# Beyond CREA: evolutionary patterns of non-allometric shape variation and divergence in a highly allometric clade of murine rodents

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**Abstract**

The shared functions of the skull are thought to result in common evolutionary patterns in mammalian cranial shape. Craniofacial evolutionary allometry (CREA) is a particularly prominent pattern where larger species display proportionally elongate rostra and smaller braincases than smaller relatives. It was recently proposed that CREA arises from the biomechanical effects of cranial scaling when diets are constant, so that it should only appear in species with similar cranial functionality. Thus, deviations from CREA should be consistent with changes in cranial biomechanical function, for example due to dietary change. Here, we test this using 3D geometric morphometric analysis in a dataset of Australian murine crania, which are highly allometric. Specifically, we contrast ordinated allometric and non-allometric variation, cranial integration/modularity, and divergence patterns over time. This confirmed that dietary specialists are not part of the sample’s common allometric variation. Interestingly, CREA-like shape variation potentially related to posture also appeared in allometry-free PCA. Integration among cranial modules was higher, and modularity lower, with size included. Size contributed substantially to divergence of shape over time, but both size-included and allometry-free shape variation peaked at only 2-4 million years of divergence. Our results support the hypothesis that CREA is a composite pattern arising from selection on cranial function, with size-mediated stabilising selection a dominant process leading to high integration and limited shape divergence. However, CREA does not appear to represent a constraint because, as predicted, substantial non-allometric shape variation occurs alongside it, particularly where dietary specialisation reduces selection on gnawing function.

**Keywords:** allometry, CREA, geometric morphometrics, integration, modularity, Muridae, stabilizing selection

## Background

The skull is arguably the most functionally diverse interface between a mammal and its environment. It is employed in the acquisition and mastication of food, receives the majority of sensory input, and carries the large and heavy brain. The evolution of mammalian cranial diversity is therefore assumed to be heavily influenced by the various selection regimes acting on cranial function. Possibly for this reason, cranial morphology across mammals displays some common patterns of evolutionary variation. The most widely discussed of these is the tendency of larger mammals to display longer rostra and smaller braincases relative to smaller species, particularly in closely related species (Cardini, Polly, Dawson, & Milne, 2015). This pattern, termed craniofacial evolutionary allometry (CREA), has been found in a diverse range of vertebrates representing 11 different orders, especially those of mammals (Bright, Marugán-Lobón, Cobb, & Rayfield, 2016; Cardini, 2019; Cardini et al., 2015).

Mitchell, Sherratt, and Weisbecker (2024) suggested that CREA is likely a product of bite force allometry and phylogenetic niche conservatism. Briefly, closely related species tend to have similar diets and are thus likely to encounter foods with similar mechanical properties, such as hardness. For similar food items, a small and a large species therefore have to apply the same absolute bite force, but the mechanical demand on the cranium will be lower for the larger species simply because of its larger size. This would allow larger species to sacrifice some capacity for bite force generation in their craniofacial architecture in response of other selective pressures. This trade-off appears to most commonly manifest as the CREA pattern of more gracile crania (Mitchell et al., 2024). Independently, the negative scaling of brain and orbit size relative to body mass tends to reduce the relative size of vault and orbital area, potentially increasing the length of the nasomaxillary region and the appearance of facial elongation (Mitchell et al., 2024). Under this scenario, morphological shifts in cranial morphology that deviate from the CREA pattern are only predicted to occur in association with substantial changes in ecology that alter bite force demands, regardless of body size.

Among mammals, rodent skulls among the most striking cases of allometry coinciding with a CREA pattern of shape variation. A previous study (Marcy et al., 2020) showed that a sample of mostly Australian rodents, diverging as early as ten million years ago, has a highly conserved slope of allometry explaining over a third of their overall shape variation. The shape variation explained by allometry is also aligned with CREA, both in the definition adopted in Marcy et al. (2020; referring to a longer/elongated rostrum/muzzle/snout) and Mitchell et al. (2024; referring to hyperallometric gracilization more generally and involving the braincase). This supports previous work suggesting that the strong allometry occurs as a result of stabilising selection on the rodent gnawing apparatus, which is highly specialized but allows for substantial dietary breadth (Druzinsky, 2015; Marcy et al., 2020; Zelditch & Swiderski, 2023). It might also explain the clade’s unique overall shape but slow-evolving morphological evolution through time (Goswami et al., 2022). Support for stabilizing selection on cranial function, rather than any intrinsic developmental constraints, comes from studies showing that cranial growth allometry varies substantially among rodents, such that similar specialisations can arise from different growth patterns (Segura, Flores, & Deferrari, 2023; Wilson, 2013). Moreover, Marcy et al. (2020) showed that species whose shape appeared most distinct from the common evolutionary allometric pattern tended to be ecological specialists with distinct diets and locomotor modes. This is consistent with Mitchell et al.’s (2024) hypothesis that CREA can be confounded by abrupt shifts in dietary hardness, and also highlights that variation beyond allometric scaling is an important part of investigation.

The rodent sample assessed in Marcy et al. (2020) offers an ideal opportunity for differentiating allometric and non-allometric variation because it combines allometrically highly uniform species with clear deviations from the common pattern. These include the carnivorous rodents, but also a group of ecological specialists with distinct non-diet related locomotor modes, the hopping mice *(Notomys*) and the rabbit-rat (*Conilurus penicillatus*). The latter group is of interest because of their conspicuous “facial tilt” of the anterior cranium, an adaptation resulting in an expansion of their field of view while hopping or bounding (Kraatz & Sherratt, 2016). This makes them intriguing cases to examine in the allometry-free morphospace, as their rostral shapes may depend on the degree to which the facial tilt is independent from CREA.

Selection on functions that result in shape variation independent of CREA is expected to be more apparent in some parts of the skull but not others (e.g. the maxillary region of carnivorous species; the back of the skull in hopping or bounding species). A key question is therefore how different parts of the skull co-evolve, and whether there are size-independent patterns alongside CREA which allow the evolution of cranial areas away from the main allometric line. This is conceivable because allometry explained a large amount (36%), but not the majority, of cranial shape variation (Marcy et al., 2020). This unexplained variation might be related to species-specific variation without any particular evolutionary patterning (such as vicariance or founder effects), but it could also be attributable to evolutionary processes resulting in non-CREA patterns. Understanding how the different parts of the cranium relate to each other in evolution can be investigated using assessments of cranial integration (covariation between modules; Klingenberg, 2009) and modularity (the degree of independence of shape variation within a module relative to the others; Klingenberg, 2009). Strong allometry in the rodent sample means that we should expect all parts of the skull to evolve as one integrated structure, which corresponds with low modularity (i.e. no differences in evolutionary behavior across modules) and high integration between modules (i.e. all modules co-varying strongly) (Bookstein, 2015; Klingenberg, 2009). Because allometry manifests as the co-variation of shape with size, its removal is expected to reduce the levels and strength of integration.

Conversely, if the allometric pattern is paired with morphological divergence in directions not aligned with the common allometric trajectory, we expect higher modularity (where parts of the skull evolve independently) and lower integration between modules (where modules co-vary less), particularly in the allometry-free space.

If diversification of rodent crania is bounded by functional allometric constraints, we should expect to see an Ornstein-Uhlenbeck- type pattern of shape divergence through time in allometric and allometry-free contexts. We would thus expect the morphological distances among species to be associated with the evolution of optimal shapes within the samples’ maximum size range. By contrast, if the allometry-free shape variation relates to the capacity of the cranium to diverge independently of allometry, we would expect to see the highest morphological distances between species that have ecological specializations with divergent functional requirements, compared to those whose shapes follow the expected CREA pattern.

In this study, we test our expectations on the relationships between allometric and non-allometric shape variation on Marcy et al’s (2020) sample of Australian murids. We ask if the removal of size from the dataset completely removes variation due to CREA; whether the allometry-free dataset confirms our prediction of higher modularity and lower integration of cranial modules; and whether size-independent shape variation should show maximum shape disparity occurring between distantly-related species and/or species with divergent functional requirements (as opposed to allometric shape spaces, where maximum shape disparity should occur between species with divergent body sizes).

## Methods

We used Marcy et al’s (2020) previously published dataset of 37 Australian rodent species (317 individuals) that were landmarked with a protocol of 60 fixed landmarks, 141 curve semi-landmarks, and 124 patch semi-landmarks. These were subjected to a generalized Procrustes analysis (GPA) with subsequent removal of the asymmetric component (details in Marcy et al. 2020 and implemented in the github repository associated with this study). Ecological information on diet and locomotion for each species was taken from Breed and Ford (2007). All analyses were performed in R (v.3.6.1) (R Core Team, 2023)**,** using the packages *geomorph* (Adams, Collyer, Kaliontzopoulou, & Baken, 2022; Baken, Collyer, Kaliontzopoulou, & Adams, 2021), *mvMORPH* (Clavel, Escarguel, & Merceron, 2015), *landvR* (Guillerme & Weisbecker, 2019)*,* and *vegan* (Oksanen et al., 2022)*.*

*Testing evolutionary modes*

To assess whether the crania in our sample follow an Ornstein-Uhlenbeck (OU) pattern of evolution, as predicted by our hypothesis of stabilizing selection, we used mvMORPH to fit models of Brownian Motion (BM), Ornstein-Uhlenbeck (OU), and also Early Burst (EB). The EB test computes a scenario of rapid initial radiation with subsequent decrease in diversification (Clavel et al., 2015). We also fitted gls models of allometry under the three evolutionary scenarios to find out which evolutionary mode fits the evolution of allometry best. To identify the best model for shape evolution and allometric fits, we compared the Generalized Information Criterion for each of the fits by calculating their relative probabilities (W scores; Burnham & Anderson, 2002). For any cases of OU/EB models being more likely, we also used simple correlation analysis to assess how strongly residuals from models of OU/EB and BM evolution were correlated. This is relevant because the downstream analyses of integration and modularity are only available in *geomorph* in the context of Brownian Motion models.

*Comparisons of allometry among modules*

We tested the expectation outlined in the introduction that CREA reflects

arises out of the negative allometry of braincase and orbits and positive allometry of rostral length by assessing allometry of each module. This was done after each the module configurations of the raw data to a separate GPA and asymmetry removal and comparing the amount of variation explained by the log-transformed centroid sizes from each GPA. To confirm that allometric patterns were not influenced by the different sizes and landmark numbers of each modules, the analyses were also re-run using the centroid sizes of the full landmark dataset.

### Comparing the distribution of species in morphospace through PCA scores

In order to visually assess the allometric and non-allometric morphospaces, we performed principal component analyses (PCA) on three different shape datasets of mean species shapes, and visualised each morphospace with plots of the first two principal components (PCs). The first, termed here ‘full shape dataset’ is based on a conventional generalised Procrustes analysis, and includes the allometric component of shape. Second, the ‘shape residual dataset’ includes the components of shape that remain once allometric shape is removed and it provides a ‘size-less’ or ‘allometry-free’ comparison of the mean species shapes. The shape residuals were obtained from a phylogenetically-informed linear generalised least squares model using random permutations implemented by the *RRPP* package (M. L. Collyer & Adams, 2018; M. L. Collyer & Adams, 2019). When residuals wereadded to the consensus shape derived from the GPA, the shape variationcould be compared visually to the full shape dataset. Third, we repeated the PCA for the shape residual dataset after removing the four hopping mice (genus *Notomys*). We did this because we expected their bipedal posture to exaggerate some features of shape variation in the PCA and the resulting morphospace plots.

### Assessment of allometric vs. allometry-free shape variation via heat maps

In order to visualise and assess allometric shape variation in the full shape dataset, we created heatmaps showing the magnitude of landmark displacements using *landvR* functions (Guillerme & Weisbecker, 2019; Weisbecker et al., 2019). We compared three different visualizations of allometry. First, using fitted allometric shapes estimated by Procrustes linear models (also using random permutations as per RRPP) across the entire sample. However, variation characterised through ordination or allometric analysis provides summaries of parts of the variation, which do not always reflect actual specimens (Weisbecker et al., 2019).. We therefore also visualised the mean configurations of the smallest native species (the delicate mouse, *Pseudomys delicatulus*) and the largest (the giant white-tailed rat, *Uromys caudimaculatus*), as determined by mean centroid size. Third, to illustrate the similarity in shape variation along PC1 to the two previous visualizations of allometric variation, we visualised the hypothetical shapes for PC1 minimum and maximum.

To compare the allometric shape change to the ‘isometry-free or ‘allometry-free’ shape variation, we produced heatmaps from the shape residual dataset visualizing the minimum and maximum hypothetical shapes for three different PC axes. First, we produced heatmaps for PC1 and PC2 to compare the allometry-free changes to the allometric cranial changes seen in the full shape dataset. We also visualised heatmaps for the shape residual PC2 without the four species of *Notomys* in order to assess the impact of their bipedal posture on the ordinated shape variation.

### Modularity and integration in allometric and allometry-free datasets

To compare the modularity patterns across our allometric and non-allometric datasets, we separated the landmarks according to a five-module framework that followed the six modules proposed across therian mammal crania (Goswami, 2006). This included the anatomical regions of the rostrum, molar area, orbital area, vault, basicranial area, but excluded the zygomatic arch module, which was missing due to scanner limitations (Marcy, Fruciano, Phillips, Mardon, & Weisbecker, 2018). To quantify modularity, we used the *geomorph* function *phylo.modularity* (Adams, 2016; Adams & Collyer, 2019). This calculates the covariance ratio (CR) coefficient, with the numerator as covariation between modules and the denominator as covariation within modules (Adams, 2016). Therefore, highly modular structures, with higher covariation within than between modules, will have small CR values within the unit interval (Adams 2016). By contrast, structures with low modularity will have CR values close to 1.0 because the two covariation values are very similar (Adams, 2016). The modularity results were juxtaposed with analyses of integration, which use partial least squares (PLS) correlation coefficients between multiple modules to assess how much they co-vary, taking into account phylogeny (Adams & Felice, 2014). In this case, values towards 1 indicate a higher PLS coefficient. In both modularity and integration analyses, the functions include a phylogenetic context by generating a matrix of partial least squares under a Brownian motion model of evolution (Adams & Felice, 2014) that was informed by our time-calibrated ultrametric molecular phylogeny (Marcy et al., 2020; Smissen & Rowe, 2018). The resulting evolutionary covariance matrix controls for similarities between closely related species, which is needed to study macro-evolutionary patterns of modularity (Adams & Felice, 2014; Klingenberg & Marugán-Lobón, 2013). Significance was determined by randomly resampling the modules 1,000 times and comparing the random distribution of CR coefficients to the observed value.

We also expected the areas that are most affected by CREA – the rostrum and braincase – to be more integrated and less modular than the remainder of the modules. To assess this, we conducted test of integration and modularity for all pairs of modules as outlined above. We also computed comparisons of integration strength between module pairs, asking the question whether the integration between two modules is significantly higher compared to other module pairs (Adams & Collyer, 2019). Significance values of modularity between module pairs are not available in *geomorph*, so we instead performed pairwise Mantel tests on the distance matrices of PC scores within each module (Legendre & Legendre, 2012). The resulting *r* statistic indicates the degree of correlation between each module pair, with values closer to one corresponding to higher integration (Hetherington et al., 2015). If a module consistently has *r* statistics closer to zero, this indicates higher modularity, i.e. greater independence in shape variation relative to the other cranial modules. The Bonferroni correction was used to adjust for multiple comparisons (Bonferroni, 1936).

Finally, we tested for global integration of the crania in both the full shape and shape residual datasets using the *geomorph* function *globalIntegration* based on Bookstein (2015). This test distinguishes between integration and a null hypothesis for self-similarity, which is the absence of any interpretable change at any spatial scale. Self-similarity in a morphological dataset is the spatial equivalent of a temporal random walk based on Brownian motion (Bookstein, 2015). The degree of integration versus self-similarity is quantified by the regression slope between the entire sample’s (n = 37) bending energy and its partial warp variance (Bookstein, 2015; Evans, Waltz, Tagliacollo, Chakrabarty, & Albert, 2017; Sansalone et al., 2019; Young, Linde-Medina, Fondon, Hallgrímsson, & Marcucio, 2017). The null expectation of self-similarity would give a regression slope of -1 so if the slope is steeper – i.e. greater absolute value – this indicates global integration due to low independence in each cranial module relative to the other cranial modules.

### Phylogenetic vs. morphological distances between species pairs

Since the constraints on shape disparity from integration may differ in the allometric and allometry-free morphospaces, we visualised the relationship between pairwise phylogenetic and morphological distances for the full shape and shape residual datasets. We retrieved a matrix of pairwise phylogenetic distances using the *picante* function *cophenic* (Kembel et al., 2010) on our ultrametric time-calibrated phylogeny phylogeny (Marcy et al., 2020; Smissen & Rowe, 2018). Values were divided in half to give values in millions of years since last common ancestor. The pairwise Procrustes distances – i.e. morphological distances – were derived from the GPA of shapes. We then plotted every pairwise combination of the phylogenetic and morphological distances between two species in our dataset for both the full shape and shape residual datasets. We expect this to provide a broad estimate of morphological divergences with and without allometry, but there are two caveats to this method: 1) pseudoreplication due to the high volume of pairwise comparisons within the sample and 2) non-uniform sampling of time due to the phylogeny’s structure, with most coverage occurring between 0.3-4.2 Ma. We therefore interpret the results with these caveats in mind.

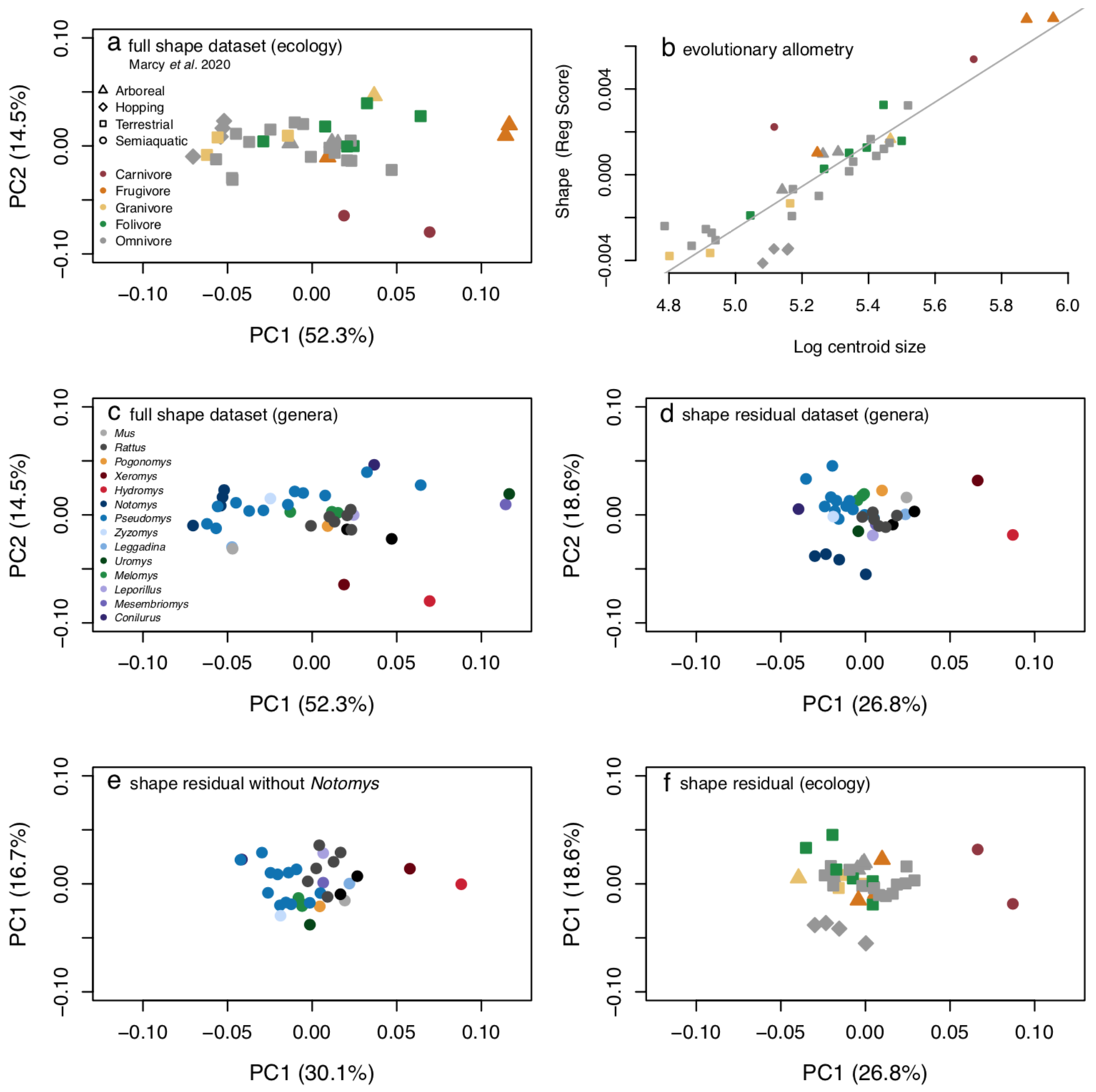
## Results

### Principal component analyses

We visually assessed whether removing allometry emphasizes different morphological patterns in the rodent crania morphospace by comparing PC1/PC2 plots and an evolutionary allometry plot of the full shape dataset (with allometry, Fig. 1a-c) to PC1/PC2 plots of the shape residual dataset (allometry-free, Fig. 1d-f). As expected, the allometry-free shape residual dataset explained less shape variation along PC1: 26.8% compared to 52% in the full shape dataset (see also Fig. S1). The PC2 axes captured similar percentages of shape variation, 14.5% and 18.6%, within the full shape and shape residual datasets, respectively. As expected, the full shape PC1 orders species by size (correlation with centroid size is 0.92; Fig. 1a vs. b), whereas neither residual shape PC1 nor PC2 distinguishes between large-bodied and small-bodied species(Fig. 1c vs. d). The divergent bipedal posture of *Notomys* was not a main driver of residual shape variation: when *Notomys* was removed, the relative positioning of species and the shape variation associated with the first two PCs remain very similar (Fig. 1d vs. e).

The species distribution along the full shape PC2 resembles the pattern along the residual shape PC1 (Fig. 1a vs. f). Both axes show the carnivorous *Xeromys myoides* and *Hydromys chrysogaster* at one extreme and a quadrupedal bounding species (the brush-tailed rabbit rat, *Conilurus penicillatus*) at the other. A correlation of 0.97 confirms the similarity between the full shape PC2 and the residual shape PC1 axes while the Mantel correlation between the two morphospaces is comparatively lower at 0.58. However, removing the full shape PC1 – and the allometric variation it captures – from the full shape morphospace and then re-performing the Mantel correlation test raises the r statistic to 0.94. This confirms that PC1 mostly captures allometric variation while the other PCs preserve other shape patterns. Nevertheless, the relationship between the full shape and shape residual axes is not such that PCresidual(n) reflects PCfull (n-1). For example, the correlation between full shape PC3 and shape residual PC2 is 0.25. This supports our expectation of the ordinations summarising different dominant patterns of shape variation in the allometry-free shape residual dataset compared to the full shape dataset.

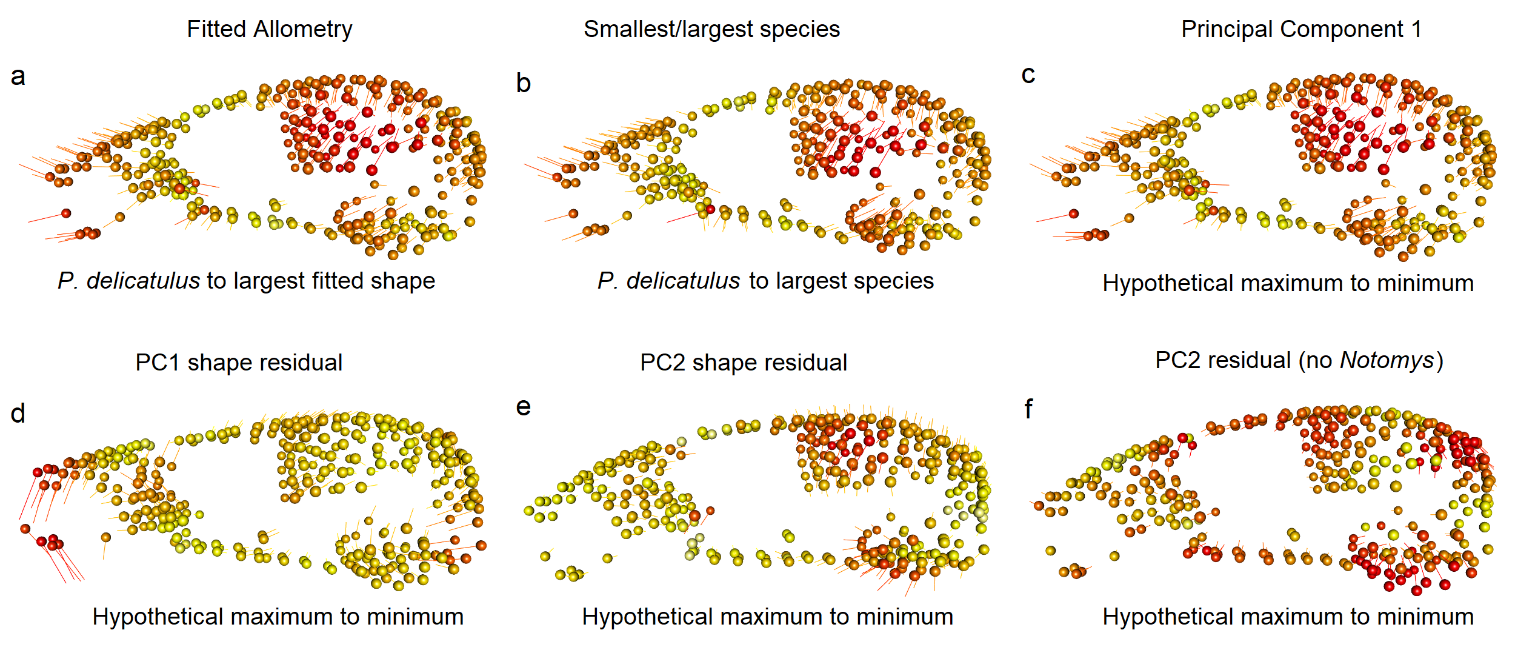
In the shape residual plot of PC1 and PC2, the majority of species cluster in the center, which is expected because the main differentiator of shape – size – is now removed. This includes the two large-bodied frugivores, which have high PC1 scores in the full shape plot and high regression scores in the evolutionary allometry plot. The allometry-free shape residual plots distinguish other ecological specialists instead, such as the two semiaquatic carnivores along PC1 and the four hopping *Notomys* species along PC2 (Fig. 1f). The PC2 maximum highlights the Australian murid most specialised for folivory, the broad-toothed rat (*Mastacomys fuscus*). All of the specialists along these extremes are in the *Pseudomys* division (sensu; Smissen and Rowe 2018), a clade of five genera from the earliest radiation of extant Australian rodents (Aplin and Ford 2014), represented here by shades of blue (legend in Fig. 1c). In the full shape dataset, most of these specialists show a degree of deviation from the common allometric line (Fig. 1b,f). The two carnivores and a specialist folivore (*Mastacomys*) plot above the line while *Notomys* appears to have a lower y-intercept for their genus-wide evolutionary allometric trajectory compared to other murids.



**Figure 1:** Differences between the hypothetical shapes captured between PC1 extremes.a, plot of PC1 and PC2 for the full shape dataset and b**,** plot of log centroid size versus the projected regression score with a gray regression line indicating the common evolutionary trajectory; data from Marcy et al. (2020) but with point shapes by locomotion and colors by diet. c,same data with point colors by genera (*Mastacomys* is within genus *Pseudomys*). d, ‘allometry-free’ shape residual dataset, e**,** shape residual dataset without *Notomys,* f, same as d with point shapes by locomotion and colors by diet.

### Landmark heatmaps

As expected, a pattern of gracilization with size is apparent in the visualization of shape variation that is associated with allometry: fitted minimum/maximum shapes, mean shapes of smallest/largest species, and shapes on the extremes of PC1 (Fig. 2a-c). Representations of larger species had lengthened rostra and smaller relative braincases compared to smaller species.



**Figure 2:** Landmark heatmaps expressing shape variation. Spheres show the mean position of landmarks for the column’s dataset, vectors show landmark displacement. Colors and lengths are calculated from relative proportions of the minimum/maximum vector lengths for each comparison, and are not equivalent across columns. a, shape differences between the shape fitted for the smallest Australian native (*P. delicatulus)* to the largest species in the sample (*U. caudimaculatus)*; b, shape differences between the mean shapes of these previous species, showing high correspondence to differences between hypothetical allometric fitted shapes; c, differences between the hypothetical shapes captured between PC1 extremes; d, differences between the hypothetical shapes captured between PC1 extremes based on allometry-free data; e, differences between the hypothetical shapes captured between PC2 extremes on allometry-free data; f, differences between hypothetical shapes between PC1 extremes after removing *Notomys.*

Removing the shape patterns that covary with size (Fig. 2d-f) also removed this gracilization pattern. Species closer to the PC1 minimum then show ventral flexion of the rostrum and anteroventral movement of the foramen magnum (Fig. 2d). However, the allometry-free PC2 heatmaps with all species highlighted shape patterns resembling the allometric variation seen in the full shape PC1 even though this pattern is not allometric, i.e. not correlated with body size (correlation = 0.11). For example, the *Notomys* species at PC1 minimum show enlarged braincases and auditory bullae, but not shortened rostra as expected under CREA (Fig. 2e). To test whether these shape patterns are an artifact driven by the four bipedal hopping *Notomys* species, we removed these from the shape residual dataset and re-calculated the heatmaps. The result showed similar regions of variation (Fig. 2f) This indicates that the bipedal hopping species are not solely responsible for the braincase and auditory bulla shape variation seen in the shape residual dataset with all species.

### Modularity and integration

As expected, the full dataset had higher levels of integration (high PLS correlation coefficient) and lower modularity (CR coefficient closer to 1) than the shape residual dataset (Fig. 3). This reflects the fact that allometric variation contains those patterns of integration that related to co-variation of all landmarks with size. As we also predicted, size-independent patterns of shape evolution exist in parallel with the size variation, with greater independence of the cranial modules suggested by the lower r-PLS and CR coefficients of the shape residual dataset. However, our Mantel tests of module PC score matrices did not confirm our expectation that the rostrum and vault module PCAs had higher correlations with each other than with others: rostrum and vault correlations in the full shape dataset had smaller r statistics (r=0.76) than that between the rostrum and the adjacent orbital region (0.81). The r statistics for the full shape dataset were all above 0.51 (Table 1, upper triangle), indicating medium-to-strong positive shape variation relationships between all modules. The molar module consistently had the lowest r statistics (r = 0.51-0.63) indicating that it is the most independent from the other modules. **R-pls values (reflecting integration) between the molar module and others were also the smallest in the analyses, small enough for the molar module and the rostral and orbital modules to approach a statistically significantly lower level of integration compared to some others (Table S1). However, none of the difference in integration were clear in terms of significance, and may just exist because the molar module has the fewest landmarks (n = 19).** Results from the residual shape dataset gave similar ratios of r statistics between modules, indicating that pairwise patterns of integration between modules do not change when allometry is removed. As expected, the absolute values decreased relative to the full shape dataset (Table 1, lower triangle), which reflects an overall decrease in shape variation when one of the major variation determinants – allometry – is removed. However, intriguingly, the correlation between residual PCAs of the vault and the rostrum is substantially higher (0.7) compared to all the other correlations (0.37 – 0.65).

A diagram of a number of objects

Description automatically generated with medium confidence

**Figure 3:** Modularity tests using the CR coefficient. **a** our five-module framework adapted from (Goswami 2006). Results from the full shape (b,d) and shape residual (c,e) datasets. Black curves are the density distribution of coefficients from 1,000 randomly drawn modules and the arrows point to the observed coefficients, which were all significant.

**Table 1:** Modularity tests using pairwise Mantel comparisons of PCA-based distance matrices of all modules, and Mantel r statistic. An r statistic of 1 indicates a strong correlation and 0 indicates no correlation. The upper triangle reports statistics for pairwise comparisons between cranial modules of the full dataset, the lower triangle reports r statistics for the residual dataset. The values in brackets are *p* values, adjusted by Bonferroni (1936) corrections for multiple comparisons.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Basicran. | Molar | Orbital | Rostrum | Vault |
| Basicran. | **64** | 0.514 (0.01) | 0.601 (0.01) | 0.719 (0.01) | 0.753 (0.01) |
| Molar | 0.367 (0.03) | **19** | 0.55 (0.01) | 0.625 (0.01) | 0.564 (0.01) |
| Orbital | 0.283 (0.06) | 0.348 (0.02) | **32** | 0.807 (0.01) | 0.731 (0.01) |
| Rostrum | 0.524 (0.02) | 0.624 (0.01) | 0.626 (0.01) | **86** | 0.762 (0.01) |
| Vault | 0.652 (0.01) | 0.45 (0.03) | 0.629 (0.01) | 0.699 (0.01) | **124** |

Consistent with the overall high integration we found in both the full and size-free datasets, the test of global integration revealed a regression slope below -1.0 (Beval\_full = -1.64; Beval\_residual = -1.55; Fig. S2), which indicates global integration across all cranial modules (Bookstein 2015) in both datasets. This means integration or a high degree of covariation among all modules might obscure any potential modular structure (Klingenberg 2009).

### Phylo-morphological distance

Our phylo-morphological distance plots (Fig. 5) asked whether the relationship between phylogenetic distance and morphological distances (i.e. Procrustes distances between the mean shapes of a species pair) increases with increasing phylogenetic distance because, as integration patterns change over time, shape covariation patterns diverge (Voje, Hansen, Egset, Bolstad, & Pélabon, 2014). As expected, all of the points closest to the origin (i.e. low phylogenetic *and* low morphological distances) are within-genus pairs. In the full shape dataset, maxima in morphological distances tend to increase with phylogenetic distance until reaching an apparent asymptote around 4.2 Ma since the last common ancestor. However, the highest divergence values involve distances of all species with the two large-bodied frugivores: *U. caudimaculatus* and the black-footed tree rat *Mesembriomys gouldii* (Fig. 4a). If these are ignored, then the dataset’s maximum morphological distance appears earlier, around 2 Ma. Furthermore, all pairs involving *Rattus*, the most recent radiation of native rodents,fall below the maximum morphological distance reached around 2 Ma by pairwise comparisons of older endemics (Fig. 5a); in other words, shape distances between *Rattus* and other Australian murids, which have divergence dates of around 10 million years, fall well within the range of morphological distances within murids. However, as noted in the methods, these results are subject to pseudoreplication because they include all possible pairwise combinations, such that each of the 37 species accounts for 36 data points. This can be seen in the vertical clustering, which represent pairwise comparisons between one species and other species with the same divergence time.

The ‘allometry-free’ shape residual pairwise comparisons were similar to the full shape dataset, with overall lower morphological distances as expected from removing allometric shape variation. The removal of allometric differences between species also has a marked effect on the spread of morphological distances at each divergence. Most conspicuously, removing allometry substantially reduces morphological distances between the large-bodied frugivores relative to other ecological specialists, so that the greatest distances between species is now at the time of divergence between the two semiaquatic, carnivorous species at 5.7 Ma (Fig. 5b). If the semiaquatic species are ignored, the remaining dataset’s maximum distances appear around 3.1 Ma, or pairwise comparisons between hopping *Notomys* species and close relatives in *Pseudomys*. Both plots show the greatest morphological divergences occurring within the old endemic species, not between more-distantly related species involving *Rattus* or *Mus*.



**Figure 5:** Phylo-morphological distance plots. Each point is a pairwise comparison with border and center colors corresponding to the two species’ genera. The x-axis is shared but the y-axes of morphological distances are not equivalent as they rely on different shape datasets: a full shape and b shape residual.

**Discussion**

In this study, we characterised the evolution of cranial shape beyond allometric patterns in a highly allometric clade of Australian murine rodents to understand the degree to which the facial gracilization that is part of the CREA pattern persists in the absence of size-related variation. As expected, removal of the allometric pattern indeed removes much of the shape variation of longer faces at larger sizes. However, a substantial part of the ordinated allometry-free shape variation – 18% - reflects differences in relative anterior braincase expansion, which are part of the CREA pattern but here appear independent of allometry. Thus, allometric patterning is not the only cause of shape variation that is commonly attributed to CREA. Removal of allometric effects thus can reveal other relevant patterns of shape variation. In the case of the murine pattern, it is possible that the brain of species with more expanded braincases along PC2 either have brain volumes that are larger than expected for their body mass – essentially reflecting the encephalization of these species (Smaers et al., 2021) – or have a different distribution of the brain tissue within the braincase (Weisbecker et al., 2021) compared to lower-scoring species. This effect serves as a reminder that comparisons of allometric and ‘allometry-free’ datasets can identify how different sources of shape variation interact to produce visible patterns of vertebrate shape diversity, even in clades with strong allometric constraints.

Allometry in mammalian crania, and the subsequent shape variation as predicted by CREA, has often been attributed to the integration of size with masticatory biomechanics (Marroig & Cheverud, 2005; Mitchell, Sherratt, Ledogar, & Wroe, 2018; Mitchell et al., 2024; Singleton, 2005; Weisbecker et al., 2019). This is probably particularly true for rodents, where high levels of allometry likely reflect constraints imposed by their highly derived gnawing function (Cox et al., 2012; Druzinsky, 2015; Ginot, Claude, & Hautier, 2018; Lessa & Patton, 1989; Marcy et al., 2020; Marcy, Hadly, Sherratt, Garland, & Weisbecker, 2016). However, the residual shape space also appears to have a biomechanical *and* allometry-independent source of shape variation: the second allometry-free axis captured CREA-like variation in relative basicranium dimensions, where the two most specialised folivore species showed shallower vaults than expected for their size. This pattern likely reflects the wider skulls and dorsally shifted temporalis muscles that increase the mechanical advantage for masticating fibrous foods, which has evolved in specialist folivores across several rodent families (Samuels, 2009).

Under the assumptions of Mitchell et al’s (2024) framework, the CREA pattern represents a tradeoff between bite force and selective pressures that shift in importance with increasing size. Importantly, such a biomechanics-focused framework allows for variation among selective pressures that result in biomechanical adaptation beyond the common CREA pattern. For example, in the case of rodents, there are potential benefits to increasing gape (Hennekam et al., 2020; Williams, Peiffer, & Ford, 2009), which might explain why carnivorous species have more elongate crania than expected for their size. Similarly, cranial morphology is expected to be determined by the toughest foods encountered by a species (Figueirido, Tseng, Serrano-Alarcón, Martín-Serra, & Pastor, 2014; Mitchell, 2019; Strait et al., 2009; Van Valkenburgh, 1989), so that frequent consumption of hard seeds and insects by the desert-living hopping generalist species (Murray, Dickman, Watts, & Morton, 1999) might explain their more robust cranial dimensions than expected for their size.

Despite evidence that the allometric pattern in our sample is determined by stabilizing selection on mastication, the allometry-free morphospaces show that this appears not to constrain the evolution of adaptations such as postural variation coinciding with ecological specializations. For example, the rabbit rat (*Conilurus penicillatus*) has the highest facial tilt of the sample, consistent with its quadrupedally bounding locomotion (Kemper 1989, Kraatz and Sherratt 2016). However, despite its unusual shape, the rabbit rat still falls along the common allometric line, thus suggesting that any stabilizing selection on mastication also permits the evolution of specialist postures. A similar pattern is seen in the bipedally hopping genus *Notomys*, whichis second in facial tilt to *Conilurus*, and to a lesser degree in *Mastacomys*. *Notomys* species do not lie on the common allometric line, but this separation is because of their derived basicranium, not their facial tilt. The inclusion of a facial tilt in *Conilurus* and *Notomys* within the common allometric pattern therefore suggests a level of flexibility to adaptations that do not interfere with masticatory function.

Visual assessment of the non-allometric shape variation provides intriguing evidence that even apparently non-allometric variation may have its origins in an underlying allometric pattern. In particular, the allometry-free PC2 axis – capturing “partial” CREA-like variation of relative braincase size – differentiates species that are adapted for different masticatory functions. This includes, specialized folivory for the broad-toothed rat *Mastacomys*, which lies above the common allometric line in the full dataset, and bipedal hopping for *Notomys*, which lies below this line. In terms of shape, *Mastacomys* displays a cranial shape like a larger murid, by having a relatively smaller braincase relative to the snout region; this is consistent with descriptions of its unusually robust skull (Breed & Ford, 2007). By contrast, *Notomys* displays a larger braincase region relative to the snout, as would be expected for a smaller murid. Despite this overall difference in multivariate intercept, the allometric slopes within the genera *Notomys* and *Mastacomys* are not significantly different from the common allometric slope Marcy et al. (2020). The changes in braincase dimension thus appear to reflect a “grade shift” of an otherwise identical allometric pattern. This further supports the expectation that bite force allometry should be expressed in similar patterns (represented by the allometric slope) in crania that have been selected for different bite forces due to dietary specialisation ((represented by the intercept differences of the allomeric slope; Mitchell et al., 2024)

The modularity and integration results reinforce the impression that evolutionary size variation results in concerted changes across the cranium, as reflected in the higher integration values in the full dataset. This may well relate to stabilizing selection due to size-related biomechanical constraints. However, in the case of murines, such constraints appear to extend beyond the allometric pattern, due to the high levels of integration retained in the residual dataset. This agrees with the observation that rodents occupy a highly distinct, slowly-evolving area in the morphospace of placental crania (Goswami et al., 2022). In addition, however, the lower integration and higher modularity of the size-free dataset reinforces the impression from the morphospaces that discrete changes in particular regions of the skull occur alongside the allometry-related pattern of shape variation. This also agrees with previous interpretations that murine crania are under strong stabilizing selection related to their derived masticatory apparatus, of which allometric patterns are just one manifestation. More generally, it highlights the importance of interpreting allometric patterns in the context of other variation, even when allometry explains the majority of shape variation (Mitchell et al., 2024).

Counter to our expectations, morphological distances between species did not correspond with increases in phylogenetic distances. Instead, the asymptotic divergence pattern of shape reinforces the concept of rodent skulls evolving around an optimal shape unless a substantial change in either size or cranial function evolves. Consistent with this, the maximum divergences correspond with our three proposed mechanisms for the evolution of shape variation in Australian murids. First, the maximum divergence in the full shape dataset involves the large-bodied frugivores, whose cranial shapes were probably facilitated by an allometric line of least resistance honed by stabilizing selection (sensu Schluter; Marcy et al., 2020). In contrast, the allometry-free maximum divergence highlights the shape distances we hypothesize occurred due to a carnivory-related release from this selection on masticatory function. Finally, the second-highest divergence in the allometry-free shape analysis involving the hopping *Notomys* likely reflects the aforementioned change in the genus-level allometric multidimensional intercept. Overall, the pattern of “spikes” of divergence in otherwise limited morphospace (as demonstrated by a plateau of diversification after 4.2-5.7 million years) suggests an evolutionary pattern most consistent with a mean-shift Ornstein-Uhlenbeck process of limited diversification around a local optimum (Harmon et al., 2010).

**Conclusions**

Characterizing the allometric and allometry-free shape variation in the cranium of Australian murine rodents has provided a useful context to recent suggestions that allometric shape variation is a biomechanics-driven process (Mitchell et al., 2024). Our study supports the predictions of this framework in several ways. One, CREA emerges as just one out of several patterns that are well-explained by established biomechanical hypotheses, highlighting the overall strong impact of biomechanics on cranial shape. Two, size appears to be only a constraint where stabilizing selection for a particular cranial function – in our case, the highly specialised gnawing apparatus of rodents - is apparent, and disappears where changes in cranial function or diet are consistent with a change of selective regime; similar deviations are seen in rodents with substantial change in mastication musculature (for example, hystricomorphs) or where extreme dietary shifts occur, such as in worm-specialists like *Paucidentomys* (Esselstyn, Achmadi, & Rowe, 2012).

One important insight is that some patterns of postural adaptation, in our case relating to facial tilt, appear to be integrated with a common allometric line, producing a shared evolutionary shape pattern for the majority of the diverse sample. This highlights how CREA itself is well-explained as an emergent property of several sub-patterns; separating these will be an important step for an even more nuanced assessment of what the CREA pattern means.

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**Declarations**

**Availability of data and materials:** The dataset of 3D specimen scans on which the landmarks are based are available on MorphoSource (https://www.morphosource.org/projects/00000C561). The dataset of landmark coordinates and the fully reproducible code for the analyses in the current study are available on DOI [10.5281/zenodo.10211695](https://doi.org/10.5281/zenodo.10211695).

**Competing interests:** The authors declare that they have no competing interests.

**Authors’ contributions:** AEM and VW conceived the study. AEM collected the data. AEM analysed the data with support from TG and VW. AEM and VW wrote the manuscript with support from TG, DRM, and MJP. VW and MJP provided supervision on the project.

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**Supporting Information**

A graph of different shapes

Description automatically generated with medium confidence**Figure0S1:** Scree plots for PCAs on the three main datasets. Scree plots show the proportion of variance explained by each individual PC. The pink line indicates 10%. The scree plot for (A) the full shape dataset with allometry, (B) the shape residual dataset (size-free or allometry-free), and (C) the shape residual dataset without *Notomys*.



**Figure0S2:** Tests of global integration.A plot of log bending energy (BEval) versus the log partial warp variance (PWvar) for our specimens, that using the method from Bookstein (2015), can distinguish global integration from self-similarity. The red line represents the null hypothesis for self-similarity with a regression slope of exactly -1 against the observed regression slopes for the (A) full shape and (B) shape residual datasets. The steeper observed slopes in both support hypotheses for global integration.