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

Ariel E. Marcy1,\*, D. Rex Mitchell2, Thomas Guillerme,3, Matthew J. Phillips4, and Vera Weisbecker2,5

**Affiliations:** 1Commonwealth Scientific and Industrial Research Organisation (CSIRO), Science Connect, Canberra, ACT 2601, Australia; 2Flinders University, College of Science and Engineering, Bedford Park SA 5042, Australia; 3University of Sheffield, school of Biosciences, Alfred Denny Building S10 2TN, United Kingdom; 4Queensland University of Technology, School of Biology & Environmental Science, Brisbane, Queensland, 4000 Australia; [\*](mailto:*amarcy2@unl.edu) ariel.marcy@csiro.au

**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 *et al.*, 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 *et al.*, 2016; Cardini, 2019; Cardini *et al.*, 2015).

Mitchell *et al.* (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 *et al.*, 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.

EXPLAIN ALLOMETRY RESIDUALS EARLY AROUND HERE

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.

If diversification of rodent crania is bounded by functional allometric constraints, whereby each size has a shape optimum, we should expect to see an Ornstein-Uhlenbeck pattern of shape divergence through time. We would also 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.

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 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) in datasets with and without size information.

Strong allometry in the rodent sample means that the skull co-evolves with size as one integrated structure, corresponding with high integration between modules (i.e. all modules co-varying strongly) (Bookstein, 2015; Klingenberg, 2009) and low modularity (i.e. low independence of modules from each other). Integration should be strongest between those modules identified as affected by CREA, including the rostrum, vault, and potentially orbital region. Removing the effects of size from the shape variation by analysing residuals of allometric regressions is therefore expected to reduce the integration of the cranium overall and between modules, and possibly also increase the degree of modularity. Analysis of residuals thus can also reveal patterns of integration that are not related to size, which would demonstrate that allometric variation is not the sole driver of cranial shape evolution.

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 *et al.*, 2022; Baken *et al.*, 2021), *mvMORPH* (Clavel *et al.*, 2015), *landvR* (Guillerme & Weisbecker, 2019)*,* and *vegan* (Oksanen *et al.*, 2022)*.* To compare allometric and modularity patterns, we separated 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 *et al.*, 2018).

*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). Note that estimates of evolutionary mode are unlikely to be very robust because our samples size is relatively small (Cooper *et al.*, 2016), which is why we expand on this test visually (see below) as well. For any cases of OU/EB models being more likely, we also used Two-Block Partial Least Squares 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. Lastly, we also investigated the most likely evolutionary mode for log-transformed centroid size through functionalities in the *Phytools* package. Note that the probabilities from *Phytools* are given as Akaike Information Criterion (AIC), which are a special case of and similar to the GICs given by the mvMORPH package (Cavanaugh & Neath, 2019).

### Visualising shape evolution

As noted above, the sample size available to us (n=37 species) makes estimations of evolutionary modes potentially unreliable. For a more direct visualization, we therefore also plotted the Procrustes distances between species against evolutionary time. For this, we retrieved a matrix of pairwise phylogenetic distances using the *picante* function *cophenic* (Kembel *et al.*, 2010) on our ultrametric time-calibrated 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 expected 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.

*Comparisons of allometry among modules*

Because CREA is expected to affect the rostral, cranial vault, and possibly orbit areas, we asked if size explained more variation in these three compared to the molar and basicranial modules. These allometry analyses were conducted after separate GPA and asymmetry removals for each module from the raw landmarks. We then compared allometries from geomorph-based PGLS regressions of shape against log-transformed centroid sizes from each GPA. To exclude a potential confounding influence on allometry analyses from the differences in landmark numbers and partition sizes between modules, we also repeated these analyses using centroid sizes from the full 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 (Collyer & Adams, 2018; 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

We used geomorph functions to assess integration (degree of co-variation) and modularity (degree to which modules evolve independently of each other) across the cranium and among modules before and after size removal. Integration was measured using 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. To quantify modularity, we used geomorph functionalities (Adams, 2016; Adams & Collyer, 2019) calculate the covariance ratio (CR) coefficient, with the numerator as covariation between modules and the denominator as covariation within modules. Therefore, highly modular structures, with higher covariation within than between modules, will have small CR values within the unit interval. By contrast, structures with low modularity will have CR values close to 1.0 because the two covariation values are very similar (Adams, 2016). 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 coefficients to the observed value. We also compared the R-PLS and CR values among modules in the full *vs.* the residual dataset. Additionally, to assess if the removal of the integrating influence of allometry results in a significant reduction in cranial integration, we also conducted comparisons of integration strengths implemented in *geomorph;* these derive statistical significances from comparisons between effect sizes of pairs of PLS analyses (Adams & Collyer, 2016).

As with the allometry analyses, we expected the areas most affected by CREA (rostrum, braincase, and possibly orbits) to be more integrated with each other than the remainder of the modules. We tested this expectation by comparing the level and relative strength of integration for all pairs of modules as outlined above, and again also asked if integration is reduced significantly between pairs of modules in the full vs. the residual dataset (Adams & Collyer, 2019).

The CR-coefficent-based modularity analyses in geomorph are designed to detect significant modular structure under a specific hypothesis of modularity (Adams & Collyer, 2019); unlike integration analyses, modularity comparisons are therefore not designed to compare differences in the level of modularity between different datasets such as ours, which have the same hypothesized modular structure. To still obtain an assessment of whether individual modules are more independent of each other (i.e. modular) after size correction, we 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. Note that this analysis has the caveat of being without phylogenetic adjustment. The Bonferroni correction was used to adjust for multiple comparisons (Bonferroni, 1936).

Lastly, also 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 sample’s (n = 37) bending energy and its partial warp variance (Bookstein, 2015; Evans *et al.*, 2017; Sansalone *et al.*, 2019; Young *et al.*, 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.

## Results

*Modes of Evolution*

Generalized Least Squares (GLS) models of shape variation alone and shape allometry were most likely under the assumption of Ornstein-Uhlenbeck evolution; in both cases, the (W-score of 1 compared to 0 for the Brownian-Motion (BM) and Early Burst (EB) models. By contrast, a Brownian-Motion model was more likely for the evolution of size, although both OU and EB models also have a moderate likelihood.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Brownian Motion | Ornstein-Uhlenbeck | Early Burst |
| Shape GIC | -446170 | **-447701** | -446167 |
| Shape W | 0 | **1** | 0 |
| log(Centroid size) AIC | **-4.802** | -2.875 | -2.802 |
| log(Centroid size) W | **0.572** | 0.22 | 0.21 |
| Shape~log(Csize) GIC | -433056 | **-434260** | -433054 |
| Shape~Loc(Csize) W | 0 | **1** | 0 |

**Table 1:** Generalised or Akaike Information criterion scores (GIC/AIC) and W-scores of relative probabilities of different modes of evolution for models of shape, log- transformed centroid size, and shape evolution with log(centroid size) as predictor variable.

As OU-structured evolutionary modes are not implemented in our analyses of modularity and integration, which are based on BM, we further asked how well the residuals of the OU *versus* BM models of shape and allometry evolution corresponded by comparing residual coordinates of shape from both models using Two-Block Partial Least Squares. In both cases, the r-PLS value was 1, suggesting that no substantial difference to BM-based analyses is to be expected.

### 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 *et al.*, 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*.

A graph of different shapes

Description automatically generated

**Figure 1:** 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.

### Principal components analyses

Comparing the variation and species distribution from the first two Principal Components (PC) of the full and residual datasets shows that the removal of allometric shape variation substantially reduces the amount of variation in the dataset that is aligned with PC1 (26% vs. 52%). 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 (with a correlation of PC1 to size of 0.92). The species distribution along the full shape PC2 resembles the pattern along the residual shape PC1 (Fig. 1a vs. c) in that 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. Removal of size thus mostly removes the shape information of PC1 from the residual dataset, with PC2 of the full dataset correlating at 0.97 with PC1 of the residual dataset. Similarly, a mantel test of the distance matrices between species derived from the full-dataset PCA without PC1 *versus* the distance matrices from PCA of the residual dataset showed a very high correlation (0.94). This shows that size removal does not impact much on the distribution of shape variation beyond PC1. The distinctive shape of the cranium of *Notomys*, arising from itsbipedal posture, 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 similar (Fig. 1c vs. d).

In the shape residual plot of PC1 and PC2, the majority of species cluster in the center. This includes the two large-bodied frugivores, whose shape lies on the common line of allometry (Fig. 1b). The allometry-free PC plots separate out other ecological specialists instead, such as the two semiaquatic carnivores along PC1 and the four hopping *Notomys* species along PC2 (Fig. 1f). The PC2 minimum 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 (Smissen & Rowe, 2018), a clade of five genera from the earliest radiation of extant Australian rodents (Aplin & Ford, 2014).

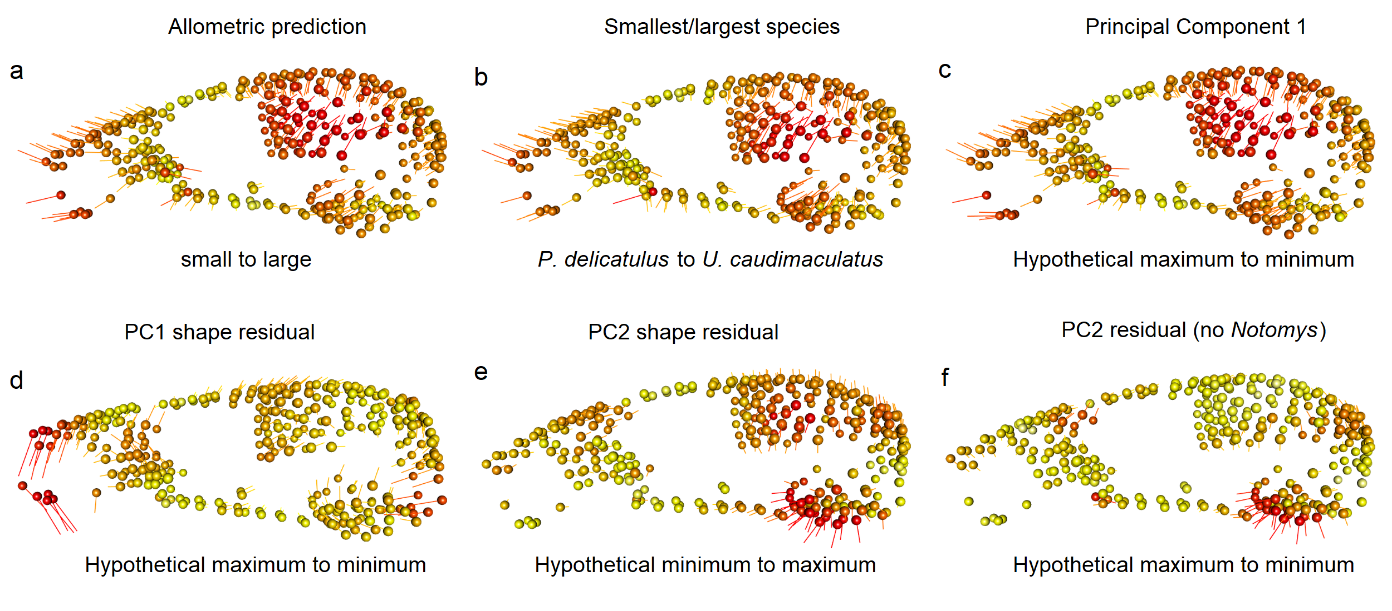
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**Figure 2**. Shape variation related to size and after size removal.a, Plot of PC1 and PC2 for the full shape dataset and b**,** plot of log centroid size versus the projected regression score with a gray regression line indicating the common evolutionary trajectory as per Marcy et al. (2020); c,‘allometry-free’ shape residual dataset; d, shape residual dataset without *Notomys*, which mainly just switches the sign of the PC scores*.*

### Landmark heatmaps

As expected, a pattern of gracilization with size is apparent in the visualization of shape variation that is associated with allometry (Fig. 2a-c). Predicted shapes for larger species had lengthened rostra and smaller relative braincases compared to smaller species (Fig. 2a), a pattern also clearly visible in comparisons of mean shapes between smallest and largest species in the dataset and predicted shapes for high and low PC1 scores for the full dataset.



**Figure 3:** 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 individual images. **a**, shape differences between the shape fitted for mean centroid size of the smallest (*P. delicatulus)* to the largest (*U. caudimaculatus)* species in the sample; **b**, shape differences between the mean shapes of these two species; **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.*

As expected, removing the shape variation that covaries with size (Fig. 2d-f) also removed the CREA-aligned 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 resemble some of the allometric variation. For example, the *Notomys* species at PC1 minimum show dorsally expanded braincases and ventrally expanded auditory regions, but not shortened rostra as expected under CREA (Fig. 2e). Removing the four bipedal hopping species of *Notomys* reduced this pattern somewhat to highlight just the expansion of the bulla, but as with the PC1/2 plots of Fig. 1, the result showed similar regions of variation (Fig. 2f). This indicates that the bipedal hopping species, despite their distinctive morphology, do not dominate the variation displayed by both PCAs.

### 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) because it contains the co-variation of shape with size. As we also predicted, size-independent patterns of shape evolution exist in parallel with allometric variation of shape, with greater independence of the cranial modules suggested by the lower r-PLS and CR coefficients of the shape residual dataset.

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**Figure 4:** 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.

Assessment of pairwise integration between modules revealed that integration between the rostrum and the cranial vault was the highest among all module pairs, even though the two modules are not neigbouring each other. This strong association seems to capture the emphasis of the allometric pattern on concomitant rostral elongation with narrowing of the vault (Fig. 2). Furthermore, the orbital region has high integration values relative to both rostrum and vault, consistent with our prediction that orbital size might also play a role in the evolution of allometric variation. Lastly, a strong association between the rostrum and the basicranium is notable and was not part of our predictions.

Removal of size resulted in several changes in r-pls values, but not all of these related to reduced integration and only some were detected as significant differences in integration strength (Table 2). All instances of significant integration strength differences relate to the vault with all other modules. The greatest difference in effect size reflects a dramatic drop in integration between the vault and the rostrum, followed by a more moderate drop in r-PLS value between vault and orbits and vault and molars. Intriguingly, an *increase* in integration between vault and basicranium is also detected as a significant change in integration strength, while a very large drop in R-pls value between rostrum and basicranium is just outside the significance cut-off (*p=*0.06).

**Table 2:** phylogenetically informed integration, expressed as r-PLS values, between the full shape (lower triangle) and residual shape (upper triangle) of modules. All r-PLS values were significant at *p* < 0.05 except for the integration between residuals of orbital and molar modules. For P-value tables, see **Supplementary Table X**. Arrows in the upper triangle indicate whether the r-PLS values of residual integration analyses are higher (↑) or lower (↓) than the full-shape r-PLS values. Numbers in the diagonal are landmark numbers for each partition.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Basicranium | Molar | Orbital | Rostrum | Vault |
| Basicranium | 64 | 0.59↓ | 0.8↑ | 0.71↓ | 0.88↑ |
| Molar | 0.69 | 19 | (0.52) ↓ | 0.82↑ | 0.69↓ |
| Orbital | 0.77 | 0.70 | 32 | 0.76↓ | 0.85↓ |
| Rostrum | 0.89 | 0.77 | 0.86 | 86 | 0.79↓ |
| Vault | 0.85 | 0.77 | 0.88 | 0.94 | 124 |

**Table 3:** Integration strength comparisons between pairs of modules, including effect sizes (Z) and significance of strength differences (*p*).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Z full | Z residual | Z difference | *p* |
| basicran x molar | 3.34 | 2.25 | 0.86 | 0.388 |
| basicran x orbital | 4.16 | 4.1 | 0.63 | 0.526 |
| basicran x rostrum | 4.29 | 2.98 | 1.88 | 0.06 |
| **basicran x vault** | 3.93 | 4.89 | 2.11 | **0.035** |
| molar x orbital | 3.09 | 1.44 | 1.23 | 0.217 |
| molar x rostrum | 3.81 | 4.24 | 0.45 | 0.654 |
| **molar x vault** | 3.69 | 2.74 | 2.1 | **0.036** |
| orbital x rostrum | 4.45 | 3.43 | 1.37 | 0.17 |
| **orbital x vault** | 4.1 | 4.22 | 2.41 | **0.016** |
| **rostrum x vault** | 4.94 | 3.19 | 2.48 | **0.013** |

In contrast to the mixed changes in integration patterns, modularity among all cranial partitions increases after the removal of size (Table 3), with no apparent difference as to how much integration levels change or whether there is a significant change in integration strength after size removal. This is also reflected in our Mantel tests comparison of distance matrices between species according to their PCA scores (Table 4); while remaining nearly all significant (meaning that the distribution of species in PC morphospaces remains more similar than expected at random), all partition comparisons had lower Mantel r statistics after the removal of size, with exception of the rostrum/molar comparison which remained nearly unchanged in both the CR-based modularity assessment (Table 3) and the Mantel tests (Table 4).

**Table 4:** phylogenetically informed modularity, expressed as CR coefficients, between the full shape (lower triangle) and residual shape (upper triangle) of modules. Numbers on the diagonal are landmark numbers for each partition.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Basicranium | Molar | Orbital | Rostrum | Vault |
| Basicranium | 64 | 0.56 | 0.66 | 0.82 | 0.82 |
| Molar | 0.52 | 19 | 0.60 | 0.68 | 0.69 |
| Orbital | 0.50 | 0.46 | 32 | 0.81 | 0.84 |
| Rostrum | 0.63 | 0.66 | 0.66 | 86 | 0.86 |
| Vault | 0.73 | 0.62 | 0.74 | 0.72 | 124 |

**Table 5:** 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 | 0.355 (0.03) | 0.290 (0.07) | 0.508 (0.01) | 0.674 (0.01) |
| Molar | 0.514 (0.01) | 19 | 0.351 (0.03) | 0.658 (0.01) | 0.424 (0.02) |
| Orbital | 0.601 (0.01) | 0.55 (0.01) | 32 | 0.620 (0.01) | 0.606 (0.01) |
| Rostrum | 0.719 (0.01) | 0.625 (0.01) | 0.807 (0.01) | 86 | 0.694 (0.01) |
| Vault | 0.753 (0.01) | 0.564 (0.01) | 0.731 (0.01) | 0.762 (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).

**Discussion**

In this study, sought understand the degree to which non-allometric shape variation occurs in the highly allometric clade of Australian murid rodents and their more remote invasive relatives. The comparison of datasets with and without size-related shape variation reveals the strong impact of size on shape, but also shows that substantial non-allometric variation exists in the clade, with its own signatures of shape divergence, ordinated variation, and significant levels of integration..

As expected, the evolution of murid cranial shape is consistent with our hypothesis that cranial shape evolves around an optimal configuration according to an Ornstein-Uhlenbeck (OU) process. This supports the hypothesis that the shape variation in the full dataset is under stabilizing selection according to size. Notably, the OU mode of the full shape is not merely a consequence of OU-dominated size evolution, since centroid sizes more likely evolve according to Brownian Motion.. 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).

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 *et al.*, 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 *et al.*, 2018; Lessa & Patton, 1989; Marcy *et al.*, 2020; Marcy *et al.*, 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 *et al.*, 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 *et al.*, 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 *et al.*, 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).

**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 *et al.*, 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).

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**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.