

Adaptive radiation of multituberculate mammals before the extinction of dinosaurs

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The Cretaceous–Paleogene mass extinction approximately 66 million years ago is conventionally thought to have been a turning point in mammalian evolution^{1,2}. Prior to that event and for the first two-thirds of their evolutionary history, mammals were mostly confined to roles as generalized, small-bodied, nocturnal insectivores³, presumably under selection pressures from dinosaurs⁴. Release from these pressures, by extinction of non-avian dinosaurs at the Cretaceous–Paleogene boundary, triggered ecological diversification of mammals^{1,2}. Although recent individual fossil discoveries have shown that some mammalian lineages diversified ecologically during the Mesozoic era⁵, comprehensive ecological analyses of mammalian groups crossing the Cretaceous–Paleogene boundary are lacking. Such analyses are needed because diversification analyses of living taxa^{6,7} allow only indirect inferences of past ecosystems. Here we show that in arguably the most evolutionarily successful clade of Mesozoic mammals, the Multituberculata, an adaptive radiation began at least 20 million years before the extinction of non-avian dinosaurs and continued across the Cretaceous–Paleogene boundary. Disparity in dental complexity, which relates to the range of diets, rose sharply in step with generic richness and disparity in body size. Moreover, maximum dental complexity and body size demonstrate an adaptive shift towards increased herbivory. This dietary expansion tracked

the ecological rise of angiosperms⁸ and suggests that the resources that were available to multituberculates were relatively unaffected by the Cretaceous–Paleogene mass extinction. Taken together, our results indicate that mammals were able to take advantage of new ecological opportunities in the Mesozoic and that at least some of these opportunities persisted through the Cretaceous–Paleogene mass extinction. Similar broad-scale ecomorphological inventories of other radiations may help to constrain the possible causes of mass extinctions^{9,10}.

Multituberculate mammals were a taxonomically rich^{3,11} and numerically abundant¹² clade that had originated by the Middle Jurassic epoch (approximately 165 million years (Myr) ago) and went extinct in the late Eocene (approximately 35 Myr ago)^{3,11}. They were nearly globally distributed¹³ and had a distinctive dentition consisting of procumbent incisors, blade-like premolars, molars with longitudinal rows of cusps (Fig. 1) and a predominantly posteriorly directed (palinal) chewing motion^{14,15}.

Palaeontologists have agreed for a long time that the success of multituberculate mammals was at least partly related to their highly derived dentition. Despite this, there is little consensus on the interpretations of their feeding ecology, perhaps owing to the limitations of previous approaches. For example, toothwear analysis is time intensive

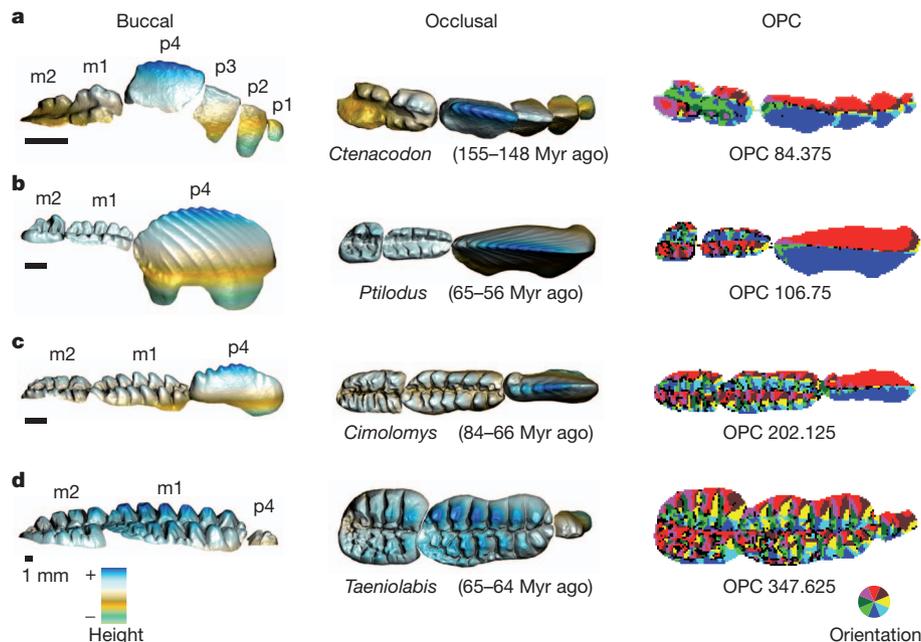


Figure 1 | **Dental and dietary diversity in multituberculate mammals.** a–d, Three-dimensional buccal–occlusal and occlusal reconstructions of multituberculate lower-right cheek tooth rows for GIS analysis: Late Jurassic plagiaulacid *Ctenacodon serratus* (a), Paleocene ptilodontoid *Ptilodus kummae* (b), Late

Cretaceous cimolomyid *Cimolomys gracilis* (c), Paleocene taeniolabidid *Taeniolabis taoensis* (d). Surface orientation map of each three-dimensional reconstruction (colour wheel indicates orientation) for OPC measurements (shown by the number of coloured patches). Clumps that are smaller than three grid points (black) are ignored. p, premolar; m, molar. Scale bars, 1 mm.

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and requires high-quality preservation¹⁵, and multituberculates lack living descendants and this hampers comparative studies. Interpretations of multituberculate feeding ecology therefore vary widely; they have been proposed to be broad herbivores, frugivores, granivores, root- and bark-eaters, egg-eaters, insectivores, carnivores and omnivores^{15–17}.

To obtain a robust and comprehensive view of multituberculate ecomorphological diversity through time, we quantified dental complexity in 41 genera using geographic information systems (GIS) analyses¹⁸ of three-dimensional crown surfaces of lower cheek teeth (Fig. 1; Supplementary Table 1). These analyses do not require cusp and facet homologies to be established, which can be a challenging task

when comparing morphologically and phylogenetically divergent taxa. Orientation patch count (OPC), a measure of dental complexity, was calculated as the number of discrete surfaces on the cheek tooth row distinguished by differences in orientation (for example, north, southwest; Fig. 1). Extant rodents, carnivorans and bats demonstrate a robust correlation between OPC and feeding ecology; OPC increases across the dietary spectrum from carnivores to omnivores to herbivores, despite many differences in specific tooth components, body size and chewing mechanics among these taxa^{18,19}. For a given clade, the standard deviation of OPC is an effective proxy for the dietary diversification and divergence in feeding function: higher standard deviation means greater dental disparity and a broader range of diets. OPC thus offers promise as a powerful tool for quantifying overall tooth shape and inferring diet in extinct taxa, such as multituberculates, that have highly derived dentitions with uncertain homology with living mammals and imprecise functional analogy.

Among the earliest multituberculates, the ‘Plagiaulacida’ are a paraphyletic assemblage of taxa with up to four simple blade-like premolars and two multi-cusped molars (Fig. 1a). OPC analyses of ‘plagiaulacids’ from the Late Jurassic through to the Early Cretaceous epoch (from 156–100 Myr ago) show low and tightly constrained dental complexity (Fig. 2a; OPC, 84–125). Their OPC values correspond to carnivory and the low end of animal-dominated omnivory among extant mammals (for example, eating both insects and fruits). Multituberculates in the early Late Cretaceous (100–84 Myr ago), which include mostly basal members of the suborder Cimolodonta, had a slightly higher mean OPC than did the ‘plagiaulacids’ but retained the low standard deviation of OPC (Fig. 2a), indicating low morphological disparity. A distinct break occurred 84–66 Myr ago, in the latest Cretaceous, when the mean OPC rose and peaked within the Campanian (mean OPC, 145) and maximum OPC and disparity sharply increased as well (Fig. 2a; OPC, 70–230). Of the 17 taxa for this interval, 5 have OPC values that are greater than 160 and 2 have OPC values that are greater than 200, corresponding to values for plant-dominated omnivory and herbivory among extant mammals, respectively (Fig. 2a).

Finally, in the early Paleocene (66–62 Myr ago) multituberculates maintained high OPC (mean OPC, 138) and disparity peaked (Fig. 2a; OPC, 70–348). The early Paleocene *Taeniolabis* from North America (Fig. 1d) had the highest OPC among multituberculates (OPC, 348), which exceeded OPC values of extant herbivorous rodents and carnivorans¹⁸. Of the 16 other early Paleocene taxa, 4 have OPC values greater than 160 and 1 has an OPC value greater than 200 (Fig. 2a). Mean OPC and disparity of OPC declined during the remainder of the Paleocene and in the early Eocene (62–49 Myr ago). *Ectypodus*, the only known genus from the middle to late Eocene (49–35 Myr ago), has a low OPC (109), corresponding to the high end of the range for extant mammalian carnivores. This sharp drop in dental ecomorphological

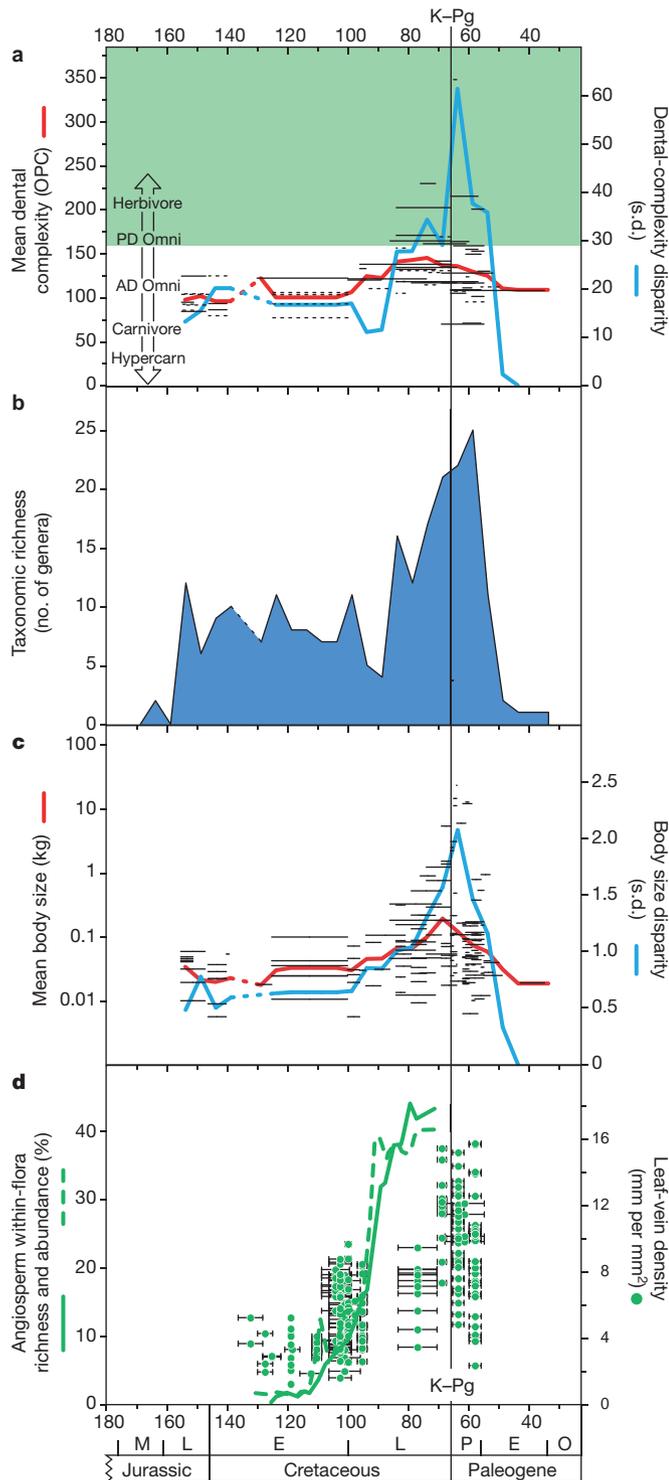


Figure 2 | Temporal patterns of multituberculate dental complexity, taxonomic richness, body size and angiosperm ecological diversification. **a**, Dental complexity (measured by OPC) for 41 multituberculate genera (solid black lines) and estimated for 24 additional genera (dashed black lines; see Supplementary Information) with mean dental complexity (OPC; red line) and disparity as standard deviations of OPC (blue line) in 5-Myr bins. Lengths of horizontal lines represent temporal ranges of taxa or uncertainties in ages of fossil localities. Labels for dietary classes are positioned at the lower end of their range based on OPC values in modern mammals¹⁸. Green shaded area represents plant-dominated omnivory (PD omni) and herbivory. **b**, Taxonomic richness is equal to the number of genera per 5-Myr bin (blue shaded area). **c**, Body-mass estimates for 156 multituberculate species (solid black lines) with geometric means (red line) and disparity (blue line) for each 5-Myr bin. **d**, Angiosperm within-flora richness (solid green line) and relative abundance (dashed green line) from ref. 24 (± 7.5 -Myr moving averages). Leaf hydraulic capacity of angiosperms as leaf-vein density (green circles) from ref. 25. The 136–131-Myr bin was excluded from all analyses (Supplementary Information). AD omni, animal-dominated omnivory; E, early; Hypercarn, hypercarnivory; M, middle; L, late; O, Oligocene; P, Paleocene.

disparity is intriguing in light of the hypothesis that some late Paleocene to early Eocene eutherian lineages (for example, rodents) competitively displaced multituberculates¹².

Variable sampling of fossils through time may influence our inferred patterns of multituberculate radiation. In particular, the relatively depauperate Jurassic and Early Cretaceous taxonomic richness of multituberculates may be partly due to the limited fossil record³. To test whether the changes in OPC through time might be sensitive to uneven sampling of fossils, we randomized the OPC value assignments for each genus. The results of 5,000 randomized mean OPC profiles through time indicate that even with additional fossil discoveries, the OPC patterns and ecological inferences that are presented here are likely to be robust (see Supplementary Fig. 8).

The overall trend of increasing multituberculate dental complexity was driven by increases in the number of cusps per molar and the relative size of molars in the cheek tooth row (Supplementary Fig. 1). This evolutionary pathway differs from that taken by ungulates, primates, rodents and lagomorphs, which responded to increased mechanical processing demands (that is, greater plant component) by evolving multi-cusped, molar-like premolars in addition to complex molars²⁰. Among several other mammalian lineages with blade-like premolars (for example, carpolestid primates and potoroid marsupials), only sthenurine marsupials re-evolved multi-cusped premolars²¹. Developmental, functional or structural constraints that are associated with the evolution of a blade-like premolar may have inhibited later evolution of more cuspidate premolars.

Patterns of generic richness and conservative estimates of body size for multituberculates (see Methods Summary) mirror the pattern of initially low dental complexity for most of the Mesozoic followed by a marked increase just before the end of the Cretaceous (84–66 Myr ago; Fig. 2b, c). From the Late Jurassic to the early Late Cretaceous, multituberculate generic richness fluctuated between 4 and 16 genera, and body mass ranged between 9 g and 105 g. A marked shift occurred near the end of the Cretaceous, with generic richness increasing from 16 to 21 genera and average body mass increasing from 67 g to 194 g, in particular, *Bubodens magnus* reached an estimated 5.25 kg (for a comparison, the alpine marmot weighs 3–8 kg). Similarly, Cenozoic generic richness and body size patterns parallel changes in dental complexity (Fig. 2b, c). However, it should be noted that body size and dental complexity are not interchangeable as predictors of diet because small multituberculates can have high OPC values, and large multituberculates can have low OPC values (Supplementary Information), conforming to previous analyses of extant taxa¹⁸. Furthermore, variation in the amount of available fossil-bearing rock through the studied interval could contribute to the pattern of generic richness²².

It is worth noting that increases in dental complexity (OPC of greater than 160) occurred in five multituberculate lineages: in the Asian Djadochtatheroidea, the North American Eucosmodontidae and Cimolomyidae (just before the end of the Cretaceous), the North American and Asian Taeniolabidae, and the North American Microcosmodontidae (in the early Paleocene). In the absence of a robust multituberculate phylogeny, it is unclear whether this represents a single evolutionary increase in tooth complexity, with multiple reversals to low complexity, or parallel increases in individual lineages or clades. This increase differs from the more commonly observed pattern, in which increased morphologic disparity precedes taxonomic diversification²³, as this increase occurred in step with an increase in multituberculate body size and taxonomic richness.

Overall, the pattern of increasing dental complexity in multituberculate mammals that pre-dates the Cretaceous–Paleogene mass extinction event contrasts with conventional ideas that mammalian evolution was suppressed during the Mesozoic era by selective pressures imposed by dinosaurs. Instead, multituberculates, the mammalian clade that co-existed for the longest time with non-avian dinosaurs, initiated an evolutionary radiation during the acme of dinosaur diversity, approximately 20 Myr before the Cretaceous–Paleogene boundary²².

This is consistent with the highly specialized adaptations that are found among several new, exceptionally well-preserved Mesozoic mammal specimens⁵ and with the timing of increased molecular divergence rates among extant mammalian lineages^{6,7}, but the multituberculates also show broad taxonomic and ecomorphologic diversification at the intraordinal level.

This adaptive response post-dated the taxonomic radiation of angiosperms but coincided broadly with increases in ecological diversity, abundance and leaf hydraulic capacities of angiosperms in the Late Cretaceous (in the Campanian and Maastrichtian)^{8,24,25} (Fig. 2d), suggesting that there is a causal link. Many angiosperms during this time were herbaceous, and had a rapid life cycle and less-effective herbivore defences compared to other seed plants and would consequently have been an attractive, protein-rich food source for herbivores²⁶. Some angiosperms had begun inhabiting a broader range of niches (trees, herbs and epiphytes) than most other plant groups and this may have enabled greater partitioning of the herbivore niche. Therefore, the multituberculate adaptive radiation, suggested by the increasing dental complexity, may have been triggered mainly by new niche space that was generated through the evolutionary and ecological radiations of angiosperms, but may have also been influenced by parallel radiations of associated non-angiosperm clades (for example, ferns²⁷), insect pollinators, dispersers and herbivores²⁸. A trophic link between angiosperms and some multituberculates is supported by our inferred dietary trend towards increased plant-dominated omnivory and herbivory among multituberculates just before the end of the Cretaceous and in the early Paleocene.

Our data also show that the dietary range of multituberculates did not decrease in response to the Cretaceous–Paleogene mass extinction event (Fig. 2a). Despite substantial taxonomic turnover of multituberculates at the Cretaceous–Paleogene boundary²⁹, they seem to have experienced little change in available food resources during one of the most severe extinction events in Earth's history. This apparent indifference of the multituberculate radiation to the Cretaceous–Paleogene event underscores the ecological selectivity of extinctions and suggests that broad-scale ecomorphological inventories of radiations may help to constrain the possible causes of extinction for other groups at the Cretaceous–Paleogene boundary^{9,10}.

METHODS SUMMARY

We scanned 48 dentitions from 41 genera of multituberculates. The three-dimensional scans of lower cheek tooth rows were analysed using GIS to quantify the number of discrete orientation patches using eight orientation directions. The mean of repeated measurements for eight rotations at multiples of 5.625° was used to reduce the effect of slight variations in the orientation of teeth. We compiled generic richness data from recent compendia and the primary literature. We estimated body mass from skull length, whenever available, and a tooth size to skull length regression formula. Detailed methods and calculations of dental complexity, generic richness and estimated body mass, data sets, measurements, calculations, randomization analyses and additional references are provided in the Supplementary Information.

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Author Information The three-dimensional scans for this study are deposited in the MorphoBrowser database (<http://morphobrowser.biocenter.helsinki.fi/>). Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this article at www.nature.com/nature. Correspondence and requests for materials should be addressed to G.P.W. (gpwilson@u.washington.edu).