The evolution of high dental complexity in the horse lineage

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The horse lineage (family Equidae) represents one of the clearest acquisitions of complicated derived dental morphology from a more generalized ancestor. Here we investigate the change in dental complexity (orientation patch count rotated, OPCR) during the evolution of key members of this group. A clear linear increase in dental complexity over evolutionary time is apparent when complexity is measured at high resolution (125 to 175 rows per tooth). The taxa examined also show a linear scaling of OPCR with varying resolution. The slope of this relationship is also a key distinguishing factor among taxa, designated as OPCRS. We found that successive increases in dental complexity are due to the addition of finer-scale morphological features. The study sets the scene for a more detailed investigation into additional members of the Equidae.

Introduction

Horses represent a poster child of adaptation and evolution, forming a clear, albeit branching, sequence of acquisition of adaptations for locomotion and feeding such as reduction in toes and increased hypsodonty (Simpson 1951, Mac-Fadden 1992, Janis 2007). One of the features that has been observed as increasing through the equid group is relative folding of the enamel bands on the secondary occlusal surface of cheek teeth (Simpson 1951, Fortelius 1985, MacFadden 1992). This morphology is interpreted as an adaptation to increased abrasiveness and/or dietary toughness experienced while feeding on monocotyledonous grasses as compared with dicotyledonous leaves, and the degree of enamel folding has been shown to correlate with body size and diet in a number of ungulate lineages,

including equids (Famoso *et al.* 2013). Mihlbachler *et al.* (2011) have also shown through mesowear analysis that equid teeth become blunter with lower relief as tooth crown height increases over evolutionary time (although not perfectly synchronised), both indicating an increase in dietary abrasion.

Previous work has shown that the complexity of the cheek tooth surface is a robust measure of broad diet (e.g. carnivory, omnivory and herbivory) in a range of modern mammalian taxa including carnivorans and rodents (Evans *et al.* 2007), dasyurid marsupials (Smits & Evans 2012), bats (Santana *et al.* 2011), primates (Ledogar *et al.* 2012, Winchester *et al.* 2014) and euarchontans (Bunn *et al.* 2011). Dental complexity is quantified as the number of separate surface patches facing different directions, or orientation patch count (OPC). This measure
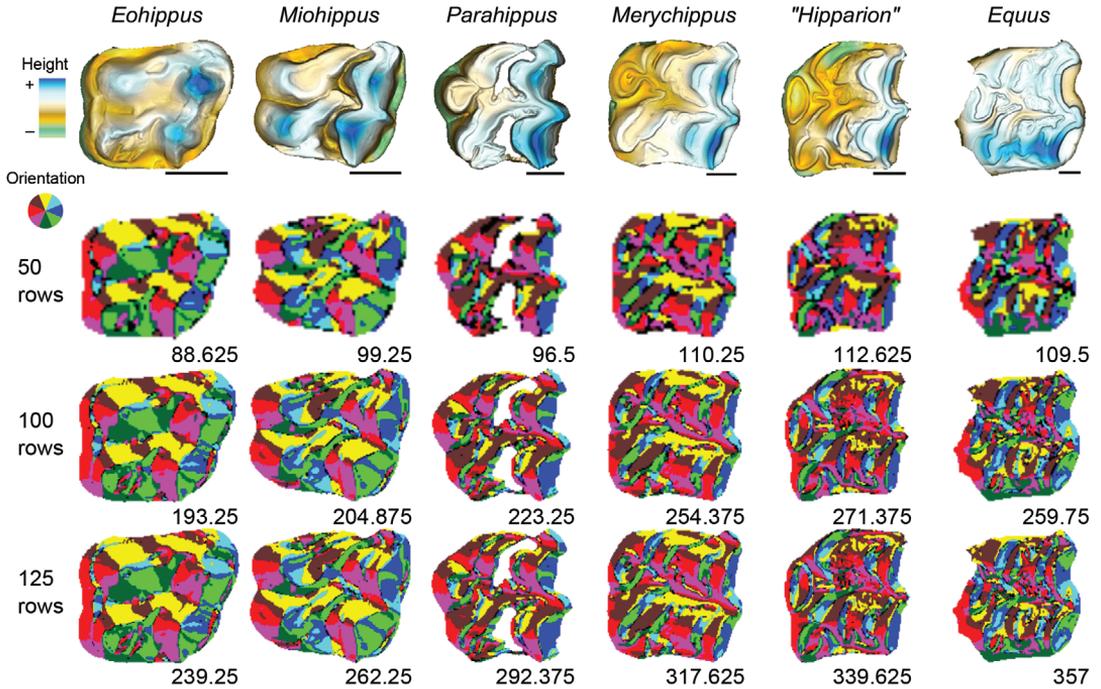
Fig. 1. 3D models of the six taxa (top row) with orientation maps at 50, 100 and 125 data rows (lower three rows). Each model is illustrated with orientation patches when first orientation is at 0°, with the OPCR score at bottom right. Scale bars = 5 mm.

has been used to interpret diet of fossil multituberculates (Wilson *et al.* 2012), plesiadapids (Boyer *et al.* 2010, 2012) and lemurs (Godfrey *et al.* 2012).

Here we examine the pattern of the evolution of dental complexity in six key equid taxa using isolated upper molars, and examine the effect of data resolution on inferences of complexity and trends in morphological evolution.

Material and methods

Six specimens from six fossil equid taxa (Table 1 and Fig. 1), ranging from the Eocene (*Eohippus* = "*Hyracotherium*"; Froehlich 2002) to the Pleistocene (*Equus*), were examined in this study. The "*Hipparion*" specimen is a hipparionine of unknown generic affinity. Most specimens show a moderate wear state; *Eohippus*

Table 1. Taxonomy and age information for specimens used in the current study. MCZ, Museum of Comparative Zoology, Harvard University.

| Taxon	Specimen number	Age of specimen (NALMA, Ma)	Age range from Fossilworks
Eohippus angustidens ("*Hyracotherium*")	MCZ 3513	Early Wasatchian 54–55	55.8–48.6 (genus)
Miohippus obliquidens	MCZ 2800	Orellan 33.7–32	37.2–24.8 (species); 37.2–15.97 (genus)
Parahippus leonensis	MCZ 7595	Early Hemingfordian 18.8–17.5	26.3–13.6 (genus)
Merychippus sp. (probably *sejunctus*)	MCZ 5150	Middle Barstovian 14.8–12.6	20.43–10.3 (genus); 20.43–13.6 (species)
"*Hipparion*" sp.	MCZ 14193	Middle Barstovian 14.8–12.6	12.7–0.01 (genus)
Equus complicatus	MCZ 16458	Pleistocene	2.59–0.01 (species)
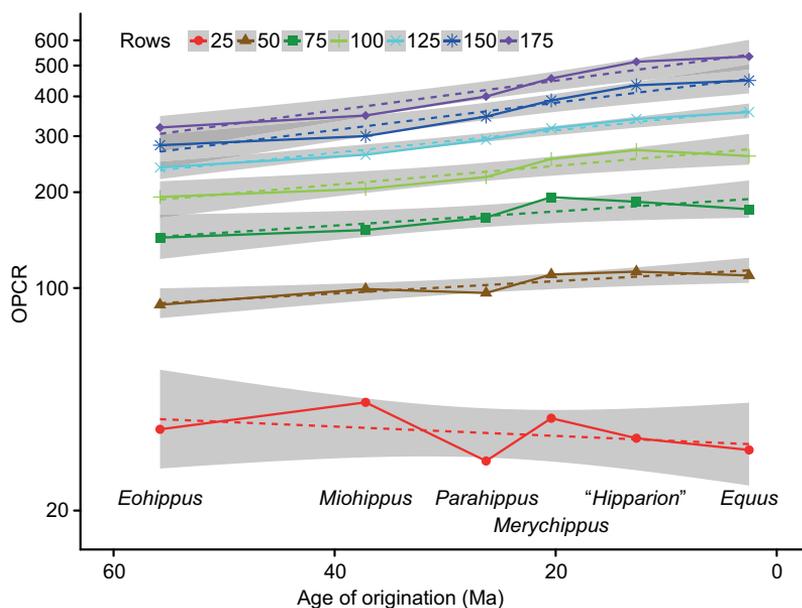
Fig. 2. OPCR (log scale) *vs.* origination age (Ma) for the six horse taxa at seven resolutions (25 to 175 data rows). Dashed lines show OLS regressions with 95% confidence intervals as grey areas.

has relatively low wear compared with the other taxa (fossil specimens of both *Eohippus* and *Mesohippus* usually show a low degree of wear; CMJ pers. obs.). Isolated molars of each taxon were 3D surface scanned at between 10 to 50 μm point spacing using a Laser Design DS2025 laser line scanner after being lightly coated with sublimated ammonium chloride. Point clouds from a variety of views were aligned and then surface meshed using Geomagic to models between 500 000 and 2.5 million polygons. Large broken surfaces of fossils were excluded from each model. Point clouds from the surface models were imported into Surfer for Windows as 2.5D surface grids using Surfer Manipulator at > 200 data rows in the anterior-posterior direction. These grids were resampled at 25, 50, 75, 100, 125, 150 and 175 anterior-posterior data rows to examine the effect of surface resolution. Surfer Manipulator was used to calculate dental complexity according to Evans *et al.* (2007). Eight equal orientation directions of 45° were used, with the first orientation direction centred at 0°. Minimum patch size was 3 (patches smaller than 3 pixels were rejected). The calculation of OPC was repeated 8 times by rotating the centre of the first orientation direction from 0° at increments of 5.625° (OPCR) to account for variation in rotation in the *x,y* plane (Wilson *et al.* 2012).

The origin of each taxon was taken as the beginning of the age range (in millions of years ago, Ma) given in the Fossilworks Database (www.fossilworks.org). Ordinary least squares (OLS) regression of age *vs.* log(OPCR) was undertaken for each resolution, and as well as a regression of log(no. of rows) *vs.* log(OPCR) for each taxon.

Results

Measured at the lowest resolution (25 rows), there is no clear trend in dental complexity over time (Fig. 2; *see* Table 2 for all statistical results). At 50 rows, there appears to be an

Table 2. Results of OLS regression for log(OPCR) *vs.* age (Ma) for each resolution (rows).

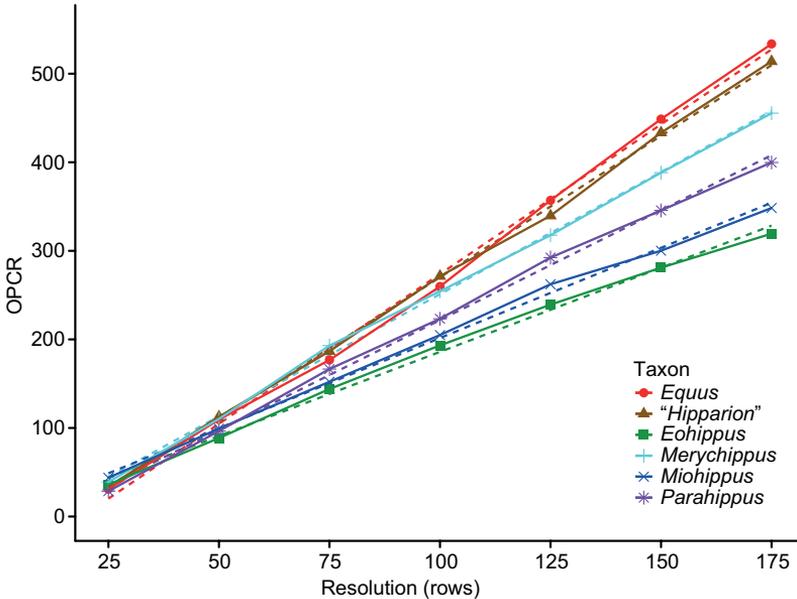| Resolution (rows)	p	Multiple r^2	Slope	Intercept
25	0.415	0.171	0.00147	1.51
50	0.0164	0.798	−0.00194	2.06
75	0.0382	0.699	−0.00222	2.28
100	0.0078	0.859	−0.00296	2.44
125	0.0003	0.974	−0.00351	2.57
150	0.0017	0.934	−0.00428	2.67
175	0.0013	0.941	−0.00466	2.74

Fig. 3. OPCR *vs.* resolution (rows) for the six horse taxa. Note the very strong linear relationship for all taxa.

increase in complexity between *Parahippus* and *Merychippus*. Teeth at 75 and 100 rows show an increasing trend until *Merychippus* and "*Hipparion*", respectively, but then show a drop in later taxa. At the highest resolutions (125, 150 and 175 rows) there is an unbroken linear increase in dental complexity over time.

When OPCR is plotted against resolution, a very strong linear relationship is apparent for all taxa (Fig. 3 and Table 3).

Discussion

At the lowest resolution used here to represent tooth shape, horses do not show an increase over time, and in fact *Equus* has lower dental com-

plexity at 25 rows than *Eohippus*. With increasing resolution, however, the finer morphological details of more derived horses are resolved in the OPCR maps, giving higher values of dental complexity.

At 50 rows, the later three taxa are higher than the earlier three. The drop in OPCR in "*Hipparion*" and *Equus* at 75 rows, and *Equus* at 100 rows, indicates that the fine morphological features of these latest taxa are only counted in the complexity analysis at the higher resolutions. When the tooth rows are represented by fewer data rows, such that the minimum size of detectable features is larger, they are too small to be counted.

These results indicate that the increase in morphological complexity leading to *Equus* is occurring at multiple spatial scales. During the evolution of the group, new features that appear on the tooth are progressively smaller. To some extent this is obvious, given that the average size of patches must decrease as they are added if the overall area remains constant (i.e., with a constant outline shape including length/width ratio). However, it reveals the resolution required to discern this extreme increase in morphological complexity that occurred in this group.

This result begs the question of what is the minimum size of features that are functionally relevant? At the highest resolution examined

Table 3. Results for OLS regression for OPCR *vs.* resolution (rows) for each taxon, including the scaling of OPCR with resolution (OPCRS).

Taxon	p	Multiple r^2	Slope (OPCRS)	Intercept
Eohippus	< 0.0001	0.996	1.90	–4.09
Miohippus	< 0.0001	0.998	2.04	–2.14
Parahippus	< 0.0001	0.998	2.48	–26.4
Merychippus	< 0.0001	0.999	2.76	–24.6
"*Hipparion*"	< 0.0001	0.999	3.19	–49.1
Equus	< 0.0001	0.997	3.38	–64.3

here (175 rows), some of the finest, low-relief enamel infoldings in "*Hipparion*" and *Equus* are not clearly differentiated in the dental complexity analysis. The distinction between these two taxa appears to include more than just this very fine enamel infolding, and so is more likely to have functional consequences. If it is true that much of this increased complexity affects performance, then it shows the resolution at which teeth should be compared to adequately capture the variation in morphological complexity.

To examine the biological implications of these results, we consider the 125 rows results, as this is the lowest resolution that shows the same pattern as the highest resolution. As expected, *Eohippus* has the lowest patch count (239). This early Eocene equid is not only brachydont, but has bunolophodont (rather than fully lophodont) cheek teeth: Mihlbachler *et al.* (2011) report a moderately high mesowear score, which they interpret as incorporating a large percentage of fruit in the diet, a conclusion which also agrees with the gross wear patterns on the teeth (Janis 1979) and microwear studies (Solounias & Semprebon 2002). The late Eocene *Miohippus* has a higher patch count (262). *Miohippus* is more fully-lophodont than *Eohippus*, although also brachydont, and the mesowear scores are low: Mihlbachler *et al.* (2011) interpret this animal as now having a more strictly folivorous (browsing) diet, but one that was relatively non-abrasive, which also agrees with the gross dental wear (Janis 1990). The early Miocene *Parahippus*, with a patch count of 292, is the first equid to show an increase in tooth crown height: this is quite moderate in this taxon (*see* Damuth & Janis 2011), but many specimens of *Parahippus* show an extremely high amount of dental wear, and relatively high mesowear scores (Mihlbachler *et al.* 2011), indicating the adoption of a more fibrous diet.

The middle Miocene *Merychippus* is the first member of the subfamily Equinae, which is the clade that is usually assumed to be hypsodont grazers, although the tooth crown height of *Merychippus* species was varied: some early species were barely more hypsodont than *Parahippus*, a few later species overlapped in crown height with the more derived equines of the late Miocene, but most were only moderately hyp-

sodont (*see* Damuth & Janis 2011). Microwear studies suggest the incorporation of grass in the diet of this genus (Solounias & Semprebon 2002). Our *Merychippus* specimen shows a patch count of 318, intermediate between *Parahippus* and the later Equinae, and their mesowear scores were similarly intermediate (Mihlbachler *et al.* 2011).

Finally, the more derived members of the Equinae, the late Miocene "*Hipparion*" (tribe Hipparionini) and the Plio-Pleistocene *Equus* (tribe Equini) show similar levels of (high) hypsodonty, although *Equus* species tend to be slightly more hypsodont than the hipparionines (which were the lineage of equids that remained persistently tridactyl) (Damuth & Janis 2011). Both hipparionines and equines usually show high mesowear scores, although there is some variability (Mihlbachler *et al.* 2011). The difference in patch count (340 in "*Hipparion*", versus 357 in *Equus*) supports other studies (e.g., Hayek *et al.* 1992) showing that hipparionines were more mixed feeders than *Equus*, although both probably lived in open habitats.

Most previous studies have used 50 rows per tooth to examine dental complexity (e.g. Smits & Evans 2012, Winchester *et al.* 2014). In light of the differences detected among equids when using higher resolutions, it is worth considering a preferred resolution for a given study. For comparisons among taxa with widely divergent diets (such as carnivory, omnivory and herbivory), it appears that 50 rows per tooth sufficiently represents the gross surface topography to distinguish them. However, when examining finer differences in diet at the higher end of dental complexity, such as distinguishing browsing and grazing, it is possible that only 50 rows per tooth does not capture the difference between them.

To decide on the appropriate resolution or resolutions, one could use a multivariate or machine learning approach where OPC and other metrics were calculated at as many resolutions as desired or possible, and then the efficacy of all resolutions were tested to see which gives the best signal for a given question. A similar approach was taken by Plyusnin *et al.* (2008) by using numerous values of each variable to calculate OPC, including minimum patch size and number of orientations. Machine learning algo-

rithms were then used to find which values were most useful in distinguishing among categories. Varying resolution was not tested by Plyusnin *et al.* (2008), it being set at about 10 000 points for a single tooth.

An important factor to consider when deciding on a resolution will be the change in OPC with resolution for a given morphology. Fig. 3 shows that there are differences in the relationship between these variables for the six morphologies examined. While they are all very close to linear ($r^2 > 0.995$ for all species) they vary in their slope by a factor of 1.8, from 1.9 in *Eohippus* to 3.38 in *Equus*. The slope of OPCR *vs.* Rows using a sufficient number of resolutions could therefore be used as another measure of complexity, signified as OPCRS ('S' representing 'slope' or 'scaling'). This measure would have in common with the measurement of fractal dimension that it is measured at multiple resolutions (e.g. Scott *et al.* 2006). However, it is instead the change in measured complexity (OPCR) with change in resolution, and so represents the scaling of complexity rather than length or area with resolution.

For the trend analysis carried out here, time series regression tends to overestimate r^2 due to autocorrelation, but relative strength between the series should be indicative of the strength of the trends, indicating a stronger trend for the highest resolution analysis. While this study is of a small sample of both taxa and individuals, this gives us preliminary results for the larger pattern.

This initial study into dental complexity of the equid lineage reveals the very high complexity reached by single teeth in later members of the group, and the extent to which tooth shape has been modified through evolution.

Acknowledgements

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