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Title: The impact of digging on craniodental morphology and integration

Running head: Rodent craniodental morphology

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ABSTRACT

The relationship between the form and function of the skull has been the subject of a great deal of research, much of which has concentrated on the impact of feeding on skull shape. However, there are a number of other behaviours that can influence craniodental morphology. Previous work has shown that subterranean rodents that use their incisors to dig (chisel-tooth digging) have a constrained cranial shape which is probably driven by a necessity to create high bite forces at wide gapes. Chisel-tooth digging rodents also have an upper incisor root that is displaced further back into the cranium compared with other rodents. This study quantified cranial shape and upper incisors of a phylogenetically diverse sample of rodents to determine if chisel-tooth digging rodents differ in craniodental morphology. The study showed that the crania of chisel-tooth digging rodents shared a similar place in morphospace, but a strong phylogenetic signal within the sample meant that this grouping was non-significant. It was also found that the curvature of the upper incisor in chisel-tooth diggers was significantly larger than in other rodents. Interestingly, most subterranean rodents in the sample (both chisel-tooth and scratch diggers) had upper incisors that were better able to resist bending than those of terrestrial rodents, presumably due to their similar diets of tough plant materials. Finally, the incisor variables and cranial shape were not found to covary consistently in this sample, highlighting the complex relationship between a species' evolutionary history and functional morphology.

Keywords: Subterranean rodents; cranial evolution; geometric morphometrics; phylogenetic comparative methods

INTRODUCTION

The relationship between the form and function of the skull and teeth in vertebrates is highly complex and has been the subject of much study over the years (e.g. Moss & Young, 1960; Herring, 1993; Weishampel, 1993; Preuschoft & Witzel, 2004). A great deal of research has focused on the process of feeding, and has sought to determine how the forces imposed by mastication shape the cranium and mandible (e.g. Maynard Smith & Savage, 1959; Turnbull, 1970; Herring & Teng, 2000; Cox, 2008). However, there are a number of other factors that can also influence the form of the skull, such as the relative size of the brain, the size and orientation of the eyes, the environment in which an animal lives, and any non-masticatory behaviours performed by the skull. Examples of such behaviours include: head-butting in goats (Farke, 2008), dam construction in beavers (Cox & Baverstock, 2016), and digging with the teeth in subterranean rodents (Samuels & Van Valkenburgh, 2009).

Subterranean rodents are a specialized group of rodents that live almost exclusively underground, and as such experience very different selection pressures to terrestrial rodents. For instance, burrowing underground requires 360-3400 times the energy of moving a similar distance above ground (Vleck, 1979; Jarvis & Bennett, 1991). This extent of energy expenditure has required the evolution of efficient methods of soil excavation. The majority of subterranean rodents show one of two types of digging method: chisel-tooth digging, using the incisors, and scratch digging, which uses only the forelimbs (Hildebrand, 1985). The ability to use incisors for digging has allowed chisel-tooth digging rodents the freedom to exploit harder soils (Lessa & Thaeler, 1989; Lessa, 1990).

Chisel-tooth digging evolved independently a number of times, including at least once in each of the six extant families of subterranean rodents (Stein, 2000). It has been noted that chisel-tooth digging rodents tend to converge in cranial shape, showing larger temporal fossae; wider and taller crania; enlarged zygomatic arches; longer rostra; more procumbent incisors and incisors that are more resistant to bending stresses (Landry, 1957; Agrawal, 1967; Samuels & Van Valkenburgh, 2009; Becerra *et al.*, 2012, 2013, 2014; McIntosh & Cox, 2016; Gomes Rodrigues *et al.*, 2016). It is thought that many of these morphological features improve bite force and gape in subterranean rodents that use their incisors to dig in hard soils (Becerra *et al.*, 2013; McIntosh & Cox, 2016; Gomes Rodrigues *et al.*, 2016).

An extensively studied modification in chisel-tooth digging rodents is the increase in upper incisor procumbency, that is, the angle of anterior projection of the upper incisors (Landry, 1957; Agrawal, 1967; Lessa & Thaeler, 1989; Lessa & Patton, 1989; Vassallo, 1998; van der Merwe & Botha, 1998; Mora *et al.*, 2003; McIntosh and Cox, 2016). Vassallo (1998) hypothesised that this increased procumbency allows for a more favourable angle of attack for breaking hard soils. Incisor procumbency in rodents is governed by overall curvature of the incisor and its placement within the rostrum (Landry, 1957; Akersten, 1981). Landry (1957) pointed out that to keep the incisor in its functional plane, the only way the procumbency can change without altering incisor morphology (incisor curvature) is by raising or lowering the posterior end of the incisor. However, rostral space in rodents is nearly completely occupied by the incisor (Fig. 1), and so this type of movement would not be possible (Landry, 1957).

The rodent cranium is a complexly integrated structure (Hallgrímsson *et al.*, 2009) and understanding how different structures covary within the cranium could potentially explain morphological diversity in some clades and constraints in others. Covariation of incisor morphology and cranial shape has never been studied in rodents, and as the incisor takes up such a large space within the craniofacial structure of rodents, it is potentially an underlying factor in chisel-tooth digging cranial convergence.

In order to address this issue, we will quantify incisor morphology in a diverse number of rodents from both terrestrial and subterranean habitats to show how chisel-tooth digging influences incisor morphology. Secondly, we will quantify cranial shape and attempt to verify the findings of Samuels & Van Valkenburgh (2009) using phylogenetic comparative methods (Felsenstein, 1985; Rohlf, 2001). Finally, we will assess how incisor morphology and cranial shape covary and determine the extent of morphological integration between the upper incisor and cranium in rodents.

MATERIALS AND METHODS

This study analysed 65 adult crania from a diverse group of rodents representing 20 genera and 11 families: Bathyergidae, Caviidae, Cricetidae, Ctenomyidae, Dipodidae, Erethizontidae, Geomyidae, Muridae, Octodontidae, Sciuridae and Spalacidae (Table 1). The study focuses on the impact of chisel-tooth digging on craniodental morphology. However, other factors such as diet, habitat and phylogeny have been shown to influence cranial and incisor morphology in rodents (Samuels, 2009; Croft *et al.*, 2011; Hautier *et al.*, 2012). In order to account for these potential additional influences, the sample contains phylogenetically distant subterranean and terrestrial rodents with different ecologies (Table 1). The specimens were scanned on an X-Tek Metris microCT scanner at the University of Hull (Medical and Biological Engineering Research Group). The resulting scans had isometric voxels with dimensions ranging between 0.01 mm and 0.07 mm.

Surface reconstructions of crania and incisors were created from the microCT scans in Avizo 8.0 (FEI, Hillsboro, Oregon). Two measures determining incisor shape (Landry, 1957) were calculated: incisor length (straight line distance from apex to tip: **a** in Fig. S1); and incisor radius of curvature (RoC), which was derived using Heron's formula (Fig. S1) from a circle fitted to three points along the dorsal midline of the incisor surface (at the apex, tip and most dorsal point of the curve). Following Lin *et al.* (2010), this method assumed that incisor growth is circular, although technically rodent incisors grow helically (Herzberg & Schour, 1941). The relationship between incisor length and RoC was assessed using ordinary least squares (OLS) regression, conducted in R (R Foundation for Statistical Computing, Vienna), to determine the overall shape of the incisor. If the gradient of the regression line fitted through the origin is 0.5, then the length is double the radius of curvature and thus the incisor is a semicircle.

Rodent incisors are long relative to their diameter (i.e. take up a large proportion of a circle) and so can be affected by bending stresses (Bacigalupe *et al.*, 2002). Second moment of area (SMA) is a geometric measurement that defines the resistance to bending of a cross-section of an object and is a good indicator of structural strength (Alexander, 1983), a potentially important property for incisors used to dig through hard soils. The SMA of each incisor at its midpoint cross-section was calculated using the BoneJ plugin (Doube *et al.*, 2010) in ImageJ (Schneider *et al.*, 2012).

The sample included a large range of body masses (*Paralomys* can be as small as 12 g, whereas *Bathyergus* can grow up to 2 kg; Nowak, 1999) and past studies on rodent incisors have shown that variables such as SMA correlate strongly with size (e.g. Verzi *et al.*, 2010). Thus, condylobasal cranial length was included as a covariate to account for scaling. Incisor morphology variables and cranial length were logged in all analyses due to size differences and the necessity to linearize variables to carry out statistical procedures.

As closely related species tend to be more similar to each other than to more distantly related species they cannot be considered as completely independent units (Felsenstein, 1985; Garland *et al.*, 2005), a prerequisite for standard statistical tests. Phylogenetic Generalized Least Squares (PGLS; Grafen, 1989; Martins & Hansen, 1997) was performed to show if any relationship between size and incisor morphology existed after phylogenetic information was incorporated into the analysis.

It is possible to test how much phylogenetic signal is present in the data, a statistical procedure that quantifies the expected covariation of species traits under a selected evolutionary model (e.g. Brownian motion) on a phylogeny (for review see Blomberg & Garland, 2002). For the univariate analyses in this study, Pagel's λ (Pagel, 1999) was used to estimate the phylogenetic signalling in the data. Pagel's λ is a scaling parameter that measures the correlation of traits relative to expected correlation under a Brownian motion model of evolution. Normally, λ ranges from zero (no phylogenetic signal and data is equivalent to a "star" phylogeny) to one (data consistent with selected phylogenetic tree under a Brownian motion model of evolution) or beyond (the evolutionary process is more orderly than Brownian motion). Pagel's λ and PGLS regressions in this study are quantified simultaneously using the method proposed by Revell (2010). Phylogenetic ANCOVA models using PGLS (to test for differences in incisor RoC relative to cranial length between chisel-tooth diggers and non-tooth diggers [including non-fossorial rodents]) were fitted to genus means of the sample using the nlme (Pinheiro *et al.*, 2016) and ape (Paradis *et al.*, 2004) packages in R. The phylogeny used in all analyses was modified from Fabre *et al.* (2012), with branch lengths in millions of years.

The cranial shape of each specimen was quantified using 3D landmark coordinates (*Geomys* was not included in this part of the study due to extensive damage of the cranium). 29 homologous landmarks were recorded from each cranial reconstruction using Avizo (Fig. 2 and Table S1). From this landmark data, variation in the shape of the crania was analysed with geometric morphometrics (for review see O'Higgins, 2000). The landmark co-ordinates were subjected to the Procrustes method of generalized least squares (GLS) superimposition (Rohlf & Slice, 1990). A principal component analysis (PCA) of genus-averaged Procrustes coordinates revealed the greatest axes of shape variation within the sample. The variance encompassed by the first ten axes is given in Table S2. Surface warps of the extreme ends of the principal components axes were also included to visualise the shape variation within the data. As Pagel's λ cannot be estimated accurately for multivariate data (Adams, 2014a), phylogenetic signal in the data was quantified by calculating the K statistic (Blomberg *et al.*, 2003), generalized to accept multivariate shape data (Adams, 2014a). Although the K statistic and λ statistic are derived differently (K is a scaled ratio of variance and λ is a scaling metric) their outcomes are normally similar i.e. <1 implies data has less phylogenetic signal than expected under Brownian motion and >1 has more phylogenetic signal than expected under Brownian motion.

A phylogenetic principal components analysis (pPCA) was also performed on the Procrustes coordinates. This analysis centres the data on the ancestral root of a phylogeny ("phylogenetic mean") and extracts principal components from the variance covariance matrix informed by phylogenetic propinquity, so that the major axes represent the major features of shape variation in the evolutionary process (Revell, 2009; Polly *et al.*, 2013; but see Uyeda *et al.*, 2015). To calculate surface warps associated with extremes of pPC axes, an average surface calculated from Procrustes coordinates is warped to an ancestral state reconstruction at the root of the phylogeny (Yang *et al.*, 1995). Appropriately scaled eigenvectors from the corresponding pPC were then used to show the shape differences along the pPC axes. The rationale for including both PCA and pPCA analyses is that PCA gives information about the distribution of the taxa in shape space, whereas the pPCA reveals the important morphological variation in the evolution of this group of rodents.

To test for differences between skull shapes of chisel-tooth diggers and non-tooth diggers, we used Procrustes ANOVA (analysis of variance) as well as phylogenetically informed Procrustes ANOVA in a phylogenetic framework under a Brownian motion model of evolution (Adams, 2014b). In this procedure, sum of squares (SS) is measured based on the SS of Procrustes distances among specimens (see Goodall, 1991), which is equivalent to a distance-based ANOVA design (Anderson, 2001). GLS superimposition, phylogenetic signal testing, principal components analysis, ANOVA and surface warps were processed using the geomorph package in R (Adams & Otárola-Castillo, 2013), and pPCA was performed using the phytools package in R (Revell, 2012).

Morphological integration and covariation of biological forms has been extensively studied using geometric morphometrics and partial least squares (PLS) (e.g. Rohlf & Corti, 2000; Bookstein *et al.*, 2003; Bastir *et al.*, 2005; Hautier *et al.*, 2012; Klingenberg, 2014). PLS quantifies the maximum amount of covariation between two sets of variables, using a correlation or covariance (for geometric morphometric studies) matrix of traits (Rohlf & Corti, 2000). This sets it apart from regression analysis which determines whether variation in the independent variable(s) can predict variation in the dependent variable. In this study, one set of variables contained the Procrustes coordinates of cranial shape. The second set of variables was the incisor measurements, incisor RoC and SMA. Generalized Procrustes analysis removes variation due to isometric scaling from the cranial shape variables, but retains allometric shape variation (Drake, 2011). Multivariate regressions of Procrustes co-ordinates on log-transformed centroid size were performed to assess the effects of allometry on cranial shape variation. Size was removed from the incisor variables when measuring covariation between cranium and incisors. Cranial length was used as the independent variable to regress against incisor variables. Regression was carried out in the PGLS framework to obtain the allometric regression vector (Revell, 2009), and residuals for taxon averages were calculated from this vector. The residuals were then used in PLS analyses with cranial shape variables to measure covariation. Multivariate regressions of cranial shape on size were performed in the geomorph package in R (Adams & Otárola-Castillo, 2013). Regressions of incisor variables on cranial length were performed in the phytools package in R (Revell, 2012).

As in the methods above, any inter-generic analysis must also account for the non-independence of the data. Incorporating phylogeny whilst quantifying morphological integration at the inter-generic level shows how morphological covariation has evolved along a tree (Klingenberg & Marugán-Lobón, 2013). Phylogenetic PLS is calculated by incorporating the evolutionary covariance matrix from PGLS to calculate PLS scores (Adams & Felice, 2014). The strength of association between cranial and incisor variables is quantified using the RV coefficient (Klingenberg, 2009). RV coefficient ranges from 0 (variables are independent) to 1 (variables are dependent). All statistical tests of covariation and association were carried out using phylogenetic PLS. However, non-phylogenetic PLS was also carried out in order to visualise morphological variation along the PLS axes, using surface warps. Phylogenetic and non-phylogenetic PLS analyses, accompanying surface warps and RV coefficient calculations were implemented in the geomorph package in R (Adams & Otárola-Castillo, 2013).

RESULTS

The relationship between upper incisor length and upper incisor RoC in each genus is displayed in Fig. 3A. Incisor shape can be changed by RoC, or by the proportion of the circle taken up by the incisor (represented by incisor length). OLS model fitted to origin (Fig. 3A) shows a positive relationship between the two variables). The gradient of the line was nearly half (0.52), which means that every upper incisor analysed in the sample was approximately semicircular in shape. As shape change was found to be so limited, all further analyses concentrated on incisor size, as represented by radius of curvature.

Fig. 3B shows the relationship between cranial length and upper incisor RoC. Firstly, to test for homogeneity between slopes (a prerequisite for ANCOVA models), an ANCOVA was conducted which included the interaction term between log cranial length (covariate) and digging method (categorical-variable). This was not significant ($P>0.05$) showing that an ANCOVA model is suitable to apply to our data. Generalized phylogenetic ANCOVA revealed that chisel-tooth digging rodents have a significantly larger upper incisor RoC ($P<0.01$) than other rodents. Phylogenetic signal in this data, measured simultaneously with PGLS model using λ, was 0.60, which is significantly different from the null hypothesis of a star phylogeny ($P<0.01$).

Fig. 3C shows the relationship between cranial length and SMA of the upper incisors. Interaction between log cranial length (covariate) and digging method (categorical variable) was significant (*P*<0.01). This meant an ANCOVA could not be applied to the data as the slopes were not similar between groups. PGLS was applied to the data instead and the residuals were examined to show the relationship between the data (Fig. 3D). The residuals of the PGLS of cranial length and upper incisor SMA show that not only do the chisel-tooth digging rodents have a relatively larger upper incisor SMA, but so do the other subterranean rodents compared to the terrestrial taxa (see Table 1 for groupings). Phylogenetic signal in this PGLS analysis was 0.51 (*P*<0.01).

Fig. 4 represents some of the variation in cranial shape using PCA (Fig. 4A) and pPCA (Fig. 4B), with both method showing a very similar pattern of shape variation. However, phylogenetic signal in the data is significant (K_{mult}=0.49, *P*<0.01). It can be seen that most of the chisel-tooth digging rodents group in the same part of the subspace (towards negative end of PC1). The only chisel-tooth digging rodent that departs from the group is *Spalacopus* (which lies positively on PC1 with respect to the rest of the chisel-tooth digging group). The non-tooth digging rodents do not group tightly and are spread over different parts of the subspace. A Procrustes ANOVA indicates that chisel-tooth and non-tooth digging groups can be distinguished in morphospace (*F*=3.57, *P*<0.01). However, a Procrustes ANOVA incorporating the phylogeny leads to non-significance between groups (*F*=2.25, *P*>0.05), unsurprising given the significant amount of phylogenetic signal in the data. Shape variation across the two PC axes is represented by the warps on the extremes of the pPC axes. Positive pPC1 scores are associated with a longer skull with a narrow, straight-sided rostrum. More negative pPC1 scores are associated with a shorter skull and a more tapered rostrum. In general, pPC2 separates chisel-tooth digging taxa from the other genera (although *Spalacopus* is again separated from the other chisel-tooth diggers). Cranial morphology varies along this axis from curved crania with short rostra (negative pPC2, chisel-tooth diggers) to flatter crania with more elongated rostra (positive pPC2, non-tooth diggers). Multivariate regressions of Procrustes co-ordinates on centroid size were not significant, indicating that allometry did not have an important effect in either the original or the phylogenetically corrected analyses.

The surface warps associated with the non-phylogenetic PLS (Fig. 5) indicate that, in this sample, elongated, narrow crania and wide rostra are associated with relatively small incisors with low SMA (negative PLS1) – these are largely non-tooth digging genera. Shorter, wider crania with narrow rostra are associated with relatively large incisors with high SMA. These are found at the positive end of cranial and incisor PLS1 and are mostly chisel-tooth digging rodents. It is particularly notable that, in this sample, bathyergids and spalacids appear to be covarying in a similar manner. However, using a phylogenetic PLS analysis, the covariation between cranial and incisor morphology was not found to be significant.

DISCUSSION

This study has shown that there is a clear correlation between size (RoC) of the upper incisor and digging method in rodents (Fig. 3B), although there is also a moderate phylogenetic signal in the data ($\lambda = 0.60$). Despite the seemingly complicated relationship between phylogeny and ecology in the evolution of incisor RoC, it is clear that chisel-tooth digging rodents have acquired a larger incisor RoC for their size compared to rodents that do not use their incisors to dig. Landry (1957) assessed upper incisor RoC in a phylogenetically diverse group of rodents and concluded that a large upper incisor RoC (and arc length) is required to improve upper incisor procumbency. McIntosh & Cox (2016) showed that, within the

Bathyergidae, chisel-tooth digging species have a craniomandibular morphology that facilitates a wide gape. A wide gape coupled with more procumbent incisors gives a more effective angle of attack (Mora *et al.*, 2003) and enables the incisor tip to be in contact with the soil throughout a complete gape motion, hence removing a larger amount of soil relative to a rodent with less procumbent incisors.

Increasing procumbency via an increase in the RoC of the upper incisor requires the root of the incisor to be further displaced into the pterygoid region of the skull. The cranium is a complex structure which plays host to the brain and other sensitive sensory structures, and as the cranium is highly integrated (Cheverud, 1982; Hallgrímsson *et al.*, 2007; Klingenberg & Marugán-Lobón, 2013), any cranial morphological change could have an effect on these systems. Alternatively, increasing incisor procumbency could be achieved by moving the incisor root forward whilst keeping the RoC constant, and decreasing the arc length of the incisor (see Landry, 1957, for further discussion). This would mean the root of the incisor would not be required to expand further back into the skull. Another strategy could be to increase the length of the rostrum to incorporate the larger incisor, as seen in species of *Ctenomys* (Mora *et al.*, 2003), but this would result in a loss of mechanical efficiency of the major masticatory muscles (McIntosh & Cox, 2016). Long incisors originating further back in the skull, as seen in chisel-tooth diggers, may well be an adaptation for the use of incisors for digging in hard soils. An elongated incisor within the rostrum gives a larger surface area in contact with the skull that can then dissipate the larger forces generated at the tip during chisel-tooth digging (Landry, 1957; Becerra *et al.*, 2012).

SMA, an indicator of bending strength, did not correlate in the same way as incisor RoC. Firstly, studying the residuals of the analysis (Fig. 3D), it is clear that this variable does not show differences between chisel-tooth digging rodents and non-tooth digging rodents. Instead, it seems that the difference lies between the subterranean and terrestrial rodents. The subterranean rodents have a larger incisor SMA for their size compared with the terrestrial rodents. For example, one of the (relatively) largest incisor SMAs was measured in *Geomys*, a subterranean, scratch digging rodent. Subterranean rodent diets are mostly made up of geophytes and other subterranean plants, which tend to be hard and fibrous materials (see Busch *et al.*, 2000). Therefore, it appears that subterranean rodents have adapted to resist the increased pressure at the incisor tip due to their hard food diet by making the incisor more resistant to bending. Incisor morphology has been shown to strongly correlate with diet in caviomorph rodents (Croft *et al.*, 2011). We propose that subterranean rodent incisors are resistant to bending due to their hard food diets, but chisel-tooth digging rodents also have an adaptation to deal with the additional forces exhibited during incisor digging in hard soils by lengthening their incisors to dissipate these forces.

It is clear from examining both PCA and pPCA plots (Figs. 4A and B, respectively) that cranial shape has significantly converged in chisel-tooth diggers. The tight grouping of chisel-tooth digging rodents shows homoplasy (Polly *et al*., 2013), as phylogenetically distant spalacids and bathyergids group closely together, even after phylogenetic information has been incorporated in the pPCA plot. The exception to this trend is *Spalacopus* which, although a chisel-tooth digging rodent with a relatively large incisor RoC (Fig. 3B), does not have a similar cranial shape to other chisel-tooth diggers. The cranium of *Spalacopus* is more rounded with a wider rostrum than that of the bathyergid and spalacid chisel-tooth diggers. This could be due to the arrangement of the incisors in the cranium of *Spalacopus*, which are located in alveolar sheaths that are lateral to the cheek teeth and thus avoid the internal cranial space (Lessa, 1990). Other chisel-tooth diggers do not have this lateralization of the alveolar sheath and incorporate the incisor alveolus into internal cranial spaces, potentially constraining cranial shape. However, only one specimen of *Spalacopus* was available for this study, and further research on a greater number of specimens is needed to address this issue thoroughly.

Convergence of cranial shape with digging methods has already been shown in rodents (Samuels & Van Valkenburgh, 2009) and was also found in this study (Procrustes ANOVA, $P<0.01$). However, Samuels & Van Valkenburgh (2009) did not take into account phylogenetic similarity between species. In this sample, the phylogenetic signal of cranial shape was significant ($K=0.49$, $P<0.01$). This was a surprising result given the amount of convergence of chisel-tooth digging crania shown in the morphospace in both principal component methods (Figs. 4A and B) and the fact that chisel-tooth digging has arisen independently at least three times in the sample used (Fig. 3D). However, when phylogeny of the data is included in the analysis, chisel-tooth digging crania are not dissimilar to non-tooth digging crania (phylogenetically informed Procrustes ANOVA, $P>0.05$). This result is likely driven by the phylogenetic distribution of chisel-tooth diggers within the sample. Eight of the nine chisel-tooth digging genera belong to one of two families, the Bathyergidae and the Spalacidae). The remaining genus, *Spalacopus*, does not converge on the same cranial morphology of the other chisel-tooth diggers. Phylogenetic comparative methods reduce the weighting of taxa that are more closely related relative to taxa that are phylogenetically more distant. So, despite the large phylogenetic distance between bathyergids and spalacids (two families whose last common ancestor may have been in the Cretaceous; Fabre *et al.*, 2012), the sample here may not have been wide enough to pick up on any biological signal.

The multivariate regression of Procrustes co-ordinates on log centroid size did not find a significant allometric component to the variation of the cranial shape in the sample. This was true whether or not phylogenetic information was incorporated into the analysis. This result is in direct contradiction to Gomes Rodrigues *et al*. (2016) who found a high significance (P<0.001) in the regression of cranial shape component on log centroid size. However, it should be noted that the sample of Gomes Rodrigues *et al*. (2016) was taken exclusively from the Ctenohystrica, whereas the sample here covers all parts of the rodent family tree. Alternatively, the difference between our result and that of Gomes Rodrigues *et al*. (2016) may simply be a lack of statistical power as a consequence of the relatively low sample size in our analysis.

When phylogenetic information was incorporated into the PLS analysis, the covariation between cranial and incisor morphology was not found to be significant. This result indicates that, from an evolutionary perspective, cranial and incisor covariation is not consistent, even though from a strictly morphological perspective, there appears to be some association between incisor and cranial form (Fig. 5). The fact that chisel-tooth digging spalacids and bathyergids group fairly tightly in the non-phylogenetic PLS plot (Fig. 5) suggests that, at least in these two families, tooth digging may be driving the convergent evolution of both large, wide incisors and short, wide crania, perhaps to resist the high forces generated by tunnelling. However, the lack of significant association in the phylogenetic PLS analysis suggests that the incisor and cranium do not covary over evolutionary time across all rodents, and may in fact be separate modules (although, as above, this may be a result of low statistical power owing to the relatively limited sample size). Such modularity has been proposed to provide evolutionary flexibility in the face of different functional pressures (Wagner & Altenberg, 1996; Kirschner & Gerhart, 1998), which may explain how chisel-tooth digging has been able to evolve independently in several families of subterranean rodents.

In summary, this study has shown that digging method is associated with skull morphology in rodents. Chisel-tooth digging clearly imposes a set of functional demands that have led to the convergent evolution of particular cranial and incisor morphologies. However, covariation between the incisor and cranium is not consistent throughout the rodents, suggesting that these two elements may not be tightly integrated, and may in fact be able to respond flexibly to different selection pressures. Overall, the results indicated a complex interplay between phylogeny and function driving the evolution of skull and tooth shape in rodents.

DATA ACCESSIBILITY

All numerical data deposited at http://figshare.com/authors/Philip_Cox/6179885

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Table 1. List of genera analysed including specimen number, diet and mode of digging. Abbreviations for dietary categories: O, omnivore; GH, generalist herbivore; SH, specialist herbivore. Dietary categories follow method of Samuels (2009). **Subterranean rodent genera are in bold**.

Family	Genus #	Genus	N	Diet	Digging mode
Bathyergidae	1	***Bathyergus***	10	SH	Scratch
	2	***Cryptomys***	1	SH	Chisel-tooth
	3	***Fukomys***	9	SH	Chisel-tooth
	4	***Georychus***	3	SH	Chisel-tooth
	5	***Heliophobius***	10	SH	Chisel-tooth
	6	***Heterocephalus***	5	SH	Chisel-tooth
Caviidae	7	*Cavia*	2	SH	
Cricetidae	8	*Paralomys*	1	GH	
Ctenomyidae	9	***Ctenomys***	1	SH	Scratch
Dipodidae	10	*Dipus*	1	GH	
Erethizontidae	11	*Erethizon*	1	SH	
Geomyidae	12	***Geomys***	1	SH	Scratch
	13	***Thomomys***	1	SH	Scratch
Muridae	14	*Rattus*	2	O	
Octondontidae	15	*Octodon*	1	GH	
	16	***Spalacopus***	1	SH	Chisel-tooth
Spalacidae	17	***Cannomys***	1	SH	Chisel-tooth
	18	***Rhizomys***	3	SH	Chisel-tooth
	19	***Tachyoryctes***	4	SH	Chisel-tooth
Sciuridae	20	*Sciurus*	7	O	

FIGURES

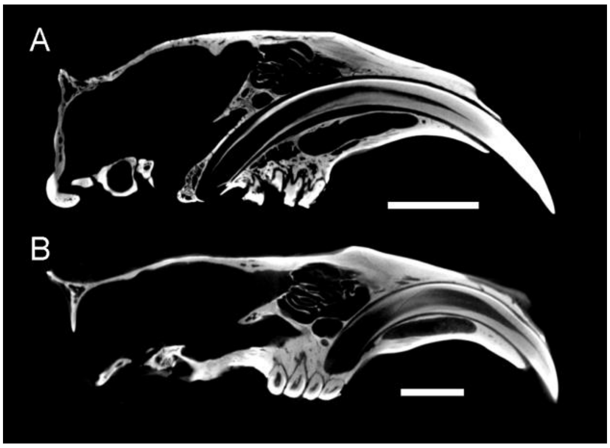


Fig. 1. Parasagittal slice of CT scan in two subterranean rodents: A, chisel-tooth digging *Georychus capensis*; and B, scratch digging *Bathyergus suillus*. Notice the posterior displacement of the incisor root in *Georychus capensis* compared with *Bathyergus suillus*. Scale bars = 10 mm.

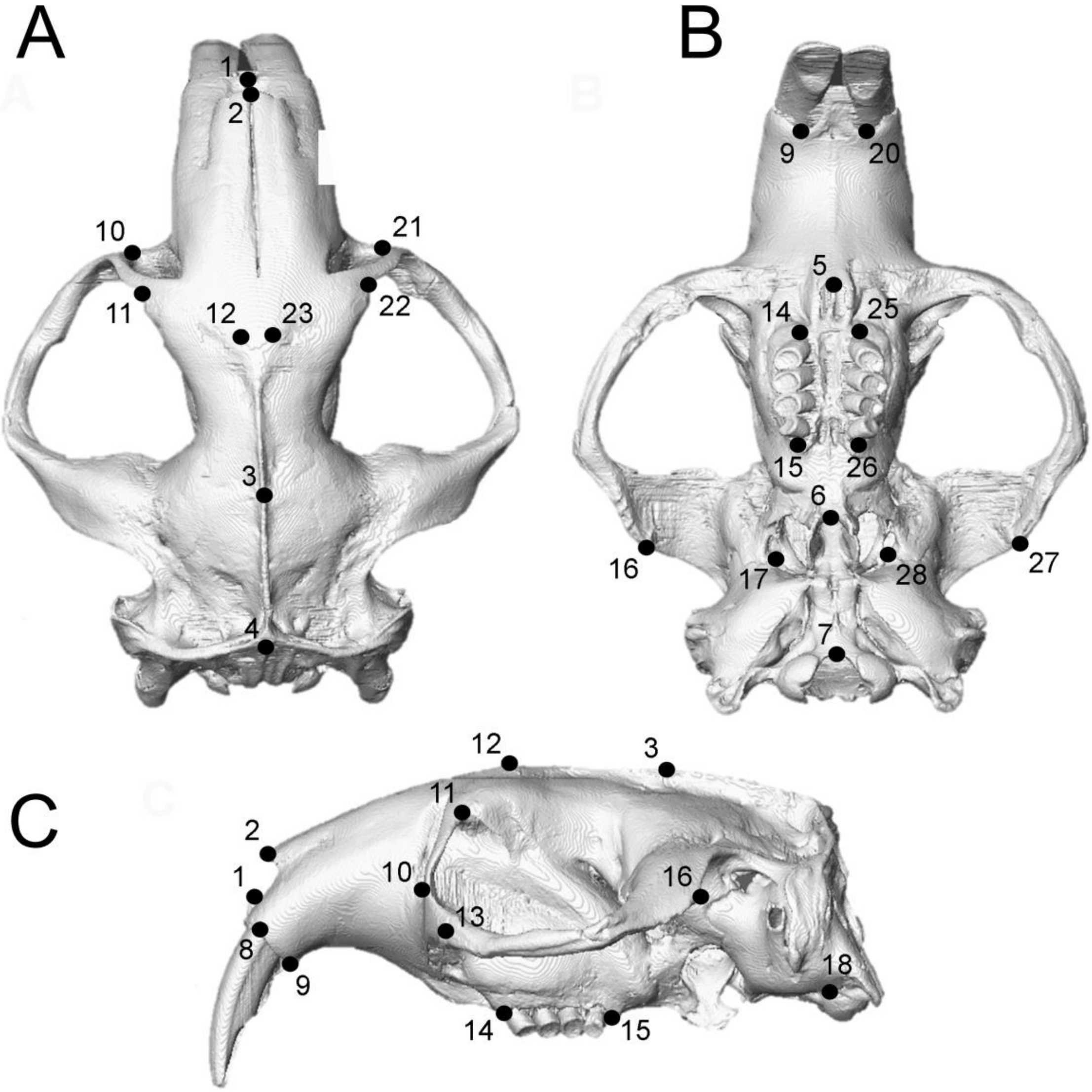


Fig. 2. Landmark configuration represented on *Fukomys mechowi*. A, dorsal view. B, ventral view. C, lateral view. See Table S1 for corresponding landmark numbers and descriptions.

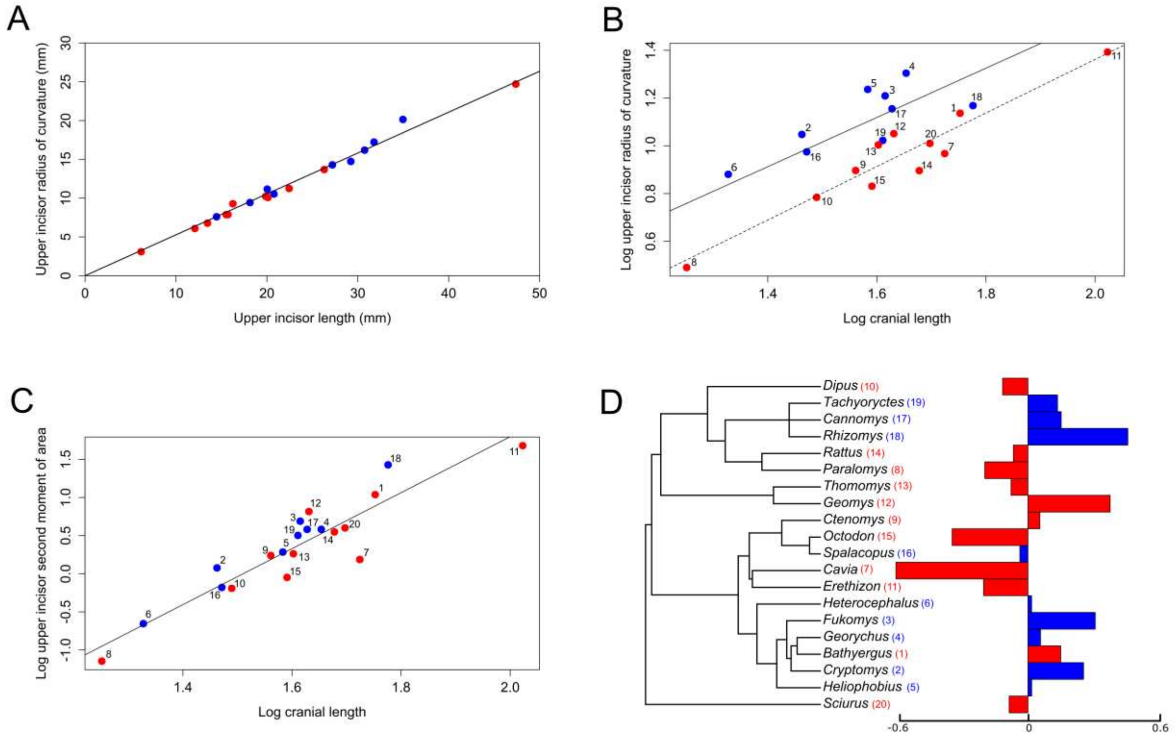


Fig. 3. A, OLS model fitted through origin showing the relationship between upper incisor length and upper incisor RoC. B, Phylogenetic ANCOVA representing the relationship between cranial length, upper incisor RoC and digging method. C, PGLS representing the relationship between cranial length and upper incisor second moment of area. D, Phylogeny of data with accompanying SMA residual values from PGLS of cranial length and upper incisor SMA. Chisel-tooth digging genera are in blue. Non-tooth digging genera are in red (including non-fossorial species). Corresponding genus numbers are given in Table 1.

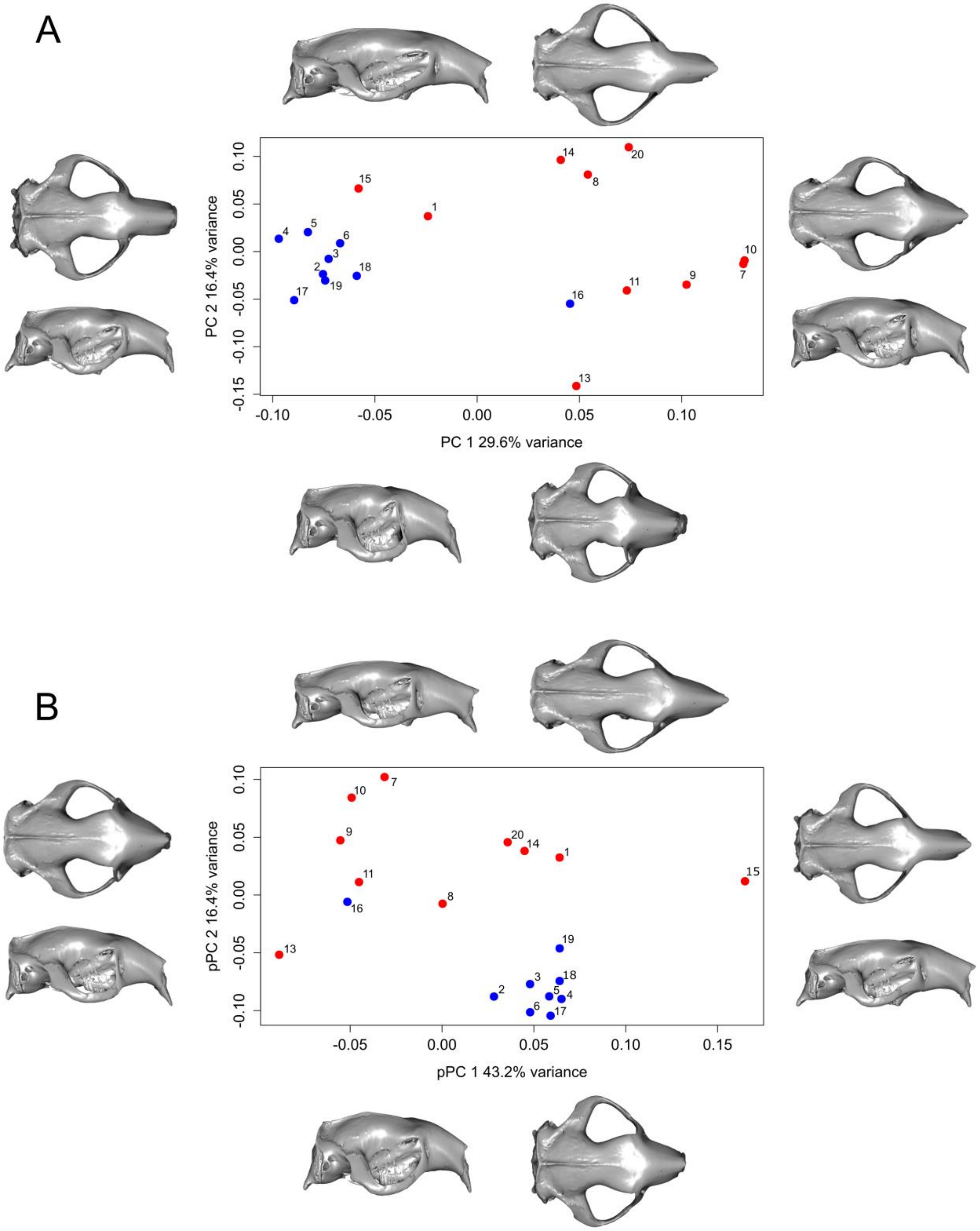


Fig. 4. A, Principal components analysis with associated virtual deformations representing shape variation at the extreme ends of PC1 and PC2. B, Phylogenetic principal components analysis with associated virtual deformations representing non-phylogenetic shape variation at the extreme ends of pPC1 and pPC2 axes. Chisel-tooth digging genera are in blue. Non-

tooth digging genera are in red (including non-fossorial species). Corresponding genus numbers are given in Table 1.

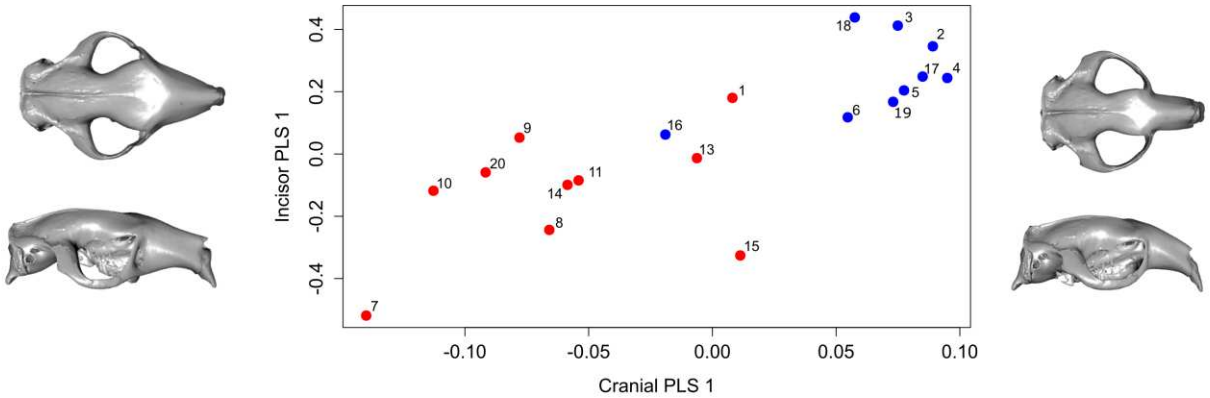
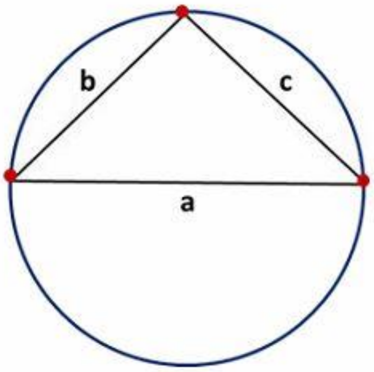


Fig. 5. Partial least squares analysis showing cranial shape and incisor covariation across PLS1 (accounts for 93.7% squared total covariance). Virtual deformations of the cranium shown at the extreme ends of cranial PLS1. Chisel-tooth digging genera are in blue. Non-tooth digging genera are in red (including non-fossorial species). Corresponding genus numbers are given in Table 1.

SUPPORTING INFORMATION



$$\text{RoC}= \frac{abc}{\sqrt{(2a^2b^2 + 2b^2c^2 + 2c^2a^2 - a^4 - b^4 - c^4)}}$$

Figure S1. Measuring incisor RoC. The length of the incisor (L) is measured using the base of the triangle (a). Red points represent the 3 landmarks placed on the surface of the incisor.

Table S1. Cranial landmarks used in geometric morphometric analysis.

Number	**Landmark definition**
1	Midpoint of ventral margin of nasal opening
2	Anteriormost point on internasal suture
3	Bregma
4	Posteriormost point on dorsal midline
5	Midpoint between anterior extremities of incisive foramina
6	Posteriormost midline point on palatine
7	Midline point on ventral margin of foramen magnum
8 & 19	Dorsalmost point on incisal alveolar margin
9 & 20	Posteriormost point on incisal alveolar margin
10 & 21	Lateralmost point on margin of infraorbital foramen
11 & 22	Dorsalmost point on orbital margin
12 & 23	Posteriormost point of naso-frontal suture
13 & 24	Anteriormost point of maxillo-jugal suture
14 & 25	Anterior extremity of cheek tooth row
15 & 26	Posterior extremity of cheek tooth row
16 & 27	Posterior tip of zygomatic arch
17 & 28	Posteriormost point of foramen ovale
18 & 29	Lateralmost point of hypoglossal foramen

Landmarks 1-7 recorded from midline, landmarks 8-18 recorded on left side of skull, landmarks 19-29 recorded on right side of skull.

Table S2. Variance represented by first 10 principal components of non-phylogenetically informed and phylogenetically informed analyses.

Principal component	Non-phylogenetic		Phylogenetic	
	Percentage variance	Cumulative variance	Percentage variance	Cumulative variance
1	29.51	29.51	43.20	43.20
2	16.47	45.98	16.35	59.55
3	11.59	57.56	9.05	68.61
4	10.04	67.60	6.08	74.68
5	7.34	74.94	4.57	79.25
6	5.54	80.48	3.58	82.83
7	4.41	84.90	3.09	85.92
8	3.75	88.64	2.61	88.53
9	2.60	91.24	2.50	91.04
10	2.23	93.47	1.99	93.03