

Research



Cite this article: Zurowski C, Jamniczky H, Graf D, Theodor J. 2018 Deletion/loss of bone morphogenetic protein 7 changes tooth morphology and function in *Mus musculus*: implications for dental evolution in mammals. *R. Soc. open sci.* **5**: 170761. <http://dx.doi.org/10.1098/rsos.170761>

Received: 4 October 2017

Accepted: 24 November 2017

Subject Category:

Biology (whole organism)

Subject Areas:

evolution/genetics/developmental biology

Keywords:

dental morphology, dental function, BMP7, inhibitory cascade, geometric morphometrics

Author for correspondence:

Jessica Theodor

e-mail: jtheodor@ucalgary.ca

Electronic supplementary material is available online at <https://dx.doi.org/10.6084/m9.figshare.c.3949618>.

Deletion/loss of bone morphogenetic protein 7 changes tooth morphology and function in *Mus musculus*: implications for dental evolution in mammals

Chelsey Zurowski¹, Heather Jamniczky², Daniel Graf³ and Jessica Theodor¹

¹Biological Sciences, University of Calgary, 2500 University Drive NW, Calgary, Alberta, Canada T2N 1N4

²Cell Biology and Anatomy, University of Calgary, 3330 Hospital Drive NW, Calgary, Alberta, Canada T2N 4Z6

³Dentistry and Medical Genetics, University of Alberta, 116 Street and 85 Avenue, Edmonton, Alberta, Canada T6G 2R3

CZ, 0000-0002-3445-1948; JT, 0000-0002-5159-7041

Quantifying regulatory gene effects on dental morphology and function has implications for the underlying mechanisms that generated dental diversity in mammals. We tested the hypothesis that regulatory gene expression changes lead to differences in molars using a neural crest knockout of bone morphogenetic protein 7 (BMP7) in *Mus musculus*. Three-dimensional geometric morphometric methods were used to quantify the shape of the molar toothrow. BMP7 mutants have extra cusps on the first upper and lower molars, and alterations in cusp orientation and morphology. Furthermore, significant shape differences between control and mutant were found for upper and lower toothrows. Mutant mice also exhibited differences in attrition facets, indicating functional changes that could lead to advantages in chewing new food resources and eventually niche diversification. The size ratio of the molars in the toothrow remained unchanged, implying that BMP7-induced changes in molar size ratio are a result of knocking out epithelial, rather than neural crest, expression of BMP7. Our results indicate that changes in BMP7 expression are sufficient to alter the morphology and function of the toothrow,

1. Introduction

Dental characters, such as the shape and size of the molars and their cusps, reveal aspects of phylogeny, taxonomy and ecology that inform most of what is known about mammalian evolution [1,2]. The clade is particularly notable for the widespread presence of convergent dental morphologies in both fossil and recent forms [3], making mammalian teeth an intriguing model for the study of the developmental-genetic architecture of adaptive change [4]. Previous work has elucidated the occurrence, timing and influence of regulatory genes throughout tooth development, the effects of such genes on the final morphology of molars and the relationship of extant molar forms to the forms found in extinct taxa [5–10]. While the relationship between form and function has important evolutionary implications, there remains a substantial gap in our understanding of the functional outcomes of morphological variation in mammal molars.

Bone morphogenetic proteins (BMPs) are a family of regulatory genes that belong to the transforming growth factor β family of extracellular signalling molecules, and that are active throughout tooth development [11] upstream of the Smad, MAPK and PI3K/Akt pathways [12]. Bone morphogenetic protein 7 (BMP7) is expressed throughout mouse tooth development in both the epithelium and the underlying neural crest-derived mesenchyme [12]. BMP7 null mice exhibit severe craniofacial and dental malformations and die shortly after birth, while BMP7 heterodeficient mice show molar size ratio differences and morphological differences in their dentition [12–15]. Here, we test the hypothesis that BMP7 conditional knockout mice demonstrate both phenotypic and correlated functional changes stemming from the reduction of neural crest-derived BMP7.

2. Material and methods

2.1. Specimens

Previously collected specimens of control and 1245fl/fl:gTomtg/+;wnt1Cre+ (BMP7 Δ) mice, background strain 129SvJ/C57BL/6 [12,16] backcrossed to C57Bl/6] for more than eight generations, were used in this study. *Bmp7*^{tm1.1Dgra} mice backcrossed to more than 10 generations of C57Bl6/J were crossed to wnt1-Cre [16] to induce neural crest-specific deletion of *Bmp7* (*Bmp7*^{ncko}).

All specimens were fed and housed under identical conditions. Specimens were sacrificed and their heads were preserved. A total of 24 control and 26 mutant specimens were either frozen fresh at -80°C , or preserved using 1% paraformaldehyde and then washed using phosphate-buffered saline (PBS) and stored at 4°C in PBS. Specimens were distributed among five age sets: 21, 53, 60, 75 and 121 days, and spontaneous death specimens, and will be referred to as 1, 2, 3, 4, 5, and E, respectively.

2.2. Scanning

Specimens were scanned using a Skyscan 1173 micro-computed tomography (μCT) instrument (Kontich, Belgium) at the University of Calgary and then reconstructed (NRECON, v. 1.6.6.0; $19.46\ \mu\text{m}$ average pixel size, image size: 2240×2240 pixel). Three-dimensional surface models of the skulls and the upper and lower tooththrows were produced using Amira and exported as polygon model files (.ply) (v. 5.6.0, FEI Visualization Sciences, Hillsborough, OR, USA).

2.3. Landmarking and statistical analysis

Landmark-based geometric morphometric methods were used to quantify morphological variation within and between control and mutant tooththrows. Two landmark datasets were developed to capture both cusp and outline morphologies, and consisted of both fixed and sliding semilandmarks (figure 1; electronic supplementary material, SI1). Each specimen was landmarked three times for each set by the same observer on different days in order to quantify intra-observer error, and these replicates were then averaged and subjected to Procrustes transformation for analysis. Principal components analysis (PCA) was performed to determine the major axis of variation present in each dataset [17]. Differences in

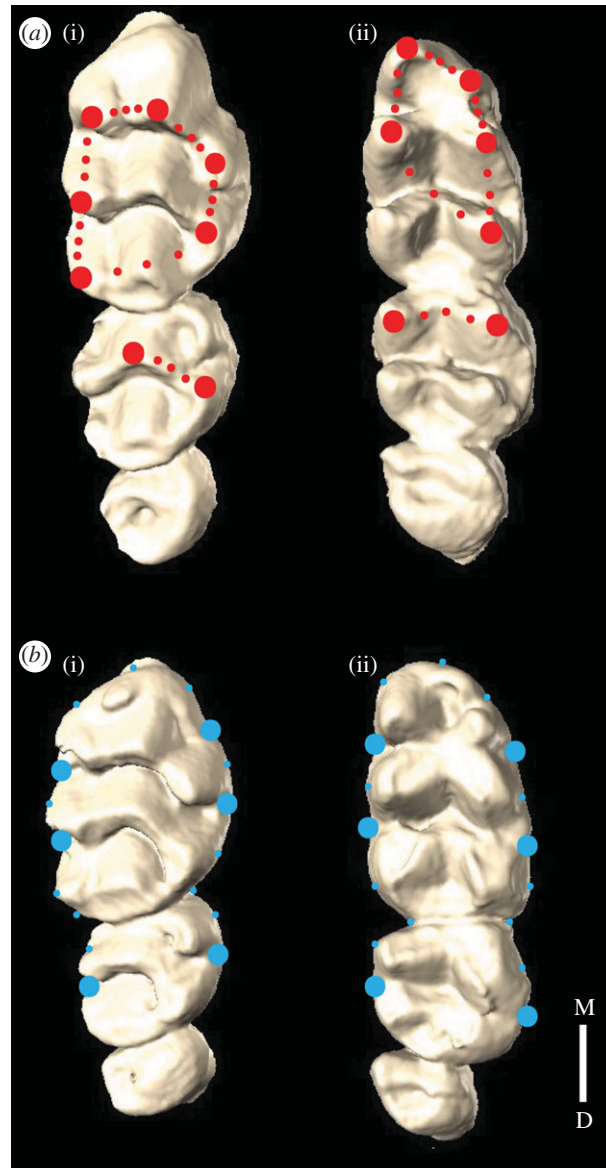


Figure 1. The location of the fixed (large circles) and sliding semilandmarks (small circles) of the cusp (red) (a) and outline (blue) (b) landmark sets shown on control upper (i) and lower (ii) right tooththrows. M, mesial; D, distal.

shape between mutant and control specimens were quantified using a morphological disparity test and discriminant function analysis (DFA). Morphological disparity estimates group variance and through permutation estimates statistical significance of between-group shape variance [18]. DFA describes shape differences between groups by the linear axis of greatest variation [19]. The first and third semilandmarks between the fixed landmarks were excluded in the cusp landmark sets to accommodate the requirements of fewer variables than specimens in the DFA. Finally, to examine the relationship between size and shape, a regression of shape score on centroid size was performed for each transformed dataset [20]. All landmarking and statistical analyses were executed using the Geomorph package (v. 3.0.1; [21]) in RSTUDIO (v. 00.99.893, R v. 3.3.0 GUI 1.68 Mavericks build, R Core Team 2016, [22]).

2.4. Wear facets

Two mutant and two control specimens from age sets 1, 3 and 5 were skinned and skeletonized following scanning. Photographs of the right lower tooththrows were taken using a Dino-Lite Edge imaging system (AM4815ZTL Dino-Lite Premier; AnMo Electronics Corporation, Taiwan) and the wear facets were

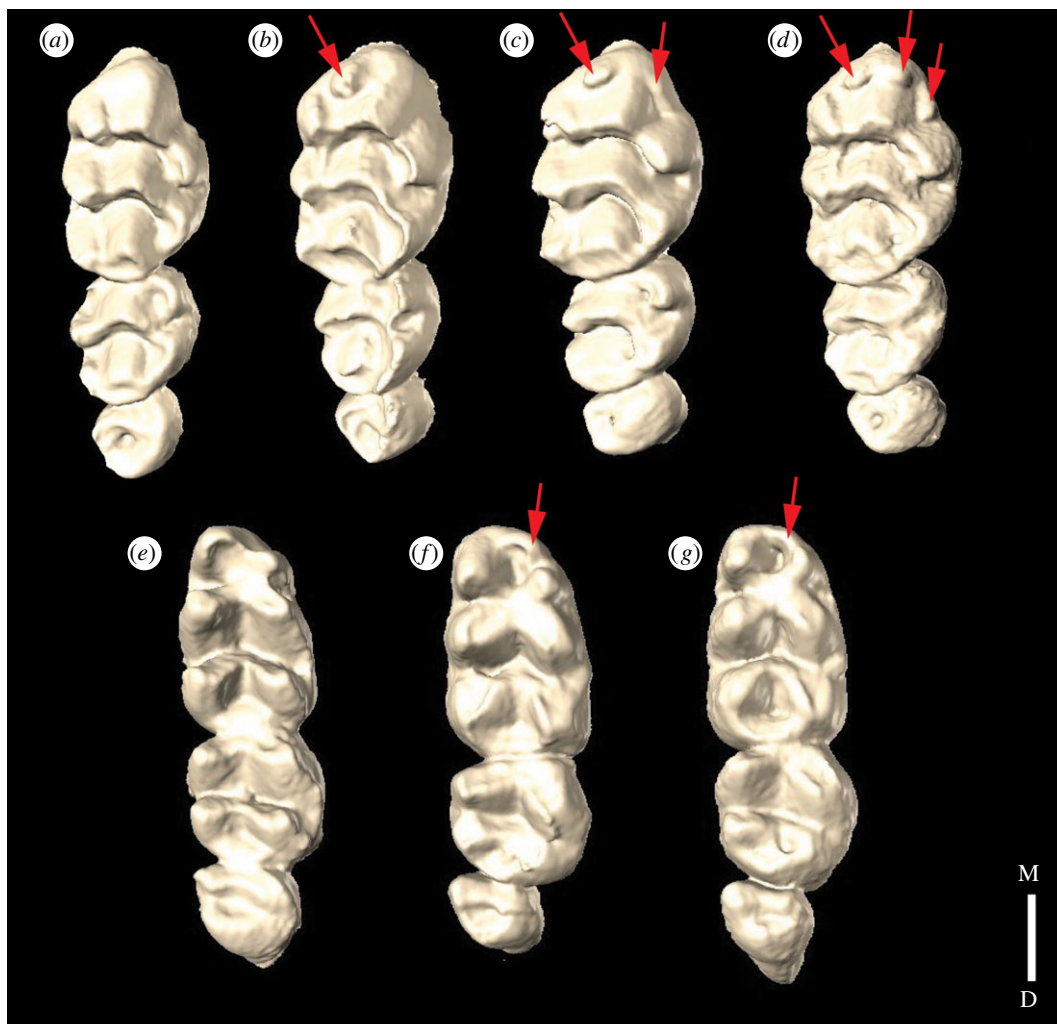


Figure 2. Comparison of control upper (*a*) and lower (*e*) tooththrows with BMP7 conditional knockout upper tooththrow morphotypes (*b–d*) and lower tooththrow morphotypes (*f,g*). Red arrows point to accessory cusp(s) on the mutant tooththrows. M, mesial; D, distal.

subsequently manually outlined in ADOBE ILLUSTRATOR CS6 (v. 16.0.3). Facet morphology was then qualitatively compared.

2.5. Description

A detailed description of the mutant specimens, along with additional figures, can be found in the electronic supplementary material, SI1.

3. Results

Mutant molars were short and broad both mesiodistally and buccolingually and were observed to have extra cusp(s) on the first upper and lower molars (figure 2; electronic supplementary material, SI1). Multiple morphotypes of the ‘extra cusp’ morphology were present in both the upper and lower first molars (figure 2; electronic supplementary material, SI1).

Three-dimensional geometric morphometric analyses confirmed shape differences between mutants and controls in each of the landmark sets and exposed differences in attrition facet development (figure 3). Specimens were clustered by genotype and age set in both upper and lower cusp landmark PCA plots, with the age sets clustering consecutively from youngest to oldest (figure 3). In PCA plots for the upper and lower outline landmark sets, specimens were only clustered by genotype (electronic supplementary material, SI2). Morphological disparity tests revealed significant differences between

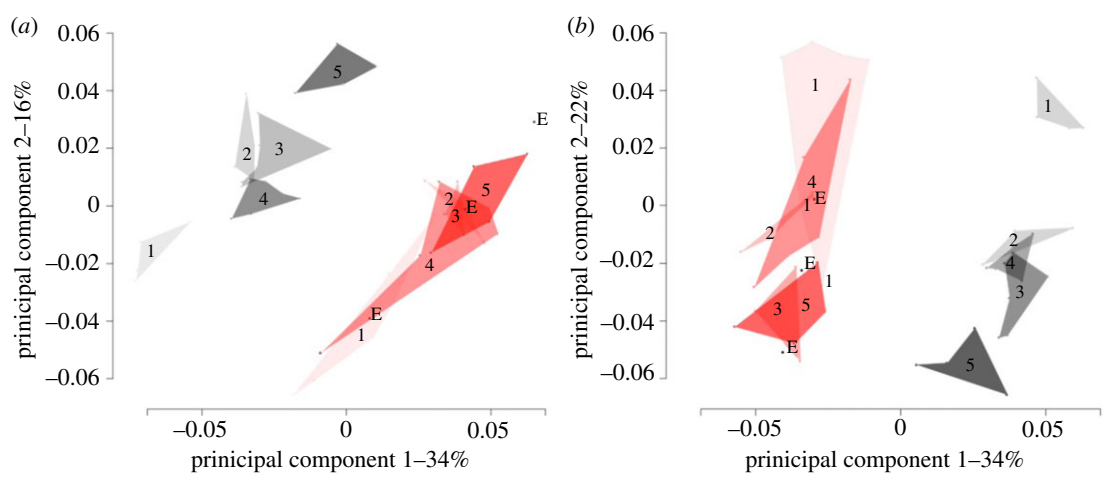


Figure 3. PCA plots for the (a) upper and (b) lower cusp landmark sets. Controls are pictured in grey and mutants in red with labels for age set and lines delineating the morphospace each age set occupies.

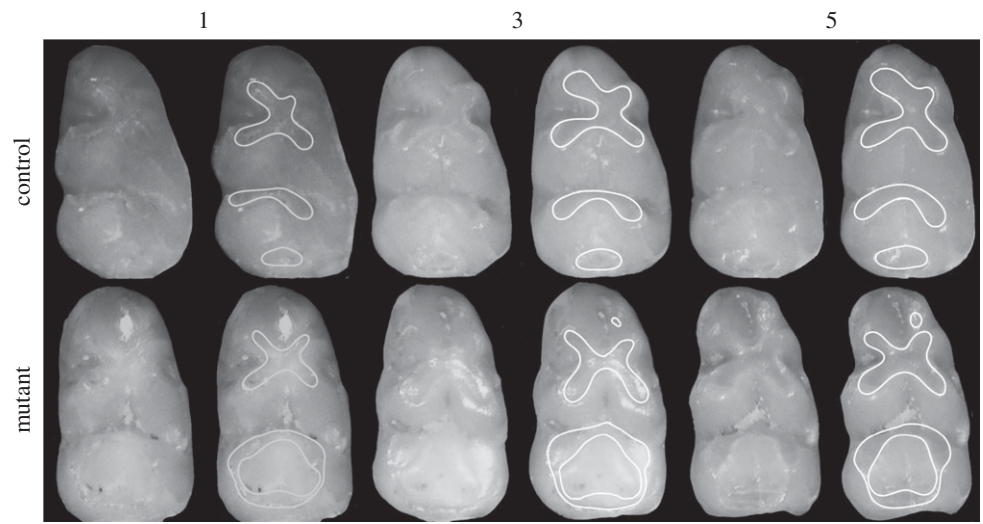


Figure 4. Comparison of wear facet morphology in mutant and control skeletonized specimens from age sets 1, 3 and 5. Each specimen is shown twice, both with and without outlines of the wear facets. Control specimens are pictured on the top row and BMP7 mutants on the bottom row. Age sets are labelled along the top.

mutant and control specimens for only the lower cusp landmark set (electronic supplementary material, S13). For all landmark sets, DFA consistently categorized specimens by genotype, but not by age (electronic supplementary material, S13). Regression analyses returned significant results for only the lower cusp and outline landmark sets (electronic supplementary material, S13).

Attrition facets on the m1 in mutant mice were visually different in morphology and exhibited accelerated wear progression patterns in all age sets examined (figure 4). In addition to large and broad wear surfaces, there was fusion between the facets on the second chevron and talonid, resulting in a large distal-facing wear surface that merged over the entoconid and metaconid (figure 4).

4. Discussion

Neural crest-derived mesenchymal cells contributing to molars express BMP7 in the placode and bell stages of tooth development [12]. This expression occurs in the underlying mesenchyme and the dental papilla, which are precursors to odontoblasts [12]. Reducing the BMP7 expression in these cells resulted in molar morphology that differed from controls using both qualitative and quantitative methods (figure 2; electronic supplementary material, S11).

4.1. Neural crest-derived bone morphogenetic protein 7 and dental morphogenesis in mice

Morphological differences in mutant molars suggest that a lack of mesenchymal BMP7 leads to differences in the distribution and timing of the secondary enamel knot development and/or modifications in differential growth at the enamel dentine junction (EDJ) where the main shape of the tooth is formed [6]. BMPs have been shown to be important in cellular motility and for the ability of cells to form apical and basal surfaces [23,24]. Lack of neural crest-derived BMP7 in mutants could disrupt cellular migration and organization, leading to morphological differences in the EDJ and the final molar shape, and the potential for novel molar form and function. Histological examination during molar development is necessary to determine the effects at the cellular level.

4.2. Functional consequences of changes in molar morphology

Cusp morphology is important in understanding the evolution of function in murine molars, and tooth wear is a complex process that greatly influences the shape of the cusps [25,26]. Morphological changes in the first molars are especially important within Murinae, as the first molars are the antagonistic set responsible for the majority of mastication [25]. Additionally, differences in attrition facet shape, size and distribution imply alterations in the way molars occlude, along with changes in molar function [27].

Similar morphologies to those observed here can be observed within many other members of Murinae, in mice with alterations in different regulatory genes, and as a spontaneous morphology in control specimens from other studies [9,28–35]. The occurrence of similar molar form within Murinae suggests the hypothesis that BMP7 may be a source of evolutionary important variation in dentition. For instance, accessory cusps and bigger molars, such as those observed in mutant specimens, can contribute to a large overall wear surface that may allow for processing of different food types [9,36].

Together, mutant molar and facet morphology correlate with the low-cusped molars and broad wear surfaces that are typical of mammalian dietary adaptations to frugivory [37]. This implies that the resulting morphological and functional differences owing to expression changes in BMP7 may lead to advantages in chewing alternate food resources and potentially niche diversification. Further testing with the food of varying hardness is required to confirm the potential to occupy a new niche.

4.3. Genomic organization and evolutionary implications

BMPs are highly conserved proteins with activity in many different tissues, which limit the possibility for changes to the protein itself as an evolutionary mechanism. However, it has been shown that the genomic organization of several members of the BMP family is modular. The genes are located in gene deserts that contain numerous long-range *cis*-regulatory enhancer sequences to control cell- and tissue-specific gene expression [38]. There is evidence that BMP7 is regulated in a similar manner [15]. The principle of enhancer modules makes changes to gene expression at a particular site relatively easy without affecting expression at other sites. Differences in BMP7 expression specifically in developing molars could thus have been achieved by such a mechanism.

4.4. Alignment with the inhibitory cascade model

The inhibitory cascade model (ICM) is a developmental model that states that mesial molars suppress the size of more distal molars, resulting in a gradient $M1 > M2 > M3$ [8]. No differences in the molar surface area ratios were observed between BMP7 conditional knockouts, where BMP7 expression was reduced in the neural crest-derived mesenchyme but not in epithelial tissues, and the control mice. Occlusal surface area ratio differences in the molars of BMP7 heterodeficient mice, where both epithelial tissue and neural crest cells in the molars had reduced expression of BMP7 [14], suggest that if the ICM is correct, BMP7 contributes to the size gradient by its expression in epithelial tissues and not in the neural crest.

Although no differences were observed in the molar size ratios, the mesial portion of the molars, especially in the M1, possessed higher amounts of morphological variability. Molar development proceeds sequentially with the M1 developing first, after which the M2 develops from the distally extending dental lamina [8]. In theory, this would allow morphological variation and novel morphologies on the mesial portion of the tooth, as it would be less likely to influence the development of subsequent molars.

5. Conclusion

Knockout of neural crest-derived BMP7 causes both morphological and functional changes in mouse teeth, showing that suppression of regulatory genes is a feasible means to generate variation in mammalian dentition. The changes observed here correlate with morphologies observed within Muridae and may correspond to dietary changes that allow for niche diversification.

Further research studies into cell dynamics in the tooth bud, along with the functional consequences of the final morphology in BMP7 conditional knockout mice, are needed to elucidate the relationship between regulatory gene expression patterns and mammalian dental evolution.

Ethics. Mice were maintained at the animal facilities of the University of Zurich and the University of Alberta. Animal sample collection at the University of Zurich was approved by the local veterinary authorities (permit 98/2011, Veterinäramt Zürich) in compliance with Swiss federal law (TSchG, TSchV) and cantonal by-laws in full compliance with the European Guideline 86/609/EC. This authority approval also included ethical approval. Animal sample collection at the University of Alberta was approved by the Research Ethics office of the University of Alberta (Animal Use and Care Committee protocol AUP1149) in compliance with the guidelines by the Canadian Council of Animal Care. Scanning of animal specimens was approved by the Animal Care and Use Committee at the University of Calgary (protocol no. AC14-0191).

Data accessibility. Our data are deposited at Dryad: <http://dx.doi.org/10.5061/dryad.70585> [39].

Author contributions. D.G. developed the BMP7 knockout mice. C.Z., H.J. and J.T. developed the analytical protocol. C.Z. carried out the scans, measurements and analyses. All authors contributed to the writing of the manuscript.

Competing interests. We declare we have no competing interests.

Funding. This research was supported by NSERC Discovery Grants to J.M.T. (331286-2011) and H.J. (418249-2012). D.G. received funding from the Swiss National Science Foundation (31003A_135530) and the University of Alberta.

Acknowledgements. We thank T. Barry, C. Barron-Ortiz, J.D. Pardo and G.L. Powell for their helpful discussions.

References

- Gingerich PD. 1979 Paleontology, phylogeny, and classification: an example from the mammalian fossil record. *Syst. Biol.* **28**, 451–464. (doi:10.2307/sysbio.28.4.451)
- Jernvall J, Hunter JP, Fortelius M. 1996 Molar tooth diversity, disparity, and ecology in cenozoic ungulate radiations. *Science* **274**, 1489–1492. (doi:10.1126/science.274.5292.1489)
- Jernvall J, Thesleff I. 2012 Tooth shape formation and tooth renewal: evolving with the same signals. *Development* **139**, 3487–3497. (doi:10.1242/dev.085084)
- Polly PD. 2013 Evolution: stuck between the teeth. *Nature* **497**, 325–326. (doi:10.1038/nature12099)
- Cheverud JM. 1996 Developmental integration and the evolution of pleiotropy. *Am. Zool.* **36**, 44–50. (doi:10.1093/icb/36.1.44)
- Jernvall J. 2000 Linking development with generation of novelty in mammalian teeth. *Proc. Natl Acad. Sci. USA* **97**, 2641–2645. (doi:10.1073/pnas.050586297)
- Harjunmaa E *et al.* 2014 Replaying evolutionary transitions from the dental fossil record. *Nature*. **512**, 44–48. (doi:10.1038/nature13613)
- Kavanagh KD, Evans AR, Jernvall J. 2007 Predicting evolutionary patterns of mammalian teeth from development. *Nature* **449**, 427–432. (doi:10.1038/nature06153)
- Charles C, Pantalacci S, Peterkova R, Tafforeau P, Laudet V, Viriot L. 2009 Effect of eda loss of function on upper jugal tooth morphology. *Anatom. Record* **292**, 299–308. (doi:10.1002/ar.20804)
- Rodrigues HG *et al.* 2013 Roles of dental development and adaptation in rodent evolution. *Nat. Commun.* **4**, 2504. (doi:10.1038/ncomms3504)
- Massagué J. 2012 TGF β signalling in context. *Nat. Rev. Mol. Cell Biol.* **13**, 616–630. (doi:10.1038/nrm3434)
- Zouvelou V, Luder H-U, Mitsiadis TA, Graf D. 2009 Deletion of BMP7 affects the development of bones, teeth, and other ectodermal appendages of the orofacial complex. *J. Exp. Zool. Part B* **312B**, 361–374. (doi:10.1002/jez.b.21262)
- Jena N, Martin-Seisdedos C, McCue P, Croce CM. 1997 BMP7 null mutation in mice: developmental defects in skeleton, kidney, and eye. *Exp. Cell Res.* **230**, 28–37. (doi:10.1006/excr.1996.3411)
- Asahara M, Saito K, Kishida T, Takahashi K, Bessho K. 2016 Unique pattern of dietary adaptation in the dentition of Carnivora: its advantage and developmental origin. *Proc. R. Soc. B* **283**, 20160375. (doi:10.1098/rspb.2016.0375)
- Saito K *et al.* 2016. Effects of Usag-1 and Bmp7 deficiencies on murine tooth morphogenesis. *BMC Dev. Biol.* **16**, 14. (doi:10.1186/s12861-016-0117-x)
- Danielian PS, Muccino D, Rowitch DH, Michael SK, McMahon AP. 1998 Modification of gene activity in mouse embryos *in utero* by a tamoxifen-inducible form of Cre recombinase. *Curr. Biol.* **8**, 1323–1326. (doi:10.1016/S0960-9822(07)00562-3)
- Jolliffe I. 2002 *Principal component analysis*. New York, NY: John Wiley & Sons, Ltd.
- Zelditch ML, Swiderski DL, Sheets HD. 2012 *Geometric morphometrics for biologists: a primer*. New York, NY: Academic Press.
- Blackith RE, Reyment RA. 1971 *Multivariate morphometrics*. New York, NY: Academic Press.
- Loy A, Mariani L, Bertelletti L, Tunsei L. 1998 Visualizing allometry: geometric morphometrics in the study of shape changes in the early stages of the two banded sea bream, *Diplodus vulgaris* (Perciformes, Sparidae). *J. Morphol.* **237**, 137–146. (doi:10.1002/(SICI)1097-4687(199808)237:2<137::AID-JMORS>3.0.CO;2-Z)
- Adams DC, Otárola-Castillo E. 2013 Geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Methods Ecol. Evol.* **4**, 393–399. (doi:10.1111/2041-210X.12035)
- Allaire J. 2012 *RStudio: integrated development environment for R*. Boston, MA: RStudio.
- de Jesus Perez VA *et al.* 2011 BMP promotes motility and represses growth of smooth muscle cells by activation of tandem Wnt pathways. *J. Cell Biol.* **192**, 171–188. (doi:10.1083/jcb.201008060)
- Saitoh M *et al.* 2013 Basolateral BMP signaling in polarized epithelial cells. *PLoS ONE* **8**, e62659. (doi:10.1371/journal.pone.0062659)
- Lazzari V, Tafforeau P, Aguilar J-P, Michaux J. 2008 Topographic maps applied to comparative molar morphology: the case of murine and cricetine dental plans (Rodentia, Muroidea). *Paleobiology* **34**, 46–64. (doi:10.1666/06052.1)
- Tatsumi C, Okamoto K. 2013 Molecular genetic investigation of dental attrition in mice. *Pediatr. Dent. J.* **23**, 22–31. (doi:10.1016/j.pdj.2013.03.004)
- Butler PM. 1980 Functional aspects of the evolution of rodent molars. *Palaeovertebrata: Mémoire Jubilaire R. Lavocat* **9**, 249–262.
- López Antónanzas R. 2009 First *Potwamus* from the Miocene of Saudi Arabia and the early phylogeny of murines (Rodentia: Muroidea). *Zool. J. Linn. Soc.* **156**, 664–679. (doi:10.1111/j.1096-3642.2008.00494.x)
- Renaud S, Auffray JC. 2013 The direction of main phenotypic variance as a channel to evolution: cases in murine rodents. *Hystrix* **24**, 1–9. (doi:10.4404/hystrix-24.1-6296)

30. Kimura Y, Jacobs LL, Flynn LJ. 2013 Lineage-specific responses of tooth shape in murine rodents (Murinae, Rodentia) to late Miocene dietary change in the Siwaliks of Pakistan. *PLoS ONE* **8**, e76070. (doi:10.1371/journal.pone.0076070)
31. Misonne X. 1969 African and indo-Australian Muridae: evolutionary trends. *Annales, Musée Royale de l'Afrique Centrale, Sciences Zoologiques* **132**, 1–XXVII.
32. Marangoni P, Charles C, Tafforeau P, Laugel-Haushalter V, Joo A, Bloch-Zupan A, Klein OD, Viriot L. 2015 Phenotypic and evolutionary implications of modulating the ERK-MAPK cascade using the dentition as a model. *Sci. Rep.* **5**, 11658. (doi:10.1038/srep11658)
33. Qiu Z, Storch G. 1990 New murids (Mammalia: Rodentia) from the Lufeng hominoid locality, late Miocene of China. *J. Vertebr. Paleontol.* **10**, 467–472. (doi:10.1080/02724634.1990.10011829)
34. Denys C, Didier Missoup A, Nicolas V, Fülling O, Delapré A, Bilong CF, J Taylor PJ, Hutterer R. 2014 African highlands as mammal diversity hotspots: new records of *Lamottemys okuensis* Petter, 1986 (Rodentia: Muridae) and other endemic rodents from Mt Oku, Cameroon. *Zoosystema* **36**, 647–690. (doi:10.5252/z2014n3a6)
35. Pagès M, Fabre P-H, Chaval Y, Mortelliti A, Nicolas V, Wells K, Michaux JR, Lazzari V. 2015 Molecular phylogeny of South-East Asian arboreal murine rodents. *Zool. Script.* **45**, 349–364. (doi:10.1111/zsc.12161)
36. Renaud S, Pantalacci S, Auffray J-C. 2011 Differential evolvability along lines of least resistance of upper and lower molars in island house mice. *PLoS ONE* **6**, e18951. (doi:10.1371/journal.pone.0018951)
37. Strait SG. 1993 Differences in occlusal morphology and molar size in frugivores and faunivores. *J. Hum. Evol.* **25**, 471–484. (doi:10.1006/jhev.1993.1062)
38. Pregizer S, Mortlock DP. 2009 Control of BMP gene expression by long-range regulatory elements. *Cytokine Growth Factor Rev.* **20**, 509–515. (doi:10.1016/j.cytogfr.2009.10.011)
39. Zurowski C, Jamniczky H, Graf D, Theodor J. 2017 Data from: Deletion/loss of bone morphogenetic protein 7 changes tooth morphology and function in *Mus musculus*: implications for dental evolution in mammals. Dryad Digital Repository. (<http://dx.doi.org/10.5061/dryad.70585>)