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Breakdown of brain–body allometry and the encephalization of birds and mammals

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The allometric relationship between brain and body size among vertebrates is often considered a manifestation of evolutionary constraints. However, birds and mammals have undergone remarkable encephalization, in which brain size has increased without corresponding changes in body size. Here, we explore the hypothesis that a reduction of phenotypic integration between brain and body size has facilitated encephalization in birds and mammals. Using a large dataset comprising 20,213 specimens across 4,587 species of jawed vertebrates, we show that the among-species (evolutionary) brain–body allometries are remarkably constant, both across vertebrate classes and across taxonomic levels. Birds and mammals, however, are exceptional in that their within-species (static) allometries are shallower and more variable than in other vertebrates. These patterns are consistent with the idea that birds and mammals have reduced allometric constraints that are otherwise ubiquitous across jawed vertebrates. Further exploration of ontogenetic allometries in selected taxa of birds, fishes and mammals reveals that birds and mammals have extended the period of fetal brain growth compared to fishes. Based on these findings, we propose that avian and mammalian encephalization has been contingent on increased variability in brain growth patterns.

Understanding the role of constraints in adaptive evolution is a long-standing challenge in evolutionary biology[1–3]. The allometric relationship between brain size and body size has been a particularly common example in debates about the causes and consequences of evolutionary constraints[4–11]. Brain–body allometry can be described by a power law, whereby brain size = a(body size)^b. This law is usually expressed in logarithmic form as the following standard linear allometric equation: log(brain size) = log(a) + $b$log(body size), where a is a scalar (intercept) and b is the allometric exponent (slope)[7]. Depending on the level of comparison, brain–body allometry reflects variation among developmental stages (ontogenetic allometry), among adult individuals within a species (static allometry) and among species (evolutionary allometry). These three types of allometry can be causally related, such that static allometry is determined by ontogenetic allometry, and evolutionary allometry is determined by ontogenetic and static allometry[12,13]. Limited evolvability in ontogenetic and static allometry can therefore generate evolutionary constraints.

For over a century, it has been recognized that evolutionary brain–body allometry explains a large fraction of brain size variation across vertebrates[14,15]. This pattern is typically explained by physiological scaling[4,16,17] and developmental constraints[6,7]. However, birds and mammals have evolved a substantially larger brain for a given body size, or larger relative brain size (that is, encephalization), compared to other vertebrates[4,5]. Despite prolific research to understand their unrivalled encephalization and intelligence[18–20], it remains unclear how birds and mammals have undergone such evolutionary changes in brain size under allometric constraints that are considered to be universal across vertebrates. Theoretical arguments[8–10] suggest that a decoupling of phenotypic integration between brain and body size, which is expressed by a reduction in static allometric exponents, can mitigate allometric constraints and potentiate encephalization. The variability of evolutionary allometric exponents in carnivores[21] and increased among-species variances of relative brain size in cetaceans and primates[22–24] suggest a decoupling in the relationship between brain and body size in large-brained lineages. However, an adequate test for the decoupling hypothesis requires a comparison of static allometries across a wide range of taxa. Here, we compile the most extensive brain- and body-mass dataset to date, consisting of 20,213 observations of adult individuals from 4,587 species across jawed vertebrates, to test the decoupling hypothesis for avian and mammalian encephalization. Using phylogenetic comparative methods, we compare the pattern of brain–body evolutionary allometry across major vertebrate forms and ask how macroevolutionary patterns are related to static allometries. We further aim to elucidate the potential underlying mechanisms of the decoupling process by comparing ontogenetic brain–body allometries across taxa.

Results

We estimated evolutionary allometries by fitting $\log_{10}$–$\log_{10}$ regressions of brain mass against body mass using phylogenetic generalized least squares (PGLS) models, whereby the residual variance is modelled according to Brownian motion[25] with phylogenetic heritability (λ)[26]. As illustrated in Fig. 1, mammals and birds occupy a section of morphospace well above ray-finned fishes, reptiles and

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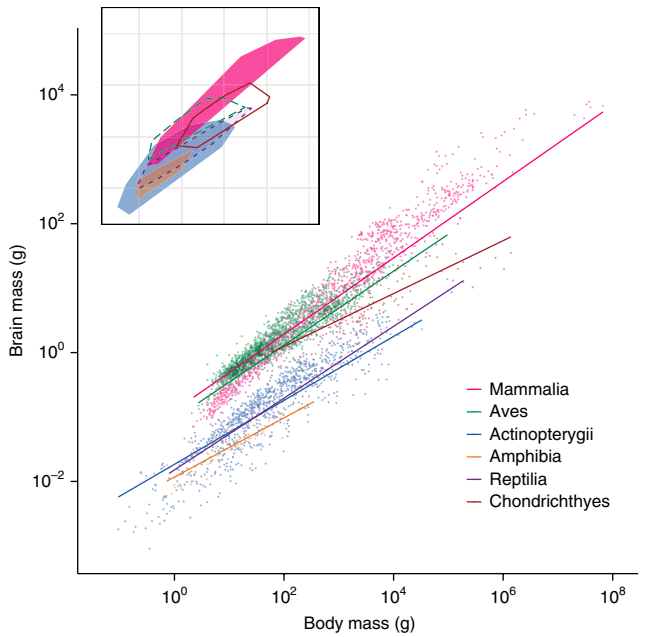

Fig. 1 | Brain–body evolutionary allometry of six vertebrate classes. Class-level brain–body allometries of six major vertebrate lineages are shown in different colours (*x* and *y* axes are in $\log_{10}$ scales). Points represent species means and unbroken lines are least square regressions accounting for phylogenetic relatedness among species. The inset shows minimum convex polygons of the morphospace occupied by Actinopterygii ($N=963$), Amphibia ($N=86$), Aves ($N=1902$), Chondrichthyes ($N=147$), Mammalia ($N=1409$) and non-avian reptiles (Reptilia, $N=79$).

amphibians. Cartilaginous fishes share some morphospace with birds and mammals, but the overlap is confined to small-bodied species. Despite these obvious shifts in relative brain size, the evolutionary allometric scaling relationships are broadly similar across all classes, with allometric exponents varying between 0.41 and 0.59. Moreover, 66–92% of among-species variance in brain mass is explained by body mass within each class, with the following phylogenetic regression slope ± s.e.m. values: Actinopterygii, 0.50 ± 0.01, $r^2=84\%$; Amphibia, 0.46 ± 0.03, $r^2=88\%$; Aves, 0.57 ± 0.01, $r^2=88\%$; Chondrichthyes, 0.41 ± 0.02, $r^2=66\%$; Mammalia, 0.59 ± 0.01, $r^2=89\%$; and non-avian reptiles, 0.56 ± 0.02, $r^2=92\%$ (Supplementary Table 1).

To assess whether the allometric relationships apparent at the high taxonomic level reflect patterns at lower levels, we examined how brain–body allometry varies at different taxonomic levels. For each class, evolutionary allometries were estimated across four taxonomic levels (that is, Class, Order, Family and Genus) using the PGLS approach, provided at least six species were available (see Methods). We estimated the static (within-species) brain–body allometric exponent (that is, the slope) for species with at least ten individuals, controlling for sex and method of measuring brain size. Figure 2 shows that the tight and steep evolutionary allometries at the class level are largely maintained throughout taxonomic levels in all groups, but birds and mammals show a dramatic drop in allometric exponents at the static level. This is in stark contrast to amphibians, cartilaginous fishes, ray-finned fishes and reptiles, for which static and evolutionary allometries at the class level are remarkably similar (Fig. 2b). We also investigated the pattern via analyses based on the node age of phylogenies and confirmed that phylogenetic age differences among similar taxonomic levels across classes do not affect our results (Supplementary Fig. 1).

The differences in static slopes between classes could be artefacts of a more narrow age and size range in lineages generally exhibiting determinate growth (birds and mammals) compared to lineages generally exhibiting indeterminate growth (amphibians, cartilaginous fishes, reptiles and teleost fishes). This could lead to a stronger attenuation of estimated allometric slopes in birds and mammals due to measurement error[27–29]. To evaluate this effect, we computed the reliability ratio[27,28], which revealed that the variance in body mass due to measurement errors would have to be 37% in birds and 47% in mammals in order to explain the difference between static slopes and evolutionary slopes by measurement error alone (Supplementary Table 2). We regard such levels of measurement variance as implausible. To further verify that steeper static slopes in fishes are not due to wider size ranges, we reared guppies (*Poecilia reticulata*) in the laboratory and measured brain and body mass of male individuals that were between 111 and 119 days old. This strictly age-controlled data revealed a brain–body static allometric exponent of $b=0.39\pm0.03$ (Supplementary Fig. 2), which is substantially steeper than the averages of birds (0.14 ± 0.02) and mammals (0.13 ± 0.01), but similar to the average of fishes (0.44 ± 0.02). Overall, the breakdown of static brain–body allometry in birds and mammals is unlikely to be an artefact of the differences in adult body size range among classes.

To identify if and how the decoupling of brain–body phenotypic integration may have contributed to encephalization, we considered two aspects of intraspecific variation in relative brain size: the conditional variance of brain size relative to body size[12,30] and the difference between the static and the evolutionary allometric slope of the clade that the species belongs to (Δ_{slope}). The Δ_{slope} measures how well static allometry is aligned with evolutionary allometry and reflects the strength of narrow-sense allometric constraints[12]. The conditional variance is a measure of variance in brain size independent of body size[12]. If the genetic variance–covariance structure (G-matrix) is patterned similarly to the phenotypic variance–covariance structure (P-matrix), this measurement represents the conditional evolvability[30] of brain size. Available data of the G-matrix for the brain–body size relationship have shown high correspondence between correlation structures at the genetic and phenotypic levels[10] (mouse, 82%; rat, 85%). In addition, the genetic correlation between adult brain and body size is much higher in fishes[31] ($r=0.89$) compared to humans[9] ($r=0.64$), mice[10] ($r=0.38$) and rats[10] ($r=0.15$), which globally matches our results based on the P-matrix. The heritability of brain size depends on the studied species, sex, populations and method of heritability estimation. For example, consider the following estimations: 82% in baboon[32], 45–48% in guppy[33], 66–97% in human[34], 60–70% in mouse[10], 60–75 % in rhesus macaque[35], 32% in three-spined stickleback[31] and 49% in zebra finch[36]. These estimates can all be considered high relative to the median heritability of 28% for previously reported cubic (that is, mass or volume) size measurements[37]. Thus, there is some phenotypic variation that is not explained by genetic variation (that is, P-matrix and G-matrix are dissimilar in matrix size), but phenotypic and genetic correlations may be very similar (that is, P-matrix and G-matrix have a similar matrix orientation).

Decoupling can facilitate evolution in relative brain size either by increasing the conditional variance of brain size or by shifting the static slope so that brain size can evolve in new directions along the static slope (Fig. 3). We investigated the role of these two evolutionary scenarios using a phylogenetic comparative method based on an Ornstein–Uhlenbeck (OU) model[38] that accounts for measurement error in both predictor and response variables. We fitted the OU model to the residual variances of the brain–body evolutionary allometry (that is, "the constraint model"[39]) to 52 clades of birds, mammals and teleost fishes (Supplementary Fig. 3). The stationary variances of these models (Supplementary Table 3) represent the expected amount of variation in relative brain size across species of

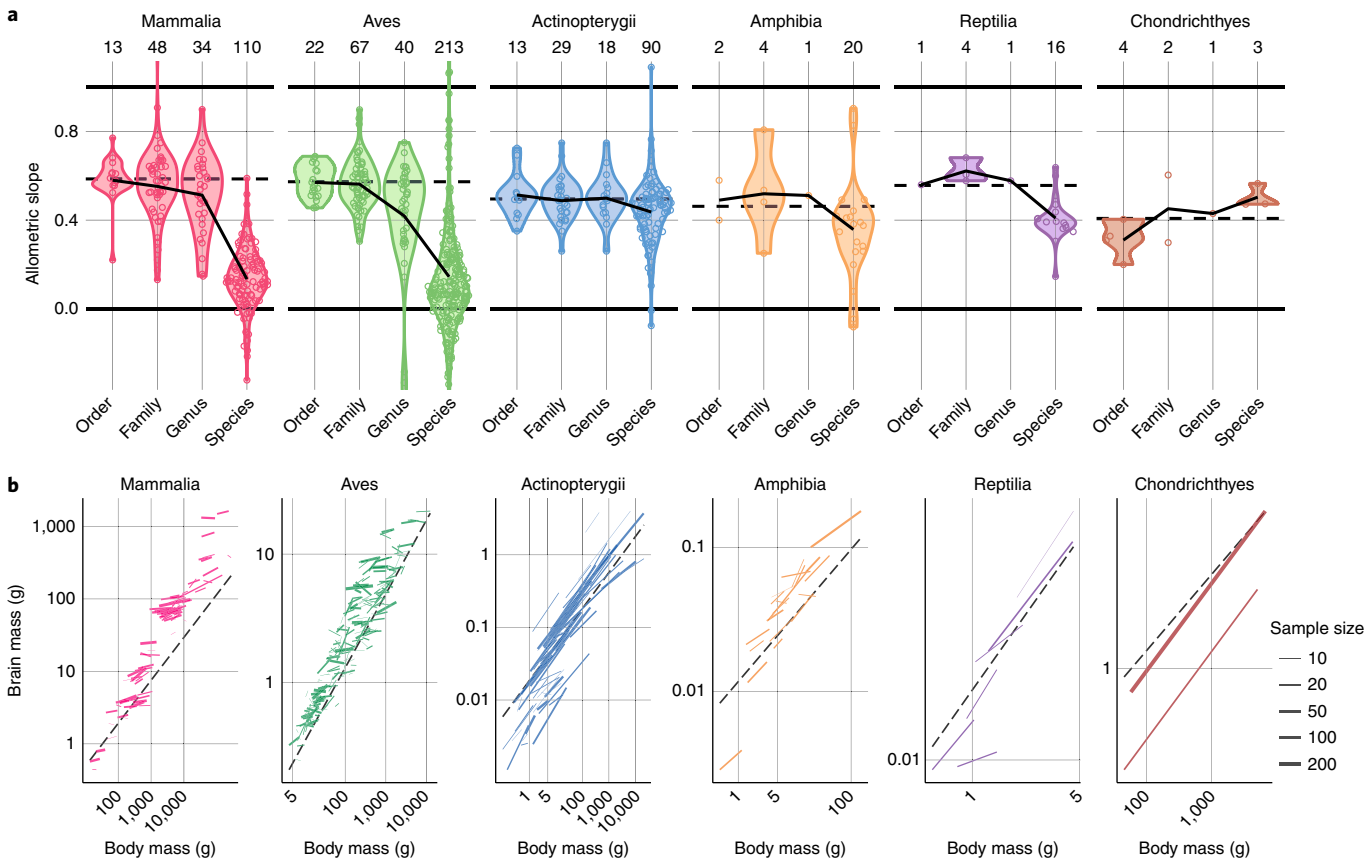

Fig. 2 | Allometric exponents at different taxonomic levels across vertebrate classes. a, Evolutionary allometric exponents at the class level (broken horizontal lines) decay at the static (within-species) levels in mammals and birds. In contrast, in ray-finned fishes, amphibians, reptiles and cartilaginous fishes, exponents remain similar throughout taxonomic levels. Numbers of clades or species at each taxonomic rank are shown above each panel. Violin plots represent the distributions of allometric exponents. Overlaid points are observations (clades or species) and unbroken lines connect the mean exponents. Values of no allometry (0) and isometry (1) are marked with unbroken horizontal lines. **b**, Plots show the static allometries (unbroken lines) and evolutionary allometries at the class level (broken lines) in six major vertebrate forms. Body mass (*x* axis) and brain mass (*y* axis) are shown in $\log_{10}$ scales. In mammals and birds, static allometries are shallow and unrelated to evolutionary allometries at the class level, but static and evolutionary allometries are parallel to each other in other lineages. The thickness of the unbroken lines indicates the sample size used to estimate static allometries (number of adult individuals within species, median = 15, range = 10–251).

each clade when they evolve under the inferred OU process for an infinite time period[38]. We then related the stationary variances with either the conditional variance of brain size or the absolute value of Δ_{slope} ($|\Delta_{slope}|$), which are the weighted averages over available parameters within each clade. Under a strict form of allometric constraints, evolutionary changes are bounded to follow trajectories imposed by static allometries[11,12]. If this is the principal mode of macroevolution in relative brain size (Fig. 3d), the stationary variance is predicted to be positively associated with $|\Delta_{slope}|$. Alternatively, across-species divergence in relative brain size can arise from the evolution of mean brain size relative to body size (that is, the intercept of static allometry). Under this scenario (Fig. 3b), we predict a positive association between the conditional variance and the stationary variance.

Figure 4 shows the stationary variance plotted against the averages of within-species conditional variance and $|\Delta_{slope}|$ of the clade. For birds and mammals, a positive relationship between stationary variance and conditional variance of brain size is revealed (Fig. 4a), meaning that relative brain size diverges more in clades that are represented by species with large intraspecific variance in relative brain size. There is, however, no relationship between stationary variance and $|\Delta_{slope}|$ (Fig. 4b). These results indicate that the variability of static allometric intercepts, but not the variability of slopes, constrains divergence of relative brain size across species in birds and mammals. The opposite pattern appears in teleost fishes, with no relationship between stationary variance and the conditional variance, but a positive relationship between stationary variance and $|\Delta_{slope}|$ (Supplementary Table 4). Hence, in teleost fishes, there has been more evolution of relative brain size in clades for which the static slope deviates more from the evolutionary slope. These patterns are consistent with the idea that static allometry acts as a constraint on brain-size evolution in fishes[11], as well as with the idea[8,22,23] that this constraint has become relaxed in birds and mammals. As illustrated in Fig. 4c, this reduction is not reflected in the amount of conditional variance, but the static slope of birds and mammals is approximately twice as variable as that in fishes (Fig. 4d, Supplementary Table 5). Hence, the determinant of the strength of allometric constraints may be the variability of static brain–body allometric slopes.

One remaining challenge to address is understanding how static allometry has become more evolvable in birds and mammals relative to teleost fishes. Variation in static allometric slopes is determined by variation in the parameters of ontogenetic allometries[12,13]. Thus, to identify potential underlying mechanisms that generate the variation in evolvability of static slopes, we collected published data of brain and body mass from fetal to adult life stages in eight species of birds, teleost fishes and mammals and compared their

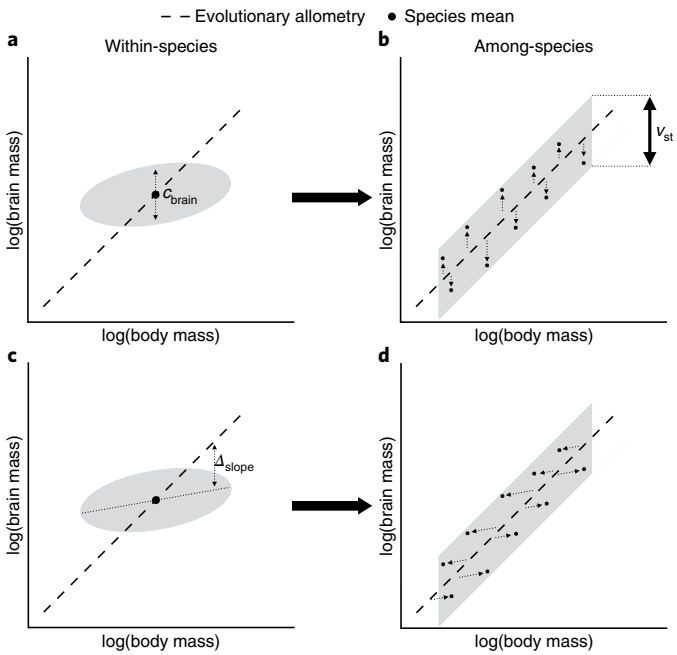

Fig. 3 | Schematic illustration of two possible relationships between within- and among-species variation. a, The ability of relative brain size to respond to directional selection on brain size is described by the conditional variance of brain size (c_{brain}, double-headed arrow) estimated from within-species variance–covariance structure (grey ellipse)**. b**, One possible scenario is that the residual variance of brain–body among-species allometry at evolutionary equilibrium (v_{st}, width of grey column) evolves as a result of changes in brain size without body size evolution (broken arrows). Evolutionary potential of this change is estimated by c_{brain}. **c**, Alternatively, evolution of relative brain size can be modelled as evolution in brain size along the static allometry. In this case, the difference between static and evolutionary allometry (Δ_{slope}, double-headed arrow) measures the degree to which brain size can evolve in new directions along the static slope. **d**, This aspect of variance generates divergence in relative brain size (v_{st}) along static allometric slopes (broken arrows). Therefore, how this evolutionary trajectory results in divergence of relative brain size depends on Δ_{slope}.

ontogenetic allometries (Fig. 5). This analysis showed that all species exhibited a biphasic developmental trajectory, characterized by steep slopes during the initial stage (rapid-growth phase) followed by more shallow slopes (slow-growth phase) that corresponded well to the static allometry of the species. The ontogenetic allometric exponents during the rapid-growth phase of red seabream (slope ± s.e.m. value of 1.02 ± 0.08) and common carp (0.83 ± 0.02) are similar to those of chicken (0.89 ± 0.08) and the mammalian average (0.88 ± 0.07), indicating a striking conservatism in the pattern of early brain growth across Gnathostomata (Supplementary Table 6). The brain growth trajectory of fish species differs from birds and mammals by exhibiting steeper slopes at the slow-growth phase and smaller body sizes at cessation of the rapid-growth phase (Fig. 5b). We further revealed that the exponent of the slow-growth phase and body mass at the breakpoint are tightly linked (r^2 = 88.7%). Therefore, the increased evolvability of static allometry in birds and mammals could have its mechanistic basis in a more variable rapid-growth phase.

Discussion

Our study supports the idea that birds and mammals have relaxed allometric constraints that are otherwise ubiquitous across Gnathostomata. Furthermore, our results suggest that this reduction of constraint has been achieved through an increased variability of the rapid brain growth phase in birds and mammals. Such an ontogenetic shift needs to coincide with corresponding adjustments in energy turnover to fuel the high cost of brain growth[40–42], and we propose that parental care[41–43] acts as a possible mediator of the increased evolvability of ontogenetic processes. The variational independence of brain size from body size may have laid the foundation for the exceptional encephalization of birds and mammals.

The taxon-level effect of brain–body allometry. Previously, the exponent (slope) of brain–body size evolutionary allometry was thought to depend on the taxonomic level at which it is estimated (the taxon-level effect[44,45]). Typically, slopes fitted to the genus level were between 0.2 and 0.4, but if slopes are fitted to higher taxonomic levels, they ranged up to ~0.75[6,44]. Employing a formal phylogenetic comparative analysis, we found that the taxon-level effect is absent, with the possible exception of a decrease at the genus level in birds (Fig. 2; Supplementary Table 1). However, even the avian genus average (0.42) is larger than the hypothetical upper margin at the genus level (0.4). In addition, according to the reliability ratio of 0.938 (Supplementary Table 2), the average slope of birds at the genus level would be ~0.448 if measurement errors in body size were controlled for. Furthermore, the same analyses based on the node age of phylogenies reveal that many bird genera are only 1 million years old, which is substantially younger than the youngest clades in other vertebrate classes (Supplementary Fig. 1). This suggests that the slight decrease of evolutionary allometric slopes in birds at the genus level is likely to reflect a taxonomic bias, whereby closely related bird populations are more frequently classified as species than other vertebrate classes. Therefore, by accounting for phylogenetic relatedness[26,46], measurement errors[27–29] and taxonomic bias, our pan-Gnathostomata analysis provides little support for the taxon-level effect in brain–body evolutionary allometric scaling.

The mechanistic basis of brain–body size scaling. Given the reduction of static allometries in birds and mammals, it is puzzling why the evolutionary allometric exponents are still so high and consistent in these lineages (Fig. 2). Hypotheses to explain brain–body evolutionary allometric exponents fall into two broad categories: those based on physiological scaling and those based on developmental constraints. The two most popular physiological scaling hypotheses predict an exponent of 0.67 (that is 2/3) based on surface-to-volume ratio[4,14] or an exponent of 0.75 (that is 3/4) based on maternal basal metabolic rate (Kleiber's law[16,17]). The exponents of evolutionary brain–body allometry in birds and mammals in our analyses are generally much lower than is predicted by either of these two hypotheses (0.5–0.6; Supplementary Table 1). Therefore, the physiological scaling laws are unlikely to explain the constancy of evolutionary brain–body allometric exponents in birds and mammals. An alternative explanation is that the developmental mechanisms are hard to evolve; therefore, evolutionary allometries follow trajectories of static and ontogenetic allometries[6,7,11–13]. Even when the phenotypic covariance between brain and body size at the adult stage is low (that is, static allometry is shallow), we showed that the allometric exponents can change during ontogeny (Fig. 5). Selection on early growth periods will therefore generate high evolutionary allometric exponents regardless of the brain–body covariance structure at later growth periods[10]. Moreover, the striking similarity of early-stage ontogenetic allometric exponents among birds, teleost fishes and mammals (Supplementary Table 6) indicates that some aspects of early-life development, such as the rate of fetal brain growth[47,48], may have limited evolvability. Although our study cannot preclude the possibility that functional adaptations rather than constraints produced the evolutionary allometric exponents of 0.5–0.6, we are unaware of any explanations that consistently generate slopes in this range[39]. Therefore, available evidence

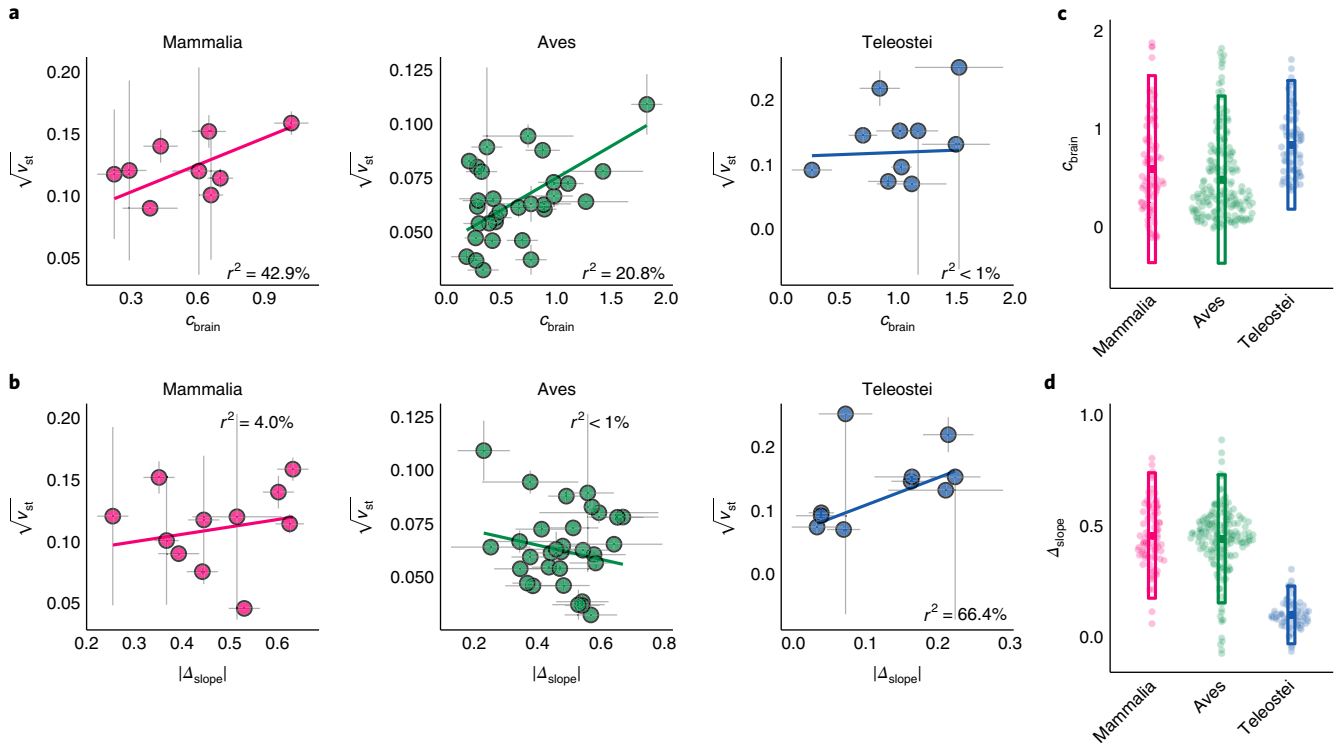

Fig. 4 | Among- and within-species variation in relative brain size. a, Among-species variation in log relative brain size ($\sqrt{v_{st}}$), measured as the stationary variance of the fitted OU process of the selected clade, is regressed against the conditional variance averaged over species within the clade for mammals (slope ± s.e.m. of 7.23 ± 3.43), birds (3.23 ± 0.43) and teleost fishes (0.68 ± 4.25). **b**, The same regression against the average absolute difference between evolutionary and static allometry ($|\Delta_{slope}|$) for mammals (0.06 ± 0.01), birds (−0.03 ± 0.005) and teleost fishes (0.43 ± 0.10). Error bars show standard errors of c_{brain} and $|\Delta_{slope}|$ along the horizontal axes, and approximate 95% confidence intervals of the stationary variance along the vertical axes (see Methods). **c**, Comparison of conditional variances among birds, mammals and teleost fishes. There is no evidence that mammals and birds have larger conditional variances than fishes (mean ± s.e.m. of 0.563 ± 0.075 for birds, 0.595 ± 0.041 for mammals, and 0.924 ± 0.083 for teleost fishes). **d**, Comparison of Δ_{slope} among birds, mammals and teleost fishes. The Δ_{slope} of birds and mammals are more variable than those of fishes (predicted standard deviation in Δ_{slope} at the tip of phylogeny of 0.149 for birds, 0.141 for mammals and 0.072 for teleost fishes). Bars indicate the average conditional variance and the Δ_{slope} measured as the predicted central state ('optimum') for an OU model of evolution fitted to each variable, and ±2 s.d. according to the predicted tip variance of the fitted OU processes. Details of estimated parameters are shown in Supplementary Table 5.

supports the developmental constraints hypothesis[2,7,49] as a tentative explanation for the cause of brain–body allometric relationships at higher taxonomic levels.

Variational constraints at a macroevolutionary timescale. We found that the macroevolution of relative brain size is predictable from patterns of phenotypic variation within species (Fig. 4). In a recent survey[50], the authors concluded that there is abundant evidence for a relationship between patterns of intraspecific variation and among-species divergence despite conceptual and methodological difficulties[30]. Thus, variation at the within-species level may often generate constraints for trait evolution at the macroevolutionary timescale[51–54]. The underlying assumption of this argument is that quantitative genetic parameters such as additive genetic and mutational variance–covariance structures remain stable over a long stretch of time[8,53,54]. In the present study, we identified a relationship between intraspecific variation and among-species divergence across clades that diverged for 103.8 (birds), 96.1 (mammals) and 219.3 (teleost fishes) millions of years (Supplementary Table 5). The stability of phenotypic variance structure for such a long time might be considered untenable, because the evolution of G-matrices[55–57] and the decoupling of P- and G-matrices[58] would be possible at this timescale. However, if these issues played a major role, they should have obscured the relationship between variability and divergence. In addition, a recent demonstration of the tight match between mutational variance, genetic variance and the rate of evolution in wing morphology of the family Drosophilidae[59] indicates that the variational structure could remain stable for tens of millions of years. One explanation for these patterns is that there is a hyperstable niche in the macroevolutionary adaptive landscape[54,60] on which mutational, genetic, phenotypic and environmental variance–covariance structures all adapt to generate a similar pattern. It is possible that early phases of rapid brain growth have limited evolvability and may accordingly be a determinant of macroevolutionary stability. The breakdown of this limitation may have enabled birds and mammals to explore novel regions of the morphospace and associated ecological niches.

Conclusion

The hypothesis that brain size evolves through changes in developmental mechanisms[61–63] is a widely appreciated view in evolutionary neurobiology[5]. Although this idea relies on the existence of developmental constraints[2,7,49], the view of development as an evolutionary constraint has recently been superseded by a more dynamic view whereby development is considered evolvable and structured to facilitate adaptations[41,42,56]. Our study highlights that the evolvability of developmental mechanisms is better viewed as a property that requires interrogation rather than being taken for granted. Why the developmental machinery can evolve more easily in some lineages compared to others is an important remaining question to address

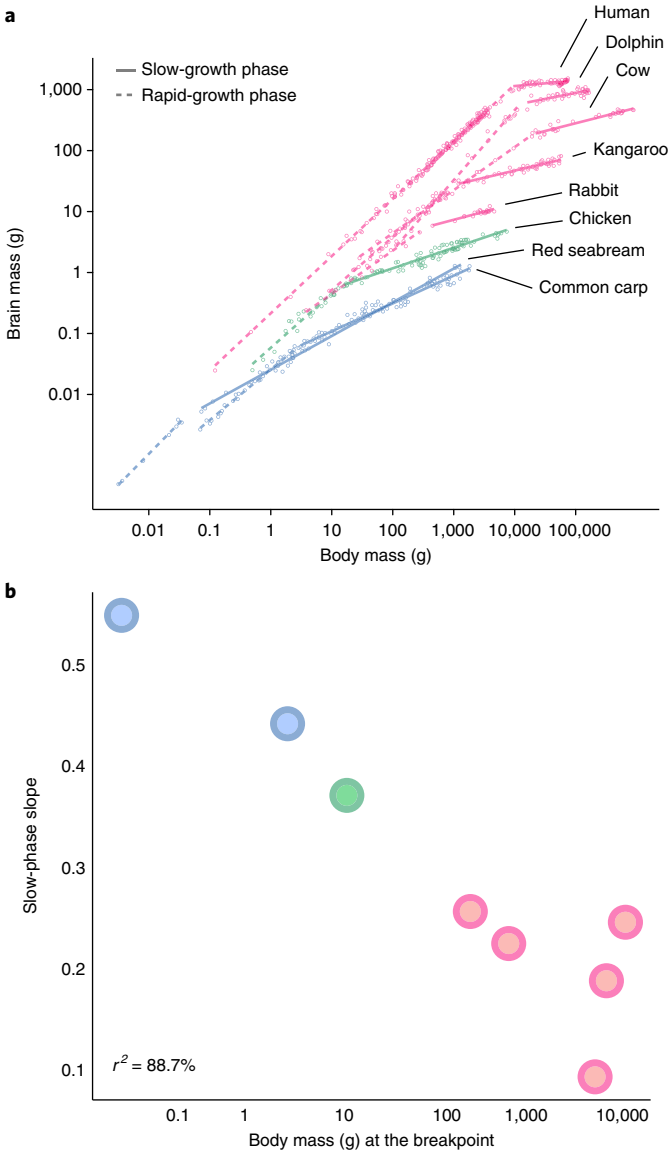

Fig. 5 | Ontogenetic brain–body allometry of eight vertebrate species. **a**, Allometric growth from fetal to adult stage reveal step-wise changes in allometric slopes through ontogeny in human (*H. sapiens*), striped dolphin (*S. coeruleoalba*), cow (*B. taurus*), eastern grey kangaroo (*M. giganteus*), European rabbit (*O. cuniculus*), chicken (*G. gallus*), red seabream (*P. major*) and common carp (*C. carpio*). Colours indicate the class as follows: magenta, mammals; green, birds; and blue, fishes. Unbroken lines and broken lines show the ontogenetic allometric exponents during the slow-growth phase and the rapid-growth phase, respectively. The axes are in $\log_{10}$ scales. **b**, The relationship between body mass at the breakpoint between rapid- and slow-growth phases and the allometric slope during the slow-growth phase (Supplementary Table 6). Colours are as stated for **a**.

for our understanding of vertebrate brain size evolution and morphological diversity.

Methods

Data assembly and screening. Our dataset is a compilation of published (51%) and unpublished (49%) data of body mass (g), brain mass (g) or brain volume (ml). The unpublished data were obtained from two independent sources, one of which was collected by J.E. (JE data) and the other by A.N.I. (ANI data). The JE data were collected from individual birds that were either hunted or died of natural causes in Denmark over the past three decades. Measurements of brain mass and body mass were made by one person (J.E.) using a digital scale to the nearest milligram after dissection. The ANI data are composed of specimens from the following museums: the Australian National Wildlife Collection, the Museum of Victoria, the South Australian Museum, the Queensland Museum, the Australian Museum, the Field Museum of Natural History, the Bernice P. Bishop Museum, the Royal Alberta Museum, the Louisiana State University Museum of Zoology, and the National Museum of Natural History. Brain volumes were estimated by filling the endocranial cavity with lead shot as described previously[64]. All endocranial volume measurements for ANI data were made by one person (A.N.I.). The method has been shown to be reliable in estimating brain mass in birds[64]. Body mass data were also recorded for all museum specimens, when available on specimen tags. The rest of our data were collected from published sources, and our references include an online database of fishes[65], three datasets across vertebrates[66–68], two datasets of primates[69,70], a curated compilation of published data across mammals[22], and primary research (see specific references in datafiles in the figshare repository https://doi.org/10.6084/m9.figshare.6803276). We screened all data to meet the following criteria: (1) measurements are from sexually mature adult individuals; (2) data were obtained from its original source; and (3) brain data were reported with body mass collected for the same individual(s). We included the data from previous publications[71,72] even though they combined original brain volume data with body mass data from published sources and therefore violated our screening criteria (3). This was because the large sample size (10–15 specimens per sex-species category) used to estimate mean brain volumes in these studies was deemed to provide a sufficiently reliable estimate of species- and sex-specific values. We recorded sample size and standard deviations whenever possible. When sample size was not reported, we made the most conservative estimate. This means that we assigned the sample size as $N=1$ when no information was available and as the minimum possible sample size when the range of sample size was reported for a given dataset. Data of brain size in birds and mammals were reported either in volume (ml) or mass (g), while the rest of our brain size data were reported in mass. We converted volume to mass using the known density of brain tissue of 1.036 g ml^{-1} that has been shown to hold in mammals[73] and birds[74]. These procedures resulted in 20,213 matching observations of brain mass and body mass based on measurements of 31,007 adult individuals across 4,587 species of vertebrates.

Phylogenetic framework. All analyses were performed in R[75] v.3.4.0. We based our comparative analyses on the phylogenetic trees obtained from published sources[76–81]. Phylogenies of mammals[77], reptiles[81] and ray-finned fishes[78] were used unchanged from original trees. For the bird phylogeny, we sampled 1,000 "Ericson" backbone phylogenies containing 9,993 species from http://www.birdtree.org[76] and retained the maximum clade credibility tree using TreeAnnotator[82] v.2.4.5. Phylogenies of Amphibia[79] and Chondrichthyes[80] were time calibrated with fossil constraints[83] using penalized likelihood in the program r8s[84] v.1.81. We imposed five fossil constraints for Amphibia and one fossil constraint for Chondrichthyes (Supplementary Table 1). We estimated the best smoothing parameter to be 10 (Amphibia) and 18 (Chondrichthyes) using cross-validation, and used these parameters to calibrate phylogenies. Taxonomic identities of both phylogeny and data were matched to the most updated taxonomic names according to the Integrated Taxonomic Information System (http://www.itis.gov, accessed 14 May 2017) using the taxize[85] package v.0.8.4.

Estimating static allometric slopes. To reliably estimate within-species (static) brain–body allometry, the dataset was further screened as follows. We first centred group means with respect to sex (as male, female or unrecorded for both brain and body mass) and measurement method (either mass or volume only for brain mass) to the global species means to account for group-specific mean differences. We then estimated static allometry separately for all species with at least 10 individuals ($N=10,359$ observations for 439 species) using ordinary least squares (OLS) of $\log_{10}$ brain mass against $\log_{10}$ body mass. Outliers were removed based on the model residual (squared residual ≥0.04) and cook's D[86] (cook's $D>1$). This operation was repeated until no further observations were removed. In total, 139 observations (1.34% of the original subset) were removed as outliers and 178 observations were removed because the sample size became <10 after outlier removal. The retained subset ($N=10,042$ observations for 418 species) was then used to estimate our static allometric slopes. Finally, we added 25 OLS static allometric slopes reported in the literature (Supplementary Table 2). Overall, we obtained static allometric slopes for 443 species.

Estimating evolutionary allometric slopes. We estimated the brain–body allometric slope across species (evolutionary allometry) using the full dataset without the 139 outliers identified in the first step of the previous section ($N=20,074$ individuals for 4,586 species) with two alternative approaches. First, we estimated evolutionary allometry at four taxonomic levels (Class, Order, Family and Genus) using PGLS, whereby the residual variance was modelled according to Brownian motion[25] with phylogenetic heritability (λ)[26]. Phylogenetic heritability, also known as Pagel's λ[46], is an estimate of the degree to which the phenotypic values of related taxa are explained by their phylogenetic relatedness. Regressions were performed on sex-pooled species means of $\log_{10}$ brain mass against $\log_{10}$ body mass, which were both weighted averaged by the sample size of each observation,

using nlme[87] and geiger[88] packages. This was done for all taxonomic units between Class and Genus represented by at least six species. Phylogenies were cropped each time to match the included species. The phylogenetic heritability for each dataset was estimated by maximum likelihood. Second, we split lineages at each internal node of the phylogeny and fitted PGLS to all subsets with at least six species descended from the focal node using the same approach as described above.

Summarizing the brain–body allometry through time. To investigate the variation in allometric exponents across different temporal scales[44,45], we estimated the averages of exponents at each taxonomic level and compared these across five taxonomic levels (Class, Order, Family, Genus and within-species). To account for heterogeneity in taxon age across classes, we plotted the evolutionary allometric exponent at each node against node age (that is, the age of the most recent common ancestor).

Assessing static allometry in the guppy. To determine the influence of extended age and size range on static allometry in lineages exhibiting indeterminate growth compared to lineages exhibiting determinate growth, we assessed a strictly age-controlled static allometry in the guppy *P. reticulata*. Animals were reared as described previously[35] and brain mass (mg) and standard length (mm) were measured for 218 male individuals that were between 111 and 119 days old (Supplementary Table 3). The experiment was performed in accordance with ethical applications approved by the Stockholm Ethical Board (reference number: N173/13, 223/15 and N8/17). These applications are consistent with the Institutional Animal Care and Use Committee guidelines. Standard length was converted to mass using the following equation: $\text{mass}_{(\text{mg})} = 0.0283 \times \text{standard length}_{(\text{mm})}^{2.899}$. This equation for conversion was derived from data of 210 adult male guppies of the same laboratory population. The static allometric slope was assessed with OLS regression of $\log_{10}$ brain mass against $\log_{10}$ body mass.

Measuring the potential for bias in allometric slope due to measurement error. A potential source of bias in the allometric slope is the measurement error in body size[27–29]. We examined the effect of measurement error using the reliability ratio (k)[27,28]. At among-species levels, the observations are species means and error variance corresponds to the estimation variance of these means. Hence, k is the ratio of variance in mean log body mass across species belonging to a given clade ($\text{var}_{\text{observed}}$) over the total variance. That is, the sum of $\text{var}_{\text{observed}}$ and a weighted average of intraspecific variance in log body mass for all species of the focal clade represented by at least ten individuals. Accordingly, we calculated k at each taxonomic unit from Class to Genus levels. At the within-species (static) level, observations are of individual specimens and error variance corresponds to the variance over repeated body-size measurements of the same specimen. Since this repeatability is unknown in our data, we retrospectively examined how much error would be necessary if the observed static slopes are generated purely from measurement error. We calculated the error variance necessary to explain the observed static slopes from empirical k of static slope over evolutionary allometric slope of a clade with similar body form and ecological niches (see subsequent sections for details) and the intraspecific variance in log body mass estimated from species within the clade.

Describing within-species variance in relative brain size. We described the within-species variation of relative brain size in two ways that correspond to two different hypotheses for how within- and among-species variation are related (Fig. 3). The first statistic is the conditional variance of brain size (c_{brain}), which is the residual variance of static allometry computed on the natural log scale[12]. The second is the difference in the static allometric slope and the evolutionary allometric slope estimated at the proximate taxonomic level using the SLOUCH method (Δ_{slope}, see the next section for details of estimating evolutionary allometry). Under the assumption that phenotypic variation is structured similarly to genetic variation[10,31–35], the conditional variance is a measure of evolvability in brain size when body size is under stabilizing selection. The Δ_{slope} measures how well evolutionary changes along the static allometry corresponds to the actual evolutionary allometry, and is a measure of the strength of allometric constraints[12].

Estimation of among-species divergence in relative brain size. We employed the OU model of evolution[89] to parameterize among-species divergence in relative brain size. OU models can describe processes of brain-size evolution under allometric constraints[39] as the following stochastic differential equation:

$$\mathrm{d}y = -\alpha(y-\theta)\mathrm{d}t + b\mathrm{d}x + \sigma\mathrm{d}W$$

In this model, $\mathrm{d}y$ is the change in log brain mass over an infinitesimal time step $\mathrm{d}t$. The parameter θ determines the intercept of evolutionary allometry and α describes the rate of pull towards the evolutionary allometry. The parameter b describes the exponent of evolutionary allometry that scales the change in log brain mass with the change in log body mass, $\mathrm{d}x$, that follows an independent white-noise process. The term $\sigma\mathrm{d}W$ describes a white noise with independent, normally distributed random changes with mean zero and variance σ^2. Our focal parameter is the stationary variance ($v_{\text{st}} = \sigma^2/2\alpha$), which describes the expected amount of divergence in residual variance of the fitted brain–body evolutionary allometry (that is, relative brain size) when traits evolve for a long time under a constant allometric constraint[38]. It is therefore important that our estimates of v_{st} are made within groups of species with similar brain–body scaling and selective regime. To achieve this, we selected 52 clades between Family and Order levels that are represented by species of similar body form and ecological niches (Supplementary Fig. 3). Due to the constraint of sample size, sufficient data were available only for birds (Class Aves), mammals (Class Mammalia) and teleost fishes (Infraclass Teleostei). We fitted the stochastic linear OU model[38] to each of these 52 clades with phylogenies cropped to match the included taxa, and estimated parameters (α, σ^2) with a maximum-likelihood algorithm implemented in the package SLOUCH[38] v.2.0.0 (the source code is presented in the Supplementary Code file). We incorporated measurement errors in both predictor ($\log_{10}$ body mass) and response ($\log_{10}$ brain mass) variables by including the square of standard errors as an estimate of sampling variance of the mean. When a standard error was not available, we estimated the error as the weighted average of available standard errors of species at the closest taxonomic rank[39]. The estimated evolutionary allometry was then used to evaluate Δ_{slope}. Our OU-based approach can be viewed as a general model of phylogenetic regression that includes the commonly used Brownian motion as a special case: as α approaches zero, the OU model converges to a linear model with a Brownian motion residual variance structure. Thus, when small α is favoured, an OU stationary phase that describes v_{st} is not reached. To account for this, we calculated the expected trait variance at the tip of phylogeny (at $t=1$) as $v_{\text{st}} \times (1\text{-}e^{-2\alpha t})$. This asymptotes at v_{st} when t (the total length of phylogeny) exceeds several phylogenetic half-lives[89] ($\ln 2/\alpha$), meaning that the OU process is approximately stationary at the tip of phylogeny. This contrasts with the case when phylogenetic half-lives are longer than the root to tip length (that is, α is small), in which case it gives the expected variance at present ($t=1$) under Brownian motion[90], while actual variances increases with time following the Brownian motion process ($\sigma^2 t$). Note that the recently debated issue over the inability to statistically distinguish OU and Brownian motion models[91] is not a concern for our study because of the following reasons: we did not perform model selection comparing OU and Brownian motion models; we controlled for measurement error in brain and body mass; and we based our argument on model parameters (that is, phylogenetic half-life and stationary variance).

Assessing the link between across- and within-species variances. For each of the 52 clades for which we estimated the stationary variance and evolutionary allometry, we calculated corresponding c_{brain} and absolute difference between static and evolutionary allometry ($|\Delta_{\text{slope}}|$) as weighted averages over available parameters for species within the clade. Using SLOUCH, we fitted linear models with the square-root of stationary variance ($\sqrt{v_{\text{st}}}$) as a response variable and c_{brain} or $|\Delta_{\text{slope}}|$ as predictor variables separately for birds, mammals and teleost fishes. Stationary variances were square-root transformed ($\sqrt{v_{\text{st}}}$) to provide isometric scaling with evolvability[50]. We entered the root age of each clade as a fixed covariate. Measurement errors were included as squared standard errors of c_{brain} and $|\Delta_{\text{slope}}|$, and the approximate 95% confidence interval of v_{st} estimated from the maximum likelihood (ML) support range calculated as follows (Supplementary Table 3): $((\sqrt{v_{\text{st}}}$ at the upper ML support region $- \sqrt{v_{\text{st}}}$ at the lower ML support region$)^2/8)$. In these analyses, we used a non-phylogenetic setting (that is, the phylogenetic half-life was set to zero) because the sample size in each dataset was too small to meaningfully estimate phylogenetic structures in the model residuals. For validation, we also ran a model in which the phylogenetic half-life and the stationary variance are estimated; the outcome confirmed that phylogeny did not influence our results (Supplementary Table 7).

Evaluation of phylogenetic mean of c_{brain} and variance of Δ_{slope}. In order to assess the phylogenetic central state of c_{brain} and variance of Δ_{slope}, we fitted a single-optimum OU model using SLOUCH. For each parameter, we fitted the model with corresponding phylogenies to estimate the central state (θ) and the stationary variance ($\sigma^2/2\alpha$) of inferred OU processes. For descriptive purposes, we also assessed the mean and variance in the static slope itself. In fitting OU to the Δ_{slope} and the static slope, we included the measurement error as square of standard errors of static slopes. The stationary variance was translated into the expected amount of variance at the tip as described earlier, and evaluated as the variance per 100 million years based on the age of the most-recent common ancestor for each dataset.

Comparison of ontogenetic brain–body allometries across species. To explore ontogenetic brain–body allometry, we assembled additional datasets of brain and body mass from fetal to adult stages in human (*Homo sapiens*), striped dolphin (*Stenella coeruleoalba*), cow (*Bos taurus*), eastern grey kangaroo (*Macropus giganteus*), European rabbit (*Oryctolagus cuniculus*), chicken (*Gallus gallus*), red seabream (*Pagrus major*) and common carp (*Cyprinus carpio*) from published sources[48,92–94] (Supplementary Table 3). We modelled the ontogenetic series with two log-linear regressions and one breakpoint between these. The breakpoint and regression parameters before and after the breakpoint were determined by maximum likelihood using a segmented regression method[95] implemented in the

segmented package. The body mass at the breakpoint and the ontogenetic slope before and after the breakpoint was then compared across species.

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

Code availability. The computer code to run SLOUCH (v.2.0.0) is provided in the Supplementary Code file.

Data availability. The data that support the findings of this study are available in the Supplementary Information, Supplementary Data files and in the figshare repository[96] (data of brain mass, brain volume, body mass and phylogeny): https://doi.org/10.6084/m9.figshare.6803276.

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References

1. Futuyma, D. J. Evolutionary constraint and ecological consequences. *Evolution* **64**, 1865–1884 (2010).
2. Gould, S. J. *The Structure of Evolutionary Theory* (Harvard Univ. Press, Cambridge, 2002).
3. Amundson, R. *The Changing Role of the Embryo in Evolutionary Thought: Roots of Evo-Devo* (Cambridge Univ. Press, Cambridge, 2005).
4. Jerison, H. J. *Evolution of the Brain and Intelligence* (Academic Press, New York, 1973).
5. Striedter, G. F. *Principles of Brain Evolution* (Sinauer Associates, Sunderland, 2005).
6. Gould, S. J. Allometry in primates, with emphasis on scaling and the evolution of the brain. *Contrib. Primatol.* **5**, 244–292 (1975).
7. Huxley, J. S. *Problems of Relative Growth* (Methuen & Co., London, 1932).
8. Lande, R. Quantitative genetic-analysis of multivariate evolution, applied to brain–body size allometry. *Evolution* **33**, 402–416 (1979).
9. Grabowski, M. Bigger brains led to bigger bodies?: The correlated evolution of human brain and body size. *Curr. Anthropol.* **57**, 174–196 (2016).
10. Riska, B. & Atchley, W. R. Genetics of growth predict patterns of brain-size evolution. *Science* **229**, 668–671 (1985).
11. Tsuboi, M. et al. Evolution of brain–body allometry in Lake Tanganyika cichlids. *Evolution* **70**, 1559–1568 (2016).
12. Voje, K. L., Hansen, T. F., Egset, C. K., Bolstad, G. H. & Pelabon, C. Allometric constraints and the evolution of allometry. *Evolution* **68**, 866–885 (2014).
13. Pelabon, C. et al. On the relationship between ontogenetic and static allometry. *Am. Nat.* **181**, 195–212 (2013).
14. Snell, O. Die abhängigkeit des hirngewichtes von dem körpergewicht und den geistigen fähigkeiten. *Eur. Arch. Psychiatry Clin. Neurosci.* **23**, 436–446 (1892).
15. Yopak, K. E. Neuroecology of cartilaginous fishes: the functional implications of brain scaling. *J. Fish. Biol.* **80**, 1968–2023 (2012).
16. Martin, R. Relative brain size and basal metabolic-rate in terrestrial vertebrates. *Nature* **293**, 57–60 (1981).
17. Kleiber, M. *The Fire of Life: An Introduction to Animal Energetics* (John Wiley & Sons, New York, 1961).
18. Benson-Amram, S., Dantzer, B., Stricker, G., Swanson, E. M. & Holekamp, K. E. Brain size predicts problem-solving ability in mammalian carnivores. *Proc. Natl Acad. Sci. USA* **113**, 2532–2537 (2016).
19. MacLean, E. L. et al. The evolution of self-control. *Proc. Natl Acad. Sci. USA* **111**, E2140–E2148 (2014).
20. Roth, G. & Dicke, U. Evolution of the brain and intelligence. *Trends Cogn. Sci.* **9**, 250–257 (2005).
21. Finarelli, J. A. & Flynn, J. J. Brain-size evolution and sociality in Carnivora. *Proc. Natl Acad. Sci. USA* **106**, 9345–9349 (2009).
22. Boddy, A. M. et al. Comparative analysis of encephalization in mammals reveals relaxed constraints on anthropoid primate and cetacean brain scaling. *J. Evol. Biol.* **25**, 981–994 (2012).
23. Holekamp, K. E., Swanson, E. M. & Van Meter, P. E. Developmental constraints on behavioural flexibility. *Phil. Trans. R. Soc. B* **368**, 20120350 (2013).
24. Montgomery, S. H. et al. The evolutionary history of cetacean brain and body size. *Evolution* **67**, 3339–3353 (2013).
25. Felsenstein, J. Phylogenies and the comparative method. *Am. Nat.* **125**, 1–15 (1985).
26. Lynch, M. Methods for the analysis of comparative data in evolutionary biology. *Evolution* **45**, 1065–1080 (1991).
27. Riska, B. Regression-models in evolutionary allometry. *Am. Nat.* **138**, 283–299 (1991).
28. Hansen, T. F. & Bartoszek, K. Interpreting the evolutionary regression: the interplay between observational and biological errors in phylogenetic comparative studies. *Syst. Biol.* **61**, 413–425 (2012).
29. Pagel, M. D. & Harvey, P. H. The taxon-level problem in the evolution of mammalian brain size—facts and artifacts. *Am. Nat.* **132**, 344–359 (1988).
30. Hansen, T. F. & Houle, D. Measuring and comparing evolvability and constraint in multivariate characters. *J. Evol. Biol.* **21**, 1201–1219 (2008).
31. Noreikiene, K. et al. Quantitative genetic analysis of brain size variation in sticklebacks: support for the mosaic model of brain evolution. *Proc. R. Soc. B* **282**, 20151008 (2015).
32. Rogers, J. et al. Heritability of brain volume, surface area and shape: an MRI study in an extended pedigree of baboons. *Hum. Brain Mapp.* **28**, 576–583 (2007).
33. Kotrschal, A. et al. Artificial selection on relative brain size in the guppy reveals costs and benefits of evolving a larger brain. *Curr. Biol.* **23**, 168–171 (2013).
34. Peper, J. S., Brouwer, R. M., Boomsma, D. I., Kahn, R. S. & Poll, H. E. H. Genetic influences on human brain structure: a review of brain imaging studies in twins. *Hum. Brain Mapp.* **28**, 464–473 (2007).
35. Cheverud, J. M. et al. Heritability of brain size and surface-features in rhesus macaques (*Macaca-Mulatta*). *J. Hered.* **81**, 51–57 (1990).
36. Airey, D. C., Castillo-Juarez, H., Casella, G., Pollak, E. J. & DeVoogd, T. J. Variation in the volume of zebra finch song control nuclei is heritable: developmental and evolutionary implications. *Proc. R. Soc. B* **267**, 2099–2104 (2000).
37. Hansen, T. F., Pelabon, C. & Houle, D. Heritability is not evolvability. *Evol. Biol.* **38**, 258–277 (2011).
38. Hansen, T. F., Pienaar, J. & Orzack, S. H. A comparative method for studying adaptation to a randomly evolving environment. *Evolution* **62**, 1965–1977 (2008).
39. Grabowski, M., Voje, K. L. & Hansen, T. F. Evolutionary modeling and correcting for observation error support a 3/5 brain–body allometry for primates. *J. Hum. Evol.* **94**, 106–116 (2016).
40. Mink, J. W., Blumenschine, R. J. & Adams, D. B. Ratio of central nervous-system to body metabolism in vertebrates—its constancy and functional basis. *Am. J. Physiol.* **241**, R203–R212 (1981).
41. Barton, R. A. & Capellini, I. Maternal investment, life histories, and the costs of brain growth in mammals. *Proc. Natl Acad. Sci. USA* **108**, 6169–6174 (2011).
42. Isler, K. & van Schaik, C. P. The expensive brain: a framework for explaining evolutionary changes in brain size. *J. Hum. Evol.* **57**, 392–400 (2009).
43. Iwaniuk, A. N. & Nelson, J. E. Developmental differences are correlated with relative brain size in birds: a comparative analysis. *Can. J. Zool.* **81**, 1913–1928 (2003).
44. Martin, R. D. & Harvey, P. H. in *Size and Scaling in Primate Biology* (ed. Jungers, W. L.) Ch. 8 (Springer, New York, 1985).
45. Nealen, P. M. & Ricklefs, R. E. Early diversification of the avian brain: body relationship. *J. Zool.* **253**, 391–404 (2001).
46. Pagel, M. Inferring the historical patterns of biological evolution. *Nature* **401**, 877–884 (1999).
47. Halley, A. C. Minimal variation in eutherian brain growth rates during fetal neurogenesis. *Proc. R. Soc. B* **284**, 20170219 (2017).
48. Halley, A. C. Prenatal brain–body allometry in mammals. *Brain Behav. Evol.* **88**, 14–24 (2016).
49. Raff, R. A. *The Shape of Life: Genes, Development, and the Evolution of Animal Form* (Univ. Chicago Press, Chicago, 1996).
50. Bolstad, G. H. et al. Genetic constraints predict evolutionary divergence in Dalechampia blossoms. *Phil. Trans. R. Soc. B* **369**, 20130255 (2014).
51. Svensson, E. & Calsbeek, R. (eds) *The Adaptive Landscape in Evolutionary Biology* (Oxford Univ. Press, Oxford, 2012).
52. Walsh, B. & Blows, M. W. Abundant genetic variation plus strong selection = multivariate genetic constraints: a geometric view of adaptation. *Annu. Rev. Ecol. Evol. Syst.* **40**, 41–59 (2009).
53. Arnold, S. J., Pfrender, M. E. & Jones, A. G. The adaptive landscape as a conceptual bridge between micro- and macroevolution. *Genetica* **112**, 9–32 (2001).
54. Arnold, S. J., Burger, R., Hohenlohe, P. A., Ajie, B. C. & Jones, A. G. Understanding the evolution and stability of the G-matrix. *Evolution* **62**, 2451–2461 (2008).
55. Jones, A. G., Arnold, S. J. & Burger, R. Evolution and stability of the G-matrix on a landscape with a moving optimum. *Evolution* **58**, 1639–1654 (2004).
56. Pavlicev, M., & Cheverud, J. M. Constraints evolve: context dependency of gene effects allows evolution of pleiotropy. *Annu. Rev. Ecol. Evol. Syst.* **46**, 413–434 (2015).
57. Jones, A. G., Burger, R. & Arnold, S. J. Epistasis and natural selection shape the mutational architecture of complex traits. *Nat. Commun.* **5**, 3709 (2014).
58. Willis, J. H., Coyne, J. A. & Kirkpatrick, M. Can one predict the evolution of quantitative characters without genetics? *Evolution* **45**, 441–444 (1991).
59. Houle, D., Bolstad, G. H., van der Linde, K. & Hansen, T. F. Mutation predicts 40 million years of fly wing evolution. *Nature* **548**, 447–450 (2017).
60. Williams, G. C. *Natural Selection: Domains, Levels, and Challenges* (Oxford Univ. Press, New York, 1992).

61. Finlay, B. L. & Darlington, R. B. Linked regularities in the development and evolution of mammalian brains. *Science* **268**, 1578–1584 (1995).
62. Striedter, G. F. & Charvet, C. J. Developmental origins of species differences in telencephalon and tectum size: morphometric comparisons between a parakeet (*Melopsittacus undulatus*) and a quail (*Colinus virgianus*). *J. Comp. Neurol.* **507**, 1663–1675 (2008).
63. Koyabu, D. et al. Mammalian skull heterochrony reveals modular evolution and a link between cranial development and brain size. *Nat. Commun.* **5**, 3625 (2014).
64. Iwaniuk, A. N. & Nelson, J. E. Can endocranial volume be used as an estimate of brain size in birds? *Can. J. Zool.* **80**, 16–23 (2002).
65. Froese, R. & Pauly, D. (eds) *FishBase* (2016); http://www.fishbase.org
66. Crile, G. & Quiring, D. P. A record of the body weight and certain organ and gland weights of 3690 animals. *Ohio J. Sci.* **40**, 219–260 (1940).
67. Hrdlička, A. *Brain Weight in Vertebrates* Vol. 3 (Smithsonian Institution, 1905).
68. Mangold-Wirz, K. Cerebralisation und ontogenesemodus bei eutherien. *Acta Anat.* **63**, 449–508 (1966).
69. Isler, K. et al. Endocranial volumes of primate species: scaling analyses using a comprehensive and reliable data set. *J. Hum. Evol.* **55**, 967–978 (2008).
70. Hrdlička, A. Weight of the brain and of the internal organs in American monkeys with data on brain weight in other apes. *Am. J. Phys. Anthropol.* **8**, 201–211 (1925).
71. Gittleman, J. L. Carnivore brain size, behavioral ecology, and phylogeny. *J. Mammal.* **67**, 23–36 (1986).
72. Matějů, J. et al. Absolute, not relative brain size correlates with sociality in ground squirrels. *Proc. R. Soc. B* **283**, 20152725 (2016).
73. Blinkov, S. M. & Glezer, I. A. I. *The Human Brain in Figures and Tables: A Quantitative Handbook* (Basic Books, New York, 1968).
74. Starck, J. M. Zeitmuster der Ontogenesen bei nestflüchtenden und-nesthockenden Vögeln. *Cour. Forsch. Inst. Senckenb.* **114**, 1–319 (1989).
75. R Core Team *R: A Language and Environment for Statistical Computing* v.3.4.0 (R Foundation for Statistical Computing, Vienna, 2017).
76. Jetz, W. et al. Global distribution and conservation of evolutionary distinctness in birds. *Curr. Biol.* **24**, 919–930 (2014).
77. Bininda-Emonds, O. R. P. et al. The delayed rise of present-day mammals. *Nature* **446**, 507–512 (2007).
78. Rabosky, D. L. et al. Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. *Nat. Commun.* **4**, 1958 (2013).
79. Pyron, R. A. & Wiens, J. J. A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Mol. Phylogenet. Evol.* **61**, 543–583 (2011).
80. Velez-Zuazo, X. & Agnarsson, I. Shark tales: a molecular species-level phylogeny of sharks (Selachimorpha, Chondrichthyes). *Mol. Phylogenet. Evol.* **58**, 207–217 (2011).
81. Zheng, Y. C. & Wiens, J. J. Combining phylogenomic and supermatrix approaches, and a time-calibrated phylogeny for squamate reptiles (lizards and snakes) based on 52 genes and 4162 species. *Mol. Phylogenet. Evol.* **94**, 537–547 (2016).
82. Bouckaert, R. et al. BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Comput. Biol.* **10**, e1003537 (2014).
83. Benton, M. J. et al. Constraints on the timescale of animal evolutionary history. *Palaeontol. Electron.* **18**, 1–106 (2015).
84. Sanderson, M. J. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Mol. Biol. Evol.* **19**, 101–109 (2002).
85. Chamberlain, S. A. & Szöcs, E. taxize: taxonomic search and retrieval in R. *F1000Res.* **2**, 191 (2013).
86. Cook, R. D. & Weisberg, S. *Residuals and Influence in Regression* (Chapman and Hall, New York, 1982).
87. Pinheiro, J. B. D., DebRoy, S., Sarkar, D. and R Core Team *nlme: Linear and Nonlinear Mixed Effects Models* v.3.1.131 (2017).
88. Harmon, L. J., Weir, J. T., Brock, C. D., Glor, R. E. & Challenger, W. GEIGER: investigating evolutionary radiations. *Bioinformatics* **24**, 129–131 (2008).
89. Hansen, T. F. Stabilizing selection and the comparative analysis of adaptation. *Evolution* **51**, 1341–1351 (1997).
90. Martins, E. Estimating the rate of phenotypic evolution from comparative data. *Am. Nat.* **144**, 193–209 (1994).
91. Boettiger, C., Coop, G. & Ralph, P. Is your phylogeny informative? Measuring the power of comparative methods. *Evolution* **66**, 2240–2251 (2012).
92. Oikawa, S. & Itazawa, Y. Relative growth of organs and parts of the carp, *Cyprinus carpio*, with special reference to the metabolism–size relationship. *Copeia* **1984**, 800–803 (1984).
93. Kawabe, S., Matsuda, S., Tsunekawa, N. & Endo, H. Ontogenetic shape change in the chicken brain: implications for paleontology. *PLoS ONE* **10**, e0129939 (2015).
94. Oikawa, S., Takemori, M. & Itazawa, Y. Relative growth of organs and parts of a marine teleost, the porgy, *Pagrus-Major*, with special reference to metabolism–size relationships. *Jpn. J. Ichthyol.* **39**, 243–249 (1992).
95. Muggeo, V. M. Segmented: an R package to fit regression models with broken-line relationships. *R News* **8**, 20–25 (2008).
96. Tsuboi, M. et al. Brain mass and body mass datasets and phylogenies linked to brain–body allometry and the encephalization of birds and mammals. Figshare fileset. https://doi.org/10.6084/m9.figshare.6803276 (2018).

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Author contributions

M.T., A.N.I. and N.K. conceived the study and wrote the manuscript. M.T., B.T.K., J.E., A.K., K.E.Y., S.P.C., A.N.I. and N.K. collected the data. M.T., W.v.d.B., B.T.K., K.L.V. and N.K. designed analytical protocols, and M.T., W.v.d.B. and B.T.K. analysed the data. All authors provided input to the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	No software was used for data collection.
Data analysis	We used R version 3.4.0. The source code of a program "SLOUCH" is available at (https://github.com/kopperud/slouch) and as a supplementary data file.

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All data and source code that support the finding of our study are available either as supplementary materials or as an archived dataset in Dryad.

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☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

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Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	Our research is a phylogenetic comparative study based on existing data. Our data consist of 20,293 observations of brain and body mass across 4,587 species and dated molecular phylogenies.
Research sample	Our study is based on existing datasets and a lab population of the guppy (Poecilia reticulata). The data is brain mass (gram) and body mass (gram). The source of datasets are briefly described in the manuscript and a complete description (taxa, measurements, sample size, standard deviation, original source, sex, age and notes) is supplied in dataset deposited in Dryad.
Sampling strategy	We used all available data and no attempts were made to predetermine sample size.
Data collection	Brain and body size of the guppy were collected by one person (Alexander Kotrschal) using a digital weight. All the rest of our data were obtained from existing datasets.
Timing and spatial scale	Since our goal is to gain a global view on how brain-body allometry within- and across-species are related, we collected all available data relevant to our question irrespective of timing and spatial scale.
Data exclusions	From all published datasets that we have examined, we exclusively included the data that meets the following criteria: 1) data are taken from sexually-mature adult individuals evaluated either by direct description in the source or inferred from body size, ii) data represent original measurements of samples recorded by authors of the data source, and iii) brain mass are reported with body mass collected from the same individual(s). These initial screenings were made to ensure that we estimate allometies at a comparable life stage and that the data will be reported with full transparency. In addition, in our assessment of the static allometry, we excluded 1.34 % of the relevant subset of the data based on model residuals and cook's distance with pre-established exclusion criteria, as described in the text.
Reproducibility	We performed no experiments to reproduce.
Randomization	Our study does not have groups to allocate samples. Thus, randomization is not relevant to our study.
Blinding	The majority of our study is based on existing data, thus blinding is not applicable for this subset of the data. With respect to the data collected from a lab population of the guppy, the data were collected blindly to our study question.

Did the study involve field work? ☐ Yes ☒ No

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other organisms

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

Laboratory animals	Guppies (Poecilia reticulata). Sex and age of individuals are presented in Supplementary Material Table S3.

Wild animals	The study does not involve wild animals.
Field-collected samples	The study does not involve field-collected samples.