7 *Using geometric morphometrics to study the mechanisms that pattern primate dental variation*

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7.1 Introduction

In the late twentieth century, a shift in morphological analyses emerged. Rather than focusing on linear measurements and qualitative descriptions of shape, it became possible to describe quantitatively and compare morphology (Adams et al. 2004). While the dilemma of oversimplifying linear measurements had long been recognized, it was not until a new method, "geometric morphometrics" (GM), was developed that morphologists could finally analyze the shape between linear end points quantitatively (Rohlf and Marcus 1993).

The quantification and analysis of shape offered by GM can provide insight into the essential biological questions, as has been demonstrated for many skeletal phenotypes, including teeth. In this chapter we introduce various GM approaches and review ways they have been applied to studying mammalian teeth, highlighting work on primates.

One area of research receiving more and more attention is the use of morphological variation within and between populations to elucidate developmental mechanisms, and thereby inform on the evolution of these mechanisms. Teeth play an important role. As our knowledge of the genetic organization of dental development expands, opportunities for exploring the relationship between these processes and the shape of the adult dentition using GM increase.

Following this research vein, we provide a review of our current knowledge of tooth developmental genetics, with special emphasis on the hierarchical structure of the dentition. We then highlight several studies that used GM to

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test hypotheses about dental development. We present our own study of shape variation in the tooth row of the *Colobus guereza* to illustrate the insights into mammalian tooth development that can be gained.

7.2 Geometric morphometric approaches

There are two approaches taken with GM: those that are landmark based and those that are not. Landmark-based studies incorporate coordinate-based (e.g., generalized Procrustes analyses, GPA) or coordinate free approaches (e.g., Euclidean distance matrix analysis, EDMA). Non-landmark-based approaches include eigenshape and Fourier analyses. In the following we review morphometric techniques most commonly applied to the study of shape in the dentition.

7.2.1 Generalized Procrustes analysis (GPA)

An important contribution to the field of morphometrics came from the technique of superimposition (Boas 1905), in which pairs of corresponding landmarks on two or more objects are directly contrasted. This method was quantified more specifically by Phelps (1932), who suggested measuring the difference between superimposed cranial forms using the Euclidean distance between landmarks, thus paving the way for future techniques like EDMA (discussed later). Sneath (1967) introduced the least-squares method of superimposition in which the landmarks of two specimens are arranged to minimize the sum of squared distances between loci. Gower (1975) generalized Sneath's pair-wise comparison for simultaneous translation, scaling, reflection, and rotation of multiple forms. Siegel and Benson (1982) followed by demonstrating that with least-squares superimposition, high variation around one or several landmarks would be distributed across the configuration, potentially obscuring shape differences between specimens. To avoid this problem, Siegel and Benson (1982) introduced a resistant-fit model, which limits the effects of regions of large variation on the fit of unchanged regions.

Today, the most widely used method of superimposition is generalized Procrustes analysis (GPA), a method of superimposition in which shapes are compared by configuring the centroid of each to an origin and scaling to a common size (Bookstein 1986; Rohlf and Slice 1990). Each shape is then rotated to a position that minimizes the squared distances between homologous landmarks (Gower 1975; Rohlf and Slice 1990).

There are some limitations to GPA. One requirement is that all landmarks be homologous and consistently identifiable across specimens (Zelditch et al.

2004). This can be limiting when studying anatomy with few such landmarks or in instances where landmarks are missing (e.g., worn teeth). A further criticism is that it cannot investigate shapes that contain presence/absence characters (Zelditch et al. 2004). Recently, however, Gómez-Robles et al. (2011) demonstrated the ability of GM techniques to investigate evolutionary novelties. Looking at two-dimensional occlusal morphologies of multiple hominid species to identify the presence (and subsequent loss) of a fifth cusp, they show that GPA is capable of discerning structures with evolutionary novelties by using sliding semilandmarks and only a single landmark associated with the novelty.

7.2.2 Euclidean distance matrix analysis (EDMA)

In 1991, Lele described an alternative method for studying group variation in two- and three-dimensional shapes. EDMA calculates Euclidean distances between all plotted landmarks on an object and compares it to a similarly landmarked object by calculating ratios of between-landmark distances in a matrix. EDMA is independent of rotation, position, and reflection of an object (Lele and Richtsmeier 1991).

EDMA, though useful in analyzing variation in shapes between groups, is not without limitations. Because EDMA techniques do not scale and use actual distances between points, an average shape will be more influenced by larger specimens (Rohlf 2000). Rohlf (2000) also describes inadequacies in the statistical power. Further, EDMA techniques are not easily visualized (see Richtsmeier et al. 2002 for discussion).

7.2.3 Eigenshape and Fourier analyses

In 1965, Lu was one of the first to recognize the ability to define curves of biological forms mathematically. He applied a harmonic analysis with three-way Fourier equations to investigate shape of the human face. A parametric approach was developed by Kuhl and Giardina (1982): elliptical Fourier analysis. Here, separate harmonics plot as ellipses and sum to the original polygonal approximation. Contemporary with the refinement of Fourier analysis was the introduction of eigenshape analysis. The latter involves comparisons of shapes of outlines of specimens by deriving a set of orthogonal shape patterns using eigenfunctions (Lohman 1983). While eigenshape chooses the optimal orthogonal function, Fourier analysis must choose from a limited number of harmonic functions (Lohman 1983).

7.3 Application of GM to studying dental variation

All of the preceding techniques have been employed in studies of dental shape variation to address a variety of biological questions. While many contributions have focused on primates, we include other mammalian groups in our review as those studies help us better understand the applicability of using GM on the primate dentition.

7.3.1 Taxonomy

Most GM work on the dentition has addressed variation in tooth shape between one or more groups; oftentimes the purpose is to distinguish taxa or identify population- or sex-specific differences in form.

7.3.1.1 Primate studies

The use of GPA to study tooth shape is dominated by work on hominoid evolution. One exception is the study of Kondo and Natori (2004), who superimposed landmarks corresponding to grooves and cusps of the occlusal surface of macaque molars to identify sex differences in shape and centroid size.

Within the hominid-focused literature, in the first of a series of papers, Martinón-Torres et al. (2006), performed a diagnostic analysis of species of *Homo* by superimposing landmarks and semilandmarks on internal crown surface features and the crown outline of LP4s. They identified a primitive-to-derived gradient from asymmetrical to more symmetrical outlines, following a general trend of dental reduction. Using the same methods, Goméz-Robles and colleagues (2007) continued by examining UM1 shape across *Homo*, distinguishing the distally displaced lingual cusps and large hypocone of Neandertals and relatively round external outline in modern humans. Finally, Goméz-Robles et al. (2008) compared LM1s across a sample that included *Australopithecus* and identified a trend of increased outline symmetry and talonid reduction from early to recent hominids, a result consistent with their study of the lower fourth premolar (Martinón-Torres et al. 2006).

Xing et al. (2009) applied GPA of landmarks and semilandmarks to compare mandibular premolar shape in Zhoukoudian *Homo erectus* with representatives of *Australopithecus*, African early *Homo*, Asian *Homo erectus* outside Zhoukoudian, European Pleistocene fossil hominids, and recent Chinese. They observed the preservation of several primitive hominid traits in Zhoukoudian specimens, including asymmetrical crown outlines and pronounced grooves

on the buccal side of the crown, and a high degree of disparity from European Pleistocene specimens.

Liu and colleagues (2010) assessed the taxonomy of the Pleistocene Jianshi hominins (China) by superimposing crown landmarks and semilandmarks of three Jianshi postcanine teeth with comparative fossil teeth from hominids from Europe, Africa, and Asia. Their analysis found that the degree of symmetry and cusp patterns of the Jianshi teeth resembled Asian early and middle Pleistocene hominid teeth, but not those of *Australopithecus*; the latter had a wider variation in crown shape relative to Asian hominids.

Variation in the shape of the enamel-dentine junction (EDJ) has been captured using superimposition of two-dimensional landmarks from histological sections in human lower molars (Smith et al. 2006). Skinner et al. (2008) expanded upon this technique by combining GPA with micro-CT to analyze EDJ shape differences of mandibular molars between *Australopithecus robustus* and *Australopithecus africanus*. Variation in EDJ shape, as captured by three-dimensional landmarks along the marginal ridge connecting dentine horns and along the curve of the cervix, successfully distinguished the two taxa (Skinner et al. 2008). Skinner et al. (2009) also showed that superimposition of LM1 and LM2 EDJ shapes can distinguish species and subspecies within *Pan*. The authors found significantly different shapes in dentine horn height and position, as well as dentine crown height and crown base shape, between *P. paniscus* and *P. troglodytes* and between the subspecies *P. t. troglodytes* and *P. t. versus*.

EDMA has also been successfully employed in studies of dental variation across different primate species. In analyzing the dental morphology of extant *Homo sapiens*, *Gorilla gorilla*, *Pan troglodytes*, and 19 Sterkfontein Member 4 hominid molars, Hlusko (2002) collected cross-sectional and occlusal landmarks and analyzed Euclidean distances between them. She found significant cross-sectional shape differences between first and second molars. It was concluded that metameric variation can provide functional and developmental information previously unattainable from fossils and tooth shape more generally.

Olejniczak et al. (2004) investigated evolutionary relationships between extant primates by analyzing Euclidean distances between nine landmarks on maxillary molars. They demonstrated the ability of dentine shape to deduce the relationships among hominoid, cercopithecoid, and ceboid primates accurately. Additionally, EDMA has been used to support the close relationship of late Miocene hominoids and orangutans; Liu et al. (2001) analyzed seven landmarks on three molars, showing that cross sections of specimens from Yuanmou, China, are more similar to those of great apes than of humans. Further, they demonstrated strong affinities between the late Miocene hominoids, *Lufengpithecus lufengensis*, *Sivapithecus*, and Yuanmou.

EDMA has also been used to study morphological variation in other aspects of dental form, such as dental arch variation. Ferrario et al. (1993a, 1993b, 1994) calculated Euclidean distances between seven landmarks on each hemirow of the mandibular and maxillary tooth rows to assess asymmetry and shape dimorphism between men and women.

Landmark-free techniques have also been used to study hominid dental morphology. Examining LP2s between Neandertals and modern humans, Bailey and Lynch (2005) applied elliptical Fourier analysis to show that Neandertal cheek teeth have truncated mesiolingual lobes, producing asymmetrical teeth. This character state was found to be derived and the symmetrical LP2s in modern humans are primitive.

7.3.1.2 Nonprimate studies

Compared to the fairly straightforward taxon diagnoses exemplified by the preceding work, larger sample sizes for nonprimate taxa, particularly for fossil assemblages, enabled researchers to ask more complex questions about species relationships. Differentiation between taxonomic groups based on dental landmark data is particularly prevalent in the study of extant and fossil rodents. Van Dam (1996) demonstrated the utility of GM tooth shape analysis for classification of fossil murids. The author collected landmark data to quantify the degree of stephanodonty, a complex, ridged crown structure, in the UM1. A comparable approach was used by Janžekovič and Kryštufek to separate species of rock mice of the genus *Apodemus* on the basis of the shape of the upper molar row (Janžekovič and Kryštufek 2004; Kryštufek and Janžekovič 2005). Similarly, Wallace (2006) used LM1 shape to differentiate between two species of the vole *Microtus*. Macholán (2006) used a combination of landmarks and sliding semilandmarks to capture shape variation in the outline of the UM1 of extant and extinct members of the genus *Mus*.

Pavlinov and colleagues have used landmark-based analyses to classify a variety of taxa. Their earliest work explored occlusal shape variation in the UM3s of subspecies of the vole *Alticola argentatus* (Pavlinov et al. 1994; Pavlinov 1999). Pavlinov (2001) compared UM1 shape across seven genera from the rodent family Gliridae followed by studies on upper tooth row shape in eight species of brown-toothed shrews of the genus *Sorex* (Pavlinov 2004a, 2004b) and upper postcanine tooth row shape among island and mainland populations of the Eurasian polar fox, *Alopex lagopus* (Pavlinov and Nanova 2008).

Polly and colleagues have utilized landmarks to quantify variation in tooth shape in the context of paleophylogeography and dating of species divergences. Polly (2001, 2003b) reconstructed phylogeographic relationships between fossil and modern samples of the European shrew *Sorex araneus* using lower

first molar shape. Polly (2002) also examined the relationship between the amount of LM1 shape divergence and the amount of divergence time separating Paleogene viverrid carnivoran populations. More recently, Polly and colleagues (2005) used landmarks to assess the degree of occlusal fit between UM1s and LM1s in sixteen species of bats.

Marcolini and colleagues have shown that landmark-based and Fourier outline analyses of tooth shape can be taxonomically informative in rodents. They examined LM1 occlusal surface and enamel-dentine junction shape in a fossil vole, *Ogmodontomys*, using landmarks and semilandmarks (Marcolini et al. 2009). Marcolini (2006) used Fourier analysis to decompose LM1 contours of six species of extinct *Mimomys* from the Pliocene and Pleistocene.

Hurth et al. (2003) distinguished six separate Plio-Pleistocene *Mimomys* species also on the basis of Fourier data from the LM1. Other explorations of systematics in the fossil record using Fourier methods include Renaud and colleagues' (1996, 1999b) study of UM1 shape in Pliocene lineages of the rodent *Stephanomys* and Miocene murines, as well as Angelone's (2008) study of the LP1 of *Prolagus*, a fossil lagomorph. Leroy et al. (2004) developed criteria for classifying fossil shrews based on Fourier analysis of UM1-UM2 and LM1-LM2 as well as the UP2 of three extant species of the genus *Crocidura*.

Cucchi and colleagues (2009, 2011) have studied the taxonomic significance of the shape of pig mandibular molars in island Southeast Asia and China. Fourier analysis of the outline of the LM3 was used to investigate the history of pig domestication on the basis of Holocene *Sus* remains from Malaysia (Cucchi et al. 2009). Occlusal cusp landmarks and outline semilandmarks of the LM3 were used in a separate study on Neolithic and modern pigs from China (Cucchi et al. 2011). Cucchi (2008) also implemented Fourier analysis in the taxonomic identification of a house mouse LM1 associated with a Late Bronze Age Mediterranean shipwreck. Additionally, house mouse lower molar shape has been contrasted in Canary Island and continental populations using Fourier analysis (Michaux et al. 2007).

Molar outline shape can also be quantified using eigenshape analysis, as demonstrated by Polly's (2003a) study of living and fossil marmots. The author used shape divergence in the outline of LM3s to reconstruct phylogenetic relationships among more than a dozen subspecies of the genus *Marmota*.

7.3.2 Ecology and adaptation

The potential for drawing connections between tooth shape and environmental factors, specifically diet, using GM has been well demonstrated across vertebrates generally, but it has not been widely employed to study dental adaptation

in primates. In one of the only primate adaptation studies, White (2009) collected landmarks corresponding to the cusps of lower molars in modern lemurs, lorises, and tarsiers to contrast tooth shape across a range of dietary strategies. Frugivorous and graminivorous taxa were distinguished from folivores and insectivores on the basis of relative cusp orientation, with omnivores being intermediate between these three groups.

The evolution of tooth shape with regard to selection by environmental factors in nonprimates has been studied extensively using landmark- and outline-based methods. Polly (2004) performed stochastic computer simulations of four different evolutionary modes for UM1 shape in the shrew *Sorex araneus*, including randomly fluctuating selection, directional selection, stabilizing selection, and genetic drift. Comparison with real shrew molar landmark data identified randomly fluctuating selection as the predominant mode. Wood and colleagues (2007) addressed evolutionary stasis in the dentition of the condylarth *Ectocion* over changing environmental conditions in the Paleocene-Eocene. They collected occlusal surface landmarks and outline semilandmarks for the LP2, LM1, and LM3 and tested variation in tooth shape over time against a null model of a random walk.

Rychlik and colleagues (2006) used a landmark approach to study the effects of sympatry on the shape of the cranium, mandible, and UM1 of two species of Polish water shrew (genus *Neomys*). Partial least squares (PLS) analysis of shape and geoclimatic data showed similar ecophenotypic responses for the two species when sharing the same environment. Piras et al. (2009) used the $\delta^{18}O$ isotope record as a proxy for climate in their study of the relationship between environment and LM1 shape in extinct populations of the vole *Terricola savii*. A temporal trend correlated with $\delta^{18}O$ was detected in univariate analyses of shape based on a combination of landmarks and semilandmarks. An analysis of LM1 shape in extant populations of *T. savii* revealed a similar relationship (Piras et al. 2010). McGuire (2010) addressed the relationship between climate and LM1 shape in the vole *Microtus californicus*. Shape variation drawn from landmarks on the molar occlusal outline was analyzed in the context of geography using PLS. A gradient of shape difference between northwest and southeast California populations was identified, revealing a significant climate signal.

Caumul and Polly (2005) investigated the relationship between environmental factors and cranium, mandible, and LM3 shape in Eurasian marmots. After capturing molar outline shape using semilandmarks, the authors used path analysis to determine the percentage of shape variation explained by the effects of diet, habitat, elevation, temperature, precipitation, body size, and mitochondrial deoxyribonucleic acid (mtDNA) genetic divergence. Stynder (2009) also used landmark and semilandmark data from multiple teeth to study niche

partitioning among fossil hyenas. Comparison of the outlines of the crowns of the LP1, LP2, and LM1 suggested differences in the degree of carnivory among four late Miocene/early Pliocene species.

Ledevin and colleagues (2010a) analyzed variation in the occlusal outline of UM1, UM3, and LM1s of Quaternary European lineages of the bank vole (*Myodes*) using Fourier analysis. A subsequent Fourier analysis of the UM3 identified season of trapping and related wear patterns as a source of shape variation of the same order of magnitude as biogeographic variation (Guéréchau et al. 2010). A third study of UM3 outline in *Myodes* identified another source of variation secondary to trapping season and wear: the presence or absence of a fourth lingual triangle (Ledevin et al. 2010b). The authors attributed this variation to the space available to the molar at the posterior end of the row, suggesting an epigenetic factor, such as maternal health, as the source.

Renaud and colleagues implemented Fourier analysis of molar outline to explore the relationship between tooth shape and a wide range of ecological factors, including geography, diet, climate, habitat and age. Renaud (1999) began by examining Um1 and LM1 outline shape across the geographic range of the African murine rodent *Oenomys*. Next, the authors studied these same teeth in the Miocene murine *Paraethomys* with respect to the climatic record (Renaud et al. 1999a). Renaud and van Dam (2002) also examined Miocene murine molar outline variation, concentrating on morphological evolution associated with a dietary shift from granivory to herbivory. Renaud and colleagues then drew upon this work to characterize the diet of the extinct lava mouse *Malpaisomys* from the Canary Islands (Renaud and Michaux 2004) and later of an entire lineage of rodents spanning a nine-million-year interval in the Neogene (Renaud et al. 2005). More recently they looked at the effects of sex and age (Renaud 2005), as well as adaptation to insular conditions, in the UM1 outline of island and mainland *Apodemus sylvaticus* wood mice (Renaud and Michaux 2007).

7.3.3 Pathology and forensics

GM has also been applied to investigations of asymmetry and malocclusion in the human dentition. Schaefer et al. (2005) investigated dental arch asymmetry in a modern inbred population from an isolated island in the Adriatic Sea. They plotted landmarks along the buccal surface of mandibular and maxillary teeth and compared shapes with a more heterogeneous population in mainland Croatia. Fluctuating asymmetry (FA, asymmetry between either side of the midline) was higher in the inbred population, suggesting genetic and environmental factors play a part in the asymmetry of these populations.

Nie and Lin (2006) compared dental arch forms between normal occlusion and Class II Division 1 malocclusion groups by performing EDMA on distances between landmarks along the maxillary and mandibular arches. Banabilh et al. (2008) used GPA to compare arch morphologies in Asian adults with obstructive sleep apnea (OSA) with normal Asian adults. They compared landmarks on cusps and incisor edges and found that maxillary arches in adults with OSA were narrower in the transverse plane of the incisor and canine region; they also found that mandibular arches in people with OSA were narrower in the anterior-posterior plane of the premolar and molar regions.

It is worthwhile to note the GM contributions to bite mark identification in forensic analyses. Kieser et al. (2007) analyzed bite marks of fifty orthodontics casts to determine their uniqueness. By plotting landmarks and semilandmarks, they show the incisal surfaces of the mandibular and maxillary dentitions are unique. Bush and colleagues (2011) examined human bite marks on cadavers and anterior dentition casts to determine whether these pieces of evidence were reliable in identifying a particular dentition. Using incisal surface landmarks in two-dimensional scans of bite marks, they analyzed variation caused by an experimental biting of skin. They show that skin distortion resulted in several distinct patterns and suggest using caution when attempting individual identification. In the context of fossil assemblages, Benazzi and colleagues (2011) assessed the use of Fourier analysis on interproximal wear facet shape to identify and match isolated tooth crowns; however, they concluded that this approach should only be used with other analyses to determine MNI.

7.4 Tooth development

In addition to questions of taxonomy, ecology, pathology, and forensics, dental researchers are increasingly motivated to understand how teeth develop as structures, linking phenotypic variation to the developmental processes that underlie it. As teeth vary in shape in many dimensions (e.g., tooth type, occlusal surface, cross section, wear pattern) and on several hierarchical levels (e.g., shape of individual tooth, collective shape of all teeth belonging to a single type, or shape of entire tooth row), GM can be valuable for quantifying phenotypic variation at these scales and consequently help elucidate underling developmental mechanisms.

Most of what is known about the genetic mechanisms specifying and patterning the dentition is known from laboratory mouse and chicken models. Although the mouse dentition is relatively specialized (and reduced) compared to most mammals (Figure 7.1), we focus on the mouse model as it presents the

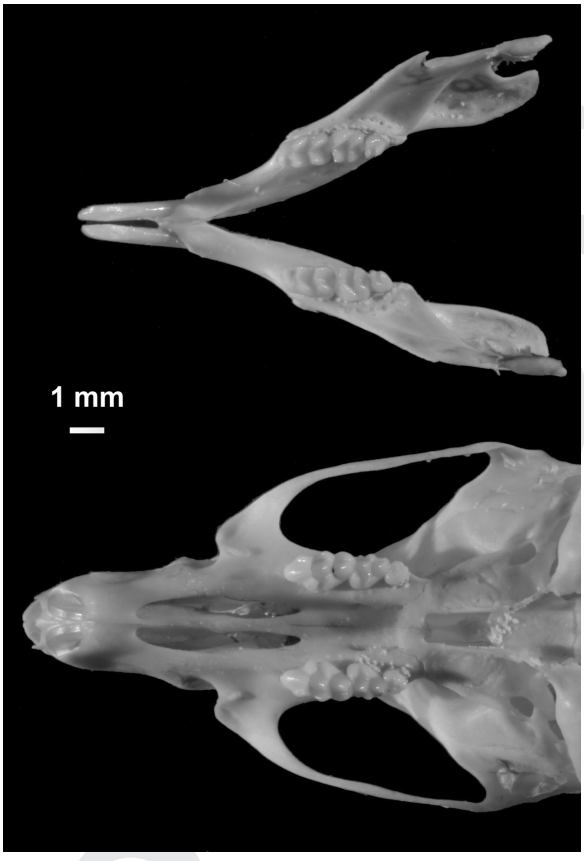

Figure 7.1. Mouse maxillary and mandibular dentitions. Note the highly derived and reduced dental formula of one incisor and three molars.

most complete picture of development and is referred to for human odontogenesis (e.g., McCollum and Sharpe 2001).

The overview of tooth and early craniofacial development that follows is focused on setting up hypotheses that can be tested through GM. For additional overviews of odontogenesis we refer the reader to Michon (2011), Cobourne and Sharpe (2010), Simmer et al. (2010), Lesot and Brook (2009), Mitsiadis and Graf (2009), and Salazar-Ciudad (2008).

7.4.1 From stomodeum to cap stage

Consideration of mouse tooth development needs to begin at least 4 embryonic days prior to any histological evidence of teeth; cell fate this early on appears

to influence later odontogenic potential. The stomodeum, or embryonic mouth, forms where head surface ectoderm and gut endoderm meet. Between E8.5 (the 8.5th mouse embryonic day in utero) and E9.5, cell death at this interface connects the feeding opening to the developing digestive tract (Poelmann et al. 1985). Sonic hedgehog in the pharyngeal endoderm induces *Fgf8* in what will become the mouth epithelium, with *Bmp4* expressed and acting as an inhibitor in the adjacent, nonmouth ectoderm (Haworth et al. 2004, 2007; Shigetani et al. 2000). These *Fgf8* and *Bmp4* expression domains are then maintained by *Pitx2* in the stomodeal ectoderm throughout early mouth patterning (Liu et al. 2003; Ohazama et al. 2010). Their expression is essential for the maintenance of downstream gene expression in assembling dental arch mesenchyme and determining oral/aboral and dorsoventral patterning.

Anterior-posterior patterning in the dental arches comes from migrating streams of neural crest mesenchyme, which leave the developing brain and form pharyngeal arches at E8.5–E9.75 (Serbedzija et al. 1992). These arches are serially homologous primordia arranged rostrocaudally that give rise to the jaws and throat structures (recently reviewed in Graham 2008; Kulesa et al. 2010). The identity of these arches is somewhat influenced before migration by the nested expression of *Hox* genes along the body axis (Minoux and Rijli 2010).

In the early embryo, mesiodistal polarity within each arch arises from dorsoventral patterning of the pharyngeal arches. During E8.25–E9, endothelin-1 from the endoderm activates nested patterns of *Dlx* genes along the dorsoventral axis of the pharyngeal arch mesenchyme via a hypothesized signaling gradient (Benouaiche et al. 2008; Creuzet et al. 2005; reviewed in Minoux and Rijli 2010). The mandibular prominences are distinguished molecularly from the maxillary prominences by a *Dlx5/6*-driven developmental program and receipt of the endothelin-1 signal (Sato et al. 2008). Other genes are differentially expressed in the maxilla and mandible as a result of differential *Dlx* expression (Minoux and Rijli 2010). The frontonasal mass neural crest, which also contributes to the maxillary incisor region, is less well characterized.

At mouse E10 and E10.5, the early broad expression of transcription factors and signaling molecules becomes more localized within the oral epithelium and mesenchyme. By E11, a primary epithelial band forms, a stripe of slightly thickened epithelium along both maxillary and mandibular arches, from which the dental lamina arises (Jernvall and Thesleff 2000; Nanci 2008). This dental lamina region expresses *Shh* and *Pitx2* (Keränen et al. 1999), a restriction of the former stomodeal expression domains of these molecules (Mucchielli et al. 1997).

The physical creation of individual teeth from epithelial and mesenchymal tissue layers occurs through the process of morphogenesis. There is a developmental shift from an instructive epithelium (Lumsden 1988) to instructive

mesenchyme around E11.5, after which genes expressed in the developing dental mesenchyme and papilla direct tooth morphogenesis (Kollar and Baird 1969, 1970; Mina and Kollar 1987). Later, we present the major events of tooth morphogenesis through the cap stage only.

The first morphological signs of individual tooth morphogenesis have been reported as early as mouse E10.5, with the thickening of the dental epithelium in foci (Kratochwil et al. 1996; Mucchieli et al. 1997). Each thickening, called a tooth placode, is hypothesized to be induced by epithelium-induced *Bmp4* and *ActivinβA* in the mesenchyme (Jernvall and Thesleff 2000), with a half-day delay between molars and incisors (Ruch 1984). Most studies show that localized mesenchymal gene expression has begun under these thickenings by E11.5 (Bitgood and McMahon 1995; Ferguson et al. 1998; Vainio et al. 1993), at which time the placode itself appears to be acting as a signaling center for members of the *FGF*, *BMP*, *Shh*, and *Wnt* families to begin the transition to mesenchymal control over tooth morphogenesis (Jernvall and Thesleff 2000).

The transition from placode to bud stage (around E12.5) is one of degree, where continued proliferation of the epithelium into the underlying mesenchyme creates a mass of epithelial cells intruding into the condensing mesenchyme. *Shh* expression in the epithelium is required for proper cell proliferation (Cobourne et al. 2001; Dassule et al. 2000; Hardcastle et al. 1998), while *FGF*, *BMP*, and *Wnt* ligands expressed there are orchestrating the reciprocal epithelial-mesenchymal interactions (Chen et al. 2009; Neubüser et al. 1997; Zhang et al. 2000). By late bud stage (around E13.5), the enamel knot is morphologically visible (see later discussion).

Cap stage marks the beginning of crown shape morphogenesis as the epithelium encircles the mesenchymally derived dental papilla. Histologically, cap stage is diagnosed by formation of the inner and outer dental enamel epithelia, with lingual and labial sides of the inner dental epithelium separated by the enamel knot. The enamel knot is a collection of nonproliferative cells acting as a signaling center for crown formation (Jernvall et al. 1994; Vaahtokari 1996). It expresses *FGFs* to direct proliferation of epithelial cells and growth of the dental papilla, *BMPs* to maintain nonproliferation in the enamel knot, as well as *Shh*, *Wnts*, and members of several other signaling pathway families (Jernvall et al. 1998; Jernvall and Thesleff 2000; Kettunen and Thesleff 1998; Thesleff et al. 2001). Enamel knots disappear by apoptosis mediated by *Bmp4* and *jagged 2*, a Notch family ligand (Jernvall et al. 1998; Mitsiadis et al. 2010). Cap stage also marks the beginning of cellular differentiation in the tooth germ.

Shh function appears to be independent of many of the other signaling pathway genes in epithelial-mesenchymal interactions, in the enamel knot, and in later morphogenesis, but it is essential for proper growth of the lingual

epithelium, for the dental cord connecting a tooth germ to oral epithelium, and for overall tooth size (Dassule et al. 2000). *Pitx2* is also critical for epithelial morphogenesis at these stages, affecting aspects of tooth orientation and/or downgrowth in the jaw, as well as cap formation (Liu et al. 2003).

The dental papilla is crucial for creating tooth shape because it is a substrate for the epithelium to fold and proliferate around and because it can induce enamel knots, apparently quite late into development (E17 in mouse transplant experiments; Kollar and Baird 1970). It is the enamel knot that seems to direct epithelial folding and proliferation to create crown shape (Jernvall et al. 1998, 2000), and the induction of primary enamel knots and, importantly, secondary enamel knots in multicusped tooth types is dependent on signals from the papilla. Secondary enamel knots express a subset of the same genes as primary enamel knots, but the domains of these genes are less restricted; some like *Shh* and *Fgf9* connect secondary enamel knots (Dassule et al. 2000; Kettunen and Thesleff 1998). The fate of primary enamel knot cells in relation to secondary enamel knots is currently under debate, depending on what markers are used (Cho et al. 2007; Coin et al. 1999; Jernvall et al. 2000; Lesot and Brook 2009; Matalová et al. 2005; Peterková et al. 2002; Shigemura et al. 1999).

Primary enamel knots sit at the cusp tips of singly cusped teeth and form the crown base in multicusped teeth in all mammals examined (Järvinen et al. 2008; Jernvall et al. 1998, 2000; Moustakas et al. 2011; Torres et al. 2008; Yamanaka et al. 2010). Secondary enamel knots form cusp tips in molars and prefigure species-specific molar morphologies (voles: Jernvall et al. 2000; Keränen et al. 1998; possum: Moustakas et al. 2011).

7.4.2 Patterning the dental arcade

There is a fair amount of imprecision in the developmental genetics literature regarding what is called a developing individual tooth compared to an odontogenic field; however, it is clear that the potential odontogenic areas of the dental arch set up the arrangement of individual tooth primordia, creating the mammalian dental formula. We will provide an overview of what is understood about the four critical steps in patterning the mammalian (and primate) dentition: (1) the location and size of the dental lamina, (2) the specific location of dental placodes along the lamina, (3) the identity of the tooth (i.e., incisor or molar), and (4) variation within a tooth class (i.e., variation between first, second, and third molars).

Most mammals have only one row of teeth around the dental arcade, a different situation than in other vertebrates such as cichlid fish (Fraser et al. 2008). In the mouse, expression of *Wnt7* in the nondental epithelium is thought to play

a role in restricting *Shh* expression to tooth-forming sites (Sarkar et al. 2000). Zhang and colleagues (2009) found that *Osr2*, an inhibitor of mesenchymal *Bmp4* expression, is required to pattern teeth into a single tooth row in mice.

Individual tooth placode initiation along the dental lamina is an area of active research and involves a feedback mechanism of *Wnt* and *Shh* signaling (Ahn et al. 2010; Cho et al. 2011; Järvinen et al. 2006; Liu et al. 2008). This has been modeled as a reaction-diffusion process for embryonic alligator teeth (Kulesa et al. 1996) and mouse molars (Cho et al. 2011) and is similar to that invoked for feather patterning (Jiang et al. 1999, 2004) and hair follicle initiation (Sick et al. 2006). Phenotypes produced by manipulations of the candidate pathways at this point in development, however, are more complex than changes in tooth placode size, number, or spacing; this outcome may reflect later roles for these genes or the existence of other mechanistic effects of candidate genes

In a heterodont dentition, tooth type varies along the arcade, a fate developmentally encoded in mesenchymal cells prior to morphogenesis (prior to mouse E11). There are currently two ideas for how tooth type is determined, or, rather, how tooth shapes are patterned along the arcade. These ideas are not mutually exclusive although we describe them individually. The first is the Homeobox Code Hypothesis and the second involves specification of odontogenic fields.

The Homeobox Code Hypothesis (Sharpe 1995; Thomas and Sharpe 1998) can be viewed as a culmination of the many patterning processes prior to the dental lamina stage. It describes a group of transcription factors regionalized in partially overlapping domains in the mouse oral mesenchyme, which play a role in determining tooth type (incisors vs. molars). Genes invoked in the Homeobox Code Hypothesis are expressed long before physical signs of tooth development, and the effects of these genes on tooth type may not play out until later.

Evidence supporting this hypothesis comes from the transcription factor *Barx1*, which is normally expressed in the proximal oral mesenchyme. When *Barx1* is experimentally misexpressed in presumptive incisor regions at E10, teeth that ultimately develop are molariform (Tucker et al. 1998). Miletich et al. (2005) hypothesize that *Barx1* is responsible for activating a morphogenetic pathway instructing mesenchyme to form multicuspid teeth. Recently, Munne et al. (2010) challenged this interpretation, arguing that the *Barx1*-molariform tooth may be a fusion of small incisorlike teeth created by the breakup of enlarged mouse incisor placodes into multiple closely spaced placodes.

Barx1 expression is, however, inactivated in the maxillary molars with the double knockout of *Dlx1* and *Dlx2*, two dorsoventral patterning genes central to the Homeobox Code Hypothesis (Thomas et al. 1997; see earlier discussion). In these double knockouts, maxillary molars arrest before bud stage

(Section 7.4.1), as a result of regional misspecification of odontogenic mesenchyme as chondrogenic mesenchyme (Qiu et al. 1997; Thomas et al. 1997). As these experiments demonstrate, there are tooth type-specific defects involving the interaction of molar specification and dental arch patterning pathways, more minor adjustments of which could result in modular tooth type-related variation.

The second idea for how the tooth row is patterned concerns specification of odontogenic fields. The subsequent initiation of tooth primordia may take place where dental lamina intersects with fields of molecular signaling (Jernvall and Thesleff 2000). One hypothesis for how this field specification occurs is that morphogenesis of the embryonic jaw due to cell proliferation shifts and expands distinct epithelial domains of *Fgf8* and *Bmp4*. Opposing spatial signals from these two molecules around E10–E10.5 create patches of more localized mesenchymal gene expression, as occurs with *Pax9* (Neubüser et al. 1997). The result of these newly restricted expression patterns is the specification of incisor and molar fields, one of each per jaw quadrant (in mice), which distinguish regions of the dental arch able to form teeth from those that have lost the ability and will become other oral structures.

There is a developmental delay in specification of mandibular incisor and molar fields, with incisor fields specified only after a subtle shift in the inhibitory *Bmp4* expression pattern coupled to changes in shape and size of the developing jaw (Neubüser *et al.* 1997; Ruch 1984). Because of the changes during this delay, incisor and molar fields may acquire slightly different characteristics. There may also be intrinsic differences between maxillary incisor and molar fields; incisor fields are specified partly on the nasal processes, derived mostly from the frontonasal mass, whereas molars are specified entirely on the maxillary processes (Kriangkrai et al. 2006a 2006b; Peterková et al. 1993; Yamanaka et al. 2007; Yamanaka and Uemura 2010). The incisor field also includes lip furrow primordia (Dassule et al. 2000; Kollar and Baird 1970).

Both developmental models for tooth type specification propose hypotheses based on empirical developmental genetics for at least two tooth developmental modules: incisors and molars. Given that GM enables exploration of various combinations of landmarks, the dental phenotype can be defined in multiple ways. As such, methods for identifying which landmark combinations most accurately reflect such developmental modules can be provided and enable researchers to determine how pervasive such a pattern of phenotypic variation is across mammals and other vertebrates.

Turning to variation within a tooth class, developmental genetics studies have been restricted to the molars, because of the reduced rodent dental formula. From studies of third molar development in mice, it is clear that the odontogenic fields specified at early stages only specify the first molar and

the more mesial teeth (Chlastaková et al. 2011). The second and third molars bud off the molars mesial to them, and their size, and possible constraint on shape, is highly determined by the balance of activating and inhibiting signals received from the mesial tooth (Catón and Tucker 2009; Grewal 1962; Grüneberg 1965; Kavanagh et al. 2007).

The limited number of developmental genetics studies of animals with premolars indicates that molars form from a posterior budding of the dental lamina; it has been suggested that they arise from a premolar field specified at homologous stages (Järvinen et al. 2009; Yamanaka et al. 2007; Yamanaka and Uemura 2010). There is a time delay in the development of these more distal molars, as well as a different jaw ossification environment, which may also contribute to differences in these molars.

In summary, both of the current ideas for how the tooth type is patterned suggest that incisors may have some degree of developmental distinction from the molars. The embryological events observed in animals with premolars suggest there may be overlap in mechanisms underlying premolars and molars. We will now explore evidence from experimental developmental genetics for such developmental modules.

7.4.3 Evidence for molar and incisor developmental modules

The existence of tooth type-specific knockouts early in development suggests that mouse tooth type (incisor versus molar) is already set prior to any morphological signs of tooth development. While there are numerous lines of evidence from development for distinct molar and incisor modules in mice, we will highlight four.

In *Lhx6/7* double mutants, molar teeth arrest before any sign of morphogenesis, resulting in the elimination of an entire tooth class by early mesenchymal patterning genes (Denaxa et al. 2009). Most of the localized markers for epithelial-mesenchymal interaction are unaffected, and incisor morphogenesis is normal (Denaxa et al. 2009). The authors interpret these results as a failure of molar placode specification, although many of the known inductive interactions seem to be occurring.

Activin βA is a mesenchymally expressed signaling factor, just under molar and incisor fields and induced by *Fgf8* (Ferguson et al. 1998). Knockout mice arrest at bud stage, but maxillary molars are unaffected because no signaling occurs there from activin βA or any other TGFβ molecule (Ferguson et al. 2000). Activin βA is critical for signaling back to the epithelium by E11.5 for the later bud stage to cap stage progression in all teeth but the maxillary molars (Ferguson et al. 1998). These results suggest that something intrinsic to the

maxillary molar epithelium or mesenchyme by E11.5 is different from other teeth, though that difference has not been identified.

In *Dlx1/2* knockout mutants, E11.5 maxillary molars form epithelial thickenings but do not progress beyond that stage like other teeth (Thomas et al. 1997). The combined loss of these early patterning genes prevents the action of proliferative signals between the epithelium and mesenchyme of any of the maxillary molars, reinforcing the idea that early mesenchymal expression domains can constrain later tooth developmental mechanisms in a tooth-specific fashion.

Recent reevaluation of gene expression also identified several genes previously thought to be exclusively endodermal; however, they are now known to be expressed in the early proximal mesenchyme and are proposed to influence molar tooth fate (Ohazama et al. 2010; Shigetani et al. 2000; Thomas et al. 2000).

7.4.4 Evidence for molar and premolar developmental modules

Careful histological observations have detected rudimentary tooth buds in the maxilla and mandible of mice and voles that may provide information about development of premolars (Keränen et al. 1999; Peterková et al. 2002; Prochazka et al. 2010). Most of these rudimentary tooth buds regress by apoptosis, but the LM1 in mice (not voles) absorbs one of these rudiments onto the anterior portion of the tooth (Peterková et al. 2002; Prochazka et al. 2010; Witter et al. 2006). *Spry* mutants (and others, reviewed in D'Souza and Klein 2007) maintain these rudiments, which have been said to resemble ancestral premolars (Kangas et al. 2004; Peterková et al. 2005, 2006; Prochazka et al. 2010). It is currently unclear how mechanisms involved in the formation and regression of such tooth buds might apply to mammals lacking a diastema and possessing more tooth types. In a later section we show how GM analyses of tooth row shape variation suggest that the Old World monkey *Colobus guereza* appears to reflect an incisor versus postcanine field, and within the postcanine field a premolar and molar field.

7.4.5 Evidence for mechanisms that cause variation within a tooth class (molars)

While each individual tooth is relatively independently controlled in terms of morphogenesis by its own signaling center (Jernvall and Thesleff 2000), genes controlling morphogenesis are shared across all teeth; thus, changes in the way they function may produce shape phenotypes in all teeth in the tooth row, or all teeth within a particular tooth class.

Ectodysplasin signaling is a key feature of enamel knot formation and maintenance during crown formation (Laurikkala et al. 2001; Pispa et al. 1999; Tucker et al. 2000, 2004). While much remains to be understood about this pathway (Charles et al. 2009a), there are dosage-dependent and X-inactivation-related effects on cusp lateral spacing in mutants of this pathway, as well as effects on tooth size (Charles et al. 2009a; Grüneberg 1966; Kangas et al. 2004; Kristenová et al. 2002). Small regulatory changes in genes such as ectodysplasin could create dental polymorphism within species and explain the evolution of a wide variety of tooth morphologies among mammals, including cusp reduction and the presence of longitudinal lophs (Kangas et al. 2004).

Ectodin (*Sostdc1*) mutants form longitudinal lophs on the buccal sides of cheek teeth, caused by a reduction in intercusp regions of the crown (Kassai et al. 2005). Decreasing *Fgf3* dosage in the primary enamel knot and mesenchyme increases cusp fusion and longitudinal lophs that resemble the morphological evolution in rodents from primitive fossil forms such as *Democricetodon* and the stem murine *Potwarmus* (Charles et al. 2009b). These authors also found that molar teeth in humans deficient in *Fgf3* are missing hypocones and have only three cusps, a morphology that resembles *Bahinia*, a primitive anthropoid primate from Asia; it is possible that *Fgf3* levels may play a role in repeated evolution of the hypocone across mammals.

More generally, morphodynamic models for crown formation were proposed that link gene expression and signaling of enamel knots to cell proliferation in the developing germ and physical and mechanical constraints of the developing enamel organ. Computational models with these parameters have shown the ability to replicate cusp morphologies of a wide variety of mammalian molars (Jernvall 2000; Osborn 2008; Salazar-Ciudad and Jernvall 2002, 2010).

Several genes have been identified that influence cusp height. Downregulating *Wnt* or *Bmp4* at this stage results in flattened molar cusps, due to reduction of *Bmp4*-directed *p21* expression in secondary enamel knots (Liu et al. 2008; Tabata et al. 2002). *Wnt* knockout mice have expanded ectodin expression, but reducing ectodin expression also results in broad, flat molars (Kassai et al. 2005; Liu et al. 2008). Follistatin knockout mice, which have elevated levels of *TGFβ*-family signaling, display blunted molar cusps that are not angled mesially because of a failure of asymmetric cell proliferation in each cusp (Wang et al. 2004). Additionally, in the possum *Monodelphis domestica*, teeth with tall, sharp cusps (the canine, premolars, and molars) express *Fgf10* in their primary enamel knots, whereas in lower-cusped teeth like incisors and all mouse teeth *Fgf10* is limited to the mesenchyme (Moustakas et al. 2011).

Pitx1 knockout mice have mandible-specific cusp anomalies. There is a single cusp on the LM1 that is shorter in length than the maxillary molars

(Mitsiadis et al. 2008). There is also an incompletely penetrant LM1-LM2 fusion, hypothesized to be a result of a LM2 developmental delay. While upper and lower teeth are often differentially affected in knockout mice, this is the only example in which only the mandible is affected at this late developmental stage. Although the mechanisms behind these defects are not well understood, apoptosis and proliferation seemed normal, but *Barx1* was somewhat downregulated in the mandibular molar mesenchyme (Mitsiadis et al. 2008).

These studies, combined with the observations of molar development, suggest that variation in the molar row may be patterned by mechanisms specific to molars, and as such the molar series in and of itself is a cohesive phenotype, as opposed to just three separate teeth. Quantitative genetic analyses of baboon dental variation provide additional evidence to the same (Hlusko et al. 2004).

7.5 GM studies on tooth development

Researchers have utilized GM to study development of the dentition in several contexts, including use of superimposition to measure phenotypes from perturbations of development at the genetic level. Most research has taken a more indirect approach, using GM data to test models of development patterning or to explore developmental constraint and modularity, on the basis of the developmental genetic literature. Primate and nonprimate work are discussed later.

7.5.1 Developmental genetics

Keller and colleagues (2007a, 2007b, 2008) examined the effects of in utero exposure to the toxicant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on development of the murine mandibular molar row. Landmarks from left and right molars were used to assess fluctuating asymmetry in control and TCDD-exposed mice. Genotypic effects were identified in a mixed-model ANOVA of shape variation, but a subsequent quantitative trait locus (QTL) analysis did not reveal any different gene action between groups (Keller et al. 2007a). Investigation of the *Ahr* locus, which codes for the receptor through which TCDD acts, did demonstrate an influence on how TCDD affects molar shape (Keller et al. 2007b). The authors were able to identify the amount and timing of TCDD exposure that produced shape alterations in LM1 and LM2 on the basis of the effects of TCDD dosage on different inbred strains of mice with TCDD-sensitive *Ahr* alleles (Keller et al. 2008).

7.5.2 Prediction of tooth shape variation based on developmental models

The patterning cascade model of cusp development, where position, size, and shape of the earliest-forming cusps affect variation in later-forming cusps (Jernvall 2000; Polly 1998; Salazar-Ciudad and Jernvall 2002), was tested by Skinner and Gunz (2010) in their study of accessory cusps in chimpanzee lower molars. The authors collected three-dimensional landmarks and semi-landmarks from micro CT models of the EDJ surface to identify correlations between molar crown shape and the presence of a sixth, accessory cusp (C6). Their results indicate that C6 frequency is higher in larger molars and variation is correlated with the location and size of later-forming cusps; the results support their hypothesis that C6 formation would increase with tooth bud size and the accompanying decrease in inhibiting gene products in the bud. Skinner and Gunz (2010) propose that extra cusps beyond C6 represent the same iterative developmental process that produced primary cusps and caution against treating such cusps as traits independent of overall tooth size or adjacent cusp morphology in cladistics.

7.5.3 Developmental constraint

Polly used two Paleogene lineages of viverrid carnivorans (1998) and five modern shrew populations (2005) to explore patterns of dental phenotypic correlation and developmental constraint. Using landmark data from carnivoran LM1s, the author found variability in cusp position significantly correlated with timing of cusp initiation, as well as the amount of intercusp growth and evolutionary change (Polly 1998). The loose local developmental constraint on molar shape suggested by these findings (Polly 1998) was also supported by computer modeling of phenotypic covariance due to developmental interactions in the LM1 of the common shrew (Polly 2005). Polly (2005) concluded that because only a small proportion of the covariance could be explained by development, it is likely that more proximate factors play a larger role in the evolution of molar shape variance.

Renaud et al. (2006) explored internal developmental constraint on molar shape by relating phenotypic covariance and the direction of morphological evolution in fossil rodents, as described by Fourier analysis. They identified an evolutionary "line of least resistance" corresponding to the direction of greatest intraspecific variation and contrasted two lineages: *Apodemus*, which epitomizes this trajectory over the last ten million years, and *Stephanomys*, a pronounced departure from the "line of least resistance" attributable to a

powerful environmental degradation. Functional constraints were considered along with development in a subsequent study of covariation in the molar row of *Mus* and *Apodemus* (Renaud et al. 2009). Strong covariation between adjacent teeth within the molar row was attributed to developmental processes, while strong covariation between occluding molars was explained by functional constraints. It is important to note that the two rodents exhibited a conserved pattern of covariation, despite having diverged more than ten million years ago (Renaud et al. 2009).

Constraint on development (and ultimately tooth morphology) due to the physical space available in the jaw was addressed by Boughner and Dean (2004). The staggered order of molar crown mineralization in the baboon was contrasted with overlap in the cusp mineralization process in chimpanzees, and three-dimensional landmark data from mandibles and molar crypts, crowns, and roots were used to explore the relationship between molar development and jaw space. Contrary to the authors' expectations, trajectories of molar row shape change were indistinguishable across the baboon and two species of chimpanzee; little correlation was seen between relative size of the mandible and the spacing and pattern of development of the molars.

7.5.4 Modularity

Insights to development can also be gained through an examination of covariation between traits and their underlying shared genetic effects. Integrated units identified in this manner are referred to as developmental modules and are characterized by their independence from other modules (Klingenberg 2008). Modularity has been explored in detail using GM in the primate cranium (e.g., Bastir and Rosas 2005; Goswami and Polly 2010) and has recently been addressed in the dentition by several GM studies in mice and voles (discussed later).

Workman and colleagues (2002) collected landmarks from the right and left mandibular molar rows of genotyped inbred mouse strains to identify QTLs associated with tooth row size and shape. They found more QTLs for molar shape than molar centroid size; however, the effects of these QTLs were spread across all three molars, suggesting no individual molar represents a genetically distinct developmental unit. The authors also noted that the QTLs for molar shape were many of the same QTLs identified for mandible shape and cranial dimensions in earlier studies. Leamy and colleagues (2005) explored the genetic basis for FA in the mandibular molar row in the same inbred mice. Their QTL study revealed only two loci affecting shape FA but many combinations of locus pairs exhibiting epistasis for size and shape FA.

Dental modularity and integration between teeth have also been studied by Laffont and colleagues (2009), who collected landmarks from outlines of the three lower molars in the vole species *Microtus arvalis*. The authors assessed covariation in shape between molars and performed a PLS analysis to test the two hypotheses that each molar constitutes a semiindependent module or that the three molars are a single integrated block. Although it was recognized that the molars collectively constitute an integrated unit at the scale of the mandible, three individualized molar modules were identified. Interestingly, covariation was higher between LM1 and LM2 than between either LM1 and LM3 or LM2 and LM3, supporting some developmental independence of the latter molar.

7.6 Case study: GM analysis of hierarchical dental development in *Colobus guereza*

The results of Laffont et al. (2009) bring to light an important methodological consideration for studies of the developmental basis of dental variation, particularly modularity, in primates. In the case of the voles, or any rodents in the superimposition studies reviewed previously, selection and placement of landmarks on the dentition are fairly straightforward given that only two tooth types are present in the jaw, and these types are physically separated by a sizable diastema. In other words, options for landmark configurations include those on a single tooth (i.e., single molar) or on all teeth of a certain type (i.e., molar row), but not landmarks on the entire tooth row because of spatial interruption by the diastema.

We use an example from our own studies of the dentition of Old World monkeys to show that primates can be a useful model for exploring modularity, and consequently, development of the mammalian dentition. The full heterodont dentition of primates not only is a more primitive mammalian configuration compared to that of mice, but presents a greater number of landmark configuration options: with the presence of additional tooth types in the canine and premolar teeth, and without a large diastema, shape can be studied at many hierarchical levels in the primate tooth row. In addition to individual teeth and tooth types, it is possible to examine landmark configurations encompassing any adjacent teeth, including the entire tooth row or the anterior and postcanine dentitions.

The choice of landmark configuration implemented should be tailored to the hypothesis being tested, and it should not be assumed that variation captured in a superimposition at one hierarchical level of the dentition would be the same at a different level. GPA depicts landmark configuration variation

as a composite of the coordinated movements of each landmark, following the resistant-fit least squares function described earlier (Gower 1975; Rohlf and Slice 1990; Siegel and Benson 1982; Sneath 1967); thus, the introduction of new landmarks on additional teeth or a decrease in landmarks as teeth are removed from a configuration will have an effect on the observed shape change at each remaining landmark. To illustrate this effect, we present the superimposition of occlusal landmarks from multiple hierarchical levels of the maxillary dentition of the eastern black and white colobus monkey, *Colobus guereza*. This study serves as a caution to tailor configurations to hypotheses; however, it also provides an example of the utility of GM for testing hypotheses of variation and modularity, based on the multiple different shape analyses afforded by the one-time collection of a large set of landmarks.

7.6.1 Materials and methods

Landmarks were collected from 75 crania curated at the American Museum of Natural History (New York), Cleveland Museum of Natural History (Ohio), and National Museum of Natural History (Smithsonian Institution). We restricted study to adult monkeys with fully erupted third molars to control for ontogenetic variation. Our sample contained 43 males and 32 females.

Specimens were photographed using a Nikon D80 camera with a Nikkor AF-S 105 mm micro lens such that each specimen was oriented with the postcanine occlusal surface in the focal plane. Two-dimensional landmark data were collected from the photographs with the digitizing program tpsDig 2.10 (Rohlf 2006). A total of 93 landmarks were collected, representing overall dental arch shape, but also including shape information for groups of teeth within the row as well as individual teeth. Landmarks are illustrated in Figure 7.2. After bilateral landmarks (all except midline incisor) were reflected across the midline and averaged using program BigFix6 (Sheets 2001a), the total number of landmarks for analyses was 47.

We implemented GPA of the landmark configurations in the program CoordGen6 (Sheets 2001b), followed by principal components analysis (PCA) using PCAGen6 (Sheets 2001c). The PC axes correspond to eigenvectors of the variance-covariance matrix for the shape data, and eigenvalues are proportional to the variance explained by the PCs (Zeldtich et al. 2004). GPA and PCA were carried out at three hierarchical levels: (1) entire tooth row, including 47 landmarks on the incisors, canine, premolars, and molars; (2) postcanine dentition, including 39 landmarks on the premolars and molars; and (3) molar row, including 31 landmarks on the three molars.

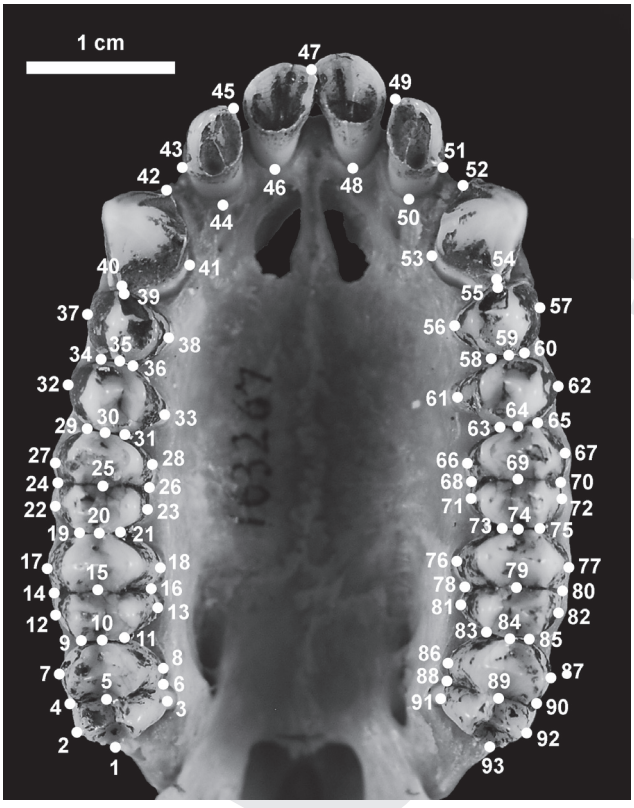

Figure 7.2. Photograph of *Colobus guereza* maxillary dentition illustrating the 93 landmarks collected in the study.

7.6.2 PCA results at three hierarchical levels

The percentage of the total shape variation explained by PC1 is greatest in the analysis at the level of the whole row (36.1 percent), followed by the postcanine level (22.8 percent) and smallest at the molar row level (18.2 percent). In addition, deformation in shape associated with PC1 differs across shared teeth in all three levels. Figure 7.3 illustrates these differences between premolars and molars at the tooth row and postcanine levels and between molars at all levels. In the whole row configuration, the greatest dimension of variation involves a mesiodistal contraction of the entire postcanine dentition, relative to translation and rotation of the anterior dentition. Note that the magnitude of vectors on the incisors and canine are some of the largest in the entire configuration, suggesting that variation in these teeth may be driving the PC1 trend.

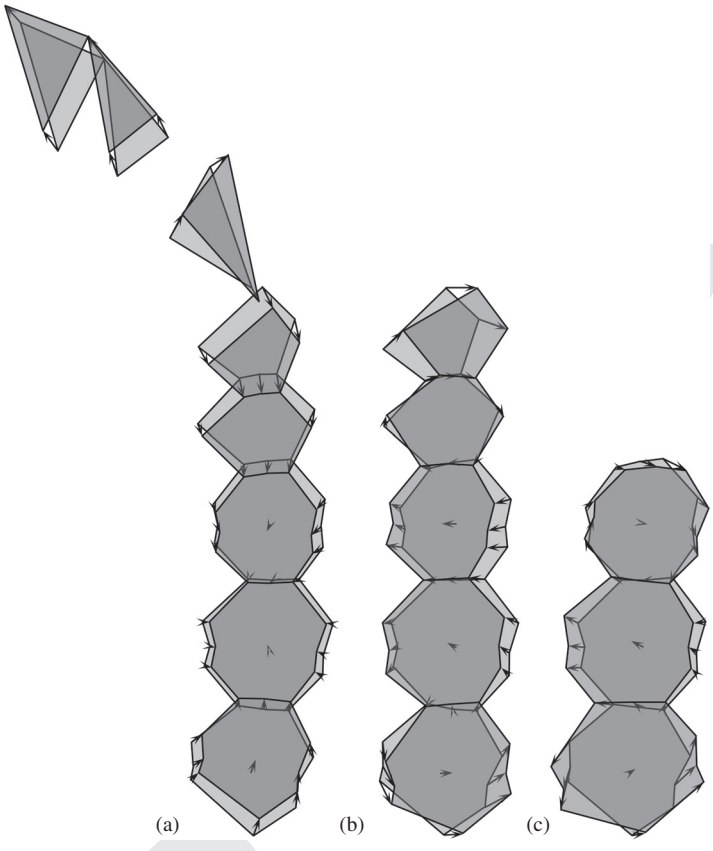

Figure 7.3. Deformations representing the first principal component of shape variation in the maxillary dentition of *Colobus guereza* at the levels of the whole tooth row (a), the postcanine teeth (b), and the molar row (c). Shape change is represented by vectors on landmarks, indicating the deformation from the mean shape (light gray tooth polygons) in one direction along the first principal component axis (dark gray tooth polygons).

Using the coordinated movement of teeth within PC1 as a means of identifying possible modular units, the postcanine dentition, which expands or contracts as a single unit relative to shape changes in the anterior dentition, stands out as a strong candidate. We then analyzed this restricted set of landmarks representing only the postcanine teeth. PC1s for the postcanine (Figure 7.3b) and molar row configurations (Figure 7.3c) present different patterns of shape variation. At the postcanine level, premolars are shifted buccally together, while the first and second molars translate lingually and the third molar rotates distally and buccally. At the level of the molar row, each molar is characterized

by a different movement. In summary, the postcanine module identified at the tooth row level is broken down into a premolar module and a separate module composed of the first two molars, with the third molar appearing independent. At the level of the molar row, the coordination between the first two molars is not observed, suggesting independence between each individual tooth.

The shape deformations represented by PC2 demonstrate similar relationships (Figure 7.4). In the whole tooth row configuration, PC2 (14.7 percent of variation explained) represents variation in the mesiodistal position of incisors and the buccolingual position of premolars, all relative to the canine, accompanied by buccal translation of the premolars and first molar and lingual translation of the third molar. In other words, the greatest dimension of variation within the tooth row includes a clockwise rotation of the entire postcanine dentition, relative to an increase in canine width and a mesial translation of the incisors. Note again that the magnitudes of vectors on the incisors are some of the largest in the entire configuration and hence may be driving the PC2 trend as well.

A postcanine module is also suggested by PC2 at the tooth row level, but, as seen for PC1, the integrated movement of teeth at lower hierarchical levels suggests smaller modules within the tooth row as well. At the level of the postcanine dentition, PC2 (10.9 percent explained) depicts extreme mesial compression of the first premolar and buccal translation of the second premolar, in contrast to the coordinated mesiodistal expansion of the molar row as a single unit (Figure 7.4b). PC2 at the molar row level (13.8 percent explained) presents a different aspect of variation, in which molars vary together in buccolingual width (Figure 7.4c)).

7.6.3 Modularity in the maxillary dentition of an Old World monkey

On the basis of the coordinated movement of teeth within deformations corresponding to the first and second PCs of shape variation from our GM analysis, we identified several levels of phenotypic modularity. Analysis of landmarks across the entire tooth row consistently demonstrated a dissociation between movement of the anterior and postcanine dentitions, suggesting that each corresponds to a separate module. This phenotypic module corresponds to expectations from the Homeobox Code Hypothesis (Sharpe 1995; Thomas and Sharpe 1998), combined premolar/molar odontogenic field specification in development (Järvinen et al. 2009; Yamanaka et al. 2007; Yamanaka and Uemura 2010), and evidence from quantitative genetic analyses of mice and baboons (Hlusko et al. 2011).

Similarly, when only landmarks on the postcanine teeth were analyzed, the coordinated movement of the premolars could be distinguished from shape

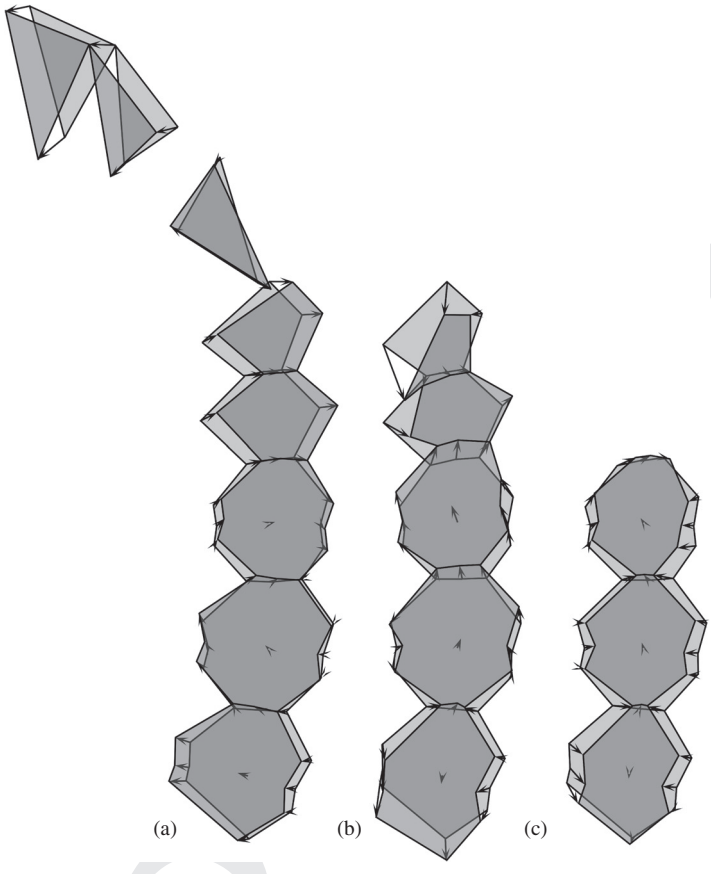

Figure 7.4. Deformations representing the second principal component of shape variation in the maxillary dentition of *Colobus guereza* at the levels of the whole tooth row (a), the postcanine teeth (b), and the molar row (c). Shape change is represented by vectors on landmarks, indicating the deformation from the mean shape (light gray tooth polygons) in one direction along the second principal component axis (dark gray tooth polygons).

change in the molars, indicating that within the postcanine module there exists some independence between tooth types. This also follows evidence from quantitative genetic analyses of baboons (Hlusko et al. 2011; Hlusko and Mahaney 2009) and suggests that some genetic distinction between premolars and molars may characterize all Old World Monkeys.

Integration within the molar row is more complicated: at the levels of the postcanine dentition and molar row, independence between molars, particularly in the case of the third molar, was observed in the first PCs, while in the

second PCs, which account for a smaller portion of the total shape variation, molars change shape in a coordinated fashion, reinforcing the idea of a single molar row module. Quantitative genetic analyses of variation in baboon molar cusp orientation suggest that there may be modules within and across the molar row (Hlusko et al. 2004). Combining this quantitative genetics work with what is coming out of mouse developmental genetics, and the possibilities inherent in the significant amount of primate skeletal material around the world, many interesting research directions are ripe for exploration.

7.7 Conclusions

One of the essential questions in the study of skeletal morphology concerns the definition of "phenotype," not in the classic sense of relationship between genes and environment, but in terms of how one should define a phenotype at the anatomical level to address a research question most accurately (e.g., Hlusko 2004; Houle 2001; Wagner and Laubichler 2001). "The phenotype" is often a proxy for understanding how genes, environment, and evolution interacted and is therefore a fluid concept that depends on the research question. For example, a question about function may necessitate a view of the hominid pelvis and hip joint as one interrelated unit (e.g., Lovejoy et al. 1999), whereas a question about how selection or drift resulted in loss of the third molar in marmosets and tamarins requires an investigation of either the mechanisms that pattern variation within the molar series specifically or the length of the dental lamina at the level of the tooth row. Which is the more representative phenotype?

GM is a powerful tool in that it enables the phenotype to be defined variably, and experimentally. As such, definition of "the phenotype" can be explored at multiple levels and for multiple research aims. Primates offer a particularly useful taxonomic group within mammals for this type of research given their geographic breadth and diversity, and the fortuitous assemblage of specimens in museum collections. Add in the depth of our understanding about developmental genetics of mammalian teeth, and GM analyses of primate dental variation will be a fruitful tool in evolutionary biology for many years to come.

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