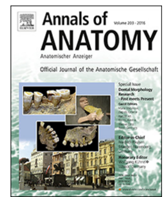
Annals of Anatomy 203 (2016) 3–11

Contents lists available at ScienceDirect

Annals of Anatomy

journal homepage: www.elsevier.com/locate/aanat



Elucidating the evolution of hominid dentition in the age of phenomics, modularity, and quantitative genetics

Leslea J. Hlusko*

Department of Integrative Biology, University of California Berkeley, 3040 Valley Life Sciences Building, MC-3140, Berkeley, CA 984720, United States

ARTICLE INFO

Article history:
Received 20 December 2014
Received in revised form 17 May 2015
Accepted 18 May 2015

Keywords:
Australopithecus
Morphological integration
Neanderthals
Primates
Paleontology
Taurodont
Enamel thickness

ABSTRACT

An organism's anatomy is the result of millions of years of interplay between DNA sequence, developmental processes, the environment, and evolutionary forces. The anatomical sciences are consequently highly integrative and interdisciplinary. That said, reaching across all of the relevant disciplines can be a daunting task because scientific publications are produced today at an astounding rate. This manuscript brings together insights from the quantitative genetic analysis of dental variation into the study of human evolutionary odontology within the context of genomics, genetic modularity, and phenomics. It primarily advocates the use of quantitative genetics to not only identify QTLs, but also to assess the patterns of genetic covariance that underlie phenotypic covariance, thereby enabling us to conceptualize phenotypic variation as a reflection of the underlying genetic mechanisms. By highlighting three phenotypes of importance within the study of human evolution (patterning of the dental arcade, enamel thickness, and taurodontism), it is demonstrated how an integrated consideration of quantitative genetics, genomic analyses, and paleontology can bring us to more detailed hypotheses about the evolution of the hominid clade.

© 2015 Elsevier GmbH. All rights reserved.

Contents

1. Introduction 3
2. Framework: quantitative genetic analysis of human and non-human primate dental variation 4
3. Phenotypes reconsidered 5
   3.1. Patterning of the dental arcade 5
   3.2. Enamel thickness variation 6
   3.3. Taurodont molars of the Neanderthals 8
4. Conclusions 9
   Conflicts of interest 9
   Acknowledgments 9
   References 9

1. Introduction

Almost 20 years ago, in a discussion of complex diseases, Schork (1997) made the distinction between the discovery of genes and the characterization of the effects of genes. He differentiated between bottom-up approaches (such as identifying candidate genes and developmental genetics) and top-down approaches (such as genomic analyses). He expressed the need for something in the middle – a connection between genes, gene-by-gene interactions, and the resultant phenotypes. He defined *phenomics* as the "delineation of connections among various genes, gene products, intermediate phenotypes, and clinical endpoints" (Schork, 1997: S107). Unfortunately, Schork's (1997) definition did not persevere, as *phenomics* has morphed into a term referring to large-scale efforts to collect phenotype data (Houle et al., 2010, credited the phenotypic definition to Soulé's (1967) phenetic analysis of lizards; for the use of the term within paleontology see Burleigh et al., 2013, and in craniofacial biology, Yong et al., 2014).

Despite the lack of a general term for it, the relationship between the genetic material that passes from generation to generation and

* Tel.: +1 5107082632.
E-mail address: hlusko@berkeley.edu

http://dx.doi.org/10.1016/j.aanat.2015.05.001
0940-9602/© 2015 Elsevier GmbH. All rights reserved.

the phenotypes upon which selection operates is one of the most fascinating, complex, and little understood phenomena faced by modern anatomists. And yet it is one of the most essential elements for understanding the evolutionary processes that underlie the variation within and among species. What the morphologist needs is *phenomics* as Schork (1997) originally intended. There are currently two routes for addressing this middle ground, namely bottom-up and top-down approaches.

The first works from development forward through ontogeny to link genotype to phenotype. Over 20 years of developmental genetics research on mice underlies a fairly sound understanding of the genes necessary and sufficient to make mammalian teeth, and how this likely relates to human dental variation (Jheon et al., 2013; Thesleff, 2014; Lesot et al., 2014). This research demonstrates that the dentition is a hierarchical organ, with genetic patterning mechanisms operating across the dental arches, along the tooth row, within tooth types, and within a tooth (e.g., Jernvall and Thesleff, 2000; Stock, 2001). Three-dimensional embryological studies reveal the temporo-spatial dynamics of signaling centers, including transient rudimentary tooth primordia in mouse diastema where teeth were evolutionarily lost (Peterkova et al., 2014). However, it is widely recognized that the mechanisms required to make an organ are not necessarily the same mechanisms that underlie its phenotypic variation.

As our understanding of developmental genetics improves, so does our ability to mathematically model the effects of developmental parameters on ultimate morphology (Félix, 2012). Specifically for teeth, Salazar-Ciudad (2012) and colleagues (Salazar-Ciudad and Jernvall, 2007, 2010) have explored what *in silico* teeth look like when different components of a gene network – modeled after mouse molar development – are incrementally adjusted. This has led to the identification of key parameters whose alteration results in computer-generated morphological variation that is reminiscent of what is seen in living organisms. These results provide hypotheses about which or genetic activator and inhibitor parameters may be most critical for influencing population and species level variation. A drawback to this approach, however, is the vast amount of research needed to elucidate the gene pathways prior to being able to construct a model. New gene sequencing approaches make this less of an obstacle than it was just a few years ago. For example, gene expression can now be studied with ChIP-seq, through which protein-DNA binding is assessed in vivo (Park, 2009), and with RAD-seq that targets selected genomic regions of interest, facilitating research on species for which whole genome sequences are not available (see overview by Van Dijk et al., 2014). However, the majority of organ systems are not yet available for such modeling approaches, including the dental arcade and bony skeleton.

The second, top-down approach is founded within genetic and genomic analyses that statistically identify loci associated with population-level and species-level dental variation (e.g., Pillas et al., 2010; Geller et al., 2011; Haga et al., 2013), and the likelihood of selection on these loci (e.g., Horvath et al., 2014). While these analytical methods identify loci that have statistically significant influences on phenotypic variation, they cannot elucidate the mechanism through which these loci accomplish this. Consequently, such results are difficult to incorporate into our understanding of the evolution of the associated anatomical structures.

Quantitative genetic analyses tend to be viewed exclusively in terms of their QTL (quantitative trait loci) results. However, the power of recently developed analytical methods enables sophisticated exploration of phenotypic variation that is, in my opinion, greatly underappreciated. Quantitative genetics relies on the principle that phenotypic variance observed within a population can be decomposed into variance due to genetic effects, variance due to non-genetic effects, and variance due to covariate effects (Falconer, 1981). For populations of related individuals, the genetic variance is structured by a matrix of kinship coefficients (e.g., Almasy and Blangero, 1998). Through a maximum likelihood approach, various parameters can be estimated essentially through linear regressions (see details of the analytical methods my colleagues and I use in Hlusko et al., 2006). Phenotypic *correlations* can similarly be decomposed into genetic and non-genetic components, providing the opportunity to assess the structure of the genetic variance that underlies phenotypes as we currently define them. This means that we can use quantitative genetics to not only identify QTLs, but also to assess the patterns of genetic covariance that underlie phenotypic covariance, enabling us to conceptualize phenotypic variation as a reflection of the underlying genetic mechanisms, or rather, the genetic architecture.

The justification for this approach builds in part on the concept of *morphological integration* (Olson and Miller, 1958) – that phenotypic traits will be tightly correlated when they share a common developmental pathway and/or ultimate function. These integrated units, or *modules* were initially identified through phenotypic correlation and were later refined by the ability to estimate genetic correlations (Ehrich et al., 2003; Cheverud et al., 1997; Klingenberg et al., 2001; Mezey et al., 2000; Leamy et al., 1999; Cheverud, 1988). This brings us to *modularity* (Wagner and Altenberg, 1996), which incorporates evidence of the mechanisms that underlie morphological integration. Modules can be classified as genetic (defined by a matrix of genetic covariances), developmental (defined by mechanistically correlated precursors of a trait), functional (defined by a trait's interaction with other body parts performing a particular function), or evolutionary in character (defined by observation of correlated change over time) (Klingenberg, 2008). These module types are not mutually exclusive and can influence each other, resulting in phenotypic covariation (Cheverud, 1996). More recently, researchers have started to assess large number of phenotypes in single-gene null mutants to search for pleiotropic effects, results that further support the interpretation that phenotypic variation is highly modular (e.g., Wang et al., 2010; see Paaby and Rockman, 2013 for a critical discussion of "pleiotropy").

Given that these heritable patterns of covariance may be stable over reasonably long periods of evolutionary time (Lande, 1979, 1980), genetic covariance may bias phenotypic response to selection through *lines of least evolutionary resistance* (LLER) (Schluter, 1996, 2000). Schluter (1996, 2000) found that evolution among closely related species tended to follow the trajectory defined by the genetic covariance structures more so than the phenotypic covariance structure. From this, he concluded that genetic architecture creates a pathway of least resistance along which evolution will typically travel unless perturbed by strong natural selection. Marroig and Cheverud (2005) found a similar result when studying cranial variation across 16 extant genera of New World Monkeys (NWM), suggesting that the genetic architecture of cranial size has been stable for at least 26 million years of NWM radiation. My colleagues and I found that Old World Monkey dental variation within populations, between species, and across genera similarly varies along trajectories defined by patterns of genetic covariance (Grieco et al., 2013). Because the genetic modularity seen in baboons does not necessarily similarly characterize the human dentition, I now quickly review the deep scientific literature on quantitative genetic analyses of human and non-human primate dental variation.

2. Framework: quantitative genetic analysis of human and non-human primate dental variation

There is close to a century of quantitative genetics research on human dental variation (see detailed review in Rizk et al., 2008).

Early analytical efforts utilized twin, sibling, and parent-offspring relationships to assess inheritance of dental caries and orthodontic disorders (Bachrach and Young, 1927; Moore and Hughes, 1942) and later to explore tooth size, cusp variation, timing of eruption, and occlusion (Lundström, 1948; Ludwig, 1957). Inter-populational differences, familial aggregation, and more sophisticated analyses of immediate family patterns of inheritance continued to yield evidence for genetic influences on tooth size variation (Horowitz et al., 1958; Niswander and Chin, 1965; Townsend and Brown, 1978a,b; Townsend, 1980). Analyses of morphological variants, such as Carabelli's trait and incisor shoveling tended to evince low or non-significant heritabilities (Garn et al., 1966; Turner, 1967; Alvesalo et al., 1975; Scott, 1980), but this may have been a result of the difficulty in quantifying the trait more so than a true signal of the biology, because other studies reported quite high heritability estimates (Skrinjaric et al., 1985; Blanco and Chakraborty, 1976; Portin and Alvesalo, 1974). However, analyses that incorporated multiple nonmetric traits also returned low heritabilities (Mizoguchi, 1977). Sex effects and maternal effects were then incorporated into the models (Garn et al., 1967, 1980; Sirianni and Swindler, 1973, 1974). Complex segregation analyses further refined the genetic and non-genetic effects on tooth size (Nichol, 1990).

When maximum likelihood estimation was incorporated into quantitative genetics in the 1990s, the additive genetic and non-genetic contributions to phenotypes such as Carabelli's trait (Townsend et al., 1992) and tooth size (Dempsey et al., 1995) could be refined. Twin studies have since provided the most recent insights about other factors that contribute to variance in the dentition (reviewed in Townsend et al., 2009b). These include intrauterine hormone effects (Ribeiro et al., 2013a), epigenetic effects (Townsend et al., 2005; Hughes et al., 2014), birth weight (Apps et al., 2004), and tooth emergence (Hughes et al., 2007; Bockmann et al., 2010). The combined results of these studies conclusively demonstrate that variation in the size and shape of the human dentition is significantly influenced by the additive effects of genes.

Over the last 15 years, my colleagues and I have undertaken quantitative genetic analyses of dental variation in a pedigreed breeding population of baboons at the Southwest National Primate Research Center. We have elucidated shared genetic effects across a plethora of dental phenotypes (Grieco et al., 2013; Hlusko and Mahaney, 2003, 2007, 2009; Hlusko et al., 2004a,b, 2006, 2007, 2011; Koh et al., 2010). Whereas most of the previous work cited above searched for additive genetic effects on the variance of one phenotype, our research on baboon dental variation focused on and explored genetic correlations between various ways to measure the phenotype. Our goal has been to describe/partition phenotypic variation such that it better reflects the underlying genetic architecture. We find that genetic correlations between seemingly homologous features often cross individual teeth and the dental arcades, but can be genetically independent on the same crown (such as the orientation of the molar lophs/lophids, Hlusko et al., 2004b, and molar cusp areas, Hlusko et al., 2007; Koh et al., 2010). Tooth linear dimensions have significant genetic correlation on the same crown, but their patterns of genetic interrelatedness with body size are distinct. For example, ~20% of the variation in buccolingual width results from the same genes that influence crown-rump length, but none of the variation in mesiodistal length is (Hlusko et al., 2006). This phenomenon may also explain the earlier observation that buccolingual widths for human molars are more highly heritable than are mesiodistal lengths (Alvesalo and Tigerstedt, 1974), given that statue is highly heritable and is genetically correlated with molar crown width. We also find that variation in baboon enamel thickness has no genetic correlation with tooth size nor is it sexually dimorphic (unless, of course, you scale it with a phenotype that is; Hlusko et al., 2004a).

Our subsequent study of extant phenotypic variation across the Old World Monkeys provides strong evidence that the genetic architecture we elucidated is conserved (Grieco et al., 2013). Given that we also found evidence of conserved genetic modularity between mice and baboons (Hlusko et al., 2011), it is likely that these genetic modules similarly underlie variation across mammals more broadly, including humans. In support of this inference, reviews of the quantitative genetic analysis of human dental variation reveal no results that are incongruous with the baboon study (Rizk et al., 2008). My laboratory is currently testing this hypothesis in detail and will be publishing results in the near future.

Selection operates on the phenotype, but phenotypic evolution can only happen according to how the genetic architecture structures its variance/covariance. By accommodating how we define phenotypes so that they better reflect the underlying genetic architecture, we will dramatically improve the information about evolution that can be extracted from phenotypic evidence–extant and extinct.

3. Phenotypes reconsidered

Arguing that genotype-phenotype thinking must be integrated into hominid evolutionary studies is not new (e.g., Hlusko, 2004). However, given the advances across the biological sciences over the last decade, the time is right to revisit and further encourage the endeavor. This paper highlights three phenotypes of importance within the study of human evolution – patterning of the dental arcade, enamel thickness, and taurodontism – and demonstrates how an integrated consideration of quantitative genetics, genomic analyses, and paleontology can bring us to more detailed hypotheses about the evolution of dental anatomy within our lineage.

3.1. Patterning of the dental arcade

Patterns of phenotypic covariance suggest that teeth of the same category (incisor, canine, premolar, and molar) covary as a result of genetic patterning. One of the most distinct examples of this phenomenon across mammals is the extreme variation in the size of the incisors without a correlative size change in the post-canine dentition. Perhaps the best example of this within the Hominidae (reviewed in Ungar, 2011) is the genus *Australopithecus*, species of which existed in Africa 4–2.5 million years ago. These species reveal a remarkable range of relative size variation between the anterior (incisors and canines) and post-canine dentition (premolars and molars) (see Fig. 1).

Butler (1939) applied Huxley and De Beer (1934)'s concept of biological fields to observed differences between the incisor, canine, and molar tooth categories, which was then expanded to accommodate humans with a premolar field (Dahlberg, 1945). In the *field theory*, specialized fields that equate to tooth categories are established early in development. At this point in developmental time, Butler proposed that all tooth primordia are identical. Subsequently, a substance diffuses through the growing region within each field, resulting in minor shape variations due to the different exposure to the diffused substance.

An alternative hypothesis, the *clone* model, proposes that the patterns are self-generated within the tooth primordia themselves rather than defined by external fields (Osborn, 1978). Whereas tooth primordia are identical at the start, the ultimate differences between teeth result from the differing times of initiation – tooth identity, shape, and size gradients are the result of growth. While the field and clone models of tooth development have a rich academic history, including recent updates by Townsend et al. (2009a), these models were derived from patterns of phenotypic data that

Fig. 1. Demonstration of dental arcade patterns in the hominid fossil record. The six replicas of fossil mandibles shown here represent, from the top left corner clockwise: AL 400-1a, *Australopithecus afarensis*; Sts 52b, *A. africanus*; Natron (Peninj), *A. boisei*; Qafzeh Hominid 9, *Homo sapiens*; Kebara 2, Neanderthal; SK 23, *A. robustus*. These are all shown at the same scale. The bottom panel shows the right side of each mandible (except, a mirror-image of the left is shown for AL 400-1a) scaled so that the first molar is the same mesiodistal length. The anterior teeth (incisors and canine) are shaded pink, premolars in blue, and molars in green to highlight the size variation in the modules described in the text.

are hard to reconcile specifically with our current understanding of developmental genetics. Therefore, it is perhaps worthwhile to set these historical frameworks aside and focus on evidence generated from quantitative genetic analyses.

Quantitative genetic analyses of tooth size across the dental arcade reveal that variation in the anterior and postcanine dentitions is genetically independent in both mice and baboons, indicative of two distinct genetic modules (Hlusko and Mahaney, 2009; Hlusko et al., 2011). Multiple mammalian lineages have evolved dramatic anterior versus posterior tooth size and proportional differences through time. Furthermore, the genetic architecture of mice and monkeys (last shared common ancestor ~69 myr; Eizirik et al., 2001). These facts led my colleagues and I to hypothesize that this genetic architecture characterizes mammal dentitions generally. These two independent genetic modules would have facilitated the differential functions of teeth in the front of the mouth versus the back of the mouth, a phenomenon that has played an important role in the diversification of mammals over the Cenozoic (Rose and Archibald, 2005).

My colleagues and I also found evidence of genetic submodularity within the post-canine dentition of baboons (Hlusko and Mahaney, 2009; Hlusko et al., 2011). Here, genetic correlations are statistically significant between premolar and molar size, and genetic correlations tend to be higher within each tooth class. We interpret this as evidence of incomplete pleiotropy between premolars and molars, and find that this covariance structure is evident in phenotypic data from across the OWMs (Grieco et al., 2013).

A recent phenotypic analysis aimed at identifying modularity in the primate maxillary dentition found that premolars and molars appear to be submodules of a larger and hierarchically superior module (Ribeiro et al., 2013b). While this work reaffirms our result, it is unfortunate that they did not consider our quantitative genetic analyses of modularity in the primate dental arcade (e.g., Hlusko et al., 2011; Grieco et al., 2013). Our evidence of genetic correlations between premolars and molars in the maxilla (Hlusko et al., 2011) likely underlies their observation that molar sizes increase to compensate the evolutionary loss of anterior premolars (Ribeiro et al., 2013a,b).

Observations such as the anterior versus post-canine tooth size patterns in *Australopithecus* suggest that the genetic modularity of baboons also characterizes the hominid clade, including our own species lineage. How this may have structured phenotypic evolution is a promising research direction. For example, during the Pleistocene, the genus *Homo* was geographically widespread across Africa, Europe, and Asia. The dental variation of these populations reflects a similar pattern of apparent independence between the anterior and postcanine dentitions, but in this instance the phenotypes are more qualitative than quantitative. Martinón-Torres et al. (2007)'s overview characterized early Pleistocene African populations of *Homo* as having simpler incisor and molar morphologies compared to those of populations outside of Africa, and the opposite phenomenon was observed for the premolars. Analyses that attempt to elucidate the genetic architecture of the more qualitative anatomies (such as incisor shoveling and Carabelli's cusp) are needed in order to better understand their potential response to selection versus genetic drift. Given that this pattern of variation anecdotally mimics the genetic modularity of tooth size, this is a highly promising research direction that is currently not being widely pursued.

As has long been recognized, the highest order of dental variation is that of the tooth categories – incisors, canines, premolars, and molars. These tooth types are underlain by genetic modularity, with some modules genetically independent and others with evidence of incomplete pleiotropy. This genetic architecture provides the framework for all of the other variation along the dental arcade. Whether these genetic modules influence other dental phenotypes, and how, will be a key area of research for the future. We now explore two phenotypes of specific concern within human evolution, bearing in mind the genetic influences on the patterning of the dental arcade.

3.2. Enamel thickness variation

Thirty years ago Martin (1985) studied differences in molar enamel thickness among various extant and extinct hominoid taxa and, in so doing, stimulated considerable research into enamel thickness variation. Techniques for studying enamel thickness have ranged from histological sections to micro-CT scans, and assessments in 2- and 3-dimensions (e.g., Macho, 1994; Schwartz, 2000;

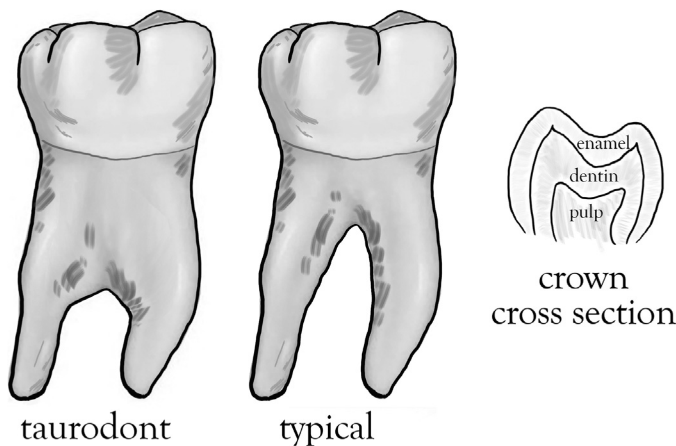


Fig. 2. Sketches to demonstrate the phenotypes discussed in Sections 3.2 and 3.3. See text for more detail.

Kono, 2004; Suwa and Kono, 2005; Smith et al., 2005; Olejniczak et al., 2008; Pampush et al., 2013).

The use of small sample sizes of extant and fossil species in the early studies led to simplified but ultimately inaccurate phylogenetic reconstructions. Enamel thickness increases from gorilla to chimpanzee to orangutan to humans, the latter with the relatively thickest enamel among these extant hominoids. Phenotypic expression of relatively and/or absolutely "thick," "thin," or "intermediate" enamel were then used in taxonomic identification (reviewed in Smith et al., 2012). Arguments over the earliest member of the hominid clade included debates over thick/hominid-like versus thin/chimpanzee-like enamel (Leakey et al., 1995). As sample sizes increased so did our understanding of ranges of variation (e.g., Smith et al., 2012), but even today the misleading elegance of such dichotomous phenotypic simplicity continues to be employed (e.g., Horvath et al., 2014; Zanolli, 2014).

An important element in the assessment of enamel thickness is the need to standardize across a range of body sizes. As such, enamel thickness data are presented in two ways: (1) actual enamel thickness (AET) defined as the average distance between the enamel–dentin junction and the outer enamel surface across the section; and (2) relative enamel thickness (RET) which is AET scaled by the size of the dentin cap (see Fig. 2). These two different ratios yield fairly different patterns of variation. For example, AET data indicate that gorillas have thicker enamel than do chimpanzees, but RET results show the opposite (Olejniczak et al., 2008, and references therein).

When the genetic architecture of population-level enamel thickness variation is considered, RET is a very different phenotype than what anatomists had originally intended (e.g., Martin, 1985). A decade ago, my colleagues and I estimated the additive genetic contribution to the phenotypic variance in molar enamel thickness (AET, but measured a little bit differently) within a pedigreed population of baboons, i.e., heritability (Hlusko et al., 2004a). Our estimation of the heritability of enamel thickness variation indicates that within this one captive population of baboons, 32–44% of the phenotypic variance is due to the additive effects of genes, with essentially no contribution from covariates. This means that the genes influencing population level variation in AET are independent of the genes that influence tooth size and/or sex. From this, we modeled theoretical response to selection (following Lande, 1976). We estimated that enamel thickness could theoretically double in 250,000 years with a culling of fewer than 4 individuals in 10,000 each generation, a fairly low selective pressure (Hlusko et al., 2004a: 231). Equally important to a consideration of enamel thickness is our quantitative genetic analysis of tooth size. We found that ~20% of the variation in molar buccolingual width is due to the same genes that influence crown-rump length in this population of baboons – tooth size and body size are influenced by overlapping genetic effects, or incomplete pleiotropy (Hlusko et al., 2006). And, whereas buccolingual width and mesiodistal length have a genetic correlation close to 0.50 (Hlusko et al., 2011), this suite of genes does not include the genes that also influence body size.

Returning to RET versus AET data, scaling enamel thickness with a phenotype that genetically correlates with body size – and thus sexual dimorphism–creates a composite phenotype that does not represent enamel thickness variation as selection operates on it. Given that sexual dimorphism is remarkably greater in gorillas than in chimpanzees (100% and 30%, respectively; Nowak, 1991: 503, 506), RET data characterize a biological phenomenon that is quite distinct from enamel thickness. For extinct taxa with varying degrees of sexual dimorphism that are still debated (e.g., Reno et al., 2010), the use of a composite phenotype is even more problematical.

This example shows that quantitative genetics can help to define phenotypes that represent the evolutionary biology we intend to capture. Such elucidation enables us to move past muddled, uncertain interpretations and onto the next phase of the scientific endeavor. For example, Smith et al. (2012) report on the most extensive survey of enamel thickness variation within the genus *Homo*. Their main conclusion is that Neanderthals had markedly greater dentin areas and as such, thinner enamel compared to *H. sapiens*. The authors cautiously state that it is "tempting" to interpret absolutely and relatively thin enamel as a derived trait in Neanderthals (Smith et al., 2012: 400). In light of our quantitative genetic characterization of enamel thickness, I would argue that the interplay between the two histological tissues and their differing pleiotropic effects (or lack thereof) is much more interesting than relatively thinner enamel in Neanderthals. I elaborate on this in Section 3.3.

At other times, interesting results are overlooked when the genetic architecture is not considered. For example, Kato et al. (2014) assessed intra- and interspecific variation in macaque molar enamel thickness – a genus with a wide geographic range that exploits a diversity of habitats that the authors highlight as analogous to hominids. Enamel thickness measurements for 386 molars representing six species yielded no sexual dimorphism in AET or RET. Although it is not clear how large their sexed-sample is for each species, this result stands out as particularly interesting. RET in baboons is sexually dimorphic because it is non-dimorphic AET scaled with the sexually dimorphic trait of body size. If the pattern observed in macaques is bolstered by expanded samples, the genetic architecture of dentin and body size must differ between macaques and baboons – despite the fact that macaques are also sexually dimorphic. Macaque males are approximately 50% larger than females in overall body size (Nowak, 1991: 471). A further exploration of sexual dimorphism in macaques may reveal important biological differences from other OWMs, furthering our understanding of the biology of primate sexual dimorphism.

As morphological studies of enamel thickness can be elevated in terms of accuracy and applicability by a consideration of the underlying quantitative genetic architecture, so too can genomic analyses. It is impossible to discuss the genetics of enamel without mentioning *Amelogenesis Imperfecta*, a phenotype of hypomineralized tooth enamel that results from a variety of genetic mutations, about half of which are unknown and the other half mapped to the genes *AMEL*, *ENAM*, *FAM83H*, *WDR72*, *KLK4*, and *MMP20*, some of which are X-linked and others autosomal (Seow, 2014). Degradation of the *enamelin* (*ENAM*) gene has been shown to underlie the loss of enamel in four orders of placental mammals that have enamel-less taxa (Meredith et al., 2009), demonstrating that the pathology associated with dysfunction of *ENAM* in humans also

underlies the convergent loss of enamel multiple times in evolution.

Enamelin (ENAM) is involved in the formation and elongation of enamel crystallites (Moradian-Oldak, 2012), and has been found to associate with variation in enamel thickness and quality in primates (Kelley and Swanson, 2008). Horvath et al. (2014) more recently searched for positive selection across hominoids in four genes involved in enamel formation (*AMELX*, *AMBN*, *ENAM*, and *MMP20*). They found no evidence of selection in the protein-coding regions but did find evidence of selection in flanking regions of *MMP20* and *ENAM* in humans and *MMP20* in chimpanzees, suggesting that there has been selection on the regulatory regions enabling rapid evolutionary changes in enamel thickness for these two taxa. In discussing the results, the authors note complicating factors, such as not knowing why, or how, or even if tooth size and enamel thickness are correlated. "*A full understanding of the complex relationship between tooth size and enamel thickness requires more work to determine the appropriate way to measure enamel thickness, particularly when the goal is to integrate genomic, developmental, and phenotypic data across multiple species*." (Horvath et al., 2014: 83). And while they cited our quantitative genetic analysis, it is unfortunate that they did not explore their interpretations in light of our actual results.

For example, let's consider the candidate genes *MMP20* and *ENAM* in more detail. *MMP20 (Matrix metalloproteinase 20)*, along with *KLK4 (kallikrein 4)* produce proteases that remove the proteins produced by the ameloblasts from the enamel matrix, clearing space for the apatite crystals to form (Bartlett, 2013; Zhu et al., 2014). *KLK4* is secreted by the ameloblasts during the final stage of development and serves as a protease to remove partially hydrolyzed matrix proteins from the enamel so that the rod and interred crystallites can grow and ultimately fill in the space. *MMP20* is present in trace amounts during the preceding secretory stage, when ameloblasts form tall columnar cells with Tomes' processes at their apical tips. Although its function is unclear, evidence indicates that it is a tooth-specific protease because enamel is the only affected phenotype in mouse and human mutants (Bartlett, 2013). Additionally, the only mammals with a non-functional MMP20 sequence also lack enamel (Meredith et al., 2011). In contrast to this tooth-specific protease, *ENAM* and the other extracellular matrix proteins are obviously important in enamel development but also have functional significance in bone development and may fine-tune the biochemical make-up of mineralization in other parts of the skeleton such as the calvarium, where it is expressed slightly at mouse postnatal day 3 (but it is interesting to note that other extracellular matrix proteins AMEL, AMTN and ODAM are expressed much more than is *ENAM*, and for as many as 35 days postnatal in the mouse; Atsawasuwan et al., 2013).

Following on this, *MMP20* may be the more likely candidate for influencing the narrowly defined enamel thickness phenotype, and perhaps demonstrates a parallel molecular pathway through which chimpanzees and humans differentially adapted from their last common ancestor. On the other hand, while *ENAM* frameshift mutations correlate with enamel-less placental mammals (Meredith et al., 2009), this and other enamel-related genes may be interesting candidates for the human-derived cranial expansion associated with our large brains, because its flanking regions reveal evidence of selection only in the highly encephalized human in the study by Horvath et al. (2014).

To date, all of the genetic evidence on enamel thickness variation bolsters our original interpretation from quantitative genetics (Hlusko et al., 2004a), that enamel thickness variation is influenced through relatively simple genetic effects. Pampush et al. (2013) found that enamel thickness has a positive association with lifetime dietary wear across 17 primate species and may have evolved to resist wear as well as fracture. Given the high degree of wear on many hominid fossil dentitions, this may well have been the strong selective force to which a fairly simple genetic architecture could readily respond. Additionally, enamel development may be another example of just how significantly chimpanzees have diverged from last common ancestor with humans (e.g., White et al., 2009, 2015; Hughes et al., 2010). Since our original publication (Hlusko et al., 2004a,b), various studies of enamel thickness have since tentatively reached the same conclusions. However, by not considering the genetic architecture revealed by our work, these authors' interpretations focused on results that further bolster ours. As a consequence, they overlooked particularly interesting results that can take the investigation of this phenotype to the next level.

3.3. *Taurodont molars of the Neanderthals*

Professional scientists and the public alike have long been fascinated by the Neanderthals since they were discovered over 150 years ago, and because they lived so recently alongside our own species (Trinkaus and Shipman, 1993). Our last mtDNA common ancestor with Neanderthals was perhaps as far back as 600,000 years ago (Krings et al., 1997). The now-extinct lineage was subsequently episodically isolated on peninsular Europe over the course of three glacials. Despite the possibility that some Neanderthals and modern humans may have intermingled their DNA on occasion (Sankararaman et al., 2012), Neanderthals evolved a distinct set of morphological traits thought to be the result of cold adaptation and genetic drift (Weaver, 2009).

Among the numerous dental features that distinguish Neanderthals from modern humans are molars with root stems that extend apically prior to root bifurcation, more so than is seen in other hominids (Gorjanović-Kramberger, 1907, 1908), a condition referred to as taurodontism (Keith, 1913) (Fig. 2). Paleoanthropologists have long pondered whether or not this feature is related to genetic drift or natural selection. Two recent studies explored the latter. Kupczik and Hublin (2010) report that the Neanderthal larger root-to-crown and dentin volumes relative to the Pleistocene or recent *H. sapiens* anatomy may be evidence of distinct occlusal loading. But a more recent finite element analysis explored various loads and strains to digital models of taurodont molars, but found no biomechanical differences between taurodont and non-taurodont molars, ruling out one possible source of selective advantage (Benazzi et al., 2014).

In the discussion of molar enamel thickness (Section 3.2), I noted that quantitative genetic analyses of baboon dental variation revealed genetic independence between enamel thickness and crown size (Hlusko et al., 2004a). In contrast, molar buccolingual width and body size share pleiotropic effects (crown-rump length, Hlusko et al., 2006). Because enamel thickness is not genetically correlated with body size, a logical but as-yet untested assumption is that the dentin and pulp cavity are so correlated. This genetic architecture provides a hypothesis about taurodontism in Neanderthals.

Silvent et al. (2013) report on 55 motifs within the *Dentin matrix acidic phosphoprotein 1 protein* (DMP1) that have been conserved for 220 million years of mammalian evolution. Large mutations in (or loss of) DMP1 results in rickets, osteomalacia, and dentin defects because it is expressed in dentin mineralization (George et al., 1993) and in osteoblasts (MacDougall et al., 1998; Kamiya and Takagi, 2001). DMP1 is part of the small integrin-binding ligand N-linked glycoprotein (SIBLING) family of proteins (Staines et al., 2012), one of the non-collagenous proteins involved in forming the organic component of bone. Three of the five SIBLING proteins are expressed in bone and dentin, DMP1, BSP, and DSPP. Over-expression of DMP1 results in narrow growth plates with

accelerated mineralization and increased bone turnover (see references in Staines et al., 2012).

Among the Neanderthals' derived postcranial features are long bones with notably thick cortical bone and a generally more robust skeleton (Weaver, 2009). Perhaps the derived dentin and root shape of Neanderthal postcanine teeth are due to pleiotropic effects of selection favoring skeletal robusticity through one or more of these three proteins within the SIBLING family. This is an easily testable hypothesis that may have been neglected due to the lack of inclusion of the phenomic perspective.

We know that Neanderthal growth differed from that of modern humans. For example, tooth mineralization indicates that one Neanderthal juvenile individual had been alive for eight years but had an eruption pattern of 10–11 years of age according to the human standard (which may have contributed to the relatively thinner enamel; Smith et al., 2007). Brain growth also appears to have been accelerated in Neanderthal infants (Ponce de León et al., 2008). This accelerated mineralization of the teeth and faster brain growth rate early in infancy may be causally related to energetic conservation more systemically due to thermal stress (Mateos et al., 2014). From 1 to 6 years of age, Neanderthal children are interpreted to have been smaller and to have had slower growth rates than do modern humans of the same age, and as such, Neanderthal children are estimated to have had slightly lower basal and energy costs (Mateos et al., 2014). It will be fascinating to explore the degree to which such metabolic shifts and their concomitant effects were genetically influenced and/or environmentally induced.

4. Conclusions

The morphological variation preserved by the fossil record is essentially the only source of knowledge we have about what extinct animals looked like, where and when they lived, what they were doing, and who they were doing it with. Ancient DNA has yielded some insights into phenotypic evolution (Kirsanow and Burger, 2012), such as the sequencing of the *MC1R* gene from Neanderthal genome that indicated that this individual was likely fair-skinned and red-headed (Lalueza-Fox et al., 2007). But until we fully grasp the relationship between genotype and phenotype, fossils and the skeletal anatomy that they preserve are the primary data that can inform us about the who-what-when-where-and-why of a lineage's evolutionary history. In order to advance our understanding of human evolution, we need to improve our ability to infer evolution from skeletal variation.

Given the tremendous advances in developmental genetics, genomics, GWAS, and other related disciplines, myriad genetic insights have been gained into bone biology. However, these top-down and bottom-up approaches tend to leave anatomists with little that they can actually apply in their research. We are in dire need of a middle ground approach. Quantitative genetics provides this critical link.

The three phenotypes discussed here are not independent of each other, and a consideration of them simultaneously shows just how pervasive their interconnectedness is. To add yet another example, Smith et al. (2012) report that Neanderthals have relatively larger dentin caps, but this feature is restricted to the postcanine dentition and is not seen in the incisors – all three of the phenotypes essentially wrapped into one phenotypic observation. This demonstrates that no one definition of a dental phenotype is appropriate for all research questions.

However, there is no need to throw our hands in the air in exasperation and return to the assumptions of independence, as has been done previously (e.g., within cladistics, Strait et al., 1997). The black-box of development no longer has to be a stumbling block. New analytical tools within the realm of quantitative genetics can elucidate the genetic architecture of phenotypic variation – the population level anatomical variation that lies at the heart of evolution by natural selection.

Conflicts of interest

None.

Acknowledgments

I would like to express my deep gratitude to M. Mahaney, my collaborator on all of the quantitative genetic analyses of baboon dentition variation. Numerous other colleagues and students have contributed significantly to my understanding of the dentition, and I would especially like to acknowledge: T. Grieco, A. Lainoff, J. Moustakas, O. Rizk, G. Suwa, and T. White. M. Huffman, C. Schmitt, T. White, and two anonymous reviewers provided helpful comments on earlier versions of this manuscript. My research reported herein was supported by the US National Science Foundation BCS Award nos. 0500179, 0130277, and 0616308, and two Research Experience for Undergraduates supplements. National Institutes of Health, National Center for Research Resources P51 RR013986 supports the Southwest National Primate Research Center.

References

Almasy, L., Blangero, J., 1998. Multipoint quantitative-trait linkage analysis in general pedigrees. Am. J. Hum. Genet. 62, 1198–1211.
Alvesalo, L., Nuutilla, M., Portin, P., 1975. The cusp of Carabelli: occurrence in first upper molars and evaluation of its heritability. Acta Odont. Scand. 33, 191–197.
Alvesalo, L., Tigerstedt, M.A., 1974. Heritabilities of human tooth dimensions. Hereditas 77, 311–318.
Apps, M., Hughes, T., Townsend, G.C., 2004. The effect of birthweight on tooth size variability in twins. Twin Res. 7, 415–420.
Atsawasuwan, P., Lu, X., Ito, Y., Chen, Y., Gopinathan, G., Evans, C.A., Kulkarni, A.B., Gibson, C.W., Luan, X., Diekwisch, T.G.H., 2013. Expression and function of enamel-related gene products in calvarial development. J. Dent. Res. 92, 622–628.
Bachrach, F.H., Young, M.A., 1927. A comparison of the degree of resemblance in dental characters shown in pairs of twins of identical fraternal types. Br. Dent. J. 21, 1293–1304.
Bartlett, J.D., 2013. Dental enamel development: proteinases and their enamel matrix substrates. ISRN Dent. 2013, Article ID 684607.
Benazzi, S., Nguyen, H.N., Kullmer, O., Hublin, J.-J., 2014. Exploring the biomechanics of taurodontism. J. Anat., http://dx.doi.org/10.1111/joa.12260 (Epub ahead of print).
Blanco, R., Chakraborty, R., 1976. The genetics of shovel shape in maxillary central incisors in man. Am. J. Phys. Anthropol. 44, 233–236.
Bockmann, M.R., Hughes, T.E., Townsend, G.C., 2010. Genetic modeling of primary tooth emergence: a study of Australian twins. Twin Res. Hum. Genet. 13, 573–581.
Burleigh, J.G., Alphonse, K., Alverson, A.J., Bik, H.M., Blank, C., Cirranello, A.L., Cui, H., Daly, M., Dietterich, T.G., Gasparich, G., Irvine, J., Julius, M., Kaufman, S., Law, E., Liu, J., Moore, L., O'Leary, M.A., Passarotti, M., Ranade, S., Simmons, N.B., Stevenson, D.W., Thacker, R.W., Theriot, R.W., Todorovic, S., Velazco, P.K., Walls, R.L., Wolfe, J.M., Yu, M., 2013. Next-generation phenomics for the Tree of Life. PLoS Curr., June 26; 5.
Butler, P.M., 1939. Studies of the mammalian dentition, differentiation of the postcanine dentition. Proc. Zool. Soc. Lond. B 109, 1–36.
Cheverud, J.M., 1988. A comparison of genetic and phenotypic correlations. Evolution 42, 958–968.
Cheverud, J.M., 1996. Developmental integration and the evolution of pleiotropy. Am. Zool. 36, 44–50.
Cheverud, J.M., Routman, E.J., Irschick, D.K., 1997. Pleiotropic effects of individual gene loci on mandibular morphology. Evolution 51, 2004–2014.
Dahlberg, A.A., 1945. The changing dentition of man. J. Am. Dent. Assoc. 32, 676–690.
Dempsey, P.J., Townsend, G.C., Martin, N.G., Neale, M.C., 1995. Genetic covariance structure of incisor crown size in twins. J. Dent. Res. 74, 1389–1398.
Ehrich, T.H., Vaughn, T.T., Koreishi, S.F., Linsey, R.B., Pletscher, L.S., Cheverud, J.M., 2003. Pleiotropic effects on mandibular morphology I. Developmental morphological integration and differential dominance. J. Exp. Zool. B: Mol. Dev. Evol. 296, 58–79.
Eizirik, E., Murphy, W.J., O'Brien, S.J., 2001. Molecular dating and biogeography of the early placental mammal radiation. J. Hered. 92, 212–219.
Falconer, D.S., 1981. Introduction to quantitative genetics. Longmans, London.
Félix, M.-A., 2012. Evolution in developmental phenotype space. Curr. Opin. Genet. Dev. 22, 593–599.

Garn, S.M., Lewis, A.B., Kerewsky, R.S., Dahlberg, A.A., 1966. Genetic independence of Carabelli's trait from tooth size or crown morphology. Arch. Oral Biol. 11, 745–747.

Garn, S.M., Lewis, A.B., Swindler, D.R., Kerewsky, R.S., 1967. Genetic control of sexual dimorphism in tooth size. J. Dent. Res. 46, 963–972.

Garn, S.M., Osborne, R.H., Alvesalo, L., Horowitz, S.L., 1980. Maternal and gestational influences on deciduous and permanent tooth size. J. Dent. Res. 49, 142–143.

Geller, F., Feenstra, B., Zhang, H., Shaffer, J.R., Hansen, T., Esserlind, A.L., Boyd, H.A., Nohr, E.A., Timpson, N.J., Fatemifar, G., Paternoster, L., Evans, D.M., Weyant, R.J., Levy, S.M., Lathrop, M., Smith, G.D., Murray, J.C., Olesen, J., Werge, T., Marazita, M.L., Sørensen, T.I., Melbye, M., 2011. Genome-wide association study identifies four loci associated with eruption of permanent teeth. PLoS Genet. 7, e1002275.

George, A., Sabsay, B., Simonian, P.A., Veis, A., 1993. Characterization of a novel dentin matrix acidic phosphoprotein. Implications for induction of biomineralization. J. Biol. Chem. 268, 12624–12630.

Gorjanović-Kramberger, D., 1907. Die Kronen und Wurzeln der Mahlz€ahne primigenius und ihre genetische Bedeutung. Anat. Anz. 31, 97–138.

Gorjanović-Kramberger, D., 1908. Über prismatische Molarwurzeln rezent er und diluvialer Menschen. Anat. Anz. 32, 401–413.

Grieco, T.M., Rizk, O.T., Hlusko, L.J., 2013. A modular framework characterizes micro- and macroevolution of Old World monkey dentitions. Evolution 67, 241–259.

Haga, S., Nakaoka, H., Yamaguchi, T., Yamamoto, K., Yi, K., Samoto, H., Ohno, T., Katayama, K., Ishida, H., Park, S.B., Kimura, R., Maki, K., Inoue, I., 2013. A genome-wide association study of third molar agenesis in Japanese and Korean populations. J. Hum. Genet. 58, 799–803.

Hlusko, L.J., 2004. Perspective: integrating the genotype and phenotype in hominid paleontology. Proc. Natl. Acad. Sci. U. S. A. 101, 2653–2657.

Hlusko, L.J., Mahaney, M.C., 2003. Genetic contributions to expression of the baboon cingular remnant. Arch. Oral Biol. 48, 663–672.

Hlusko, L.J., Mahaney, M.C., 2007. A multivariate comparison of dental variation in wild and captive populations of baboons (*Papio hamadryas*). Arch. Oral Biol. 52, 195–200.

Hlusko, L.J., Mahaney, M.C., 2009. Quantitative genetics, pleiotropy, and morphological integration in the dentition of *Papio hamadryas*. Evol. Biol. 36, 5–18.

Hlusko, L.J., Suwa, G., Kono, R., Mahaney, M.C., 2004a. Genetics and the evolution of primate enamel thickness: a baboon model. Am. J. Phys. Anthropol. 124, 223–233.

Hlusko, L.J., Maas, M.L., Mahaney, M.C., 2004b. Statistical genetics of molar cusp patterning in pedigreed baboons: implications for primate dental development and evolution. J. Exp. Zool. B: Mol. Dev. Evol. 302, 268–283.

Hlusko, L.J., Lease, L.R., Mahaney, M.C., 2006. The evolution of genetically correlated traits: tooth size and body size in baboons. Am. J. Phys. Anthropol. 131, 420–427.

Hlusko, L.J., Do, N., Mahaney, M.C., 2007. Genetic correlations between mandibular molar cusp areas in baboons. Am. J. Phys. Anthropol. 132, 445–454.

Hlusko, L.J., Sage, R.D., Mahaney, M.C., 2011. Evolution of modularity in the mammalian dentition: mice and monkeys share a common dental genetic architecture. J. Exp. Zool. B: Mol. Dev. Evol. 316, 21–49.

Horowitz, S.L., Osborne, R.H., DeGeorge, F.V., 1958. Hereditary factors in tooth dimensions, a study of the anterior teeth of twins. Angle Orthod. 28, 87–93.

Horvath, J.E., Ramachandran, G.L., Fedrigo, O., Nielsen, W.J., Babbitt, C.C., St Clair, E.M., Pfefferle, L.W., Jernvall, J., Wray, G.A., Wall, C.E., 2014. Genetic comparisons yield insight into the evolution of enamel thickness during human evolution. J. Hum. Evol. 73, 75–87.

Houle, D., Govindaraju, D.R., Omholt, S., 2010. Phenomics: the next challenge. Nat. Rev. Genet. 11, 855–866.

Hughes, T.E., Bockman, M.R., Gotjamanos, T., Gully, N., Richards, L.C., Townsend, G.C., 2007. Strong genetic control of emergence of human primary incisors. J. Dent. Res. 86, 1160–1165.

Hughes, J.F., Skaletsky, H., Pyntikova, T., Graves, T.A., van Daalen, S.K., Minx, P.J., Fulton, R.S., McGrath, S.D., Locke, D.P., Friedman, C., Trask, B.J., Mardis, E.R., Warren, W.C., Repping, S., Rozen, S., Wilson, R.K., Page, D.C., 2010. Chimpanzee and human Y chromosomes are remarkably divergent in structure and gene content. Nature 463, 536–539.

Hughes, T.E., Seow, K., Bockmann, M., Mihaildis, S., Lekkas, D., Ranjitkar, S., Pinkerton, S., Brook, A., Townsend, G.C., 2014. The teeth and faces of twins: providing insights into dento-facial development and oral health for practicing oral health professionals. Aust. Dent. J. 59 (Suppl 1), 101–116.

Huxley, J.S., De Beer, G.R., 1934. The Elements of Experimental Embryology. The University Press, Cambridge.

Jernvall, J., Thesleff, I., 2000. Reiterative signaling and patterning during mammalian tooth morphogenesis. Mech. Dev. 92, 19–29.

Jheon, A.H., Seidel, K., Biehs, B., Klein, O.D., 2013. From molecules to mastication: the development and evolution of teeth. WIREs Dev. Biol. 2, 165–182.

Kamiya, N., Takagi, M., 2001. Differential expression of dentin matrix protein 1, type I collagen and osteocalcin genes in rat developing mandibular bone. Histochem. J. 33, 545–552.

Kato, A., Tang, N., Borries, C., Papakyrikos, A.M., Hinjde, K., Miller, E., Kunimatsu, Y., Hirasaki, E., Shimizu, D., Smith, T.M., 2014. Intra- and interspecific variation in macaque molar enamel thickness. Am. J. Phys. Anthropol. 155, 447–459.

Keith, A., 1913. Problems relating to the teeth of the earlier forms of prehistorical man. Proc. R. Soc. Med. 6, 103–119.

Kelley, J.L., Swanson, W.J., 2008. Dietary change and adaptive evolution of enamelin in humans and among primates. Genetics 178, 1595–1603.

Kirsanow, K., Burger, J., 2012. Ancient human DNA. Ann. Anat. 194, 121–132.

Klingenberg, C.P., Leamy, L.J., Routman, E.J., Cheverud, J.M., 2001. Genetic architecture of mandible shape in mice: effects of quantitative trait loci analyzed by geometric morphometrics. Genetics 157, 785–802.

Klingenberg, C.P., 2008. Morphological integration and development modularity. Ann. Rev. Ecol. Evol. Syst. 39, 115–132.

Koh, C., Bates, E., Broughton, E., Do, N.T., Fletcher, Z., Mahaney, M.C., Hlusko, L.J., 2010. Genetic integration of molar cusp size variation in baboons. Am. J. Phys. Anthropol. 142, 246–260.

Kono, R.T., 2004. Molar enamel thickness and distribution patterns in extant great apes and humans: new insights based on a 3-dimensional whole crown perspective. Anthropol. Sci. 112, 121–146.

Krings, M., Stone, A., Schmitz, R.W., Krainitzki, H., Stoneking, M., Pääbo, S., 1997. Neanderthal DNA sequences and the origin of modern humans. Cell 90, 19–30.

Kupczik, K., Hublin, J.-J., 2010. Manibular molar root morphology in Neanderthals and Late Pleistocene and recent Homo sapiens. J. Hum. Evol. 59, 525–541.

Lalueza-Fox, C., Römpler, H., Caramelli, D., Stäubert, C., Catalano, G., Hughes, D., Rohland, N., Pilli, E., Longo, L., Condemi, S., de la Rasilla, M., Fortea, J., Rosas, A., Stoneking, M., Schöneberg, T., Bertranpetit, J., Hofreiter, M., 2007. A melanocortin 1 receptor allele suggests varying pigmentation among Neanderthals. Science 318, 1453–1455.

Lande, R., 1976. Natural selection and random genetic drift in phenotypic evolution. Evolution 30, 314–334.

Lande, R., 1979. Quantitative genetic analysis of multivariate evolution, applied to brain: body size allometry. Evolution 33, 402–416.

Lande, R., 1980. Genetic variation and phenotypic evolution during allopatric speciation. Am. Nat. 116, 463–479.

Leakey, M.G., Feibel, C.S., McDougall, I., Walker, A., 1995. New four-million-year-old hominid species from Kanapoi and Allia Bay, Kenya. Nature 376, 565–571.

Leamy, L., Routman, E., Cheverud, J.M., 1999. Quantitative trait loci for early and late developing skull characters in mice: a test of the genetic independence model of morphological integration. Am. Nat. 153, 201–214.

Lesot, H., Hovorakova, M., Peterka, M., Peterkova, R., 2014. Three-dimensional analysis of molar development in the mouse from the cap to bell stage. Aust. Dent. J. 59 (Suppl 1), 81–100.

Ludwig, F.J., 1957. The mandibular second premolars: morphological variation and inheritance. J. Dent. Res. 36, 263–273.

Lundström, A., 1948. Tooth size and occlusion in twins. Doctoral dissertation. University of Uppsala, Stockholm.

MacDougall, M., Gu, T.T., Luan, X., Simmons, D., Chen, J., 1998. Identification of a novel isoform of mouse dentin matrix protein 1: spatial expression in mineralized tissues. J. Bone Miner. Res. 13, 422–431.

Macho, G.A., 1994. Variation in enamel thickness and cusp area within human maxillary molars and its bearing on scaling techniques used for studies of enamel thickness between species. Arch. Oral Biol. 39, 783–792.

Marroig, G., Cheverud, J.M., 2005. Size as a line of least evolutionary resistance: diet and adaptive morphological radiation in New World monkeys. Evolution 59, 1128–1142.

Martin, L.B., 1985. Significance of enamel thickness in hominoid evolution. Nature 314, 260–263.

Martinón-Torres, M., Bermúdez de Castro, J.M., Gómez-Robles, A., Arsuaga, J.L., Carbonell, E., Lordkipanidze, D., Manzi, G., Margvelashvili, A., 2007. Dental evidence on the hominin dispersals during the Pleistocene. Proc. Natl. Acad. Sci. U. S. A. 104, 13279–13282.

Mateos, A., Goikoetxea, I., Leonard, W.R., Martín-González, J.A., Rodríguez-Gómez, G., Rodríguez, J., 2014. Neanderthal growth: What are the costs? J. Hum. Evol. in press.

Meredith, R.W., Gatesy, J., Murphy, W.J., Ryder, O.A., Springer, M.S., 2009. Molecular decay of the tooth gene enamelin (ENAM) mirrors the loss of enamel in the fossil record of placental mammals. PLoS Genet. 5 (9), e1000634.

Meredith, R.W., Gatesy, J., Cheng, J., Springer, M.S., 2011. Pseudogenization of the tooth gene enamelysin (MMP20) in the common ancestor of extant baleen whales. Proc. Biol. Sci. 278, 993–1002.

Mezey, J., Cheverud, J.M., Wagner, G., 2000. Is the genotype-phenotype map modular? A statistical approach using mouse QTL data. Genetics 156, 305–311.

Mizoguchi, Y., 1977. Genetic variability in tooth crown characters: analysis by the tetrachoric correlation method. Bull. Natl. Sci. Mus. D 3, 37–62.

Moore, G.R., Hughes, B.O., 1942. Familial factors in diagnosis, treatment, and prognosis of dentofacial disturbances. Am. J. Orthod. Oral Surg. 28, 603–639.

Moradian-Oldak, J., 2012. Protein-mediated enamel mineralization. Front. Biosci. 17, 1996–2023.

Nichol, C.R., (Doctoral dissertation) 1990. Dental Genetics and Biological Relationships of the Pima Indians of Arizona. Arizona State University, Tempe.

Niswander, J.D., Chin, S.C., 1965. The effects of inbreeding on tooth size in Japanese children. Am. J. Hum. Genet. 17, 390–398.

Nowak, R.M., 1991. Walker's Mammals of the World, 5th edition, Volume I. Johns Hopkins University Press, Baltimore.

Olejniczak, A.J., Tafforeau, P., Feeney, R.N.M., Martin, L.B., 2008. Three-dimensional primate molar enamel thickness. J. Hum. Evol. 54, 187–195.

Olson, E.C., Miller, R.L., 1958. Morphological Integration. University of Chicago Press, Chicago.

Osborn, J.W., 1978. Morphogenetic gradients: fields vs clones. In: Butler, P.M., Joysey, K.A. (Eds.), Development, Function and Evolution of Teeth. Academic Press, New York, pp. 171–201.

Paaby, A.B., Rockman, M.V., 2013. The many faces of pleiotropy. Trends Genet. 29, 66–73.

Pampush, J.D., Duque, A.C., Burrows, B.R., Daegling, D.J., Kenney, W.F., McGraw, W.S., 2013. Homoplasy and thick enamel in primates. J. Hum. Evol. 64, 216–224.
Park, P.J., 2009. ChIP-seq: advantages and challenges of a maturing technology. Nat. Rev. Genet. 10, 669–680.
Peterkova, R., Hovorakova, M., Peterkova, M., Lesot, H., 2014. Three-dimensional analysis of the early development of the dentition. Aust. Dent. J. 59, 55–80.
Pillas, D., Hoggart, C.J., Evans, D.M., O'Reilly, P.F., Sipilä, K., Lähdesmäki, R., Millwood, I.Y., Kaakinen, M., Netuveli, G., Blane, D., Charoen, P., Sovio, U., Pouta, A., Freimer, N., Hartikainen, A.L., Laitinen, J., Vaara, S., Glaser, B., Crawford, P., Timpson, N.J., Ring, S.M., Deng, G., Zhang, W., McCarthy, M.I., Deloukas, P., Peltonen, L., Elliott, P., Coin, L.J., Smith, G.D., Jarvelin, M.R., 2010. Genome-wide association study reveals multiple loci associated with primary tooth development during infancy. PLoS Genet. 6, e1000856.
Ponce de León, M.S., Golovanova, L., Doronichev, V., Romanova, G., Akazawa, T., Kondo, O., Ishida, H., Zollikofer, C.P., 2008. Neanderthal brain size at birth provides insights into the evolution of human life history. Proc. Natl. Acad. Sci. U. S. A. 105, 13764–13768.
Portin, P., Alvesalo, L., 1974. The inheritance of shovel shape in maxillary central incisors. Am. J. Phys. Anthropol. 41, 59–62.
Reno, P.L., McCollum, M.A., Meindl, R.S., Lovejoy, C.O., 2010. An enlarged postcranial sample confirms *Australopithecus afarensis* dimorphism was similar to modern humans. Philos. Trans. R. Soc. Lond. B: Biol. Sci. 365, 3355–3363.
Ribeiro, D.C., Brook, A.H., Hughes, T.E., Sampson, W.J., Townsend, G.C., 2013a. Intrauterine hormone effects on tooth dimensions. J. Dent. Res. 92, 425–431.
Ribeiro, M.M., de Andrade, S.C., de Souza, A.P., Line, S.R.P., 2013b. The role of modularity in the evolution of primate postcanine dental formula: integrating jaw space with patterns of dentition. Anat. Rec. 296, 622–629.
Rizk, O.T., Amugongo, S., Mahaney, M.C., Hlusko, L.J., 2008. The quantitative genetic analysis of primate dental variation: History of the approach and prospects for the future. In: Irish, J.D., Nelson, G.C. (Eds.), Technique and Application in Dental Anthropology. Cambridge University Press, Cambridge, pp. 317–348.
Rose, K.D., Archibald, J.D., 2005. The Rise of Placental Mammals: Origins and Relationships of the Major Extant Clades. Johns Hopkins University Press, Baltimore.
Salazar-Ciudad, I., 2012. Tooth patterning and evolution. Curr. Opin. Genet. Dev. 22, 585–592.
Salazar-Ciudad, I., Jernvall, J., 2007. The economy of tinkering mammalian teeth. Novart. Found. Symp. 284, 207–216.
Salazar-Ciudad, I., Jernvall, J., 2010. A computational model of teeth and the developmental origins of morphological variation. Nature 464, 583–586.
Sankararaman, S., Patterson, N., Li, H., Pääbo, S., Reich, D., 2012. The date of interbreeding between Neanderthals and modern humans. PLoS Genet. 8, e1002947.
Schluter, D., 1996. Adaptive radiation along genetic lines of least resistance. Evolution 50, 1766–1774.
Schluter, D., 2000. The Ecology of Adaptive Radiation. Oxford University Press, New York.
Schork, N.J., 1997. Genetics of complex disease: approaches, problems, and solutions. Am. J. Respir. Crit. Care Med. 156, S103–S109.
Schwartz, G.T., 2000. Taxonomic and functional aspects of the patterning of enamel thickness distribution in extant large-bodied hominoids. Am. J. Phys. Anthropol. 111, 221–244.
Scott, G.R., 1980. Population variation of Carabelli's trait. Hum. Biol. 52, 63–78.
Seow, W.K., 2014. Developmental defects of enamel and dentine: challenges for basic science research and clinical management. Aust. Dent. J. 59, 143–154.
Silvent, J., Sire, J.-Y., Delgado, S., 2013. The Dentin Matrix Phosphoprotein 1 (DMP1) in the light of mammalian evolution. J. Mol. Evol. 76, 59–70.
Sirianni, J.E., Swindler, D.R., 1973. Inheritance of deciduous tooth size in *Macaca nemestrina*. J. Dent. Res. 52, 179.
Sirianni, J.E., Swindler, D.R.,1974. Tooth size inheritance in Macaca nemestrina. In: Symposium Volume of the Fifth International Congress of Primatology. S. Krager, Basel, pp. 12–19.
Skrinjaric, M., Slaj, M., Lapter, V., Muretic, Z., 1985. Heritability of Carabelli's trait in twins. Colleg. Anthropol. 9, 177–181.
Smith, T.M., Olejniczak, A.J., Martin, L.B., Reid, D.J., 2005. Variation in hominoid molar enamel thickness. J. Hum. Evol. 48, 575–592.
Smith, T.M., Toussaint, M., Reid, D.J., Olejniczak, A.J., Hublin, J.-J., 2007. Rapid dental development in a Middle Paleolithic Belgian Neanderthal. Proc. Natl. Acad. Sci. U. S. A. 104, 20220–20225.
Smith, T.M., Olejniczak, A.J., Zermeno, J.P., Tafforeau, P., Skinner, M.M., Hoffmann, A., Radovčić, J., Toussaint, M., Kruszynski, R., Menter, C., Moggi-Cecchi, J., Glasmacher, U.A., Kullmer, O., Schrenk, F., Stringer, C., Hublin, J.-J., 2012. Variation in enamel thickness within the genus *Homo*. J. Hum. Evol. 62, 395–411.
Soulé, M., 1967. Phenetics of natural populations I. Phonetic relationships of insular populations of the side-blotched lizard. Evolution 21, 584–591.
Staines, K.A., MacRae, V.E., Farquharson, C., 2012. The importance of the SIBLING family of proteins on skeletal mineralization and bone remodeling. J. Endocrinol. 214, 241–255.
Stock, D.W., 2001. The genetic basis of modularity in the development and evolution of the vertebrate dentition. Philos. Trans. R. Soc. Lond. B: Biol. Sci. 356, 1633–1653.
Strait, D.S., Grine, F.E., Moniz, M.A., 1997. A reappraisal of early hominid phylogeny. J. Hum. Evol. 32, 17–82.
Suwa, G., Kono, R.T., 2005. A micro-CT based study of linear enamel thickness in the mesial cusp section of human molars: reevaluation of methodology and assessment of within-tooth, serial, and individual variation. Anthropol. Sci. 113, 273–289.
Thesleff, I., 2014. Current understanding of the process of tooth formation: transfer from the laboratory to the clinic. Aust. Dent. J. 59, 48–54.
Townsend, G.C., Brown, T., 1978a. Inheritance of tooth size in Australian Aboriginals. Am. J. Phys. Anthropol. 48, 305–314.
Townsend, G.C., Brown, T., 1978b. Heritability of permanent tooth size. Am. J. Phys. Anthropol. 49, 497–504.
Townsend, G.C., 1980. Heritability of deciduous tooth size in Australian Aboriginals. Am. J. Phys. Anthropol. 53, 297–300.
Townsend, G.C., Richards, L.C., Brown, T., Burgess, V.B., Travan, G.R., Rogers, J.R., 1992. Genetic studies of dental morphology in South Australian twins. In: Smith, P., Tchernov, E. (Eds.), Structure, function and evolution. Freund Publishing House, London, pp. 501–518.
Townsend, G.C., Hughes, R.L., Pinkerton, S., Schwerdt, W., 2005. Epigenetic influences may explain dental differences in monozygotic twin pairs. Aust. Dent. J. 50, 95–100.
Townsend, G.C., Harris, E.F., Lesot, H., Clauss, F., Brook, A., 2009a. Morphogenetic fields within the human dentition: a new, clinically relevant synthesis of an old concept. Arch. Oral Biol. 54 (Suppl. 1), S34–S44.
Townsend, G.C., Hughes, T., Luciano, M., Bockman, M., Brook, A., 2009b. Genetic and environmental influences on human dental variation: a critical evaluation of studies involving twins. Arch. Oral Biol. 54S, 45–51.
Trinkaus, E., Shipman, P., 1993. The Neanderthals. Alfred A. Knopf, New York.
Turner, C.G.I.I., 1967. Dental genetics and microevolution in prehistoric and living Koniag Eskimo. J. Dent. Res. 46, 911–917.
Ungar, P.S., 2011. Dental evidence for the diets of Plio-Pleistocene hominins. Am. J. Phys. Anthropol. Suppl. 53, 47–62.
Van Dijk, E.L., Auger, H., Jaszczyszyn, Y., Thermes, C., 2014. Ten years of next-generation sequencing technology. Trends Genet. 30, 418–426.
Wagner, G.P., Altenberg, L., 1996. Complex adaptations and the evolution of evolvability. Evolution 50, 967–976.
Wang, Z., Liao, B.-Y., Zhang, J., 2010. Genomic patterns of pleiotropy and the evolution of complexity. Proc. Natl. Acad. Sci. U. S. A. 107, 18034–18039.
Weaver, T.D., 2009. The meaning of Neanderthal skeletal morphology. Proc. Natl. Acad. Sci. U. S. A. 106, 16028–16033.
White, T.D., Asfaw, B., Beyene, Y., Haile-Selassie, Y., Lovejoy, C.O., Suwa, G., WoldeGabriel, G., 2009. Ardipithecus ramidus and the paleobiology of early hominids. Science 326, 75–86.
White, T.D., Lovejoy, C.O., Asfaw, B., Carlson, J.P., Suwa, G., 2015. Neither chimpanzee nor human, *Ardipithecus* reveals the surprising ancestry of both. Proc. Natl. Acad. Sci. U. S. A. 112, 4877–4884.
Yong, R., Ranjitkar, S., Townsend, G.C., Smith, R.N., Evans, A.R., Hughes, T.E., Lekkas, D., Brook, A.H., 2014. Dental phenomics: advancing genotype to phenotype correlations in craniofacial research. Aust. Dent. J. 59, 39–47.
Zanolli, C., 2014. Molar crown inner structural organization in Javanese Homo erectus. Am. J. Phys. Anthropol., http://dx.doi.org/10.1002/ajpa.22611 (Epub ahead of print).
Zhu, L., Haichuan, L., Witkowska, E., Huang, Y., Tanimoto, K., Li, W., 2014. Preferential and selective degradation and removal of amelogenin adsorbed on hydroxyapatites by MMP20 and KLK4 *in vitro*. Front. Physiol. 5, 268.