# 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 *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 are among the most striking cases of allometry coinciding with a CREA pattern. 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 morphological evolution through time (Goswami *et al.*, 2022), and finds that radical changes in shape, such as island gigantism, are possible within species along the allometric line (Schlis-Elias & Malaney, 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 departed most 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 patterns can be “interrupted” by abrupt shifts in dietary hardness, and also highlights that variation beyond allometric scaling is an important part of investigation.

Examples include the specialized *Mastacomys fuscus*, whose diet nearly entirely consists of grass (Calaby & Wimbush, 1964; Green *et al.*, 2014) which is highly abrasive to dentition (e.g. Winkler *et al.*, 2019). Another deviation from the common allometric line is seen in the two carnivorous sister species, the Rakali *Hydromys chrysogaster* and water mouse *Xeromys myoides,* and a group of ecological specialists with distinct non-diet related locomotor modes, the hopping mice *(Notomys*) and the rabbit-rat (*Conilurus penicillatus*). The hopping mice are 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.

The rodent sample of Marcy *et al.* (2020) offers an ideal opportunity for differentiating allometric and non-allometric variation (via analysis of residuals from regressions of shape against size), because it includes a predominance of allometrically highly uniform species with clear deviations from the common pattern. For example, it allows us to test if diversification of rodent crania is bounded by functional allometric constraints, whereby each size has a shape optimum. In this case, 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, and without much functional constraint, we would expect a Brownian-motion process and find the greatest morphological distances – and differentiation in shape - between species that have ecological specializations with divergent functional requirements. This effect should also be visible in ordinations of shape, both in terms of the distribution of species in Principal Components morphospaces and the shape variation associated with this distribution.

In addition, the biomechanical processes leading to CREA are expected to be reflected in high allometry and high levels of co-variation in cranial areas that are expected to vary most under CREA – the rostrum, cranial vault, and potentially orbital region (Mitchell *et al.*, 2024). By contrast, 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 depending on cranial function (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 selection that is unrelated to CREA.

Our above predictions can be tested through 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 the skull co-evolves with size as one integrated structure, so that the integration between modules (i.e. their co-variance) (Bookstein, 2015; Klingenberg, 2009) is expected to be higher and and modularity (i.e. independence of modules from each other) potentially lower in datasets with shape information contained. Integration should also 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 the expectations outlined above on the relationships between allometric and non-allometric shape variation on Marcy et al’s (2020) sample of Australian murids. Contrasting the shape variation of the dataset with allometry-free residual shape data, we characterize the mode of evolution, ordinated morphospace distribution, integration and modularity in the whole cranium and its modules. This provides the first investigation targeting the interpretation of shape variation after allometry removal, which serves to test the predominance of biomechanical drivers of mammalian cranial shape variation.

## 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. The species included 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).

*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, and additionally the most likely mode of evolution of the residuals of that model. To identify the best modes, we compared the Generalized Information Criterion for each of the fits by calculating their relative probabilities (W scores; Burnham & Anderson, 2002). Lastly, we also investigated the most likely evolutionary mode for log-transformed centroid size through functionalities in the *Phytools* package, by calculating W scores of the Akaike Information Criterion (AIC) outputs.

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.

### Visualising shape evolution through phylo-morphological distance plots

As noted above, the sample size available to us (n=37 species) makes estimations of evolutionary modes potentially unreliable. We therefore also visualized change of shape relative to time by plotting 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.

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

In order to visually assess distribution of species in 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.

### Allometry, disparity, modularity and integration

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 analyses can be done after separate GPA/asymmetry removals for each module; however, this risks not capturing allometric size change between modules, which is an important part of our question. We therefore here report the results of allometry analyses of shape versus log-transformed centroid size for each module based on joint GPA and separate GPAs, each with an asymmetry removal step included.

To further contextualise effect sizes of allometry per module, we compared disparity among modules (from a joint GPA) before and after adjustment of size. This was done by computing each module’s disparity and adjusting this value by the number of landmarks. The aim here was to understand how variable individual modules were in the raw dataset, and whether these relationships change after adjustment by size.

The impact of allometry on shape variation was further contextualized by assessing integration (degree of co-variation) and modularity (degree of module independence) across the cranium and between modules. Integration was measured using partial least squares (PLS) correlation coefficients between multiple modules, taking into account phylogeny (Adams & Felice, 2014); 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.

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 between modules in the full *vs* the residual dataset as 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; e.g. 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

*Evolutionary mode*

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.

|  |  |  |  |
| --- | --- | --- | --- |
| DONE | Brownian Motion | Ornstein-Uhlenbeck | Early Burst |
| Shape GIC | -446170 | **-447701** | -446167 |
| Shape W | 0 | **1** | 0 |
| Shape~log(Csize) GIC | -433056 | **-434260** | -433054 |
| Shape~log(Csize) W | 0 | **1** | 0 |
| Shape residuals GIC | -475814 | **-476364** | -475812 |
| Shape residual W | 0 | **1** | 0 |
| log(Csize) AIC | **-4.802** | -2.875 | -2.802 |
| log(Csize) W | **0.572** | 0.218 | 0.21 |

**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. We thus also proceeded with allometric residuals of BM-based allometric models.

### Visualising shape evolution through phylo-morphological distance plots

Our phylo-morphological distance plots (Fig. 1) 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. 1a). 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. 1a); 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. 1b). 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.

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

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 19.8%, 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. 2 a 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. 2c 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. 2b). 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. 2c). Two species of specializing in the consumption of grasses, the broad-toothed rat (*Mastacomys fuscus*) and XXX, are separated by low PC 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).

A group of graphs with different colored squares

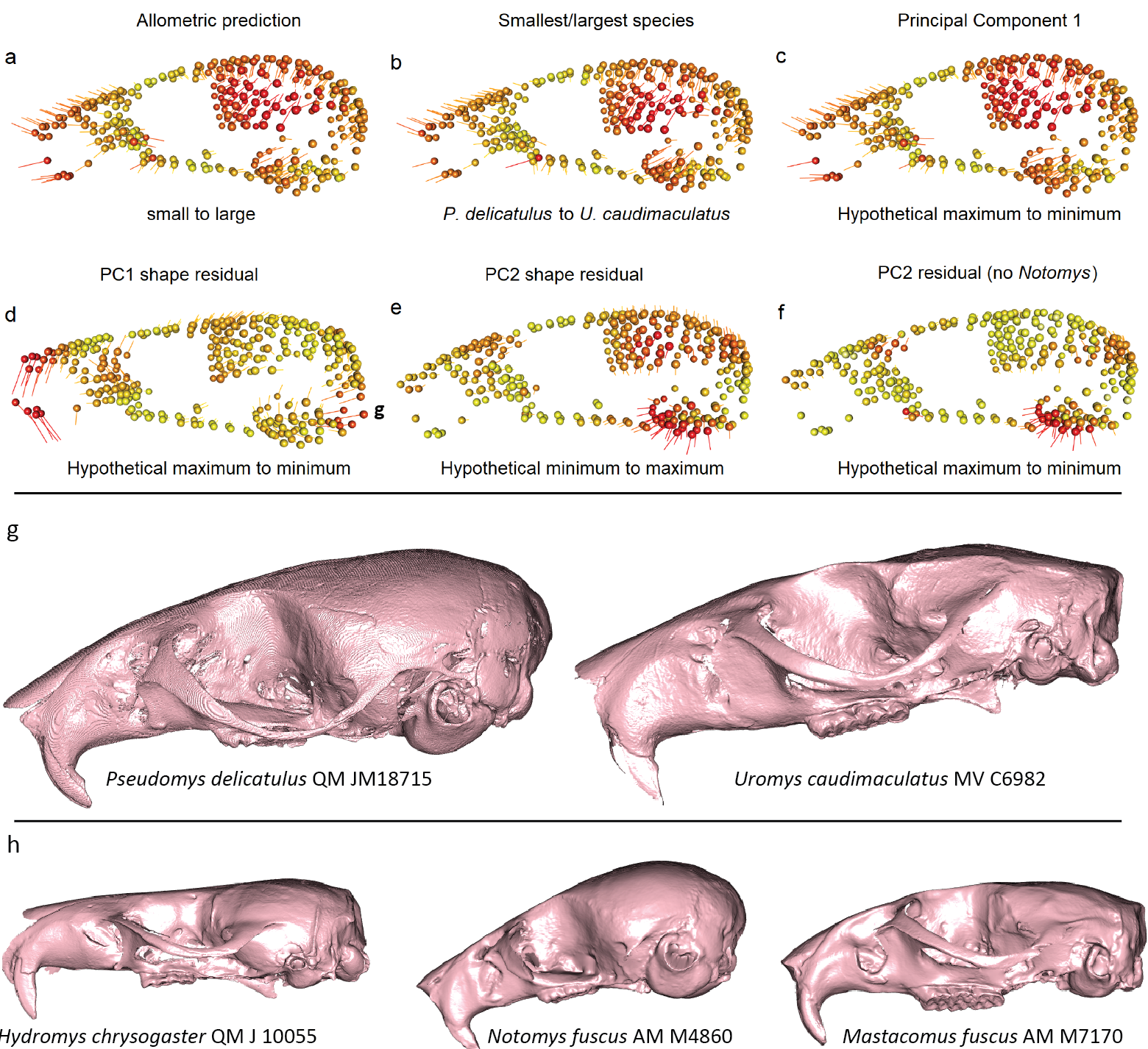
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**Figure 2**. Shape variation related to size and after size removal.a, Plot of PC1 and PC2 for the full shape dataset and b**,** plot of log centroid size versus the projected regression score with a gray regression line indicating the common evolutionary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

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

As expected, a pattern of relatively longer rostra and smaller brain cases with size is apparent in the visualization of shape variation that is associated with allometry (Fig. 3a-c). This is 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. Visually comparing meshes of representatives from the smallest and largest species provides visual confirmation that the allometric and ordinated variation reflects major differences between the crania (Fig. 3g) and is not an artifact of Procrustes superimposition (Klingenberg, 2021).

As expected, removing the shape variation that covaries with size (Fig. 3d-f) also removed the CREA-aligned patterns. Species closer to the PC1 maximum, such as the carnivorous *H. chrysogaster* (Fig. 3h) then show a straighter anterior rostra/incisor regions and dorsoposterior displacement of the foramen magnum (Fig. 3d, h). 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. 3e and mesh in 3h). This coincides with other differences, like variation in the dorsal maxillary region (3e, compare *Notomys* and *Mastcomys* in Fig. 3h). 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. 3f). This indicates that the bipedal hopperd, despite their distinctive morphology, do not dominate the variation in both PCAs.



**Figure 3:** Landmark heatmaps expressing shape variation and examples of meshes. **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.* 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. **g,** comparison between the smallest *versus* largest species in the sample (reflecting the variation seen in 3a-c); **f**, examples of species on extremes of residual PC1 (*H. chrysogaster*), and residual PC1/2 (*N. fuscus, M. fuscus).* Not to scale.

### Allometry, disparity, modularity and integration

The amount of variation explained by size varied widely among modules (Table 2), and had larger effect sizes and R2 valuse in the joint GPA compared to separate GPAs. Surprisingly, the rostral module was revealed as having the least of its variation explained by size, despite its extensive variation predicted by allometric fit heatmaps (Fig. 3); in fact, the association between rostral shape an rostral centroid size just below the significance threshold.

**Table 2:** Phylogenetically adjusted generalized least squares analysis *(PGLS)* of module shape *vs* log-tranformed centroid sizes for joint (top) and separate (bottom) GPAs. SS, sum of squares;

MS, Mean Squares; R2, R-squared value; F – F values; Z, Z scores (effect sizes from F values); *p*, p-value (probability of significant association at *p* < 0.05 based on 10,000 permutations).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | SS | MS | Rsq | F | Z | *p* |
| Joint GPA | | | | | | |
| Basicranium | 0.002 | 0.133 | 0.13 | 5.38 | 3.31 | **0.000** |
| Molar | 0 | 0.09 | 0.09 | 3.47 | 2.7 | **0.002** |
| Orbital | 0.001 | 0.206 | 0.21 | 9.07 | 3.59 | **0.000** |
| Rostrum | 0.003 | 0.074 | 0.07 | 2.79 | 2.29 | **0.007** |
| Vault | 0.01 | 0.263 | 0.26 | 12.49 | 3.79 | **0.000** |
| Separate GPA | | | | | | |
| Basicranium | 0.01 | 0.085 | 0.09 | 3.26 | 2.69 | **0.002** |
| Molar | 0.025 | 0.139 | 0.14 | 5.63 | 3.74 | **0.000** |
| Orbital | 0.027 | 0.196 | 0.2 | 8.51 | 3.37 | **0.000** |
| Rostrum | 0.005 | 0.038 | 0.04 | 1.37 | 1.44 | 0.077 |
| Vault | 0.021 | 0.184 | 0.18 | 7.9 | 4.14 | **0.000** |

Substantial differences in disparity (adjusted by landmark numbers) exist among modules (based on joint GPA). The cranial vault and rostrum contain the greatest disparity, but after size adjustment, the disparity in the vault drops by nearly 60% whereas all other modules experience far less reduction in disparity, such that the rostrum has the greatest disparity after size removal.

**Table 3:** Disparity (measured as Procrustes variance of landmarks divided by number of landmarks) for each module before (Full dataset) and after allometry removal (Residuals), as well as the amount of reduction in disparity as a percentage of the full dataset disparity (% reduction). Disparity values were multiplied by 10,000 for ease of interpretation.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Full dataset | Residuals | % reduction |
| Basicranium | 0.107 | 0.077 | 28 |
| Molar | 0.082 | 0.065 | 20.7 |
| Orbital | 0.103 | 0.067 | 35 |
| Rostrum | 0.114 | 0.08 | 29.8 |
| Vault | 0.149 | 0.063 | 57.7 |

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. 4) 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 and higher CR coefficients of the shape residual dataset.

A diagram of a number of objects

Description automatically generated with medium confidence

**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 (Table 4, lower triangle). Unlike the allometry analyses, this is consistent with the allometric pattern on concomitant rostral elongation with relative reduction of the vault (refer to Fig. 3). 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. Strong co-variation between rostrum and basicranium is notable and was not part of our predictions.

Removal of size resulted in several changes in r-pls values (Table 4, upper triangle), but not all of these related to reduced integration and only some were detected as significant differences in integration strength (Table 5). 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 4:** 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 5:** Integration strength comparisons between pairs of modules, including effect sizes (Z) and significance of strength differences (*p*).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Z full | Z residual | Z difference | *p* |
| basicranium x molar | 3.34 | 2.25 | 0.86 | 0.388 |
| basicranium x orbital | 4.16 | 4.1 | 0.63 | 0.526 |
| basicranium x rostrum | 4.29 | 2.98 | 1.88 | 0.06 |
| **basicranium 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 6, 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 7); 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 6 and the Mantel tests (Table 7).

**Table 6:** 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 7:** 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 |

**Discussion**

In this study, we 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.

Allometry in mammalian crania, and the associated 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). The greater likelihood of an Ornstein-Uhlenbeck (OU) pattern of limited diversification around a local optimum (Harmon *et al.*, 2010) reinforces this impression, particularly because size itself most likely evolves according to BM. However, the OU- mode of evolution is also most likely in our residual dataset, foreshadowing the finds of our downstream analyses that allometric variation is only one manifestation of the overarching impact of biomechanical adaptation on the rodent cranium.

An OU pattern is also suggested by plotting morphological divergences against time, which plateau around 2 million years. Of particular interest here are several “spikes” of morphological divergence where a substantial change in either size or cranial function evolved . In the full shape dataset, this corresponds to the evolution of unique– but allometrically expected - shapes of the large-bodied frugivores (sensu Schluter; Marcy *et al.*, 2020). Changes of lesser magnitude are then revealed by the allometry-free data, where divergences are greatest where the dietary shift to carnivory selected for a fundamentally different masticatory action (Freeman & Lemen, 2008; Satoh & Iwaku, 2006), and where the hopping *Notomys* display a change in the genus-level allometric multidimensional intercept (discussed further below).

The OU-patterned evolution of shape in allometric and allometry-free contexts is also reflected in the fact that the cranium and all but one pair of modules remain significantly and sometimes slightly more integrated after removal of allometry, confirming our expectation that allometric patterns are just one manifestation of cranial adaptation and do not represent a singular constraining process. The extremely high integration between the rostrum and the cranial vault in the full dataset is predicted by CREA, and notable because it supports the hypothesis of Mitchell et al. (2024) that CREA is a mosaic arising from different mechanisms: bite force allometry affecting the rostral area and hypo-allometry of the brain affecting the cranial vault. Consistent with CREA patterns being driven mostly by allometry, the integration between the vault and the rostrum also drops most dramatically after the removal of size. However, the significant integration remaining between all but one module pair after size removal again emphasizes that other sources of covariation clearly shape cranial variation in Australian rodents.

As already discussed in Marcy et al. (2020), the allometric prediction and variation among PC1 extremes support the existence of a CREA pattern of rostral elongation and relative reduction of braincase size. However, the gracilization that is part of the expected CREA pattern is not as apparent in our sample as it is in other mammals (Mitchell *et al.*, 2024) because the rostral elongation coincides with a dorsal expansion of the rostrum. Visual assessment of the cranial meshes in Fig. 3 suggests that this might be a unique feature of rodent cranial allometry related to the ever-growing incisors, which extend much further into the dorsal rostrum compared to other mammals and are known to dominate the rostral shape of rodents (Marcy *et al.*, 2016).

While our ordinations, visualisations of shape change, and integration results are all consistent with the existence of a CREA pattern, a challenge arises through the very low (even non-significant, in separate GPAs) amount of variation in the rostrum that is attributable to size. Interpreting this unexpected result represents a challenge and we can only offer some suggestions here. One possibility is that the visualisations of Procrustes-superimposed landmark variation are victim to the “Pinocchio-Effect”, where variation in the tip of a triangular shape – such as most vertebrate rostra - is exaggerated (summarized in Klingenberg, 2021). However, this should also affect the statistical analyses of the rostral module; moreover, comparisons of cranial meshes demonstrate that the differences in rostral shape between small and large species are real and substantial. It is probably relevant that rostral shape is clearly shaped by more processes (e.g. variation in angle as seen in residual PC1), than the cranial vault whose variation and disparity are strongly driven by size. A strong association between the substantial allometric component of vault variation and low allometric component of rostrum variation would then explain the high integration values and emphasis on rostral shape variation in the visualisations, and also account for the drastically and significantly lower strength of integration between the two after size removal.

The Australian muroids in our sample display relatively few dietary specialisations, which prevented statistical analyses of their influence on cranial variation. However, a focus on the distribution of these species’ cranial shape in the full and residual shape morphospaces is consistent with known hypotheses of mammalian cranial biomechanics. Residual PC1 emphasizes the angle at which the rostrum and incisors are situated relative to the remainder of the cranium. This will result in a changed gape and leverage of incisor biting, an effect known to change gape and bite force in lower jaw of rodents (Freeman & Lemen, 2008). The carnivorous species, whose crania are least adapted to the extensive gnawing action typical of other rodents, have the straightest rostra resulting in widest gape, which is consistent with the benefits of larger gapes in carnivorous rodents (Hennekam *et al.*, 2020; Satoh & Iwaku, 2006; Williams *et al.*, 2009). By contrast, the specialized grass-feeding *Mastacomys* and granivore *Conilurus penicillatus* score low on both the residual PC1 and 2, reflecting curved anterior rostra and overall smaller cranial vaults. This is consistent with finds of 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) and leads to more robust cranial dimensions in this species (Breed & Ford, 2007) and other folivores (Barbero *et al.*, 2023). 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 generalists *Notomys* and *Conilurus* (Murray *et al.*, 1999) might explain their more robust cranial dimensions than expected for their size.

Another, potentially non-biomechanical effect emerging from the ordination of shape residuals is a tendency of the dorsal cranial vault to expand dorsally together with a ventral expansion of the basicranium on PC2, resulting in overall braincase expansion. This may be related to changes in the proportions between cranium and brain size, either through increase or decrease in encephalization (Smaers *et al.*, 2021) or a different distribution of brain tissue within the braincase (Weisbecker *et al.*, 2021). The genera where this effect is most obvious – *Mastacomys* (with a relatively small braincase) and *Notomys* (with a relatively large braincase) indeed have large residuals, despite having slopes of static allometry that are not significantly different from the common 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)

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 stabilizing selection on mastication permits the evolution of specialist postures. A similar pattern is seen in the bipedally hopping genus *Notomys*, whichis second in facial tilt to *Conilurus*. *Notomys* species do not lie on the common allometric line, but this separation is because of their basicranium and vault shape, not their facial tilt. The inclusion of a facial tilt in *Conilurus* and *Notomys* within the common allometric pattern therefore confirms the hypothesis that CREA – patters related to specific parts of the cranium, without representing a constraint on the entire skull.

**Conclusions**

This agrees with the observation that rodents occupy a highly distinct, slowly-evolving area in the morphospace of placental crania (Goswami *et al.*, 2022), and 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). 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.