Creating diversity in mammalian facial morphology: a review of potential developmental mechanisms

Kaoru Usui and Masayoshi Tokita

Abstract
Mammals (class Mammalia) have evolved diverse craniofacial morphology to adapt to a wide range of ecological niches. However, the genetic and developmental mechanisms underlying the diversification of mammalian craniofacial morphology remain largely unknown. In this paper, we focus on the facial length and orofacial clefts of mammals and deduce potential mechanisms that produced diversity in mammalian facial morphology. Small-scale changes in facial morphology from the common ancestor, such as slight changes in facial length and the evolution of the midline cleft in some lineages of bats, could be attributed to heterochrony in facial bone ossification. In contrast, large-scale changes of facial morphology from the common ancestor, such as a truncated, widened face as well as the evolution of the bilateral cleft possessed by some bat species, could be brought about by changes in growth and patterning of the facial primordium (the facial processes) at the early stages of embryogenesis.

Keywords: Mammals, Craniofacial morphology, Diversity, Transgenic mice, Bats, Facial processes, Neural crest, Ectomesenchyme, Bone, Orofacial cleft

Morphological diversity in mammalian faces
Mammals (class Mammalia) are one of the major groups of vertebrates, containing over 5400 living species as well as abundant extinct species [1–4]. Living mammals consist of three major clades: monotremes (order Monotremata), marsupials (infraclass Marsupialia), and placentals (infraclass Placentalia; Fig. 1). Recent phylogenetics, including comparative phylogenomic studies, have lead to a general consensus concerning the deeper branches of the mammalian evolutionary tree, for example identifying four major clades within placentals: Xenarthra, Afrotheria, Laurasiatheria, and Euarchontoglires [5–11].

Mammals have evolved diverse morphologies to adapt to a wide range of ecological niches [3, 4]. The morphological diversity of mammalian heads is especially remarkable, possibly due to the head's fundamental role in sensing, communication, and feeding [12–18] (Fig. 1). For example, both long- and short-faced taxa are recognized in each mammalian group (Fig. 1). Craniofacial morphology in mammals has been quantitatively evaluated in each group by comparative morphological analyses, including modern geometric morphometrics (summarized in Table 1).

However, the genetic and developmental mechanisms underlying the diversification of mammalian craniofacial morphology remain largely unknown. In this review, we compiled the recent findings in the developmental genetics of mice, a model mammalian species, to attempt to deduce the potential diversification mechanisms of mammalian facial morphology. We also introduce the results of previous studies in which a strong correlation between the number of nucleotide tandem repeats within the Runx2 gene and the facial length in some placental mammals was reported. Finally, we focus on bats (order Chiroptera), which display a substantial degree of craniofacial diversity and discuss their potential as a model for understanding the evolution of mammalian craniofacial morphology.

*Correspondence: masayoshi.tokita@sci.toho-u.ac.jp
Department of Biology, Faculty of Science, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan

© The Author(s) 2018. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Molecular and cellular mechanisms creating diversity in facial morphology uncovered by mouse transgenesis

Mouse transgenesis is a powerful tool to infer the function of genes related to vertebrate morphogenesis. We examine the phenotypes of transgenic mice to gain insights into the molecular and cellular mechanisms that produce morphological variation in mammalian faces. We focused on two developmental events: (1) growth and patterning of the facial primordium and (2) ossification of the facial bones that lead to a shortened face and the orofacial cleft (Table 2).

Growth and patterning of the facial primordium

Formation of mammalian faces begins at the pharyngula stage of embryogenesis, through growth and fusion of the five facial processes: the frontonasal process (FNP), medial nasal processes (MNPs), lateral nasal processes (LNPs), maxillary processes (MAXs), and mandibular processes (MANs) [19]. In the facial development of mice, FNP first expands anteriorly in a nine-day-old embryo (E9.0). Subsequently, MNPs and LNPs start to bulge out from the FNP at E10.0. These two processes surround the nasal placodes, MNP surrounds its medial aspect, and LNP surrounds its lateral aspect. During the same embryonic stage, MAXs begin to bulge anteriorly covering the ventrolateral aspect of the FNP. MAXs and the FNP continue to grow and fuse to each other in later stages to form the upper jaw. Paired MANs begin to grow anteriorly at E9.0 and fuse to one another at the midline to form the mandible [19, 20].

The early patterning of the mammalian face is regulated by migration and proliferation of the neural crest-derived mesenchyme (ectomesenchyme hereafter) [19,
### Table 1  Diversity of craniofacial morphology in mammals and recent studies evaluating this diversity using landmark-based geometric morphometrics

| Clade          | Remarks on diversity of craniofacial morphology                                                                 | Landmark-based geometric morphometric studies |
|---------------|------------------------------------------------------------------------------------------------------------------|---------------------------------------------|
| Monotremata   | All extant monotremes have a toothless bill covered by electro- and mechano-receptors. The platypus has a flat, widened, duck-like bill. Echidna bills are more pointed, slender compared to platypus bills | None                                        |
| Marsupialia   | The viscerocranium, which includes the early-ossifying bones of the oral region, is morphologically less diverse than in placentals. The level of disparity of late-ossifying neurocranium is equivalent with that in placentals. This suggests that the ossification of marsupial oral bones is more constrained compared to placentals | [102–105]                                  |
| Xenarthra     | Armadillo skulls are elongated anteroposteriorly and flattened dorsoventrally. The zygomatic arch is complete, differing from those of another xenarthran lineage, Pilosa. The dentoary bone is thin and long. Variation in skull shape is only described in the family Pampatheriidae which is an extinct group of Cingulata. Skull shape is highly conserved among extant members. The suborder Vermilingua (anteaters) has a specialized skull for eating small insects; the skull is highly elongated and has no tooth. Its pointed rostrum encases a long tongue. The zygomatic arch is incomplete |
| Cingulata     | Armadillo skulls are elongated anteroposteriorly and flattened dorsoventrally. The zygomatic arch is complete, differing from those of another xenarthran lineage, Pilosa. The dentoary bone is thin and long. Variation in skull shape is only described in the family Pampatheriidae which is an extinct group of Cingulata. Skull shape is highly conserved among extant members. The suborder Vermilingua (anteaters) has a specialized skull for eating small insects; the skull is highly elongated and has no tooth. Its pointed rostrum encases a long tongue. The zygomatic arch is incomplete |
| Pilosa        | The suborder Forivora (sloths), which consists of Bradypodidae (three-toed sloth) and Megalonychidae (two-toed sloth), has a short, high skull with a strongly reduced rostrum. The zygomatic arch is robust but incomplete. The skulls of three-toed and two-toed sloths are distinct to one another according to morphometric analyses. Three-toed sloth skulls have a relatively shortened rostrum and no diastema. The suborder Vermilingua (anteaters) has a specialized skull for eating small insects; the skull is highly elongated and has no tooth. Its pointed rostrum encases a long tongue. The zygomatic arch is incomplete |
| Afrotheria    | Aardvark skulls are elongated anteroposteriorly, accompanied by long and slender dentoary bones. The nasal bone is triangular in shape. The frontal bones expand dorsally in front of the orbit as a result of a highly developed nasal chamber. The zygomatic arch is complete but slender. There is no postorbital bar |
| Tubulidentata | Macroscelidea species have a tall, dome-shaped cranium. The zygomatic arch is complete. The rostrum is long. Macroscelidae consists of two subfamilies: Rhynchocyoninae and Macroscelidinae. Rhynchocyoninae species have a relatively large skull with nasal bones having partially ossified tips. The bony palate is not perforated. Macroscelidinae species have a relatively smaller skull and wholly cartilaginous nasal bone tips. The bony palate has some holes |
| Macroscelidea | Macroscelidea species have a tall, dome-shaped cranium. The zygomatic arch is complete. The rostrum is long. Macroscelidae consists of two subfamilies: Rhynchocyoninae and Macroscelidinae. Rhynchocyoninae species have a relatively large skull with nasal bones having partially ossified tips. The bony palate is not perforated. Macroscelidinae species have a relatively smaller skull and wholly cartilaginous nasal bone tips. The bony palate has some holes |
| Afroasorica   | Aforosoricida consists of two families: Tenrecidae (tenrecs) and Chrysochloridae (golden moles). Tenrec skulls have a long, slender rostrum. The jugal bone is absent and the orbital bone is usually small. The skull of golden moles is abruptly conical, its anterior portion is pointed, and its posterior portion widened. The zygomatic arch is formed by an elongated process of the maxilla, and the occipital area contains the tabular bones, which are not typical in mammals. Tenrec skulls are less morphologically diverse than those of golden moles. It is suggested that the similarities in skull morphology among the speciose genus Microgale masks morphological diversity among the rest of the family |
| Hyracoidia    | All four extant hyrax species have short skulls and deep dentoary bones. The skull has a postorbital bar, which is sometime complete (Dendrohyrax) and sometime incomplete (Heterohyrax and Procavia) | None                                        |
| Proboscidea   | All extant elephant species (Loxodonta and Elephas) have short, tall skulls which are pneumatized particularly in the cranial roof, thereby reducing cranium weight. Skulls bear two tusks derived from the second incisors of the upper jaw |
| Sirenia       | The skulls of Sirenia species are highly specialized for aquatic life, including adaptations such as deep dentoary bones. Sirenia consists of two families: Dugongidae and Trichechidae. In Dugongidae skulls, the premaxilla bones are relatively larger, the nasal bones are absent, and the nasal cavity is shortened. In Trichechidae skulls, the premaxilla bones are small, the nasal bones are present, and the nasal cavity is elongated. Within Trichechidae, Trichechus inunguis is distinct in skull shape. The skull shape of T. senegalensis, T. manatus manatus, and T. m. latirostris are more similar to each other. Within T. manatus, geographic variations in skull morphology, perhaps caused by geographic isolation, are reported |
| Laurasiatheria| Disparity in skull morphology among eulipotyphlans may be explained by phylogeny rather than ecology. In the genus Sorex, similarities and differences in skull shape between species are proportional to the phylogenetic distance between them. Similarly, the degree of morphological variation in the dentoary bone between talpid species corresponds to the phylogenetic distance between the species |
| Eulipotyphla  | Perissodactyla skulls are adapted to an herbivorous diet. Extant Perissodactyla consists of three families: Equidae, Tapiridae, and Rhinocerotidae, and all have a long skull with an elongated face and large cheek teeth adapted for grinding coarse vegetation. Equid skulls are generally flat in a mediolateral direction, with long, deep rostrums. The skulls of the Tapiridae have a well-developed sagittal crest, rostrally positioned orbital bones, and a small cranium with a reduced posterior region. Rhinocerotidae have a thickened, enlarged nasal bone which extends anteriorly beyond the anterior margin of the premaxilla bone. The occipital bone is unusually high where the neck muscles attach to sustain the heavy head | None |
Table 1 (continued)

| Clade          | Remarks on diversity of craniofacial morphology                                                                 | Landmark-based geometric morphometric studies |
|----------------|---------------------------------------------------------------------------------------------------------------|---------------------------------------------|
| Chiroptera     | Bat skulls are morphologically highly diverse. However, the degree of morphological disparity in skull shape is not the same among taxa. The family Pteropodidae, which lost the ability to echolocate, have large orbits accompanied with a well-developed postorbital bar. The rostrum is morphologically uniform despite variation in diet between species. The family Phyllostomidae shows a high level of variation in skull morphology explained by a diversity of diets. Nectarivorous species possess an elongated face while frugivorous species have a shortened face. Skull morphology of the family Vespertilionidae is highly conserved, although it is the most speciose group in the order | [82, 85, 86, 94, 120, 121] |
| Carnivora      | Carnivoran skulls are characterized by an expanded braincase in which the frontal-parietal suture is located posteriorly relative to the postorbital constriction, as well as fully or partially ossified ectotympanic bones that are firmly fused to the skull. Carnivoran skulls are highly varied corresponding to different diets. In general, felid species have a shorter rostrum for production of higher bite force, while canid species typically have a longer rostrum with a large nasal chamber associated with a well-developed olfactory sense. The pinnipeds, semiaquatic marine mammals, usually have a short rostrum, and enlarged orbits | [53, 122–146] |
| Pholidota      | All extant pangolin species have a long, narrow, toothless skull. The dentary bone is narrow and slender as well. The surface of the cranium is smooth without any ridges or crests. The zygomatic arch is present but incomplete. The postorbital bar is absent | None |
| Cetartiodactyla| The skulls of Cetartiodactyla usually have a long rostral portion. The postorbital bar is always present. When horns are present, they are most often formed on the frontal bones. The extant Cetartiodactyla consists of the suborder Suina (pigs and peccaries), the infraorder Cetacea (whales), the infraorder Ancondonta (hippos), and the suborder Ruminantia (cows, goats giraffes, deers etc.). Suiforme skulls are distinct from those of other cetartiodactyls, having a posteriorly extended squamosal bone that contacts the exoccipital bone. Ancondontids have a tall skull with high-positioned orbits, enlarged as well as task-like canines and incisors. Ruminantids bear antlers or horns that are often large and complex in shape. The mastoid bone is exposed between the squamosal and occipital bones. Cetaceans have a highly modified skull caused by posterior migration of the nostrils. The premaxilla and maxilla bones form the roof of the rostrum. Enlarged occipital bones occupy the posterior part of the skull. The nasal and parietal bones are highly reduced in size | [147–157] |
| Euarchontoglires|                                                                                                                |                                             |
| Scandentia     | Treeshrews have a unique, prominent hole in the zygomatic arch. The postorbital bar is well developed and contacts the zygomatic arch. There is variation in skull morphology within Tupaiid genus that might be due to the geographic barriers between populations. For example, island populations have a smaller skull than continental ones | [158–160] |
| Rodentia       | Rodent skulls are unique, bearing a single pair of persistently growing incisors in the upper and lower jaws. The orbital cavity is located dorsal to the cheek teeth. The zygomatic arch fuses to the maxilla in line with the first cheek teeth. The vertical ramus of the dentary bone is enlarged and provides the area for insertion of the masseter muscle. Rodentia consists of three suborders: Myomorpha, Sciuromorpha, Hystrixomorpha. Myomorpha have enlarged temporal bones where a large temporal muscle attaches. The muscle produces high mastication power using cheek teeth. Sciuromorpha have a large vertical ramus of dentary bone where the masseter muscle attaches. This produces a high power in biting using incisors. Hystrixomorpha have a large infraorbital foramen in their skull. Both phylogenetic and ecological factors influence the determination of skull morphology in rodents. In Hystricomorphids (e.g., guinea pigs, porcupines, and spiny rats), phylogenetic constraints are more important than ecological factors in generating morphological variation of the dentary bone. On the other hand, morphological variation of skulls is mainly brought about by ecological factors. Hystricomorphids living in open habitats, such as guinea pigs, have upward-facing orbits and a wide basicranium. Hystricomorphids living in woody areas, such as spiny rats, have more laterally facing orbits and a narrow basicranium | [161–194] |
| Lagomorpha     | Rabbits have a fenestrated skull which is unique among mammals. The fenestration (lattice-like bone) is seen in the proximalateral part of the rostrum. Morphological disparity of skull morphology in the family Leporidae is mainly explained by differences in the degree of facial tilt among species | [195–198] |
| Primates       | Skull morphology is very different between haplorhines and strepsirhines, mainly in relative skull length and width and facial depth. Haplorhines tend to have a mediolaterally wide as well as dorsoventrally tall skull. Strepsirhines have a narrower, shallower skull, an elongated face, and a narrower snout. Intraspecific variation in skull shape has been studied in several groups of primates, including Cercopithecoidae and Hominidae | [199–214] |
| Dermoptera     | The two extant colugo species have skulls with large front-facing orbits that improve binocular vision. The position of three pairs of upper incisors is shifted laterally, and the second upper incisors are transformed into a canine-like shape. The first two lower incisors are broad and form a comb-like shape | None |
Mice with genetic defects related to the migration or proliferation of the ectomesenchyme possess a shortened face [22–25] and/or cleft lip (CL) occasionally accompanying the cleft palate (CP) [19, 26–28]. Several major signaling pathways, including BMP, FGF, Shh, and Wnt signaling pathways, are associated with outgrowth and fusion of the facial processes [19]. Repression of the up-stream component genes of these signaling pathways (e.g., Bmp4, Fgf8, Shh, and Wnt3) leads to a truncated face [19, 22, 24, 29, 30]. Recent papers have reported that migration of ectomesenchyme in the heads of mouse embryos are directly regulated by Wnt5a, a ligand of non-canonical Wnt signaling pathway [22, 25, 31, 32]. Alteration of the level of neural crest-specific Wnt5a expression (by both knockout and over-expression) results in a widened, shortened face [25, 33]. In Wnt5a conditional knockout mice, the migration pattern of the ectomesenchyme that later occupies the internal space of the facial processes is altered from that in control wild type mice [25]. The change in the ectomesenchyme migration pattern was attributed to the disruption of the directionality of cell division [25]. The induction of the internal facial structures (e.g., cartilage, bones, sensory compartments, muscles, glands, and teeth) was not influenced, and the lower jaw’s volume in the Wnt5a conditional knockout mouse was almost equivalent to that of the control mouse [25]. These results suggest that Wnt5a could play a crucial role in generating a shortened, widened face (truncated face) as naturally seen in koalas, sloths, the great apes, and cats through regulating the ectomesenchyme's migration pattern, which in turn governs growth and organization of the facial processes (Fig. 1).

Disruptions in the growth and fusion of the facial processes also cause CL with or without CP (collectively called ‘CL/P’) [26–28]. A fusion of the facial processes first occurs between LNP and MNP, followed by a fusion of LNP and MAX. Finally, the anterior ends of both MAX and MNP are fused to one another. Fusion of the facial processes is initiated by contact of the epithelium of each facial process through proper organization of the facial processes [19]. Subsequently, the epithelial seam between coadjacent facial processes disappears due to apoptosis. Fusion of the MNP and the MAX and fusion of the MNP and the LNP are defective in mutants of the genes (e.g., Bmp4, Bmpr1a, Tcfap2a, Sox11, and Wnt9b) that regulate

### Table 2: The genes involved in shortening the face and making the orofacial cleft in mouse

| Gene   | Mutant | Phenoype       | Protein function                          | Signaling pathway | References |
|--------|--------|----------------|-------------------------------------------|-------------------|------------|
| Bmp2/4| Wnt1Cre; Bmp4f/f; Bmp4f/f | Truncated face | Signaling molecule BMP | [30] |
| Bmpr1a| NestinCre; Bmpr1a−/f  | CL/P            | Receptor BMP | [215] |
| Ctnnb1| Ctnnb1f/f; Cect   | Truncated face | Transcription factor, regulation of cell–cell adhesion Wnt | [22] |
| Mtx1  | Mtx1−/−   | Truncated face | Transcription factor BMP | [23] |
| Ptc1  | Wnt1Cre; Ptc1f/f | CL              | Receptor Hh | [216] |
| Smo   | Wnt1Cre;Smo−/c | Truncated face | Receptor Hh | [217] |
| Tflap2a| Tflap2a Neo(hypomrpha)/Null model | CL/P            | Transcription factor FGF Notch BMP | [218] |
| Wnt3  | Wnt3−/−  | CL/P            | Signaling molecule Wnt | [219] |
| Wnt9b | Wnt9b−/cF1  | CL/P            | Signaling molecule Wnt | [220] |
| Wnt5a | Wnt5a−/− | Truncated face | Signaling molecule Wnt | [25, 31, 32] |
| Bmpr1a| Os2-Cre;Bmpr1a−/f | SMCP            | Receptor BMP | [38] |
| Fblin5| Fblin5−/− | Shortened face | Secreted extracellular matrix protein MAPK-Erk | [35] |
| Fgf8  | Osr2Cre;Rosa26R-Fgf8 | CP              | Growth factor FGF | [221] |
| Kif3a | Wnt1Cre;Kif3a−/f | CP              | Motor protein Hh | [222] |
| Mtx1  | Mtx1−/−  | Shortened face | Transcription factor BMP | [223, 224] |
| Mn1   | Mn1−/−   | Shortened face | Transcription coregulator N/A | [225] |
| Shox2 | Shox2−/− | CP              | Transcription factor N/A | [226] |
| Tbx22 | Tbx22−/− | SMCP            | Transcription factor BMP | [39] |
| Tgfb2 | Wnt1Cre; Tgfb2−/f | CP              | Receptor TGF-β | [227] |
apoptosis within the epithelium as well as outgrowth and organization of the facial processes. Failure of these facial processes fusing accompanies CL/P [26].

**Ossification of the facial bones**
The palate of mammals separates the oral cavity from the nasal cavity and is subdivided into the anterior bony hard palate (palatal bones) and posterior soft palate [34]. The formation of the palate (palatogenesis) proceeds in two steps, the primary and secondary palate formations. In mouse development, the primary palate is formed by the fusion of the MAXs and MNPs at E11.5. Subsequently, the secondary palate is formed through three consecutive events. First, a pair of palatal shelves is formed by an uplift of the tongue at E11.5. Second, at E14.5, each palatal shelf grows medially above the tongue through ‘palatal shelf elevation’ [34]. Third, the left and right palatal shelves meet and fuse at the midline at E15.0 with fusion completing at E17.0. Palatal bones (anterior premaxilla derived from the ectomesenchyme of the primary palate, and central maxilla and posterior palatine that are derived from the ectomesenchyme of the secondary palate) begin to form at E14.5.

In contrast to defects in facial process development that produce an extremely shortened face (see the previous section), defects in facial bone formation, which occur in later phases of facial development, lead to a shortened face with milder dysmorphology. For example, *Fbln5* knockout mice exhibit decreased outgrowth of the premaxilla bones during postnatal stages, compared to control wild type mice [35]. Fibulin-5 is an extracellular matrix protein deposited as a fibrous matrix in neural crest-derived craniofacial suture mesenchyme and plays a role as a regulator of cellular function such as cell proliferation [35, 36]. While premaxilla-maxilla suture mesenchyme in *Fbln5* knockout mice were capable of differentiating into osteoblasts, suture cells in the mutant were less proliferative, suggesting fibulin-5 is indispensable for the regulation of facial suture mesenchymal cell proliferation required for craniofacial skeletal morphogenesis [35]. External facial morphology of adult *Fbln5* knockout mice is almost normal, although facial length is slightly shortened compared to the control [35].

Defected facial bone development also leads to a submucous cleft palate (SMCP). SMCP is a clinical subgroup of CP. While CP is characterized by the whole palate (including both bones and epithelium) separated at the midline, SMCP is characterized by incomplete fusion of left and right palatal bones at the midline without cleft formation in the oral epithelium covering the bones. In mouse transgenesis, SMCP is only observed in the region between left and right maxilla bones. Only two genes that cause SMCP have been reported to date, *Bmpr1a* and *Tbx22*. In *Osr2-IresCre;Bmpr1adF* transgenic mice, *Bmpr1a* was specifically knocked out in the tissue constructing the secondary palate. Osr2, whose promoter sequence was used for tissue/time-specific *Bmpr1a* knockout, is uniquely expressed in secondary palate morphogenesis in mice (see [37] for detail). The tissue-specific inactivation of *Bmpr1a* causes reduction of mesenchymal condensation in the anterior part of the secondary palate which subsequently differentiates into the maxilla bones [38]. Expression of Runx2, Osterix, and *Dlx5*, genes encoding transcriptional factors for bone development, is severely down-regulated in the anteromedial part of the secondary palate of *Osr2-IresCre;Bmpr1adF* transgenic mice. As a result, elongation of the maxilla bones toward the midline is blocked, resulting in a cleft between the left and right maxilla bones [38]. Tbx22 is a transcription factor required for palatal bone formation [39]. Tbx22 knockout embryos bear a CP or SMCP accompanied by delayed osteoblast differentiation and hypotrophic maxilla bones [39].

To our knowledge, elongation of the face in transgenic mice compared to wild type mice has not been reported to date. In fish and birds, longer and more pointed jaws or beaks are formed by up-regulation of calmodulin signaling [40–43]. In mammals, however, the function of calmodulin signaling in facial development is poorly understood. Runx2 may regulate facial length in mammals. We briefly review the correlation between facial length and the variation of glutamine/alanine tandem repeats within Runx2 in the next section.

**The number of Runx2 tandem repeats and mammalian facial length**
There are long- and short-faced taxa in each mammalian group, and both face types show a high degree of diversity and evolvability in facial length (Fig. 1). Runx2 (Runt-related transcription factor 2) is an important transcription factor protein that plays multiple roles in bone development (e.g., osteoblast differentiation) in vertebrates including mammals [44–46] (reviewed in [47]). Runx2 enhances early osteoblast differentiation but inhibits terminal osteoblast differentiation [48]. Therefore, up-regulation of Runx2 leads to accelerated (via early onset of osteoblast differentiation) and extended (via delayed termination of osteoblast differentiation) bone development, while down-regulation of Runx2 results in delayed, shortened bone development [48, 49].

The Runx2 protein contains a highly conserved RUNT DNA binding domain and a repetitive glutamine (Q) and alanine (A) domain [46, 50]. Changes to the tandem repeat glutamines to alanines ratio (QA ratio), calculated by dividing the number of consecutive glutamines by the number of consecutive alanines within Runx2, alter
transcriptional activity of Runx2 and its target genes [49, 51].

The Runx2 QA tandem repeat ratio is correlated with facial length variation in carnivores [49, 52, 53]. Species with higher QA ratios have longer faces [49] (Fig. 2). In contrast, a lower QA ratio leads to lower transcriptional activity of Runx2 and results in short-faced carnivorans [49] (Fig. 2). This suggests that the QA ratio is associated with allometric variation in carnivore facial length and the timing of facial bone (e.g., premaxilla, maxilla, nasal, jugal, vomer, palatine, and dentary) ossification. A similar pattern has been reported in primates [54].

Conversely, there is no correlation between the Runx2 QA tandem repeat ratio and facial length in xenarthrans and afrotherians [55], and marsupials [51]. Although marsupials display variation in facial length roughly equivalent to that observed in placentals (Fig. 1), almost no variation is observed in the nucleotide sequence of glutamine/alanine repeats in Runx2 [51]. The extreme conservation of nucleotide sequence and the QA ratio in marsupials may heavily constrain the timing of facial bone ossification in marsupial species [51]. These results suggest that the variations of facial length in xenarthrans, afrotherians, and marsupials are brought about by distinct molecular mechanisms. For example, a missense mutation in the gene Bmp3 (that encodes a growth factor, Bone morphogenetic protein 3) causes brachycephaly (shortened head) in domestic dogs [56]. We recommend further research concerning the role of morphogenetic genes such as Bmp3 to improve our understanding of the mechanisms generating facial length variation in mammals other than carnivorans and primates.

**Bats: a model for understanding the diversification of mammalian craniofacial morphology**

As reviewed in section II, our understanding of mammalian facial development mechanisms has been informed by studies of laboratory mice. However, the developmental mechanisms that produce facial morphology in non-model, wild mammal species have been only partially understood, perhaps due to difficulties in obtaining embryonic materials for analyses. More is understood about the molecular and cellular mechanisms underlying diversification of facial (beak) morphology in non-model bird species thanks to a series of evo-devo studies of Darwin’s finches, one of the most famous examples of adaptive radiations in vertebrates [40, 57–62]. Although model mammals help us to understand the basic mechanisms of mammalian morphogenesis, studying non-model species is necessary to identify other molecular and cellular mechanisms that lead to the morphological evolution of this group of vertebrates (including humans). Here, we focus on bats as a potential model for understanding evolution of mammalian craniofacial morphology.

Bats (order Chiroptera) are the second largest group of mammals after rodents [2, 63]. More than 1300 extant bat species are known, classified into 20 families [63]. Recent molecular phylogenetic studies [64–67] identified two major clades within bats, the Yinpterochiroptera and Yangochiroptera (Fig. 3). Chiropterans are distributed worldwide in all but the coldest regions [63], probably facilitated by the evolution of flight [68–80]. Although largely neglected by biologists, diversity in bat facial morphology is astonishing. This diversity reflects their adaption to various environments and highly impressed Ernst Haeckel, an influential comparative embryologist and an artist in nineteenth century
New World leaf-nosed bats (family Phyllostomidae) are especially known for their incredible facial diversity [82, 83]. Phyllostomid facial length is strongly correlated with diet [84–86]. For example, frugivorous species (e.g., the wrinkle-faced bat, *Centurio senex*) have a truncated, widened face that exerts a high bite force. In contrast, nectarivorous species (e.g., the mexican long-tongued bat, *Choeronycteris mexicana*) have a long, narrow face that helps them to insert their rostrum into flowers. However, the molecular and cellular mechanisms that regulate the facial length of bats and are responsible for generating existing diversity in craniofacial morphology are poorly understood.

Bats have a unique morphological feature in the rostral part of the upper jaw, an orofacial cleft on the premaxilla and maxilla bones that is anatomically similar to that observed in humans with congenital anomalies [87, 88]. There are two types of chiropteran orofacial cleft, midline and bilateral clefts. The midline cleft evolved only once in the common ancestor of Rhinolophidae and Hipposideridae. Character mapping was based on Orr et al. [88].
Midline clefts are U-shaped clefts present between two premaxilla bones that are highly reduced in size (Fig. 6). Each premaxilla bone bears two permanent incisors and is completely fused to the maxilla bone posteriorly. The inner space of the cleft is occupied with a robust, translucent, fibrous membrane. The bilateral cleft is only seen in Rhinolophidae and Hipposideridae (Fig. 3). In this cleft type, the premaxilla bone, which bears a single diminutive incisor, is separated from the laterally located maxilla bone by a cleft. The cleft is filled with fibrous connective tissue. The posterior margin of the medially fused premaxilla bones is loosely connected to the maxilla bones with fibrous connective tissue.

Bat orofacial clefts may contribute to reduction of returning echolocation signal interference, modulation of nasal acoustic emissions, increasing oral gape to facilitate capture of large prey, reduction of overall weight, and increase of olfactory ability [88]. However, the molecular and cellular mechanisms underlying orofacial cleft development in bats and the degree to which development of the two cleft types is similar are currently unknown.

Few studies have investigated the molecular mechanisms related to craniofacial diversity in bats. One such study by Phillips et al. [89] focused on Pax9, a transcription factor that plays an important role in vertebrate craniofacial and dental development. The authors compared

---

Fig. 4 Diversity of craniofacial morphology in bats. Left, a picture drawn by Ernst Haeckel, an influential comparative embryologist and artist [81]. Right, the silhouettes of the bat species illustrated in the Haeckel’s picture: (1) lesser long-eared bat (*Nyctophilus geoffroyi*), frontal view of the head; (2) brown long-eared bat (*Plecotus auratus*), frontal view of the head; (3) brown long-eared bat, entire body; (4) lesser false vampire bat (*Megaderma spasma*), frontal view of the head; (5) big-eared woolly bat (*Chiropterus auritus*), lateral view of the head; (6) Tomes’s sword-nosed bat (*Lonchochirina aunita*), caudo-lateral view of the head; (7) Tomes’s sword-nosed bat, frontal view of the head; (8) Mexican funnel-eared bat (*Natalus stramineus*), frontal view of the head; (9) Antillean ghost-faced bat (*Mormoops blainvillei*), frontal view of the head; (10) flower-faced bat (*Anthops ornatus*), high magnification of noseleaf; (11) greater spear-nosed bat (*Phyllostomus hastatus*), frontal view of the head; (12) thumbless bat (*Furipterus horrens*), frontal view of the head; (13) greater horseshoe bat (*Rhinolophus ferrumequinum*), frontal view of the head; (14) wrinkle-faced bat (*Centurio senex*), frontal view of the head; (I) spectral bat (*Vampyrum spectrum*), frontal view of the head.
nucleotide sequences of the 3′ untranslated region (UTR) of Pax9 among phyllostomids, vespertilionids, and other mammalian orders and identified four Musashi-binding elements (MBE) within conserved regions of the 3′ UTR [89]. The number of MBEs in morphologically diverse phyllostomid bats varied but was invariant in morphologically similar vespertilionid bats with the exception of a Murina species [89]. Because the number of MBEs may affect the expression level of Pax9, the authors proposed that the evolution of Pax9 regulation may be a contributing mechanism to the radiation of craniofacial morphological diversity in bats [89]. Although this study provides valuable insight into a potential genetic mechanism underlying the evolution and diversification of craniofacial morphology in phyllostomid bats, our understanding of the fundamental facial development mechanisms is far from complete.

Because convergence or parallel evolution of morphological traits in vertebrates is often brought about by identical genetic mechanisms (e.g., [90–93]), common mechanisms might regulate facial length even in bats (superorder Laurasiatheria) and rodents (superorder Euarchontoglires; Table 2).

In mice, a shortened face without apparent facial bone defects is mainly brought about by a decrease in proliferation and differentiation of the ectomesenchyme which later differentiates into osteoblasts [35]. In addition, facial length variation observed in carnivorans and primates are correlated with the level of activity of Runx2, which influences facial bone development duration [49]. Therefore, facial length variation in bats could be attributed to differences in the duration of facial bone development among species. For example, nectarivorous bats (e.g., Choeronycteris mexicana) have a relatively longer face. In this case, the duration of facial bone development might be extended, giving facial bones time to enlarged, especially anteriorly (Fig. 5). Conversely, insectivorous or omnivorous bats (e.g., Macrophyllum macrophyllum) have a relatively shorter face. Here, the period of facial bone development may be shortened leading to earlier completion of facial bone growth and preventing further anterior elongation (Fig. 5). Indeed, heterochronic shift in formation and growth of the palatal bones may produce variations of craniofacial morphology in phyllostomid bats [94]. Sears supposed that the diversity of palate shapes along phyllostomids is the result of relatively subtle evolutionary changes in later rather than earlier developmental event. Although it is likely that Runx2 plays a crucial role in producing facial length diversity in carnivorans and primates [49, 52–54], its function in chiropteran craniofacial development has yet to be identified and warrants further investigations.

The truncated face of Wnt5a conditional knockout mice is brought about by the disruption of ectomesenchyme migration within the facial processes [25]. Notably, some phyllostomid bats (e.g., Centurio senex) possess...
an extremely truncated face that shares multiple characteristics with \textit{Wnt5a} knockout mice faces. Therefore, facial morphology in these bat species might be derived from changes in expression of the genes that control direction of migration of the ectomesenchyme through regulating the directionality of cell division within the facial processes (Fig. 5). It would be interesting to compare \textit{Wnt5a} activity and expression pattern in facial ectomesenchyme among chiropteran species.

The orofacial clefts observed in bats are morphologically categorized as SMCP. They are probably brought about by changes in premaxilla and maxilla bone formation. As we introduced in section II, \textit{Osr2-IresCre:Bmpr1a} \cite{38} mice have a cleft between paired maxilla bones \cite{38}. If \textit{Bmpr1a} expression is specifically inactivated in the primary palate region using a similar transgenic technique (e.g., using a promoter of the gene that is uniquely expressed in the primary palate in gene knockout), a cleft may appear between paired premaxilla bones that are derived from the ectomesenchyme distributed within the primary palate. Considering this, the midline cleft in bats, which is present between two premaxilla bones, could be explained by domain-specific repression or down-regulation of \textit{Bmpr1a} in the ectomesenchyme within the primary palate (instead of the secondary palate) that later gives rise to the premaxilla bones (Fig. 6). Because \textit{Bmpr1a} is a receptor of the growth factor, the down-regulation of \textit{Bmpr1a} may decrease the degree of ossification of the premaxilla bone through heterochrony (shorter and/or delayed ossification of the bone compared to the ancestor) and may result in such a small-scale morphological change in the tip of the face.
The formation of the bilateral cleft could be much more complicated, perhaps associated with extensive alterations of the developmental program. The premaxilla bones are derived from the ectomesenchyme distributed within the primordium of the primary palate, while the maxilla bones are derived from that of the secondary palate. Therefore, in the facial development of bat species bearing the bilateral cleft, the relative position of the primary and secondary palates might be changed through alterations in formation and organization of the facial processes from those in bat species without orofacial cleft. We speculate that the bilateral cleft developed through the following three steps (Fig. 6). First, the ectomesenchyme occupying the secondary palate expanded its distribution antero-medially and restricted the space for primary palate development at the tip of the face. Second, the osteoblasts derived from the ectomesenchyme distributed within the anterior part of the secondary palate differentiated into bone and made anterior projection of the maxilla bones surrounding the premaxilla bone laterally. Thus, the position of the premaxilla bone became restricted at the center of the tip of the face. Third, inhibition of ossification at the suture between the medially positioned premaxilla and laterally positioned maxilla bones left the unossified area between the two bones as a cleft.

Orofacial clefts occur as a craniofacial anomaly in humans at a relatively high frequency (approximately 1 in 700 live births) [88]. Investigating the mechanisms behind orofacial cleft formation in bats may contribute not only to understanding the reason why this cranial feature, which usually occurs as a skeletal pathology in other mammals groups including humans, appears as a normal phenotype in bats, but also to developing novel therapies against human orofacial cleft.

In the last 15 years, several studies have described in detail the overall embryonic development [95–100] and specifically wing development of bat species where embryos could be obtained [68–77, 79, 80]. We believe that examination of bat facial development and its comparisons among the species provide profound insights into the molecular and cellular bases of craniofacial morphology diversification in mammals.

Conclusions
In this paper, we have reviewed recent advances in understanding how mammalian faces are formed and discussed how these data are being applied to make new hypotheses about the diversity creation in mammalian craniofacial morphology. Small-scale changes in facial morphology from the ancestor, such slight changes in facial length and the evolution of the midline cleft in some lineages of bats could be attributed to heterochrony in facial bone ossification. In contrast, large-scale changes in facial morphology from the ancestor, such as a truncated, widened faces, as well as the evolution of the bilateral cleft in some bat species, could be brought about by changes in growth and patterning of the facial primordium (the facial processes) at the early stages of embryogenesis. Significant work remains to be done to test these hypotheses.

Abbreviations
CL: cleft lip; CP: cleft palate; FNP: frontonasal process; LNP: lateral nasal process; MAN: mandibular process; MAX: maxillary process; MBE: Musashi-binding elements; MNP: medial nasal process; SMCP: submucous cleft palate.

Authors’ contributions
All authors contributed to the writing and editing of the manuscript. Both authors read and approved the final manuscript.

Acknowledgements
We thank Dr. Shin-ichiro Kawada (National Museum of Nature and Science, Tokyo) and Dr. Hiroko Kudo-Hirotani (Kanagawa Prefectural Museum of Natural History) for arrangement of the stuffed specimens of mammals, Mr. Jason Preble for proofreading the early version of the manuscript, and all members of the laboratory of Vertebrate Diversity and Evolution, Toho University for helpful discussions.

Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
Not applicable.

Consent for publication
Not applicable.

Ethics approval and consent to participate
Not applicable.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 26 February 2018   Accepted: 25 May 2018

Published online: 14 June 2018

References
1. Kemp TS. The origin and evolution of mammals. Oxford: Oxford University Press; 2005.
2. Wilson DE, Reeder DM. Mammal species of the world. 3rd ed. Baltimore: Johns Hopkins University Press; 2005.
3. Vaughan TA, Ryan JM, Czaplewski NJ. Mammalogy. 6th ed. Burlington: Jones & Bartlett Learning; 2013.
4. Feldhamer GA, Drickamer LC, Vessey SH, Merritt JF, Krajewski C. Mammalogy: adaptation, diversity, ecology. 4th ed. Baltimore: Johns Hopkins University Press; 2014.
5. Meredith RW, Janečka JE, Gatesy J, Ryder OA, Fisher CA, Teeling EC, Goodbla A, Ezirik E, Simão TL, Stadler T, Rabosky DL, Honeycutt RL, Flynn JJ, Ingram CM, Steiner C, Williams TL, Robinson TJ, Burk-Herrick A, Westerman M, Ayoub NA, Springer MS, Murphy WJ. Impacts of the cretaceous terrestrial revolution and KPg extinction on mammal diversification. Science. 2011;334:521–4.
6. Dos Reis M, Inoue J, Hasegawa M, Asher RJ, Donoghue PC, Yang Z. Phyllogenomic datasets provide both precision and accuracy in
estimating the timescale of placental mammal phylogeny. Proc Natl Acad Sci. 2012;109:14942–7.

7. Song S, Liu L, Edwards SV, Wu S. Resolving conflict in eutherian mammal phylogeny using phylogenomics and the multispecies coalescent model. Proc Natl Acad Sci. 2012;109:14942–7.

8. O’Leary MA, Bloch J, Flynn JJ, Gaudin TJ, Giallombardo A, Giannini NP, Goldberg SL, Kraatz BP, Luo ZX, Meng J, Ni X, Novacke MJ, Perini FA, Randall ZS, Rougier GW, Sargis EJ, Silcox MT, Simmons NB, Spaulding M, Velazco PM, Weksler M, Wible JR, Ciranne AL. The placental mammalian ancestor and the post-K-Pg radiation of placentals. Science. 2013;339:662–7.

9. Mitchell KJ, Pratt RC, Watson LN, Gibb GC, Llamas B, Kasper M, Edson J, Hopwood B, Male D, Armstrong KN, Meyer M, Hofreiter M, Austin J, Donnellan SC, Lee MS, Phillips MJ, Cooper A. Molecular phylogeny, biogeography, and habitat preference evolution of Marsupials. Mol Biol Evol. 2014;31:2322–30.

10. Foley NM, Springer MS, Teeling EC. Mammal madness: is the mammal tree of life not yet resolved? Philos Trans R Sci. 2016;371:20150140.

11. Tarver JE, Dos Reis M, Mirarab S, Moran RJ, Parker S, O’Reilly JE, King BL, O’Connell MJ, Asher RJ, Warnow T, Peterson KJ, Donoghue PC, Pisani D. The interrelationships of placental mammals and the limits of phylogenetic inference. Nat Bio Rev Biol. 2016;8:330–44.

12. Marcus E, Hingst-Zaheer E, Zäher H. Application of landmark morphometries to skulls representing the orders of living mammals. Hystrix. 2000;11:27–47.

13. Brugmann SA, Kim J, Helms JA. Looking different: understanding diversity in facial form. Am J Med Genet A. 2006;140:2519–21.

14. Goswami A. Cranial modularity shifts during mammalian evolution. Am Nat. 2006;168:270–80.

15. Porto A, de Oliveira FR, Shirai LT, De Conto V, Marroig G. The Evolution of modularity in the mammalian skull: morphological integration patterns and magnitudes. Evol Biol. 2009;36:118–35.

16. Koyabu D, Maier W, Sánchez-Villagra MR. Paleontological and developmental evidence resolve the homology and dual embryonic origin of a mammalian skull bone, the interparietal. Proc Natl Acad Sci USA. 2012;109:14075–80.

17. Cardina A, Polly PD. Larger mammals have longer faces because of size-related constraints on skull form. Nat Commun. 2013;4:2458.

18. Koyabu D, Werneburg I, Morimoto N, Zollikofer CP, Forasiepi AM, Endo H, Kimura J, Ohdachi SD, Truong Son N, Sánchez-Villagra MR. Mammalian skull heterochrony reveals modular evolution and a link between cranial development and brain size. Nat Commun. 2014;5:3625.

19. Jiang R, Bush JO, Lidral AC. Development of the upper lip: morphogenetic and molecular mechanisms. Dev Dyn. 2006;235:1152–66.

20. Tehiler K. The house mouse: atlas of embryonic development. New York: Springer; 1989.

21. O’Rahilly R, Müller F. The development of the neural crest in the human. J Anat. 2007;211:335–51.

22. Medio M, Yeh E, Popelut A, Babajko S, Berdal A, Helms JA. Wnt/β-catenin signaling and activation of canonical Wnt signaling during midfacial morphogenesis in mice. Dev Dyn. 2008;235:1484–50.

23. Alleged regulates a dose-dependent transcriptional program to control craniofacial development. Development. 2012;139:709–19.

24. Bakker ERM, Raghoebir L, Franken PF, Helvenstein W, van Gurp L, Meijlink F, van der Valk MA, Rottier RJ, Kuipers EJ, van Veelen W, Smits R. Induced Wnt5a expression perturbs embryonic outgrowth and intestinal elongation, but is well-tolerated in adult mice. Dev Biol. 2012;369:91–100.

25. Ho HY, Susman MW, Bikoff JB, Ruy YK, Jonas AM, Hu L, Kuruvilla R, Greenberg ME. Wnt5a-Ror-Dishevelled signaling constitutes a core developmental pathway that controls tissue morphogenesis. Proc Natl Acad Sci USA. 2012;109:4044–51.

26. Amerongen RV, Fuerer C, Muzatii M, Nusse R. Wnt5a can both activate and repress Wnt/β-catenin signaling during mouse embryonic development. Dev Biol. 2012;369:101–14.

27. Bush JO, Jiang R. Palatogenesis: morphogenetic and molecular mechanisms of secondary palate development. Development. 2012;139:828.

28. Noda K, Nakamura T, Komatsu Y. Fibulin-5 deficiency causes developmental defect of premaxillary bone in mice. Biochem Biophys Res Commun. 2015;361:1–4.

29. Yanagisawa H, Schubert BM, Breken RA, Schmelzer A, Tsukahara S, Fibulin-5, an integrin-binding macromolecular protein: its function in development and disease. J Cell Commun Signal. 2009.3:337–47.

30. lan Y, Ovitt CE, Cho E, Maltby KM, Wang Q, Jiang R. Odd-skipped related 2 (Osir2) encodes a key intrinsic regulator of secondary palate growth and morphogenesis. Development. 2004;131:3207–16.

31. Baek JA, Lan Y, Liu H, Maltby KM, Mishina Y, Jiang R. Bmpr2a signalizing plays critical roles in palatal shelf growth and palatal bone formation. Dev Biol. 2011;350:520–31.

32. Pauw E, Hoshino A, Bentley L, Prajapati S, Keller C, Hammond P, Martinez-Barbera J, Moore GE, Stanier P. Bsh2x mouse have a submucous cleft palate due to reduced palatal bone formation and also display ankyloglossia and choanal atresia phenotypes. Hum Mol Genet. 2009;18:4171–9.

33. Abzhanov A, Kuo WP, Hartman C, Grant BR, Grant PR, Tabin CJ. The calmodulin pathway and evolution of elongated beak morphology in Darwin’s finches. Nature. 2006;442:563–7.

34. Parsons JK, Albertson RC. Roles for Bmp4 and Cam1 in shaping the jaw: an anatomical study. J Anat. 2002;201:427–36.

35. Komori T. Regulation of skeletal development by the Runx family of transcription factors. J Cell Biochem. 2005;95:445–53.

36. Knief U, Schleizhe H, Kumpfensab E, Eliegren H, Forstreiner W. QTL and quantitative genetic analysis of beak morphology reveals patterns of standing genetic variation in an Estrildid finch. Mol Ecol. 2012;21:3704–17.

37. Gunter H, Koppe mann C, Meyer A. Revisiting de Beer’s textbook example of heterochrony and jaw elongation in fish: calmodulin expression reflects heterochronous growth, and underlies morphological innovation in the jaws of belonoid fishes. EvoDevo. 2014;5:8.

38. Suda T, Kobayashi K, Jimi E, Udagawa N, Takahashi N. The molecular basis of osteoblast differentiation and activation. Novartis Found Symp. 2001;232:235–50.

39. Komoni T. Regulation of skeletal development by the Runx family of transcription factors. J Cell Biochem. 2005;95:445–53.

40. Schroeder TM, Jensen ED, Westendorf JJ. Runx2: A master organizer of gene transcription in developing and maturing osteoblasts. Birth Defects Res C Embryo Today. 2005;75:213–25.

41. Komoni T. Regulation of skeletal development by the Runx family of transcription factors. J Cell Biochem. 2005;95:445–53.

42. Knief U, Schleizhe H, Kumpfensab E, Eliegren H, Forstreiner W. QTL and quantitative genetic analysis of beak morphology reveals patterns of standing genetic variation in an Estrildid finch. Mol Ecol. 2012;21:3704–17.

43. Gunter H, Koppe mann C, Meyer A. Revisiting de Beer’s textbook example of heterochrony and jaw elongation in fish: calmodulin expression reflects heterochronous growth, and underlies morphological innovation in the jaws of belonoid fishes. EvoDevo. 2014;5:8.

44. Suda T, Kobayashi K, Jimi E, Udagawa N, Takahashi N. The molecular basis of osteoblast differentiation and activation. Novartis Found Symp. 2001;232:235–50.

45. Komoni T. Regulation of skeletal development by the Runx family of transcription factors. J Cell Biochem. 2005;95:445–53.

46. Schroeder TM, Jensen ED, Westendorf JJ. Runx2: A master organizer of gene transcription in developing and maturing osteoblasts. Birth Defects Res C Embryo Today. 2005;75:213–25.

47. Tomita K, Chaeychomsri W, Siruntawineti J. Skeletal gene expression in the temporal region of the reptilian embryos: implications for the evolution of reptilian skull morphology. Springerplus. 2013;2:336.

48. Tomita K, Runx2, a multifunctional transcription factor in skeletal development. J Cell Biochem. 2002;82:4171–9.
52. Fondon JW 3rd, Garner HR. Molecular origins of rapid and continuous morphological evolution. Proc Natl Acad Sci USA. 2004;101:18058–63.

53. Fondon JW 3rd, Garner HR. Detection of length-dependent effects of tandem repeat alleles by 3-D geometric decomposition of craniofacial variation. Dev Genes Evol. 2007;217:79–85.

54. Ritzman TB, Banovich N, Buss KP, Guida J, Rulka B, Mundy NI, Asheville RU, Bradley B. RUNK2 tandem repeats and the evolution of facial length in placental mammals. BMC Evol Biol. 2012;12:103.

55. Pointer MA, Kamilar JM, Warmuth V, Chester SGB, Delsuc F, Mundy NI, Abzhanov A, Protas M, Grant BR, Grant PR, Tabin C. Bmp4 and morphological evolution of beaks in Darwin’s Finches. Science. 2004;305:1462–5.

56. Fritz JA, Brancale J, Tokita M, Burns KJ, Hawkins MB, Abzhanov A, Brenner MP. Scaling and shear transformations capture beak shape variation in Darwin’s finches. Proc Natl Acad Sci USA. 2010;107:3356–60.

57. Mallarino R, Grant PR, Grant BR, Herrel A, Kuo WP, Abzhanova A. Two developmental modules establish 3D beak-shape variation in Darwin’s finches. Proc Natl Acad Sci USA. 2011;108:4057–62.

58. Mallarino R, Campás O, Fritz J, Burns K, Weeks OG, Brenner MP, Abzhanov A. Closely related bird species demonstrate flexibility between beak morphology and underlying developmental programs. Proc Natl Acad Sci USA. 2012;109:16222–7.

59. Fritz J, Brancale J, Tokita M, Burns KJ, Hawkins MB, Abzhanov A, Brenner MP. Shared developmental programme strongly constrains beak shape diversity in songbirds. Nat Commun. 2014;5:3700.

60. Tokita M, Yano W, James HF, Abzhanov A. Cranial shape evolution in extant bats (Chiroptera). Mol Biol Evol. 2005;22:1869–86.

61. Shi JJ, Rabosky DL. Speciation dynamics during the global radiation of flowering plants. Proc Natl Acad Sci USA. 2012;109:16222–7.

62. Shi JJ, Rabosky DL, Rimbault M, Decker B, Kidd JM, Sood R, Boyko AR, Fondon JW 3rd, Wayne RK, Bustamante CD, Ciruna B, Ostrander EA. Variation of BMP3 contributes to dog breed skull diversity. PLoS Genet. 2012;8:1–11.

63. Fenton MB, Simmons NB. Bats a world of science and mystery. Chicago: Cambridge University Press. 2012;385–409.

64. Eick GN, Jacobs DS, Matthee CA. A nuclear DNA phylogenetic perspective on the evolution of echolocation and historical biogeography of extant bats (Chiroptera). Mol Biol Evol. 2005;22:1869–86.

65. Teeling EC, Springer MS, Madsen O, Bates P, O’Brien SJ, Murphy WJ. Molecular analyses elucidate the evolutionary relationships of bats. Curr Biol. 2007;17:20150481.

66. Teeling EC, Springer MS, Madsen O, Bates P, O’Brien SJ, Murphy WJ. A molecular phylogeny for bats illuminates biogeography and the fossil record. Science. 2005;307:580–4.

67. Giannini NP, Simmons NB. The Chiropteran Premaxilla: a reanalysis of morphological variation and its phylogenetic interpretation. Am Museum Novit. 2007;3585:1–44.

68. Orr DJA, Teeling EC, Puechmaille SJ, Finarelli JA. Patterns of orofacial clefing in the facial morphology of bats: a possible naturally occurring model of cleft palate. J Anat. 2016;229:569–77.

69. Phillips CD, Butler B, Fondon JW, Mantilla-Meluk H, Baker RJ. Contrastive evolutionary dynamics of the developmental regulator PAX9, among bats, with evidence for a novel post-transcriptional regulatory mechanism. PLoS ONE. 2013;8:e57649.

70. Phillips CD, Butler B, Fondon JW, Mantilla-Meluk H, Baker RJ. Contrasting evolutionary dynamics of the developmental regulator PAX9 among bats, with evidence for a novel post-transcriptional regulatory mechanism. PLoS ONE. 2013;8:e57649.

71. Phillips CD, Butler B, Fondon JW, Mantilla-Meluk H, Baker RJ. Contrasting evolutionary dynamics of the developmental regulator PAX9 among bats, with evidence for a novel post-transcriptional regulatory mechanism. PLoS ONE. 2013;8:e57649.

72. Phillips CD, Butler B, Fondon JW, Mantilla-Meluk H, Baker RJ. Contrasting evolutionary dynamics of the developmental regulator PAX9 among bats, with evidence for a novel post-transcriptional regulatory mechanism. PLoS ONE. 2013;8:e57649.

73. Phillips CD, Butler B, Fondon JW, Mantilla-Meluk H, Baker RJ. Contrasting evolutionary dynamics of the developmental regulator PAX9 among bats, with evidence for a novel post-transcriptional regulatory mechanism. PLoS ONE. 2013;8:e57649.

74. Phillips CD, Butler B, Fondon JW, Mantilla-Meluk H, Baker RJ. Contrasting evolutionary dynamics of the developmental regulator PAX9 among bats, with evidence for a novel post-transcriptional regulatory mechanism. PLoS ONE. 2013;8:e57649.

75. Phillips CD, Butler B, Fondon JW, Mantilla-Meluk H, Baker RJ. Contrasting evolutionary dynamics of the developmental regulator PAX9 among bats, with evidence for a novel post-transcriptional regulatory mechanism. PLoS ONE. 2013;8:e57649.

76. Phillips CD, Butler B, Fondon JW, Mantilla-Meluk H, Baker RJ. Contrasting evolutionary dynamics of the developmental regulator PAX9 among bats, with evidence for a novel post-transcriptional regulatory mechanism. PLoS ONE. 2013;8:e57649.

77. Phillips CD, Butler B, Fondon JW, Mantilla-Meluk H, Baker RJ. Contrasting evolutionary dynamics of the developmental regulator PAX9 among bats, with evidence for a novel post-transcriptional regulatory mechanism. PLoS ONE. 2013;8:e57649.

78. Phillips CD, Butler B, Fondon JW, Mantilla-Meluk H, Baker RJ. Contrasting evolutionary dynamics of the developmental regulator PAX9 among bats, with evidence for a novel post-transcriptional regulatory mechanism. PLoS ONE. 2013;8:e57649.

79. Phillips CD, Butler B, Fondon JW, Mantilla-Meluk H, Baker RJ. Contrasting evolutionary dynamics of the developmental regulator PAX9 among bats, with evidence for a novel post-transcriptional regulatory mechanism. PLoS ONE. 2013;8:e57649.

80. Phillips CD, Butler B, Fondon JW, Mantilla-Meluk H, Baker RJ. Contrasting evolutionary dynamics of the developmental regulator PAX9 among bats, with evidence for a novel post-transcriptional regulatory mechanism. PLoS ONE. 2013;8:e57649.

81. Phillips CD, Butler B, Fondon JW, Mantilla-Meluk H, Baker RJ. Contrasting evolutionary dynamics of the developmental regulator PAX9 among bats, with evidence for a novel post-transcriptional regulatory mechanism. PLoS ONE. 2013;8:e57649.

82. Phillips CD, Butler B, Fondon JW, Mantilla-Meluk H, Baker RJ. Contrasting evolutionary dynamics of the developmental regulator PAX9 among bats, with evidence for a novel post-transcriptional regulatory mechanism. PLoS ONE. 2013;8:e57649.

83. Phillips CD, Butler B, Fondon JW, Mantilla-Meluk H, Baker RJ. Contrasting evolutionary dynamics of the developmental regulator PAX9 among bats, with evidence for a novel post-transcriptional regulatory mechanism. PLoS ONE. 2013;8:e57649.

84. Phillips CD, Butler B, Fondon JW, Mantilla-Meluk H, Baker RJ. Contrasting evolutionary dynamics of the developmental regulator PAX9 among bats, with evidence for a novel post-transcriptional regulatory mechanism. PLoS ONE. 2013;8:e57649.

85. Phillips CD, Butler B, Fondon JW, Mantilla-Meluk H, Baker RJ. Contrasting evolutionary dynamics of the developmental regulator PAX9 among bats, with evidence for a novel post-transcriptional regulatory mechanism. PLoS ONE. 2013;8:e57649.
Lau AC, Asahara M, Han SY, Kimura J. Sexual dimorphism of the Eurasian otter (Lutra lutra) in South Korea. Craniodental geometric morphometry. J Vet Med Sci. 2016;78:1007–11.

de Moura Bubadué J, Cáceres N, Dos Santos Carvalho R, Meloro C. Ecogeographical Variation in Skull Shape of South-American Canids: Abiotic or Biotic Processes? Evol Biol. 2016;63:145–59.

Bertolini F, Gandolfi B, Kim ES, Haase B, Lyons LA, Rothschild MF. Sexual dimorphism of the Eurasian otter (Lutra lutra) in East Asia. J Vet Med Sci. 2017;79:144–52.

Lau AC, Asahara M, Han SY, Kimura J. Geographic variation of craniodental morphology of the Eurasian otter (Lutra lutra) in Japan waters. Fish Sci. 1995;61:555–8.

Monteiro-Filho ELA, Monteiro LR, Reis SF. Skull shape and size divergence in dolphins of the genus Sotalia: a tridimensional morphometric analysis. J Mammal. 2002;83:125–34.

Amaral AR, Coelho MM, Marugán-Lobón J, Rohlf FJ. Cranial shape differentiation in three closely related delphinid cetacean species: Insights into evolutionary history. Zoology. 2009;112:38–47.

Gutstein CS, Cozzuol MA, Vargas AO, Suárez MA, Schultz CL, Rubilar-Roy C, Gales N, Hilário OC. Geometric morphometrics, neural networks and diagnosis of sibling Tetracerus species (Rodentia, Gerbillinae). Biol J Linn Soc. 2002;77:319–27.

Klingenberg CP, Leamy LJ, Routman EJ, Cheverud JM. Genetic architecture of mandible shape in mice: effects of quantitative trait loci analyzed by geometric morphometrics. Genetics. 2001;157:785–802.

Dobigny G, Baylac M, Denys C. Geometric morphometrics, neural networks and diagnosis of sibling Taterillus species (Rodentia, Gerbillinae). Biol J Linn Soc. 2002;77:319–27.

Klingenberg CP, Mebus K, Auffray JC. Developmental integration in a complex morphological structure: how distinct are the modules in the mouse mandible? Evol Dev. 2003;5:522–31.

Klingenberg CP, Leamy LJ, Cheverud JM. Integration and modularity of quantitative trait locus effects on geometric shape in the mouse mandible. Genetics. 2004;166:1909–21.

Zelditch ML, Lundrigan BL, Garland T Jr. Developmental regulation of skull morphology. I. Otoconic dynamics of variance. Evol Dev. 2004;6:194–206.

Kawakami M, Yamamura K. Cranial bone morphometric study among mouse strains. BMC Evol Biol. 2008;8:73.

Fornell R, Cordeiro-Estrada P, De Fretas TR. Skull shape and size variation in Neomys minutus (Rodentia: Ctenomyidae) in geographical, chromosomal polymorphism, and environmental contexts. Biol J Linn Soc. 2010;101:705–20.

Boell L, Tautz D. Micro-evolutionary divergence patterns of mandible shapes in wild house mouse (Mus musculus) populations. BMC Evol Biol. 2011;11:306.

Cow PG, Fagan MJ, Rayfield EJ, Jeffery N. Finite element modelling of squirrel, guinea pig and rat skulls: using geometric morphometrics to assess sensitivity. J Anat. 2011;219:696–709.

Jamnicky HA, Hallgrímsson B. Modularity in the skull and cranial vasculature of laboratory mice: implications for the evolution of complex phenotypes. Evol Dev. 2011;13:28–37.

Burgio G, Baylac M, Heyer E, Montagutelli X. Exploration of the genetic organization of morphological modularity on the mouse mandible using a set of interspecific recombinant congenic strains between C57BL/6 and mice of the Mus spretus species. Genes Genomes Genet. 2012;2:1257-68.

Hauter L, Lebrun R, Cox PG. Patterns of covariation in the masticatory apparatus of hystricognathous rodents: implications for evolution and diversification. J Morphol. 2012;273:1319–37.

Paradis MR, Raj MT, Bougner JC. Jaw growth in the absence of teeth: the developmental morphology of edentulous mandibles using the p63 mouse mutant. Evol Dev. 2013;15:268–79.

Gonzalez PN, Kristensen E, Morck DW, Boyd AS, Hallgrímsson B. Effects of growth hormone on the ontogenetic allometry of craniofacial bones. Evol Dev. 2013;15:133–45.

Christians JK, de Zwaan DR, Fung SH. Pregnancy associated plasma protein A2 (PAPP-A2) affects bone size and shape and contributes to natural variation in postnatal growth in mice. PLoS ONE. 2013;8:e56260.

Casanovas-Vilar I, van D. Comparative analysis of the subterranean rodent Ctenomys bicolor (Rodentia: Ctenomyidae) in geographical, chromosomal polymorphism, and environmental contexts. Biol J Linn Soc. 2002;77:319–27.

Stolz JF, Gonçalves GL, Leipnitz L, Freitas TR. DNA-based and geometric morphometric analysis to validate species designation: a case study of the subterranean rodent Ctenomys bicolor. Genet Mol Res. 2013;12:5023–37.

Anderson PS, Renaud S, Rayfield EJ. Adaptive plasticity in the mouse mandible. BMC Evol Biol. 2014;14:85.

Parsons TE, Weinberg SM, Khaksarfard K, Howie RN, Rayfield EJ, Elsalanty M, Yu JC, Cray JJ Jr. Craniofacial shape variation in Twist1−/− mutant mice. Anat Rec (Hoboken). 2014;297:286–33.

Pallares LF, Harr B, Turner LM, Tautz D. Use of a natural hybrid zone for assessing sensitivity. J Anat. 2011;219:696–709.

Sargis EJ, Woodman N, Morningstar NC, Bell TN, Olson LE. Skeletal variation of the skull of the bottlenose dolphin, Tursiops truncatus (Montagu, 1821) in geographical, chromosomal polymorphism, and environmental contexts. Biol J Linn Soc. 2002;77:319–27.
Walsh RE, Aprigio Assis AP, Patton JL, Marroig G, Dawson TE, Lacey EA. Morphological and dietary responses of shipmunks to a century of climate change. Glob Chang Biol. 2016;22:3233–52.

Pallares LF, Turner LM, Tautz D. Craniofacial shape transition across the house mouse hybrid zone: implications for the genetic architecture and evolution of between-species differences. Dev Genes Evol. 2016;226:173–86.

Quintela FM, Fornel R, Freitas TR. Geographic variation in skull shape. Ge D, Lv X, Xia L, Huang C, Yang Q. Geometric morphometric analysis of adult Scapteromys tumidus (Cricetidae, Sigmodontinae): isolation-by-distance plus environmental and geographic barrier effects? An Acad Bras Cienc. 2016;88:451–66.

Percival CJ, Liberton DK, Pardo-Manuel de Villena F, Spritz R, Marcucio R, Hallgrímsson B. Genetics of murine craniofacial morphology: diallel analysis of the eight founders of the Collaborative Cross. J Anat. 2016;228:96–112.

Vasiliev AG, Bolshakov VN, Evdokimov NG, Sineva NV. Morphological diversity of mole voles mono- and polymorphic populations. Does Chernov’s “compensation principle” work within a population? Dokl Biol Sci. 2016;468:118–21.

Pavlíčev M, Mitteroecker P, Gonzalez PM, Rolian C, Jamniczky H, Villena FP, Marcucio R, Spritz R, Hallgrímsson B. Development Shapes a Consistent Inbreeding Effect in Mouse Crania of Different Line Crosses. J Exp Zool B Mol Dev Evol. 2016;326:474–88.

Singh N, Albert FW, Plyusnina I, Trut L, Pääbo S, Harvati K. Facial shape differences between rats selected for tame and aggressive behaviors. Plos ONE. 2017;12:e0175043.

Abromav SA, Lopatina NV, Litvinov NV. Cranial size and shape variation in isolated populations of the Olkhon mountain voles (Alticola olchonensis Litvinov, 1960). Zoologia (Jena). 2017;123:91–100.

Caurus R, Polly PD. Phylogenetic and environmental components of morphological variation: skull, mandible, and molar shape in marmots (Marmota, Rodentia). Evolution. 2005;59:2460–72.

Ge D, Dv X, Xia L, Huang C, Yang Q. Geometric morphometric analysis of postnatal size and shape changes in the cranium of cape hare (Lagomorpha, Leporidae, Lepus capensis). Acta Theriologica Sinica. 2012;32:12–24.

Kraatz BP, Serrrat E, Bumacod N, Wedel MJ. Ecological correlates to cranial morphology in Leporidae (Mammalia, Lagomorpha). PeerJ. 2015;3:e8444.

Ge D, Yao L, Xia L, Zhang Z, Yang Q. Geometric morphometric analysis of skull morphology reveals loss of phylogenetic signal at the generic level in extant lagomorphs (Mammalia: Lagomorpha). Contributions to Zoology. 2015;84:267–84.

Kraatz B, Serrrat E. Evolutionary morphology of the rabbit skull. PeerJ. 2016;4:e2453.

De León MS, Zollkofper CPE. Neandertal cranial ontogeny and its implications for late hominin diversification. Nature. 2001;412:534–8.

Singleton M. Patterns of cranial shape variation in the Papionini (Primates: Cercopithecidae). J Hum Evol. 2002;42:547–78.

Hallgrimsson B, Willmore K, Dorval C, Cooper DM. Craniofacial variation in isolated populations of the Olkhon mountain vole (Alticola olchonensis Litvinov, 1960). Zoology (Jena). 2017;123:91–100.

Wu W, Gu S, Sun C, He W, Xi E, Li Y, Ye W, Qin C, Chen Y, Xiao J, Liu C. Dynamics of the Facial Skeleton and Mandible in Pan troglodytes. J Hum Evol. 2010;314:663–83.

Brugmann SA, Allen NC, Mekonnen Z, Madan E, Helms JA. A primary cilia-dependent etiology for midline facial disorders. Hum Mol Genet. 2010;19:1577–92.

Sakataki I, Maas R. Msx1 deficient mice exhibit cleft palate and abnormalities of craniofacial and tooth development. Nat Genet. 1994;6:348–56.

Zhang Z, Song Y, Zhao X, Zhang X, Fermin C, Chen Y. Rescue of cleft palate in Msx1-deficient mice by transgenic bmp4 reveals a network of BMP and Shh signaling in the regulation of mammalian palatogenesis. Development. 2002;129:4135–46.

Meester-Smoo MA, Vermeij MJ, van Helmond MJ, Molin AJ, van Wely KH, Heiman AC, Vermeij-Keenes C, Riegeham PH, Zwanthoff EC. Targeted disruption of the Msx1 oncogene results in severe defects in development of membranous bones of the cranial skeleton. Mol Cell Biol. 2005;25:4229–36.

Yu L, Gu S, Alappat S, Song Y, Yan M, Zhang X, Zhang G, Jiang Y, Zhang Z, Zhang Y, Chen Y. Sho2-deficient mice exhibit a rare type of incomplete clefting of the secondary palate. Development. 2005;132:4397–406.

Ito Y, Itoy OE, Chylit A, Han J, Biragis P, Nakajima A, Shuler CE, Moses HL, Chai Y. Conditional inactivation of Tgfbr2 in cranial neural crest cells causes cleft palate and calvaria defects. Development. 2003;130:5269–80.

Lack JB, Van Den Bussche RA. Identifying the confounding factors in resolving phylogenetic relationships in Vespertilionidae. J Mammal. 2010;91:1345–48.