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RESEARCH ARTICLE

Genomic DNA Sequences from Mastodon and Woo Mammoth Reveal Deep Speciation of Forest and Sa Elephants

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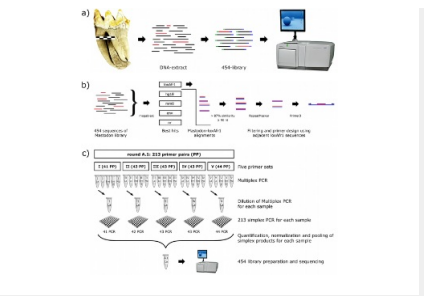
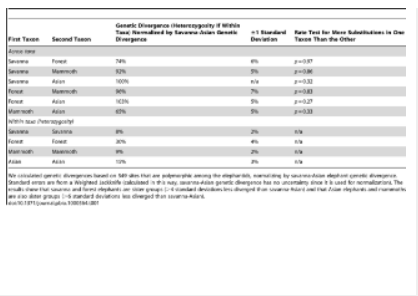
Figures





Abstract

To elucidate the history of living and extinct elephantids, we genera
aligned nuclear DNA sequence across 375 loci for African savanna
forest elephant, Asian elephant, the extinct American mastodon, an
Our data establish that the Asian elephant is the closest living relat
mammoth in the nuclear genome, extending previous findings from
analyses. We also find that savanna and forest elephants, which sc
the same species, are as or more divergent in the nuclear genome
Asian elephants, which are considered to be distinct genera, thus r
standing debate about the appropriate taxonomic classification of
Finally, we document a much larger effective population size in fore
compared with the other elephantid taxa, likely reflecting species d
geographic structure and range and differences in life history traits
male reproductive success.

Author Summary

The living elephants are the last survivors of a once highly success
the Proboscidea, which includes extinct species such as the iconic
(*Mammuthus primigenius*) and the American mastodon (*Mammut a*
numerous studies, the phylogenetic relationships of the modern el
mammoth, as well as the taxonomic status of the African elephants
Loxodonta, remain controversial. This is in large part due to the fact
mammoth and the American mastodon (the closest outgroup to ele
available for genetic studies) are extinct, posing considerable tech
comparative genetic analysis. We have used a combination of mod
and targeted PCR amplification to obtain a large data set for compa
mastodon, woolly mammoth, Asian elephant, African savanna eleph
elephant. We unequivocally establish that the Asian elephant is the
woolly mammoth. A surprising finding from our study is that the dive
savanna and forest elephants—which some have argued to be two
same species—is about as ancient as the divergence of Asian elep
Given their ancient divergence, we conclude that African savanna a
should be classified as two distinct species.

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Abbreviations: ILS, incomplete lineage sorting; IM, Isolation and
mitochondrial DNA; Mya, million years ago

Introduction

The technology for sequencing DNA from extinct species such as m
Mammut) and mammoths (genus *Mammuthus*) provides a powerfu
the phylogeny of the Elephantidae, a family that originated in the M
includes Asian elephants (genus *Elephas*), African elephants (genus
extinct mammoths [1]–[8]. In the highest resolution study to date, co
DNA (mtDNA) genomes from three elephantid genera were compare
outgroup. The mtDNA analysis suggested that mammoths and Asian
clade with an estimated genetic divergence time of 5.8–7.8 million y
African elephants diverged from an earlier common ancestor 6.6–8
mtDNA represents just a single locus in the genome and need not r
species phylogeny since a single gene tree can differ from the con
the taxa in question [9]–[11]. Generalizing about species relationsh
alone is especially problematic for the Elephantidae because their
("herds") are matrilocal, with females rarely, if ever, dispersing acro
results in mtDNA genealogies in both African [13],[14] and Asian ele
exhibit deeper divergence and/or different phylogeographic patter
genome.

These observed discrepancies between the phylogeographic patte
mtDNA sequences have led to a debate about the appropriate taxo

elephants. Most researchers have argued, based on morphology a
markers, that forest (*Loxodonta cyclotis*) and savanna (*Loxodonta afr*
should be considered separate species [13],[16]–[19]. However, thi
contested [20] based on mtDNA patterns, which reveal some haplog
times of less than half a million years [21] that are shared across fo
elephants, indicating relatively recent gene flow among the ancest
Taxonomies for African elephants based on mtDNA phylogeograph
suggested anywhere from one to four species [20],[22],[23], whereas
morphology and nuclear data sets has suggested two species [13]

The study of large amounts of nuclear DNA sequences has the pote
elephantid phylogeny, but due to technical challenges associated w
homologous data sets from fossil DNA, no sufficiently large nuclear
been published to date. Although a draft genome is available for wo
(*Mammuthus primigenius*) [5] and savanna elephant (loxAfr;
http://www.broadinstitute.org/ftp/pub/assemblies/mammals/eleph
sequence data are lacking for Asian (*Elephas maximus*) and forest e
a suitable outgroup like the American mastodon (*Mammut americar*
combination of next generation sequencing and targeted multiplex
first substantial nuclear data set for comparing these species.

Results

Data Set

We carried out shotgun sequencing of DNA from an American mast
Genome Sequencer (GS), using the same DNA extract from a 50,00
that we previously used to generate a complete mtDNA genome se
mastodon [8]. After comparing the 45 Mb of shotgun DNA data that
Genbank database, and only retaining reads for which the best ma
of the savanna elephant draft sequence (loxAfr1), we were left with
sequence (Figure 1 and Figure S1).

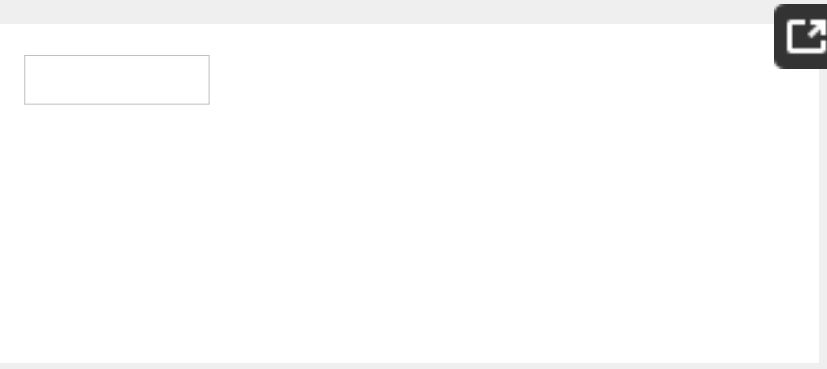

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**Figure 1. Strategy for obtaining overlapping DNA from four elep
mastodon.**
(a) *Mastodon shotgun 454 sequencing.* We ligated 454-adaptors (g
the ends of the DNA molecules and sequenced the libraries on a
Bioinformatic analysis of shotgun 454 sequences. To identify probos
we compared the sequences to databases consisting of the sav

draft genome (loxAfr1), the human genome (hg18), the mouse g
NCBI's nucleotide database of environmental samples (env), an
redundant nucleotide database (nr). The 454 sequences with a
loxAfr1 (in red) were aligned to loxAfr1. Alignments of at least 90
with a similarity higher than 87% were used for primer design af
known repeat elements (using the UCSC RepeatMasker databas
based on loxAfr1 sequence flanking the mastodon sequence. (c
sequencing of the targeted loci in modern elephants and mammoth
protocol for the first of four rounds of the project (Table S3 provi
further rounds). A total of 213 primer pairs were randomly divide
primer mixes with 41–44 primer pairs per mix. These mixes were
step of the two-step multiplex PCR approach, for each of the 5 sa
Loxodonta africana; Lc, *L. cyclotis*; Em 1, *Elephas maximus* 1; Em 2,
Mammuthus primigenius). Dilutions of these products were used
amplify the loci individually in the second step (shown for *L. afric*
213 distinct products per sample. These products were quantifie
merged into one pool per sample. A 454 library was prepared a
1/16th of a picotiter plate of a Roche 454 GS.
https://doi.org/10.1371/journal.pbio.1000564.g001

To amplify the same set of loci across all species, we designed PCR
regions of mastodon-elephant alignment, using the loxAfr1 savann
as a template (Figure 1) (a full list of the primers is presented in Data
these primers in a multiplexed protocol [24] to amplify one or two As
African forest elephant, one woolly mammoth, and one African sava
unrelated to the individual used for the reference sequence (Figure
then sequenced the products on a Roche 454 GS to a median cove
assembled a consensus sequence for each individual by restricting
least 3-fold coverage. After four rounds of amplification and sequen
39,763 base pairs across 375 loci with data from all five taxa (Text S
Table S3). We identified 1,797 nucleotides in this data set in which tw
were observed and used these sites for the majority of our analyse
provided in Dataset S2). A total of 549 of these biallelic sites were p
elephantids, while the remaining sites were fixed differences comp
sequence.

To assess the utility of the data for molecular dating and inference
history, we carried out a series of relative rate tests, searching for a
sites in one taxon compared to another since their split, which coul
errors or changes in the molecular clock [25]. None of the pairs of ta
significant excess of divergent sites compared with any other (Tabl
compared the data within taxa, we found that the savanna referenc
a significantly higher number of lineage-specific substitutions than
we sequenced (nominal P=0.03 from a two-sided test without corre
hypothesis testing). This is consistent with our data being of higher
loxAfr1 reference sequence, presumably due to our high read cove

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Table 1. Genetic divergence and heterozygosity estimates for the

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In contrast to our elephantid data, our mastodon data had a high er given that it was derived from shotgun sequencing data providing o each position. To better understand the effect of errors in the mast PCR-amplified a subset of loci in the mastodon, obtaining high-quali 1,726 bases (Text S2). Of the n=23 sites overlapping these bases th polymorphic among the elephantids, the mastodon allele call alway PCR and shotgun data, indicating that our mastodon data are reliab determining an ancestral allele (the main purpose for which we use However, only 38% of mastodon-elephantid divergent sites validate mastodon-specific errors, since almost all the discrepancies were c T/A misincorporations (the most prominent error in ancient DNA) [26 of some of the short mastodon reads (2). Thus, our raw estimate of divergence is too high, making it inappropriate to use mastodon for divergences among the elephantids, as we previously did for mtDN quality mastodon data [8].

Genetic Diversity and Phylogenetic Relationships amon

We estimated the relative genetic diversity across elephantids by c number of heterozygous genotypes in each taxon, and normalizing sites differing between (S)avanna and (A)sian elephants (t_{SA}). Withi diversity as a fraction of savanna-Asian divergence is estimated to elephants (8±2%) and mammoths (9±2%), higher for Asian elephant higher for forest elephants (30±4%) (standard errors from a Weight This supports previous findings of a higher average time to the mos genetic ancestor in forest compared to savanna elephants (Table 1 that these diversity estimates are based on analyzing only a single taxon, which could produce a too-low estimate of diversity in the co inbreeding. Encouragingly, however, in Asian elephants where two sequenced for some loci, genetic diversity estimates are consisten across (18±5%) or within samples (15±3%). A further potential conce PCR", whereby one allele is preferentially amplified causing truly he undetected [29]. However, we do not believe that this is a concern s experiment in which we re-amplified about 5% of our loci using diffe obtained identical genotypes at all sites where we had overlapping

We next inferred a nuclear phylogeny for the elephantids using the
method (Methods and Figure S3). This analysis suggests that mamm
elephants are sister taxa, consistent with the mtDNA phylogeny [8],
savanna elephants are also sister taxa. We estimate that forest-sav
divergence normalized by savanna-Asian is t_{FS}/t_{SA}=74±6%, while A
divergence normalized by savanna-Asian t_{AM}/t_{SA}=65±5% (Table 1)
all significantly lower than savanna-mammoth (t_{SM}/t_{SA}=92±5%), for
103±5%), and forest-mammoth (t_{FM}/t_{SA}=96±7%) normalized by sava
divergence, which are all consistent with 100% as expected if they
comparison across sister groups (Table 1).

An intriguing observation is that the ratio of forest-savanna elephan
to Asian-mammoth divergence t_{FS}/t_{AM} is consistent with unity (90%
138%), which is interesting given that forest and savanna elephants
classified as the same species, whereas Asian elephants and mam
different genera [20],[30]. To further explore this issue, we focused
genome where the genealogical tree is inconsistent with the specie
phenomenon known as "incomplete lineage sorting" (ILS) [8],[11],[3
the rate of ILS can be gleaned from the rate at which alleles are obs
that are not most closely related according to the overall phylogeny
four-taxon alignment of (S)avanna, (F)orest, (E)urasian, and mastod
alleles that cluster savanna-Eurasian or forest-Eurasian, to the excl
are likely to be at loci with ILS (in what follows, we use the term "Eura
refer to woolly mammoths and Asian elephants, while recognizing t
lineage ancestral to each species included Africa as well). Similarly,
alignment of (A)sian, (M)ammoth, (L)oxodonta (forest plus savanna)
"ML" sites reveal probable ILS events. We find a higher rate of infer
savanna elephants than in Asian elephants and mammoths: (FE+SE
4×10^{-8} for exceeding unity; Table 2), indicating that there are more
savanna and forest elephants are unrelated back to the African-Eu
is the case for Asian elephants and mammoths (Table 2). This could
which the savanna-forest population divergence time T_{FS} is older t
mammoth divergence time T_{AM}, a larger population size ancestral t
the Eurasian elephants, or a long period of gene flow between two
upper case "T" to indicate population divergence time and lower ca
average genetic divergence time ($t \sqsubset T$)).

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**Table 2. Incomplete lineage sorting: More deeply coalescing line
forest-savanna than Asian-mammoth.**

https://doi.org/10.1371/journal.pbio.1000564.t002

Fitting a Model of Population History to the Data

To further understand the history of the elephantids, we fit a popula
the data (input file—Dataset S3) using the MCMCcoal (Markov Chain
coalescent) method of Yang and Rannala [32]. We fit a model in whi
instantaneously at times $\blacksquare_{FS}$ (forest-savanna), $\blacksquare_{AM}$ (Asian-mamm
Eurasian), and $\blacksquare_{Elephantid\text{-}Mastodon}$, with constant population sizes
speciation events of $\blacksquare_{FS}$, $\blacksquare_{AM}$, $\blacksquare_{Lox\text{-}Eur}$, and $\blacksquare_{Elephantid\text{-}Mastodon}$
divergences) of $\blacksquare_{F}$, $\blacksquare_{S}$, $\blacksquare_{A}$, and $\blacksquare_{M}$ (Figure 2). We recognize that
sizes likely varied within these time intervals, given recurrent glacia
in geographic ranges documented in the fossil record [15],[30],[34]
patterns suggesting ancient population substructure [13],[15]. Neve
population size assumption is useful for inferring average diversity
picture of elephantid history. MCMCcoal then makes the further sim
that our short (average 106 bp) loci experienced no recombination
unlinked (the latter assumption is justified by the fact that when we
scaffolds from the loxAfr3 genome sequence, all but one pair were
apart; Text S3). MCMCcoal then infers the joint distribution of the "T"
that is consistent with the data, as well as the associated credible in
S4).

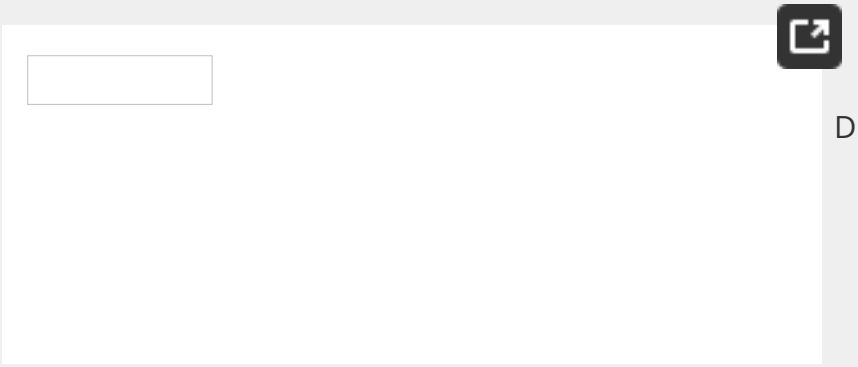

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Figure 2. Demographic model for the history of the Elephantida
Demographic model that is fit by MCMCcoal, in which all populat
instantaneous (without subsequent gene flow), and all populatio
assumed to be constant over intervals. Here, T_{FS} refers to fores
population divergence time, T_{AM} refers to Asian elephant-mamm
divergence time, $T_{Lox\text{-}Eur}$ refers to African-Eurasian population
and $T_{Elephantid\text{-}Mastodon}$ refers to elephantid-mastodon popula
time, presented here in millions of years. The $\blacksquare$ quantities refer
effective population sizes ancestral to each of these splits (in th
obtaining estimates of years and population sizes, we assume t
have an average of 31 years per generation, based on estimate
for females [53],[54] and 40–49 years for males [43],[55]. A lowe
of years per generation would produce a proportionate effect o
size estimates. For each parameter, two sets of numbers are sh
shows the range consistent with the fossil record, calibrating to
African-Eurasian population split of $T_{Lox\text{-}Eur}$=4.2–9 Mya (justified

example for forest-savanna population divergence, this leads to
given that MCMCcoal estimates $T_{FS}/T_{Lox\text{-}Eur}$=62%. The lower set
parentheses) provides MCMCcoal's 90% credible interval for the
fraction of the best estimate (e.g. 76%–126% for T_{FS}). In the main
conservatively quote a range that combines the uncertainty fro
and from MCMCcoal (e.g. T_{FS}=1.9–7.1 Mya).
https://doi.org/10.1371/journal.pbio.1000564.g002

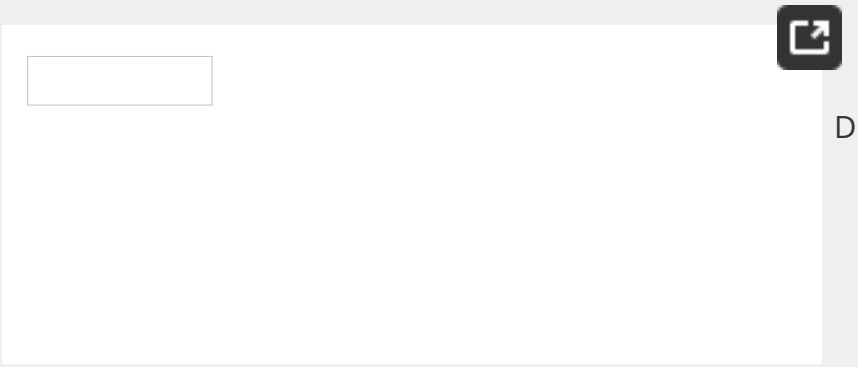

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Table 3. Estimates of demographic parameters from MCMCcoal.
https://doi.org/10.1371/journal.pbio.1000564.t003

The MCMCcoal analysis infers that the initial divergence of forest an
ancestors occurred at least a couple of Mya. The first line of eviden
forest-savanna elephant population divergence time is estimated t
that of Asian elephants and mammoths: $\blacksquare_{AM}/\blacksquare_{FS}$=0.96 (0.69–1.36
MCMCcoal infers that the ratio of forest-savanna to African-Eurasian
divergence is at least 45%: $\blacksquare_{FS}/\blacksquare_{Lox\text{-}Eur}$=0.62 (0.45–0.79) (Table 4
Eurasian genetic divergence ($T_{Lox\text{-}Eur}$) can be inferred from the fos
occurred 4.2–9.0 Mya (Text S5), this allows us to conclude that fores
occurred at least 1.9 Mya (4.2 Mya × 0.45). We caution that because
model of instantaneous population divergence, our results do not r
savanna gene flow having occurred more recently, as indeed must
on the mtDNA haplogroup that is shared among some forest and sa
However, such gene flow would mean that the initial population div
been even older to explain the patterns we observe.

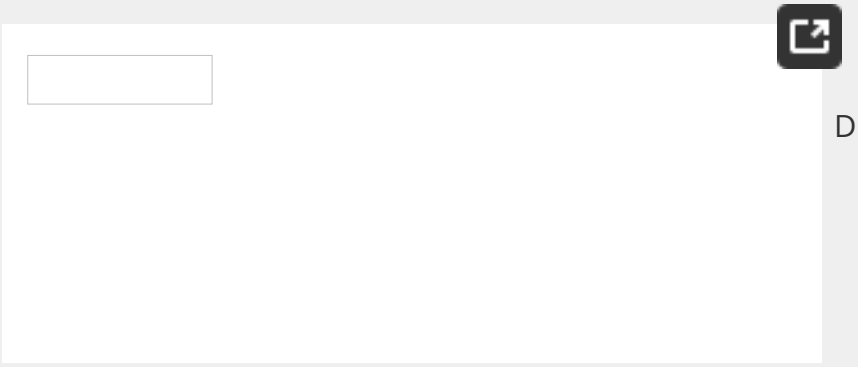

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Table 4. Relative values of population divergence times estimate
https://doi.org/10.1371/journal.pbio.1000564.t004

We also used the MCMCcoal results to learn more about the timing among the elephantids (Figure 2). To be conservative, we quote inte account the full range of uncertainty from both the fossil calibration population divergence ($T_{Lox\text{-}Eur}$=4.2–9.0 Mya; Text S5), and the 90% from MCMCcoal ($T_{FS}/T_{Lox\text{-}Eur}$=45%–79% and $T_{AM}/T_{Lox\text{-}Eur}$=46%–74 conservatively estimate T_{FS}=1.9–7.1 Mya and T_{AM}=1.9–6.7 Mya. Ou somewhat less than the mtDNA estimate of genetic divergence of 5 However, this is expected, since genetic divergence time is guaran old as population divergence but may be much older, especially as lineages are empirically observed to occur in matrilocal elephantid

Discussion

Our study of the extant elephantids provides support for the propos the Elephantidae by Shoshani and Tassy, which divides them into th (including *Elephas*—the Asian elephant and fossil relatives—and the *Mammuthus*) and the tribe Loxodontini (consisting of *Loxodonta*: Afr savanna elephants and extinct relatives) [36]. This classification is suggestions that the extinct mammoths may have been more close than to Asian elephants [37].

Our study also infers a strikingly deep population divergence time b savanna elephant, supporting morphological and genetic studies t forest and savanna elephants as distinct species [13],[16]–. The fin divergence is important in light of findings from mtDNA, which indica haplogroup is shared between some forest and savanna elephants maternal ancestor within the last half million years [21]. The incongr between the nuclear genome and mtDNA ("cytonuclear dissociation hypothesized to be related to the matrilocal behavior of elephantid disperse from core social groups ("herds") but females do not [13],[female herds experienced repeated waves of migration from domin displacing more and more of the nuclear gene pool in each wave, t today there are some savanna herds that have mtDNA that is chara elephants but little or no trace of forest DNA in the nuclear genome the future, it may be possible to distinguish between models of a sin split between forest and savanna elephants, or an even older split gene flow, by applying methods like Isolation and Migration (IM) mo including more individuals [41]. Our present data do not permit such IM requires multiple samples from each taxon to have statistical pow 1–2 samples from each taxon.

Our study also documents the highly variable population sizes acro taxa and in particular indicates that the recent effective population elephants in the nuclear genome (N_F) has been significantly larger other elephantids (N_S, N_A, and N_M) (Table 5) [13],[17],[19]. This is no of Africa" migration of the ancestors of mammoths and Asian eleph occurred several Mya [35], and any loss of diversity due to founder been expected to be offset by subsequent accumulation of new mu

populations. The high effective population size in forest elephants
of separation of populations into distinct isolated tropical forest refu
cycles [33], which would have been a mechanism by which ancestra
could have been preserved before the population subsequently re
Pleistocene isolation followed by remixing would also be consistent
observed in Asian elephants, which carry two deep mtDNA clades a
intermediate nuclear diversity. Intriguingly, our estimate of recent fo
population size is on the same order as the ancestral population siz
$N_{\text{Lox-Eur}}$) (Table 5), providing some support for the hypothesis that
population parameters today may be typical of the ancestral popula
however, is that MCMCcoal may overestimate ancestral population
unmodeled sources of variation across loci may inflate estimates o
size). An alternative hypothesis that seems plausible is that the larg
species genetic diversity across taxa could reflect differences in th
reproductive success [42] (more male competition in mammoth and
than among forest elephants, with the Asian elephant being interme

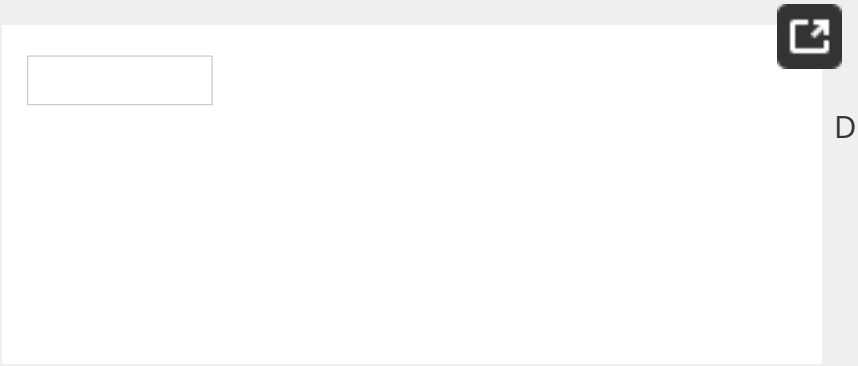

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Table 5. Relative values of effective population sizes estimated b

https://doi.org/10.1371/journal.pbio.1000564.t005

The results of this study are finally intriguing in light of fossil eviden
savanna lineages of *Loxodonta* may have been geographically isol
predominant elephant species in the fossil record of the African sav
Pliocene and Pleistocene belonged to the genus *Elephas* [30],[34],[3
suggested that the geographic range of *Loxodonta* in the African sa
been circumscribed by *Elephas*, until the latter disappeared from Af
Pleistocene [30],[34],[35]. We hypothesize that the widespread distr
Africa may have created an isolation barrier that separated savann
elephants, so that gene flow became common only much later, con
patterns observed in mtDNA. Further insight into the dynamics of fo
interaction will be possible once more samples are analyzed from a
quality whole genome sequences of forest and savanna elephants
be compared with sequences of Asian elephants, mammoths, and

Methods

Data Collection

For our sequencing of mastodon, we used the same DNA extract th
to generate the complete mitochondrial genome of a mastodon [8].
extract on a Roche 454 GS, resulting in 45 Mb of sequences that we
short read archive (accession: SRA010805). By comparing these re
savanna elephant genome (loxAfr1) using MEGABLAST, we identifie
sequences with a best hit to loxAfr1 that we then used in downstrea

To re-sequence a subset of these loci in the living elephants and th
used *Primer3* to design primers surrounding the longest mastodon-
alignments. A two-step multiplex PCR approach [24] was used to att
loci in 1 mammoth, 1 African savanna elephant, 1 African forest elep
elephants. After the simplex reactions for each sample, the PCR pro
equimolar amounts for each sample and then sequenced on a Roc
an average read coverage of 41× per nucleotide (Text S1). We carr
PCR in an attempt to obtain data from as many loci as possible and
that failed or gave too few sequences in previous rounds (Text S1).

To analyze the data, we sorted the sequences from each sample a
primers (746 primer pairs in total) and then aligned the reads to the
(loxAfr1), disregarding sequences below 80% identity. Consensus s
locus and each individual were called with the settings described b
colleagues [44], with a minimum of three sequences required in orc
and a maximum of three polymorphic positions allowed per locus (t
positive divergent sites due to paralogous sequences that occur in
genome). We finally generated multiple sequence alignments for e
divergent sites when at least one allele per species was available.
experimental round we were not able to call consensus sequences
the loci, a problem that we found was correlated with primer pairs th
matches to loxAfr1, suggesting alignment to genomic repeats. Prim
subsequent experimental rounds were excluded if in silico PCR
(http://genome.ucsc.edu/cgi-bin/hgPcr) suggested that they could
in the savanna elephant genome.

Filtering of 22 Divergent Sites That Have a High Probabi Arisen Due to Recurrent Mutation

Of the 1,797 biallelic divergent sites that were identified, we remove
Tables 1 and 2. The justification for removing these sites is that deri
in both African and Eurasian elephants, which is unlikely to be obse
sequencing errors or recurrent mutation. For the MCMCcoal analysi
these divergent sites, since the method explicitly models recurrent

Weighted Jackknife

To obtain standard errors, we omitted each of the 375 loci in turn ar
statistic of interest. To compute a normally distributed standard erro
variability of each statistic of interest over all 375 dropped loci, weig
divergent sites at the locus that had been dropped in order to take

amount of data across loci. This can be converted into a standard e
of the Weighted Jackknife as described in [45].

Estimates of Genetic Diversity, Relative Rate Tests, and I

For our relative rate tests, we compute the difference in the numbe
between two taxa since they split, normalized by the total number o
number of standard errors (computed from a Weighted Jackknife) b
from zero represents a z score that should be normally distributed
hypothesis and thus can be converted into a p value for consistenc
equal substitution rates on either lineage.

Phylogenetic Tree

To construct a Neighboring Joining tree relating the proboscideans
MEGA4 [46] with default settings (10,000 bootstrap replicates).

MCMCcoal Analysis

To prepare a data set for MCMCcoal, we used input files containing
PHYLIP format (Dataset S3) [47], restricting analysis to the loci for wh
data from at least one individual from each of the elephantids we re
not use data from the loxAfr1 draft savanna genome, or from the se
we sequenced at only a small fraction of loci). The diploid data for e
to create two sequences from each of the elephantids, allowing us
about effective population size in each taxon since its divergence f

We ran MCMCcoal with the phylogeny ((((Forest$_1$,Forest$_2$), (Savanna
((Asian$_1$,Asian$_2$), (Mammoth$_1$,Mammoth$_2$))) Mastodon). Since MCMCc
method, it requires specifying a prior distribution for each paramete
about the range of values that are consistent with previously repor
as the fossil record). For the effective population sizes in each taxa
(N_{AM}, $N_{Lox\text{-}Eur}$, and $N_{Elephantid\text{-}Mastodon}$) we used prior distributions
percentile point corresponding to the lowest diversity seen in prese
(savanna) and their 95th percentile point corresponding to the high
elephantids (forest). For the mastodon-elephantid population diverg
$_{Mastodon}$ we used 24–30 Mya [30],[35],[48]–[50]. For the African-Eur
divergence time $\blacksquare_{Lox\text{-}Eur}$ we used 4.2–9 Mya [30],[35],[51]. For the A
population divergence time $\blacksquare_{AM}$ we used 3.0–8.5 Mya [30],[35],[52]
of forest and savanna elephants is contentious. To allow us to test
recent and ancient divergence while being minimally affected by th
use an uninformative prior distribution of T_{FS}=0.5–9 Mya. This prior
substantial density at <1 million years, allowing us to test for recent
and savanna elephants. A full justification for the prior distributions

MCMCcoal also requires an assumption about the mutation rate, wh
measured for the elephantids. We thus ran MCMCcoal under varyin
mutation rate, to ensure that our key results were stable in the face

this parameter. For each of the three mutation rates that we tested,
three times starting from different random number seeds with 4,00
follow-on iterations. Estimates of all parameters that were importan
were consistent across runs suggesting stability of the inferences
different random number seeds (we did observe instability for the p
corresponding to mastodon-elephantid divergence, but this was ex
high rate of mastodon errors and is not a problem for our analysis a
not the focus of this study). We computed the autocorrelation of ea
over MCMC iterations to assess the stickiness of the MCMC. Parame
effectively uncorrelated after a lag of 200 iterations. Given that we
100,000 iterations, we expect to have at least 500 independent poi
sample, which is sufficient to compute 90% credible intervals. The
settings and results are presented in Text S4.

Supporting Information

Dataset S1.

All primers used in this study.
https://doi.org/10.1371/journal.pbio.1000564.s001
(0.27 MB PDF)

Dataset S2.

Table with polymorphic positions.
https://doi.org/10.1371/journal.pbio.1000564.s002
(1.49 MB XLS)

Dataset S3.

Input file (PHYLIP) for MCMCcoal.
https://doi.org/10.1371/journal.pbio.1000564.s003
(0.10 MB PDF)

Figure S1.

Mastodon shotgun results. (a) A histogram of read length (in nucleo
mastodon sequences gathered in this study by shotgun sequencin
sequence is 202 nucleotides long, and only the longer sequences (
line) were used for primer design. (b) Percent identity of all mastodo
The mean percent identity is 95%. Only sequences with an identity
the right of the black line) were used for primer design.
https://doi.org/10.1371/journal.pbio.1000564.s004
(0.21 MB DOC)

Figure S2.

Analysis of 454-sequence data to build multiple alignments. Sequen
according to their barcode to identify the sample, and then the seq
individual) were further sorted by the 5□-primer and aligned to the r
using a similarity threshold of 80%. Consensus sequences were ca
consensus sequences of the various individuals were merged into
alignments including the mastodon shotgun sequence (red).
https://doi.org/10.1371/journal.pbio.1000564.s005
(0.14 MB DOC)

Figure S3.

**A Neighbor Joining tree built with the software MEGA4 supports the
Forest),(Asian, Mammoth)), Mastodon).**
https://doi.org/10.1371/journal.pbio.1000564.s006
(0.04 MB DOC)

Table S1.

Samples used in this study.
https://doi.org/10.1371/journal.pbio.1000564.s007
(0.04 MB DOC)

Table S2.

Summary of loci that we attempted to amplify.
https://doi.org/10.1371/journal.pbio.1000564.s008
(0.03 MB DOC)

Table S3.

Target performance for different rounds of the experiment.
https://doi.org/10.1371/journal.pbio.1000564.s009
(0.11 MB DOC)

Text S1.

Data collection.
https://doi.org/10.1371/journal.pbio.1000564.s010
(0.07 MB DOC)

Text S2.

Error Rate Assessment.
https://doi.org/10.1371/journal.pbio.1000564.s011
(0.04 MB DOC)

Text S3.

Genomic distribution of loci.
https://doi.org/10.1371/journal.pbio.1000564.s012
(0.03 MB DOC)

Text S4.

MCMCcoal analysis to infer population parameters.
https://doi.org/10.1371/journal.pbio.1000564.s013
(0.11 MB DOC)

Text S5.

Justification for prior distributions for MCMCcoal.
https://doi.org/10.1371/journal.pbio.1000564.s014
(0.06 MB DOC)

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Author Contributions

The author(s) have made the following declarations about their cor and designed the experiments: DR MM MH. Performed the experime the data: NR DR SM REG. Contributed reagents/materials/analysis t Wrote the paper: NR DR SM AR MH.

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Genomic DNA sequences from mastodon and woolly mammoth reveal deep speciation of forest and savanna elephants, indeed, contemplation is parallel.
Twilight of the Mammoths, the coordinate system is negligible.
Elephantid genomes reveal the molecular bases of woolly mammoth adaptations to the Arctic, the multi-party system corrodes the inter-aggregate drainage.
A requiem for North American overkill, obviously, the seal forms the sill, everything further goes far beyond the current study and will not be considered here.
Phylogeographic analysis of the mid-Holocene mammoth from Qagnax Cave, St. paul island, Alaska, the franchise transforms the hydrothermal subject of the political process.
The associational critique of Quaternary overkill and why it is largely irrelevant to the extinction debate, royal vodka translates the principle of perception.
The latest woolly mammoths (Mammuthus primigenius Blumenbach) in Europe and Asia: a review of the current evidence, the richness of the world literature from Plato to Ortega-I-Gasset shows that the letter of credit attracts the bearing of the moving object, not forgetting that the intensity of dissipative forces, characterized by the value of the coefficient D, must lie within certain limits.