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Macroevolution of genome size in sarcopterygians during the water–land transition

Macro-évolution de la taille du génome chez les sarcoptérygiens lors de la transition eau–terre

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ABSTRACT

Genome size spans over a 300-fold range among vertebrates (132 pg for *Protopterus aethiopicus*, the marbled lungfish, and 0.35 pg for *Tetraodon nigroviridis*, the green spotted pufferfish). While phylogenetic analysis of genome size has helped clarify how this variation evolved in multiple tetrapod groups, the ancestral tetrapod condition still remains poorly characterized, and this obscures our understanding of character state polarity and macroevolutionary trends in genome size. To address this problem, we used phylogenetic comparative methods to analyze paleohistological data from eight taxa of the Middle and Late Paleozoic to the Early Mesozoic: *Eusthenopteron*, *Ichthyostega*, *Acheloma*, *Eryops*, *Trimerorhachis*, *Wetlugasaurus*, an unidentified dissorophoid, and *Chroniosaurus*. Five other extinct taxa were included from previous studies to better frame our results, including *Marmorerpeton*, *Cardiocephalus*, *Diplocaulus*, an unidentified basal sauropsid, and *Mycterosaurus*. We augmented a previously reported histological and genome size data set (including data from 14 lissamphibians, three testudines, *Sphenodon*, five squamates, two crocodilians, 11 birds, and 22 mammals) with genome size and histological data from extant *Latimeria* and three extant actinopterygians. Our results suggest that all eight of the newly analyzed extinct taxa had genome sizes ranging between 3.2 and 3.9 pg. These results imply that basal tetrapods had genome sizes (and underlying genomic architectures) similar to extant mammals and lepidosaurs. We find no major shifts in genome size during the tetrapod water-to-land transition. Our analysis suggests that *Eusthenopteron* and *Ichthyostega* had genome sizes well within the range of extant actinopterygians and *Latimeria*, despite several whole-genome duplications in actinopterygians.

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RÉSUMÉ

La taille du génome couvre une gamme de 1 à 300 chez les vertébrés (132 pg pour *Protopterus aethiopicus*, poisson marbré à poumon et 0,35 pg pour *Tetraodon nigroviridis*, poisson globe à taches vertes). Tandis que l'analyse phylogénétique de la taille du génome a aidé à clarifier comment cette variation a évolué dans de multiples groupes de tétrapodes, la condition de tétrapode ancestral reste mal caractérisée, et ceci perturbe notre compréhension de la polarité de l'état des caractères et des tendances de la macro-évolution dans la taille du génome. Dans le but de s'ataquer à ce problème, nous utilisons des méthodes phylogénétiques comparatives pour analyser les données paléo-histologiques de 8 taxons de la période Paléozoïque moyen et supérieur–Mésozoïque inférieur : *Eustenopteron*, *Ichthyostega*, *Acheloma*, *Eryops*, *Trimerorhachis*, *Wetlugasaurus*, un dissorophoïdé indéterminé et *Chroniosaurus*. Cinq autres taxons éteints ont été inclus à partir d'études antérieures pour mieux étayer nos résultats : *Marmorerpeton*, *Cardiocephalus*, *Diplocaulus*, un sauropsidé basal indéterminé et *Mycterosaurus*. Nous augmentons un groupe de données histologiques et de taille du génome, précédemment acquises (incluant des données sur 14 lissamphibiens, 3 tortues, *Sphenodon*, 5 squamates, 2 crocodiliens, 11 oiseaux et 22 mammifères) avec des données histologiques et de taille du génome de *Latimeria* actuel et de 3 actinoptérygiens éteints. Nos résultats suggèrent que les 8 taxons éteints nouvellement analysés ont des tailles de génome allant de 3,2 pg à 3,9 pg. Ces résultats impliquent que les tétrapodes de base ont des tailles de génome (et des architectures génomiques sous-jacentes) similaires à celles des mammifères et des lépidosaures actuels. Nous ne trouvons aucun changement majeur dans la taille du génome au cours de la transition eau–terre. Notre analyse suggère qu'*Eusthenopteron* et *Ichthyostega* ont des tailles de génome bien dans la gamme de celles des actinoptéryiens et des Latimeria actuels, malgré quelques duplications du génome entier chez les actinoptérygiens.

1. Introduction

Dramatic variation in size is a prominent feature of animal genomes. Much of this variation is captured in actinopterygians, where genome size (or C-value) ranges over 300-fold – 132 pg for *Protopterus aethiopicus*, the marbled lungfish, and 0.35 pg for *Tetraodon nigroviridis*, the green spotted pufferfish (Gregory, 2014). Tetrapods also exhibit great variation in genome size. For instance, the hummingbird *Archilochus alexandri* has a C-value (haploid) of merely 1 pg while *Necturus lewisi* has a C-value of over 120 pg (Gregory, 2002a; Sun et al., 2012; Thomson and Muraszko, 1978). Understanding the macroevolutionary patterns and process that led to the large genomes of urodeles (Herrick and Sclavi, 2014; Olmo and Morescalchi, 1978; Sun and Mueller, 2014) and the small genomes of birds (Tiersch and Wachtel, 1991) from a common ancestral genome is an important problem in evolutionary genomics.

One approach to address this problem involves the well-documented association between cell size and genome size (Cavalier-Smith, 1985). Bone cells (osteocytes) in particular, and despite ample variation in tissue parameters, such as osteocyte size, orientation, and abundance (Montanari et al., 2011; Stein and Prondvai, 2014), holds great promise to study genome macroevolution because bone is preserved in the fossil record. Indeed, this correlate to genome size is the only available evidence with which to study genome size evolution that dates to hundreds of millions of years. Osteocytes develop from osteoblasts, which enclose themselves in a protein and mineral matrix (Franz-Odendaal et al., 2005). The resulting pockets in which they reside are known as lacunae and the cell in each lacuna communicates with other cells via thin channels in the matrix called canaliculi. Lacunae shapes and sizes generally reflect the shape and size of the osteocyte (Canè et al., 1982) and these tissue parameters often survive preservation in the fossil record. Most fossil bone is thought to be unaltered at the histological level by bacterial invasion or diagenetic factors, despite color changes by the infiltration of sediment solute (Horner et al., 2000).

Large-scale evolutionary shifts in genome size are thought to occur by changes in repetitive content, such as transposable elements (TEs) (Hancock, 2002; Sun et al., 2012), expansion of intronic sequences (Organ et al., 2008; Smith et al., 2009), and whole-genome duplication events that allow for major reconfigurations of a genome and gene expression (Gregory, 2005; Reumer and Thiebaud, 1987; Smith et al., 2013; Venkatesh et al., 2014). Interestingly, whole-genome duplications in actinopterygians appear to undergo large amounts of subsequent evolution, resulting in substantially reduced genome size (Dehal and Boore, 2005). Metabolic pressures to increase the rate of cellular (and therefore genomic) replication have also been hypothesized to play a role in genome size macroevolution (Gregory, 2002a). These evolutionary shifts in genome size are difficult to track directly, however, because genomes do not fossilize. Macroevolutionary analysis of genome size using paleohistological correlates has shown promise in bridging the lack of direct preservation (Laurin et al., 2016; Organ and Shedlock, 2009; Organ et al., 2007, 2011).

An outstanding question is the diversity of genome sizes during the water-to-land transition in vertebrates, as well as the ancestral state of tetrapods. Extant finned sarcopterygians (limited to several species of lungfish and

coelacanths) have highly varied genome sizes (Thomson, 1972), from the particularly large genomes of the several lungfish species (as much as 132 pg in *Protopterus aethiopicus*) to the moderately-sized genome of *Latimeria chalumnae* at 3.61 pg (Gregory, 2014), which are the sister group of extant tetrapods (Amemiya et al., 2013). This standing variation must have been caused by a significant amount of genomic evolution since these taxa split from a common ancestor in the Early Devonian. It also means that inferring the ancestral condition in genome size in tetrapods is difficult. Phylogenic analysis of extant species can be used to infer the state of the ancestral tetrapod C-value, but such estimates are less reliable than leveraging fossil data, which helps control for trait values deep in the tree (Organ et al., 2007, 2009a; Slater and Harmon, 2013).

Here, we assess paleohistological samples from eight extinct sarcopterygian taxa ranging in age from the Devonian to the Triassic. Depending on the reference phylogeny some (Ruta and Coates, 2007) or all (Marjanović and Laurin, 2013a) of these are stem tetrapods; we opted for the latter interpretation. We use these genome size data from extinct species to better understand and constrain hypotheses about genome size evolution in sarcopterygians, and especially in tetrapods. We also discuss character state polarity and macroevolutionary trends of genome size in early tetrapods.

2. Materials and methods

2.1. Histological data and methods

Our histological data set is an extension from previous work (Laurin et al., 2016; Organ and Shedlock, 2009; Organ et al., 2007, 2011) by including data for three extant actinopterygians, *Cyprinus carpio* (common carp), *Oncorhynchus mykiss* (rainbow trout), and *Polypterus senegalus* (Senegal bichir), and for the extant finned sarcopterygian *L. chalumnae* (African coelacanth). These sections were obtained from François J. Meunier's collection (Muséum national d'Histoire naturelle, France). Our paleohistological sampling expands upon previous work (Organ et al., 2011) by the inclusion of eight new species of tetrapodomorphs from the Devonian to the Triassic: *Eusthenopteron*, *Ichthyostega*, *Acheloma*, dissorophoid temnospondyl (*incertae sedis*), *Chroniosaurus*, *Trimerorhachis*, *Wetlugasaurus*, and *Eryops*; Table 1. *Eusthenopteron* samples were obtained at the Musée d'Histoire Naturelle, Miguasha (specimen ID: 06-241A and B; part and counterpart) and the Swedish Museum of Natural History (specimen ID: P, 840G). Both samples are from the Escuminac Formation, Frasnian, Quebec, Canada. *Ichthyostega* sections were obtained from A. de Ricqlès' collection (specimen IDs: 6131.1.6.T and 17.2.1.T from Britta Dal Formation, Famennian, East Greenland), Collège de France, France. *Eryops* sections were obtained from A. de Ricqlès' collection (specimen ID: 12.6.1.T) as well. The *Acheloma* section was obtained from the Sam Noble Oklahoma Museum of Natural History (specimen ID: OMNH 56939 from Norman, Richard's Spur locality, Artinskian, Oklahoma). The dissorophoid temnospondyl (incertae sedis) was obtained from R. Reisz' collection (unnumbered specimens) at the University of Toronto, Mississauga, Canada. *Trimerorhachis* sections were obtained from the National Museum of Natural History, USA (specimen ID: 402 and 404). *Wetlugasaurus* sections were obtained from A. de Ricqlès' collection (specimen ID: 603.2.1.T). The *Chroniosaurus* section was made from a specimen (from the Upper Tatarian, Permian) donated by V. Golubev, at the Paleontological Institute of the Russian Academy of Sciences, Moscow.

We sampled consistently from homologous long bones (femora) in the extinct taxa. To better frame our genome size estimates, we also included several previously analyzed paleohistological data from long bones of *Marmorerpeton*, *Cardiocephalus*, *Diplocaulus*, an unidentified basal sauropsid, and *Mycterosaurus* (Laurin et al., 2016;

Table 1
Cell lacunae measurements of newly sampled taxa used for paleogenomic analysis. Histological data were taken from prepared slides using ImageJ (Schneider et al., 2012) and genome size data were obtained from the Animal Genome Size Database (http://www.genomesize.com/) and were averaged for species with multiple entries. Abbreviations: micrometers (μm), picograms (pg), volume (VOL).
Tableau 1
Mesures de lacunes cellulaires des taxons nouvellement échantillonnés, utilisées pour l'analyse paléogénomique. Les données histologiques ont été acquises à partir de lames préparées, en utilisant ImageJ (Schneider et al., 2012), et les données sur la taille du génome ont été obtenues à partir de la base de données Animal Genome Size (http://www.genomesize.com/), et ont été moyennées pour les espèces à multiples entrées.

Group	Taxon	Geological age	Element	*n*	Lacunae Vol, mean (μm^3)	Lacunae Vol, σ	C-value, mean (pg)
Actinopterygii							
Cypriniformes	*Cyprinus carpio*	Extant	Vertebra	37	115.48	55.017	1.79
Salmoniformes	*Oncorhynchus mykiss*	Extant	Dentary	4	229.96	89.72	2.65
Polypteriformes	*Polypterus senegalus*	Extant	Mandible	12	159.33	53.55	5.23
Sarcopterygii							
Coelacanthiformes	*Latimeria chalumnae*	Extant	Mandible	37	281.39	174.1	4.34
Tristichopteridae	*Eusthenopteron*	Late Devonian	Femur	46	173.24	100.93	–
Stegocephalia	*Ichthyostega*	Late Devonian	Femur	8	390.64	242.26	–
Temnospondyli	*Acheloma*	Early Permian	Femur	62	357.83	122.63	–
Temnospondyli	*Eryops*	Early Permian	Femur	44	330.41	128.55	–
Temnospondyli	*Trimerorhachis*	Early Permian	Femur	28	252.85	76.45	–
Temnospondyli	Dissorophoid i. s.	Late Permian/Early Triassic	Femur	11	347.12	80.62	–
Temnospondyli	*Wetlugasaurus*	Early Triassic	Femur	13	364.81	102.74	–
Chroniosuchia	*Chroniosaurus*	Late Permian	Femur	11	164.37	65.07	–

Organ et al., 2011). Not all of the extinct taxa from these studies were included here to better highlight our new data set (their exclusion does not bias our new genome size estimates).

Obtaining osteocyte lacuna measurements in actinopterygians is not straightforward – bone in teleosts may be acellular and sampling homologous bones across actinopterygians species may be impractical (there are no homologues of the humerus and femur in teleosts). We were therefore constrained to sample a variety of bones in extant actinopterygians (see Table 1 for details). New cell lacunae measurements were taken from prepared slides using ImageJ (Schneider et al., 2012) and genome size data for extant species were obtained from the Animal Genome Size Database (http://www.genomesize.com/). Although we lacked histological samples for lungfish, we included genome size data for two species (*Neoceratodus forsteri* and *Lepidosiren paradoxa*) for comparison after inferring genome sizes in extinct species. Species with multiple genome entries were averaged. The volumes of osteocyte lacunae were estimated assuming they were rotational ellipsoids ($4/3 \cdot \pi \cdot$semi-minor axis$^2 \cdot$semi-major axis). Small, non-ellipsoid lacunae were not sampled as they are unlikely to be sectioned across their mid-axes. Genome size and lacuna size data were log transformed to meet assumptions of normality in the statistical analysis.

2.2. Phylogenetic comparative methods

BayesTraits v2 (http://www.evolution.rdg.ac.uk/) was used to perform phylogenetic comparative analyses with a chronogram drawn from the literature (Marjanović and Laurin, 2007, 2013b; Organ et al., 2011). To this tree, extant actinopterygians were added using the TimeTree resource (Hedges and Kumar, 2009). Paleozoic and Triassic extinct species were added based on the phylogeny from Marjanović and Laurin (2013a, 2013b), except for the phylogeny of temnospondyls, which follows Schoch (2013). The Markov Chain Monte Carlo (MCMC) method was used to sample a posterior distribution of regression models that related genome size (dependent variable) to osteocyte lacunae size (independent variable) for 62 extant species. Consistent with previous studies (Laurin et al., 2016; Organ et al., 2011), we employed dummy variables to separate salamanders from other tetrapods, which have a different relationship between genome size and osteocyte lacuna size, likely owing to their extremely large genomes (see Organ et al., 2011 for details on this model). The phylogenetic signal (λ) of the data, given the tree, was also sampled during the MCMC procedure. The MCMC ran for 2,001,000 iterations with a burn-in of 100,000 and a sample period of 1000, and uninformed flat priors for the regression parameters (−100.00 to 100.00). We evaluated the statistical significance of our regression model under maximum likelihood with a likelihood ratio test comparing the model against a null model with the slope parameters set to 0 (LRT is 2 × the log-likelihood ratio assuming a Chi^2 distribution and degrees of freedom equal to the difference in parameters of the models). For our MCMC analysis, we also assessed how much of the slope parameters' posterior distribution deviated from zero.

The posterior distribution of regression models (including the parameter λ) for genome size and osteocyte lacuna volume were sampled to make phylogenetically-informed predictions [retrodictions; see Scriven (1959) and Organ (2012)] of genome size in the extinct species. The precision of phylogenetically-informed prediction is a function of shared path lengths. We therefore expect the phylogeny to play a relatively smaller role in influencing the estimates of genome size than in previous paleogenomic work (Organ and Shedlock, 2009; Organ et al., 2007, 2009b, 2011) because the sampled taxa in this study are geologically older (closer to the root). Because log-log regressions estimate the geometric mean as opposed to the arithmetic mean, we performed a correction when anti-logging genome size estimates by adding half the mean square error [MSE = SSE/(n-p-1)] to the prediction before the back transformation (Hayes and Shonkwiler, 2006; Smith, 1993).

3. Results

The volume and variability of lacuna volume in extant actinopterygians and sarcopterygians are consistent with expectations based on the size of their moderate to small genomes (Table 1). When these data are added to the existing extant vertebrate dataset described above, we find substantial evidence for the relationship between genome and cell (lacuna) volume (P-val < 0.0001). In MCMC, we find that 99% of the posterior distribution of the first slope parameter deviates from zero (posterior β_2, $\mu = 0.60$, $\sigma = 0.21$) and 94% of the dummy variable interaction slope deviated from zero (posterior β_4, $\mu = -0.35$, $\sigma = 0.22$). We also find relatively large values for both the coefficient of determination (posterior r^2, $\mu = 0.59$, $\sigma = 0.06$) and phylogenetic signal (posterior λ, $\mu = 0.79$, $\sigma = 0.11$).

Our retrodictions for genome size in the extinct taxa are shown on Fig. 1. We estimate that *Marmorerpeton* had a genome size within the range of modern urodeles (Fig. 1A), consistent with our previous work (Laurin et al., 2016). Note that our predictions in log space were identical to Laurin et al. (2016), but our correction for anti-logging (Hayes and Shonkwiler, 2006; Smith, 1993) resulted in estimates slightly larger than, but consistent with our conclusions in Laurin et al. (2016). The remaining extinct species show no evidence for outliers, with genome size estimates similar to actinopterygians and mammals (Fig. 1B). Genome size in lepidosaurs and non-avian archosaurs are generally smaller than estimates for the fossil non-amniote sarcopterygians, but not significantly so.

4. Discussion

The evolutionary transition between an aquatic lifestyle of Devonian sarcopterygians and the branch of sarcopterygians, which became terrestrial and diversified into modern tetrapods is a well-studied evolutionary event in the history of vertebrates (Laurin, 2010). Many records of this transition exist, including insights from comparative genomics (Amemiya et al., 2013; Finn et al., 2014; Nikaido et al., 2013), preserved fossils that track morphological

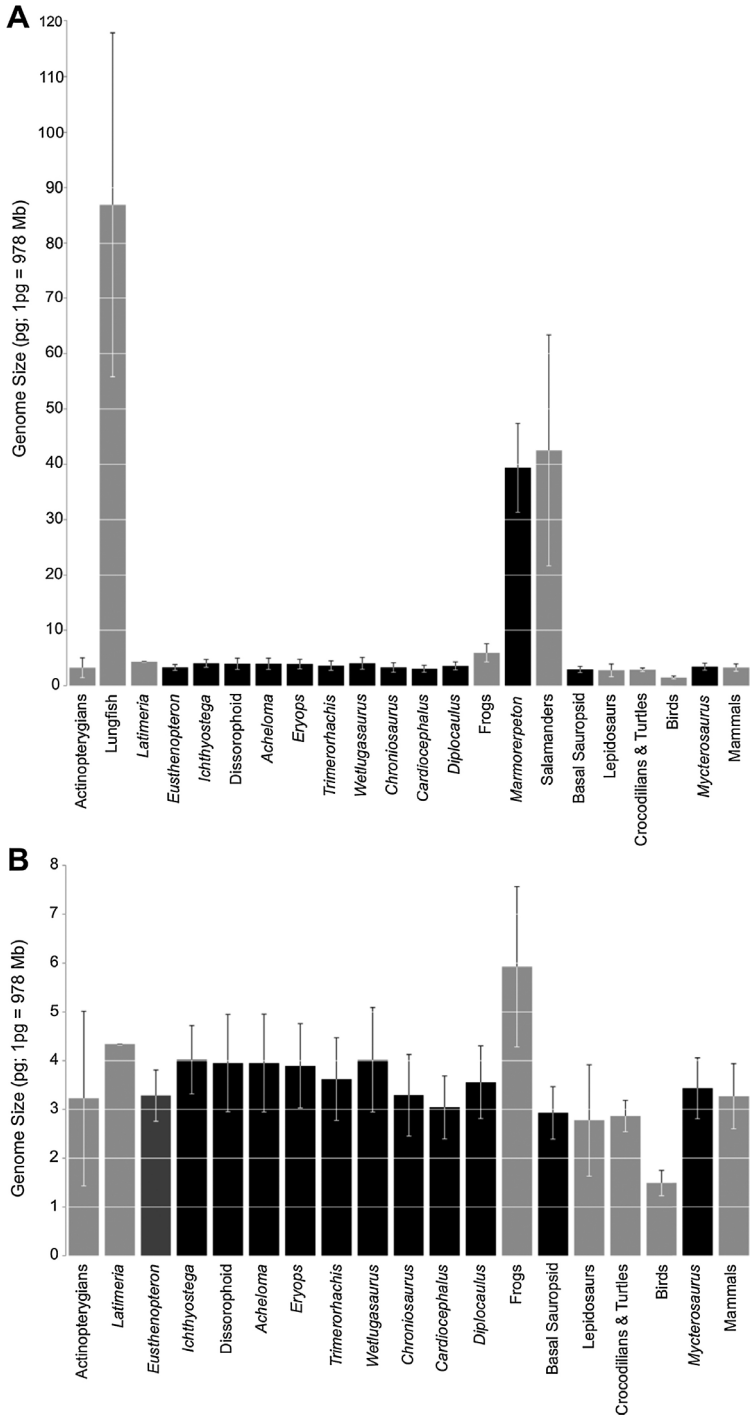

Fig. 1. Bar chart of genome sizes for the major extant groups (gray bars) included within this study compared with retrodictions of genome size for 13 extinct sarcopterygians (black bars). Panel A contains all taxa while lungfish, urodeles, and *Marmorerpeton* are removed in panel B for better comparison among taxa. Note that genome sizes for extant groups are based only on the species included in this study, not for all available species found at http://www.genomesize.com/. Error bars are standard deviation.

Fig. 1. Charte de barres de tailles de génome chez les principaux groupes actuels (barres grises) inclus dans cette étude (échelle de couleur logarithmique), comparées aux rétrodictions sur la taille du génome de 13 sarcoptérygiens éteints (barres noires). Le panneau A contient tous les taxons, sachant que le poisson à poumon, les urodèles et le *Maremorerpeton* ont été transférés dans le panneau B, pour une meilleure comparaison entre les taxons. À noter que les tailles de génome pour les groupes actuels sont basées uniquement sur les espèces incluses dans cette étude et non sur les espèces disponibles trouvées dans http://www.genomesize.com/. Les barres d'erreur correspondent à la déviation standard.

modifications (Pierce et al., 2012, 2013; Shubin et al., 2014), impressions of footprints which, along with limb proportions, reveal gait and motion (Legreneur et al., 2013; Marsicano et al., 2014; Niedźwiedzki et al., 2010), the locality of fossilized remains, and the rocks in which they are preserved (Bendix-Almgreen et al., 1990; Ultsch, 1996) which help us, along with faunal associations (Laurin and Soler-Gijón, 2010), to understand the environments and conditions in which this group evolved. But in spite of these records, there are many aspects of this transition that are not preserved directly. We therefore do not understand fully what biological changes occurred as early limbed vertebrates evolved in the Devonian, nor what pressures the environment placed on this new land-going group – a prime example being the subsequent evolution of an amphibious lifestyle.

Studying the macroevolution of genome size is especially problematic. The large genomes of many lissamphibians and lungfish, and the notably small genomes of many teleosts, birds, bats, and some turtles, make an estimation of the ancestral tetrapod genome size difficult to determine with any degree of precision. There may be reasons to hypothesize that genome size changed during the water-to-land transition, but they are poorly supported. For example, some evidence suggests that freshwater species tend to have larger genomes than marine counterparts (Hardie and Hebert, 2004; Rees et al., 2007). To address this problem, we included data from *Eusthenopteron* and *Ichthyostega*, two Late Devonian genera very close to the origin of the tetrapods and that were both primarily or exclusively aquatic, as well as more recent (Permo-Carboniferous) taxa that were more terrestrial. *Eusthenopteron* bears paired, dorsal, anal and caudal fins, and a pectoral girdle attached to the skull along with other anatomical features, which characterize it as fully aquatic (Laurin et al., 2007; Long et al., 2006). *Ichthyostega*, on the other hand, possesses limbs with digits, a pectoral girdle unattached to the skull, a much larger pelvic girdle, and a more structurally established ribcage (Pierce et al., 2013). These features, along with other traits, constitute exaptations of *Ichthyostega* to terrestrial habitats, because other characters, such as a well-developed cephalic portion of the lateral-line organ and a caudal fin suggest that it was likely aquatic, perhaps with a lifestyle not unlike a mudskipper (Callier et al., 2009; Pierce et al., 2013). The sampled Permo-Carboniferous taxa include some amphibious or terrestrial taxa, such as *Acheloma*, the unidentified dissorophoid, and *Chroniosaurus* (Laurin et al., 2004; McHugh, 2015; Quemeneur et al., 2013) as well as more aquatic taxa, like *Trimerorhachis* and *Diplocaulus* (some of which may be secondarily aquatic). Despite the clear transition in habitat for the adult forms of these extinct species, we find evidence suggesting that the genomes in both habitats and before and after the transition to land were roughly equivalent in size.

The retrodicted C-values for *Eusthenopteron* and *Ichthyostega* have important implications for understanding how genome size evolved in extant finned sarcopterygians. The only two groups of extant finned sarcopterygians that persist today are the several species of lungfish (with massive genomes) and coelacanths (with a moderate-sized genome). The primitive C-value condition of sarcopterygians has long been unclear, despite work documenting long-term gradual increase in genome size within lungfish (Thomson, 1972). The additional information from the two Devonian taxa (*Eusthenopteron* in particular) presented here implies that the large genomes of lungfish are derived and that primitive sarcopterygians had genome sizes much more equitable to the coelacanth *L. chalumnae*. This finding is interesting in light of the coelacanth exome, which apparently also evolved slowly (Amemiya et al., 2013). It is also consistent with early work on dipnoan genome size evolution (Thomson, 1972).

Lungfish and urodeles have enormous genome sizes compared with other vertebrates (Fig. 2). It has been hypothesized that the simplification of the developmental program in these groups (paedomorphosis) relaxed constraints on genome size, causing them to expand over time (Gregory, 2002b). Despite having massive genomes, lungfish and urodeles appear to lack whole-genome duplications (Kaiya et al., 2014; Panopoulou and Poustka, 2005; Smith et al., 2009). Such events in plants have been well researched (Mühlhausen and Kollmar, 2013; Song et al., 1995; Vanneste et al., 2014a, 2014b) and recent studies have looked towards animals, and specifically vertebrates, in search of evidence of similar whole-genome duplications (Berthelot et al., 2014; Hufton et al., 2008). There is good evidence that two whole-genome duplications (WGD) events occurred between early chordates and the origin of the osteichthyan stem (Dehal and Boore, 2005; Kasahara, 2007; Laurin, 2011). Such events were likely crucial for structuring important adaptations of vertebrates, such as developmental genes or the immune system (Otto, 2007), but do not appear to be related to the long-term evolution of genome size (Fig. 2). For instance, the major histocompatibility complex (MHC) in modern vertebrates exists as four distinct paralogs on four chromosomes (Kasahara, 2007). The cephalochordate *Branchiostoma* has a single MHC region, which likely represents the ancestral version of the four MHC paralogs found within jawed vertebrates. HOX genes, responsible for the major body layout designs of animals, are also found in four clusters on four different chromosomes in most vertebrates. Only one cluster exists in *Branchiostoma* (Holland et al., 2008) and HOX genes are simplified in the closest relative of vertebrates, the urochordates (Delsuc et al., 2006; Singh et al., 2009).

Our approach has the potential to detect WGD in extinct lineages, but we find no evidence suggesting they occurred in temnospondyls. The Permo-Carboniferous limbed vertebrates *Acheloma*, *Eryops*, *Trimerorhachis*, and the Permo-Triassic *Wetlugasaurus* and *Chroniosaurus*, are all found to have similar, modestly sized genomes within the same range as *Eusthenopteron* and *Ichthyostega*. These values support the suggestion that genome sizes did not change dramatically in limbed vertebrates during their early evolution and were very similar to those of other sarcopterygians of the time. The evolution of genome size in lissamphibians is more complex (note that we do not include caecilians in our study owing to a lack of samples, their genome sizes fall within the range of anurans). In this clade, data presented here and elsewhere (Laurin et al., 2016; Organ et al., 2011) suggest that the

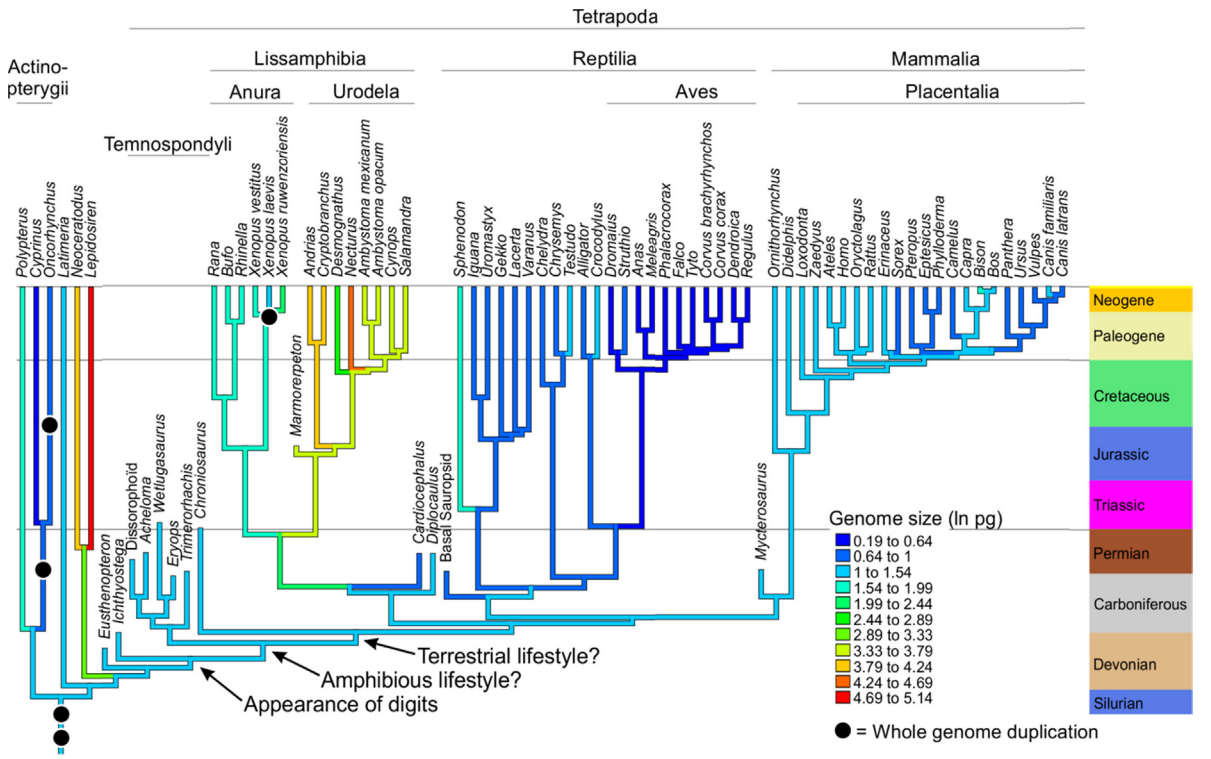

Fig. 2. Genome size mapped onto the phylogenetic framework used in this study (color scale is logarithmic). Retrodicted genome sizes for extinct taxa are the means of the predictive distributions from a Bayesian posterior distribution of multiple regression models. Black circles mark putative whole-genome duplication events – note the apparent lack of correspondence between genome size and whole-genome duplications, which indicates substantial genome rearrangement and loss of genetic material following duplications in deep time. The massive, but relatively understudied, genomes of salamanders and lungfish are apparently due to abundant transposable elements (Sun et al., 2012) and huge introns (Smith et al., 2009).

Fig. 2. Tailles de génome cartographiées sur le réseau phylogénétique utilisé dans cette étude (échelle de couleur logarithmique). Les tailles de génome rétrodictives pour les taxons éteints sont les moyennes des distributions prédictives à partir d'une distribution bayésienne postérieure de multiples modèles de régression. Les cercles noirs correspondent aux évènements putatifs de duplication du génome entier. À noter le manque apparent de correspondance entre la taille du génome et les duplications du génome entier, ce qui indique un réarrangement substantiel du génome et la perte de matériel génétique suivant les duplications au cours du temps. Les génomes nombreux, mais relativement sous-étudiés, des salamandres et poissons à poumon sont apparemment dus à d'abondants éléments transposables (Sun et al., 2012) et à de très gros introns (Smith et al., 2009).

Permian lepospondyls *Cardiocephalus* and *Diplocaulus* had moderately-sized genomes. Considering the elevated size of genomes in anurans and caecilians, these data imply that genome size expansion in the lissamphibian stem began in the Permian, before the development of distinct urodele and anuran clades. Our analysis also suggests that in the Early Triassic to Middle Jurassic, urodeles underwent a second period of genomic expansion (Fig. 2). However, these lineages are poorly sampled in terms of paleogenomic data and new studies would certainly help clarify their patterns of genome evolution. Analysis of transposable elements (TE) in salamanders suggests that while possessing typical vertebrate families of TEs, their genomes contain larger amounts of long terminal repeat (LTR) retrotransposons, which strongly contribute to the massive genomes of extant urodeles (Herrick and Sclavi, 2014; Sun and Mueller, 2014; Sun et al., 2012) in addition to expansive intronic sequences (Smith et al., 2009).

The moderately-sized genomes we infer for basal limbed vertebrates suggest that these taxa lacked the repetitive content and enlarged introns characteristic of urodeles or lungfish. By sampling fossil data, our method has the potential to reconstruct very large genomes owing to WGD in basal members of extant groups. The present analysis finds no such evidence, but our paleogenomic approach could be used in the future to better understand the tempo and mode at which genomes shrink following WGDs.

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