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ORIGINAL PAPER

Are Hypsodonty and Occlusal Enamel Complexity Evolutionarily Correlated in Ungulates?

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Abstract The spread of grasslands and cooling climate in the Miocene contributed to an increasingly abrasive diet for ungulates. This increase in abrasiveness is proposed to select for both hypsodonty and increasing complexity of occlusal enamel bands. If these traits evolved in response to strong selection to resist tooth wear while feeding in grassland habitats, we might expect them to have evolved in a correlated fashion. If, on the other hand, there was a developmental or physiological constraint, or if selection was not strong on total enamel production, we would expect species to have evolved one or the other of these traits at a time, producing an uncorrelated, or even inversely correlated, pattern of trait evolution. To test these hypotheses, we examined the Occlusal Enamel Index (OEI) and Hypsodonty Index (HI) of 773 ungulate teeth. We tested the dependence of OEI on HI for the orders Artiodactyla and Perissodactyla using phylogenetic generalized least squares regression (PGLS). The two traits are not significantly correlated in the PGLS, for Artiodactyla and Perissodactyla. Despite their physical proximity, close functional utility, and conventional correlation, our results reject the hypothesis that HI and OEI are evolutionarily linked in these lineages, suggesting that selection to resist tooth wear was not so strong as to drive the overall evolutionary trajectory of both these traits at the same time.

Keywords Hypsodonty · Occlusal enamel complexity · Artiodactyla · Phylogenetic generalized least squares regression · Equini · Hipparionini

Introduction

The evolution of different dietary strategies (e.g., browsing vs. grazing) among ungulates has had a strong effect on their dental morphology (Damuth and Janis 2011; Mihlbachler et al. 2011; Famoso et al. 2013). Increased amounts of abrasive material (e.g., grass phytoliths and/or exogenous grit) in ungulate diets are thought to create a selective pressure for an increase in the relative height of the tooth (hypsodonty) and the complexity of enamel on the chewing surface (occlusal enamel complexity; Damuth and Janis 2011; Jardine et al. 2012; Famoso et al. 2013). Because both hypsodonty and high enamel complexity appear to have evolved as a response to maximizing tooth longevity by increasing the functionally available amount of enamel as an adaptation for oral processing of grasses (Molnar and Gantt 1977; Hoppe et al. 2004), we hypothesize that they are evolutionary complements that evolved to accommodate the heavy dental wear typical of a diet high in abrasive ingesta. If this were the case, we might expect hypsodonty and enamel complexity to have evolved in a correlated fashion. Alternatively, hypsodonty and enamel complexity may offset changes in one another and thus could

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have evolved independently or, if there is a developmental tradeoff, in an inversely correlated manner. If the evolution of hypsodonty and occlusal enamel are strongly inversely correlated, ungulates would seem to be constrained by a conservation of total enamel volume in the tooth. Such a constraint, if apparent, would play an important role in shaping the range of ungulate evolutionary responses to changes in dietary grit. Additionally, the case might just be that increasing enamel complexity achieves the same result as extreme hypsodonty and there is not necessarily selective pressure to evolve both.

In grazing taxa, there is an important interplay between the constraints of maintaining a flat occlusal profile to support the shearing function of the teeth and overall resistance to abrasion which would seem from first principles to engender a strong positive selection for both increasing hypsodonty and increasing enamel complexity in any taxa occupying this ecospace. Supporting this line of reasoning, Famoso and Davis (2014) found preliminary evidence supporting such a conservation of enamel volume in fossil horses, with hipparionin horses showing consistently higher enamel complexity than equin horses, opposite of their relative hypsodonty values (compared to means from Mihlbachler et al. (2011) and Famoso et al. (2013)). Here we test these two hypotheses (correlated evolution of hypsodonty and enamel complexity vs. a tradeoff between hypsodonty and enamel complexity) using phylogenetically-informed least squares regression analyses.

Methods

Hypsodonty and occlusal enamel complexity have been quantified using indices that isolate allometric changes in tooth morphology: Hypsodonty Index (HI) and Occlusal Enamel Index (OEI). HI (Van Valen 1960) is unworn crown height divided by width, while OEI (Famoso et al. 2013) is the total enamel band length divided by the square root of tooth area. HI values for artiodactyls were obtained from Janis (1988), Sponheimer et al. (1999), Badenhorst and Plug (2003), Meachen (2005), Mendoza and Palmqvist (2008), Louys et al. (2012), and Jordana et al. (2014). Upper first molar (M^1) HI for extinct horses were obtained from original data for MacFadden (1988, personal communication April 2002), and from Forsten (1975), MacFadden (1984, 1994), Hulbert (1988a,b), Mihlbachler et al. (2011), and Famoso et al. (2013). HI values are assigned based on species or in some cases genera, and not obtained directly from individual specimens, because of the necessity of measuring HI on unworn teeth; therefore, for some genera, every species shares the same HI value (Famoso et al. 2013). OEI values for both artiodactyls and perissodactyls were obtained from Famoso et al. (2013). The average OEI was calculated for each species. By normalizing these traits to a body size proxy (tooth width or area) as discussed by Famoso et al. (2013), functional changes are emphasized over size-related changes.

Morphological characteristics cannot be considered to vary independently among evolutionarily related taxa (Felsenstein 1985; Harvey and Pagel 1991). Treating the values as independent variables may improperly raise the degrees of freedom in the statistical model, yielding increased probabilities of Type I errors (Martins and Garland 1991; Garland et al. 1992, 1993). We therefore employed phylogenetic generalized least squares regression (PGLS; Grafen 1989) using Pagel's λ (Pagel 1997) to incorporate the estimated phylogenetic covariance structure from the regression, as implemented in the caper package version 0.5 (Orme et al. 2011) for R version 3.0.2 (R Core Team 2013). In this case λ is a measure

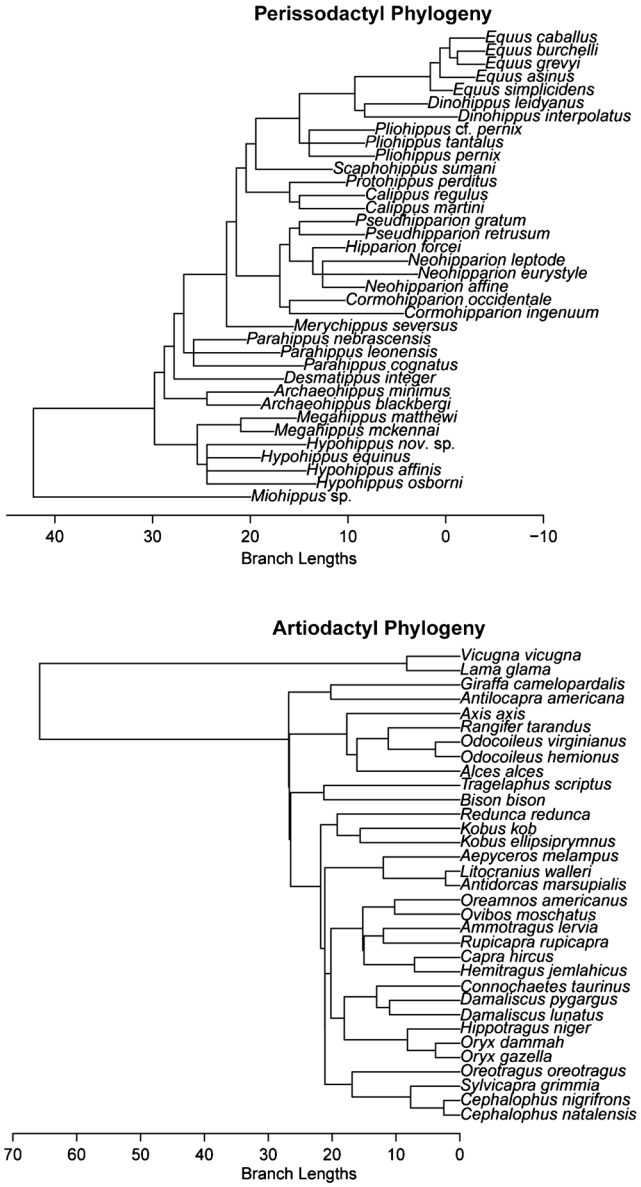

Fig. 1 Phylogenies used in this study. The perissodactyl tree is a time-scaled informal supertree. The artiodactyl tree is an example of one of the 101 tree topologies used in this study

Table 1 Summary statistics for phylogenetic regressions

Model	λ	95 % CI Lower	95 % CI Upper	*t*-values	*p*-values
Perissodactyla	0.541	0.006	0.865	1.015	0.317
Artiodactyla (mean)	0.783	0.233	0.959	−0.075	0.925

of the phylogenetic signal of the residuals from the regression (Revell 2010), which is the degree to which the variance in the residuals between species can be explained by the phylogeny. When λ equals 0, there is no phylogenetic signal and when λ= 1, the phylogenetic signal is equal to that found under Brownian motion.

We used an informal supertree for equids derived from the congruent phylogenies of MacFadden (1998), Kelly (1998), and Hulbert (1993) (Fig. 1). Because the supertree has no branch lengths, we time calibrated the tree using the timePaleoPhy function with type="zlba" to account for zero length branches in the paleotree package version 1.8.2 (Bapst 2012) in R. First and last occurrence data for each species were obtained from the Paleobiology Database (http://paleobiodb.org/) on November 18, 2014. We used 101 artiodactyl trees based on the topology of Fritz et al. (2009), which had polytomies resolved using a constant rates birth-death process following the methods of Kuhn et al. (2011) and are presented in Price and Hopkins (2015). An example of one of the 101 trees is presented in Fig. 1. PGLS was performed on all 101 artiodactyl trees and the mean of each statistic was used for comparison. The R code, dated artiodactyl tree, uncalibrated tree of perissodactyls, first and last occurrence data for perissodactyls, OEI and HI data for artiodactyls, and OEI and HI data for perissodactyls used in this analysis may be found in Online Resources 1, 2, 3, 4, 5, and 6.

Results and Discussion

The λ calculated from both of our PGLS analyses was closer to 1 indicating that there is phylogenetic signal and the PGLS is appropriate (Table 1). Our PGLS was not significant for Perissodactyla or Artiodactyla (Table 1) and the slope of the line of regression was slightly positive for Perissodactyla and near zero for Artiodactyla (Fig. 2). Of the 101 PGLS analyses conducted on the set of randomly resolved trees for artiodactyls, none of the tree topologies were significant.

This study is the first to investigate the coevolution of hypsodonty and occlusal enamel complexity in artiodactyls and equids. We reject the hypothesis that HI and OEI are evolutionarily correlated (Fig. 2, Table 1). Additionally, these ungulates do not appear to have conserved the amount of enamel in their teeth, which would have produced a significant negative correlation between OEI and HI. This result contrasts sharply with Famoso and Davis (2014), who found consistently higher HI values for equin horses and consistently higher OEI values for hipparionin horses. Our result combined with this previous finding suggest a single origin for each of the tribes' characteristic HI/OEI ratios. That is, the two tribes hit on different, successful adaptations for resisting dietary grit rather than displaying a continuous range of values reflecting a conservation of enamel production. The previous study employed nested MANOVAs to control for taxonomic relationships, but that method cannot control for evolutionary interdependence at the same level of complexity as PGLS. We see a result opposed to Famoso and Davis (2014) in our PGLS regressions (Fig. 2), which show no significant correlation between OEI and HI. In the end, the details of species-level phylogenetic relationships have a strong enough control on these traits that we cannot take their correlations at face value. True, hipparionin horses never get as hypsodont as equin horses, and equin horses never get as complex enamel as hipparionin horses, so the overall pattern of different solutions

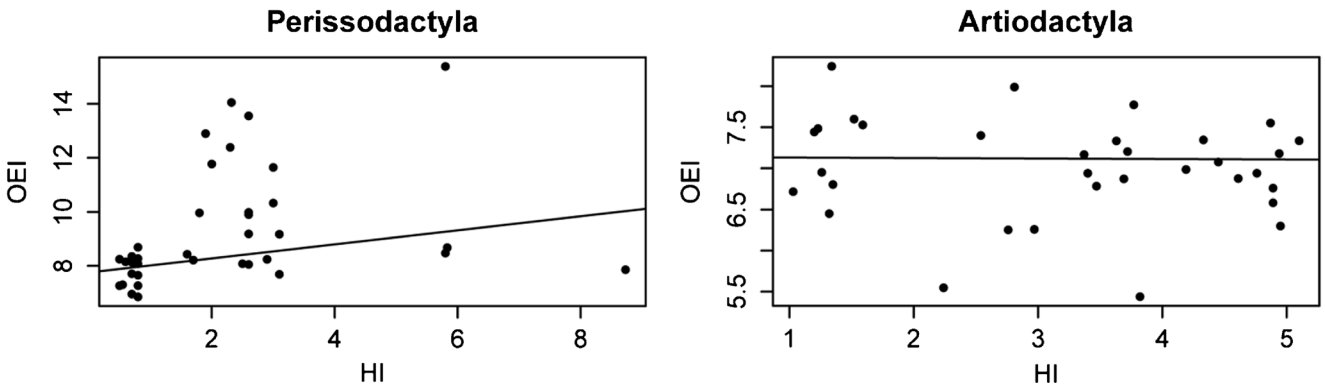

Fig. 2 Results of our phylogenetic generalized least square regression (PGLS) for Perissodactyla and Artiodactyla. The *p*-values for these regressions are not significant. The PGLS for Artiodactyla corresponds to the phylogeny presented in Fig. 1

in the two clades holds, but we cannot assert that these two traits are evolving in correlated or anticorrelated fashion to produce the clade-level pattern.

Interestingly, PGLS on the 101 artiodactyl topologies yielded no significant results. It is unusual that the PGLS presents us with no significant results for any of the artiodactyl topologies (binomial probability of 0.005 assuming a type one error rate of 0.05). This perhaps can be interpreted as an unusually discordant relationship between OEI and HI in artiodactyls. In the end, we can conclude that the PGLS shows no significant correlation for artiodactyls, suggesting completely independent evolution of enamel complexity and tooth crown height.

It is worth noting here that we are using two proxies for volume of enamel in the tooth, HI and OEI, and that the relative thickness and orientation of the occlusal enamel bands are not considered. Because our question in this study is ultimately about the volume of tooth enamel, an important future test would be to use a volume estimation derived from micro CT scans of teeth. Another interesting line of investigation is to examine the orientation of the enamel bands in addition to the total volume of enamel available (Heywood 2010; Kaiser et al. 2010; Fraser and Rybczynski 2014).

The standard HI works well for PGLS analysis because it is calculated on the basis of the most hypsodont available unworn tooth for each species. However, Hoppe et al. (2004) determined that the adult molars of modern horses are still growing while the teeth are in wear, thus making it impossible to get a true HI unless a composite tooth is calculated as was done with some lagomorphs (Bair 2007, 2011). Consequently, there can be no sensible variation in HI within a species. On the other hand, OEI can be calculated for all available teeth within a species; so for the purposes of this study; we used species averages for our PGLS. It would be interesting to perform PGLS on the coefficients of variation (V) for traits within species. In this way, one could come to understand how the variation within a trait has evolved. We have not included such an analysis here because we cannot calculate a V for HI under the current paradigm. Ultimately, OEI is more useful than HI for some types of analyses because it can be calculated for all available specimens.

Additionally, from studies on the development of mammalian teeth, it is known that tooth crown height and overall morphology (occlusal enamel bands in our case here) are controlled by different developmental pathways and proteins (Dassule et al. 2000; Tummers and Thesleff 2003; Harjunmaa et al. 2012; Salazar-Ciudad and Jernvall 2013). Results from evolutionary development experiments on the teeth of mice indicate that occlusal complexity is controlled by interactions between three unlinked genes. Consequently, increasing occlusal complexity is unlikely to result from evolutionary drift alone (Harjunmaa et al. 2012; Salazar-Ciudad and Jernvall 2013). Assuming a homologous system controls ungulate enamel band complexity, we can reasonably conclude that our results for complexity were shaped by selective pressure. The evolutionary independence of HI from OEI, then, presents a new hypothesis for evolutionary development work: how are these two characters controlled in these groups?

In the end, our results indicate that the ungulate lineages we investigated are not maintaining a constant volume of enamel within their cheek teeth. Rather, these lineages show no relationship between complexity and hypsodonty, and are likely influenced by other factors, such as regional variation in abrasiveness of diet or stochastic availability of variation for selection which would manifest as species level variation. In conclusion, while the specific diets of these taxa are significantly related to each of these traits independently and in combination (Famoso et al. 2013), there is no clear evolutionary connection between hypsodonty and occlusal enamel complexity.

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