

Natural selection on molar size in a wild population of howler monkeys (*Alouatta palliata*)

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Dental traits have long been assumed to be under selection in mammals, based on the macroevolutionary correlation between dental morphology and feeding behaviour. However, natural selection acting on dental morphology has rarely, if ever, been documented in wild populations. We investigated the possibility of microevolutionary selection on dental traits by measuring molar breadth in a sample of *Alouatta palliata* (mantled howler monkey) crania from Barro Colorado Island (BCI), Panama. The age at death of the monkeys is an indicator of their fitness, since they were all found dead of natural causes. Howlers with small molars have significantly decreased fitness as they die, on average, at an earlier age (well before sexual maturity) than those with larger molars. This documents the existence of phenotypic viability selection on molar tooth size in the BCI howlers, regardless of causality or heritability. The selection is further shown to occur during the weaning phase of *A. palliata* life history, establishing a link between this period of increased mortality and selection on a specific morphological feature. These results provide initial empirical support for the long-held assumption that primate molar size is under natural selection.

Keywords: dentition; fitness; microevolution; selection; viability; weaning

1. INTRODUCTION

The evolutionary relationships of fossil mammals are estimated based largely on dental morphology, due to the frequent preservation of teeth and their apparently strong phylogenetic signal. Molar size, in particular, has been a key character for palaeomammalian systematics, especially in primates (Hlusko *et al.* 2002). As such, it is a long and widely held assumption that at least some aspects of dental morphology, such as molar size, are generally subject to natural selection in mammals, including primates (Osborn 1907). Currently, the evidence for such selection is limited to the macroevolutionary correlation of dental mor-

phology and feeding behaviour across taxa (e.g. Kay 1975; Swindler 2002). To our knowledge, selection on a dental trait has not been demonstrated in a natural primate population (Endler 1986; Kingsolver *et al.* 2001), as previous attempts using palaeontological samples were confounded by temporal and taphonomic issues (Marcus 1969; Perzigian 1975). One of the authors (K.M.) initiated and oversaw the collection of a skeletal sample that, by virtue of being a 'found dead' collection, allows for microevolutionary tests of natural selection on dental traits. We carried out such a test and report the main result.

2. MATERIAL AND METHODS

(a) *Howler monkey crania*

Our study is based on a sample ($n=215$) of *Alouatta palliata* (mantled howler monkey) crania from monkeys found dead on Barro Colorado Island (BCI), Panama between 1986 and 1996 (DeGusta & Milton 1998; Jones *et al.* 2000). BCI is one of the most studied tracts of tropical forest in the world, and abundant information is therefore available on numerous aspects of the environment and fauna (e.g. Milton 1980; Leigh & Wright 1990). Howlers on BCI are free ranging and are not provisioned, hunted or otherwise manipulated by humans, nor are they subject to vertebrate predation (DeGusta & Milton 1998). While the skeletal sample is biased against recovery of immature individuals, there is absolutely no expectation or evidence of a dental-size recovery bias within age categories (DeGusta & Milton 1998).

(b) *Theoretical issues in identifying selection*

Since all monkeys were found dead of natural causes, their age at death is an indicator of their fitness. Individuals that die before reaching sexual maturity have lower fitness (zero) than monkeys that live to adulthood (potential for non-zero fitness). In this sample, then, a correlation between age-at-death and dental morphology is potential evidence of mortality-based (viability) selection on the dentition (Endler 1992).

However, such a correlation can be caused by factors other than selection acting directly on the studied trait. First, environmental variation can create correlations between fitness values and traits that, while selectively neutral, are also influenced by such variation (Rauscher 1992). Quantitative methods exist for detecting environmental variation effects on selection (Rauscher 1992), but require data rarely available from skeletal remains (e.g. breeding values). Theoretically, however, it is unlikely that highly mobile howler monkeys, effectively confined to BCI, should experience significant intrapopulation environmental variation (though this is admittedly speculative). Furthermore, since dental morphology is determined early in life and is not thereafter remodelled (except by mechanical wear), such variation would have to occur in the early stages of life (i.e. before the animal reached *ca.* 1 year of age). All crania were recovered from within 6 km², further indicating that significant environmental variation is unlikely in this sample. Finally, even if such variation is responsible for a correlation between a trait and fitness, there is still phenotypic selection despite the fact that it will not lead to evolutionary change (cf. Kruuk *et al.* 2002). This highlights the important distinction between phenotypic selection and the evolutionary response to selection (Arnold & Wade 1984).

The inference of selection from a statistical correlation between a trait and survival can also be confounded by linkages between that trait and others. Specifically, the trait of interest may not be under direct selection, but instead be genetically or functionally linked to a trait that is under such selection (Lande & Arnold 1983). Quantitative methods exist for determining the relative contributions of individual traits to overall fitness, but these require that the trait(s) directly under selection are included as one of the measured traits (Lande & Arnold 1983; Arnold & Wade 1984). For primates, this would clearly require the simultaneous inclusion of numerous variables (behavioural, physiological, genetic and morphological), whose measurement would itself substantially impact upon the population. In addition, most such variables are not available from skeletal material. Furthermore, when considering phenotypic selection (rather than the evolutionary response to that selection), there is no distinction between 'direct' and 'indirect' selection—if a trait is correlated with fitness, then it is under selection (Endler 1992).

As such, it is clear that a significant correlation between a dental trait and age-at-death in the howler sample would be evidence of phenotypic selection on that trait (Endler 1986). Lacking a range of other data, as discussed above, it is not currently possible to predict the evolutionary response (if any) to such selection. To identify

Table 1. Width of maxillary permanent first molar by age stage in the BCI howler sample.

age stage	age in months ^a	no. of individuals	mean (mm)	median (mm)	s.d.	range (mm)
2	6–12	16	7.09	7.64	1.06	5.31–8.22
3	12–24	21	7.56	7.44	0.56	5.94–8.41
4	24–36	16	7.61	7.63	0.37	7.01–8.42
5	> 36	162	7.75	7.74	0.39	6.95–9.10

^a Chronological correlations are approximate (DeGusta & Milton 1998).

whether such a correlation exists in our sample requires that we measure an appropriate dental trait and estimate the age of each individual in the sample.

(c) *The dental trait, ageing and sexing*

The dental trait we measured in the BCI *A. palliata* sample was the buccolingual width of the permanent maxillary first molar (UM1). Tooth crowns do not grow once fully formed, and all individuals in our sample have fully formed UM1s. Thus, any size differences between age stages are not due to growth (drastically reducing the problem of environmental variance, as discussed above). The buccolingual width was selected for measurement because it is at least partially heritable (Jernvall & Jung 2000; Hlusko *et al.* 2002) and is not altered by occlusal wear in BCI *A. palliata* (Jones *et al.* 2000). The measurement error was negligible (1.7%). The UM1 was chosen for study because it is one of the first permanent teeth to erupt, thus permitting its longitudinal examination. The other early erupting teeth in *A. palliata* are incisors, which are frequently missing in the BCI sample and whose dimensions are altered by occlusal wear. A number of specimens in the BCI sample also lack the mandible, so study of the lower first molar (LM1) is limited by sample size. Even so, the limited results from the LM1 are congruent with the results reported here for the UM1.

Specimens were assigned to one of five dental eruption age stages (1–5, youngest to oldest): 1—only deciduous dentition erupted; 2—permanent I1, I2 and M1 erupted; 3—permanent M2 erupted; 4—permanent P2, P3 and P4 erupted; and 5—permanent C and M3 erupted. A tooth was considered erupted if its crown penetrated the alveolar plane. Dental eruption stages are a robust relative aging method, and the approximate correspondence between the dental age stages and age in months (DeGusta & Milton 1998) is given in table 1. Sexual maturity clearly occurs at stage 5 in BCI *A. palliata* (DeGusta & Milton 1998). Data on the timing of tooth crown formation (amelogenesis) in *Alouatta* indicate that the UM1 crown begins to form before birth (Tarrant & Swindler 1973) and is probably completed some weeks following birth.

Adult specimens were sexed based on canine dimorphism, but the sex of immature skulls is, at present, unknown. Since male BCI howlers have significantly larger mean UM1 breadth than females (Jones *et al.* 2000), differences in sex ratios across immature age-stage sub-samples can introduce a spurious trend in molar size. However, since the degree of sexual dimorphism in this characteristic is known for this population (mean difference of 0.25 mm; Jones *et al.* (2000)), we can determine whether a difference in male-to-female ratios across age-stage sub-samples is responsible for any significant correlations detected.

3. RESULTS

The youngest howlers (stage 2, about 6–12 months old) have significantly smaller UM1s than the other age stages using the *t*-test with Bonferroni's correction (figure 1; table 1). There are no other statistically significant differences in UM1 size between age stages (table 2). The absolute mean differences in UM1 breadth between age stage 2 and the older age stages are much greater than 0.25 mm (table 1) which, as discussed above, means that differing sex ratios between the age stages cannot explain this difference.

The smaller mean size in age stage 2 is due to the presence of specimens with very small molars in that age group, rather than the absence of larger individuals (figure 2). Of the six smallest UM1 breadths in the sample, the

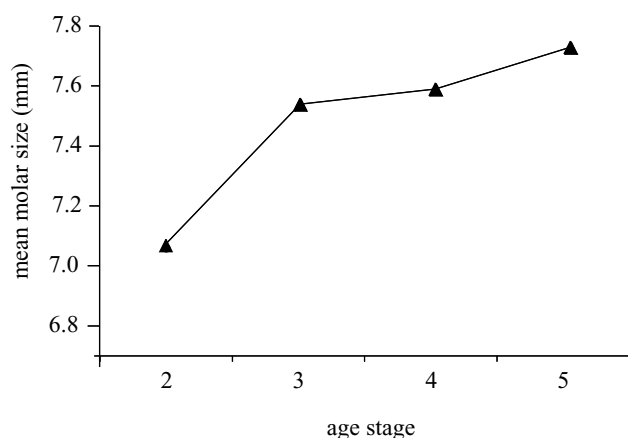


Figure 1. A graph of mean molar size (buccolingual breadth of maxillary first molar) by age stage (2–5, youngest to oldest) for a sample of 215 *Alouatta palliata* (howler monkey) crania from Barro Colorado Island, Panama.

five smallest are from age stage 2 individuals and the sixth is from an age stage 3 individual (figure 2). There is a gap of 1 mm in the distribution of UM1 breadths between these six individuals (less than 5.9 mm) and the remaining 209 individuals in the sample (greater than 6.94 mm). The six smallest crania were recovered in four different years, spanning the decade of collection, ruling out a cohort effect. Since molars do not grow once fully formed (Jernvall & Jung 2000), and since all individuals in our sample have fully formed and erupted UM1s, the difference in mean UM1 size between age stage 2 howlers and older howlers cannot be attributed to growth.

4. DISCUSSION

Upper first molar breadth is correlated with age-at-death in BCI howlers. Specifically, BCI howlers with the smallest molars have greatly increased mortality at about 6–12 months of age. This corresponds with the weaning period in *A. palliata* (particularly for males; Clarke (1990)), which is known to be a time of frequent infant mortality in *A. palliata* (especially for males; Clarke (1990)). Interestingly, however, molar size is not at all correlated with longevity beyond that age. It might have been expected that there would be a consistent trend of 'larger molars = longer survival'. Instead, we have shown that once the very smallest individuals are weeded out at age stage 2 (suggesting stabilizing selection), molar size no longer correlates with age-at-death (figures 1 and 2). This documents the presence of an early selective bottleneck in BCI howlers, associated with weaning, that selects against individuals with small molars, thus linking a life-history

Table 2. Comparisons of mean differences in maxillary permanent first molar breadth between age stages using the *t*-test with Bonferroni's correction.

comparison	mean difference ^a	<i>p</i> value ^b
age stage 2 and 3	-0.46	0.005*
age stage 2 and 4	-0.51	0.003*
age stage 2 and 5	-0.69	< 0.0001*
age stage 3 and 4	-0.05	0.755
age stage 3 and 5	-0.20	0.085
age stage 4 and 5	-0.14	0.258

^a Difference is first listed age stage minus second.

^b Asterisks denote significant comparisons (adjusted alpha value of 0.008).

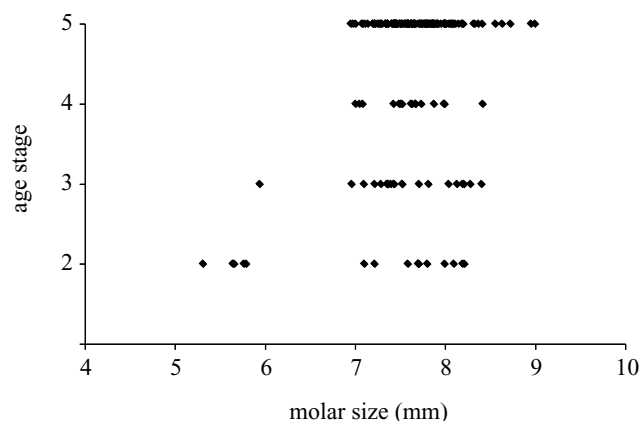


Figure 2. A plot of individual molar sizes (buccolingual breadth of maxillary first molar) by age stage (2–5, youngest to oldest) for a sample of 215 *Alouatta palliata* (howler monkey) crania from Barro Colorado Island, Panama.

episode long known to have high mortality with a specific morphological feature.

The correlation between small molar size and decreased fitness means that molar size is under phenotypic selection, specifically viability selection, regardless of causality or heritability (as discussed above). Even so, since BCI *A. palliata* are generally folivorous (Milton 1980), expanded molar size has plausible adaptive value as one of the factors influencing rate of food processing and caloric intake. Therefore, molar size is unlikely to be adaptively neutral and the documented selection is unlikely to be entirely due to the correlation of molar size with some other factor under selection. However, molar size is also unlikely to be entirely uncorrelated with other factors under selection. Maternal nutritional status (itself a result of numerous factors) is likely to play an important role in determining molar size, since the UM1 crown begins to form shortly after birth. Overall size may also be a factor, though this cannot be tested directly in the BCI sample since howler crania grow throughout age stages 2–5. The notable gap (1 mm, or *ca.* 13% of total size) in the distribution between the smallest molars and the rest of the sample may indicate the influence of a more discrete variant.

These results document phenotypic viability selection on molar tooth size in the BCI *A. palliata* population. To

our knowledge, this is the first time that a dental trait has been shown to be under selection in a wild primate population. This selection is further shown to occur during the weaning phase of *A. palliata* life history, providing a documented link between this period of increased mortality and a specific morphological feature. In total, these results provide initial support for the long-held assumption that aspects of mammalian dental morphology are under natural selection.

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