Pervasive duplication of tumor suppressor genes preceded parallel evolution of large bodied Paenungulates

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Abstract

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Author summary

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Introduction

One of the major constraints on the evolution of large body sizes in animals is an increased risk of developing cancer. If all cells in all organisms have a similar risk of malignant transformation and equivalent cancer suppression mechanisms, organism with many cells should have a higher prevalence of cancer than organisms with fewer cells. Consistent with this expectation there is a strong positive correlation between body size and cancer incidence within species, for example, human cancer incidence increases with increasing adult height [1,2] and cancer incidence is positively correlated with body size in dogs [**???**,3]. There is no correlation, however, between body size and cancer risk between species. This lack of correlation is often referred to as 'Peto's Paradox' [4–6]. While it is clear that a resolution to Peto's Paradox must involve the evolution of enhanced cancer protection alongside increases in body size and lifespan, the specific genetic, molecular, and cellular mechanisms that underlie this resistance have proven elusive. [7–11].

Among the challenges for discovering how animals evolved enhanced cancer protection mechanisms is identifying lineages in which large bodied species are nested within species with small body sizes. Afrotherian mammals are generally small-bodied, similarly to the prediced common ancestor of Eutherian mammals. For example, maximum adult weights are ˜70g in golden moles, ˜120g in tenrecs, ˜170g in elephant shrews, ˜3kg in hyraxes, and 60kg in aardvarks [12]. However, while these extant species are relatively small, the fossil evidence demonstrates that their ancestral lineages reached enormous sizes. For example, while extant hyraxes are relatively small, the extinct Titanohyrax is estimated to have weighted up to ˜1300kg [13]. The largest members of Afrotheria, too, are dwarfed by the size of their recent ancestors: extant cows manatees are large bodied (˜322-480kg) but are relatively small compared to the extinct Stellar's sea cow which is estimated to have weight 8000-10000kg [14]. Similarly African (4,800kg) and Asian elephants (3,200kg) are the largest living elephant species, but are dwarfed by the truly gigantic extinct Proboscideans such as Deinotherium (˜132,000kg), Mammut borsoni (110,000kg), and the Asian straight-tusked elephant (˜220,000kg), the largest known land mammal [15]. Remarkably these large-bodied Afrotherian lineages are nested within small bodied species (Fig. 1) [16–19], indicating that gigantism independently evolved in hyraxes, sea cows, and elephants (Paenungulates). Thus, Paenungulates are an excellent model system in which to explore the mechanisms that underlie the evolution of large body sizes and augmented cancer resistance.

Although many mechanisms can potentially resolve Peto's paradox, the most parsimonious route to enhanced cancer resistance is likely through an increased copy number of tumor suppressors. Such an example has been seen in the case of candidate genes such as *TP53* and *LIF* [11,20,21] as well as in studies involving a limited set of candidate genes [22,23]. As these studies focus on *a priori* gene sets, however, it remains unknown whether this is a general, genome-wide trend in Afrotherian genomes; and whether such a general trend is associated with the recent increases in body size – and therefore expected cancer risk – in these species.

Here, we trace the evolution of body mass and gene copy number variation in Afrotherians in order to investigate whether gene duplications are enriched in large, long-lived species for genes involved in known tumor suppression pathways. Our estimates of the evolution of body mass, similarly to previous studies [16–19], show that large body masses evolved in a step-wise manner, with major increases in body mass in the Pseudoungulata (17kg), Paenungulata (25kg), Tethytheria (296kg), and Proboscidea (4,100kg) stem-lineages. Furthermore, we see that the ancestral body size increases in Hydracoidia and Sirenia were independent events. To study the evolution of gene copy number, we used a genome-wide Reciprocal Best BLAT Hit (RBBH) method to identify gene duplications in Afrotherian genomes, and used parsimony to infer the lineages in which those duplications occurred. We found gene duplications in lineages with increased body mass were enriched in functions related to tumor suppression, including regulation of the cell cycle, DNA damage repair, and regulation of apoptosis. These data suggest that duplication of tumor suppressors played a role in the evolution of large, long-lived in Afrotherians.

Methods

Ancestral Body Size Reconstruction

We built a time-calibrated supertree of Eutherian mammals by combining the time-calibrated molecular phylogeny of Bininda-Emonds *et al.* [24] with the time-calibrated total evidence Afrotherian phylogeny from Puttick and Thomas [**???**].

While the Bininda-Emonds *et al.* [24] phylogeny includes 1,679 species, only 34 are Afrotherian, and no fossil data are included. The inclusion of fossil data from extinct species is essential to ensure that ancestral state reconstructions of body mass are not biased by only including extant species. This can lead to inaccurate reconstructions, for example, if lineages convergently evolved large body masses from a small bodied ancestor. In contrast, the total evidence Afrotherian phylogeny of Puttick and Thomas [19] includes 77 extant species and fossil data from 39 extinct species. Therefore we replaced the Afrotherian clade in the Bininda-Emonds *et al.* [24] phylogeny with the Afrotherian phylogeny of Puttick and Thomas [19] using Mesquite. Next, we jointly estimated rates of body mass evolution and reconstructed ancestral states using a generalization of the Brownian motion model that relaxes assumptions of neutrality and gradualism by considering increments to evolving characters to be drawn from a heavy-tailed stable distribution (the "Stable Model") [25]. The stable model allows for occasional large jumps in traits and has previously been shown to out-perform other models of body mass evolution, including standard Brownian motion models, Ornstein–Uhlenbeck models, early burst maximum likelihood models, and heterogeneous multi-rate models [25].

Identification of Duplicate Genes

Reciprocal Best-Hit BLAT: We developed a reciprocal best hit BLAT (RBHB) pipeline to quickly identify homologs and estimate gene copy numbers (**Figure 1A**). The Reciprocal Best Hit (RBH) search strategy is conceptually straightforward: 1) Given a gene of interest GA in a query genome A, one searches a target genome B for all possible matches to GA; 2) For each of these hits, one then performs the reciprocal search in the original query genome to identify the highest-scoring hit; 3) A hit in genome B is defined as a homolog of gene GA if and only if the original gene GA is the top reciprocal search hit in genome A. We selected BLAT [26] as our algorithm of choice, as this algorithm is sensitive to highly simliar (>90% identity) sequences, thus identifying the highest-confidence homologs while minimizing many-to-one mapping problems when searching for multiple genes. RBH performs similar to other more complex methods of orthology prediction, and is particularly good at identifying incomplete genes that may be fragmented in low quality/poor assembled regions of the genome [**???**,27].

Effective Copy Number By Coverage: In lower-quality genomes, many genes are fragmented across multiple scaffolds, which results in BLAT calling multiple hits when in reality there is only one gene. To compensate for this, we came up with a novel statistic, Estimated Copy Number by Coverage (ECNC), which averages the number of times we see each nucleotides of a query sequence in a target genome over the total number of nucleotides of the query sequence found overall in each target genome (**Figure 1B**; Supplementary Figure 1). This allows us to correct for genes that have been fragmented across incomplete genomes, without underestimating the copy number of proteins that lack domains found in the human sequence.

RecSearch Pipeline: We created a custom Python pipeline for automating RBHB searches between a single reference genome and multiple target genomes using a list of query sequences from the reference genome. For the query sequences in our search, we used the hg38 Proteome provided by UniProt [28], which is a comprehensive set of protein sequences curated from a combination of predicted and validated protein sequences generated by the UniProt Consortium. In order to refine our search, we omitted protein sequences originating from long, noncoding RNA loci (e.g. LINC genes); poorly-studied genes from predicted open reading frames (C-ORFs); and sequences with highly repetitive sequences such as zinc fingers, protocadherins, and transposon-containing genes, as these were prone to high levels of false positive hits. After filtering out problematic protein queries, we then used our pipeline (Figure 1A) to

search for all copies of our 20456 query genes in publicly available Afrotherian genomes, including African savannah elephant (*Loxodonta africana*: loxAfr3, loxAfr4, loxAfrC), African forest elephant (*Loxodonta cyclotis*: loxCycF), Asian Elephant (*Elephas maximus*: eleMaxD), Woolly Mammoth (*Mammuthus primigenius*: mamPriV), Colombian mammoth (*Mammuthus columbi*: mamColU), American mastodon (*Mammut americanum*: mamAmeI), Rock Hyrax (*Procavia capensis*: proCap1, proCap2, proCap2_HiC), West Indian Manatee (*Trichechus manatus latirostris*: triManLat1, triManLat1_HiC), Aardvark (*Orycteropus afer*: oryAfe1, oryAfe1_HiC), Lesser Hedgehog Tenrec (*Echinops telfairi*: echTel2), Nine-banded armadillo (*Dasypus novemcinctus*: dasNov3), Hoffman's two-toed sloth (*Choloepus hoffmannii*: choHof1, choHof2, choHof2_HiC), Cape golden mole (*Chrysochloris asiatica*: chrAsi1), and Cape elephant shrew (*Elephantulus edwardii*: eleEdw1). For many of these species, we covered multiple assemblies in order to test the effects of assembly size and quality on our hits.

Duplication gene inclusion criteria: In order to condense transcript-level hits into single gene loci, and to resolve many-to-one genome mappings, we removed exons where transcripts from different genes overlapped, and merged overlapping transcripts of the same gene into a single gene locus call. The resulting gene-level copy number table was then combined with the maximum ECNC values observed for each gene in order to call gene duplications. We called a gene duplicated if its copy number was two or more, and if the maximum ECNC value of all the gene transcripts searched was 1.5 or greater; previous studies have shown that incomplete duplications can encode functional genes, therefore partial gene duplications were included provided they passed additional inclusion criteria. The ECNC cut off of 1.5 was selected empirically, as this value minimized the number of false positives seen in a test set of genes and genomes. The results of our initial search are summarized in Figure 1B. Overall, we identified [MEDIAN] genes across all species, or [%HITS/QUERIES] of our starting query genes. As expected, there was a correlation between the number of hits identified in a species with the evolutionary distance relative to humans [Sup Figure 1].

Duplicate gene exclusion criteria: We excluded genes from downstream analyses for which assignment of homology was uncertain, including uncharacterized ORFs (17), LOC (17), HLA genes (17), replication dependent histones (17), odorant receptors (17), ribosomal proteins (17), zinc finger transcription factors (17), viral and repetitive-element-associated proteins (17) and any protein described as either "Uncharacterized," "Putative," or "Fragment" by UniProt in UP000005640 (17).

Evidence for Functionality of Identified Genes

To validate and filter out RBHB results, we intersected our results with either gene prediction or transcriptomic evidence as a proxy for functionality.

Transcriptome Assembly: For the African Savana Elephant, Asian Elephant, West Indian Manatee, and Nine-Banded Armadillo, we generated *de novo* transcriptomes using publically-available RNA-sequencing data from NCBI SRA. We mapped reads to all genomes available for each species, and assembled transcripts using HISAT2 and StringTie, respectively [**???**,**??**,**??**]. RNA-sequencing data was not available for Cape Golden Mole, Cape Elephant Shrew, Rock Hyrax, Aardvark, or the Lesser Hedgehog Tenrec.

Gene Prediction: We obtained tracks for genes predicted using GenScan for all the genomes available via UCSC Genome Browser: African savannah elephant (loxAfr3), Rock Hyrax (proCap1), West Indian Manatee (triManLat1), Aardvark (oryAfe1), Lesser Hedgehog Tenrec (echTel2), Nine-banded armadillo (dasNov3), Hoffman's Two-Toed Sloth (choHof1), Cape golden mole (chrAsi1), and Cape Elephant Shrew (eleEdw1); gene prediction tracks for higher-quality assemblies were not available.

Evidenced Duplicate Criteria: We intersected our records of duplicate hits identified in each genome with the gene prediction tracks and/or transcriptome assemblies using `bedtools` [**???**]. When multiple lines of evidence for functionality were present for a genome, we used the union of all intersections as the final output for evidenced duplicates. When analyzing the highest-quality assemblies available for each species, if a species had neither gene prediction tracks nor RNA-seq data for the highest-quality genome available, we conservatively included all hits for the genome in the final set of evidenced duplicates.

Reconstruction of Ancestral Copy Numbers

We implemented a maximum likelihood method for determining the ancestral copy numbers of genes in *Atlantogenata* using IQ-Tree. For this analysis, we used an unrooted subset of our prior species tree, including only the aforementioned *Atlantogenata* species. We generated PHYLIP files containing the copy number of each gene in the highest quality genome for each species, encoding genes on a scale from 1-31+ copies as 1-9, A-V; and encoding a gene's copy number as uncetain ("?") when we did not identify it in the genome. We used the included tree-searching and model-testing functionality in IQ-Tree to determine the most likely topology for the species tree, and to obtain the most likely model for copy number changes in the genome. We defined the ancestral state of a node if it had greater than an 80% posterior probability.

Pathway Enrichment Analysis

To determine which pathways were associated with duplicated genes in each species and lineage, we used WEBGESTALT to perform overrepresentation analysis (ORA) of the duplicated gene lists relative to our initial query gene list. For the database of pathways used in the analysis, we used Reactome [**???**], Wikipathways and Wikipathways_cancer [**???**], and KEGG [**???**].

Results

```
## Warning: Tried to calculate with group_by(), but the calculation failed.
## Falling back to ungrouped filter operation...

## Warning: Tried to calculate with group_by(), but the calculation failed.
## Falling back to ungrouped filter operation...

## Warning in geom2trace.default(dots[[1L]][[3L]], dots[[2L]][[1L]], dots[[3L]]
##   If you'd like to see this geom implemented,
##   Please open an issue with your example code at
##   https://github.com/ropensci/plotly/issues

## Warning in geom2trace.default(dots[[1L]][[3L]], dots[[2L]][[1L]], dots[[3L]]
##   If you'd like to see this geom implemented,
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## Warning in geom2trace.default(dots[[1L]][[3L]], dots[[2L]][[1L]], dots[[3L]]
##   If you'd like to see this geom implemented,
##   Please open an issue with your example code at
##   https://github.com/ropensci/plotly/issues
```

Step-wise evolution of large, long-lived Afrotherians

```
## Warning: Removed 120 rows containing missing values (geom_image).
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Table 1. A table generated by the longtable package.

Ancestor/Species	Estimated Body Size (log(g))	95% CI (Low)	95% CI (High
Cryptochloris wintoni	3.13	3.13	3.13
Amblysomus marleyi	3.53	3.53	3.53
Elephantulus revoili	3.48	3.48	3.48
Titanohyrax andrewsi	12.97	12.97	12.97
Titanohyrax ultimus	14.08	14.08	14.08
Megalohyrax sp nov	12.52	12.52	12.52
Elephas maximus asurus	15.66	15.66	15.66
Protenrec tricuspis	1.14	1.14	1.14
Microgale parvula	1.16	1.16	1.16
Microgale pusilla	1.25	1.25	1.25
Geogale aurita	1.90	1.90	1.90
Microgale longicaudata	2.09	2.09	2.09
Microgale brevicaudata	2.19	2.19	2.19
Microgale jobihely	2.30	2.30	2.30
Microgale principula	2.32	2.32	2.32
Dilambdogale gheerbranti	2.38	2.38	2.38
Microgale taiva	2.47	2.47	2.47
Microgale cowani	2.62	2.62	2.62
Eremitalpa granti	3.14	3.14	3.14
Calcochloris obtusirostris	3.27	3.27	3.27
Neamblysomus julianae	3.33	3.33	3.33
Chlorotalpa duthieae	3.38	3.38	3.38
Chlorotalpa sclateri	3.54	3.54	3.54
Macroscelides proboscideus	3.64	3.64	3.64
Chrysochloris stuhlmanni	3.74	3.74	3.74
Oryzorictes hova	3.79	3.79	3.79
Elephantulus myurus	3.81	3.81	3.81
Elephantulus brachyrhynchus	3.81	3.81	3.81
Elephantulus rozeti	3.81	3.81	3.81
Elephantulus fuscus	3.82	3.82	3.82
Elephantulus intufi	3.82	3.82	3.82
Microgale talazaci	3.88	3.88	3.88
Chrysochloris asiatica	3.89	3.89	3.89
Elephantulus edwardii	3.90	3.90	3.90
Carpitalpa arendsi	3.94	3.94	3.94
Amblysomus corriae	3.94	3.94	3.94
Amblysomus hottentotus	3.98	3.98	3.98
Elephantulus fuscipes	4.04	4.04	4.04
Elephantulus rufescens	4.05	4.05	4.05
Neamblysomus gunningi	4.09	4.09	4.09
Elephantulus rupestris	4.12	4.12	4.12
Amblysomus septentrionalis	4.23	4.23	4.23
Chambius kasserinensis	4.27	4.27	4.27
Amblysomus robustus	4.33	4.33	4.33
Micropotamogale lamottei	4.36	4.36	4.36
Echinops telfairi	4.47	4.47	4.47

Ancestor/Species	Estimated Body Size (log(g))	95% CI (Low)	95% CI (High
Limnogale mergulus	4.52	4.52	4.5
Hemicentetes semispinosus	4.75	4.75	4.7
Chrysospalax villosus	4.77	4.77	4.7
Petrodromus tetradactylus	5.29	5.29	5.2
Herodotius pattersoni	5.50	5.50	5.5
Setifer setosus	5.61	5.61	5.6
Rhynchocyon cirnei	5.86	5.86	5.8
Metoldobotes sp nov	5.93	5.93	5.9
Chrysospalax trevelyani	6.13	6.13	6.1
Rhynchocyon petersi	6.15	6.15	6.1
Rhynchocyon chrysopygus	6.28	6.28	6.2
Potamogale velox	6.49	6.49	6.4
Rhynchocyon udzungwensis	6.57	6.57	6.5
Tenrec ecaudatus	6.75	6.75	6.7
Dasypus sabanicola	7.05	7.05	7.0
Tolypeutes matacus	7.11	7.11	7.1
Dasypus septemcinctus	7.30	7.30	7.3
Zaedyus pichiy	7.31	7.31	7.3
Dasypus hybridus	7.31	7.31	7.3
Chaetophractus villosus	7.61	7.61	7.6
Chaetophractus nationi	7.67	7.67	7.6
Heterohyrax brucei	7.78	7.78	7.7
Cabassous centralis	7.92	7.92	7.9
Seggeurius amourensis	7.98	7.98	7.9
Procavia capensis	8.01	8.01	8.0
Dendrohyrax dorsalis	8.06	8.06	8.0
Microhyrax lavocati	8.13	8.13	8.1
Bradypus tridactylus	8.23	8.23	8.2
Bradypus torquatus	8.27	8.27	8.2
Dasypus novemcinctus	8.37	8.37	8.3
Euphractus sexcinctus	8.43	8.43	8.4
Choloepus hoffmanni	8.47	8.47	8.4
Bradypus variegatus	8.49	8.49	8.4
Tamandua tetradactyla	8.52	8.52	8.5
Cyclopes didactylus	8.53	8.53	8.5
Choloepus didactylus	8.71	8.71	8.7
Thyrohyrax meyeri	8.78	8.78	8.7
Saghatherium bowni	9.13	9.13	9.1
Dasypus kappleri	9.23	9.23	9.2
Thyrohyrax domorictus	9.30	9.30	9.3
Dimaitherium patnaiki	9.57	9.57	9.5
Phosphatherium escuilliei	9.62	9.62	9.6
Saghatherium antiquum	9.73	9.73	9.7
Thyrohyrax litholagus	10.01	10.01	10.0
Myrmecophaga tridactyla	10.26	10.26	10.2
Myorycteropus africanus	10.27	10.27	10.2
Selenohyrax chatrathi	10.73	10.73	10.7
Priodontes maximus	10.82	10.82	10.8
Orycteropus afer	10.87	10.87	10.8
Antilohyrax pectidens	10.93	10.93	10.9
Bunohyrax fajumensis	11.32	11.32	11.3

Ancestor/Species	Estimated Body Size (log(g))	95% CI (Low)	95% CI (High
Afrohyrax championi	11.32	11.32	11.3
Geniohyus mirus	11.33	11.33	11.3
Prorastomus sirenoides	11.49	11.49	11.4
Elephas antiquus falconeri	11.51	11.51	11.5
Pachyhyrax crassidentatus	11.81	11.81	11.8
Megalohyrax eocaenus	11.95	11.95	11.9
Elephas cypriotes	12.21	12.21	12.2
Bunohyrax major	12.36	12.36	12.3
Titanohyrax angustidens	12.48	12.48	12.4
Daouitherium rebouli	12.80	12.80	12.8
Arcanotherium savagei	12.89	12.89	12.8
Dugong dugon	12.92	12.92	12.9
Trichechus senegalensis	13.03	13.03	13.0
Trichechus inunguis	13.08	13.08	13.0
Protosiren smithae	13.20	13.20	13.2
Numidotherium koholense	13.23	13.23	13.2
Omanitherium dhofarensis	13.35	13.35	13.3
Trichechus manatus	13.44	13.44	13.4
Moeritherium spp	13.82	13.82	13.8
Phiomia spp	13.89	13.89	13.8
Elephas maximus	15.02	15.02	15.0
Barytherium spp	15.20	15.20	15.2
Mammuthus primigenius	15.27	15.27	15.2
Mammut borsoni	16.49	16.49	16.4
Mammuthus trogontherii	16.38	16.38	16.3
Loxodonta africana	15.35	15.35	15.3
Loxodonta cyclotis	15.37	15.37	15.3
Palaeoloxodon antiquus	16.14	16.14	16.1
Palaeoloxodon namadicus	16.81	16.81	16.8
Mammut americanum	15.61	15.61	15.6
Mammuthus columbi	15.71	15.71	15.7
Hydrodamalis gigas	15.72	15.72	15.7
Atlantogenata	5.55	4.06	7.9
Afrotheria	5.55	4.05	7.9
Afrosorcida	4.35	2.58	6.1
Macroscelidae	5.27	3.98	6.8
Pseudoungulata	9.76	5.21	12.7
Paenungulata	10.13	7.24	13.0
Tethytheria	12.60	10.25	13.8
Proboscidae	15.23	14.22	16.2
Elephantidae	15.49	14.89	16.1
Elephantina	15.51	15.08	15.9
Mammuthus	15.54	15.24	15.8
Loxodontini	15.55	15.02	16.1
Loxodona	15.72	15.16	16.3
Xenarthra	7.57	5.96	9.1

To trace the evolutionary history of body mass and lifespan in Afrotherians, we built a time-calibrated supertree of Eutherian mammals combining 1,679 species from Bininda-Emonds et al [24] with a total evidence Afrotherian phylogeny including 77 extant and fossil data from 39 extinct species [19]. Fossil data from extinct species were

included to ensure that ancestral state reconstructions of body mass in Afrotherians were not biased by only including extant species, which can lead to inaccurate reconstructions, for example, if lineages multiple lineages evolved large body masses from a small bodied ancestor. We jointly estimated rates of body mass evolution and reconstructed ancestral states using a generalization of a Brownian model of character evolution, which allows for occasional large jumps in traits (stable model) and out performs standard Brownian motion and Ornstein?Uhlenbeck models of character evolution [25].

Similar to previous studies of Afrotherian body size [19,25], we found that the body mass of the Afrotherian ancestor was inferred to be small (0.26kg, 95% CI: 0.31-3.01kg) and that substantial accelerations in the rate of body mass evolution occurred coincident with a 65× increase in body mass in the stem-lineage of *Pseudoungulata* (17kg), a 1.5× increase in body mass in the stem-lineage of *Paenungulata* (25kg), a 12× increase in body mass in the stem-lineage of *Tehthytheria* (296kg), and a 14× increase in body mass in the stem-lineage of *Proboscidea* (4,100kg; Figure 1). The ancestral *Hyracoidea* was inferred to be relatively small (2.86-15.71kg), and rate accelerations were coincident with independent body mass increases in large hyraxes such as *Titanohyrax andrewsi* (67× increase in body mass). While the body mass of the ancestral *Sirenian* was inferred to be large (61-656kg), a rate acceleration occurred coincident with a 10× body mass increase in Stellar's sea cow. Rate accelerations also occurred coincident with 36× body mass reduction in the stem-lineage of the dwarf elephants *Elephas* (*Palaeoloxodon*) *falconeri* and *Palaeoloxodon cypriotes*. These data suggest that gigantism in *Afrotherians* evolved step-wise, from small to medium bodies in the *Pseudoungulata* stem-lineage, medium to large bodies in the *Tehthytherian* stem-lineage and extinct hyraxes, and from large to exceptionally large bodies independently in the *Proboscidean* stem-lineage and Stellar's sea cow (Figure 1).

Pervasive duplication of tumor suppressors during the origins of large bodied Afrotheirans

```
## Warning: Column ‘Genome‘ joining factor and character vector, coercing into
## character vector

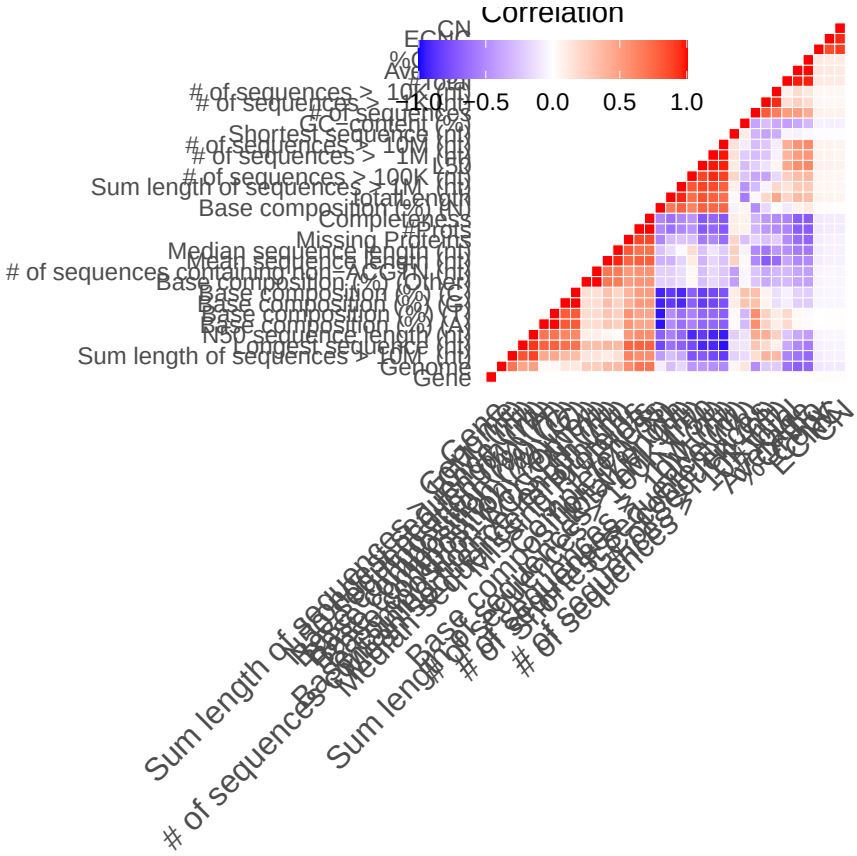
## Warning in melt(., na.rm = T): The melt generic in data.table has been passe
## matrix and will attempt to redirect to the relevant reshape2 method; please
## that reshape2 is deprecated, and this redirection is now deprecated as well.
## To continue using melt methods from reshape2 while both libraries are attach
## e.g. melt.list, you can prepend the namespace like reshape2::melt(.). In the
## next version, this warning will become an error.

##                                       Var1 Var2        value
## 1                                     Gene Copy  0.011177676
## 2                                   Genome Copy -0.104365173
## 3       Sum length of sequences > 10M  (nt) Copy -0.074960529
## 4                      Longest sequence (nt) Copy -0.067231077
## 5                  N50 sequence length (nt) Copy -0.050741921
## 6                  Base composition (%) (A) Copy -0.001502967
## 7                  Base composition (%) (T) Copy -0.001379093
## 8                  Base composition (%) (G) Copy -0.043271156
## 9                  Base composition (%) (C) Copy -0.042268177
## 10             Base composition (%) (Other) Copy -0.050228064
## 11 # of sequences containing non-ACGTN (nt) Copy -0.050621596
## 12                Mean sequence length (nt) Copy -0.074983744
```

```
## 13              Median sequence length (nt) Copy -0.074897040
## 14                        Missing Proteins Copy -0.079692396
## 15                                  #Prots Copy -0.101300994
## 16                             Completeness Copy -0.101321644
## 17                Base composition (%) (N) Copy  0.019768158
## 18                               totalLength Copy  0.067454870
## 19       Sum length of sequences > 1M  (nt) Copy  0.051354838
## 20               # of sequences > 100K (nt) Copy  0.056049593
## 21                                    L50 Copy  0.082175611
## 22               # of sequences >   1M (nt) Copy  0.074461957
## 23               # of sequences >  10M (nt) Copy  0.075679451
## 24                   Shortest sequence (nt) Copy -0.013216232
## 25                           GC-content (%) Copy -0.064591581
## 26                           # of sequences Copy  0.076630197
## 27               # of sequences >   1K (nt) Copy  0.047474518
## 28               # of sequences >  10K (nt) Copy  0.045583001
## 29                                  #Total Copy  0.116391876
## 30                                Average Copy  0.124341009
## 31                                %Ortho Copy  0.120616153
## 32                                  Gene ECNC  0.011137324
## 33                                Genome ECNC -0.090522003
## 34      Sum length of sequences > 10M  (nt) ECNC -0.061380983
## 35                    Longest sequence (nt) ECNC -0.053145168
## 36                N50 sequence length (nt) ECNC -0.040007282
## 37                Base composition (%) (A) ECNC  0.004626621
## 38                Base composition (%) (T) ECNC  0.004862588
## 39                Base composition (%) (G) ECNC -0.037787699
## 40                Base composition (%) (C) ECNC -0.037045693
## 41             Base composition (%) (Other) ECNC -0.040274294
## 42 # of sequences containing non-ACGTN (nt) ECNC -0.040587311
## 43                 Mean sequence length (nt) ECNC -0.061569667
## 44               Median sequence length (nt) ECNC -0.060928944
## 45                        Missing Proteins ECNC -0.062209031
## 46                                  #Prots ECNC -0.084046941
## 47                             Completeness ECNC -0.084067608
## 48                Base composition (%) (N) ECNC  0.013486259
## 49                               totalLength ECNC  0.061175951
## 50       Sum length of sequences > 1M  (nt) ECNC  0.046387082
## 51               # of sequences > 100K (nt) ECNC  0.049326449
## 52                                    L50 ECNC  0.070430706
## 53               # of sequences >   1M (nt) ECNC  0.063725329
## 54               # of sequences >  10M (nt) ECNC  0.062895861
## 55                   Shortest sequence (nt) ECNC -0.012113794
## 56                           GC-content (%) ECNC -0.066249117
## 57                           # of sequences ECNC  0.070289641
## 58               # of sequences >   1K (nt) ECNC  0.042151677
## 59               # of sequences >  10K (nt) ECNC  0.041222984
## 60                                  #Total ECNC  0.102797584
## 61                                Average ECNC  0.108543367
## 62                                %Ortho ECNC  0.104394790
## 63                                  Gene   CN  0.013089940
## 64                                Genome   CN -0.092501845
```

```
## 65      Sum length of sequences > 10M  (nt)   CN -0.061610123
## 66                    Longest sequence (nt)   CN -0.052591263
## 67                 N50 sequence length (nt)   CN -0.038770715
## 68                 Base composition (%) (A)   CN  0.006139187
## 69                 Base composition (%) (T)   CN  0.006354445
## 70                 Base composition (%) (G)   CN -0.036741673
## 71                 Base composition (%) (C)   CN -0.035971303
## 72              Base composition (%) (Other)   CN -0.041544748
## 73 # of sequences containing non-ACGTN (nt)   CN -0.041868348
## 74                 Mean sequence length (nt)   CN -0.063786387
## 75               Median sequence length (nt)   CN -0.063541494
## 76                          Missing Proteins   CN -0.063703014
## 77                                    #Prots   CN -0.085286259
## 78                              Completeness   CN -0.085306203
## 79                 Base composition (%) (N)   CN  0.012122309
## 80                                totalLength   CN  0.060342667
## 81       Sum length of sequences > 1M  (nt)   CN  0.044889905
## 82                # of sequences > 100K (nt)   CN  0.047874628
## 83                                      L50   CN  0.069838784
## 84                # of sequences >   1M (nt)   CN  0.062839574
## 85                # of sequences >  10M (nt)   CN  0.062215472
## 86                    Shortest sequence (nt)   CN -0.013168670
## 87                           GC-content (%)   CN -0.066875760
## 88                           # of sequences   CN  0.073563144
## 89                # of sequences >   1K (nt)   CN  0.044231140
## 90                # of sequences >  10K (nt)   CN  0.042436480
## 91                                    #Total   CN  0.105113732
## 92                                  Average   CN  0.110764419
## 93                                   %Ortho   CN  0.106456398
```

between genome quality scores-1.pdf




```
## Warning: Column ‘Genome‘/‘BestGenome‘ joining factor and character vector,
## coercing into character vector

## Warning: ‘as.tibble()‘ is deprecated as of tibble 2.0.0.
## Please use ‘as_tibble()‘ instead.
## The signature and semantics have changed, see ‘?as_tibble‘.
## This warning is displayed once every 8 hours.
## Call ‘lifecycle::last_warnings()‘ to see where this warning was generated.

## Warning: Removed 1 rows containing missing values (geom_text).


## Warning: Table 2
```

Enhanced cancer suppression may have evolved through many mechanisms; among the most parsimonious is an increase in the copy number of genes with tumor suppressor functions. Previous studies focusing on candidate gene studies, for example, have identified increased copy number of the tumor suppressors *TP53* and *LIF* in elephants [**???**,11,21–23]. Therefore, in order to test whether this was a pervasive phenomena genome-wide in *Afrotherians*, we used a Reciprocal Best Hit BLAT (RBHB) approach to infer gene copy number in *Afrotherian* and *Atlantogenatan* genomes (Fig. 2A). Because RBHB-like approaches can over-estimate copy number when genes are fragmented or incorrectly assembled across multiple scaffolds, we also inferred copy number using a complementary method that quantifies the ratio between observed and expected gene coverage per nucleotide (ECNC) (Fig. 2B; Sup. Fig. 2A-D). By only including nucleotides from the query sequence that were observed in the target genome,

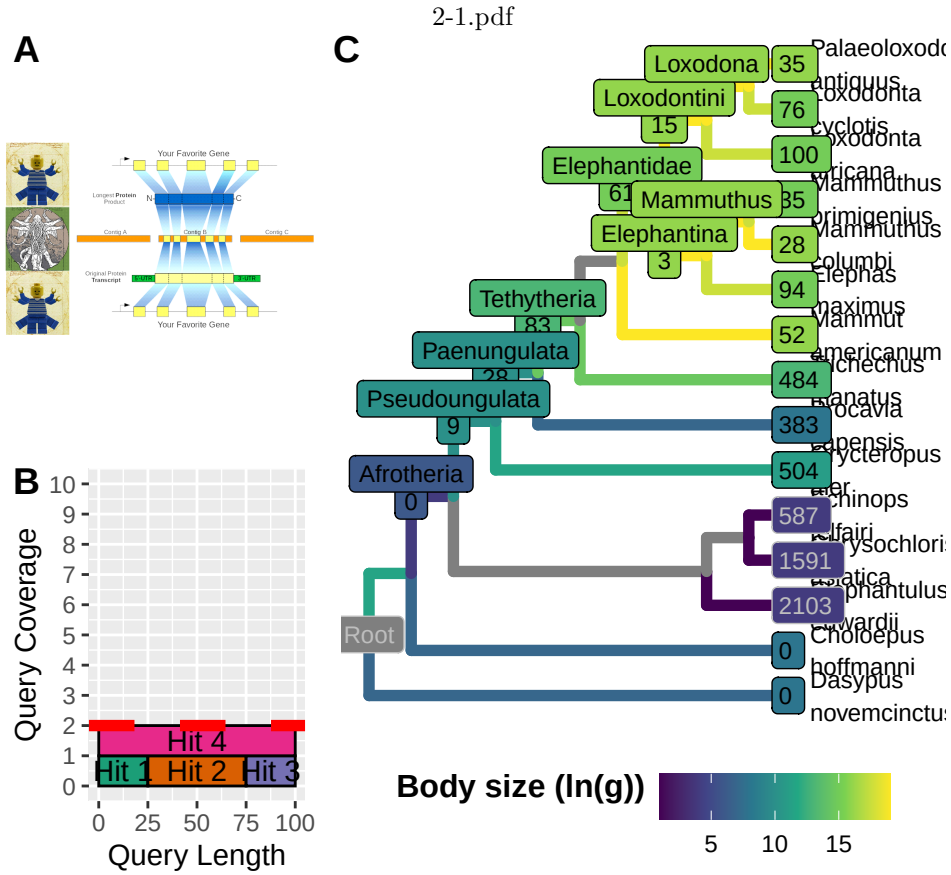


Fig 1. Figure 2: A Reciprocal Best-Hit BLAT pipeline for identifying gene copy number in other genomes. A) A graphic summary of the reciprocal best-hit strategy. B) Estimated Copy Number by Coverage. C)

we also correct for partial hits where some or all of the homologs of a gene have diverged from the human homolog.

Because our sequence database included various protein transcripts for each gene, in order to obtain gene-level copy number information and elimate any many-to-one mappings of hits, we labeled each exon of every reciprocal best hit (RBH) with the gene corresponding to the query transcript and merged all overlapping exons; next, we eliminated any many-to-one exons that resulted from the previous step. Finally, we reassembled the gene loci based on the original transcript starts and ends, and the collapsed exon data, obtaining the full sequence of each RBH locus. Genes were considered to be duplicated if its copy number via RBHB was greater than or equal to 2, and the maximum ECNC among all transcripts prior to filtering was greater than or equal to 1.50. This cutoff of ECNC was selected to account for truncated gene duplications, which have been shown to be functional in various examples [**???** examples of this].

To reconcile the Atlantogenatan phylogeny with duplication events, we used maximum likelihood to reconstruct likely ancestral copy numbers for each gene at each node in the phylogeny. To define the copy number of a gene, we conservatively used the lesser value between the RBHB hit count, and the ECNC value rounded to the nearest whole number. In order to perform

Next, in order to select genes and duplicates which were likely functional, we omitted any hits that were not supported by either the gene prediction method GenScan, or by at least one transcript assembled from publically-available RNA-seq data.

We describe the number of genes that increased in each lineage in *Atlantogenata* in Figure 2. Among the genes that increased in copy number in the elephant lineage are TP53 and LIF, as previously described. Furthermore, we identify

Duplications that occured recently in Probodiscea are enriched for tumor suppressor pathways

In order to infer the functional consequences of these gene duplications, we tested if duplicate genes were enriched in specific pathways relative to our initial query set of genes. We used

Retroduplication of EEF1A1 in the stem-lineage of Tehthytheria

```
## Warning: Fig 3: EEF1A1
```

Previous studies have identified genes whose transgenic overexpression delays aging related phenotypes and extends lifespan. Overexpression of the eukaryotic translation elongation factor 1 alpha 1 (EEF1A1) gene, for example, extends lifespan in transgenic Drosophila. Like most mammalian genomes, we found that Afrotherian genomes encoded numerous EEF1A1 retrogenes (Fig. XA). While the majority of these retrogenes have premature stop codons and insertions/deletions characteristic of pseudogenes, at least 13 Tehthytherian-specific EEF1A1 retrogenes have the potential to encode functional proteins (Fig. XB). Unfortunately it is difficult to determine if these retrogenes are transcribed because they have relatively high sequence similarity (83-88%), suggesting evidence of transcription using RNA-Seq data is likely an artifact. Indeed, nearly all of the EEF1A1 retrogene transcripts assembled by StringTie were confined to their gene body and UTRs and thus lacked unique sequences. The EEF1A1RTG13 transcript, however, initiates within an upstream MLT1B retrovirus-like MaLR transposable element and includes unique 5?- and 3?-UTR sequences, indicating at least one EEF1A1 retrogene is transcribed (Fig. XC). EEF1A1

Candidate gene studies, for example, have identified functional duplicates of the tumor suppressors TP53 and LIF in elephants. In a larger candidate gene study, Caulin et al. characterized the copy number of 830 known tumor-suppressor genes across 36 mammals and identified 382 putative duplicates, including duplicates in species with large body sizes and long life-spans. however, the probability of developing cancer is similar for small, short-lived mammals such as mice and for large, long-lived mammals such as elephants.

In stark contrast, genome-wide studies of unusually large or long-lived species such as the bowhead whale (Keane et al., 2015), Myotid bats (Seim et al., 2013; Zhang et al., 2013), naked mole rat (Kim et al., 2011), and blind mole rat (Fang et al., 2014) did not find an over representation of tumor suppressors among duplicate genes.

A genomic analysis of genetic changes associated with the evolution of enhanced cancer resistance in the elephant lineage has yet to be performed. Thus it is not clear if the duplication of TP53 and LIF reflects a general pattern of tumor suppressor duplication in the elephant lineage, unlike other lineages that resolved Peto?s paradox, or from the kinds of ascertainment biases common in candidate gene studies.

Concerted duplication of TP53 and TP53-related genes towards *Probodiscea*

```
## Warning: Fig4
```

Step-wise reduction of intrinsic cancer risk in large, long-lived Afrotherians

```
## # A tibble: 253 x 6
##     parent  node branch.length label                       lnSize Lifespan
##      <int> <int>         <dbl> <chr>                        <dbl>    <dbl>
##   1    135     1     40.1      Geogale.aurita                   2    1.72
##   2    140     2    122.       Limnogale.mergulus               5    2.30
##   3    140     3      0.135    Microgale.taiva                  2    1.72
##   4    139     4     33.5      Microgale.parvula                1    1.52
##   5    138     5      0.595    Microgale.brevicaudata           2    1.72
##   6    142     6      0.571    Microgale.cowani                 3    1.91
##   7    142     7      1.07     Microgale.jobihely               2    1.72
##   8    145     8      0.772    Microgale.longicaudata           2    1.72
##   9    145     9      0.173    Microgale.principula             2    1.72
##  10    144    10     34.3      Microgale.pusilla                1    1.52
##  11    143    11     61.4      Microgale.talazaci               4    1.76
##  12    136    12     22.8      Oryzorictes.hova                 4    2.14
##  13    147    13      7.75     Echinops.telfairi                4    2.94
##  14    147    14     12.5      Setifer.setosus                  6    2.65
##  15    148    15      4.68     Hemicentetes.semispinosus        5    0.993
##  16    148    16     79.5      Tenrec.ecaudatus                 7    2.16
##  17    150    17      2.82     Micropotamogale.lamottei         4    2.14
##  18    150    18    103.       Potamogale.velox                 6    2.50
##  19    151    19     69.8      Protenrec.tricuspis              1    1.52
##  20    151    20      2.21     Dilambdogale.gheerbranti         2    1.72
##  21    157    21      0.454    Carpitalpa.arendsi               4    2.14
##  22    158    22      3.26     Neamblysomus.gunningi            4    2.14
##  23    158    23      5.72     Neamblysomus.julianae            3    1.91
##  24    162    24      0.976    Amblysomus.corriae               4    2.14
```

```
##  25   162   25     1.38     Amblysomus.robustus              4   2.14
##  26   161   26     0.524    Amblysomus.septentrionalis       4   2.14
##  27   160   27     0.0152   Amblysomus.hottentotus           4   2.14
##  28   159   28     3.79     Amblysomus.marleyi               4   2.14
##  29   163   29     0.321    Chlorotalpa.duthieae             3   1.91
##  30   163   30     0.0874   Chlorotalpa.sclateri             4   2.14
##  31   165   31    62.8      Chrysospalax.trevelyani          6   2.50
##  32   165   32     0.130    Chrysospalax.villosus            5   2.30
##  33   164   33    13.4      Calcochloris.obtusirostris       3   1.91
##  34   153   34     9.65     Eremitalpa.granti                3   1.91
##  35   167   35     3.34     Chrysochloris.asiatica           4   2.14
##  36   167   36     5.78     Cryptochloris.wintoni            3   1.91
##  37   166   37     0.333    Chrysochloris.stuhlmanni         4   2.14
##  38   175   38     0.926    Elephantulus.brachyrhynchus      4   2.14
##  39   175   39     0.118    Elephantulus.rufescens           4   2.07
##  40   176   40     1.15     Elephantulus.intufi              4   2.23
##  41   176   41     0.319    Elephantulus.rupestris           4   2.14
##  42   177   42     0.237    Elephantulus.edwardii            4   2.14
##  43   177   43     0.953    Elephantulus.myurus              4   2.14
##  44   179   44    10.5      Elephantulus.rozeti              4   1.97
##  45   179   45    24.6      Petrodromus.tetradactylus        5   2.30
##  46   178   46    14.2      Macroscelides.proboscideus       4   2.16
##  47   181   47     1.93     Elephantulus.fuscipes            4   2.14
##  48   182   48     0.678    Elephantulus.fuscus              4   2.14
##  49   182   49     1.10     Elephantulus.revoili             3   1.91
##  50   184   50     0.403    Rhynchocyon.chrysopygus          6   2.40
##  51   185   51     3.30     Rhynchocyon.cirnei               6   2.50
##  52   185   52     4.33     Rhynchocyon.udzungwensis         7   2.69
##  53   183   53     2.13     Rhynchocyon.petersi              6   2.50
##  54   170   54    15.9      Metoldobotes.sp..nov             6   2.50
##  55   186   55    11.8      Chambius.kasserinensis           4   2.14
##  56   186   56    11.6      Herodotius.pattersoni            5   2.30
##  57   188   57     6.59     Orycteropus.afer                11   3.39
##  58   188   58     0.569    Myorycteropus.africanus         10   3.27
##  59   194   59     1.86     Dendrohyrax.dorsalis             8   2.88
##  60   195   60     1.64     Heterohyrax.brucei               8   2.88
##  61   195   61     0.000068 Procavia.capensis                8   2.69
##  62   193   62     1.15     Thyrohyrax.domorictus            9   3.08
##  63   192   63     3.55     Thyrohyrax.meyeri                9   3.08
##  64   198   64    28.6      Thyrohyrax.litholagus           10   3.27
##  65   201   65     2.29     Pachyhyrax.crassidentatus       12   3.66
##  66   201   66     1.45     Bunohyrax.fajumensis            11   3.47
##  67   202   67     5.44     Geniohyus.mirus                 11   3.47
##  68   202   68    11.4      Bunohyrax.major                 12   3.66
##  69   203   69     0.235    Megalohyrax.eocaenus            12   3.66
##  70   203   70     7.21     Megalohyrax.sp..nov             13   3.86
##  71   207   71     0.0699   Titanohyrax.andrewsi            13   3.86
##  72   207   72    34.6      Titanohyrax.ultimus             14   4.05
##  73   206   73     0.0353   Titanohyrax.angustidens         12   3.66
##  74   205   74    13.7      Antilohyrax.pectidens           11   3.47
##  75   204   75     0.194    Afrohyrax.championi             11   3.47
##  76   209   76     2.90     Saghatherium.antiquum           10   3.27
```

```
##  77   209   77    15.0   Selenohyrax.chatrathi          11   3.47
##  78   208   78    15.8   Saghatherium.bowni              9   3.08
##  79   211   79     2.82  Seggeurius.amourensis           8   2.88
##  80   211   80     0.733 Microhyrax.lavocati             8   2.88
##  81   210   81    18.2   Dimaitherium.patnaiki          10   3.27
##  82   220   82     5.81  Elephas.maximus                15   4.18
##  83   222   83     6.12  Elephas.antiquus.falconeri     12   3.66
##  84   222   84     1.90  Elephas.cypriotes              12   3.66
##  85   221   85     0.340 Elephas.maximus.asurus         16   4.44
##  86   223   86    16.0   Mammuthus.trogontherii         16   4.44
##  87   224   87     0.910 Mammuthus.columbi              16   4.44
##  88   224   88     2.17  Mammuthus.primigenius          15   4.24
##  89   225   89     1.28  Loxodonta.africana             15   4.17
##  90   227   90     0.0149 Palaeoloxodon.antiquus        16   4.44
##  91   227   91    12.8   Palaeoloxodon.namadicus        17   4.63
##  92   226   92     3.72  Loxodonta.cyclotis             15   4.24
##  93   228   93    15.3   Mammut.borsoni                 16   4.44
##  94   228   94     0.951 Mammut.americanum              16   4.44
##  95   216   95     3.64  Phiomia.spp                    14   4.05
##  96   230   96     0.0280 Omanitherium.dhofarensis      13   3.86
##  97   230   97     7.29  Arcanotherium.savagei          13   3.86
##  98   229   98    73.6   Barytherium.spp                15   4.24
##  99   214   99     5.71  Moeritherium.spp               14   4.05
## 100   231  100   326.    Phosphatherium.escuilliei      10   3.27
## 101   232  101     2.29  Numidotherium.koholense        13   3.86
## 102   232  102     0.742 Daouitherium.rebouli           13   3.86
## 103   235  103     5.85  Dugong.dugon                   13   4.29
## 104   235  104   173.    Hydrodamalis.gigas             16   4.44
## 105   237  105     0.688 Trichechus.inunguis            13   3.69
## 106   237  106     1.39  Trichechus.manatus             13   4.23
## 107   236  107     0.574 Trichechus.senegalensis        13   3.86
## 108   238  108    13.6   Prorastomus.sirenoides         11   3.47
## 109   238  109    33.7   Protosiren.smithae             13   3.86
## 110   241  110     0.640 Choloepus.didactylus           9   3.61
## 111   241  111     0.316 Choloepus.hoffmanni            8   3.71
## 112   242  112     0.0297 Bradypus.torquatus            8   2.88
## 113   243  113     0.483 Bradypus.tridactylus           8   2.88
## 114   243  114     0.512 Bradypus.variegatus            8   2.88
## 115   245  115    10.4   Tamandua.tetradactyla          9   2.94
## 116   245  116    41.0   Myrmecophaga.tridactyla        10   3.43
## 117   244  117     2.15  Cyclopes.didactylus            9   3.08
## 118   249  118    15.0   Euphractus.sexcinctus          8   3.10
## 119   249  119     5.54  Zaedyus.pichiy                 7   2.53
## 120   250  120     0.0899 Chaetophractus.nationi        8   2.88
## 121   250  121     0.418 Chaetophractus.villosus        8   3.23
## 122   251  122   268.    Priodontes.maximus             11   2.71
## 123   252  123    16.0   Tolypeutes.matacus             7   3.61
## 124   252  124     0.248 Cabassous.centralis            8   2.88
## 125   253  125     4.05  Dasypus.hybridus               7   2.69
## 126   253  126    74.1   Dasypus.kappleri               9   3.08
## 127   253  127    14.7   Dasypus.novemcinctus           8   3.10
## 128   253  128    12.2   Dasypus.sabanicola             7   2.69
```

```
## 129    253   129      4.44     Dasypus.septemcinctus            7    2.82
## 130    130   130     NA        Atlantogenata                    2    1.72
## 131    130   131      0.000982 Afrotheria                       6    2.50
## 132    131   132     44.5      Afrosorcida                      4    2.11
## 133    132   133     11.8      <NA>                             4    2.11
## 134    133   134      0.420    <NA>                             4    2.11
## 135    134   135     10.1      <NA>                             3    1.91
## 136    135   136      0.373    <NA>                             3    1.91
## 137    136   137      2.96     <NA>                             3    1.91
## 138    137   138      2.69     <NA>                             3    1.91
## 139    138   139      0.450    <NA>                             2    1.72
## 140    139   140      3.36     <NA>                             3    1.91
## 141    137   141      0.153    <NA>                             3    1.91
## 142    141   142      0.116    <NA>                             2    1.72
## 143    141   143      0.222    <NA>                             2    1.72
## 144    143   144      0.771    <NA>                             2    1.72
## 145    144   145      0.109    <NA>                             3    1.91
## 146    134   146     38.5      <NA>                             5    2.30
## 147    146   147      1.81     <NA>                             3    1.91
## 148    146   148      5.26     <NA>                             3    1.91
## 149    133   149      4.28     <NA>                             3    1.91
## 150    149   150     52.3      <NA>                             5    2.30
## 151    149   151     15.6      <NA>                             3    1.91
## 152    132   152     12.0      <NA>                             4    2.11
## 153    152   153      0.0295   <NA>                             4    2.11
## 154    153   154      0.173    <NA>                             3    1.91
## 155    154   155      0.0141   <NA>                             3    1.91
## 156    155   156      0.107    <NA>                             4    2.11
## 157    156   157      0.00544  <NA>                             3    1.91
## 158    157   158      0.102    <NA>                             3    1.91
## 159    156   159      0.150    <NA>                             3    1.91
## 160    159   160      0.483    <NA>                             3    1.91
## 161    160   161      0.321    <NA>                             3    1.91
## 162    161   162      0.00743  <NA>                             4    2.11
## 163    155   163      2.12     <NA>                             3    1.91
## 164    154   164      0.774    <NA>                             4    2.11
## 165    164   165     18.6      <NA>                             5    2.30
## 166    152   166      0.260    <NA>                             3    1.91
## 167    166   167      0.155    <NA>                             3    1.91
## 168    131   168      0.00016  <NA>                             6    2.50
## 169    168   169      2.49     Macroscelidae                    5    2.30
## 170    169   170      0.105    <NA>                             5    2.30
## 171    170   171      4.23     <NA>                             5    2.30
## 172    171   172      6.20     <NA>                             3    1.91
## 173    172   173      0.977    <NA>                             3    1.91
## 174    173   174      0.448    <NA>                             4    2.11
## 175    174   175      0.349    <NA>                             4    2.11
## 176    174   176      0.176    <NA>                             4    2.11
## 177    173   177      1.62     <NA>                             4    2.11
## 178    172   178      0.155    <NA>                             3    1.91
## 179    178   179      0.180    <NA>                             4    2.11
## 180    171   180      0.100    <NA>                             5    2.30
```

```
## 181    180   181     11.2      <NA>                    3    1.91
## 182    181   182     12.0      <NA>                    3    1.91
## 183    180   183     30.6      <NA>                    3    1.91
## 184    183   184      2.34     <NA>                    3    1.91
## 185    184   185      0.0165   <NA>                    3    1.91
## 186    169   186      4.53     <NA>                    5    2.30
## 187    168   187    546.       Pseudoungulata         10    3.27
## 188    187   188     12.9      <NA>                   10    3.27
## 189    187   189      4.42     Paenungulata           10    3.27
## 190    189   190      6.90     <NA>                    5    2.30
## 191    190   191      1.47     <NA>                   10    3.27
## 192    191   192     17.9      <NA>                    9    3.08
## 193    192   193      0.00758  <NA>                    9    3.08
## 194    193   194     19.5      <NA>                    5    2.30
## 195    194   195      2.67     <NA>                    5    2.30
## 196    191   196      5.61     <NA>                    3    1.91
## 197    196   197      8.46     <NA>                    3    1.91
## 198    197   198      0.602    <NA>                   11    3.47
## 199    198   199      8.81     <NA>                   12    3.66
## 200    199   200      0.347    <NA>                    3    1.91
## 201    200   201      0.177    <NA>                   12    3.66
## 202    200   202      0.584    <NA>                    3    1.91
## 203    199   203      8.90     <NA>                   12    3.66
## 204    197   204      9.93     <NA>                   11    3.47
## 205    204   205      1.23     <NA>                   12    3.66
## 206    205   206     22.2      <NA>                    3    1.91
## 207    206   207     10.2      <NA>                   13    3.86
## 208    196   208      6.63     <NA>                    3    1.91
## 209    208   209      1.16     <NA>                   10    3.27
## 210    190   210     22.7      <NA>                    9    3.08
## 211    210   211      8.32     <NA>                    8    2.88
## 212    189   212    187.       Tethytheria            13    3.86
## 213    212   213      3.40     <NA>                    3    1.91
## 214    213   214      6.37     <NA>                   13    3.86
## 215    214   215      1.15     <NA>                   14    4.05
## 216    215   216     13.4      <NA>                    3    1.91
## 217    216   217     30.3      Proboscidae             3    1.91
## 218    217   218      2.21     Elephantidae           15    4.24
## 219    218   219      0.0116   Elephantina            16    4.44
## 220    219   220      0.128    <NA>                   15    4.24
## 221    220   221      0.369    <NA>                    3    1.91
## 222    221   222    400.       <NA>                    3    1.91
## 223    219   223      0.645    <NA>                    3    1.91
## 224    223   224      0.467    Mammuthus              16    4.44
## 225    218   225      0.108    Loxodontini            16    4.44
## 226    225   226      0.862    Loxodona               16    4.44
## 227    226   227      6.09     <NA>                    3    1.91
## 228    217   228      9.63     <NA>                    3    1.91
## 229    215   229      0.197    <NA>                    3    1.91
## 230    229   230      2.47     <NA>                    3    1.91
## 231    213   231      0.128    <NA>                   13    3.86
## 232    231   232      0.268    <NA>                    3    1.91
```

```
## 233    212   233      0.309    <NA>                    12    3.66
## 234    233   234     16.4      <NA>                     3    1.91
## 235    234   235      0.530    <NA>                     3    1.91
## 236    234   236      0.134    <NA>                     3    1.91
## 237    236   237      0.145    <NA>                     3    1.91
## 238    233   238      3.59     <NA>                     3    1.91
## 239    130   239    125.       Xenarthra               11    3.47
## 240    239   240     14.6      <NA>                     3    1.91
## 241    240   241      3.04     <NA>                     3    1.91
## 242    240   242      0.0660   <NA>                    11    3.47
## 243    242   243      0.112    <NA>                    11    3.47
## 244    239   244     15.1      <NA>                     3    1.91
## 245    244   245     21.7      <NA>                     9    3.08
## 246    239   246      0.958    <NA>                    10    3.27
## 247    246   247      0.0602   <NA>                     3    1.91
## 248    247   248      0.0914   <NA>                     3    1.91
## 249    248   249      0.000579 <NA>                     8    2.88
## 250    248   250      0.000908 <NA>                    11    3.47
## 251    247   251      0.217    <NA>                     3    1.91
## 252    251   252      0.0523   <NA>                    11    3.47
## 253    246   253      0.140    <NA>                     8    2.88
```

```
## Warning: Fig5
```

The dramatic increase in body mass and lifespan in some Afrotherian lineages implies those lineages evolved reduced cancer risk. To infer the magnitude of these reductions we estimated differences in cancer risk between small bodied, short-lived species and large bodied, long-lived species as well as for reconstructed ancestral Afrotherians. Following [**???**] we estimate the intrinsic cancer risk as the product of risk associated with body mass and lifespan. Differences in cancer susceptibility due to body mass differences between species can be approximated simply as the fold difference in body mass (D) between species [**???**]. The risk of developing cancer also increases in proportion to the sixth power of age and is approximated by the formula Ct^6, in which the proportionality constant C that determines susceptibility to cancer induction is multiplied by the sixth power of the age in years, t [**???**,**??**,**??**]. Thus we can estimate differences in the intrinsic cancer risk between species as $D(Ct^6)$. We inferred that susceptibility to cancer induction (C) was reduced 3.40x10ˆ3-fold in the ancestor of Pseudoungulata, 7.20$\times$10ˆ3-fold in the ancestor of Paenungulata, 8.81x10ˆ5-fold in the ancestor of Tehthytheria, 1.46x10ˆ8-fold in the ancestor of Proboscidea, and 1.28x10ˆ6-fold in the ancestor of Sirenia relative to the ancestral Afrotherian (Table 3). What biological mechanisms underlie the evolution of thousand- to hundred million-fold reductions in cancer susceptibility during the origins of Afrotherians, which are essential for large body size and long lifespan to evolve

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