

Adaptive evolution toward larger size in mammals

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Short Title: Adaptive evolution towards larger size in mammals

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Abstract

The notion that large body size confers some intrinsic advantage to biological species has been debated for centuries. Using a novel phylogenetic statistical approach that allows the rate of body size evolution to vary across a phylogeny, we find a long-term directional bias towards increasing size in the mammals. This pattern holds separately in ten of eleven orders for which sufficient data are available and arises from a tendency for accelerated rates of evolution to produce increases – but not decreases – in size. On a branch-by-branch basis, increases in body size have been more than twice as likely as decreases, yielding what amounts to millions and millions of years of rapid and repeated increases in size away from the small ancestral mammal. These results are the first evidence from extant species that are compatible with Cope’s rule – the pattern of body size increase through time observed in the mammalian fossil record. We show that this pattern is unlikely to be explained by several non-adaptive mechanisms for increasing size, and most likely represents repeated responses to new selective circumstances. By demonstrating that it is possible to uncover ancient evolutionary trends from a combination of a phylogeny and appropriate statistical models, we illustrate how data from extant species can complement paleontological accounts of evolutionary history, opening up new avenues of investigation for both.

Significance Statement

There is a long-held notion that bigger body sizes are intrinsically advantageous. We demonstrate an overwhelming tendency for rapid morphological change to lead to larger body size in 10 of the 11 largest mammal orders. Our results suggest that mammals have consistently evolved towards larger size, most likely as a response to selection pressure. This is the first evidence from extant taxa that is compatible with the pattern of increasing body size through time observed in the mammalian fossil record. By accommodating variation in the rate of evolution into studies of size change, we demonstrate that it is possible to detect and reconstruct accurate historical evolutionary processes by taking advantage of the wealth of data available from extant species.

\body

Introduction

The idea that large size confers some intrinsic advantage has lingered in the psyche of biologists for centuries. Researchers have proposed that bigger body sizes can increase tolerance to environmental extremes (1), reduce mortality (2) and enhance predation success (3), among other advantages. In support of these conjectures, analyses from a range of different taxonomic groups demonstrate that within populations larger individuals have significantly enhanced survival, fecundity and mating success (4, 5). If these advantages are general and have played out over long timescales it could explain the existence of Cope's rule (6) – a broad trend towards increasing size through time (4, 5, 7).

Mammals evolved from a relatively small common ancestor over 165 million years ago (8-10) and went on to form one of the largest and most successful vertebrate radiations in the earth's history. Mammals vary greatly in size – spanning almost eight orders of magnitude. This implies that some groups have experienced much greater evolutionary change in size from the ancestral form than others. Indeed, the mammalian fossil record provides the clearest evidence in support of Cope's rule over long evolutionary timescales (6, 11, 12).

In spite of this, evidence for Cope's rule remains elusive from studies of extant data alone (13-15), including studies of the mammals (16). A possible reason for the discrepancy between paleontological and extant data might be that conventional comparative methods for studying trends within extant data implicitly assume homogenous evolutionary patterns and processes. When these assumptions are violated it renders the homogenous modelling approach at best incomplete and at worst a source of potential bias in the study of historical evolutionary change – for example, reconstructions of probable ancestral values can be biased towards average or intermediate values (17, 18) which would thereby mask long-term evolutionary trends that are apparent from the fossil record.

Previously we have shown that rates of body size evolution in the mammals routinely violate the assumption of homogeneity (19) but how these rate changes might be related to size itself has not been studied. If changes towards larger size in the mammals have consistently occurred at rates that differ from changes to smaller size then reconstructed ancestral states accounting for these rate differences may track more closely the observed fossil record. This would allow the detection of size-related evolutionary trends from extant data.

Here we apply a novel statistical phylogenetic approach for reconstructing mammalian evolutionary history that allows the rate of evolution to vary throughout a phylogenetic tree without prior knowledge or specification of where and when rate-shifts occurred. We use this method to test for size-related biases in rates of morphological change and ask whether accounting for any such bias allows us to predict a generalised pattern of size increase in the mammals in line with that observed in the fossil record. Finally, we consider whether a size-related bias in the rate of morphological evolution can help to choose among the several macro-evolutionary processes that have been suggested to give rise to Cope's rule.

Results and Discussion

Because the rate of morphological evolution has varied considerably among the mammals throughout their history, branch lengths measured in time can over- or under-estimate the amount of change expected under a homogeneous Brownian motion model (19). We therefore scale time by an amount reflecting the rate of morphological evolution along individual branches of the mammalian phylogeny (19, *SI*) – longer *rate-scaled branches* have experienced more change than would be expected given their length in time (*Methods*). If body size increase has been disproportionately favoured, we expect to find that longer rate-scaled branches are linked to larger increases in size throughout the phylogeny. If this has been repeated over many branches, we expect to find them associated with a long-term historical trend towards increasing size (6).

Across all mammals we find a significant positive relationship between *path-wise rates* (the sum of all rate-scaled branches along the evolutionary path leading to individual species, *Methods*) and body size (likelihood ratio [*D*] test, compared to a homogenous Brownian motion model: $D = 359.85$, $p < 0.001$, $df = 2$, Fig. 1A; this holds in all of 500 randomly selected trees from the posterior distribution of rate-scaled phylogenies). Allowing the slope of the relationship between size and path-wise rate to vary among orders (Fig. 1B, separate-slopes model) significantly improves on the model relying on a single common slope ($D = 252.24$, $p < 0.001$, $df = 31$; this also holds in all of 500 randomly selected trees from the posterior distribution of rate-scaled phylogenies), and reveals that the positive relationship is maintained separately within 10 of 11 mammalian orders (Fig. 1B, Table S1): the only exception is the marsupial order Diprotodontia, where the path-wise rate is largest in the evolutionary paths leading to smaller species (Fig. 1B).

We visualize the importance of detecting variation in the rate of evolution by simulating body sizes from the separate-slopes regression model (*Methods*) and from a conventional homogenous Brownian motion model assuming a single uniform rate of change. The separate-slopes model simulates values which symmetrically bracket the observed body size distribution (Fig. 2A). By comparison, the homogeneous model systematically overestimates small sizes and underestimates large sizes (Fig. 2B). This poor fit to the real data arises by virtue of the homogenous model missing the historical bias towards rapid rates leading to larger size.

Using our separate-slopes model we infer the ancestral body size at each internal node of the mammalian phylogeny (*Methods*, Fig. 3A). The tendency for body size increase can be studied quantitatively by finding the difference in body size from the start to the end of each branch of the phylogeny ($N = 5233$). We term these differences *phylogenetic ancestor-descendant* (PAD) comparisons to contrast with the paleontological approach where *fossil ancestor-descendant* (FAD) comparisons (12, 20, 21) are made between the sizes of taxonomically paired species found in the fossil record.

Our PAD comparisons demonstrate that not only are size increases more common they also tend to be greater in magnitude and occur at a faster rate when compared to body size decreases (Fig. 3). Of the 5233 PAD comparisons, 3496 or 66.8% showed an increase in size (exact binomial test, $p < 0.001$). On average, descendant species are $0.10 \pm 0.004 \log_{10}$ units,

or $6\pm 0.25\%$ larger than their ancestors (Fig. 3B), although this figure varies between 1.4-16.9% in individual orders ($-3.8\pm 0.63\%$ in Diprotodontia, Table S2). These figures compare favourably to results from paleontological data where North American Cenozoic mammals are, on average, 9% larger than their ancestors (12).

We find that on a branch-by-branch basis the largest increases in size are associated with the fastest rates of evolution (Fig. 3C, $\beta = 0.015$, $p < 0.001$). One argument for such a pattern is based on the premise that phyletic increases in size arise simply as a consequence of evolutionary divergence away from a small ancestral value, where there is some lower physiological limit on size (22, 23). In this scenario, a taxon's "*maximal potential adaptive zone*" (22) is always skewed such that larger species will evolve and that those species will be specialized (6, 22).

We use our PAD comparisons to test for the presence of a lower bound by drawing on ideas developed in the paleontological literature (12, 21, 23, 24) whilst explicitly accounting for shared ancestry. If some lower boundary on size is enforced, we expect most ancestor-descendant size changes to be positive when the ancestral size is near to that limit; it is only possible to get larger. But as the ancestral state moves away from this limit, we predict that the distribution of body size change will become increasingly centred about zero i.e. size decreases are equally likely as size increases (24). Taken over all branches of the phylogeny, this therefore predicts a negative relationship between a branch's ancestral size and the average body size change observed along that branch (12, 21): when ancestral size is small changes will tend to be positive, but when ancestral size is large size can change in either direction.

We do not find the predicted negative relationship (Fig. 3D; SI). Instead, we find that size change actually slightly increases in magnitude when ancestral size is larger (Fig. 3D, $\beta = 0.020$, $p < 0.001$). This pattern is also found in the paleontological data using FAD comparisons (12). To retain the idea that some physiological lower limit could produce these PAD results and results from paleontological data (12), proponents would have to invoke a new physiological lower limit for each new species that comes into existence. Why, or according to what processes these mysterious and dynamically shifting constraints arise impose a steep hill for this explanation to climb.

The notion that 'adaptive zones' litter the morphological landscape has often been wielded as a driver for large-scale macro-evolutionary patterns (25-29). With this view, one might expect fast evolutionary rates to be the result of shifts from one zone to another or in the position of the adaptive peak through time (26, 29-31). If the occupation of new adaptive zones is constantly associated with changes toward large size or there is some sort of continuously moving optima such that large size is favoured, this view would be consistent with the pattern we observe here – although there is nothing in the patterns we observe that requires the existence of discrete adaptive zones.

It has been suggested that large-bodied species may have an inherently faster rate of evolution owing to the relaxation of some unspecified size-linked constraint (26, 32) (e.g. genetic, developmental or biomechanical). If such constraints were operating we would expect to observe that larger-bodied species change disproportionately more along the branches of the phylogeny than smaller-bodied ones. This leads to the prediction that the variance of body size change should be positively correlated with ancestral size: small-bodied species change less than larger-bodied ones. We calculated the variance for all PAD comparisons ($\sigma^2_{\Delta \log_{10} \text{ body size}}$, $N = 5233$), after adjusting for the regression to the mean artefact (*SI*, 12, 33). We then regressed log-transformed $\sigma^2_{\Delta \log_{10} \text{ body size}}$ onto log-transformed ancestral size (i.e. size reconstructed at the start of a branch) across all branches of the phylogeny. We do not find the expected positive relationship ($\beta = -0.017$, $t = -1.47$, $p = 0.14$, see Fig. 3D). Therefore, and in agreement with previous work (34, 35), we see no reason to invoke the release of constraints as a force driving rate variation or size changes in mammals.

A possible difficulty for our model is that it predicts that mammals will become increasingly and indefinitely larger over long periods of time even though there must be some physical limit on the maximum size a terrestrial vertebrate can attain. Usefully, it seems that mammals have not reached those limits: even the largest ever-known terrestrial mammals (36, 37) fall well below the proposed maximum masses for terrestrial animals of between 20 thousand to 1 million kg (38, 39). If extant mammals had reached their maxima this would be reflected in additional parameters (quadratic effects) in our model that would account for a slowing of the trajectory towards increasing size but, at least for now, this is not necessary (*SI*).

A second difficulty is that if large body size is continuously favoured one would expect that there must have come a point at which it was advantageous for species to become small – exploiting niches made available by continued size increases in competing taxa. In fact, size reduction was common in the evolutionary history of mammals (1737 of our PAD data or 33.2%) and often occurred at rapid rates (40, Fig. 3C). For example, there was rapid evolutionary change towards body size decrease in the branch leading to extant bats, although subsequent evolution within this group returned to a general pattern of body size increase (Fig. 3, Fig. 1B). In the special case of Diprotodontia, it appears that rapid changes resulting in smaller size dominated, although we do still observe some large body size increases in this group. A possible explanation for this pattern is that these species might have become smaller in response to nutrient-poor environments in Australian habitats (41, 42).

The consistent signal for directional evolutionary change in size implies a relatively small common ancestor of mammals. Previously, ancestral state reconstruction in the face of such a trend has been problematic; conventional comparative methods make it impossible to detect evolutionary trends using extant data. Incorporating fossils into a phylogeny improves ancestral state estimates (43-46) but here we test a long-positing suggestion that it is possible to infer from extant data alone the existence of ancient forms whose size or shape is not intermediate to the range of present diversity (17). If our characterization of

mammalian size evolution is a good description of the historical processes that led to contemporary mammal species, we should be able to infer ancestral states that are closer to those observed in the fossil record than estimates derived from conventional homogenous models without using fossil data. These expectations are borne out (Fig. 3A). We estimate that the ancestral size at the root of therian mammals was 24.5g. This falls within the fossil body size range (20-25g) of *Eomaia scansoria* (10), which has been recently suggested to lie close to the root of all placental and marsupial mammals (9). In contrast, the homogenous Brownian motion model reconstructs the ancestral body size to be greater than 600g – more than an order of magnitude too large.

It may be wrong to assume that fossil species are directly ancestral to extant groups (47). Accordingly, we reconstructed body sizes for 65 unique fossil taxa (Table S3) that represent the oldest or basal members of several mammal groups (*Methods*). Homogenous Brownian motion reconstructions of these taxa yield sizes that are systematically larger than paleontological estimates (Fig. 4A, $t = 4.68$, $p < 0.001$). In contrast, the separate-slopes regression model reconstructs body sizes that do not differ significantly from paleontological estimates (Fig. 4B; $t = 0.76$, $p = 0.45$).

Taken together, our results demonstrate that mammals have consistently evolved towards larger size, almost certainly reflecting an adaptive response to new selective circumstances e.g. competition (48), climate changes (7, 49) or dietary specialization (11). These results are not compatible with purely passive explanations for trends through time (24, 50). Instead, rapid and repeated instances of evolutionary change towards bigger body size have consistently shaped mammalian diversity, allowing mammal species to attain larger sizes over the millions and millions of years of their evolutionary history. This represents unique support for an adaptive explanation for one of the most enduring and iconic notions in evolutionary biology – Cope’s rule. The ability to detect and characterize trends within extant taxa provides the attractive opportunity to study a broad number of taxonomic groups using the vast amounts of data available for extant species. Such analyses should be viewed as complementary to work based on fossil evidence that benefit from the ability to study morphology directly through time.

Methods

Data

We used a comprehensive time-scaled phylogenetic tree of extant mammals (8, N = 3321) along with body size data from two major databases (51, 52). Body sizes were log-transformed. Our analyses are based on the assumption that the Fritz et al (8) tree provides a relatively reliable estimate of mammalian phylogeny and divergence times. We classified the mammals into orders following Bininda-Emonds et al. 2007 (53). To measure the rate of body size evolution in our mammal dataset, we apply a recently developed phylogenetic statistical approach that detects regions of the tree that have undergone especially fast or slow rates of change (19). Our approach stretches or compresses time-measured branch lengths by an amount reflecting the inferred rate of evolution in that branch (19, *SI*): stretched branches reflect increased rates of change and compressed branches reflect regions where size has changed less than expected under background rates.

Detecting Trends

We can then use our rate-scaled branch lengths to study long-term trends. This is achieved by summing all the rate-scaled branches along the evolutionary path of a species, leading from the root to the tip. This creates *path-wise rates* – a measure of the total changes in rate a species has experienced during the course of its evolution. If elevated rates have been disproportionately associated with size increases we expect to find that species with greater path-wise rates will be larger in size. To test this idea, we regressed log body mass onto path-wise rate using phylogenetic generalized least squares (GLS) models (54, 55) in a maximum-likelihood framework.

To test for different patterns among mammal orders we allowed the relationship between path-wise rate and body size to vary among those orders where sample size was large enough for analysis ($N \geq 40$, *SI*). Owing to the small sample sizes of orders within the monophyletic superorder Afrotheria, we study Afrotheria as a single group (*SI*). As aquatic species may have different patterns and processes of body size evolution (40, 56) we allowed the magnitude of the relationship to vary for these groups (Pinnipeds, Sirenia and Cetacea). We compared nested models using the likelihood-ratio test statistic (*D*).

Reconstructing historical body sizes

We estimated ancestral body sizes at each node of the mammal phylogeny using a phylogenetic predictive modelling approach that incorporates the parameters of our separate-slopes regression model (57-59). We then tracked body size change and rates on a branch-by-branch basis across the entire phylogeny. We term these *phylogenetic ancestor-descendant* (PAD) comparisons to contrast with the paleontological method of *fossil ancestor-descendant* (FAD) comparisons (*SI*).

Using the same predictive modelling approach as for ancestral state reconstruction, we assessed how well our results could be reconciled with paleontological data by estimating the expected size of 65 unique fossil taxa given their proposed phylogenetic position (*SI*).

We compared these reconstructions to the paleontological estimates and reconstructions using conventional homogenous Brownian motion methods (*SI*).

Quantifying Constraints

We assessed whether our data fit the predictions made by the presence of a size-linked constraint using our PAD comparisons. If some unspecified constraint is acting to restrict evolutionary potential in smaller species, we would expect to see released pressure in larger species allowing for more evolutionary change – as ancestral size increases, we should observe an increase in variance around the observed change in body size ($\Delta\log_{10}$ body size). To test this, we calculated the variance in body size change ($\sigma^2_{\Delta\log_{10} \text{ body size}}$) for all PAD comparisons ($N = 5233$) across every branch of the phylogeny, after adjusting for the regression to the mean artefact (*SI*, 12, 33). We assessed whether there was a significant increase in variance with increasing ancestral size by regressing $\sigma^2_{\Delta\log_{10} \text{ body size}}$ onto log-transformed ancestral body mass. A visualization of the observed variance in body size change is shown in Fig. 3D.

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References

1. Peters RH (1986) *The ecological implications of body size* (Cambridge University Press).
2. Brown JH & Sibly RM (2006) Life-history evolution under a production constraint. *Proc Natl Acad Sci U S A* 103(47):17595-17599.
3. Hone DW & Benton MJ (2005) The evolution of large size: How does Cope's rule work? *Trends Ecol Evol* 20(1):4-6.
4. Kingsolver JG & Pfennig DW (2004) Individual-level selection as a cause of Cope's rule of phyletic size increase. *Evolution* 58(7):1608-1612.
5. Kingsolver JG & Pfennig DW (2007) Patterns and power of phenotypic selection in nature. *Bioscience* 57(7):561-572.
6. Cope ED (1896) *The primary factors of organic evolution* (Open Court Publishing Company, Chicago).
7. Hunt G, Wicaksono SA, Brown JE, & Macleod KG (2010) Climate-driven body-size trends in the ostracod fauna of the deep Indian Ocean. *Palaeontology* 53(6):1255-1268.
8. Fritz SA, Bininda-Emonds ORP, & Purvis A (2009) Geographical variation in predictors of mammalian extinction risk: big is bad, but only in the tropics. *Ecol Lett* 12(6):538-549.
9. O'Leary MA, *et al.* (2013) The placental mammal ancestor and the post-K-Pg radiation of placentals. *Science* 339(6120):662-667.
10. Ji Q, *et al.* (2002) The earliest known eutherian mammal. *Nature* 416(6883):816-822.
11. Van Valkenburgh B, Wang X, & Damuth J (2004) Cope's rule, hypercarnivory, and extinction in North American canids. *Science* 306(5693):101-104.
12. Alroy J (1998) Cope's rule and the dynamics of body mass evolution in North American fossil mammals. *Science* 280(5364):731-734.
13. Moen DS (2006) Cope's rule in cryptodiran turtles: Do the body sizes of extant species reflect a trend of phyletic size increase? *J Evol Biol* 19(4):1210-1221.
14. Pianka ER (1995) Evolution of body size: Varanid lizards as a model system. *Am Nat* 146(3):398-414.
15. Knouft JH & Page LM (2003) The evolution of body size in extant groups of North American freshwater fishes: Speciation, size distributions, and Cope's rule. *Am Nat* 161(3):413-421.
16. Monroe MJ & Bokma F (2010) Little evidence for Cope's rule from Bayesian phylogenetic analysis of extant mammals. *J Evol Biol* 23(9):2017-2021.
17. Pagel M (1999) Inferring the historical patterns of biological evolution. *Nature* 401:877-884.
18. Elliot MG & Mooers AO (2014) Inferring ancestral states without assuming neutrality or gradualism using a stable model of continuous character evolution. *BMC Evol Biol* 14(226).
19. Venditti C, Meade A, & Pagel M (2011) Multiple routes to mammalian diversity. *Nature* 479(7373):393-396.
20. Raia P, Carotenuto F, Passaro F, Fulgione D, & Fortelius M (2012) Ecological specialization in fossil mammals explains Cope's rule. *Am Nat* 179(3):328-337.
21. Alroy J (2000) Understanding the dynamics of trends within evolving lineages. *Paleobiology* 26(3):319-329.
22. Stanley SM (1973) An explanation for Cope's rule. *Evolution* 27(1):1-26.

23. McShea DW (1994) Mechanisms of large-scale evolutionary trends. *Evolution* 48(6):1747-1763.
24. Wagner PJ (1996) Contrasting the underlying patterns of active trends in morphologic evolution. *Evolution* 50(3):990-1007.
25. Uyeda JC, Hansen TF, Arnold SJ, & Pienaar J (2011) The million-year wait for macroevolutionary bursts. *Proc Natl Acad Sci U S A* 108(38):15908-15913.
26. Simpson GG (1953) *The Major Features of Evolution* (Columbia University Press).
27. Slater GJ (2013) Phylogenetic evidence for a shift in the mode of mammalian body size evolution at the Cretaceous-Palaeogene boundary. *Methods Ecol Evol* 4(8):734-744.
28. Hunt G (2012) Measuring rates of phenotypic evolution and the inseparability of tempo and mode. *Paleobiology* 38(3):351-373.
29. Hansen TF (1997) Stabilizing selection and the comparative analysis of adaptation. *Evolution*:1341-1351.
30. Estes S & Arnold SJ (2007) Resolving the paradox of stasis: models with stabilizing selection explain evolutionary divergence on all timescales. *Am Nat* 169(2):227-244.
31. Arnold SJ (2014) Phenotypic evolution: the ongoing synthesis. *Am Nat* 183(6):729-746.
32. Stanley SM (1979) Macroevolution, pattern and process.
33. Kelly C & Price TD (2005) Correcting for regression to the mean in behavior and ecology. *Am Nat* 166(6):700-707.
34. Cooper N & Purvis A (2009) What factors shape rates of phenotypic evolution? A comparative study of cranial morphology of four mammalian clades. *J Evol Biol* 22:1024-1035.
35. Smith FA, *et al.* (2004) Similarity of mammalian body size across the taxonomic hierarchy and across space and time. *Am Nat* 163(5):672-691.
36. Alexander RM (1998) All-time giants: the largest animals and their problems. *Palaeontology* 41(6):1231-1245.
37. Smith FA, *et al.* (2010) The evolution of maximum body size of terrestrial mammals. *Science* 330:1216-1219.
38. Hokkanen JEI (1986) The size of the largest land animal. *J Theor Biol* 118(4):491-499.
39. Economos AC (1981) The largest land mammal. *J Theor Biol* 89(2):211-214.
40. Evans AR, *et al.* (2012) The maximum rate of mammal evolution. *Proc Natl Acad Sci U S A* 109(11):4187-4190.
41. Orians GH & Milewski AV (2007) Ecology of Australia: the effects of nutrient-poor soils and intense fires. *Biol Rev Camb Philos Soc* 82(3):393-423.
42. Milewski A & Diamond R (2000) Why are very large herbivores absent from Australia? A new theory of micronutrients. *J Biogeogr* 27(4):957-978.
43. Finarelli JA & Flynn JJ (2006) Ancestral state reconstruction of body size in the Caniformia (Carnivora, Mammalia): the effects of incorporating data from the fossil record. *Syst Biol* 55(2):301-313.
44. Oakley TH & Cunningham CW (2000) Independent contrasts succeed where ancestor reconstruction fails in a known bacteriophage phylogeny. *Evolution* 54(2):397-405.
45. Finarelli JA & Goswami A (2013) Potential pitfalls of reconstructing deep time evolutionary history with only extant data, a case study using the Canidae (Mammalia, Carnivora). *Evolution* 67(12):3678-3685.

46. Slater GJ, Harmon LJ, & Alfaro ME (2012) Integrating fossils with molecular phylogenies improves inference of trait evolution. *Evolution* 66(12):3931-3944.
47. Foote M (1996) On the probability of ancestors in the fossil record. *Paleobiology* 22(2):141-151.
48. Benson RBJ, Frigot RA, Goswami A, Andres B, & Butler RJ (2014) Competition and constraint drove Cope's rule in the evolution of giant flying reptiles. *Nat Commun* 5.
49. Hunt G & Roy K (2006) Climate change, body size evolution, and Cope's rule in deep-sea ostracodes. *Proc Natl Acad Sci U S A* 103(5):1347-1352.
50. Gould SJ (1988) Trends as changes in variance: A new slant on progress and directionality in evolution. *J Paleontol* 62(3):319-329.
51. Jones KE, *et al.* (2009) PanTHERIA: a species-level database of life history, ecology, and geography of extant and recently extinct mammals. *Ecology* 90(9):2648-2648.
52. Ernest SKM (2003) Life history characteristics of placental nonvolant mammals. *Ecology* 84(12):3402.
53. Bininda-Emonds OR, *et al.* (2007) The delayed rise of present-day mammals. *Nature* 446(7135):507-512.
54. Pagel M (1997) Inferring evolutionary processes from phylogenies. *Zool Scr* 26(4):331-348.
55. Freckleton RP, Harvey PH, & Pagel M (2002) Phylogenetic analysis and comparative data: A test and review of evidence. *Am Nat* 160(6):712-726.
56. Schmidt-Nielsen K (1984) *Scaling: Why is Animal Size so Important?* (Cambridge University Press, Cambridge).
57. Jetz W & Freckleton RP (2015) Towards a general framework for predicting threat status of data-deficient species from phylogenetic, spatial and environmental information. *Philos Trans R Soc Lond B Biol Sci* 370(1662):20140016.
58. Organ CL, Shedlock AM, Meade A, Pagel M, & Edwards SV (2007) Origin of avian genome size and structure in non-avian dinosaurs. *Nature* 446(7132):180-184.
59. Franks PJ, Freckleton RP, Beaulieu JM, Leitch IJ, & Beerling DJ (2012) Megacycles of atmospheric carbon dioxide concentration correlate with fossil plant genome size. *Philos Trans R Soc Lond B Biol Sci* 367(1588):556-564.

Figure Legends

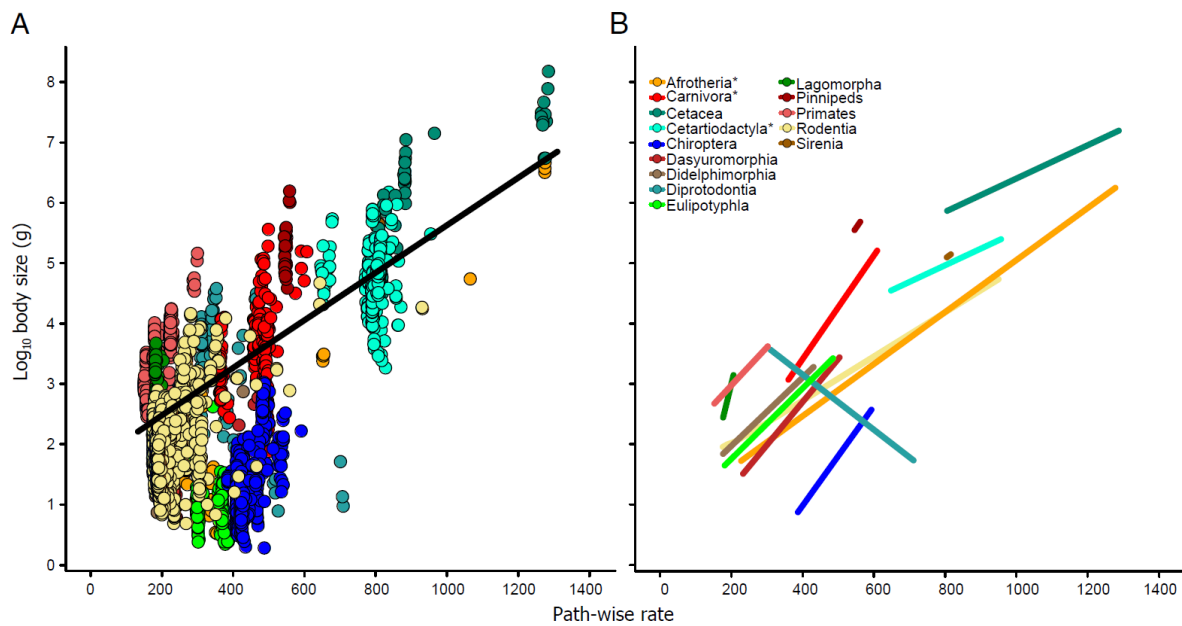


Fig. 1. Faster path-wise rates have led to larger body size in mammals. (A) The relationship across all mammals is plotted – data points are coloured by order (N = 3321). The black line is the fitted phylogenetic slope of the relationship between body size and path-wise rates (*Methods*) across all mammals. (B) The fitted phylogenetic slopes of the relationship within each of the 11 mammalian orders investigated here. Orders which contain aquatic groups are indicated by an asterisk; for these orders, only the terrestrial members are plotted. Aquatic groups are plotted separately (Cetacea, Pinnipeds and Sirenia).

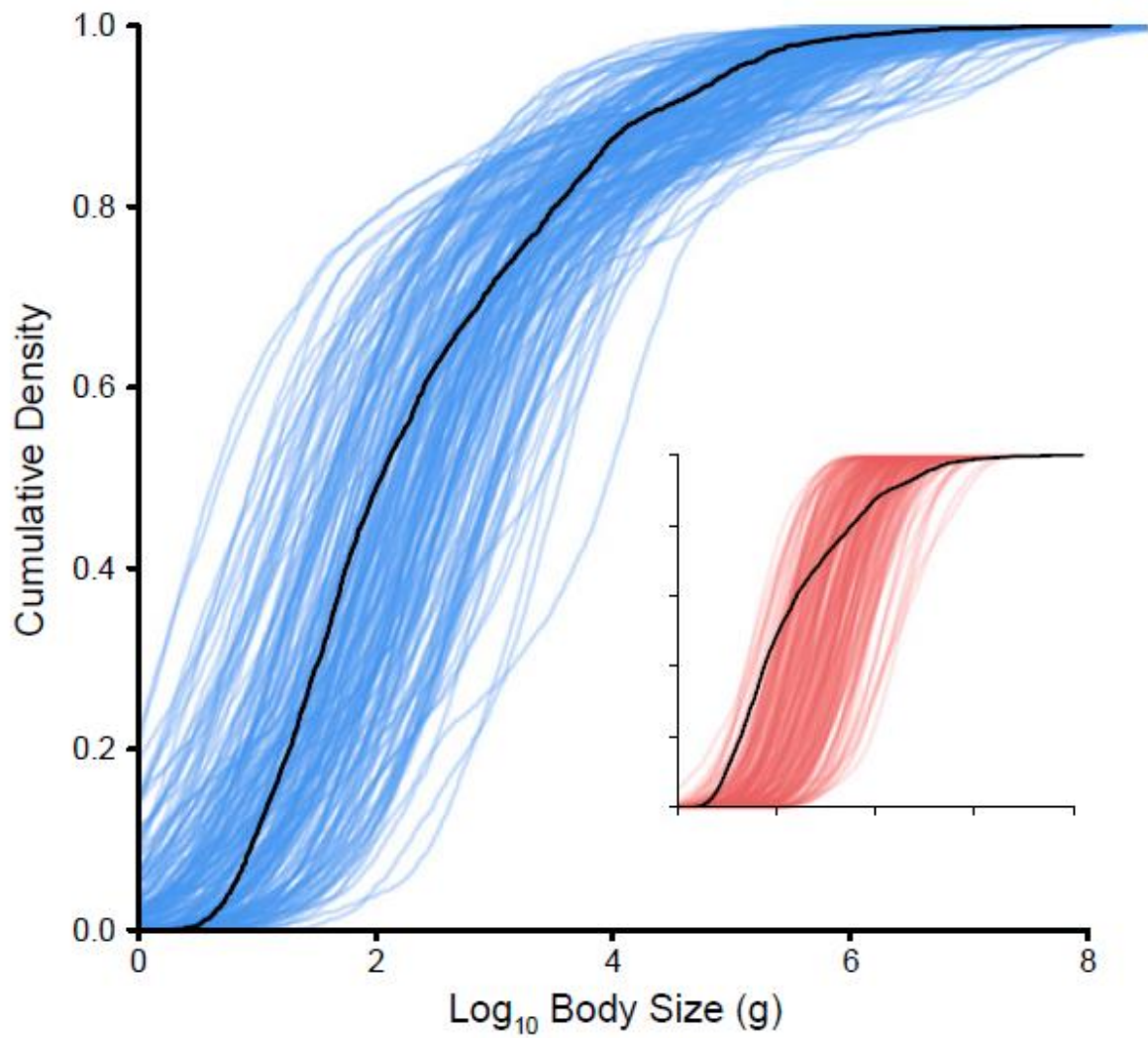


Fig. 2. Comparisons between the cumulative distribution of observed mammalian body sizes (N = 3321, black lines) and simulated data (N = 1000, coloured lines). The real data is compared to simulations generated from our separate-slopes regression model (blue lines) and a conventional homogenous Brownian motion model (red lines, inset).

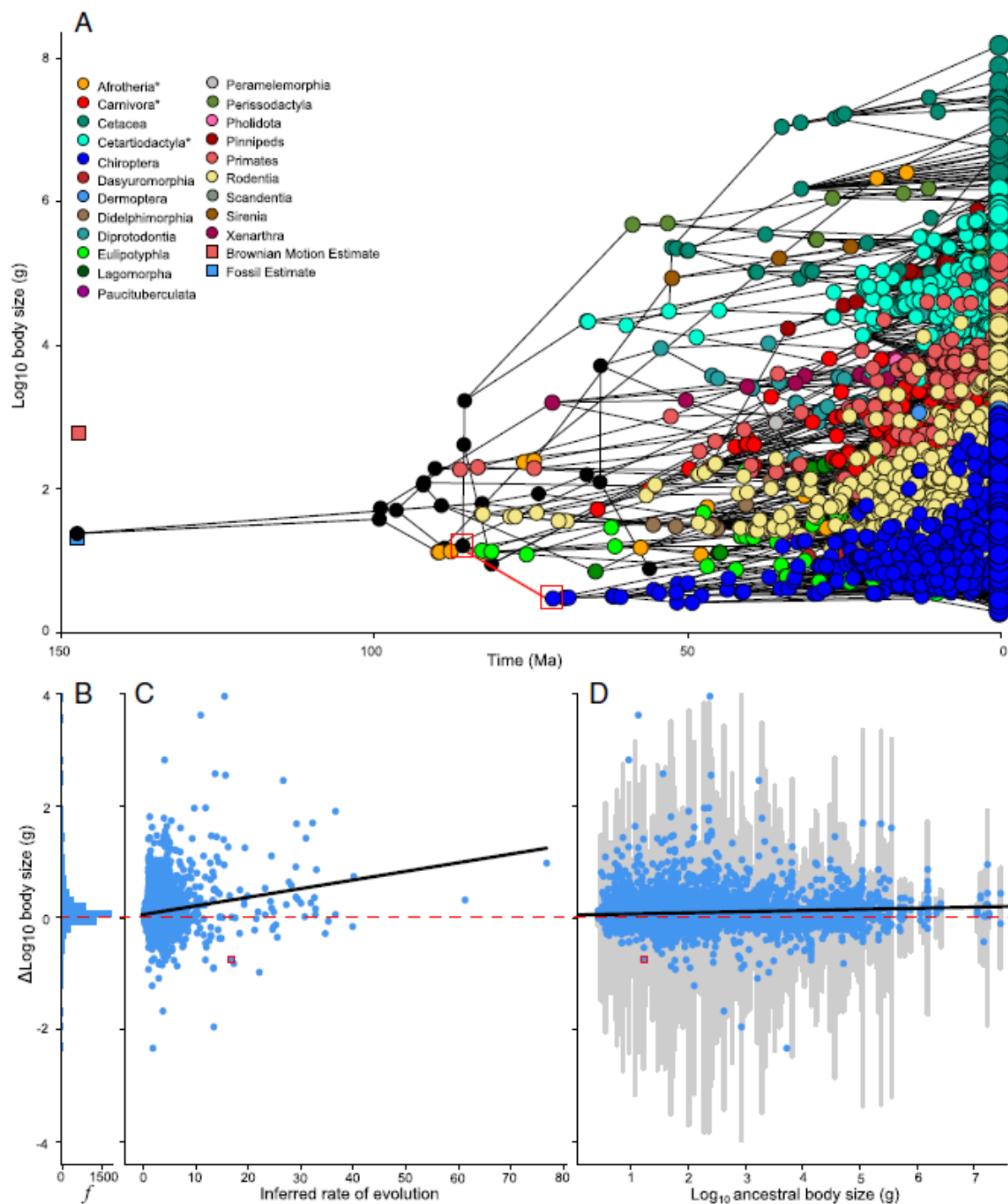


Fig. 3. Phylogenetic ancestor-descendant (PAD) comparisons and reconstructed ancestral sizes. (A) Projection of ancestral state reconstructions into a phylomorphospace (N = 5234 including all tips and internal nodes). Points are connected by phylogeny and each internal node of the tree has been reconstructed using the parameters of our separate-slopes regression model. Our estimate for the therian root (24.5g) falls within the ranges given by the paleontological data (20-25g, midpoint indicated by pale blue square). This is in contrast to the estimate made by a conventional homogenous Brownian motion model which is more than an order of magnitude too large (pale pink square, 610.7g). Orders which contain aquatic groups are indicated by an asterisk; for these orders, only the terrestrial members

are plotted. Aquatic groups are plotted separately (Cetacea, Pinnipeds and Sirenia). (B-D) PAD changes ($\Delta\log_{10}$ body size) across every branch of the mammalian phylogeny (N = 5233). The red dashed line indicates no change in size. (B) The frequency (f) distribution of $\Delta\log_{10}$ body size across individual branches. There is a significant bias towards body size increase (exact binomial test, $p < 0.001$). (C) Plot of the inferred rate of evolution along individual branches (*Methods*) against $\Delta\log_{10}$ body size. The regression line is significantly positive ($\beta = 0.015$, $p < 0.0001$). (D) Ancestral body size against body size change across individual branches. The grey bars represent the standard deviation of $\Delta\log_{10}$ body size calculated from the variance associated with each data point (*Methods*, $\sigma^2_{\Delta\log_{10} \text{ body size}}$). The regression line and the standard deviations in D have been corrected for the regression to the mean artefact (*Methods*, *SI*). The slope of the relationship between ancestral size and $\Delta\log_{10}$ body size is significantly positive ($\beta = 0.020$, $p = 0.0006$). Highlighted by a red square on each of these plots is the branch leading to modern bats

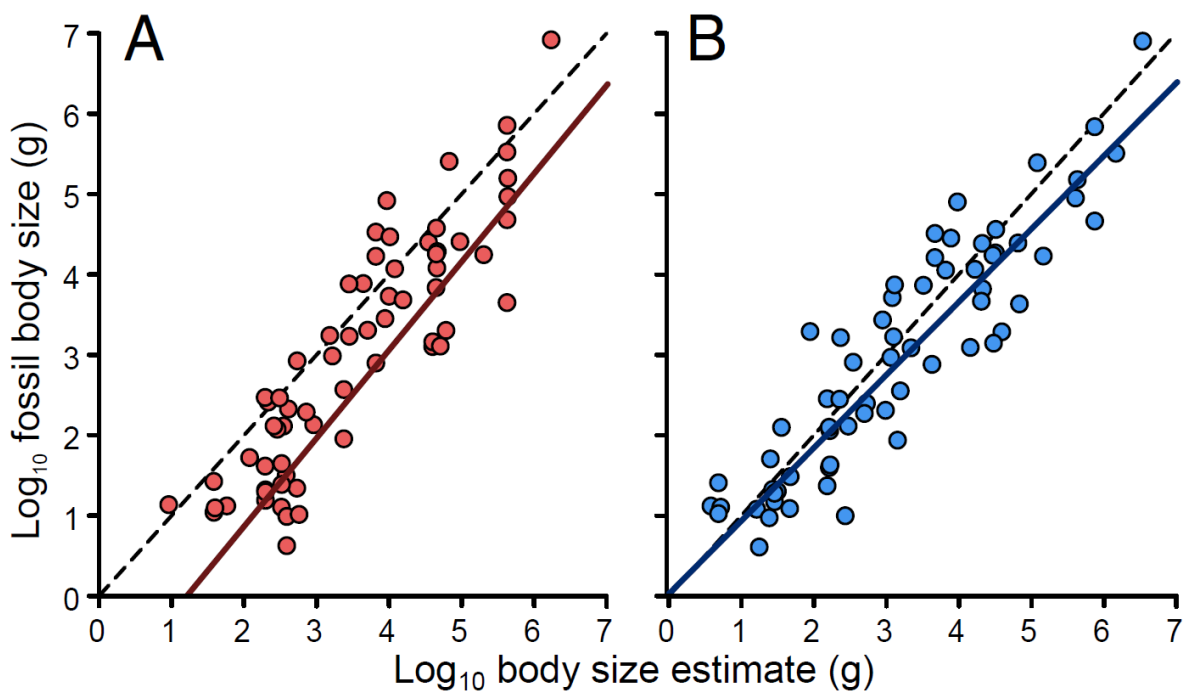


Figure 4. Comparisons of reconstructed body sizes to fossil estimates. The solid coloured lines in both plots are the predicted phylogenetic slopes from a regression model of fossil sizes as given in the paleontological literature against reconstructed values (N = 65). The dashed black lines indicate a one-to-one relationship – the expected slope if models are predicting body sizes accurately. (A) Predicted body sizes from a homogenous Brownian motion model compared to fossil estimates. (B) Predicted body sizes from our separate-slopes model in comparison to the fossil record.