Sauropod dinosaurs evolved moderately sized genomes unrelated to body size

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Sauropodomorph dinosaurs include the largest land animals to have ever lived, some reaching up to 10 times the mass of an African elephant. Despite their status defining the upper range for body size in land animals, it remains unknown whether sauropodomorphs evolved larger-sized genomes than non-avian theropods, their sister taxon, or whether a relationship exists between genome size and body size in dinosaurs, two questions critical for understanding broad patterns of genome evolution in dinosaurs. Here we report inferences of genome size for 10 sauropodomorph taxa. The estimates are derived from a Bayesian phylogenetic generalized least squares approach that generates posterior distributions of regression models relating genome size to osteocyte lacunae volume in extant tetrapods. We estimate that the average genome size of sauropodomorphs was 2.02 pg (range of species means: 1.77–2.21 pg), a value in the upper range of extant birds (mean = 1.42 pg, range: 0.97–2.16 pg) and near the average for extant non-avian reptiles (mean = 2.24 pg, range: 1.05–5.44 pg). The results suggest that the variation in size and architecture of genomes in extinct dinosaurs was lower than the variation found in mammals. A substantial difference in genome size separates the two major clades within dinosaurs, Ornithischia (large genomes) and Saurischia (moderate to small genomes). We find no relationship between body size and estimated genome size in extinct dinosaurs, which suggests that neutral forces did not dominate the evolution of genome size in this group.

Keywords: palaeogenomics; Dinosauria; genome size; genomics; Sauropodomorpha

1. INTRODUCTION

Little is known about the molecular biology and evolution of extinct organisms. The absence of molecular information for long-extinct species limits the use of molecular data in phylogenetic analyses that include fossil species, though recent research suggests that such endeavours may be possible (Organ *et al.* 2008*a*; Schweitzer *et al.* 2009). More profoundly, pervasive extinction obscures large-scale patterns of molecular evolution through deep time.

An emerging route around the latter problem is the estimation of genome size in extinct species, a genomic parameter analogous to organismal body size, which is largely determined in animals by introns and various forms of repetitive elements (Shedlock 2006). There is a tight correlation between cell size and genome size in extant vertebrates (Gregory 2001), which allows the estimation of genome size in extinct species from the size of osteocyte lacunae in palaeohistological thin sections (Organ *et al.* 2007; Organ & Shedlock 2009). Such estimations provide answers to critical questions about the genome biology of extinct species and about the macroevolution of the animal genome. For example, how are genomes maintained over long periods of time (Gregory 2005)? Is genome size adaptive or non-adaptive (Lynch & Conery 2003)? Are the well-established correlations among genome size and certain adaptations or behaviours, such as flight (Andrews *et al.* 2008; Organ & Shedlock 2009) or endothermy (Vinogradov & Anatskaya 2006), due to historical constraints? Why is there so much variation in genome size among species (Organ *et al.* 2008*b*)? Like many questions in evolutionary biology, these are difficult to fully answer without recourse to the fossil record.

Dinosaurs are a promising extinct group for palaeogenomic investigation. Dinosaurs were the pre-eminent vertebrates in most terrestrial ecosystems during a 160 Myr span from the Late Triassic until the end of the Cretaceous (Sereno 1999), evolved into a wide array of shapes and sizes, and filled many ecological niches (Brusatte *et al.* 2008). Mesozoic dinosaurs also gave rise to birds, and thus occupy a critical position as ancestors and close relatives of birds, the most speciose group of extant terrestrial vertebrates (Padian & Chiappe 1998). Practically, dinosaurs are well-studied and known from a bounty of specimens, many of which have been investigated histologically (Erickson 2005). Indeed, dinosaurs were the first long-extinct amniotes studied in a genomic context (Organ *et al.* 2007). However, these analyses were aimed at two specific issues: the evolution of genome size as related to the evolution of avian flight; and the timing

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of the genomic contraction that led to the small and constrained genomes of birds.

Previous work on dinosaur palaeogenomics (Organ *et al.* 2007) unevenly sampled Dinosauria, with only one specimen from Sauropodomorpha, one of the three main dinosaur subgroups and the sister group to the carnivorous theropods (which include birds). The largest terrestrial animals ever to have lived are sauropods, even if many estimations of body mass for these species are inflated owing to statistical artefacts (Packard *et al.* 2009). Sauropods include iconic creatures such as *Brachiosaurus* and *Apatosaurus* that reached masses of at least 16 tonnes (Packard *et al.* 2009) and perhaps as much as 80 tonnes (Peczkis 1994), with body lengths up to 40 m (Sander & Clauss 2008). Organ *et al.* (2007) hypothesized that non-avian theropods evolved smaller genomes than sauropodomorphs, despite their immense size, but were unable to address this question because of poor sampling within Sauropodomorpha. We address this hypothesis in the current study as well as the hypothesis that no relationship between genome size and body size exists in extinct dinosaurs (a non-phylogenetic analysis has found such a relationship in birds; Gregory 2005). Because of their tremendous body sizes (Peczkis 1994; Packard *et al.* 2009), sauropodomorphs provide a critical source of data to the ongoing debate about genome size evolution. Moreover, sauropodomorphs must be considered in order to comprehensively evaluate hypotheses concerning the diversity of the dinosaur genome, the timing of the contraction of genome size along the lineage leading to birds, and the neutral theory of genome evolution in extinct dinosaurs.

2. MATERIAL AND METHODS

Samples of fossil bone were thin-sectioned for the following sauropodomorph taxa: *Massospondylus carinatus*, *Barosaurus lentus*, *Janenschia robusta*, *Europasaurus holgeri*, *Phuwiangosaurus sirindhornae*, *Plateosaurus engelhardti*, *Thecodontosaurus antiquus*, *Dicraeosaurus sattleri* and *Saturnalia tupiniquim*. Phylogenetically, this group contains basal (e.g. *Saturnalia* and *Thecodontosaurus*) and derived (e.g. *Europasaurus* and *Janenschia*) sauropodomorphs that range in body size from small to colossal. The specimens used for sectioning were sub-adults and adults. Preparation of thin sections followed standard protocols (e.g. Reid 1996; Horner *et al.* 2001). Cell volumes (lacunae volume) were estimated by measuring the length and width of the largest lacunae within a given thin section (figure 1), assuming an ellipsoid shape ($4/3 \times \pi \times$ width axis radius^2 $\times$ length axis radius), as detailed in Organ *et al.* (2007). These data (table 1) were combined with cell volume and haploid genome size data (www.genomesize.com) from 38 extant species (see Organ & Shedlock 2009). Several extinct archosauromorph species were also included from Organ *et al.* (2007) and Organ & Shedlock (2009) so that the clade Archosauria was sampled evenly.

Construction of the phylogenetic framework followed Organ & Shedlock (2009), with Sauropodomorpha constructed from Upchurch *et al.* (2004, 2007) using MESQUITE v. 2.01 (Maddison & Maddison 2007) and the STRATADD package (Faure *et al.* 2006). Branch lengths are in units of time and follow the standard geological time scale (Gradstein *et al.* 2004).

Genome size and cell size measurements were logarithmically transformed and analysed, along with femur length—a proxy for body size (Christiansen & Farina 2004), in the program BAYESTRAITS (http://www.evolution.rdg.ac.uk). BAYESTRAITS generates posterior distributions of regression models for continuous characters while using phylogenetic generalized least squares (PGLS) to account for the evolutionary non-independence among the characters (Pagel 1997, 1999). Predictions of genome size were made by sampling the posterior distributions of regression models and accounting for the amount of expected covariation among the extinct and extant taxa based on the phylogenies noted above. Details about our character analysis can be found in the electronic supplementary material.

3. RESULTS

The Bayesian estimation of the correlation between osteocyte cell volume and genome size (electronic supplemental material, fig. 1) did not differ from previous work (Organ *et al.* 2007). The Markov chain reached the station phase without an extended burn-in. Moreover, plots of the regression parameters by their log-likelihood show that they are normally distributed so that the mean of the distribution is close to the maximum likelihood estimate.

The average estimated haploid genome size for the 10 sauropodomorphs in this study (table 1; figure 2) is 2.02 pg, with s.d. of 0.41. There is no apparent phylogenetic pattern in the distribution of genome size within Sauropodomorpha. That is, the genome size of basal members of the group, such as *Saturnalia* (mean estimated genome size: 1.94 pg, s.d. = 0.82), do not differ substantially from more derived members, such as *Dicraeosaurus* (mean estimated genome size: 2.21 pg, s.d. = 0.42). We found no evidence suggesting that genome size evolution evolved in a directional manner in dinosaurs (random walk model versus directional model, $p = 0.14$).

The hypothesis that genome size contraction evolved within the theropod lineage can be evaluated using our results. We find that, as originally hypothesized (Organ *et al.* 2007), theropods have smaller genomes than sauropodomorphs (phylogenetic *t*-test for difference in genome size between theropods and sauropodomorphs, $p = 0.048$). There is also a substantial difference between the genome sizes of Saurischia (Sauropodomorpha and Theropoda) and Ornithischia (phylogenetic *t*-test, $p = 0.0002$).

We find no relationship between body size and genome size in extinct Mesozoic dinosaurs ($n = 27$, PGLS $r^2 = 0.0005$, $p = 0.282$) or within sauropodomorphs ($n = 10$, PGLS $r^2 = 0.011$, $p = 0.39$). For example, the small extinct bird *Hesperornis* (femur length = 155 mm) is estimated to have had a genome nearly identical in size to that of the massive sauropod *Barosaurus* (femur length=1440 mm).

4. DISCUSSION

Previous work on genome size evolution in non-avian dinosaurs (Organ *et al.* 2007) included only one sauropodomorph taxon (*Apatosaurus*). Because estimation of ancestral states is essentially a weighted mean, taxon
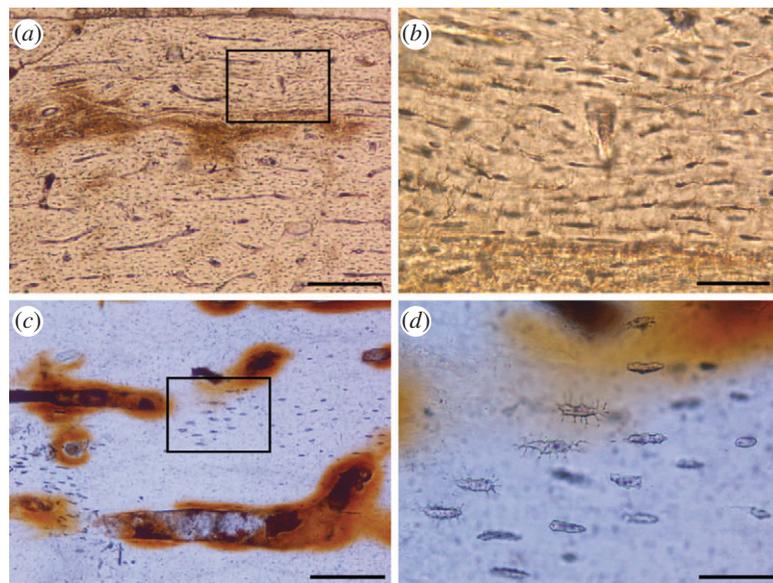
Figure 1. Thin sections of fossil dinosaur bone with preservation of vascular spaces and osteocyte lacunae. (*a*) Transverse section of a femur of the basal sauropodomorph *Thecodontosaurus antiquus*. Scale bar = 200 µm. (*b*) Magnification of selected area in (*a*). Scale bar = 50 µm. (*c*) Longitudinal section of a *Barosaurus lentus* femur. Scale bar = 200 µm. (*d*) Magnification of selected area in (*c*). Scale bar = 50 µm.

Table 1. Summary statistics of lacunae volume for 10 sauropodomorph species. These data were used to infer haploid genome size (*C*-value) using the Bayesian comparative method described in §2 and in further detail elsewhere (Organ *et al.* 2007; Organ & Shedlock 2009). Apatosaurus data is taken from (Organ *et al.* 2007). Institutional abbreviations are as follows: MCP (Museu de Ciências e Tecnologia PUCRS, Porto Alegre, Brazil), NHUB (Naturkundemuseum of the Humboldt-Universität Berlin, Germany), DFMMh/FV (Dinosaurier-Freilichtmuseum Münchehagen/Verein zur Förderung der Niedersächsischen Paläontologie (e.V.), Germany), MOR (Museum of the Rockies, Bozeman, MT), IPB (Institut für Paläontologie, Bonn, Germany).

Sauropodomorph taxon	specimen ID	skeletal element	n	cell volume average (μm^3)	cell vol s.d.	C-value (pg)	C-value s.d.
Apatosaurus	MOR 1996-05	scapula	20	130.49	52.11	2.31	0.44
Barosaurus	T16a	femur	30	57.3	43.04	1.77	0.36
Dicraeosaurus	NHUB O2	femur	30	106.69	60.78	2.21	0.42
Europasaurus	DFMMh/FV495.9	femur	30	95.29	82.31	2.13	0.41
Janenschia	F37a	femur	30	79.02	53.58	1.99	0.39
Massospondylus	Chinsamy (1993)	femur	30	106.65	46.86	2.21	0.7
Phuwiangosaurus	k4-366	femur	30	71.87	54.23	1.93	0.40
Plateosaurus	F14a	femur	30	63.68	39.92	1.82	0.30
Saturnalia	MCPV-3846	femur	37	75.93	33.50	1.94	0.282
Thecodontosaurus	IPB (no ID)	tibia	30	67.56	30.15	1.87	0.31

sampling can have a large influence on estimated trait values at nodes. This is especially true if taxa near a node are omitted and (i) these taxa have very short branch lengths connecting to the node, (ii) they have trait values far from the mean of the group, and/or (iii) the underlying model (Brownian motion or Ornstein-Ulenbeck) poorly fits the data. Several sauropodomorphs used in this study have short branch lengths leading to the node Saurischia, a major subgroup of dinosaurs that includes birds, and these have estimated genome sizes intermediate between theropod and ornithischian dinosaurs (average genome sizes: Ornithischia = 2.75 pg, Sauropodomorpha = 2.02 pg and Theropoda = 1.7 pg). Our analysis suggests that genome size within Sauropodomorpha differs little from the ancestral saurischian condition (figure 2, left panel) and we find no evidence for directional evolution that would invalidate the ancestral state reconstructions noted above (a direction model of evolution indicates that the ancestral value may lie outside the range observed in the descendants). The results also indicate that a progressive decrease in genome size occurred along the evolutionary line to birds, with substantial decrease in Saurischia after the origin of dinosaurs and again in the theropod line after saurischians split into sauropodomorphs and theropods. These results agree with Organ *et al.* (2007), who found that the small genomes of extant birds began to contract in non-avian theropods before the origin of flight.

In the wider context of reptile genomics, our results suggest that the genomes of sauropodomorphs lie in the upper range of birds (ostrich *Struthio camelus*, genome size = 2.16 pg) and near the median for non-avian reptiles (2.24 pg), which ranges from 1.05 pg in the skink
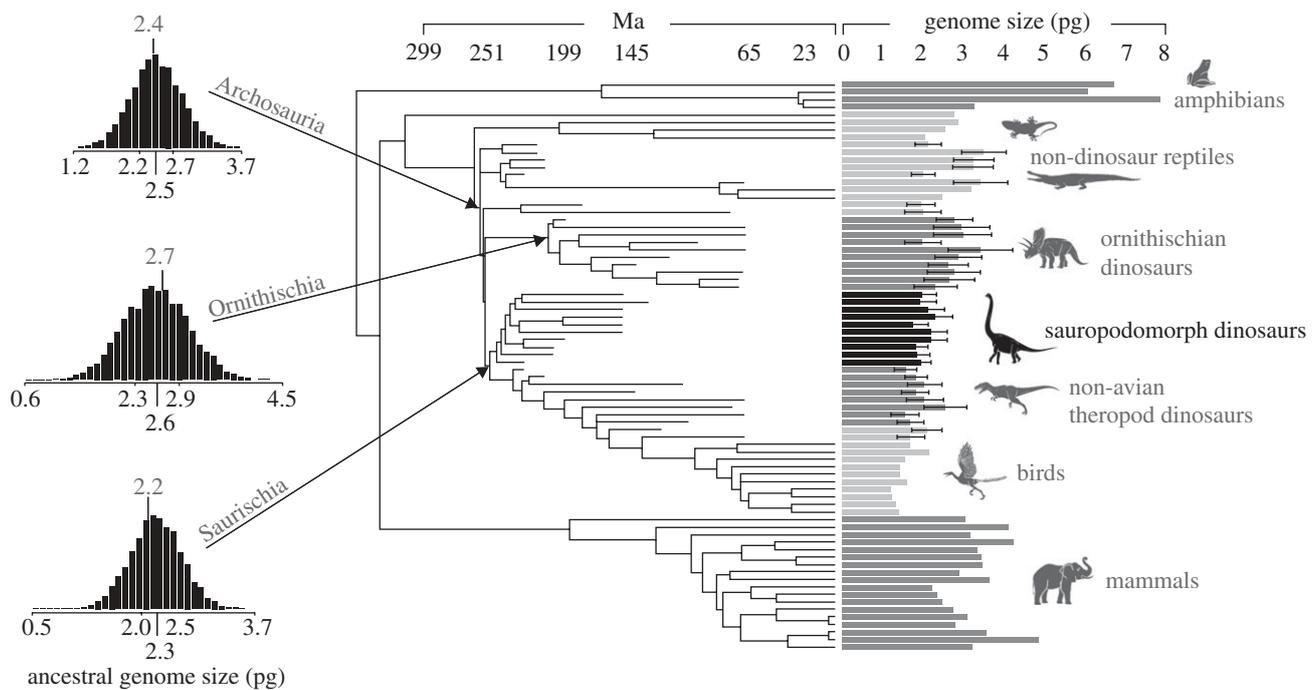
Figure 2. Estimated haploid genome size for sauropodomorph dinosaurs (black bars). For extinct taxa, the bars are the mean of the posterior prediction and the error bars are 1 s.d. The branches of the phylogeny relating the bars in the graph are drawn in units of absolute time. The distributions in the left panel are ancestral state reconstruction for Archosauria (top), Dinosauria: Ornithischia (middle) and Dinosauria: Saurishcia (bottom). The axes in the left panel are labelled with the minimum, 25 per cent quartile, median, 75 per cent quartile, and the maximum of the distributions. The grey number above the distributions is the ancestral state point estimate produced by squared change parsimony. As is clear, the two methods give largely congruent answers.

Chalcides mionecton to 5.44 pg in the Greek tortoise *Testudo graeca* (Gregory 2009). Our results also indicate that genome size variability in extinct dinosaurs, though larger than in extant birds, was not as great as that in mammals, which range from 1.73 pg for the bent-winged bat *Miniopterus schreibersi* to 8.40 pg for the red viscacha rat *Tympanoctomys barrerae* (note that the red viscacha rat is tetraploid). The average genome size for mammals is 3.37 pg (Gregory 2009). The variation in genome size within extinct Dinosauria is therefore more comparable to the variation found in non-avian reptiles than in mammals, whose diversity and dominance in modern terrestrial ecosystems is often compared with Mesozoic dinosaurs.

One of the most important and debated questions in modern evolutionary genomics is the extent to which the evolutionary forces of selection and drift shape the genome (Ellegren 2007). For instance, recent work by Lynch (summarized in Lynch 2007) provides important insights into the respective roles that selection and drift have played in forming genome architecture, and therefore genome size. These ideas may be termed 'the neutral theory of genome evolution' and they predict that genome size in small populations, in which the efficiency of selection is reduced, should expand owing to the accumulation of mutations drifting to fixation. The primary evidence for the neutral theory of genome evolution is represented by the large population sizes and small genomes of prokaryotes in contrast to the small population sizes and large genomes of eukaryotes. This contrast is made feasible because body size, which is roughly inversely proportional to population size, spans 20 orders of magnitude across prokaryotes and eukaryotes (Lynch 2007).

Unlike the prokaryote/eukaryote contrast, in living amniotes differences in body size reach around only four orders of magnitude, well exemplified by the proverbial mouse and elephant. As the largest land animals to have ever walked the earth, weighing up to 80 tonnes (Peczkis 1994), sauropodomorphs provide a critical source of data to the ongoing debate about genome size evolution. The neutral theory of genome evolution predicts that sauropodomorphs should have had relatively small populations, owing to their large body size, and therefore large genomes. The genome sizes estimated here for sauropodomorphs would support the neutral theory if genome size and body size are inversely related in animals (Lynch 2007) or if genome size expands for derived sauropods (species with the largest body sizes) relative to more primitive, smaller basal sauropodomorphs. However, our results do not support a purely neutral process of genome evolution in extinct dinosaurs. We find no correlation between genome size and body size in Mesozoic dinosaurs as predicted by the neutral theory. This agrees with recent findings in which correlations between genome size and body size were absent in extant eukaryotes using standard statistical approaches (Gregory 2005), and in extant tetrapods using phylogenetic comparative methods (Organ & Shedlock 2009). Furthermore, we do not find an expansion of genome size in larger sauropods, but rather the opposite, a slight reduction relative to the saurischian common ancestor (figure 3). However, these results do not rule out the possibility that drift played an important role in the evolution of amniote or dinosaur genomes, only that the evidence presented here does not match the predictions made by a predominately neutral model.
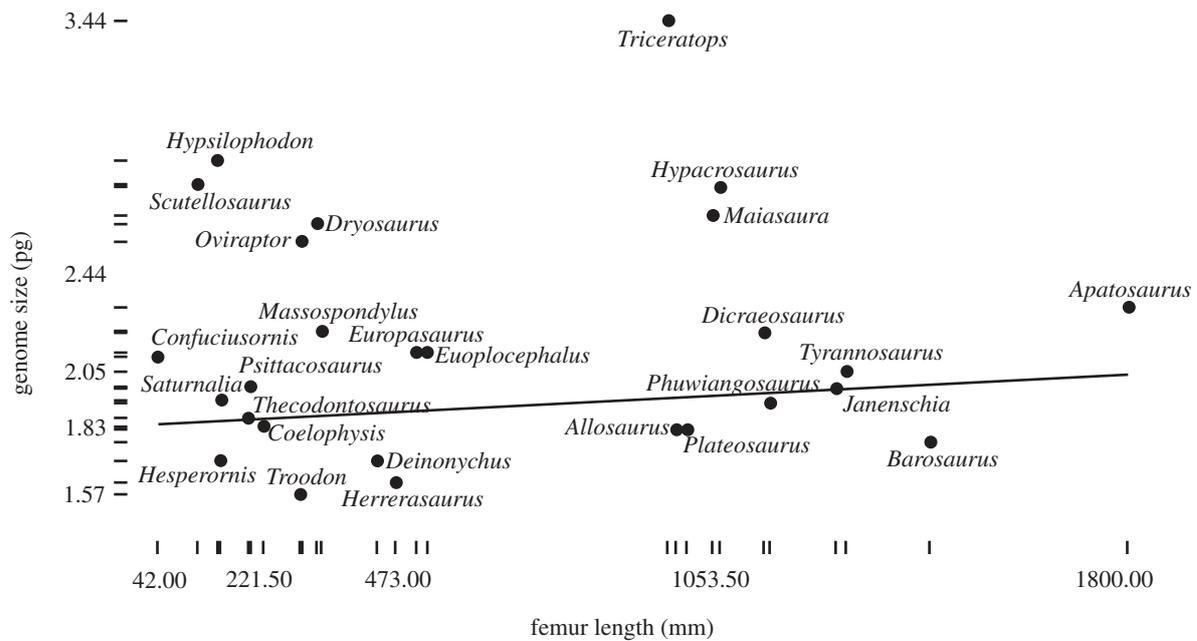
Figure 3. The relationship between genome size and body size in extinct dinosaurs. The phylogenetic generalized least-square (PGLS) regression line relating genome size to body size in 27 extinct dinosaur species is genome size = $1.84 + (0.0001 \times$ femur length), $r^2 = 0.0005$, $p = 0.282$. The axes are labelled with the quartiles of the data (minimum, 25% quartile, median, 75% quartile and maximum).

Our results, and palaeogenomics in general, permit the formation and testing of detailed and rigorous hypotheses concerning molecular and cellular information in extinct taxa. For example, because genome size correlates with cell size and cell division rate, and because these associations are thought to be causative, knowing genome size has the potential to clarify aspects of an organism's biology for which there is no direct fossil evidence. Of specific interest is the observation that genome size is correlated with metabolic rate in tetrapods generally (Vinogradov & Anatskaya 2006) and in birds particularly (Gregory 2002). This relationship has prompted speculation that the small genomes in extinct theropods and pterosaurs may be evidence of an elevated metabolic rate (Organ *et al.* 2007; Organ & Shedlock 2009). Histological work suggests that sauropods were metabolically active, given their inferred rapid growth rates (Curry Rogers 1999; Klein & Sander 2008). Yet such speculation is more difficult to offer here given the estimated genome sizes of sauropods, which lie in the range of many ectothermic lizards and palaeognath birds. Regardless, future palaeogenomic work on other extinct tetrapod groups should help clarify when repetitive elements radiated or went extinct within genomes, and whether expansions or contractions in genome size were associated with the acquisition of other traits, such as rapid growth rates or integumentary covering, each of which may be indicative of an elevated metabolism.

In this vein, genome size is an independent line of evidence that may be brought to bear on certain long-debated questions of organismal biology in extinct species and large-scale patterns of molecular evolution on lineages leading to living species. Yet there are still many unknowns regarding the relationship between genome size, cell size and cell physiology. Additional rigorous, phylogenetically based studies of many clades, both extinct and extant, are needed to clarify the issues raised above. Just as fossils can reveal patterns of anatomical character change from extinct ancestors to living descendants, fossils may also help reveal broad patterns of genome evolution over geological time scales.

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