephants into reciprocally monophyletic clades (Roca *et al.* 2001; Comstock *et al.* 2002), and that the divergence between them has been estimated as 2.6 to 5.6 Mya using nuclear markers (Rohland *et al.* 2010).

Mitochondrial (mt) DNA phylogeographic patterns among elephantids are often discordant with those of nuclear DNA markers or morphology (Debruyne 2005; Roca *et al.* 2005; Enk *et al.* 2011; Ishida *et al.* 2011; Lei *et al.* 2011). Among African elephants, mtDNA forms two deeply distinctive clades, designated clades F and S (Debruyne 2005), originating in forest and savanna elephants, respectively (Ishida *et al.* 2011). Forest elephants do not carry S

clade mtDNA, while some nuclear alleles that are common or fixed across savanna elephants are not found in forest elephants (Roca *et al.* 2005; Ishida *et al.* 2011), suggesting that forest elephant populations may be completely or almost completely isolated from gene flow from savanna elephant populations. By contrast, many African savanna elephant individuals and populations are known to carry F clade mtDNA (Debruyne 2005; Roca *et al.* 2005; Lei *et al.* 2008), although almost all of these show no evidence of carrying forest elephant nuclear alleles (Roca *et al.* 2005; Lei *et al.* 2009). This mito-nuclear incongruence has suggested that species isolation mechanisms exist that largely prevent hybrid males, but not hybrid females, from reproducing successfully among savanna elephants (Roca *et al.* 2005; Roca & O'Brien 2005; Roca *et al.* 2007). Nonetheless, based on both simulations and empirical surveys of interspecies gene flow patterns (Currat *et al.* 2008; Petit & Excoffier 2009), male-mediated dispersal in savanna elephants (Archie *et al.* 2008) would be expected to facilitate the widespread introgression of mtDNA but not nuclear alleles from forest to savanna elephant populations.

Among Asian elephants (*Elephas maximus*), two deeply divergent mtDNA clades separated 1.6 to 2.1 Mya. These mtDNA clades do not appear to correspond to recognized subspecies subdivisions (Deraniyagala 1955), or to nuclear genetic subdivisions in Asian elephants (Lei *et al.* 2011), but may instead reflect the impact of Pleistocene glacial cycles on the climate and habitats of Asia (Fernando *et al.* 2000; Fleischer *et al.* 2001; Fernando *et al.* 2003; Vidya *et al.* 2009). In an extinct elephantid, the woolly mammoth (*Mammuthus primigenius*), two clades with ca. 1 to 2 Mya divergence have been detected (Gilbert *et al.* 2008). One clade appears to be more geographically restricted than the other, and to have gone extinct earlier (Gilbert *et al.* 2008). The two woolly mammoth mtDNA clades, and further subdivisions detected within them, have provided the framework for hypotheses involving the potential

effects of speciation events, migrations, habitat changes, and glacial cycles on woolly mammoths (Barnes *et al.* 2007; Debruyne *et al.* 2008; Gilbert *et al.* 2008; Miller *et al.* 2008). Recently, woolly mammoth mtDNA was found to have introgressed into a morphologically distinctive species, the Columbian mammoth (*Mammuthus columbi*) (Enk *et al.* 2011).

Sex differences in dispersal and in the variance of reproductive success have been well documented in living elephantids (Archie *et al.* 2007; Hollister-Smith *et al.* 2007; Roca *et al.* 2007; Poole *et al.* 2011). Elephant females reaching maturity remain with their natal core social group or “herd” (Archie *et al.* 2007; Hollister-Smith *et al.* 2007) and females do not typically migrate between herds (Archie *et al.* 2007; Hollister-Smith *et al.* 2007), thus the mitochondrial genome is necessarily coupled to the geographic range of the core social group. By contrast, males leave the natal herd and mediate gene flow between herds and across the landscape (Archie *et al.* 2007; Hollister-Smith *et al.* 2007; Roca *et al.* 2007; Poole *et al.* 2011). Furthermore, male-male competition is intense, and in any generation only a small proportion of males are reproductively successful (Archie *et al.* 2007; Hollister-Smith *et al.* 2007; Rasmussen *et al.* 2008). We have previously formulated the hypothesis that these well-documented sex differences in elephants have enabled the observed phylogeographic incongruence in mitochondrial patterns (Ishida *et al.* 2011).

Since lower female dispersal and higher variance in male reproductive success would have increased mtDNA effective population sizes relative to those of nuclear loci (Hoelzer 1997; Hedrick 2007), then if the matrilineal and matrilineal social structure and intense male reproductive competition are responsible for the observed mito-nuclear incongruence (Ishida *et al.* 2011), one would predict that the ratio of coalescent dates for mtDNA relative to those of nuclear loci would have a value greater than the 0.25 expected in populations without sex

differences in dispersal or in variance in reproductive success. Here, we examine the coalescent dates of mtDNA and nuclear loci in elephantids to test our expectation that sex differences in dispersal and in the variance of reproductive success have led to disproportionately high mtDNA effective population sizes and coalescent dates relative to those of nuclear markers. We generated full mitochondrial genome sequences for *Loxodonta cyclotis*, sequencing two forest elephants that carried haplotypes corresponding to the two most divergent mtDNA subclades within the species (Debruyne 2005; Roca *et al.* 2005). We aligned them to mitogenomes representing the most divergent mtDNA clades within each of the other elephantid lineages. We examined the relationships of forest elephants to other elephantid taxa, and estimated divergence and coalescent dates using fossil calibrations. We contrasted the ratio of mtDNA to nuclear coalescent dates within and across elephantid taxa. Finally, we compared our date estimates to the timing of climate transitions, finding that elephantid evolution in Africa may have been driven by drier climates at the end of the Miocene and by the initiation of glacial cycles at the start of the Pleistocene.

Materials and Methods

Elephant samples and DNA extraction. The study was conducted in compliance with the University of Illinois Institutional Animal Care and Use Committed (IACUC) approved protocol number 09036. Samples were obtained in full compliance with required CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) and other permits. Two forest elephant DNA samples were used. One was extracted from tissue collected using a biopsy dart from a female forest elephant (designated Lcy-DS1534 or DS1534) in the Dzanga Sangha

Forest Reserve of the Central African Republic (Georgiadis *et al.* 1994). The other was extracted from a blood sample of “Coco,” a male forest elephant (*Loxodonta cyclotis*) from Sierra Leone, designated Lcy-SL0001 or SL0001, generously provided by the Paris Zoo (Parc Zoologique de Paris-Vincennes, France). Nuclear DNA sequences had previously established that both individuals were forest elephants (Roca *et al.* 2001; Roca *et al.* 2005; Capelli *et al.* 2006; Ishida *et al.* 2011); mtDNA sequences had established that SL0001 was from a sub-clade of mtDNA found in West Africa (Barriel *et al.* 1999; Eggert *et al.* 2002; Debruyne *et al.* 2003; Debruyne 2005). DNA was extracted using a kit from Qiagen (SL0001), or a standard phenol-chloroform method (DS1534) (Sambrook *et al.* 1989).

Oligonucleotide primers. The mitochondrial genome was amplified in eight long overlapping fragments. We designed eight PCR primer pairs (Supporting information Table S1) using DNA sequences identified as being conserved (Murphy & O'Brien 2007) between the mtDNA genomes of the elephant (Hauf *et al.* 1999) and the armadillo (Arnason *et al.* 1999), or as being conserved across published elephantid sequences. In addition to the PCR primers, within each of the amplified regions we designed a set of additional sequencing primers to produce forward or reverse sequences within each of the eight amplified segments (Supporting information Table S1); together these sets generated overlapping sequences spanning the entire mitochondrial genome, except for a repetitive non-coding region, containing a variable number of tandem repeats (VNTR), that has also not been reported for most previously generated proboscidean mitogenomes (Rogaev *et al.* 2006; Gilbert *et al.* 2007; Gilbert *et al.* 2008).

PCR and sequencing. Genomic DNA (ca. 50 ng) underwent amplification by PCR in a 25 µl reaction volume containing 1x GeneAmp PCR buffer II, 1.5 mM MgCl₂, 0.2 mM each of the four deoxyribonucleoside 5'-triphosphates (dATP, dCTP, dGTP, and dTTP), 0.04 unit/µl

AmpliTaq-GOLD DNA Polymerase (Applied Biosystems Inc. [ABI]), and 0.4 uM final concentration of each oligonucleotide primer. PCR was run with an initial step of 95°C for 9:45 min; with cycles of 20 sec at 94°C; followed by 30 sec at 60°C (first 3 cycles), 58°C (next 5 cycles), 56°C (5 cycles), 54°C (5 cycles), 52°C (5 cycles), or 50°C (final 22 cycles); followed by 3 min extension at 72°C; with a final extension after the last cycle of 7 min at 72°C. PCR products were enzyme-purified (Hanke & Wink 1994) and sequenced using the BigDye Terminator system (ABI). Extension products were purified using Sephadex G-50 (Amersham) and resolved on an ABI 3700 DNA Sequencer, or on ABI 3730xl capillary systems. The software *Sequencher* (Gene Codes Corporation) was used to trim and concatenate sequences and confirm open reading frames. DNA sequences have been deposited in Genbank (accession numbers: JN673263 and JN673264).

Avoidance of nuclear DNA sequences of mitochondrial origin (numts). Steps were taken to minimize the possibility of amplifying numts. Primers were designed for conserved regions (see above), which would minimize the risk of mismatches in the target mtDNA versus the primers. Amplicons were several thousand bases in length, which would avoid those numts that were shorter. Obvious indicators of numts were not observed: PCR did not produce multiple bands; there were no sites that appeared heteroplasmic with secondary peaks; open reading frames were present in all coding regions (see Supporting information Figure S1)--these would have been disrupted in many numts; sequences overlapping between amplicons were identical in the region of overlap, minimizing the possibility that primer mismatches had led to selective amplification of numts over cytoplasmic mtDNA.

Sequence alignments. Three mtDNA genome sequences have been generated for S clade African savanna elephants (Hauf *et al.* 1999; Rogaev *et al.* 2006; Murata *et al.* 2009), along with

three for Asian elephants (Rogaev *et al.* 2006; Maikaew *et al.* 2007; Arnason *et al.* 2008), nineteen for woolly mammoths (Krause *et al.* 2006; Rogaev *et al.* 2006; Gilbert *et al.* 2007; Gilbert *et al.* 2008), and one Columbian mammoth mitogenome (Enk *et al.* 2011). The mtDNA genome has also been sequenced for one proboscidean that is outside of the family Elephantidae, the extinct American mastodon (*Mammot americanum*), which diverged from the lineage leading to elephantids some 24-30 Mya (Rohland *et al.* 2007; Rasmussen & Gutierrez 2009; Rohland *et al.* 2010; Sanders *et al.* 2010). We retrieved complete mtDNA genomic sequences from GenBank for seven elephantid individuals, including three African savanna elephants (GenBank: DQ316069, NC000934, and AB443879) (Hauf *et al.* 1999; Rogaev *et al.* 2006; Murata *et al.* 2009), two Asian elephants (GenBank: NC005129.2 and AJ428946) (Rogaev *et al.* 2006; Arnason *et al.* 2008) and two woolly mammoths (GenBank: NC007596.2 and EU153453) (Krause *et al.* 2006; Gilbert *et al.* 2008). For some analyses, the mitogenomic sequence of an American mastodon (Genbank: NC_009574) (Rohland *et al.* 2007) was also included. Published genomes were aligned with the two newly generated African forest elephant sequences using the software CLUSTALW 2.0 (Larkin *et al.* 2007) in EBI Web Services (McWilliam *et al.* 2009); alignment output was visually inspected. The control region was excluded from analyses since it is subject to greater saturation and is less reliable as a molecular clock relative than other mtDNA regions (Ingman *et al.* 2000). Mismatches between the mtDNA genomes were visualized using the software Geneious (Drummond *et al.* 2010).

Phylogenetic analyses. The Akaike Information Criterion (AIC) (Akaike 1974) was implemented using the software Modeltest 3.06 (Posada & Crandall 1998) to estimate the model of DNA sequence evolution that best fit the data. This was found to be the Tamura-Nei model with invariant site heterogeneity (TrN+I), with the following parameters: Base (base frequencies

for A, C and G, with T inferred) = (0.3286 0.2523 0.1358); *Nst* (number of substitution types listed in a rate matrix) = 6 (the number of unique substitution types for this model is 3; PAUP* specifies this using a matrix with six values, four of which are identical); Rmat (rate matrix) = (1.0000 38.7425 1.0000 1.0000 55.3986 1.0000); Rates (distribution of rates at variable sites) = equal; and Pinvar (proportion of invariant sites) = 0.7045. These parameter values were used in PAUP*4.0b10 (Swofford 2002) for Neighbor Joining (NJ), minimum evolution (ME), and maximum likelihood (ML) phylogenetic methods. PAUP* was also used to infer a maximum parsimony (MP) tree. For all phylogenetic methods, exhaustive searches were conducted. Bootstrap resampling support was based on at least 100 replicates, in a full heuristic search with starting trees obtained by random stepwise addition and tree bisection-reconnection branch swapping for MP, ME and ML analyses. Bayesian phylogenetic inference was performed using BEAST v1.5.4 software (Drummond & Rambaut 2007), using the TN93 + I model of nucleotide substitution, determined using the AIC (Akaike 1974). Maximum parsimony and maximum likelihood analyses were also run using a partitioned genome (protein coding genes, rRNA+tRNA genes, H strand and L strand genes), with the AIC (Akaike 1974) used to select the model of evolution for each partition using Modeltest 3.06 (Posada & Crandall 1998). A dataset was run consisting of the entire mitogenome (excluding control region), but with each partition allowed to follow a different model of evolution; each partition was also run separately (Supporting information Text S1). In all cases inter-individual relationships were identical in all phylogenies (Supporting information Text S1).

Molecular dating. Nodal divergence dates within the tree were estimated using the software BEAST (Drummond & Rambaut 2007), with fossil estimates used as prior dates for calibration. We utilized an uncorrelated lognormal relaxed molecular clock model, which

permits the rate of molecular substitution to be uncorrelated across the tree while incorporating uncertainty in both tree topology and multiple fossil calibrations (Drummond *et al.* 2006). Fossil calibration priors were incorporated into the clock model as a normal distribution representing soft bounds (5th and 95th percentile corresponding to the minimum and maximum dates respectively), since maximum fossil calibration dates incorporated uncertainty in the fossil record (see below) (Forest 2009). We used a randomly generated starting tree. Posterior distributions were obtained by Markov chain Monte Carlo (MCMC) sampling from a total of 10,000,000 steps, with a discarded burn-in of 1,000,000; samples were drawn every 1,000 MCMC steps. Acceptable mixing and convergence to the stationary distribution were verified by inspection and plotting of posterior samples. Effective sample size values were above 200 for all parameters.

Two sets of prior date calibrations were used. The fossil calibration dates listed below were applied to one dataset that included only elephantid sequences and calibration dates, and then applied to a second dataset that included the American mastodon mitogenome as the outgroup, and also used a mastodon-elephantid calibration in addition to the elephantid calibration priors. Thus we performed a total of four analyses, two with only the elephantid mitogenomes and two with the mastodon mitogenome included as the outgroup. The two sets of prior dates used as calibrations were as follows:

(i) Our “broad” fossil calibration used the same fossil date range estimates as Rohland *et al.* (2010), except that no prior date estimates were used for the divergence between African elephant F and S mtDNA clades. Rohland *et al.* (2010) set the date range for the mastodon-elephantid divergence as 24-30 Mya. The minimum was based on Sanders *et al.* (2010), who mention the Rasmussen and Gutierrez (2009) report of fauna from the Eragaleit Beds of

Lothidok as including the earliest definitively known mammutid, dated to between >24-27 Mya (Rasmussen & Gutierrez 2009). The maximum was also based on Sanders et al. (2010), who mention a specimen from Chilga, Ethiopia (28-27 Ma) described as having mammutid features superimposed on an otherwise palaeomastodont-type molar. To this and to other estimates of maximum dates was added 2 My, to account for difficulty in recognizing differences during an initial period of divergence, and to account for inadequate fossil sampling (Rohland *et al.* 2010). The 2 My also approximates the maximum (1.8 My) of averages for elephantid species duration that had been calculated by Maglio using six intervals of one million years of time, with 3 to 14 elephantid species present within each interval (Maglio 1973).

The range for the split between *Loxodonta* and Eurasian elephantids was set as 4.2-9 Mya (we use “Eurasian” to refer to the clade including Asian elephants and woolly mammoths, while recognizing that both *Elephas* and *Mammuthus* originated in Africa, and the woolly mammoth range included North America) (Rohland *et al.* 2010). The minimum of 4.2 Mya was based on Sanders et al. (2010), who list *Elephas ekorensis*, as “the most ancient unequivocal representative of the genus *Elephas*” (Sanders *et al.* 2010). Among locations listed for this species, the oldest is 5.0-4.2 Mya (Sanders *et al.* 2010). This minimum also assumes that *Loxodonta* is paraphyletic. The maximum of 9 Mya does not assume that *Loxodonta* is paraphyletic, and is based on the older bound (7 Mya) of the age estimate of 6-7 Mya for the oldest *Loxodonta* fossil (Vignaud *et al.* 2002; Sanders *et al.* 2010), to which was added 2 Mya to the maximum date for the reasons noted above (Rohland *et al.* 2010).

The range for the split between *Elephas* and *Mammuthus* was set at 3-8.5 Mya (Rohland *et al.* 2010). Although the earliest reported fossil mammoth is *Mammuthus subplanifrons*, Sanders et al. (2010) state that “in the absence of associated crania, however, there is no certainty

that this species is a mammoth”, and that *M. africanavus* is “the earliest unambiguous evidence of the genus *Mammuthus* in Africa”. In the associated table in Sanders et al. (2010), the latter species is listed as mid-late Pliocene, with dated locales at 3.5-3.0 Mya (Sanders et al. 2010). Rohland et al. therefore took 3 Mya as the minimum date for the *Elephas-Mammuthus* divergence, which also assumes that *Elephas* is paraphyletic (Rohland et al. 2010). The maximum date for the split did not assume paraphyly for the taxa. For the questionable *M. subplanifrons*, the oldest date listed by Sanders et al. (2010) for any date range at a locale is 6 Mya, but there is an older “*Elephas nawataensis*” that dates to 4.2-6.5 Mya (Leakey & Harris 2003). Although Sanders et al. (2010) suggest that this taxon may be mis-assigned to the genus *Elephas* (Rohland et al. 2010), it was nonetheless included by Rohland et al. (2010) as the oldest potential *Elephas* fossil identified in the literature; adding 2 Mya to the maximum date for the reasons noted above provided the 8.5 Mya maximum (Rohland et al. 2010).

(ii) Our “narrow” fossil calibration range reduced the range of fossil date estimates in two ways. First we removed from consideration *Elephas nawataensis* and *Mammuthus subplanifrons* since Sanders et al. (2010) considered them to be potentially mis-assigned to their respective genera. Second, we considered all genera to be monophyletic, consistent with several fossil phylogenies (Maglio 1973; Shoshani & Tassy 1996). This made the minimum date estimate for the *Loxodonta*-Eurasian split equal to the lower bound of the age attributed to the oldest *Loxodonta* fossil, or 6 Mya (Vignaud et al. 2002; Sanders et al. 2010), while the maximum date for this divergence remained the same as for the “broad” fossil calibration. For the divergence between *Elephas* and *Mammuthus*, the oldest fossil recognized by Sanders et al. (2010) as definitely assigned to either genus is *Elephas ekorensis*. Among locations listed for this species, the oldest is 4.2-5.0 Mya (Sanders et al. 2010). Thus the maximum date for the calibration of the

split between *Elephas* and *Mammuthus* is 7.0 Mya (including two million years added for reasons noted above). The assumption of monophyly would mean that this is also the fossil used to set a date minimum for the *Elephas-Mammuthus* split, of 4.2 Mya (Sanders *et al.* 2010). The “narrow” fossil calibration uses the same calibration dates as the “broad” fossil calibration for the mastodon-elephantid split (for the dataset that included a mastodon mitogenome) (Rohland *et al.* 2010). Like the “broad” fossil calibration, it used no prior estimate for the divergence between F and S African elephant mtDNA clades.

Results

Forest elephant mitochondrial genomes

For the two forest elephants, we sequenced all 13 coding genes of the mtDNA genome, along with the ribosomal RNAs and transfer RNAs, and part of the control region. We sequenced 16,028 bp of mtDNA for forest elephant DS1534 and 16,156 bp of mtDNA for forest elephant SL0001. We generated an alignment of the two forest elephant sequences with the mitochondrial genomes of other elephantids, including all three previously sequenced mitogenomes for S clade savanna elephants. We also aligned two Asian elephant and two woolly mammoth mtDNA genomic sequences representing the deepest subdivisions present across the mtDNAs of those species (Fernando *et al.* 2000; Fleischer *et al.* 2001; Krause *et al.* 2006; Gilbert *et al.* 2008; Vidya *et al.* 2009). Although the mitogenome of a Columbian mammoth (*Mammuthus columbi*) has been recently sequenced, we did not include it in our alignment because the Columbian mammoth mitogenome had originated in the woolly mammoth lineage, and been transferred between the two species through hybridization (Enk *et al.* 2011).

A comparison of nucleotide differences across elephantid lineages is shown in Figure 1. The top panel compares elephantid mitochondrial genomes to that of forest elephant DS1534, while the bottom panel compares them to the reference savanna elephant S clade mitochondrial genome (Hauf *et al.* 1999). The mitogenomes of the *Loxodonta* individuals shown are representative of the deepest divergences present within the F and the S mtDNA clades (Debruyne 2005; Roca *et al.* 2005; Lei *et al.* 2008; Murata *et al.* 2009). It is evident that the differences between the two forest elephant F clade genomes (Figure 1, top panel) are greater than the differences that exist across the savanna elephant S clade mitochondrial genomes (Figure 1, bottom panel), consistent with an older coalescent date for F clade than for S clade mitogenomes (see below).

Phylogenetic analyses and molecular dating

Phylogenetic relationships across lineages were inferred using maximum parsimony, Neighbor-Joining, minimum evolution and maximum likelihood methods. All methods inferred the same relationships among the elephantid mitogenomes. For all methods, all nodes were supported with 100% bootstrap support. A Bayesian approach inferred the same relationships across the phylogeny (Figure 2) as the other methods, with a Bayesian posterior probability of 1.00 for all nodes. The results were consistent with previous molecular studies showing that woolly mammoths are closer to Asian elephants than to African elephants (Krause *et al.* 2006; Rogaeu *et al.* 2006; Maikaew *et al.* 2007; Rohland *et al.* 2007; Murata *et al.* 2009; Rohland *et al.* 2010). Deep divergence was also inferred for the split between the F and S clade mtDNA lineages in African elephants (Figure 2).

To estimate divergence dates, we used two sets of fossil calibration dates for nodes on the elephantid phylogeny (Table 1). Using the “broad” fossil calibration dates (see above, and Table 1), the divergence between *Loxodonta* and the other elephantids was estimated as occurring 6.06 (95% CI 3.92-8.50) Mya, while the date for the *Elephas-Mammuthus* divergence was estimated as 5.42 (95% CI 3.40-7.62) Mya (Table 1). The divergence between the forest elephant F clade mitochondrial genomes and the S clade savanna elephant genomes was estimated as 4.86 (95% CI 2.96-6.90) Mya, almost as deep as the split between Asian elephant and woolly mammoth mitogenomes (Table 1). Using the “narrow” range of fossil calibration dates (see above, and Table 1), the divergence between *Loxodonta* and the other elephantids was estimated as 6.81 (95% CI 5.43-8.23) Mya (Table 1, Figure 2), while the *Elephas-Mammuthus* divergence was estimated as 6.01 (95% CI 4.71-7.17) Mya (Table 1, Figure 2). The divergence between African elephant F and S clades was estimated as 5.51 (95% CI 4.26-7.24) Mya, or about 92% of the divergence time estimate for the mammoth-Asian elephant split (Table 1, Figure 2).

While it had been appropriate to use the mastodon as an outgroup for inferring divergence dates among elephantid genera (Rohland *et al.* 2007; Roca 2008; Rohland *et al.* 2010), we thought that for inferring the divergence between the African elephant F and S clades, fossil calibration dates for *Loxodonta*, *Elephas* and *Mammuthus* represented more recent and thus more appropriate outgroups. Nonetheless, we considered and tested whether the addition of the mastodon mitochondrial genome (Rohland *et al.* 2007) to the elephantid mitogenomic dataset might have a great effect on divergence date estimates for the elephantids. Adding the mastodon would have the benefit of polarizing the polymorphisms present across elephantids by identifying potential ancestral character states for nucleotides. This could potentially improve the accuracy of date estimates by rooting the crown of the elephantid tree. Yet when the mastodon

mitogenome and calibration dates (Table 1) were included in the analyses, there was no effect on the relative estimates for the split between African elephant F and S clades as a proportion of the estimate for the split between Asian elephants and mammoth: the ratio was 0.92 when the mastodon was not included and 0.92 when the mastodon was included (Table 1), using the narrow fossil calibration dates. Thus rooting with the mastodon appears to have left divergence ratios unchanged.

Within-taxon coalescent dates

The estimate for the F clade coalescent date was 2.57 (95% CI 2.07-3.15) Mya for the narrow fossil calibration priors when the mastodon mitogenome was included in the alignment and the mastodon-elephantid calibration date was used along with fossil calibration dates for elephantid genera (Table 1). This compared to 2.43 (95% CI 1.68-3.38) Mya estimated using the narrow fossil date priors as the F clade coalescent date with only elephantids used in the alignment or as fossil calibrations (Table 1, Figure 2). Both estimates place the initial divergence of crown group forest elephant mitogenomes as occurring near the start of the Pleistocene.

Our mtDNA coalescent estimates (Table 2) were compared to the within-taxon coalescent estimates for nuclear loci determined by Rohland et al (2010). Our mtDNA coalescent estimates were based on the most diverse mtDNA clades within each lineage. Our mtDNA coalescent estimates are likely to accurately reflect basal within-lineage coalescent dates, and not represent inadequate sampling, since sampling for all of the mtDNA lineages involved has been quite extensive, involving dozens of locations and up to hundreds of individuals from across the entire range of each taxon (Fernando *et al.* 2000; Fleischer *et al.* 2001; Krause *et al.* 2006; Gilbert *et al.* 2008; Vidya *et al.* 2009; Ishida *et al.* 2011). Likewise, nuclear coalescent estimates

are likely to be accurate since they had been calculated for each taxon using 375 loci (Rohland *et al.* 2010). In order to account for different assumptions or methods used to generate the coalescent estimates, we normalized the intra-taxon coalescent dates as a percent of the divergence estimated between Asian and African elephants (Table 2). In populations with random progeny production and no sex difference in dispersal, mtDNA is expected to have an effective population size (and hence coalescent date) one-fourth that of nuclear loci (Hedrick 2007). Yet for each elephantid taxon, the relative coalescent for mtDNA was much higher than 0.25, and in each case greater than the coalescent for nuclear DNA (Table 2). The most extreme value was estimated for the woolly mammoth, for which the coalescent, standardized to the Asian-African elephant divergence, was more than twice as old for mtDNA as for nuclear loci (Table 2). We also compared the mtDNA coalescent to the coalescent of nuclear loci that were two standard deviations above the mean nuclear coalescent estimate. The mtDNA:nuclear coalescent ratio was much higher than 0.25 even when the coalescent for the mtDNA was compared to the estimated coalescent for nuclear loci two standard deviations above the mean (Table 2).

Discussion

For a number of elephantid taxa, mtDNA phylogeographic patterns appear to be incongruent with nuclear genetic or morphological patterns (Debruyne 2005; Roca *et al.* 2005; Roca *et al.* 2007; Lei *et al.* 2008; Lei *et al.* 2009; Enk *et al.* 2011; Ishida *et al.* 2011; Lei *et al.* 2011). One hypothesis has attributed this incongruence to sex differences in reproductive competition and dispersal (Ishida *et al.* 2011). One prediction based on this hypothesis would be that the ratio of mtDNA coalescent dates to nuclear coalescent dates should be much greater than

the 0.25 expected in populations with random progeny production and no sex difference in dispersal. In elephantids, both low female dispersal and high male variance in reproductive success would tend to increase mtDNA effective population sizes relative to those of nuclear loci (Hoelzer 1997; Hedrick 2007).

Using within-taxon coalescent estimates for nuclear markers estimated by Rohland *et al.* (2010), we were able to determine that mtDNA coalescent dates were indeed older than those of nuclear loci (Table 2). The coalescent data estimates for nuclear data are likely to accurately reflect values for neutral loci in the nuclear genome since: (1) sequences had been randomly generated, so were likely to be non-coding loci not under selection; and (2) repetitive elements had been screened out, so that sequences across taxa were likely to represent orthologous and not paralogous loci (Rohland *et al.* 2010). Elephantid mtDNA coalescent dates were very high relative to nuclear coalescent dates even when the mtDNA coalescent was compared to the estimated coalescent for nuclear loci that are two standard deviations above the mean coalescent (Table 2). Thus our study provides support for the hypothesis that among elephantids coalescent dates for mtDNA relative to nuclear DNA would be much older than the 0.25 expected in populations with random progeny production and no sex difference in dispersal. The relatively ancient mtDNA coalescent dates are consistent with lower female than male dispersal and reproductive competition in elephantids, which would allow mtDNA genetic patterns to locally persist even as male dispersal would disrupt any geographic subdivisions among nuclear alleles (Wright 1943; Petit & Excoffier 2009; Ishida *et al.* 2011).

The effects of sex differences in reproductive success on effective population size (N_e) have been previously quantified (Hoelzer 1997; Hedrick 2007). As male effective population sizes are reduced, N_e drops for nuclear loci (Hoelzer 1997; Hedrick 2007). The effective

population size becomes equal for autosomal, X-linked and mtDNA loci when the male effective population size is reduced to one-seventh that for females ($N_{em} = N_{ef}/7$), i.e., when the effective population size for males comprises one-eighth that of the total effective population size of males and females combined (Hoelzer 1997; Hedrick 2007). Such a ratio could be consistent with the lack of reproductive success documented for a large proportion of males in field studies of savanna elephants (Hollister-Smith *et al.* 2007; Poole *et al.* 2011). For example, in a 22-year study at Amboseli National Park, of 89 male elephants genotyped, 53 (60%) were not found to have sired a calf; while 30% of the 119 calves examined had been fathered by just three males (Hollister-Smith *et al.* 2007). Male savanna elephants represent the extreme among mammals in the extent to which high mating and paternity success occur late in life (Hollister-Smith *et al.* 2007). Age-specific paternity peaks at 45-53 years of age (Hollister-Smith *et al.* 2007). Since fewer than 10% of males are estimated to survive to age 50 (Poole *et al.* 2011), a high proportion of males are not reproductively successful, and this high variance in reproductive success would reduce the effective population size of nuclear markers (Hoelzer 1997; Hedrick 2007).

Likewise, the lower degree of dispersal for female than male elephants would be expected to increase the effective population size and coalescent of mtDNA relative to those of nuclear loci (Wright 1943; Hoelzer 1997; Ishida *et al.* 2011). The interspecies transfer of F clade mtDNA from forest to savanna elephant populations (Debruyne 2005; Roca *et al.* 2005; Ishida *et al.* 2011) was also likely enhanced by low rates of female dispersal (Petit & Excoffier 2009), although we could disregard the effects of hybridization by considering only S clade coalescent dates among savanna elephants. When dispersal rates for males are high while females exhibit high matrilocality, the sex-biased dispersal can increase the effective population size of mtDNA

relative to nuclear DNA even to the point of causing the coalescent of mtDNA to exceed that of nuclear loci (Hoelzer 1997).

For both nuclear and mtDNA loci, coalescent estimates are low for the savanna elephant (low diversity and effective population sizes relative to other taxa); high for the forest elephant (highest diversity and effective population size); and intermediate for the Asian elephant (intermediate diversity and effective population size) (Table 2) (Rohland *et al.* 2010). Thus the rank order of genetic diversity for these three lineages was the same for mtDNA and nuclear markers (Table 2). The savanna elephant has the lowest nuclear coalescent among the elephantids (Rohland *et al.* 2010), and the mtDNA coalescent date for the savanna elephant S clade was also low relative to other taxa (Table 2). A number of authorities have suggested that the geographic range and numbers of savanna elephants expanded towards the end of the Pleistocene following the extinction of *Elephas*, which had been the predominant elephantid on the African savannas until that time (Maglio 1973; Kingdon 1979; Sanders *et al.* 2010). The relatively low coalescent values for both nuclear and S clade mtDNA in the savanna elephant (relative to other taxa) could reflect this proposed Late Pleistocene founder effect for the savanna elephant. Simulations and empirical data have shown that the interspecies transfer of alleles to a species is expected to occur as its dispersing sex colonizes a region occupied by the other species, with the other species contributing alleles transmitted by the non-dispersing sex to the colonizing species (Currat *et al.* 2008; Petit & Excoffier 2009). The transfer of F clade mtDNA from forest to savanna elephants would be consistent with this expectation, if male savanna elephants had expanded their range into formerly forest elephant habitats (Roca *et al.* 2005). The F clade mtDNA sequences shared by forest and savanna species are quite similar (Eggert *et al.*

2002; Roca *et al.* 2005), indicating that the interspecies transfer would be consistent with a Late Pleistocene event (Murata *et al.* 2009).

For the woolly mammoth, the level of nuclear diversity was low and comparable to that of savanna elephants. Yet the mtDNA coalescent date was much older for mammoths than for S clade savanna elephants (Table 2). The low nuclear genetic diversity present in the woolly mammoth could reflect high (relative to other species) levels of male reproductive competition (Rohland *et al.* 2010). Male-male competition often also leads to the evolution of sexual size dimorphism (Weckerly 1998), which is also observed in mammoths, with males about one meter taller than females (Haynes 1991). The woolly mammoth mitochondrial coalescent seems especially ancient relative to the nuclear coalescent estimate (Table 2), and the mammoth was the only species for which the mtDNA coalescent estimate (Table 1, Figure 2) was more than twice as old as those previously reported for nuclear loci (Rohland *et al.* 2010). This pattern may reflect a more ancient widespread distribution for this species, its survival in multiple glacial refugia, or the effects of the distribution of woolly mammoths across two continents that were only intermittently connected during Pleistocene glacial cycles (Barnes *et al.* 2007; Debruyne *et al.* 2008; Gilbert *et al.* 2008; Miller *et al.* 2008). These factors would be more likely to affect mtDNA than nuclear genetic patterns among elephantids since (1) male-male competition would lower effective population size for nuclear loci, reducing overall nuclear genetic diversity but not affecting mtDNA diversity, and (2) male-mediated gene flow would tend to diminish nuclear genetic differences across populations, while not affecting mtDNA differences across populations.

As part of this study, we conducted the first full sequencing, to our knowledge, of mitogenomes from forest elephants. These were, exclusive of the control region, 15,418 bp in

length for DS1534, and 15,420 bp for SL0001. The size difference was due to an insertion of 2 bp in the 12S rRNA of SL0001, not found in any other proboscidean mitogenome. The previously published savanna elephant S clade mitochondrial genome reference sequence was 15,418 bp in length (exclusive of control region) (Hauf *et al.* 1999). Since some savanna elephants carry F clade mitochondrial genomes (Debruyne 2005; Roca *et al.* 2005), savanna elephant nuclear genes involved in mitochondrial function would appear to be capable of interacting with mtDNA-coded proteins whether these derive from F clade or S clade mitochondrial genomes. Thus amino acid differences between forest F and previously sequenced savanna S clade mtDNAs (Supporting information Figure S1) would not be expected to have led to greatly altered protein function (McKenzie *et al.* 2003), although an alternative is possible. Genetic markers carried only by the non-dispersing sex are expected to be less subject to the effects of selection (Petit & Excoffier 2009). This is somewhat paradoxical, given that the higher effective population sizes among such markers (Wright 1943; Hoelzer 1997) would make fewer mutations effectively neutral (Ohta 1973). However, limited gene flow can preclude selective sweeps within species at loci carried only by the non-dispersing sex (Petit & Excoffier 2009). This may suggest that F clade mitogenomes in savanna elephants could persist even should they lead to somewhat lower levels of fitness than S clade mitogenomes.

Our results also confirmed previous reports that mammoths are closer to living Asian elephants than to African elephants (Krause *et al.* 2006; Rogaev *et al.* 2006; Maikaew *et al.* 2007; Rohland *et al.* 2007; Rohland *et al.* 2010). The split between *Elephas* and *Mammuthus* occurred 6.0 (95% CI 4.71-7.17) Mya, while *Loxodonta* F and S clades diverged 5.5 (95% CI 4.26-7.24) Mya (Figure 2). Our estimate for the divergence of F clade and S clade African elephant mitochondrial lineages is comparable to or somewhat older than previous estimates for

the divergence between forest and savanna elephants (Roca *et al.* 2001; Roca *et al.* 2005; Rohland *et al.* 2007; Murata *et al.* 2009), and suggests that the four major elephantid mtDNA lineages were established around the end of the Miocene Epoch. In Africa during this period there were cooler and more arid climates, changes in vegetation marked by a reduction in forests, and shifts in habitat zones (Cerling *et al.* 1997; Cerling *et al.* 1998; Maley 2001; Griffin 2002; Sepulchre *et al.* 2006; Bonnefille 2010; Otero *et al.* 2011). These changes may have driven the evolution not only of elephantid lineages but also of other modern mammalian lineages, including artiodactyls, perissodactyls, and primates including hominids (Cerling *et al.* 1998; Folinsbee & Brooks 2007; Roca 2008; Werdelin & Sanders 2010).

The basal divergence represented by the two sequenced forest elephant mtDNA genomes is estimated to have occurred 2.43 (95% CI 1.68-3.38) Mya, around the beginning of the Pleistocene Epoch 2.58 Mya (as recently defined to include the Gelasian Stage) (Gibbard & Head 2010). Divergence between humans and chimpanzees is comparable to the divergence across elephantid taxa, yet the coalescent date for all living human mitogenomes is under 200,000 years (Ingman *et al.* 2000). Previous studies have shown that mtDNA differences persist in elephants within and between geographic regions even in the face of changes in habitats or in nuclear genotypes (Roca *et al.* 2005; Roca & O'Brien 2005; Roca *et al.* 2007; Ishida *et al.* 2011). A close association of elephant mtDNA lineages with geographic region would be expected since females do not typically migrate away from natal social groups. Since lack of female dispersal increases the effective population size of mtDNA (Table 2), mtDNA lineages would be removed by genetic drift much less rapidly than would nuclear diversity, so that geographic partitions caused by ancient climate and habitat changes would be detectable in the mtDNA

phylogeographic patterns long after the nuclear phylogeographic partitions had been erased by male-mediate gene flow (Wright 1943; Hoelzer 1997; Petit & Excoffier 2009).

The deepest subdivision within the forest elephant F clade mtDNA could reflect the initial fragmentation of African tropical forest habitats during glacial cycles that began 2.5 Mya (Figure 2) (Maley 2001). With the caveat that locations and definitions of Pleistocene refugia for tropical forests remain controversial (Lowe *et al.* 2010), African tropical forest habitats are believed to have repeatedly fragmented during glacial cycles into a set of discontinuous refugia (Mayr & Ohara 1986; Maley 2001; DeMenocal 2004). Forest elephant populations within refugia may have been separated by intervening savanna habitats occupied by larger elephantid species (Maglio 1973; Kingdon 1979; Sanders *et al.* 2010). The two forest elephant mitogenomes sequenced were from an individual from the West African Guinean forest block and an individual from the Central African Congolian forest block, although it is not clear whether this deepest F clade subdivision was consequent to an east-west separation of the tropical forest, as currently occurs (White 1983; Salzmann & Hoelzmann 2005). Elephants carrying mtDNA haplotypes from each of the two deepest subdivisions of the F clade are currently present in populations in both West and Central Africa, an indication that some migration of elephant females across the two forest blocks occurred subsequent to the basal F-clade split (Debruyne *et al.* 2003). Also, given that gene flow in elephants is male-mediated and that the long-distance dispersal of forest elephant nuclear alleles is well documented (Roca *et al.* 2001; Roca *et al.* 2005), the partitioning of mtDNA would not necessarily imply the establishment or persistence of nuclear genetic differentiation between the two forest blocks (Roca *et al.* 2007; Ishida *et al.* 2011). Nonetheless, since the ancient coalescent for mtDNA was likely consequent to relatively low levels of competition and limited dispersal among matrilineal

females, elephant mtDNA would be likely to persistently record a phylogeographic signature of ancient climate transitions and habitat changes, such as occurred in Africa at the start of the Pleistocene.

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Conflict of interest:

The authors have declared that there is no conflict of interest.

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Data Accessibility:

DNA sequences: Genbank accession numbers *Loxodonta cyclotis* JN673263 and JN673264.

**Figure 1. Sequence differences found among the mitochondrial genomes of elephantids
with respect to African forest (LCY) or savanna (LAF) elephants.** Nucleotide comparisons
between a reference genome and those of other elephantids are shown by horizontal bars in the
top and bottom panels, with nucleotide matches represented by the white background,
substitutions indicated by black vertical lines, and indels by horizontal lines or dots. *Top panel:*

comparison of elephantid mtDNA genomic sequences to reference forest elephant DS1534. Deep within-species differences are evident in the comparison to SL0001. *Center panel*: nucleotide positions within the mtDNA genome and locations of mtDNA regions. The D-loop was excluded due to saturation of sites. *Lower panel*: comparison of elephantid mtDNA genomic sequences to reference savanna elephant NC_000934 (Hauf *et al.* 1999). Note that differences present across the savanna elephant S clade (DQ316069 and AB443879) are not as great as the differences across forest elephants shown in the top panel, reflecting the more recent coalescent date for savanna elephant S clade genomes. Panels were drawn using Geneious v5.4 (Drummond *et al.* 2010). Abbreviations: LCY: *Loxodonta cyclotis*, African forest elephant; LAF: *Loxodonta africana*, African savanna elephant; MPR: *Mammuthus primigenius*, woolly mammoth; EMA: *Elephas maximus*, Asian elephant.

Figure 2. Maximum clade probability tree inferred from the alignment of elephantid mitochondrial genome sequences. The software *BEAST* (Drummond & Rambaut 2007) was used to generate the tree, which is displayed as a chronogram. Shaded bars indicate the 95% highest posterior density as determined using two sets of calibrations. The top bar used the broadest range of calibrations based on fossil estimates (Rohland *et al.* 2010; Sanders *et al.* 2010); the lower bar relied on “narrow” set of calibration dates that excluded fossils identified as being of questionable assignment to genera by Sanders *et al.* (2010), and assumed the monophyly of elephantid genera (Maglio 1973; Shoshani & Tassy 1996; Rohland *et al.* 2010). The tree shown was based on the narrow fossil calibrations. The divergence date between forest elephant (F clade) and savanna elephant (S clade) mtDNA genomes was comparable to the divergence between Asian elephant and mammoth mtDNA genomes. For all four lineages, the two or three

840 genomes shown encompass the most basal within-lineage divergences; these indicate that forest
841 elephants have the deepest within-lineage crown-group coalescent date, while S clade savanna
842 elephants have the most recent mtDNA coalescent date. The posterior probability was 1.00 for
843 all nodes (leftmost number). The same relationships across mitogenomes were inferred in
844 PAUP* (Swofford 2002) using maximum parsimony (MP), Neighbor Joining (NJ), minimum
845 evolution (ME) and maximum likelihood (ML) methods (not shown). Bootstrap support was
846 100% for each method at each node (shown respectively from left to right, from the second
847 number at each node, for MP, NJ, ME and ML). Tree scores were: MP, 1611 variable sites, 1106
848 parsimony informative, 1 tree, length=1867, CI=0.876, RC=0.764; ME, 1 tree, score=0.12588;
849 ML, 1 tree, -ln likelihood=31041.20336. A time scale and geological epochs are shown below
850 the phylogeny. Africa in the late Miocene experienced drier climates and the retreat of forests
851 (Bonnefille 2010), and shifts in habitat zones during the Messinian (Griffin 2002). Shown also is
852 a Pliocene-Pleistocene stack of 57 globally distributed benthic $\delta^{18}\text{O}$ records, which measure
853 global ice volume and deep ocean temperature (lower values reflect lower temperatures), and
854 which illustrate the initiation of glacial cycles at the start of the Pleistocene (Lisiecki & Raymo
855 2005).

856

Figure 1

Table 1. Proboscidean fossil calibration dates and divergence time estimates, in millions of years

Calibration used:	Narrow Fossil ¹ <i>Median (95% CI)</i>	Broad Fossil ² <i>Median (95% CI)</i>
<i>Prior dates used to estimate divergence times</i>		
Elephantid-Mastodon	27.0 (24-30)	27.0 (24-30)
Loxodonta-Eurasian	7.5 (6-9)	6.6 (4.2-9)
Asian-Mammoth	5.6 (4.2-7)	5.75 (3-8.5)
Forest-Savanna	Not used	Not used
Monophyly of genera?	yes	no
<i>Divergence dates estimated using elephantid mitogenomes and priors only</i>		
Loxodonta-Eurasian	6.81 (5.43-8.23)	6.06 (3.92-8.50)
Asian-Mammoth	6.01 (4.71-7.17)	5.42 (3.40-7.62)
F clade-S clade	5.51 (4.26-7.24)	4.86 (2.96-6.90)
<i>Within-taxon coalescent dates estimated using elephantid mitogenomes and priors only</i>		
Woolly Mammoth	1.48 (0.96-2.03)	1.33 (0.78-2.08)
Asian Elephant	1.35 (0.90-1.84)	1.20 (0.66-1.84)
African Savanna S clade	0.91 (0.60-1.30)	0.80 (0.45-1.23)
African Forest F clade	2.43 (1.68-3.38)	2.13 (1.22-3.19)
<i>Divergence dates estimated by elephantid and mastodon mitogenomes and priors</i>		
Elephantid-Mastodon	25.35 (23.60-26.89)	26.19 (22.48-29.24)
Loxodonta-Eurasian	7.13 (6.29-8.06)	7.43 (6.06-8.61)
Asian-Mammoth	6.35 (5.57-7.22)	6.65 (5.39-7.80)
F clade-S clade	5.83 (4.94-6.71)	6.06 (4.85-7.17)
<i>Within-taxon coalescent dates estimated by elephantid and mastodon mitogenomes and priors</i>		
Woolly Mammoth	1.62 (1.28-2.05)	1.70 (1.20-2.17)
Asian Elephant	1.33 (1.00-1.65)	1.41 (1.07-1.79)
African Savanna S clade	0.76 (0.57-0.99)	0.81 (0.60-1.11)
African Forest F clade	2.57 (2.07-3.15)	2.69 (1.99-3.39)
African-Eurasian ratio ³ (elephantid priors only)	0.92	0.90
African-Eurasian ratio ⁴ (mastodon+elephantid priors)	0.92	0.91
F-S clade ratio ⁵ (with and without mastodon)	1.06	1.25

Footnotes:

¹Narrow date priors are fossil dates described in Rohland et al. (2010), based on Sanders et al. (2010), with questionable taxa removed and assuming monophyly

²Broad date priors are fossil dates described in Rohland et al. (2010), based on Sanders et al. (2010)

³Ratio: F clade-S clade to Asian-Mammoth divergence, estimated using only elephantid mitogenomes and priors

⁴Ratio: F clade-S clade to Asian-Mammoth divergence, estimated using elephantid and mastodon mitogenomes and priors

⁵Ratio: F clade-S clade divergence calculated using mastodon and elephantid mitogenomes and priors vs using only elephantid mitogenomes and priors

Table 2. Intra-taxon mtDNA and nuclear coalescence normalized by African-Asian elephant divergence

Taxon	mtDNA	95% CI	nuclear loci +/- 2 s.d.	mtDNA:nuclear*	mtDNA:nuclear+2s.d.
Forest elephant	36%	25-50%	30%	1.20	0.95
Savanna elephant (S clade)	13%	9-19%	8%	1.63	1.08
Woolly mammoth	22%	14-30%	9%	2.44	1.69
Asian elephant	20%	13-27%	15%	1.33	0.95

Nuclear estimates are from Rohland et al. (2010); s.d. is standard deviation
 *Expected ratio is 0.25 with random progeny production and no sex differences in dispersal

Forest elephant mitochondrial genomes reveal that elephantid diversification in Africa tracked climate transitions

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SUPPORTING INFORMATION

Supporting Information Text S1. Phylogenetic analysis of partitioned mitogenomes.

The full mitochondrial genome was partitioned into protein coding genes, rRNA+tRNA genes, H-strand genes and L-strand genes. The Akaike Information Criterion (AIC) (Akaike 1974) was implemented using the software Modeltest 3.06 (Posada & Crandall 1998) to estimate the model of DNA sequence evolution that best fit each partition. AIC parameter values were used in PAUP*4.0b10 (Swofford 2002) for maximum likelihood (ML) phylogenetic analysis, with each partition run individually. Although models cannot be applied to the maximum parsimony tree, PAUP* was also used to infer a maximum parsimony (MP) tree for each of the datasets. For both ML and MP methods, exhaustive searches were conducted and yielded topologies consistent with un-partitioned data; in each case relationships among the individuals on the tree were the same. Model parameters and tree statistics are as follows:

The model of evolution determined by Modeltest is listed here for each partition as follows: “Base” indicates the base frequencies for A, C and G, with T inferred (bases are “equal” when the model assumes frequencies are the same). “Nst” lists the number of substitution types listed in a rate matrix; the number of unique types may be inferred. This is followed by “Rmat”, the rate matrix. “Rates” indicates the distribution of rates at variable sites. “Pinvar” indicates the proportion of invariant sites:

For Protein coding genes: Tamura-Nei model with invariant site heterogeneity (TrN+I).
Base=(0.3278 0.2544 0.1307) Nst=6 (unique Nst=3) Rmat=(1.0000 39.8690 1.0000 1.0000
54.9781 1.0000) Rates=equal Pinvar=0.6883.

For rRNA and tRNA genes: Tamura-Nei model with invariant site heterogeneity (TrN+I).

Base=(0.3572 0.2134 0.1493) Nst=6 (unique Nst=3) Rmat=(1.0000 25.9384 1.0000 1.0000
56.4363 1.0000) Rates=equal Pinvar=0.8094

For H-strand: Tamura-Nei model with invariant site heterogeneity (TrN+I).

Base=(0.3271 0.2495 0.1336) Nst=6 (unique Nst=3) Rmat=(1.0000 38.1881 1.0000 1.0000
53.5181 1.0000) Rates=equal Pinvar=0.6923

For L-strand: Tamura-Nei model with invariant site heterogeneity (TrN+I).

Base=(0.3738 0.2753 0.1142) Nst=6 (unique Nst=3) Rmat=(1.0000 48.6352 1.0000 1.0000
89.7056 1.0000) Rates=equal Pinvar=0.7637

Tree scores were as follows for each partition:

For protein coding genes: MP, 1410 variable sites, 975 parsimony informative, 1 tree,
Length=1632, CI=0.878, RI=0.875, RC=0.768; ML, 1 tree, -ln likelihood=23929.12496.

For rRNA and tRNA genes: MP, 186 variable sites, 119 parsimony informative, 1 tree,
Length=216, CI=0.861, RI=0.859, RC=0.740; ML, 1 tree, -ln likelihood=6610.969593.

For H-strand: MP, 1505 variable sites, 1035 parsimony informative, 1 tree,
Length=1739, CI=0.879, RI=0.876, RC=0.771; ML, 1 tree, -ln likelihood=28913.41203.

For L-strand: MP, 93 variable sites, 61 parsimony informative, 1 tree, Length=111,
CI=0.838, RI=0.835, RC=0.699; ML, 1 tree, -ln likelihood=1924.21973.

The AIC parameter values were also used in BEAST (Drummond & Rambaut 2007) for Bayesian posterior probability estimation, using a dataset consisting of the entire mitogenome (excluding control region), but with each partition allowed to follow a different model of

evolution. Additional settings included the use of an uncorrelated lognormal relaxed molecular clock model and a randomly generated starting tree; all other parameters remained as default settings. Posterior distributions were obtained by Markov chain Monte Carlo (MCMC) sampling from a total of 10,000,000 steps, with a discarded burn-in of 1,000,000; samples were drawn every 1,000 MCMC steps. Acceptable mixing and convergence to the stationary distribution were verified by inspection and plotting of posterior samples. Effective sample size values were above 200 for parameters. Posterior probabilities were as follows:

<u>Node</u>	<u>Posterior Probability</u>
<i>Loxodonta</i> – Eurasian clade	1.00
Asian – mammoth clade	1.00
F mtDNA – S mtDNA clade	1.00
Woolly mammoth crown group	1.00
Asian elephant crown group	1.00
African savanna S clade	1.00
African forest F clade	1.00

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Table S1. Oligonucleotide primers used for PCR and sequencing of elephant mitochondrial DNA (mtDNA)

Primer name	Primer function	Primer sequence	mtDNA regions (full or partial):	PCR amplicon size (bp)
Set 1				
mtAlI-F1	PCR, Sequencing	CCCCCACGGGAWACAGCAG	12S ribosomal RNA	2282
mtAlI-F12	Sequencing	AAGTGAGCTTAATCATAACCCATGA	tRNA-Val	
mtAlI-F13	Sequencing	AATAGAGATAGTACCGCAAGGGAAT	16S ribosomal RNA	
mtAlI-F14	Sequencing	CACCTGCCTGCCCACTGACCAAG		
mtAlI-R1	PCR, Sequencing	GTCTGAACTCAGATCAGTAGGACT		
mtAlI-R11	Sequencing	CGGTCTGAACTCAGATCAGTAG		
mtAlI-R12	Sequencing	GAGACAGTTAAACCCCTCGTGTG		
mtAlI-R13	Sequencing	TGAATTTACTCGCTAATCAAGGTTGT		
Set 2				
mtAlI-F2	PCR, Sequencing	GACCTCGATGTTGGATCAGG	16S ribosomal RNA	2821
mtAlI-F21	Sequencing	CCCCAATTCTAGCCCTAACC	tRNA-Leu	
mtAlI-F22	Sequencing	GAAACTAACCGAGCCCCATT	NADH dehydrogenase subunit 1 (ND1)	
mtAlI-F23	Sequencing	CGAACCTAAACTCGAAGATTCAA	tRNA-Ile	
mtAlI-F24b	Sequencing	CACCTACACCAACACCAACCT	tRNA-Gln	
mtAlI-R2	PCR, Sequencing	TGCAAAATTCRAAGAAGCAG	tRNA-Met	
mtAlI-R21	Sequencing	AAATTATTGGGGTTAGGGGAAG	NADH dehydrogenase subunit 2 (ND2)	
mtAlI-R22	Sequencing	TGTTTTGTTTCATATTAGAGTTAGGG	tRNA-Trp	
mtAlI-R23	Sequencing	ATAATGGTGCTAATTTTTGTCAAGT	tRNA-Ala	
mtAlI-R24	Sequencing	AGTTGGTCGTATCGGAATCG	tRNA-Asn	
mtAlI-R25	Sequencing	TTTGATGAGGTTTGTGGGTAGA	tRNA-Cys	
mtAlI-R26	Sequencing	TTCGGGGTATGGGCCCGATAGC		
Set 3				
mtAlI-F3	PCR, Sequencing	AACTGGGCTCAATCTACTTCTCC	tRNA-Cys	2401
mtAlI-F31	Sequencing	TAATTGGAGGCTTTGGAACCTG	tRNA-Tyr	
mtAlI-F32	Sequencing	TCCCAGTTCTAGCAGCAGGTAT	Cytochrome c oxidase subunit I (COX1)	
mtAlI-F33	Sequencing	CTACCTTTCATGGCGGTAA	tRNA-Ser	
mtAlI-F34	Sequencing	CCTGCAGGAGGAGGAGACCCAAT		
mtAlI-R3	PCR, Sequencing	GGAATTGCRCTCKGTTTTAGACCT	tRNA-Asp	
mtAlI-R31	Sequencing	ACAAGACTAAGGAGCTAATAAGGAAAA	Cytochrome c oxidase subunit II (COX2)	
mtAlI-R32	Sequencing	TTAGAAGTAAATGCCTCTCAAATTAT		
mtAlI-R33	Sequencing	GTGATGTAGGACAATGTCTAGTGAAG		
mtAlI-R34b	Sequencing	TTGAGATTGCGATCCGTTAAT		
mtAlI-R36	Sequencing	AAGCTCGAGTGTCAACGTCTATGCC		
Set 4				
mtAlI-F4	PCR, Sequencing	CACCTCCAYGACCAYACCCTAATAAT	Cytochrome c oxidase subunit II (COX2)	2758
mtAlI-F41	Sequencing	CTGCGGAGCAAACCATAGC	tRNA-Lys	
mtAlI-F42	Sequencing	AAGAAGTCATGTGTTTTCCCTTG	ATP synthase F0 subunit 8 (ATP8)	
mtAlI-F43	Sequencing	AAATCTCACTAGCCCATCTTCTCC	ATP synthase F0 subunit 6 (ATP6)	
mtAlI-F45	Sequencing	TGGGCTGTCCCATCCCTAGGT	Cytochrome c oxidase subunit III (COX3)	
mtAlI-R4	PCR, Sequencing	TGAGTCGAAATCAYTTGTT	tRNA-Gly	
mtAlI-R41	Sequencing	TAAAGAAATAGGATCCTCATCAGTAAA	NADH dehydrogenase subunit 3 (ND3)	
mtAlI-R42	Sequencing	TGGTGAGCTCAGGTAATAGTCACTC	tRNA-Arg	
mtAlI-R43	Sequencing	AGATAATGCTCCGGTAAGAGGTC		
Set 5				
mtAlI-F5	PCR, Sequencing	GCCATCCAAGCTAATAATACAAGTCTA	NADH dehydrogenase subunit 3 (ND3)	2145
mtAlI-F51	Sequencing	CTCCAATACCTACGGACTAGACTACG	tRNA-Arg	
mtAlI-F52	Sequencing	ACATTATATTTGAAACAACGCTTATC	NADH dehydrogenase subunit 4L (ND4L)	
mtAlI-F53	Sequencing	AATACCTCTATATGGTCTCCACCTATG	NADH dehydrogenase subunit 4 (ND4)	
mtAlI-F54	Sequencing	TTTTAGCACGAGGCTTACAAC	tRNA-His	
mtAlI-R5	PCR, Sequencing	AAGCAATTTGTCTTGTGTTGTTGT	tRNA-Ser	
mtAlI-R51a	Sequencing	CGGGTAAATGAAGGTTTAATGG	tRNA-Leu	
mtAlI-R52	Sequencing	TGGTAATTCGTAGTATCCCATACCC	NADH dehydrogenase subunit 5 (ND5)	
mtAlI-R53	Sequencing	AGGAAGTATGACCTGCGCTTTA		
mtAlI-R54	Sequencing	TGTTAGAGGTAATCAGGCAAGG		
Set 6				
ND56-F1d	PCR, Sequencing	TGGTGCAACTCCARATAAAAGTAA	NADH dehydrogenase subunit 5 (ND5)	2149
ND56-3F2	Sequencing	AACAGAYGCTAACACAGCAGCTCT	NADH dehydrogenase subunit 6 (ND6)	
ND56-4F2	Sequencing	CCTAYATCAACACCTGAGCACTA	tRNA-Glu	
ND56-45F2	Sequencing	ATTTACRGTAGCAACAGAACTTAACAA	Cytochrome b (CYTB)	
ND56-5F2	Sequencing	CCCCAGAATAATCCTCACG		
ND56-R1A	PCR, Sequencing	TYACATCTCGGCAAAATATGG		
ND56-5R2	Sequencing	CTTGAGGGTCAAATGTTGTA		
ND56-35R2	Sequencing	GCACAGATAGCTGTRAATAAAGTAGTAAT		
ND56-45R2	Sequencing	TGTTAAGTTCTGTTGCTACTGYTAAATC		
Set 7				
CBCR-F1d	PCR, Sequencing	ACCATGACTAATGATCTGAAAAACC	tRNA-Glu	1938
CBCR-F2	Sequencing	TTCATAGGATAYGTCCTTCC	Cytochrome b (CYTB)	
CBCR-F3	Sequencing	KTACGCCATYCTACGATC	tRNA-Thr	
CBCR-F5	Sequencing	ATTACAATGGTCTTGTAGCCATAAA	tRNA-Pro	
CBCR-F6	Sequencing	GATAAACCATAGTCTTACATAGCACAT	D-Loop	
CBCR-R1d	PCR, Sequencing	CTCAGACGGCCATAGCTGA		
CBCR-R3	Sequencing	GGACRCCTCCTAGTTTGTGTTGTA		
CBCR-R4	Sequencing	CTTCCCTGAATACCCCTAGAAA		
CBCR-R5	Sequencing	GCTTTAATGTGCTATGTAAGACTATGG		
CBCR-R52	Sequencing	AAGCCTCCTCARATTCATTCTACTA		
Set 8				
mtAlI-F8	PCR, Sequencing	ACCCAAARCTGANATTCT	tRNA-Pro	1731
mtAlI-F82	Sequencing	GTTAATGTAGCTTAAACAAAAAGCAA	D-Loop	
mtAlI-R8	PCR, Sequencing	TGTTACGACTTGTCTCCTCT	tRNA-Phe	
mtAlI-R81	Sequencing	GACTGAATTAGCAAG	12S ribosomal RNA	
mtAlI-R82	Sequencing	ACGCCGTATGCTTATTAATTTGG		

PCR amplicon sizes are based on NC_000934

Supporting Information Figure S1. Amino acid alignments for mitochondrial proteins

across elephantids. The translated codons of 13 protein coding genes in elephantid mitogenomes were aligned for two forest elephants (DS1534 and SL0001) and seven other elephantids (GenBank numbers: NC_000934, AB443879, DQ316069, AJ428946, NC_005129, EU153453, and NC_007596) using Geneious v5.4 (Drummond et al. 2011). Conserved amino acids are shown as dots below a consensus sequence. Colors indicate the hydrophobicity of amino acid residues, with red being highly hydrophobic and blue highly hydrophilic. With few exceptions the differences between the forest elephants and the other elephantids fell into the following categories: (1) amino acid differences that did not involve change from a highly hydrophobic to a highly hydrophilic residue sequence, or vice versa; (2) amino acid substitutions that were also present in at least one other elephantid species; or (3) alternative stop codon present in at least one other elephantid species. The following comprise the only exceptions: For forest elephant DS1534, ATP6 appears to rely on an alternative start codon from the other elephantids, ND4L has a different stop codon from the other elephantids, and ND4 has Gln instead of Leu in the second amino acid position. For forest elephant SL0001, ND3 relies on an alternative start codon from the other elephantids. Both forest elephants have Tyr instead of His at position 312 of CYTB. Abbreviations: LCY: *Loxodonta cyclotis*, African forest elephant; LAF: *Loxodonta africana*, African savanna elephant; EMA: *Elephas maximus*, Asian elephant; MPR: *Mammuthus primigenius*, woolly mammoth.

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NADH dehydrogenase subunit 1 (ND1)

Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

SSKFFETXAPCLALTLALTVWAPLPMPYPILININLSLLFLAMSSLMVYSLLWSGWASNSKYALM

Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

[illegible]

Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

A P E D G L L G C S P L V S G F N V B Y S A G P F A F F F M A B Y A N L I M M N A F L V L F F M C L S X B P O X P B L S L I N F V
 C D A
 C D I
 C D M
 Y D M
 Y D M
 C D M
 C D M
 C D M
 C N M
 C N M
 C N M

Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

XKTXLILITICFLWVRASYPRERYDOLMXLILWKNFLPLTIALCMWHISXIISIACIPPOX

NADH dehydrogenase subunit 2 (ND2)

Cytochrome c oxidase subunit I (COX1)

Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-NC_007596
9. MPR-EU153453

1 10 20 30 40 50 60
M F A N R W L Y S I N H K D I G T L Y L I E G A W A G M V G T A E S I I R A E L G O P G S I L G P D O L Y N V X V I A H A F V M

V
V
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Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-NC_007596
9. MPR-EU153453

L F F M V M P I M I G G E G N W L I P L M I G A P D M A F P R M N N M S F W L L P P S F L L L A S S M V E A G X G T G W T V Y P
 A
 A
 A
 A
 I
 I
 I
 I

Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-NC_007596
9. MPR-EU153453

[illegible]

Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-NC_007596
9. MPR-EU153453

SL**P**VLAAGITMLTDRNLNT**E**DPAGGGD**P**XLYOHLFWFEGH**P**EVYLIILPGFGMV**S**HIV**T**

Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-NC_007596
9. MPR-EU153453

YSGKKKEPFGYMGVMWAMMSIGELGELVWAHHMETVGMDDVDTRAYELTSATMLIAIPITGVKVESWLA

Consensus

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2. LCY-SL0001
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5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-NC_007596
9. MPR-EU153453

P L H G G X I K W S P A M M W A L G C F L F L F L G G L T G L V A N S S L D L V L F D L Y Y V V A H F L H Y V L S M G A V L A L L
 S
 N
 N
 N
 N
 N
 N
 N
 N

Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-NC_007596
9. MPR-EU153453

GGPILHWPPPLSCGYDNLXPLWAKIQPLVMPILGVNLPPLPPHPLPLGLSCMPRRYSDYPPDAYVATINLASS

Consensus

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2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-NC_007596
9. MPR-EU153453

MGSEFLSLLVAVILMVFMWEAFASKREVSXEXELTNXEWLNGCPPPHHTEEPAYVKSNS

Cytochrome c oxidase subunit II (COX2)

Consensus

- 1. LCY-DS1534
- 2. LCY-SL0001
- 3. LAF-NC_000934
- 4. LAF-AB443879
- 5. LAF-DQ316069
- 6. EMA-AJ428946
- 7. EMA-NC_005129
- 8. MPR-EU153453
- 9. MPR-NC_007596

Consensus

- 1. LCY-DS1534
- 2. LCY-SL0001
- 3. LAF-NC_000934
- 4. LAF-AB443879
- 5. LAF-DQ316069
- 6. EMA-AJ428946
- 7. EMA-NC_005129
- 8. MPR-EU153453
- 9. MPR-NC_007596

Consensus

- 1. LCY-DS1534
- 2. LCY-SL0001
- 3. LAF-NC_000934
- 4. LAF-AB443879
- 5. LAF-DQ316069
- 6. EMA-AJ428946
- 7. EMA-NC_005129
- 8. MPR-EU153453
- 9. MPR-NC_007596

Consensus

- 1. LCY-DS1534
- 2. LCY-SL0001
- 3. LAF-NC_000934
- 4. LAF-AB443879
- 5. LAF-DQ316069
- 6. EMA-AJ428946
- 7. EMA-NC_005129
- 8. MPR-EU153453
- 9. MPR-NC_007596

ATP synthase F0 subunit 8 (ATP8)

Consensus
 1. LCY-DS1534
 2. LCY-SL0001
 3. LAF-NC_000934
 4. LAF-AB443879
 5. LAF-DQ316069
 6. EMA-AJ428946
 7. EMA-NC_005129
 8. MPR-EU153453
 9. MPR-NC_007596

Consensus
 1. LCY-DS1534
 2. LCY-SL0001
 3. LAF-NC_000934
 4. LAF-AB443879
 5. LAF-DQ316069
 6. EMA-AJ428946
 7. EMA-NC_005129
 8. MPR-EU153453
 9. MPR-NC_007596

ATP synthase F0 subunit 6 (ATP6)

Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

[illegible]

Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

[illegible]

Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

[illegible]

Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

[illegible]

Cytochrome c oxidase subunit III (COX3)

Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

LE**O**G**T**H**I**P**X**V**O**E**X**I**R**Y**G**M**I**J**F**I**L**S**E**V**L**F**F**L**G**F**E**W**A**F**E**Y**H**S**S**L**A**P**L**P**B**L**C**S**Y**N**P**P**V**G**V**Y**P**L**N**P**L**E**V**L

Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

Sequence logo for the Lysine 270 position. The top sequence is SYFFSINLVXLTSN, with positions 270 and 275 marked. Below is a sequence logo showing the conservation of residues at each position. The X at position 270 is highlighted with a blue circle.

NADH dehydrogenase subunit 3 (ND3)

Consensus

1. LCY-DS1534

2. LCY-SL0001

3. LAF-NC_000934

4. LAF-AB443879

5. LAF-DQ316069

6. EMA-AJ428946

7. EMA-NC_005129

8. MPR-EU153453

9. MPR-NC_007596

Consensus

1. LCY-DS1534

2. LCY-SL0001

3. LAF-NC_000934

4. LAF-AB443879

5. LAF-DQ316069

6. EMA-AJ428946

7. EMA-NC_005129

8. MPR-EU153453

9. MPR-NC_007596

Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

NADH dehydrogenase subunit 4L (ND4L)

Consensus

1. LCY-DS1534

2. LCY-SL0001

3. LAF-NC_000934

4. LAF-AB443879

5. LAF-DQ316069

6. EMA-AJ428946

7. EMA-NC_005129

8. MPR-EU153453

9. MPR-NC_007596

Consensus

1. LCY-DS1534

2. LCY-SL0001

3. LAF-NC_000934

4. LAF-AB443879

5. LAF-DQ316069

6. EMA-AJ428946

7. EMA-NC_005129

8. MPR-EU153453

9. MPR-NC_007596

[illegible]

NADH dehydrogenase subunit 5 (ND5)

Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

Consensus

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2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

Consensus

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2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

NADH dehydrogenase subunit 6 (ND6)

Cytochrome b (CYTB)

Consensus

1. LCY-DS1534
2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

Consensus

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2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

Consensus

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2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

Consensus

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2. LCY-SL0001
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6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

Consensus

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5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596

Consensus

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2. LCY-SL0001
3. LAF-NC_000934
4. LAF-AB443879
5. LAF-DQ316069
6. EMA-AJ428946
7. EMA-NC_005129
8. MPR-EU153453
9. MPR-NC_007596