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Genetics and the Evolution of Primate Enamel Thickness: A Baboon Model

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ABSTRACT The thickness of mammalian tooth enamel plays a prominent role in paleontology because it correlates with diet, and thicker enamel protects against tooth breakage and wear. Hominid evolutionary studies have stressed the importance of this character for over 30 years, from the identification of "*Ramapithecus*" as an early Miocene hominid, to the recent discovery that the earliest hominids display molar enamel intermediate in thickness between extant chimpanzees and *Australopithecus*. Enamel thickness remains largely unexplored for nonhominoid primate fossils, though there is significant variation across modern species. Despite the importance of enamel thickness variation to primate evolution, the mechanisms underlying variation in this trait have not yet been elucidated. We report here on the first quantitative genetic analysis of primate enamel thickness, an analysis based on 506 pedigreed baboons from a captive breeding colony. Computed tomography analysis of 44 *Papio* mandibular molars shows a zone of sufficiently uniform enamel thickness on the lateral surface of the protoconid. With this knowledge, we developed a caliper metric measurement protocol for use on baboon molars worn to within this zone, enabling the collection of a data set large enough for genetic analyses. Quantitative genetic analyses show that a significant portion of the phenotypic variance in enamel thickness is due to the additive effects of genes and is independent of sex and tooth size. Our models predict that enamel thickness could rapidly track dietary adaptive shifts through geological time, thus increasing the potential for homoplasy in this character. These results have implications for analyses of hominoid enamel thickness variation, and provide a foundation from which to explore the evolution of this phenotype in the papionin fossil record. Am J Phys Anthropol 124:223–233, 2004.

Variation in enamel thickness is widely used in the study of mammalian evolution and adaptation, ranging from Rodentia (Flynn et al., 1987; Grayson et al., 1990; Meng and Wyss, 1994) to Proboscidea (Beden, 1980; Crochet et al., 1996). Primates are no exception, though research on primate molar enamel thickness has focused primarily on anthropoids (Dumont, 1995; Gantt, 1977; Kay, 1981; Molnar and Gantt, 1977; Shellis et al., 1998; Ulhaas et al., 1999), and particularly on hominoids (e.g., Andrews and Martin, 1991; Beynon and Wood, 1986; Grine and Martin, 1988; Kono, 2002; Macho and Berner, 1993, 1994; Martin, 1985; Schwartz, 2000a,b; Schwartz et al., 1998). In anthropology, enamel thickness variation in primates has widely informed interpretative studies of function and morphology, diet and adaptation, and phylogeny (e.g., Jolly, 1970; Kay, 1981; Martin, 1985; Simons and Pilbeam, 1972; Strait et al., 1997; Szalay, 1972; Wood, 1992, 1995). Historically, enamel thickness played a prominent role in the rise and reconsideration of "*Ramapithecus*" as a hominid (Simons and Pilbeam, 1972). Today, enamel thickness remains an important character in functional and phylogenetic assessments of the earliest hominids, *Ardipithecus ramidus*, *Australopithecus anamensis*, *Orrorin tugenensis*, and *Sahelanthropus tchadensis* (Andrews, 1995; Brunet et al., 2002; Leakey et al., 1995; Senut et al., 2001; White et al., 1994). Very few studies have investigated enamel thickness in the nonhominoid primate fossil record (Benefit, 1987).

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Systematic quantification of molar enamel thickness in anthropoid primates was first attempted by Gantt (1977) and Molnar and Gantt (1977), followed by Martin (1983, 1985), who focused on hominoids. They sectioned cercopithecoid and hominoid molars through the mesial cusps, and measured thickness and areas on these sections. Since then, similar methodologies based on the destructive sectioning of teeth have been applied on ape and human material (e.g., Beynon et al., 1998; Grine and Martin, 1988; Macho and Berner, 1993; Schwartz, 2000b; Shellis et al., 1998), and on some cercopithecid and other primate molars (Dumont, 1995; Shellis et al., 1998; Ulhaas et al., 1999). Other methods of investigating enamel thickness include measuring thickness on worn molars (Benefit, 1987; Kay, 1981), on natural fractures (Beynon and Wood, 1986; Suwa et al., 1996; White et al., 1994), on radiographs (e.g., Grine et al., 2001; Harris et al., 2001; Molnar et al., 1993; Sperber, 1985; Zilberman and Smith, 1992), by three-dimensional digitizing coupled with enamel decalcification (Kono et al., 2002; Kono-Takeuchi et al., 1997), by ultrasonic pulse-echo (Huysmans and Thijssen, 2000), by initial attempts with computed tomography (CT) scanning (Conroy, 1991; Grine, 1991; Macho and Thackeray, 1992; Zonneveld and Wind, 1985), and by use of more accurate CT-based techniques (Kono, 2002; Schwartz et al., 1998; Spoor et al., 1993).

These and other studies (cited below) suggest a complex evolutionary pattern for enamel thickness, with a possible dietary component of intertaxon variation superimposed on allometric trends (Gantt, 1977; Kay, 1981; Shellis et al., 1998). Thin enamel is considered to be advantageous in maintaining sharp enamel crests that are supposedly good for shearing (e.g., Kay, 1981), although such actual functional effects remain to be demonstrated with hominoid molars that lack well-defined phase 1 wear facets. Thick enamel is likely an adaptation to resist abrasive wear (Molnar and Gantt, 1977; Teaford et al., 1996) or to withstand higher masticatory loads (Spears and Crompton, 1997; Spears and Macho, 1995), although the effectiveness of comparatively thicker enamel in the latter may not be significant (Macho and Spears, 1999). Though enamel thickness is generally thought to be responsive to both of these selective forces (Kay, 1981; Macho, 1995; Shellis et al., 1998; Teaford and Ungar, 2000), there is no clear understanding as to the relative importance of the two. The details of enamel thickness patterning are only beginning to be documented from a three-dimensional whole-crown perspective, and available data suggest the additional existence of a morphogenetic patterning of thickness not directly related to masticatory load but perhaps with internal cusp topography (Kono et al., 2002). Empirically, molar enamel thickness does appear to correlate with diet. Thin enamel is associated with softer foodstuffs, and thicker enamel correlates with hard-object feeding (Benefit, 1987; Dumont, 1995; Gantt, 1977; Kay, 1981; Ulhaas et al., 1999).

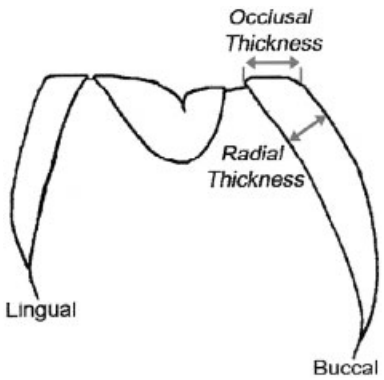

Fig. 1. Diagram of cross section through mesial loph of a worn baboon molar, demonstrating difference between occlusal and radial enamel thickness.

Despite the salience of enamel thickness in modern primate and hominoid evolutionary studies, no attempts to elucidate the genetic and environmental contributions to its variance have been made. The purpose of the present paper is to determine the relative contributions of selected factors underlying population-level variation in the trait of molar enamel thickness. The relative contributions of tooth size, genetic effects, and nongenetic effects to enamel thickness variation were estimated in captive, pedigreed baboons from the Southwest Foundation for Biomedical Research, the first such estimate in any mammal.

In order to assess the quantitative genetics of molar enamel thickness, both large sample sizes ($>$300) and accurate measures of thickness are needed. The exact quantification of enamel thickness based on sectioned molars, by necessity, has been conducted on small sample sizes due to the destructive procedure. Ideally, high-resolution CT scans of molar teeth of individuals from model populations are needed, but this is not possible at present. We therefore took the alternative of developing a sufficiently accurate and reliable externally based method of measuring molar enamel thickness in baboons.

Some earlier studies of broad taxonomic variation were based on nondestructive, externally measured estimates of enamel thickness on worn occlusal surfaces (Benefit, 1987; Kay, 1981). Such an approach necessitates a consideration of either external crown and/or enamel-dentin junction (EDJ) surface orientations. Figure 1 demonstrates how taking a width measurement of an obliquely oriented object will consistently yield imprecise overestimates. Kay (1981) attempted a measurement of enamel thickness tangential to the external surface. However, his method has not been evaluated in terms of the effects of tooth wear, configuration of the external and internal enamel surfaces, and/or measurement error.

In the present paper, we first report on a protocol for measuring radial enamel thickness on the lateral crown faces of *Papio* mandibular molars, employing a knowledge of internal tooth anatomy gained from industrial computed tomography (CT) scans made through a series of modern *Papio* molars. We demonstrate the presence of a zone of relatively uniform enamel thickness on the most buccal aspect of the baboon protoconid as seen in CT scans, which enable a sufficiently accurate measure of thickness on a range of worn molars. In hominoid molars, enamel thickness of the crown faces are less consistent, so that the same method is not generally applicable. We then measured radial enamel thickness of the buccal protoconid face on adequately worn molars of a large sample of captive, pedigreed baboons, to document lateral enamel thickness in a large sample of baboon molars. These data provide the foundation for the first-ever quantitative genetic analysis of this phenotype.

MATERIALS

CT study sample

The CT study sample consisted of 44 molars from 31 individuals of *Papio hamadryas* spp. (14 males, 11 females, 3 juveniles, and 3 of unknown sex). The mandibular specimens used for this study were from the Cleveland Museum of Natural History and the University of California at Berkeley's Museum of Vertebrate Zoology. CT scans of 34 unworn or slightly worn mandibular molars were used for determination of length and location of the region of uniform thickness. CT scans of all 44 molars were used to assess enamel thickness variation within the sample. Antimeres were not included.

Quantitative genetic analysis sample

The Southwest Foundation for Biomedical Research (SFBR) houses the world's largest captive breeding colony of baboons (>3,000), maintained in pedigrees (with all mating opportunities controlled). This pedigree structure, coupled with genetic marker maps for ~1,000 of the animals, make this colony unique and important for the quantitative genetic analysis of normal phenotypic variation in primates (Rogers et al., 2000).

Using the enamel thickness measurement protocol outlined herein, data were obtained from high-resolution plaster dental casts of 506 pedigreed baboons (*Papio hamadryas*), with a female-to-male sex ratio approximating 2:1, and ranging in age from 4.6–30 years. All procedures related to the treatment of the baboons during this study were approved by the Institutional Animal Care and Use Committee in accordance with the established guidelines (National Research Council, 1996), and are outlined in detail elsewhere (Hlusko et al., 2002).

All pedigree data management and preparation were accomplished using the computer package PEDSYS (Dyke, 1996). The 506 animals from which data were obtained were distributed across 11 extended pedigrees. The mean number of animals with data per pedigree was 44, with these animals occupying the lower two or three generations of each pedigree. Genetic management of the colony, begun over 20 years ago, allows for data collection from noninbred animals. All nonfounder animals in this study were the result of matings that were random with respect to dental, skeletal, and developmental phenotype.

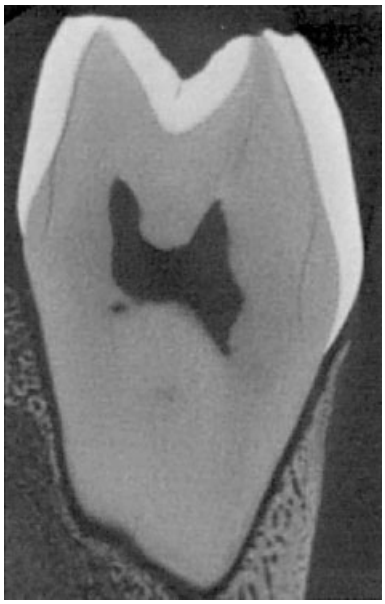

Fig. 2. CT scan through mesial loph of a baboon mandibular molar used for assessment of ZUET. Buccal is to the right. See text for details.

METHODS

CT study and definition of ZUET

The mesial portion of each mandibular molar from the CT study sample was CT-scanned using a microfocal X-ray industrial CT scanner (model TX225-Actis, Tesco) at the University Museum, University of Tokyo. Slice thickness was set at 50 μm, and images were reconstructed in a 512 × 512 matrix with a pixel size of 50 μm. Pixel size was calibrated to an accuracy of ca. 0.1% by measuring an aluminum rod of known diameter (9.996 mm). Each tooth was set so that multiple CT slices were taken parallel to a plane that bisects the "cone-like" buccal protoconid face. From the series of sections, the one that passed through the protoconid apex was chosen for measurement. This section usually passes close to, but not strictly through, the metaconid apex (Fig. 2).

Because of the high resolution of our micro-CT system, the CT value profile of the tissue interface was steep, enabling accurate measurement of enamel thickness. Measurement endpoints were defined to subpixel resolution by means of the half-maximum method (Schwartz et al., 1998; Spoor et

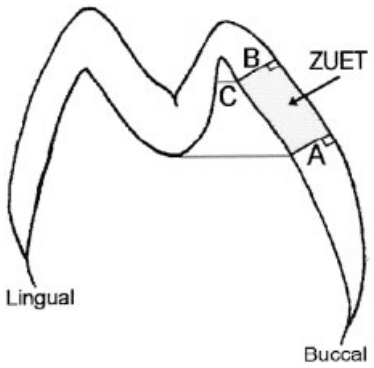

Fig. 3. Diagram of cross section through mesial loph of an unworn baboon molar, showing measurements used in assessment of the ZUET.

al., 1993), with independent threshold values extracted each for the air-enamel and enamel-dentine interfaces.

Visual inspection of the CT scans of the 34 unworn or only slightly worn molars shows an extensive region of the enamel on the buccal surface of the protoconid to be fairly uniform in thickness. These CT scans were then measured to determine and quantitatively evaluate this zone of uniform enamel thickness (ZUET).

The landmark for the inferior boundary of the uniform region was defined as a point in line with the most inferior aspect of the enamel-dentine junction (EDJ) of the intercuspal ridge on the CT section. On CT scans, a line was drawn from the EDJ of the intercuspal ridge to the buccal surface of the protoconid. This line was oriented approximately parallel to the occlusal surface of the tooth crown (Fig. 3). Though the ZUET generally extends inferiorly beyond this landmark, this is the most appropriate inferior landmark, because it is readily observable without the aid of CT scanning or removing the molar from its alveolus.

Enamel thickness was measured superiorly to the projected intercuspal ridge-EDJ line (Fig. 3, distance A) by taking 4–10 (depending on overall crown size) evenly spaced (~0.20 mm) measurements. Each thickness measurement approximated a line perpendicular to the buccal slope of the crown, thereby estimating radial enamel thickness, rather than occlusal-view enamel thickness.

Generally for papionin molars, enamel is thinner in the superiormost region of the protoconid. When thickness measurements exceeded the projected intercuspal ridge-EDJ line (Fig. 3, distance A) measure by ±0.10 mm, the uniform region was deemed to have ended (Fig. 3, distance B). For these 34 molars, the average number of measurements taken for each tooth was 6.5, and the average standard deviation 0.033 (range, 0.008–0.057). The mean differences between the superiormost vs. middle measurement and the middle versus inferiormost measurement are −0.002 mm and 0.05 mm, respectively. The latter difference is statistically significant in a paired *t*-test ($P < 0.01$). These results demonstrate that the zone is more uniform than the 0.1mm restriction applied, although a very slight decrease of thickness appears to occur toward the lower half of ZUET (see below for an evaluation of this effect). As shown below, these small but significant differences are within the range of measurement error for the caliper metric protocol. Therefore, they are beyond our current level of measurement precision, and do not hinder the analysis of enamel thickness in this study.

In order to estimate where the enamel starts to thin and the region of uniform thickness ends, the buccolingual width of the dentine was measured at the most superior point of the region of uniform thickness (Fig. 3, distance C). This measurement translates into how much of the dentine needs to be exposed in order to ensure that the region of uniform thickness has been reached by tooth wear. The dentine exposure was then conservatively translated into well-established wear stages, so that only teeth apparently worn to the ZUET level would be included in the analysis. Individuals from the population with unusual molar wear were not included in the study.

Caliper test methods

The caliper protocol was then developed and tested, using casts of the mandibular molars of 46 baboons from the SFBR sample. Adjustable-jawed calipers (Model NTD10-6″C, Mitutoyo) were used for the collection of all caliper-measured enamel thickness data used in these analyses.

This protocol is designed to measure enamel thickness on the buccal surface of the protoconid of worn *Papio* molars. Given the information from the CT scans, the boundaries of ZUET were determined. As shown in Figure 1, radial enamel thickness will return a more accurate assessment of protoconid enamel thickness than will occlusally measured enamel thickness. Therefore, when measuring the buccalmost aspect of the protoconid enamel, the calipers need to be oriented so that one of the jaws aligns with the buccal surface of the protoconid (Fig. 4). The other jaw will align with the enamel-dentine junction. The most difficult and most important aspect of this protocol is the orientation of the calipers. Each measurement was taken three times, with 24 hours passing between each measurement.

Quantitative genetic analytical methods

Quantitative genetic analyses were performed on the second molar data of the pedigreed sample population, as these were the largest data sets. Statistical genetic analyses were conducted by means of a maximum likelihood-based variance decomposition approach implemented in the computer package SOLAR (Almasy and Blangero, 1998). In these analyses, phenotypic variance

$$(\sigma_P^2)$$

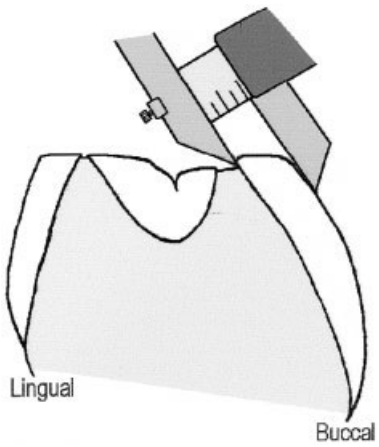

Fig. 4. Diagram of cross section through mesial loph of a worn baboon molar, showing orientation of adjustable-jawed calipers used for measurement of the ZUET.

is partitioned into components corresponding to the additive genetic

$$(\sigma_G^2)$$

and environmental (i.e., nonadditive genetic)

$$(\sigma_E^2)$$

effects. Because these components are additive, such that

$$\sigma_P^2=\sigma_G^2+\sigma_E^2,$$

we estimated the heritability, or proportion of phenotypic variance attributable to additive genetic effects, as

$$h^2=\frac{\sigma_G^2}{\sigma_P^2}.$$

Phenotypic variance attributable to nongenetic factors is estimated as $e^2 = 1 - h^2$. Additionally, we estimated the mean effects of sex, age, mesiodistal length, and mesial buccolingual width of the crown on the enamel thickness value recorded for each molar studied. Protocols for collecting length and width data are described in detail in Hlusko et al. (2002).

Significance of the maximum likelihood estimates for heritability and other parameters was assessed by means of likelihood ratio tests. Twice the difference of the maximum likelihoods of a general model (in which all parameters were estimated) and a restricted model (in which the value of a parameter to be tested was held constant at some value, usually zero) were compared. This difference is distributed asymptotically approximately as either a ½:½ mixture of χ^2 and a point mass at zero, for tests of parameters such as h^2 for which a value of zero in a restricted model is at a boundary of the parameter space, or as a χ^2 variate, for tests of covariates for which zero is not a boundary value (Hopper and Mathews, 1982). In both cases, degrees of freedom are equal to the difference in the number of estimated parameters in the two models (Boehnke et al., 1987). However, in tests of parameters such as h^2 whose values may be fixed at a boundary of their parameter space in the null model, the appropriate significance level is obtained by halving the *P*-value (Boehnke et al., 1987).

RESULTS

Establishing the measurement protocol

In the CT study, we found that the length of ZUET differs between first, second, and third molars. ZUET is approximately 0.78 mm in length for first molars (M_1s), 1.44 mm for second molars (M_2s), and 1.91 mm for third molars (M_3s). See Table 1. The average dentine exposure (Fig. 3, distance C) for M_1s is 0.77 mm, 0.95 mm for M_2s, and 0.98 mm for M_3s.

The values for the three molar positions are different because of the size differences between first, second, and third molars. However, all of these measurements translate into the same wear stage, e.g., stage 4 of Benefit (1987). Therefore, buccal protoconid radial enamel thickness measured on molars exposing this much or more dentine is expected to be within ZUET. Meanwhile, in relatively worn molars, enamel at the intercuspal ridge must be preserved in order to ensure that the buccal surface of the protoconid is still within the region of uniform thickness. Consequently, molars that can be used with this protocol must roughly fall within wear stages 4 and 7 as defined by Benefit (1987, 1993), and wear stages as scored from 3–6 by Delson (1973), including grade C and part of D.

Using these criteria for the caliper metric, we found that the error between repeated measurements of dental casts was between 2.9–5.1%, with an average of 3.98% (Table 2).

In order to assess the accuracy of the caliper method, we compared CT scan data with caliper data. Most of the molars used for the CT scan study were unworn or only slightly worn, and therefore enamel thickness could not be measured with calipers. There were five molars CT-scanned that were worn enough to measure with calipers. The discrepancy between caliper and CT-derived enamel thickness data ranged to approximately 0.13 mm (Table 3). Since the caliper method tends to overestimate fairly consistently, the precision of the caliper method is deemed reliable. The agreement between the caliper data and the CT scan data is not problematic so long as only one type of data collection method is employed for comparative enamel thickness studies.

Enamel thickness results

The distribution of mandibular molar enamel thickness values in the large pedigreed sample of baboons demonstrates that considerable population-level variation is present in this population. Standard coefficients of variation (CVs) for enamel thickness center around 10. As noted by Polly (1998), CVs

TABLE 1. Length and location of zone of uniform enamel thickness[1]

Tooth	N	Length of uniform region	Min	Max	St dev	Dentine exposure	Min	Max	St dev
M1	7	0.78	0.47	1.33	0.31	0.77	0.64	0.9	0.11
M2	9	1.44	0.93	2.18	0.36	0.95	0.28	1.53	0.37
M3	18	1.91	1.17	2.85	0.55	0.98	0.4	1.87	0.37

[1] M, molar; 1, 2, 3, position in molar row; St dev, standard deviation; Min, minimum measurement; Max, maximum measurement.

TABLE 2. Measurement error test[1]

	RM1	RM2	RM3	LM1	LM2	LM3
n	22	38	10	23	36	9
Avg measurement	1.18	1.34	1.39	1.22	1.37	1.39
Absolute avg error	0.06	0.05	0.05	0.06	0.05	0.04
% error	5.1	3.7	3.6	4.9	3.7	2.9

[1] R, right; L, left; M, molar; 1, 2, 3, position in molar row; Avg, average; % error, calculated as absolute average error divided by average measurement; measurements are in millimeters.

can be biased by measurement error when the trait is small, such as enamel thickness. Corrected CVs were also calculated as

$$CV_{corr}=100\left[\frac{\sqrt{V_{all}-V_{me}}}{X}\right]$$

(modified from Polly, 1998), where V_{all} is the variance for the trait, V_{me} is the variance of the measurement error, and X is the mean of the trait in this population. The corrected CVs range between 7.10–10.05.

Another approach suggested for comparing relative variance between traits of different magnitudes is to regress the standard deviation against the mean (Polly, 1998). However, since the effect of a constant measurement error to the standard deviation is not linear, the appropriate regression would be variance against the square of the mean. The intercept would then represent the error variance, and the slope the square of the error-free CV. When the three enamel thickness measures of the combined sex sample are examined in this way, we estimate measurement error to be 0.09 and error-free CV to be 6.8.

There is no consistent pattern of sexual dimorphism in absolute enamel thickness, but enamel thicknesses scaled with linear size measurements are systematically dimorphic, with females having relatively thicker enamel than males (Table 4). This dimorphism is reduced when the square root of the two-dimensional occlusal view area of the protoconid is used as a scaling factor (Table 4).

Our results also show that baboon molar enamel thickness has significant metameric variation. Enamel increases in absolute thickness in the more distal molars (Table 5). But when enamel thickness is scaled with linear measures of molar crown size, the metameric pattern is reversed such that first molars have relatively thicker enamel than second molars, and the pattern between second and third molars is not consistent (Table 5). The CT study also demonstrates this same metameric relationship in absolute enamel thickness (Table 6), though none of the differences between the means in these small CT samples are significantly different ($P > 0.05$).

Quantitative genetic results

Quantitative genetic analyses show that absolute enamel thickness for second mandibular molars is heritable. A significant proportion of the phenotypic variance in this trait is attributable to the additive effects of genes: for RM_2,

$$h_r^2=0.44,$$

and LM_2,

$$h_r^2=0.32$$

(Table 7). With the exception of a significant mean effect of age on RM_2 ($P = 0.046$), none of the tested covariates, including tooth length and width ($P > 0.10$), contributed significantly to the likelihoods of the genetic models for enamel thickness of the second mandibular molars in these baboons. And, in the case of RM_2, the contribution of age to the variance in enamel thickness of this tooth was small (approximately 4%).

DISCUSSION

This is the largest assessment of enamel thickness variation in primates to date, and the first quantitative genetic analysis of this trait in any mammal. Our results show that mandibular molar enamel thickness does vary in this population. Depending on the method used to assess relative variance, enamel thickness in the pedigreed population either exceeds what would be expected or falls within the range of variation seen for other dental linear dimensions (Plavcan, 1993). We found that the source of this observed enamel thickness variation has a large genetic component.

Our results show that absolute enamel thickness for second mandibular molars is heritable. Though the proportion of the phenotypic variance attributable to the effects of genes (i.e., the heritability estimate) may vary across populations due to population-specific factors that affect the genetic or environmental components to the variance, the fact of a significant genetic contribution is consistent (Wang et al., 2001). Thus the detection and characterization of a significant genetic component for enamel thickness variation are of considerably more interest than the actual heritability point estimates themselves.

TABLE 3. Comparison of caliper and CT scan enamel thickness measurements of same specimen[1]

Specimen number	Tooth	Caliper measurement	CT measurement	Difference
CMNH B1368	M1	1.23	1.107	0.123
MVZ 122416	M2	1.24	1.117	0.124
MVZ 154147	M2	1.14	1.119	0.021
MVZ 106562	M2	1.35	1.213	0.137
MVZ 154145	M2	1.3	1.330	−0.030

[1] M, molar; 1, 2, 3, position in molar row; measurements are in millimeters.

Due to the nature of tooth development and mineralization, age in these models acts as a proxy for wear, indicating that wear does affect our caliper protocol to a small extent for RM_2. In our polygenic models, age is included as a parameter for RM_2 only. No covariates were found to be significant for LM_2. This is consistent with our evaluation of minor directional differences in enamel thickness within ZUET. However, the covariate effect for RM_2 is small and only nominally significant. We consider the contribution of age (or wear) to the total phenotypic variance for this tooth to be modest.

Similarly, when the additive effects of genes on variation in enamel thickness are accounted for, neither the effects of molar crown size (crown length and width) nor of sex on enamel thickness are detectable in this sample of pedigreed baboons. While our results suggest the independence of absolute enamel thickness from crown length and width in these pedigrees, additional studies will be needed to confirm this independence and to determine its generalizability to other populations and species. If these initial indications are borne out by subsequent study, then we would conclude that different genes or suites of genes (and perhaps environmental factors) influence the development of enamel thickness and overall tooth size.

Although absolute enamel thickness is not systematically sexually dimorphic, estimates of relative enamel thickness based on linear measurements of crown size are highly dimorphic, with females having relatively thicker enamel than males (Table 4). This is because sex plays a significant role in contributing to phenotypic variance of molar crown size in this baboon population (Hlusko et al., 2002).

We also found that significant metameric variation exists in enamel thickness in this pedigreed baboon sample. The pattern of this variation depends on the use and type of scaling factor employed. Coupling the metameric variation with the sexual dimorphism of scaled enamel thickness, our results suggest that pooling data from different molar positions and unsexed individuals might confound intraspecific variation with taxonomic-level variation in systematic and evolutionary studies, as cautioned previously (Macho and Berner, 1993).

One way to mitigate the potential complications outlined above would be to use a scaling measure that is not influenced by sex and/or position. An ideal scaling measure would be one that is associated isometrically with enamel thickness, either

TABLE 4. Absolute and scaled enamel thickness data for left first, second, and third molars from pedigreed sample[1]

Measure	N	Mean	St Dv	CV	Corrected CV
Males and females					
M1	195	1.21	0.123	10.17	9.42
M2	327	1.34	0.128	9.55	8.91
M3	104	1.37	0.131	9.56	8.95
M1/MD length	158	0.117	0.012	10.26	
M2/MD length	287	0.109	0.012	11.01	
M3/MD length	67	0.090	0.012	13.33	
M1/BL width	154	0.170	0.018	10.59	
M2/BL width	284	0.148	0.016	10.81	
M3/BL width	90	0.142	0.017	11.97	
M1/Tri length	117	0.218	0.022	10.09	
M2/Tri length	205	0.202	0.022	10.89	
M3/Tri length	53	0.196	0.024	12.24	
M1/√ProtArea	115	0.325	0.036	11.08	
M2/√ProtArea	214	0.315	0.032	10.16	
M3/√ProtArea	46	0.320	0.034	10.63	
Females					
M1	172	1.21	0.123	10.17	9.42
M2**	242	1.33	0.127	9.55	8.89
M3	64	1.36	0.144	10.59	10.03
M1/MD length*	139	0.118	0.012	10.17	
M2/MD length*	212	0.112	0.012	10.71	
M3/MD length*	42	0.093	0.012	12.90	
M1/BL width*	137	0.172	0.018	10.47	
M2/BL width*	211	0.150	0.016	10.67	
M3/BL width*	56	0.146	0.018	12.33	
M1/Tri length**	102	0.219	0.022	10.05	
M2/Tri length*	151	0.206	0.022	10.68	
M3/Tri length*	32	0.203	0.024	11.82	
M1/√ProtArea	100	0.328	0.037	11.28	
M2/√ProtArea**	164	0.318	0.032	10.06	
M3/√ProtArea	33	0.322	0.036	11.18	
Males					
M1	23	1.23	0.132	10.73	10.05
M2**	85	1.37	0.129	9.42	8.79
M3	40	1.39	0.109	7.84	7.10
M1/MD length*	19	0.109	0.010	9.17	
M2/MD length*	75	0.103	0.009	8.74	
M3/MD length*	25	0.085	0.010	11.76	
M1/BL width**	17	0.157	0.016	10.19	
M2/BL width*	73	0.141	0.014	9.93	
M3/BL width*	34	0.134	0.012	8.96	
M1/Tri length**	15	0.206	0.016	7.77	
M2/Tri length*	54	0.192	0.018	9.37	
M3/Tri length*	21	0.185	0.020	10.81	
M1/√ProtArea	15	0.309	0.030	9.71	
M2/√ProtArea**	50	0.306	0.029	9.48	
M3/√ProtArea	13	0.313	0.027	8.63	

[1] Right-side data have same relationships. Top third of table includes males and females. All measurements reported in millimeters.

*Male and female differences significant at $P < 0.01$, with variances assumed equal.

**Male and female differences significant at $P < 0.05$, with variances assumed equal. CV, coefficient of variation; M, mandibular molar; 1, 2, 3, position in tooth row; MD, mesiodistal length; BL, buccolingual width; Tri, trigonid mesiodistal length; √ProtArea, square root of two-dimensional area of the occlusal view of the protoconid.

TABLE 5. Metameric variation in absolute and relative enamel thickness measurements from pedigreed population[1]

	No. of pairs	Significance	%difference between means	Direction of effect
ET				
M1 vs. M2	163	0.00	10–11	<
M2 vs. M3	84	0.00	5–6	<
ET/√P				
M1 vs. M2	97	0.03	n.s.	n.s.
M2 vs. M3	41	0.00	4–5	<
ET/MD				
M1 vs. M2	130	0.00	5	>
M2 vs. M3	52	0.00	15–17	>
ET/BL				
M1 vs. M2	125	0.00	12–13	>
M2 vs. M3	69	0.41	n.s.	n.s.
ET/Tri				
M1 vs. M2	97	0.00	6–7	>
M2 vs. M3	40	0.66	n.s.	n.s.

[1] ET, enamel thickness; MD, mesiodistal length; BL, buccolingual width; Tri, trigonid mesiodistal length; √P, square root of two-dimensional occlusal view area of protoconid; M, mandibular molar; 1, 2, 3, position in tooth row.

morphogenetically or by functional constraints. We find that absolute enamel thickness scaled with the square root of the two-dimensional occlusal view area of the protoconid is only weakly sexually dimorphic, and exhibits few to no metameric differences in this pedigreed baboon population. Of all of the scaling methods used here, this is the most appropriate to use in mixed-sex and/or mixed-position samples of baboons. However, we note that this is an empirical observation without a known underlying basis, so its applicability to other taxa remains to be investigated.

Narrow-sense heritability estimates (the estimates of the proportion of the phenotypic variance due to the additive effects of genes) have implications for finding genes for traits such as enamel thickness. Statistical power to detect and localize quantitative trait loci (QTLs) influencing variation in enamel thickness, or any other quantitative trait, is largely a function of the QTL-specific heritability (i.e., the proportion of variance in the trait attributable to the effect of the QTL), for which the heritability estimate described in this report provides the upper bound. Demonstrating that enamel thickness variation is significantly heritable is a prerequisite to searching for the genes responsible for that heritable component. A whole-genome linkage map is available for this population of baboons (Rogers et al., 2000), and the majority of animals for which we have enamel thickness measures were genotyped at the marker loci that comprise this map. We are currently undertaking a whole-genome linkage screen (Rogers et al., 1999) for enamel thickness variation.

To date, both the genes that determine variation in enamel thickness and the mechanisms by which they do so are unknown. Enamel formation is a highly heterogeneous process involving proteins from at least six different genes (including amelogenin, enamelin, and ameloblastin) (Robinson et al., 1998). Enamel pathologies such as *amelogenesis imperfecta* (AI) demonstrate the important role these genes play in enamel mineralization (Robinson et al., 1998). Several studies provide suggestive evidence that enamel thickness may be determined in part by sex-linked genetic effects. For example, sex chromosome polysomy (having an additional X or Y chromosome) in humans results in thicker enamel than is seen in controls (Alvesalo et al., 1985, 1987, 1991), whereas X monosomy in humans results in thinner enamel (Alvesalo and Tammisalo, 1981; Townsend et al., 1984). These sex-linked effects were proposed to have had roles in the evolution of hominid enamel thickness (Wood, 1995), and there is some limited genetic evidence to support this (Fincham et al., 1991).

However, though some versions of AI are sex-linked, most enamel genetic defects exhibit patterns of inheritance consistent with the localization of major loci on autosomes (Bell et al., 2001; Clark and Clark, 1933; Hart et al., 1997; Wright et al., 1993, 1996). It is also unknown whether the proteins necessary for enamel mineralization also determine ultimate enamel thickness (Robinson et al., 1998). Evidence indicating that enamel thickness is not determined by the sex chromosomes is building, such as the autosomal nature of the many pathologies, the noninvolvement of sex as a covariate in the quantitative genetic analyses of variation in baboon enamel thickness, and the nondimorphic characteristic of nonpathological enamel thickness in humans (Alvesalo and Tammisalo, 1981; Harris et al., 2001). Enamel thickness may be determined to some degree by homeobox genes that control earlier morphogenesis. Lezot et al. (2000) found that *Dlx2* (a member of the *distalless* homeobox gene family) expression in the later stages of incisor development in mice is inversely related to enamel thickness. Gene expression studies such as these help identify genes involved in enamel formation. As described above, *Dlx2* and the enamel matrix proteins are considered to be candidates for determining variation in enamel thickness.

This review of the known genetics of enamel development provides a backdrop for our quantitative genetic analysis. It is widely acknowledged that the genetic mechanisms needed to produce an organ are not necessarily the same ones that determine its normal population-level variation. Because selection operates on populations, we need to understand the genetic and nongenetic factors that produce normal variation in order to reconstruct an integrated geno- and phenotypic evolutionary history (Jernvall, 2000).

Quantitative genetics is based on the principle that the degree to which relatives are similar phenotypically is related to the genetic variation they have in common (as opposed to random environmental effects; Falconer, 1989; Lynch and Walsh, 1998).

TABLE 6. Enamel thickness measured from CT scans[1]

	N	Avg thickness	Min	Max	St dev	Breadth	Min	Max	St dev	Avg thick/ breadth	Min	Max
M1	10	1.05	0.91	1.13	0.08	7.29	6.68	8.18	0.53	0.15	0.13	0.16
M2	15	1.18	0.78	1.33	0.13	9.38	7.11	10.78	0.95	0.13	0.11	0.15
M3	19	1.28	1.12	1.55	0.11	10.82	8.72	12.51	1.14	0.12	0.1	0.14

[1] M, molar; 1, 2, 3, indicates position in molar row; St dev, standard deviation; Min, minimum measurement; Max, maximum measurement; Avg, average of all enamel thickness measurements taken along ZUET; measurements are in millimeters.

TABLE 7. Heritability estimates for second molars[1]

	Right second molar	Left second molar
N	332	336
$h_r^2 \pm$ SE	0.438 ± 0.120	0.319 ± 0.158
P-value	< 0.0001	0.0098
e^2	0.562	0.681
Covariates	Age ($b = -0.05 \pm 0.016$ SE; $P = 0.046$)	None
Covariate var	0.04	None

[1] Covariate var, proportion of phenotypic variance due to covariates; SE, standard error; *b*, regression coefficient. These analyses included sex, age, mesiodistal length, mesial buccolingual width, and distal buccolingual width as potential covariates.

Quantitative genetic analyses therefore approach developmental questions from a direction opposite that of gene expression studies. We quantify adult phenotypic variation and model the underlying genetic and nongenetic effects statistically. Modern quantitative genetic analyses models estimate the proportions that different sources of variance contribute to the total phenotypic variance (Almasy and Blangero, 1998), including nongenetic and genetic factors (such as diet and/or different alleles), and genetic and nongenetic covariance with other phenotypes (such as tooth size or sex).

Modeling experiments show that the minor modification of relatively well-known developmental pathways can produce known morphological variation between taxa as diverse as mice and voles (Salazar-Cuidad and Jernvall, 2002), demonstrating that any advance in understanding the development of one organism greatly enhances our understanding of others, especially for taxa in the same order. Therefore, understanding the genetics and evolution of enamel thickness variation in baboons helps to clarify the evolution of enamel thickness in other primate taxa, including humans. Additionally, the mechanisms underlying population-level variation were demonstrated to be directly relevant to diversity at higher taxonomic levels (Jernvall, 2000; Shubin, 2002; Stern, 2000). One of our aims is to understand the relative contributions selected factors have on enamel thickness variation across the molar crown as well as the entire dentition. The quantitative genetic analysis of enamel thickness variation in baboons reported here represents a significant step towards achieving this larger goal. However, at this point in time it is premature to speculate on how the results presented here will relate to variation in other regions of the dentition, given that we still have a very limited understanding of the mechanisms that ultimately determine enamel thickness variation. Only recently have we started to gain a fuller understanding of enamel thickness variation in three dimensions, spanning the entire molar crown (Kono et al., 2002).

Heritability estimates may also provide insights into the sensitivity of such traits to selective pressures. Assuming continuity in the relative additive genetic contribution to the variance in a trait (i.e., the heritability) between diachronic populations, the potential responsiveness of that trait to selection (natural and artificial) can be modeled (Lande, 1976). Using the model of Lande (1976), the baboon population mean for M_2 enamel thickness could theoretically double (assuming $h^2 = 0.35$ and $\sigma = 0.144$ remain constant) in approximately 50,000 generations (or ~250,000 years if a generation is estimated to be 5 years, the age at which *P. h. hamadryas* females reach sexual maturity; Melnick and Pearl, 1987), with a culling of less than 4 individuals in 10,000 each generation. Actual selection would have been more complex than this simple model, but it is inescapable that given heritabilities similar to what we observed here, large shifts in enamel thickness could result from moderate or low selective pressures over evolutionarily short periods.

Our assessment of the contribution of the additive genetic component of baboon molar enamel thickness predicts common parallel evolution (homoplasy) in this character. We report significant sequential molar variation within an individual (i.e., metameric variation) and a potentially confounding relationship with sex when scaled using linear measurements. Therefore, when used uncritically, enamel thickness has the potential to confound rather than to clarify phylogenetic studies of higher primates. Though studies of diachronic change in primate enamel thickness are highly sensitive to these factors, informative phylogenetic signals can be revealed when these concerns are taken into account. Further study of the underlying genetics of enamel thickness patterns will provide additional guidelines for the phylogenetic evaluation of enamel thickness in studies of primates, including early hominids.

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