LETTERS

High-level similarity of dentitions in carnivorans and rodents

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The study of mammalian evolution depends greatly on understanding the evolution of teeth and the relationship of tooth shape to diet. Links between gross tooth shape, function and diet have been proposed since antiquity, stretching from Aristotle[1] to Cuvier[2], Owen[3] and Osborn[4]. So far, however, the possibilities for exhaustive, quantitative comparisons between greatly different tooth shapes have been limited. Cat teeth and mouse teeth, for example, are fundamentally distinct in shape and structure as a result of independent evolutionary change over tens of millions of years[5]. There is difficulty in establishing homology between their tooth components or in summarizing their tooth shapes, yet both carnivorans and rodents possess a comparable spectrum of dietary specializations from animals to plants. Here we introduce homology-free techniques[6–8] to measure the phenotypic complexity of the three-dimensional shape of tooth crowns. In our geographic information systems (GIS) analysis of 441 teeth from 81 species of carnivorans and rodents, we show that the surface complexity of tooth crowns directly reflects the foods they consume. Moreover, the absolute values of dental complexity for individual dietary classes correspond between carnivorans and rodents, illustrating a high-level similarity between overall tooth shapes despite a lack of low-level similarity of specific tooth components. These results suggest that scale-independent forces have determined the high-level dental shape in lineages that are widely divergent in size, ecology and life history. This link between diet and phenotype will be useful for inferring the ecology of extinct species and illustrates the potential of fast-throughput, high-level analysis of the phenotype.

The overall difficulties in analysing phenotypes are in contrast with the increasing availability and efficiency of analysing genomes. Successful linking of the genotype to the phenotype requires powerful tools, or 'fast-throughput morphometrics', for screening phenotypes and identifying the relevant details of the phenotype under natural selection.

Our approach to this task develops three-dimensional shape analysis and builds on relating the amount of mechanical processing that the food requires to the gross tooth form. The direct functional demands on tooth design depend on the required degree of mechanical processing[9,10]. In turn, the degree of mechanical processing that is required depends, first, on the mass-specific metabolic requirements of the animal and, second, on the difficulty with which mechanical and chemical breakdown of different kinds of foods can be achieved. We predict that the processing capability of the tooth will increase over evolutionary time when either of these two factors increases. An effective way of increasing processing capability is to add features onto the teeth that allow more food to be divided in each occlusal stroke. If we view teeth as 'tools' for breaking down food[11], this is like adding extra tools to the tooth that function in food

breakdown. This is similar in meaning to 'breakage sites'[10]. 'Dental complexity' is then any measure of the number of features, tools or breakage sites on a tooth.

The foods of mammals vary extensively in their requirements for mechanical processing. For instance, vertebrate muscle is relatively easily digested, and so does not need to be fractured into small pieces for digestion. In contrast, the microbial digestion employed by mammals that eat fibrous plants requires efficient and repeated dental processing[12]. Thus, the proportion of muscle and analogous tissues to that of fibrous plant material gives a rough indication of the demands of the cheek teeth for mechanical processing. Although our approach allows broad-scale comparisons, it is of course a considerable simplification of the real situation in teeth, where the effectiveness of each 'tool' will vary both with its own shape and with the precise physical properties of the foods.

To test our prediction we measured cheek tooth complexity in two mammalian groups, carnivorans and rodents. Despite substantial differences in body size, chewing direction and physiology, members of both mammalian groups have independently and repeatedly evolved different dietary specializations, covering most of the range from animal to plant foods. This breadth of dietary specialization in radiations that have been distinct for at least 65 million years[5] makes carnivorans and rodents both suitable and separate tests of the association between diet and dental morphology. Individual taxa were chosen for this study on the basis of the availability of detailed dietary information from the wild, phylogenetic position, and the availability of dental material. The sample included 32 species of carnivorans (Ailuridae, Canidae, Felidae, Herpestidae, Hyaenidae, Mustelidae, Procyonidae, Ursidae and Viverridae) and 49 species of murine, sigmodontine and otomyine rodents, the first two of these being commonly known as rats and mice of the Old and New Worlds, respectively. We concentrated mainly on murine and sigmodontine rodents because they represent a major component of recent rodent diversity, with the members showing disparate and independent specializations to different diets. All scans in this study are viewable in the MorphoBrowser database, a web-accessible database with an interactive three-dimensional viewer (see Methods). We used five dietary categories to classify the species in this study, in order of increasing processing demands on the teeth: hypercarnivore, carnivore (including insectivores), animal-dominated omnivore, plant-dominated omnivore, and herbivore (specifically stem and leaf feeders, composed of grazers, browsers and mixed feeders). Whereas both mammalian groups show considerable overlap in dietary specializations, rodents lack hypercarnivores (that is, dedicated vertebrate flesh eaters) and carnivorans have few taxa in the plant-dominated omnivore and herbivore categories.

We developed fast-throughput procedures for the three-dimensional analysis of teeth. Teeth were first digitized; this was followed by

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processing of the data files to produce digital tooth shapes for the analyses. For efficient computation of the various GIS and other complexity measures, a custom computer program was written (available from the authors on request). Currently, the most time-intensive step is the three-dimensional scanning (1–3 h) and initial processing of data (less than 30 min) into the GIS format, whereas computations take only seconds for each tooth row. As scanning and data processing technologies are developing at a rapid rate, this approach will quickly become increasingly efficient.

Both upper and lower cheek tooth rows (carnassials and all molars present) were digitized with a high-resolution laser scanner, and the three-dimensional point files were converted into digital elevation models of the tooth rows (Fig. 1). We used whole cheek tooth rows because this gives a better estimation of the overall processing capacity than single teeth. The results can be normalized for the number of teeth in the row, but the basic pattern remains unchanged. Because we were interested in shape apart from size, all tooth rows were scaled to the same length. To approximate the number of 'tools' on the crown[11], we subdivided the surface of each digital elevation model into patches based first on slope orientation and then on topographic elevation. Orientation maps were generated by determining the orientation at each grid point on the topographic maps as being one of eight compass directions (for example north and southwest). The maps were divided into patches by grouping contiguous points on the same contour level or with the same orientation together as a 'patch'. Next we used three different methods for calculating dental complexity and information content for both the orientation and the topographic patches: orientation and topographic patch count (OPC and TPC), orientation and topographic patch diversity (a measure of Shannon information; OPD and TPD), and image compression ratio of surface maps (OIC and TIC; see Methods and Supplementary Information).
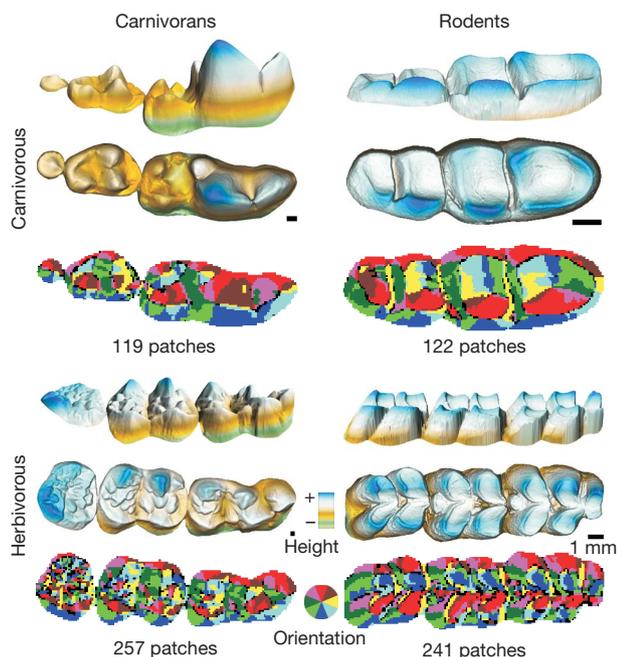
For carnivorans, OPC shows a relatively clear gradation in dental complexity from low values in hypercarnivores, intermediate in the omnivores, and highest in the herbivores (Fig. 2; $P < 0.001$ for all tests) in both the upper and lower tooth rows. Significant differences are also found between the dietary categories for TPC, OPD, TPD, OIC and TIC ($P < 0.05$ for all tests). The rodents illustrate a similar trend of dental complexity with diet (Fig. 2). Differences between the dietary categories were significant for OPC ($P < 0.01$ for lower tooth rows, $P < 0.05$ for upper tooth rows), but not for the other measures ($P > 0.05$ for all tests). We note that the better resolving power of OPC may be due to its identifying distinct functional surfaces (such as wear facets), fitting with the concept of tooth crown consisting of individual 'tools' for breaking down food[11].

When the same comparisons were made within the murines and the sigmodontines in the rodent sample, the OPC for the lower tooth row remained significantly different between the dietary categories ($P < 0.01$). This significant pattern of rodent OPC in relation to diet indicates that there remain selective pressures on molar form despite all rodents' having continuously growing incisors. Omnivore categories tend to have the largest ranges and are the least well resolved of the categories. This is perhaps expected, given that the diversity of foods that members of these two classes consume is likely to be much greater than at either end of the spectrum. Within the carnivorans, several of the families have a limited dietary range (for example, felids in hypercarnivores, and ursids in animal-dominated omnivores and herbivores), but for families that span several dietary categories (for example, mustelids) the patch count varies according to diet, indicating intrafamily and interfamily resolution.

Furthermore, when we compare the absolute values of the complexity measures between carnivorans and rodents, the ranges of dental complexity values in the two taxonomic groups overlap one another (Fig. 2). Only the carnivore diet category differs significantly between the two taxonomic groups (except for upper OPC ($P = 0.439$); Mann–Whitney U-tests; see Supplementary Information). This may reflect the fact that the carnivorous rodents are mostly invertebrate feeders, whereas the corresponding carnivorans are mostly vertebrate feeders. Indeed, if the carnivoran species that do not include moderate amounts of insects in their diet are excluded (*Crocuta crocuta*, *Gulo gulo*, *Mustela erminea*, *M. lutreola* and *M. putorius*), the mean OPC for the lower tooth row rises from 92 to 110, although this is still below that of carnivorous rodents (lower OPC mean 156, $P < 0.005$). The higher OPC of insectivores is likely to be linked to the greater development of shearing crests noted previously[13,14].

This high-level similarity between carnivoran and rodent dentitions reported here is noteworthy because the extensive differences in the low-level details of cheek tooth shape (number and position of cusps, crests and folds), number of teeth, tooth classes represented (muroids do not have canines or premolars), replacement (muroids do not have a deciduous dentition) and chewing motion[15,16] make it difficult to identify comparable landmarks and features in carnivoran and rodent teeth. In addition, body sizes, and consequently tooth sizes, are greatly different in the two groups, ranging from 5 g to 1 kg in rodents and from 90 g to 380 kg in carnivorans included in this sample. Despite these fundamental differences, our results show not only a similar tendency in relation to diet but also comparable complexity values in rodents and carnivorans for each dietary category. We interpret these results as being strongly indicative of scale-independent and phylogeny-independent effects of diet on general aspects of dental shape. It has been shown that tooth shapes can diverge relatively rapidly between populations as long as the occlusal fit is maintained[17,18], and it remains to be tested how evolutionarily labile OPC is with respect to other shape parameters.

Several anatomical and physiological characteristics have recently been shown to reflect the degree of carnivory and herbivory in disparate ranges of living mammals (for example, intracellular targeting of alanine:glyoxylate aminotransferase[19] and salivary gland

Figure 1 | Dental and dietary diversity in carnivorans and rodents. Three-dimensional buccal–occlusal and occlusal reconstructions of two carnivoran tooth rows (top left, red fox *Vulpes vulpes*; bottom left, giant panda *Ailuropoda melanoleuca*) and two rodent tooth rows (top right, golden-bellied water rat *Hydromys chrysogaster*; bottom right, Rothschild's woolly rat *Mallomys rothschildi*) for the GIS analysis. Determination of surface orientation (below each three-dimensional reconstruction, with orientation indicated by colour as shown on the colour wheel) allows the measurement of OPC (the number of coloured patches is indicated under each figure). These measures are compared with diets, namely carnivorous and herbivorous. Clumps smaller than three grid points are coloured black. Lower right tooth rows; anterior towards the right. Scale bars, 1 mm.
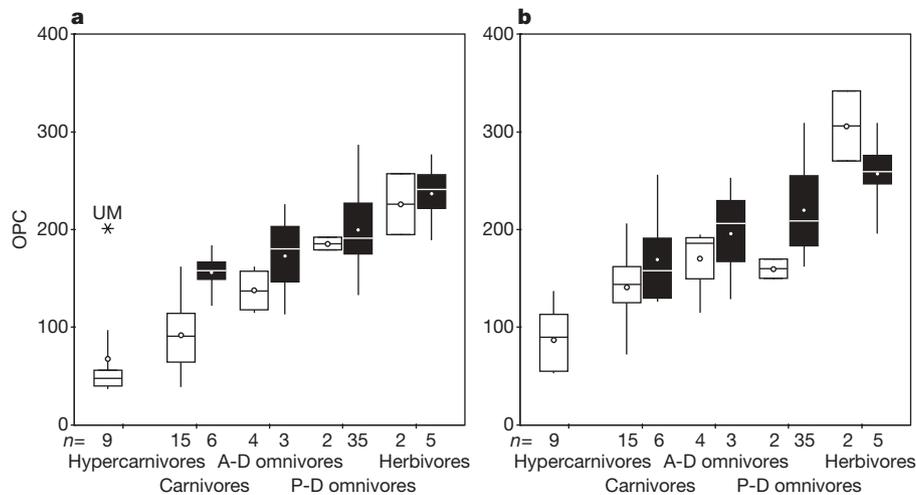
Figure 2 | Dental complexity follows diet similarly in carnivorans and rodents. Tooth complexity (measured as OPC) for five major dietary types in two taxonomically disparate groups (carnivorans (open boxes) and rodents (filled boxes)) for the lower (**a**) and upper (**b**) tooth rows. There is a consistent increase in the dental complexity when moving from hypercarnivory (meat-feeding) through animal-dominated (A-D) and plant-dominated (P-D) omnivory (meat and plants) to herbivory (plant material such as leaves and grasses). For several of the dietary categories, a similar range of values is found in both carnivorans and rodents (for example 'herbivores' in the lower tooth row and 'carnivores' in the upper tooth row). Boxes enclose 50% of observations; the median and mean are indicated with a horizontal bar and circle, respectively, and whiskers denote range, other than the one extreme outlier (UM, *Ursus maritimus*), which is indicated with an asterisk. *n*, number of species in each category.

structure[20]). However, it remains to be determined how quickly aspects of physiology and dental complexity respond to shift in diet. In our data the polar bear (*Ursus maritimus*) seems to be the main exception to the measured patterns. The relatively high dental complexity value of the highly carnivorous polar bear is likely to reflect its recent divergence from the brown bear (*Ursus arctos*), a plant-dominated omnivore, during the late Pleistocene (250–200 kyr ago), with morphological divergence perhaps only in the past 20–40 kyr (refs 21–23). Nevertheless, the upper tooth row of the polar bear still has lower dental complexity values than the other ursids in the sample (*U. arctos*, *U. americanus* and *Ailuropoda melanoleuca*), indicating that some change in the expected direction has already occurred at an extremely rapid rate. Furthermore, in comparison with other ursids, the polar bear has a substantially reduced relative tooth area (see Supplementary Information). We note, however, that whereas in our data there is a tendency towards larger tooth size in herbivores, dental complexity more completely differentiates the species according to diet and without the need for body size information (see Supplementary Information). We interpret these patterns of results to support the conclusion[10] that tooth-size–body-size predictions are best made on species groups with homologous diets.

Thus, we have shown here that in the evolution of two major mammalian groups, carnivorans and rodents, the consumption of more demanding foods has resulted in the evolution of more complex teeth in multiple independent lineages, which we could liken to the similarity of the sums irrespective of the parts. In combination with recent views of emergent properties of dental development[24], this strongly suggests that simple functional and developmental considerations may explain the bewildering diversity of tooth shapes observed in fossil and living mammals. Our results hold promise for the use of OPC in reconstructing diets of extinct taxa, even in cases where the use of living analogues is difficult because of disparate morphologies. In this respect our approach of using three-dimensional morphology is analogous to recent advances in determining three-dimensional texture of micro-wear in fossil hominins[25] and underscores the potential for fast-throughput data acquisition and analyses of living and fossil taxa.

METHODS

Diet categories. The modern species sampled from carnivorans and rodents were placed into five dietary categories (hypercarnivore, carnivore, animal-dominated omnivore, plant-dominated omnivore and herbivore) roughly reflecting the increasing demands of mechanical processing; however, for detailed discussion on mechanical properties of foods, see ref. 10. Diets were obtained from ref. 26 and monographic sources from the literature (see Supplementary Information). In this study, the 'herbivore' category is limited to stem and leaf feeders, which includes grazers, browsers and mixed feeders. Species with diets including substantial amounts of other plant material and occasional feeding on animals were placed in the 'plant-dominated omnivore' category. The wide variety of carnivorans (members of the order Carnivora) in the study cover the range from hypercarnivory (felids and some canids) through plant-dominated omnivory (bears) to herbivory (giant panda), amounting to 32 species. The rodents are largely represented by the murine and sigmodontine radiations, which account for more than 45% of rodent species diversity. Our sample includes 49 rodent species that cover a very wide dietary range.

Three-dimensional scans. One upper and one lower tooth row of each species were scanned with a Nextec Hawk three-dimensional laser scanner at between 10 and 50 μm resolution, depending on the size of the tooth row. Scans were entered into the MorphoBrowser database (http://morphobrowser.biocenter.helsinki.fi/). Teeth were oriented manually to maximize crown–base projection. For carnivorans, the carnassials (upper 4th premolar, P^4, and lower 1st molar, M_1) and all teeth posterior to them were scanned, representing between one tooth (a single M_1 in species such as *Felis silvestris*) and four teeth (P^4 to M^3 and M_1 to M_4 for *Otocyon megalotis*); for the rodents, the entire molar row, which is either two or three teeth in each jaw, was scanned. To standardize for size, each tooth row was represented by 150 data rows (typically less than half of the scanning resolution), varying in the number of columns depending on the relative width of the tooth row. To obtain functionally comparable measures of tooth shape, only carnivoran specimens with light wear were used, and for the rodents, which already have enamel-free areas on their unworn cusp tips, we standardized the wear state to a moderate level. Whereas specific functional features have been shown to be modified by tooth wear[7], general topographic measures are more stable[6,8], indicating that high-level patterns should be relatively robust to tooth wear.

Patch count, patch diversity and image compression. Initial interpolation of a regular grid of points was performed with Surfer for Windows (Golden Software, Inc.). Topographic (contour) maps were then generated with contours of twice the *x* and *y* resolutions. Using custom GIS software written by one of the authors (A.R.E.), the topographic and orientation maps were divided into patches, with a minimum patch size of three grid points. The number of these patches is the patch count for TPC and OPC maps, respectively (see Fig. 1 for example). To test for the effect of detected feature coarseness, variations in contour size (twice or four times *x* and *y* resolution, and *z* range divided by five or ten), orientation (four or eight orientations) and minimum patch size (3 or 11) were examined. To test for the effect of tooth orientation, surface with a slope of less than 5° or 10° was either voided or grouped as a separate patch. Except for some of the

coarsest levels, these variations did not affect the significance of the observed patterns (see Supplementary Information).

The following methods were used to estimate 'information content' or patch diversity of the tooth surface. TPD and OPD were calculated as $1/\sum[($patch size in grid points$)^2/($total number of grid points$)^2]$. This is a measure of information content derived from ref. 27, with the current method based on ref. 28. Each TIC and OIC map was compressed by using the JPEG and PNG algorithms, and the compression ratio was used as a measure of information content[29]. IrfanView (http://www.irfanview.com/) was used for image compression, using JPEG 80% quality (10% was also tested) and PNG compression level 6 (level 9 was also tested).

For all measures, statistical differences between dietary categories were tested with Kruskal–Wallis tests, and differences between taxonomic groups within dietary categories with the use of Mann–Whitney *U*-tests, each with a two-tailed Monte Carlo estimation of significance with 10,000 samples performed in SPSS version 11.0 (SPSS Inc.).

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