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Macroevolutionary shift in the size of amphibian genomes and the role of life history and climate

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The evolution and great diversity of genome size has been of long-standing interest to biologists, but has seldom been investigated on a broad phylogenetic scale. Here we present a comparative quantitative analysis of factors shaping genome size evolution in amphibians, the extant class of vertebrates with the largest variation in genome size. We find that amphibian genomes have undergone saltations in size, although these are rare and the evolutionary history of genome size in amphibians has otherwise been one of gradual, time-dependent variation (that is, Brownian motion). This macroevolutionary homogeneity is remarkable given the evolutionary and ecological diversity of most other aspects of the natural history of amphibians. Contrary to previous claims, we find no evidence for associations between life cycle complexity and genome size despite the high diversity of reproductive modes and the multiple events of independent evolution of divergent life cycles in the group. Climate (temperature and humidity) affects genome size indirectly, at least in frogs, as a consequence of its effect on premetamorphic developmental period, although directionality of the relationship between developmental period and genome size is not unequivocal.

Genomes vary conspicuously in size across the tree of life[1]. This size variation shows only a weak relationship with organismal complexity (the C-value enigma[2,3]), although there is an apparent smooth ranking at broad levels of organization from unicellular eukaryotes to vertebrates and plants[3,4]. Genome size correlates with the number of genes within prokaryotes, but not in eukaryotes, where size variation is determined by proportion of mobile elements, number of introns and complexity of regulatory regions[3]. The resulting variability is substantial[5,6] and, although both adaptive and non-adaptive hypotheses of genome size evolution have been postulated[7–9], the evolutionary processes underlying this staggering variation remain ambiguous[10,11].

Ultimately, differences in genome size are the result of stochastic genetic or genomic processes tolerated by the organism, most prominently polyploidization[9,12], spontaneous deletions or insertions (particularly in introns[13–15]), increases in the length of tandem repeats[16] and the copy number of transposable elements (plants[17], vertebrates[18–21], arthropods[22–24]). However, the physical size of the genome has consequences for organismal fitness and may thus be subject to selection. For example, genome size correlates with cell and nucleus size[25,26], nutrient requirements[27], life cycle complexity[28] and basal metabolic[29], cell cycle[12], tissue differentiation[30] and developmental[31–33] rates. Biosynthesis models on body temperatures and cell replication have predicted that poikilotherms should experience a negative correlation between genome size and ambient temperature[34] and that cold geological periods would have tolerated poikilotherms with large genomes. However, robustly testing the causality of such relationships on a broad taxonomic scale has proven difficult in the past due to the inter-correlation of biological traits and their phylogenetic non-independence. Substantial recent developments in comparative phylogenetic methods permit us to re-visit this problem and here we provide a macroevolutionary perspective on genome sizes in amphibians based on a much-expanded dataset.

Amphibians constitute the class of vertebrates with the largest reported variation in genome size, ranging over at least two orders of magnitude from 0.95 pg (the frog *Platyplectrum ornatum*) to 140 pg (the salamander *Necturus lewisi*). Moreover, amphibians are ancient (originating in the Devonian[35]), distributed globally and exposed to a diversity of climatic conditions. Consequently they have evolved a multitude of ecological adaptations, and display the greatest diversity of reproductive modes of any major group of tetrapods, often involving the loss of entire life history stages[36]. Although salamander (Caudata) genomes have received considerable attention because of their exceptional size, Amphibia-wide studies have been hampered by the dearth of information on caecilian (Gymnophiona) genomes. The unusually large genomes of Caudata relative to other vertebrates have been attributed to a larger number of genes and longer introns within these[15], lower deletion[14] and substitution rates[37] and, most prominently, the accumulation of transposable elements[19], but whether these processes have occurred gradually and at homogeneous rates throughout amphibian history, or as heterogeneous saltations has never been tested. Here, we measure genome size in a broad sample of Gymnophiona and augment the sampling of Anura (frogs) and Caudata to produce the largest amphibian dataset to date. We reconstruct ancestral states, test for evolutionary rate heterogeneity and whether genomes have experienced saltations in their evolution.

Temperature and precipitation predict the non-random geographic distribution of amphibians with specific life cycles[36]. Moreover, ambient temperature is a strong determinant of the physiology of poikilotherms[35], influencing rates of cellular processes including cell replication, metabolism and development[38,39]. Because faster development has been associated with reduced genome sizes in invertebrates and amphibians[32,40], ambient temperature may be a key determinant for genome size evolution. The majority of amphibian species are dependent on water for reproduction and, because arid environments typically provide only ephemeral opportunities to complete the larval phase of the amphibian life cycle, we hypothesize that such habitats will select for faster development and smaller genomes.

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Not all amphibians have an aquatic larval phase, however, and species with evolutionarily derived reproductive modes such as direct development or viviparity forego free-living larval stages altogether. Differences in reproductive mode (hereafter: life history complexity) and especially the loss of metamorphosis of free-living larvae have been linked to differences in genome size, although trends have not been rigorously tested, nor is there a universal pattern for Amphibia (direct developing Anura are proposed to have smaller genomes than species with larvae whereas the opposite trend is seen in Caudata[28,41]). Here, we test the premise that species with life cycles involving metamorphosis have larger genomes than species without multiple distinct life history stages. Furthermore, we test whether climatic variables and life-history traits are correlated with genome size and, more specifically, whether life history traits that typically vary with life history complexity such as developmental period, egg size and body size impose an optimum on genome size or whether, conversely, genome size imposes optima on life-history traits.

Results

Genome size (C-value), life-history traits and climatic variables were mapped onto an amphibian phylogeny to reconstruct their evolutionary history. This study included 464 species of amphibians, sampling 50 of the 75 currently recognized families. We obtained de novo measurements of genome sizes for 71 species based on 138 individuals, including the largest known anuran, *Conraua goliath* (snout-vent length ~25 cm) and one of the smallest, *Sooglossus sechellensis* (no bigger than 2 cm), representatives of the only known extant viviparous anuran genera, *Nimbaphrynoides* and *Nectophrynoides*, and 21 of the 23 genera of Gymnophiona represented in the phylogeny covering all currently recognized families[42,43]. Based on these estimates, Gymnophiona have genomes ranging from 2.94 to 11.78 pg, overlapping almost entirely with Anura (0.95–11.50 pg) but not with Caudata (13.89–120.56 pg). In addition, we document a new record for the smallest known amphibian genome (in the anuran *Aromobates tokuko*, 0.8 pg, 18.25% coefficient of variation).

Genome size evolution. Two recently developed methods for fitting multiple Brownian motion and/or Ornstein–Uhlenbeck models of trait evolution were employed to detect and explicitly test heterogeneity in the evolutionary processes shaping genome size evolution across amphibians and to reconstruct ancestral states under the optimally performing model. The l1ou algorithm[44] uses a lasso approach to automatically detect heterogeneity in trait histories (Ornstein–Uhlenbeck-only) and recovered three branches in the amphibian phylogeny that are likely to have experienced shifts, with descendant clades evolving towards distinct evolutionary optima (Fig. 1). These shifts occur on the stem of Caudata (magnitude of shift coefficient $\beta = 1.769$), the stem of the caudatan genus *Desmognathus* ($\beta = -2.974$) and the terminal branch for the anuran species *Alytes muletensis* ($\beta = -14.804$). However, bootstrap support for the latter two shifts was low (47 and 50%; Fig. 1), and when testing this model against three a priori hypotheses (a model with a single set of parameters (H1), a model where the three amphibian orders evolve under distinct parameters (H2) and a model where Caudata deviates from the other two orders (H3)), it did not perform significantly better (Δ phylogenetic Bayesian information criterion (pBIC) < 2) than a model with only a single shift on the Caudata stem (H3; Table 1). The single shift model (H3) was therefore preferred.

The same three a priori hypotheses were tested again using the mvMORPH algorithm[45], this time under both Brownian motion and Ornstein–Uhlenbeck processes, and a log likelihood ratio test preferred heterogeneous models (H2 and H3) over models fitting only a single set of parameters to all amphibians (H1; Table 2).

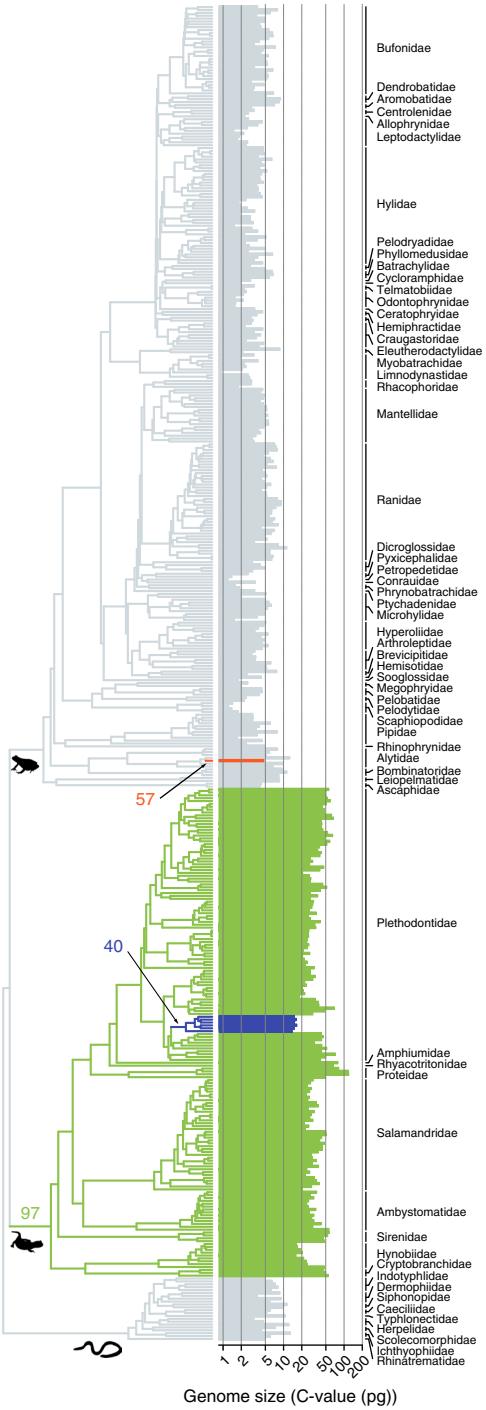

Fig. 1 | Amphibian phylogeny with clades painted to reflect shifts in Ornstein–Uhlenbeck parameters according to the best performing l1ou model for genome size evolution. Deviations from the background profile (grey) were recovered for Caudata (green), *Desmognathus* spp. (blue) and *Alytes muletensis* (orange). Branches where shifts occurred are labelled with bootstrap support for those shift placements. The adjacent bar chart depicts deviations of log-transformed genome sizes from the mean.

The best-supported model (Akaike weight (AW) = 0.803) was a Brownian motion model describing a scenario where genomes of Anura and Gymnophiona evolved under a common process from which Caudata deviated to evolve around its own set of parameters (H3; Table 2). However, the log likelihood ratio test for this two- (H3) versus a three- (H2) trait mean model did not differ

Table 1 | Comparison of single versus multiple Ornstein–Uhlenbeck optima model performance using l1ou.

	log likelihood	pBIC	ΔpBIC
Best l1ou model	296.850	−527.997	-
Singular model (H1)	268.635	−522.348	5.649
Three-order model (H2)	280.062	−512.012	15.984
Caudata model (H3)	279.445	−526.587	1.410

l1ou model support for genome size evolution comparing the best model proposed by l1ou to three hypotheses: a model with a single optimum (H1), a model where the three amphibian orders evolve towards distinct optima (H2) and a model where Caudata deviates from the other two orders with its own optimum (H3).

Table 2 | Comparison of performance of Brownian motion versus Ornstein–Uhlenbeck models using mvMORPH.

	AICc	ΔAICc	AW
Brownian motion			
Singular model (H1)	−530.222	37.232	0.000
Three-order model (H2)	−564.646	2.808	0.197
Caudata model (H3)	−567.454	-	0.803
Ornstein–Uhlenbeck			
Singular model (H1)	−528.193	39.261	0.000
Three-order model (H2)	−545.607	21.847	0.000
Caudata model (H3)	−546.708	20.746	0.000

mvMORPH model support for genome size evolution comparing three hypotheses: a model with a single set of parameters (H1), a model where the three amphibian orders evolve under distinct parameters (H2) and a model where Caudata deviates from the other two orders with its own set of parameters (H3). All the hypotheses were tested fitting a Brownian motion and an Ornstein–Uhlenbeck process and compared using corrected Akaike information criterion (AICc) and AW.

significantly (ratio = 1.289, P value = 0.525) and under these two models, the point estimates for the genome size of the ancestor of all amphibians are 4.62 pg (H3) and 5.95 pg (H2), and the estimates of the caudatan ancestor are 42.70 pg (H3) and 43.12 pg (H2; Fig. 2). Compared to simulated distributions of species' genome sizes under these two models, the empirical genomes of the caudatans *Necturus lewisi* (H2, H3) and *N. punctatus* (H2) are larger than expected and four out of the six *Desmognathus* spp. (*D. wrighti*, *D. quadramaculatus*, *D. ochrophaeus* and *D. fuscus*; H2, H3) as well as the anurans *Platyplectrum ornatum* (H2, H3) and *Petropedetes cameronensis* (H2) have smaller genomes than expected (95% confidence interval; Supplementary Information 1).

Rate dynamics. We tested whether the rate of genome evolution has been homogeneous over time and among orders, and whether the shifts in trait space detected for Caudata is the result of gradual change or saltational evolution. Rates of genome size evolution estimated using the Bayesian Analysis of Macroevolutionary Mixtures algorithm (BAMM[46]) indicated that genome size in Caudata has evolved more slowly than in Anura (Fig. 3a), with Gymnophiona showing intermediate, overlapping rates. The robustness of these rate estimates was confirmed using both the Maximum Likelihood and MCMC approach of the R package geiger v2.0.6[47], resulting in maximum likelihood rate estimates of $\beta = 5.337 \times 10^{-4}$ for Anura, $\beta = 2.548 \times 10^{-4}$ for Caudata and $\beta = 3.819 \times 10^{-4}$ for Gymnophiona (Fig. 3a). Analyses of rate changes through time using BAMM suggest that Caudata has experienced an overall decline in mean rates towards the present, whereas Anura and Gymnophiona show slight increases in rate over time with very similar trajectories (Fig. 3b).

BAMM models only estimate continuous changes in rate. To test whether genome size has experienced discrete evolutionary jumps ('Lévy processes') we used the levolution algorithm[48], which compares a null model of a single Brownian motion rate model to a model that extends the Brownian motion model with a superimposed independent Poisson jump process. The latter outperformed the null model (log likelihood ratio: 165.318, $P < 0.001$; ΔAIC: 161.318; α: 155.327; λ: 0.008; tree length: 20,207.7; Supplementary Information 2) and, under this model, the estimated genome size of the amphibian ancestor is 7.64 pg. At posterior probabilities > 0.99, levolution supported only a single jump on the stem leading to Caudata (see Supplementary Information 2 for jump posterior probabilities on all branches), but at a lower threshold (>0.9) additional smaller jumps were recovered; one on the branch leading to *Desmognathus* and nine more on terminal branches, all in Anura, for *Alytes muletensis*, *Xenopus ruwenzoriensis*, *X. vestitus*, *Dermatonotus muelleri*, *Petropedetes cameronensis*, *Lithobates grylio*, *Platyplectrum ornatum*, *Pseudophryne binronii* and *Rhinella marina*.

Life cycle complexity. Having multiple discrete life cycle stages (larval and adult stage) was previously suggested to be correlated with differences in genome size compared to amphibian species that have reduced this complexity, through evolutionary loss of either the larval stage (direct development or viviparity) or the adult stage (paedomorphosis;[28]). However, phylogenetic analysis of variance (ANOVA) supported no significant effect of life cycle complexity on genome size, regardless of how this complexity was coded (Fig. 2a: five-state coding, Anura: $F_{4,267} = 1.793$, $P = 0.526$; Caudata: $F_{3,166} = 3.940$, $P = 0.716$; Gymnophiona: $F_{2,19} = 0.371$, $P = 0.758$; Fig. 2b: larval stage present versus direct development, Anura: $F_{1,270} = 1.290$, $P = 0.556$; Caudata: $F_{1,168} = 10.038$, $P = 0.569$; Gymnophiona: $F_{1,20} = 0.347$, $P = 0.671$). Within Caudata, genome sizes of paedomorphic species are also not significantly different from those of non-paedomorphic species ($F_{1,168} = 1.012$, $P = 0.677$; Fig. 2c).

Life history and climate. Genome size has been hypothesized to be linked to evolution of life history traits and also directly or indirectly to extrinsic conditions. Using phylogenetic path analysis[49], we formally tested alternative scenarios of potential causal links among genome size with body size, egg size, duration of developmental period and temperature and seasonal duration of aquatic breeding habitats. Models with a life history/genome size relationship (model group A and B; Supplementary Information 9) substantially outweigh models without this relationship (model group C) for Anura (cumulative weights (CW) for model group A and B: 0.998), but much less so for Caudata (CW = 0.663; Supplementary Information 9). The subset of these models with a developmental period/genome size relationship were the dominant contributors, but interestingly, relative cumulative weights for models with directional effect of developmental period on genome size (Anura CW = 0.517; Caudata CW = 0.201) versus models with this effect in the opposite direction were comparable (Anura CW = 0.477; Caudata CW = 0.237; Supplementary Information 9) and thus a definitive directionality of this relationship cannot yet be established. Both orders show significant effects of temperature (negative) and aridity (positive; where high aridity index values correspond to high humidity) on the length of developmental period (models with this relationship for Anura, CW = 0.835 and Caudada, CW = 0.996). These relationships are also captured by a model averaging approach (Fig. 4) additionally highlighting a positive effect of body size on egg size in both orders and in Caudata, egg size has a significant positive effect on developmental period.

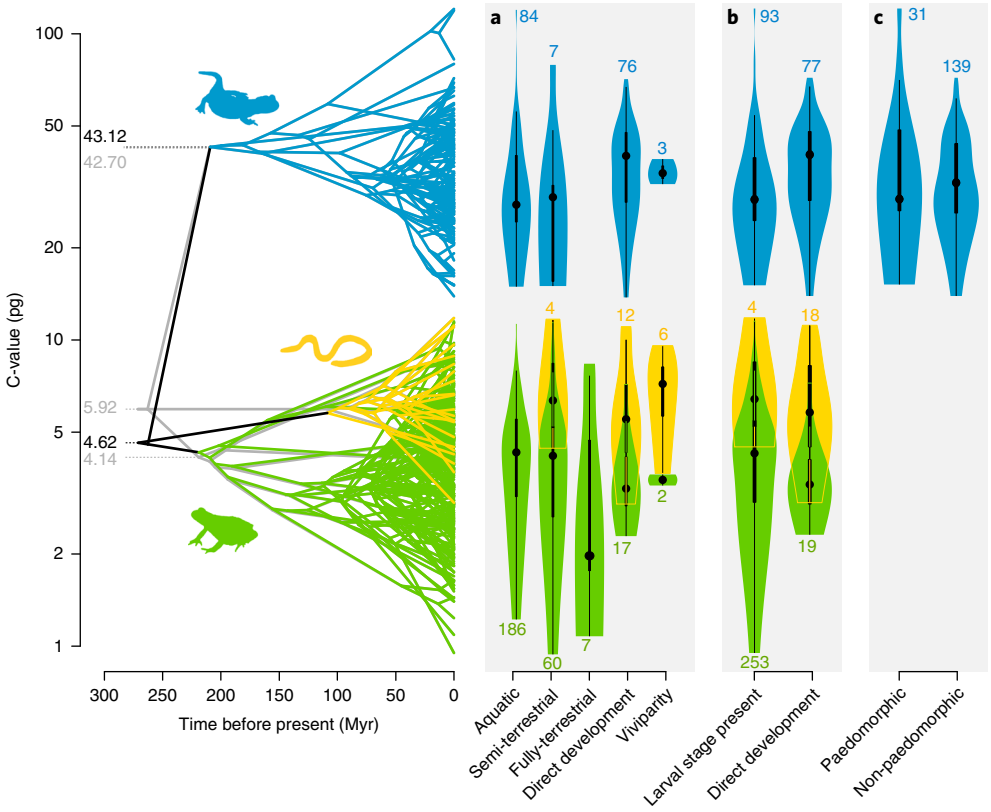

Fig. 2 | Phenogram showing ancestral state reconstructions for genome size evolution in amphibians under the two best performing mvMORPH models (two-trait mean Brownian motion in colour, three-trait mean Brownian motion underlain in grey). Values at nodes refer to genome sizes of the most recent common ancestor of the a priori specified clades for two- and three-trait mean models. **a**–**c**, Adjacent violin plots show genome size distributions for amphibians with different reproductive modes per order; Caudata (blue), Anura (green) and Gymnophiona (gold). Reproductive mode was categorized either into **a**, five or **b**, two categories and, exclusively for Caudata, **c**, whether species are paedomorphic or not. Points show medians; boxes the interquartile range; whiskers, 1× interquartile range and violin widths reflect kernel densities. Numbers over or under the violin shapes indicate sample sizes in each group.

Discussion

Genome size evolution in amphibians. By providing a macroevolutionary perspective on genome size evolution in extant amphibians for the first time across all three orders, we contribute several important insights into amphibian evolution, and more widely to the ongoing debate on the genome size enigma. Evolutionary model-fitting suggests that anuran and gymnophionan genomes have evolved under a shared Brownian motion processes, with a distinct process shaping the evolution of Caudata genomes. Under such a two-state model, the common ancestor of extant amphibians is predicted to have had a genome more similar in size to those of extant Gymnophiona and Anura, and considerably smaller than those of extant Caudata. We infer that genome size has evolved gradually as a function of time (Brownian motion) in amphibians, with only one strongly supported instance of punctuated change, on the Caudata stem (but see discussion on other possible jumps below). This saltational event was so profound that it has resulted in the largest genome size divergence in any group of vertebrates and, despite over 200 million years of largely gradual evolution, genomes of extant Caudata do not overlap in size with those of other extant amphibians.

Indirect evidence from the size of fossilized cells[50] and inferences from genome sizes of the oldest extant amphibian lineages[51] have predicted that the size of the genome of the last common ancestor of extant amphibians was in the upper range of extant Anura and substantially smaller than extant Caudata. Here we provide strong support for this using multiple comparative phylogenetic methods and a much-expanded taxon sampling, with our estimates falling between 4.62 and 7.57 pg. We find no evidence for selective directionality (Ornstein–Uhlenbeck processes) at an Amphibia-wide scale and with relatively stable evolutionary rate profiles over time in each of the amphibian orders. We show that Caudata has experienced overall lower rates of genome size evolution than other living amphibians, and that their genome gigantism occurred early and in a saltational manner (Lévy process). Palaeontological data have suggested a similar pattern[52] and genomic evidence also suggests that a single punctuated proliferation of a group of Long Terminal Repeat retrotransposons[18,53] could have caused the enlargement of caudatan genomes.

Although we conclude that the evolution of genomes in amphibians otherwise proceeded gradually, some lineages are consistently identified as outliers to a universal Brownian motion process, although with less statistical support. The levolution algorithm singled out a number of additional jump locations, some of which coincide with lineages also highlighted by the mvMORPH (*Desmognathus* spp., *Pedropedetes cameronensis*, *Platyplectrum ornatum*) and l1ou (*Desmognathus* spp., *A. muletensis*) analyses. Most consistently, species of the caudatan genus *Desmognathus* deviate from all tested models and have smaller genomes than expected under Brownian motion evolution. This lineage has received attention in previous genome size studies for having re-evolved a larval stage from a direct developing ancestor[28], although based on the present study, we argue that this need not be the causal link. Other potential jump positions and model outliers were generally confined to terminal branches and were more sensitive to taxon sampling and C-value estimates (Supplementary Information 12).

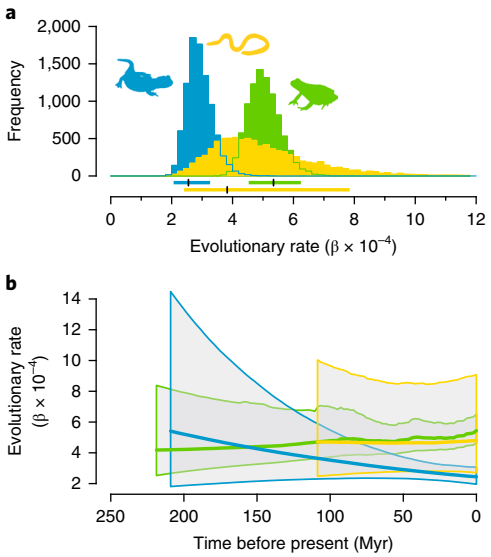

Fig. 3 | Rate estimates for genome size evolution for the three amphibian orders showing posterior distributions as estimated by BAMM showing mean rates per clade and through time. **a**, Mean rates per clade. **b**, Mean rates through time (with shading reflecting 95% confidence interval). In **a**, the horizontal range bars below the mean clade rates represent the 95% confidence interval of rates estimated using an MCMC approach in geiger with vertical black bars showing the maximum likelihood estimates. Colours correspond to Caudata (blue), Anura (green) and Gymnophiona (gold).

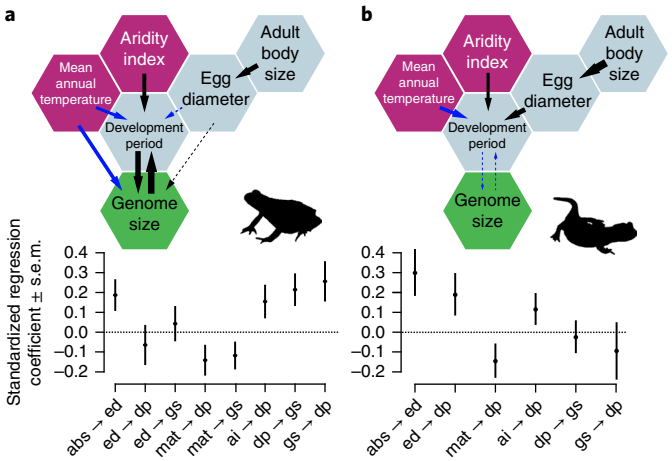

Fig. 4 | Best-scoring phylogenetic path analysis models. **a**, Anura. **b**, Caudata. Arrow colour and width represent direction and size of regression coefficient (black: positive; blue: negative) with full lines signifying coefficients significantly deviating from 0. Panels below show standardized regression coefficients with standard errors for the paths in the above model schematic. Abbreviations on *x* axis refer to variables in the schematic above.

To assess their validity, improved sampling at the species level may be required.

Genome size has been reported to correlate with life-history traits[20,35] and, in the case of amphibians, also with life history complexity[28,31], although notably with opposite trends in Anura and Caudata[28]. Here, we find no significant correlation between genome size and life history complexity in any of the three orders and conclude that neither the evolution of direct development and loss of a free larval stage, nor paedomorphosis alone can explain genome size variations. Our larger dataset and phylogenetic statistical testing therefore contradict previous claims that the presence or absence of metamorphosis is linked to differences in genome size[28,41].

Cell size and cell replication rate are directly influenced by genome size and, as a consequence, organisms with larger genomes are predicted to have longer developmental periods[7,31,54]. For example, spadefoot toads (Pelobatoidea) include rapid developing species such as *Scaphiopus* spp. with much smaller genomes than more slowly developing *Pelobates* spp., possibly as a consequence of selection for faster cell replication rates[55]. Despite a number of studies showing a relationship between the duration of embryonic period and genome size in amphibians[31,54], establishing a causal link and directionality between developmental time and genome size has previously been confounded by other intercorrelated variables and by unaccounted-for shared evolutionary history among species. Here we find that in Anura and Caudata, strong correlations exist between environment and life history, whereby both warmer temperatures and higher aridity (a proxy for shorter reproductive periods) have significant effects on reducing developmental time. In Anura, genome size is strongly linked to developmental period so that species with shorter developmental periods have reduced genome sizes, with model weights slightly favoring a scenario where natural selection is acting to limit or reduce genome size in environments where faster embryonic and larval development occur. The directionality of this relationship is not unequivocal, however. Despite previous studies reporting a positive relationship between embryonic period and genome size in specific caudatan taxa[31,54], the relationship between genome size and life-history traits is less supported in this order, with the best path analysis model excluding this relationship entirely and models without it accounting for a third of the total cumulative weights (as opposed to less than 1% in Anura). We suggest that these contrasting results may be due to the previous use of embryonic period as a non-fixed developmental point across biphasic and direct developing species (see Methods section) or may be a matter of scale, given that our dataset encompasses a greater variety of species in terms of life history and ecological diversity. We have previously observed similar erosion of apparently significant patterns for other life-history traits such as body size and egg size when expanding studies to the level of whole orders[36].

Genome size evolution may also be influenced by non-adaptive processes, a topic of ongoing debate[4,10,11,56] and not directly addressed here. An increased mutation rate leads to increased risk of deleterious effects that are removed through purifying selection. Therefore, a lower mutation rate (that is, lower mutational hazard[4]) or reducing the effect of purifying selection relative to drift through reduced effective population sizes could result in larger genomes[3]. A comparison of six Caudata and six Anura found no differences in effective population size[37], but Caudata do, however, have lower mutation rates than Anura[37,57]. Trends within orders in relation to genome sizes have so far not been explored and our own preliminary investigation into correlations between genome size and substitution rates for each of the three amphibian orders suggests that only in Anura is there a significant relationship, but contrary to expectation, genome size is positively correlated with mutation rates (Supplementary Information 11). More robust analyses are required to test the importance of mutation rates and genetic drift on amphibian genome sizes, as well as the possible effects of life history on effective population size[58].

Implications for the macroevolution of genomes. There has been debate as to whether the striking variation in genome size across the tree of life is a manifestation of the accumulation of small, gradual changes over time[59] or the result of punctuated evolutionary events[60,61]. We propose that both processes contribute, but that punctuated events are rare, although they may have a profound impact upon the overall pattern of genome size evolution when

they do occur. We speculate that the Brownian-motion-like pattern of evolution is likely to be the result of an interplay of molecular processes, predominantly changes in content of transposable elements[9], that produce traces of gradual, random changes and that rare, macro-genomic processes such as polyploidization or duplication events[62,63] or even massive alterations of activity of transposable elements[64] can result in saltational genome evolution.

The presence or absence of a larval stage is not correlated with a species' genome size for any of the three amphibian orders, but there is some evidence that life history and genome size evolution are correlated, especially in anurans, where selection can act indirectly on genome size when reducing premetamorphic developmental period.

Methods

Data compilation. The time-calibrated molecular phylogeny of Pyron[65] was used for studying the evolution of genome sizes in amphibians. Genome size estimates (picograms of DNA in haploid nuclei; C-value) were compiled for all amphibian species listed in the genome size database (www.genomesize.com) that were also represented in the phylogeny (Supplementary Information 3 and 4). Taxonomy followed Frost[66] and naming was standardized across datasets using the R package AmphiNom v1.0.0 (available from: https://github.com/hcliedtke/AmphiNom), and all synonyms were collapsed/pruned from the tree. Genome sizes for subspecies were pooled at the species level and median values were used if more than one measurement per species was available. This genome size database dataset ($n=396$ species) was augmented with new measurements ($n=68$ species; Supplementary Information 3 and 4) detailed in the section below. With the intention of producing the largest possible dataset, measurements taken using different methods and from different cell types were combined, which is known to introduce possible error[67]. To assure that pooling data in this manner did not significantly affect the conclusions of this study, parallel analyses on a subset of genome size estimates (320 species) derived using the same technique and cell type were performed (Supplementary Information 12).

Life-history data were collected from online databases and literature sources (Supplementary Information 5). To test predicted genome size correlation with life cycle complexity[28], life cycles were discretized based on whether species are (1) fully aquatic (eggs and larvae develop in water), (2) semi-terrestrial (eggs laid on land but larvae develop in water), (3) terrestrial with larvae (eggs and larvae develop outside of water), (4) terrestrial without larvae (direct development) or (5) viviparous. Caudata were additionally scored as being paedomorphic (facultative or obligate) or not. Data were collected from the same sources on continuous traits previously hypothesized[30,31,54] to correlate with genome size, namely body size (snout-vent length of adult females), egg diameter and duration of development up to metamorphosis (median hatching time plus median larval period or only hatching time, in direct developing species). We used time-to-metamorphosis (or emergence of a juvenile form) as a standardized time period that is comparable across almost all species, with the caveat that larval periods are ecologically very sensitive and investment into growth and development is decoupled.

Climate preference profiles per species were generated by randomly sampling geospatial coordinates within the species ranges defined by the IUCN red list (http://www.iucnredlist.org/) distribution polygons. These points were then projected onto the Annual Mean Temperature BioClim climatic layer (BIO1; http://www.worldclim.org) as well as the Aridity Index product from CGIAR-CSI (http://www.cgiar-csi.org/), where low index values refer to arid areas and high values to less arid areas. Annual Mean Temperature may not directly reflect temperatures experienced by amphibians during their developmental period, but is intended to provide a relative measure across species found in vastly different climates. We acknowledge this as a caveat, although this measure performed more consistently than other BioClim temperature products in preliminary tests. Sample sizes for the random generation of sampling locations ranged from 10 to 100, relative to the ranked species distribution area (log areas calculated from Mollweide equal area projected IUCN range polygons). All geospatial processing was done in R using the packages sp v1.2-5[68], raster v2.5-8[69] and maptools v0.9-2[70], and median values were used as per-species values in all subsequent analyses (S5).

Genome size determination. New empirical genome size estimates were made using Feulgen image analysis densitometry on stained cells from ethanol-preserved tissues (liver, leg muscle or tail) or dried blood smears. Sampling focused primarily on Gymnophiona to obtain data for representative species per genus present in the phylogeny ($n=21$ of 23 genera represented; suitable tissue for *Typhlonectes natans* and *Epicrionops* was not available). Additionally, selected anuran ($n=45$) and caudatan ($n=2$) species were included to achieve greater phylogenetic coverage and to increase sample sizes for underrepresented reproductive modes (for example, viviparity $n=2$ and direct development, $n=5$). In total, genomes of 138 individuals for 71 species were newly measured (Supplementary Information 7), 68 of which are represented in the phylogeny. Tissues were provided by the natural history museums of Madrid (MNCN), London (NHM) and Berlin (MfN) and the Museum of Vertebrate Zoology at Berkeley (MVZ). The laboratory procedure was based on Hardie et al.[67] and Jeffery and Gregory[71]. The full protocol is provided as Supplementary Information 6. In brief, cells were mechanically disaggregated, fixed on a microscope slide,and their nuclei stained with Schiff reagent. Stained DNA in nuclei was photographed under a Zeiss Axio Imager A1 microscope fitted with an Axiocam ERc 5 s (Carl Zeiss AG, Jena, Germany) and image optical densities of the nuclei were then estimated using the Fiji suite of imageJ[72]. A standard curve of image optical densities against genome size in picograms of seven species with known genome sizes was plotted (*Drosophila melanogaster*, *Epidalea calamita*, *Passer domesticus*, *Salamandra salamandra*, *Xenopus laevis*, *Danio rerio*, *Ichthyosaura alpestris*; linear regression $y=435821x$; adj-$R^2=0.991$, $P<0.001$; S6), from which all new genome size estimates were made. Wherever possible, three individuals per species were included to allow the calculation of mean genome sizes (full list of specimens: Supplementary Information 7).

Genome size evolution. For all analyses genome sizes were log transformed. To detect past deviations from the expected mean genome size over time and across lineages, a lasso approach implemented in l1ou[44] was used to model Ornstein–Uhlenbeck processes across the phylogeny using a random root model and pBIC model selection criterion. Additionally, three hypothetical Ornstein–Uhlenbeck models were tested; a model with a single set of parameters for all amphibians (H1), a model where the three amphibian orders evolve under distinct parameters (H2) and a model where Caudata deviates from the other two orders with its own set of parameters (H3).

The same three models were also tested in mvMORPH[45], fitting Ornstein–Uhlenbeck and Brownian motion models. Model comparison was based on size-corrected AICc and AW as well as likelihood ratio tests in cases where models had the same number of parameters. Point estimates of ancestral states at each node of the phylogeny were estimated under the best-fitting model(s) and visualized as a phenogram using phytools v0.6-20[73]. Additionally, genome size evolution was simulated 10,000 times under the best-fitting model(s) to test if any species deviate from the expected distribution of traits under the given model (that is, falling outside the 5% tails of the simulated distribution).

Rate dynamics. The Bayesian Analysis of Macroevolutionary Mixtures software (BAMM[46]) in combination with the R package BAMMtools v2.1.6[74] was used to investigate temporal rate dynamics and topological rate heterogeneities of genome sizes in amphibians. BAMM does not model jump-processes and so analyses were performed on each order separately to avoid biasing the analyses by the large deviation in genome size in Caudata. For each order, BAMM was allowed to sample every 5,000th generation of 50 million MCMC iterations, and priors were configured using the setBAMMprior function in BAMMtools. Two chains were run to check convergence of parameters, and chain diagnostics (effective sample sizes and autocorrelation) were inspected using the coda package v0.16-1[75]. Using the post-burnin dataset (discarding 10% of the chain), mean overall clade rates and mean rates through time (based on 100 slices) of genome size evolution were calculated. To evaluate the robustness of this result, we repeated the calculations of evolutionary rate under Brownian motion per amphibian order, using both a maximum likelihood and a MCMC approach with the R package geiger v2.0.6 (MCMC chain was run for 1 million generations sampling every 1,000th generation).

To test whether genome size has experienced 'evolutionary jumps' (compound or Lévy processes) and to detect the location of potential jumps, we compared model fit of a single Brownian motion process model against a model that extends the Brownian motion model with a superimposed independent Poissonian jump process, implemented in the program levolution[48]. Levolution was set to expect ten jumps (but different parameters were also tested; Supplementary Information 2) and at first the grid search option was used to find the optimal jump strength parameter α (α range and sampling frequency: 1–200/50; vectors: 5,000 burnin: 1,000; thinning: 2). The final analysis was run with α set to the optimum proposed by the grid search (α: 155.3; tree height: 20,207.7), with MCMC settings adjusted to 20,000 sampled jump configurations, with a burnin of 2,000 and thinning of 2, 1,000 maximum Expected Maximization iterations (with a stop threshold of 50) and slightly extended MCMC searches for the jump location inference (vectors: 55,000 burnin: 5,000; thinning: 5).

Life cycle complexity. A phylogenetic ANOVA with 1,000 simulations, coupled with a Holm post-hoc test in phytools, was used to test whether genome size is significantly different between species with different life cycles. Analyses were performed for each order separately, and performed once using the five-state coding outlined above and again using a derived, binary coding to distinguish between larval stage present versus no larval stage present (that is, direct development or viviparity without larva) and for Caudata exclusively, whether species were paedomorphic or not.

Life history and climate. Using the hypothesis-driven framework of PPA[49] implemented in the R package phylopath v1.0.0[76], we tested for direct and indirect effects of life-history traits and climate on genome size evolution while accounting

for phylogenetic non-independence of species. The variables included in the models were: *Environment*, mean annual temperature and aridity index; *Life history*, median female body size, median egg diameter and median developmental time and *Genome size*, median C-value. A total of 20 models with different configurations of these variables were compared (Supplementary Information 8) using the C-statistic Information Criterion (CICc) corrected for small sample sizes to evaluate the models. Models were designed so as to test directionality of effects, that is, whether selection on life-history traits imposes restrictions on genome size (model group A; Supplementary Information 8 and 9), whether genome size imposes restrictions on life-history traits (model group B), or whether there is no relationship between genome size and life-history traits (model group C). We also tested if and how environmental factors such as temperature and aridity affect both life history and genome size evolution. We primarily used cumulative model weights to discuss the importance of variables and directionality of effects, but in order to graphically display results, we calculated the average of the best performing models ($\Delta CICc < 2$), averaging path coefficients only over models where the path exists. The analyses were carried out for each amphibian order separately, including only species for which full datasets were available (118 species of Anura and 83 of Caudata). Gymnophiona had to be excluded entirely due to a dearth of the requisite life history data[77].

The phylogenetic path analysis did not include life history complexity due to the difficulty of adding categorical variables to the directed acyclic graph models and because there was no significant effect recovered using the phylogenetic ANOVA (see results). However, to complement our analysis, we performed a series of phylogenetic generalized least squares models that also include life history complexity as a categorical variable. The results largely conformed to the PPA analysis and are therefore only discussed in Supplementary Information 10.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All genome size measurements generated for this study are available in the supplementary information.

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Author contributions

H.C.L., I.G.-M., D.J.G. and M.W. were all involved in planning and executing the project. D.J.G. and M.W. contributed key samples and H.C.L. obtained de novo genome size estimates and performed data analyses with input from I.G.-M. H.C.L. wrote the first version of the manuscript, to which all other authors thereafter contributed.

Competing interests

The authors declare no competing financial interests.

Additional information

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Research sample	The research samples are C-value estimates of genome size for extant amphibians. The study is based on an existing dataset (www.genomesize.com) that was augmented with our own measurements. Life history information was primarily collected from www.amphibiaweb.org unless otherwise stated.
Sampling strategy	Due to the nature of the study, sampling strategy was limited by availability of data. Emphasis was placed on obtaining a taxonomically representative dataset.
Data collection	Data was collected through literature and online searches in addition to laboratory preparations of samples. All data was recorded by HCL.
Timing and spatial scale	Not relevant.
Data exclusions	No data was excluded from the analysis.
Reproducibility	Not relevant as there was no experimental component.
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Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	No laboratory animals were involved in this study.
Wild animals	No wild animals were involved in this study. Samples used in this study stem from museum collections.
Field-collected samples	Not relevant.