Developmentally cascading structures do not lose evolutionary potential, but compound developmental instability in rat molars

Natasha S. Vitek[1], Ella Saks[1], Amy Dong[1], Robert W. Burroughs [1], Devin L Ward[2], Emma Pomeroy[3], Malgorzata Martin-Gronert[4], Susan E. Ozanne[4]

[1] Department of Ecology & Evolution, Stony Brook University, Stony Brook, NY.

[2] Department of Anthropology, University of Toronto, Toronto, Canada

[3] Department of Archaeology and Newnham College, University of Cambridge, Cambridge, UK

[4] Metabolic Research Laboratories and MRC Metabolic Diseases Unit, Institute of Metabolic Science, University of Cambridge, Cambridge, UK

Abstract

Increasing variability down serially segmented structures, such as mammalian molar teeth and vertebrate limb segments, is a much-replicated pattern. The same phenotypic pattern has conflicting interpretations at different evolutionary scales. Macroevolutionary patterns are thought to reflect greater evolutionary potential in later-forming segments, but microevolutionary patterns are thought to reflect less evolutionary potential and greater phenotypic plasticity. We address this conflict by recalculating evolutionary potential (evolvability) from published mammalian molar data and directly measuring phenotypic plasticity from a controlled feeding experiment. Effects on lengths and widths are discordant in a way that suggests general growth

pathways have a role in phenotypically plastic dental responses to nutrition. Effects on successive trait means do not necessarily increase downstream, contrary to long-standing hypotheses. We confirm prior findings of increasing non-inherited variance downstream, showing decoupling between effects on trait mean and variance. These patterns can be explained by a cascading model of tooth development compounding the effect of anatomically hyper-local developmental instability as an influence separate from general environmental effects on the developing embryo. When evaluated in terms of evolvability, not heritability, later-developing molars are equally or more evolvable than earlier-developing molars, aligning their microevolutionary potential with macroevolutionary patterns in other serially segmented structures.

Introduction

Animal bodies contain numerous serially segmented structures, such as vertebrate teeth, limb bones, and vertebrae [1]. The semi-independent nature of serial structures makes them important systems for understanding how diverse phenotypes evolve in structures that share a significant proportion of their genetic and developmental basis [2,3].

In vertebrate limbs and teeth in particular, conflicting perspectives emerge on the relative evolutionary potential of different segments in a module. In macroevolution, where phenotype is largely understood to have a genetic basis, later-developing segments are considered to have greater evolutionary potential than early-developing segments. Evidence primarily comes from the limbs. There, later-developing, downstream segments have greater phenotypic variation and faster evolutionary rates than early-developing, upstream segments [4,5].

In microevolutionary contexts, where phenotype likely has both inherited genetic basis and non-inherited plastic basis, elements of the limbs and mammalian molars usually, but not always, share the same pattern of higher phenotypic variation in later-forming segments [6,7] (see [8,9] for counter-examples). Along the same sequences of segments, two other patterns emerge. First, successive molars generally (though not exclusively) have lower levels of heritability (h^2), or the proportion of phenotypic variance that can be attributed to additive genetic variation and can respond to selection over generations [10–12]. Second, successive molars have higher levels of fluctuating asymmetry, or non-inherited phenotypic variation usually attributed to developmental instability [13,14].

These patterns of microevolutionary variation are interpreted differently from the patterns of macroevolutionary variation. The interpretation has been most clearly characterized for mammalian molars, for which later-forming teeth are thought to be more variable because they have a longer exposure to or are more strongly affected by environmental influences [12,15], or have “diminished genetic control” [16]. The emerging interpretation is that greater phenotypic variation at the microevolutionary level either does not indicate anything about relative evolutionary potential, because it is a signal of relative amounts of non-inherited variation, or indicates less evolutionary potential, directly conflicting with the macroevolutionary perspective of greater evolutionary potential.

Put another way, the similar patterns of phenotypic variation at both micro- and macroevolutionary levels are attributed to two very different causes at each level, one inherited, producing evolvable phenotypes, and one not. To address this apparent conflict in interpretation, in this study we examine variation in serially segmented structures at the within-species, microevolutionary level. Specifically, we test the microevolutionary interpretation of reduced

evolutionary potential in successive segments (hereafter: Reduced Potential model) using the mammalian molar tooth module as a study system.

Predictions for Evolvability

One interpretation of a Reduced Potential model is that later-forming structures carry less additive genetic variation, the kind of variation that can respond to selection and evolve over generations [17]. To address this interpretation, we examined patterns of both heritability and evolvability down the molar row. Heritability has long served as a comparative metric for such purposes, but the evolvability (I_A) metric has emerged as a more appropriate, direct evaluation of evolutionary potential [18,19]. Both heritability and evolvability are calculated from quantitative genetic studies partitioning phenotypic variation into additive genetic (V_A) and other (V_E) components (note that V_E is sometimes called the environmental component but does not refer solely to an ecological environment with which a species interacts). Evolvability makes the additive genetic component comparable between traits and samples by mean-scaling evolvable variance (V_A), using the same mean-scaling logic that underlies coefficients of variation (CV) [18]. In contrast, heritability conflates potential for both an evolved response and a non-evolved, phenotypically plastic response by scaling evolvable variance by total phenotypic variance, which includes both sources of variation [20]. Given the Reduced Potential model, our approach tests:

Prediction 1: We expect to observe decreased evolvability along the molar row, similar to published patterns for heritability.

Predictions for Phenotypically Plastic Patterns

A complementary interpretation of the Reduced Potential model is that later-forming structures are more phenotypically variable because their formation is more strongly affected by their growth environment. To address this interpretation, we isolate phenotypically plastic variation and characterize its pattern along the molar row using a controlled feeding study of inbred lab rats *(Rattus norvegicus*). Phenotypically plastic variation occurs when different environments induce different phenotypes from an identical genotype [21]. Environments may refer to climatic conditions, such temperature inducing trait change [22], as well as other external conditions, such as nutrition availability [23,24], as well as highly local conditions, such as slight differences in the developmental microenvironment surrounding symmetric structures, known as developmental instability [12,25]. Resulting structures share genotypes and should be phenotypically perfectly symmetrical, but often carry a small amount of fluctuating asymmetry [26].

Different sources of phenotypic plasticity predict different specific patterns. It is important to differentiate between them to better link pattern to process. In this case, sources of plasticity must work through the developmental process that form molar teeth. We developed predictions using knowledge of molar development and its relationship to growth as well as the relationship between growth and nutrition, given our study system of nutrition's phenotypically plastic effect on molar sizes [23,27,28].

First, more generally, statistical expectations of phenotypic plasticity depend on the developmental mechanism inducing that plasticity. Specifically, trait means may not respond to the environment in the same way as trait variances (Fig. 1). An environmental condition that induces changes in developmental pathways can cause a change in trait means in a sample. For

example, poor nutrition reducing body size or changing trait proportions would affect means, but do not necessarily require a change in variance [28–30]. The same mechanism could also affect variance if, for example, later-forming segments are smaller or if a compounding effect reduces means, resulting in an increase in mean-scaled measures of variance such as CV [31]. We refer to this situation as one of decreasing environmental canalization [6]. Developmental instability is considered as a separate phenomenon. If an environmental condition increases developmental instability without inducing other changes, then the random changes will increase the variance but should not affect the mean [6,32]. Therefore, in this study we assess our expectations through both means and variances.

Second, more specifically to our study system, molar tooth width and length have potentially different relationships to nutrition and its effect on development. Width, but not length, is significantly genetically correlated with body size [33]. Molars also reach their maximum length earlier in development, before they reach maximum width [34], similarly supporting the hypothesis that length and width are determined by different sets of developmental pathways. If genetic correlations between body size and molar width, but not molar length, occur through sharing pathways that affect growth in general, and if those pathways are affected by nutrition [29,35], then we expect that molar traits more strongly correlated with body size to also be more strongly affected by nutrition than other molar traits. Therefore:

Prediction 2: We expect poor nutrition will have a stronger effect on molar width than molar length.

Third, developmental relationships between teeth may result in increasing phenotypic plasticity in downstream segments (Fig. 1). Segmented structures form from the iterative expression of similar or identical sets of developmental pathways [1,36]. Importantly, early-

forming structures have the capacity to interact with later-forming structures, producing downstream phenotypes that may differ between individuals only because of variation in phenotype of the initial segment, not because of any change in genetic basis between segments or because of different relationships of different segments to the environment [1,2]. This cascading process could compound a phenotypically plastic effect in later-forming segments, producing a stronger effect and less environmental canalization in the phenotypes of later-forming segments [6]. Alternatively, effects may not compound. Molar size phenotypes are incompletely described by a single model of compounding developmental process [37,38]. In addition, molar positions have some level of genetic independence from one another, albeit often small, implying that the sets of developmental pathways that control their growth may be slightly different [11,39]. These deviations from a cascading model fit an alternative proposal that a different effect of phenotypic plasticity on different molars is instead related to different levels of exposure, or the amount of time each tooth spends developing, implying that the molars are equally canalized against environmental effects per unit of exposure time [12,15]. Either model could explain previously observed patterns of decreasing heritability and increasing phenotypic variance down the tooth row [1,2,40,41], leading to:

Prediction 3: We expect the phenotypically plastic effect of any environmentally induced change will increase along the tooth row from M_1 to M_3.

Overall, this approach of separating expectations for evolvable phenotypic variation and plastic phenotypic variation may clarify potential connections between microevolutionary and macroevolutionary patterns, resolving apparent disconnects in interpretation.

Methods

Evolvability

To test Prediction 1, we searched the literature for reports of heritability of molar size variables that could be used to calculate evolvability so that the two measures could be compared between samples. The search was intended to capture data reflecting patterns commonly summarized in reviews (e.g., [12,15,16]), but not to be systematic. To meet minimum criteria for this purpose, publications needed to report the following for buccolingual width or mesiodistal length for at least two molar positions: (1) trait means (m), (2) trait variances (V_P), (3) trait heritability ($h^2=V_A/V_P$). From these values, we could calculate evolvability, $I_A = V_A/m^2$, from heritability using the equation: $I_A = h^2 * V_P /m^2$. Many publications did not report one or more values. To our knowledge, six samples from four publications met all three criteria [11,42–44]. Some samples are right and left sides from the same individuals, which we report separately for clarity and a first-order sense of confidence intervals, given that the two sides should be equally evolvable. To test if the overall pattern was characterized by a significant decrease in heritability or evolvability, we conducted a sign test for each metric for each successive pair of molars (M_1 vs. M_2, M_2 vs. M_3), acknowledging that with $N \leq 6$ the power of each test is limited. Tests were one-tailed, with the null hypothesis of successive molars having equal or greater heritability or evolvability.

Trait Plasticity

To test Predictions 2 and 3, we leveraged data collected from a previously conducted controlled experiment originally intended to study the effect of maternal malnutrition on insulin action and adipose tissue in male offspring of Wistar Han rats (*Rattus norvegicus*; Crl:WI[Han] strain). This type of laboratory study using inbred lines controls for genetic variation between

individuals, allowing us to assume that all phenotypic variation is due to some component of environment. Thus, by comparing phenotypic patterns between traits and between experimental groups we could study the impact of environment on traits. In this case, the main environmental variable that differed between control and experimental groups was the quality of the maternal diet throughout gestation and suckling (8% low-protein experimental diet group vs. 20% protein control diet group), and we measured its effect on offspring phenotype [45,46]. Nutritional variation is known to influence dental phenotypes, providing a high likelihood of observing environmental impacts in our study [28,29]. The time window of environmental perturbation (Day 1 of gestation, E1, through weaning on postnatal day P21) is appropriate for studying the effect of environment on rat molars because molar final size is determined during a finite window of growth and then remains unchanged for the remainder of an individual's life [47]. In rats, which develop 1-2 days slower than mice, molars initiate formation as the first molar (M_1) placode on embryonic day E13-14, then a tooth bud at E16-16 [34,47]. The M_2 tooth bud appears at E17-19, and the M_3 tooth bud is visible approximately 10 days later, at postnatal day P5-7 [2,47]. Each molar reaches an inflection point and slows growth about 5 days after the bud stage, and achieves its final size about 8 days after bud stage [34], meaning that in the rat, final M_1 size is achieved by P1-2, M_2 size by P3-4, and M_3 size by P13-15. Therefore, we consider each molar tooth to be equally exposed to a consistent environmental perturbation.

To collect phenotypic trait data, we used μCT scans of offspring sacrificed at age 3 months (N = 25 control, 16 low-protein) to generate 3D models from which linear measures could be collected. Further details of experimental design and μCT scan collection are reported in [45,46]. From μCT image stacks, volumes of the left molar row were segmented into 3D surfaces using automated thresholding in Avizo® version 2019.3 (FEI, Hilsboro, USA). Smoothing of the

region of interest was conducted in each of the three standard planes, but after a surface was extracted from that region we conducted no further smoothing. From these surfaces, mesiodistal length and buccolingual width were measured in triplicate by a single observer using MeshLab [48]. To evaluate whether measurement error might contribute an undue amount of spurious noise to our estimates of variation, as has been proposed to explain some patterns in variation across molars [31], we calculated percent measurement error for each trait [49].

We calculated CV for each experimental group separately to ensure that potentially different amounts of change in mean trait size did not spuriously influence estimates of variation. To evaluate whether CVs differed between samples, we used a bootstrap approach to construct a null distribution around the mean, resampling each treatment group with replacement 10,000 times. Reported p-values represent the proportion of bootstrap replicates in one trait that produced a CV larger or smaller than the observed value of the comparison trait, depending on the hypothesis tested. For example, one part of Prediction 3 was that M_2 length CV should be significantly greater than M_1 length CV. The test for significance was the proportion of bootstrapped M_2 length CVs that were smaller than the observed M_1 length CV. A small proportion would indicate a significant difference in variance between the two traits, under the model that the distribution of bootstrapped M_2 length CVs formed a null hypothesis against which to compare the test statistic of M_1 length CV. We tested hypotheses that CV should increase between successive widths, between successive lengths (one-tailed), that it should differ between length and width (two-tailed), and whether it differed between control and experimental groups. Low-protein CVs were used for between-trait comparisons.

To determine if it was necessary to test hypotheses of mean effect on any specific trait, we first established whether the nutritional environment had a significant effect on trait means by

comparing experimental groups. For mean trait size, we used a t-test. To compare effect between teeth, it was necessary to take the different sizes of each molar position into account ($M_1 > M_2 > M_3$), because the same magnitude of difference has a different meaning for effect on the M_1 vs. the M_3. We calculated a percent reduction statistic [mean control value – mean experimental value] / [mean experimental value] to mean-scale the effect, similar to the mean scaling performed for variation (CV) and evolvability (I_A) [18]. To evaluate whether effect size differences were significant, we used the same bootstrapping approach used to compare CVs. To test Prediction 2, we compared width to length values within each tooth. To test Prediction 3, we compared successive length values to each other and successive width values to each other. When each hypothesis was evaluated using multiple tests, we report p-values corrected using the Bonferroni approach [50]. All analyses and visualizations were conducted in R version 4.4.1 [51] using packages `dplyr`, `reshape2`, `ggplot2`, `ggthemes`, and `patchwork` [52–56].

Results

Evolvability

Evolvability (I_A) remained stable or increased along the molar row for both lengths and widths (Fig. 2). Heritability (h^2) estimates for the same samples generally, but not always, decreased down the molar row for length, and had no consistent pattern of increase or decrease for width. Sign tests for downstream decreases were not significant ($p > 0.109$), though with the statistical power associated with N=6, only a perfectly consistent pattern would have yielded a significant p-value (SI Table 1).

Trait Plasticity

Measurement error was low across traits (<2%, Table 1). All 6 traits were significantly smaller in the low-protein offspring group compared to the control offspring group (Table 1). Effect size, as measured by percent reduction from control to low-protein, was not consistent down the toothrow (Table 2, Fig. 3A). Length of M_2 was significantly more strongly affected than the length of the M_1. Widths of M_2 and M_3 were more strongly affected than width of the M_1. No other comparison between upstream and downstream effects was significant. Widths were generally more strongly affected than lengths, but only significantly so in the M_1 and M_3.

Variance, as measured by CV, did not differ significantly between the control and low-protein group, with the single exception of M_3 length. CV significantly increased down the molar row for lengths, but not for widths (Table 2, Fig. 3B). Widths were only more variable than lengths for the M_1.

Discussion

Our results largely confirm prior patterns for statistics related to variances [12]. Phenotypic variances are higher in the lengths of later-forming teeth. These new results are consistent with our collection of previously published estimates of molar length heritability, which generally decrease down the tooth row for lengths. The pattern for widths in both variance-based measures appears less consistent than has been previously recognized. However, our additional results show that molar size heritability and phenotypic variance do not reliably predict molar size evolvability, nor do they predict patterns of effect on trait means. In short, Prediction 1 was not supported, and Prediction 3 was supported for measures of variance but not trait means. The

notably different patterns between lengths and widths generally supports Prediction 2, though support is not universal and patterns in the second molar are somewhat puzzling. Our results are not attributable to measurement error, as has potentially been an issue in other studies of variation [31].

Phenotypic Plasticity in Serially Segmented Structures

Evolutionary potential in teeth may be masked by phenotypic plasticity, which may introduce both noise and conflicting signal into trait values. The discordant patterns in trait means vs. trait variances allows us to distinguish between causes of phenotypic plasticity and their potentially different relationship to environmental conditions. Increasing environmental effects on trait variances, but not trait means, is consistent with compounding developmental instability, but not compounding loss of environmental canalization (Fig. 1). We acknowledge that because the experimental design did not include observations of how long each molar took to form, given the variability in the process, we cannot test the alternative hypothesis of environmental canalization scaled by exposure time, nor can we assert that later-forming molar trait means are not more strongly affected in general outside of this study [28,57]. However, the point that the phenotypically plastic responses of trait means and variances are decoupled in later-forming molars holds regardless. Different developmental mechanisms either respond differently to the environment or respond to different components of the environment.

We therefore attribute the compounding variance to general developmental instability, rather than sensitivity to an aspect of an organism's environment, given the lack of associated compounding changes in trait means. We recognize that fluctuating asymmetry is a more standard statistic for inferring developmental instability [6,32]. Our data collection protocol did not permit us to evaluate fluctuating asymmetry in this study, but prior studies have found a

consistent pattern of downstream increase, consistent with our explanation [13,14]. The other phenomenon that would produce such an effect, antisymmetry, is not a feature of mammalian tooth sizes [26,58,59].

The pattern of compounding of developmental instability may be related to the cascading nature of molar development itself, or developmental interaction between successive molars [2,12,25]. Under this hypothesis, any stochastic developmental instability in the conditions affecting the M_1 would add on to the stochasticity affecting the M_2 itself, and so on down the tooth row. No tooth would have developmental instability less than zero, and therefore developmental instability would only ever remain stable or increase down a set of segments, resulting in higher downstream phenotypic variance if all else were equal among molars. The fluctuating nature of developmental instability would mean that this random process averages out with no accumulating downstream effect on trait means in a sample, even though the effect accumulates downstream in any given individual. The observation that control and poor-nutrition groups did not generally differ in levels of phenotypic variance supports the interpretation that compounding developmental instability may be a phenomenon separate from the effect of an organism's external environmental conditions [6,12].

In short, our results contradict the commonly held hypothesis that increasing developmental instability is mechanistically related to increasing sensitivity to environment and decreasing environmental canalization [6]. Although compounding developmental instability can potentially explain patterns of variance-related statistics, it cannot explain either the patterns of environmental canalization or of evolvability, and therefore does not support a Reduced Potential model. Instead, we recognize at least two phenotypically plastic processes at work in our study that are not mechanistically related to evolutionary potential. First, we hypothesize a model of

developmental instability compounding down the development of the molar row, explaining patterns of phenotypic variance, heritability, and fluctuating asymmetry documented in this and other studies [7,11,13]. Second, we hypothesize an independent effect of nutritional environment on organismal growth, which affects molars to the degree that they are developmentally non-independent of overall organismal growth.

Plasticity & Organismal Growth Reflected in Teeth

Prior work found stronger links between molar width and overall body size than molar length in terms of genetic correlations [33]. Further work built on these links to focus on studying more tooth-specific evolutionary patterns, rather than patterns that might reflect more mixed evolutionary signals of body size and tooth size evolution [60]. In our study, all molar size traits were significantly, phenotypically plastically reduced by poor nutrition, highlighting that no trait is fully buffered from non-evolved, size-related responses to environment, consistent with heritability estimates [11]. However, the stronger impact on widths than lengths supports the interpretation that focusing on lengths better isolates the evolution of teeth separate from evolution of other organismal properties [60].

The stronger effect of poor nutrition on molar width than length may reflect how overall organismal growth pathways are specifically expressed during tooth growth. This hypothesis would make our phenotypic patterns a potential phenocopy (sensu [6]) of evolved changes whose underlying mutations affected the same developmental pathways. In the case of teeth and growth, insulin-like growth factor (IGF) signaling and expression is a prominent candidate for future study. Insulin-like growth factors are ubiquitous during embryonic development and regulation of IGF is critical for cellular proliferation of developing tissue and organs, including dentition [34,61,62]. Poor nutrition can impact IGF regulation by reducing the rate of protein

phosphorylation, resulting in a reduction of available IGF binding proteins and potentially resulting in a negative feedback loop [63,64]. Reduced IGF results in systematically smaller sizes for developing embryos without impacting the fundamental timing or process of development due to the role of IGF in proper organogenesis [65]. By reducing phosphorylation, poor nutrition could directly influence developing tooth size without otherwise inducing significant alteration of gene expression in other developmental pathways, similar to its role in genetically-based patterning of teeth [34].

Reconciling Macro- and Microevolutionary Interpretations

Overall, we found equal or increasing evolutionary potential down the molar row in microevolutionary contexts. This interpretation is consistent with the macroevolutionary interpretation of limb segment patterns, another example of serially segmented structures. It helps reconcile prior discordant interpretations, but the reconciliation includes some nuance that bears discussion. First, mammalian molars appear to be another case where heritability is not as informative of evolutionary potential as evolvability [18,20]. Later-forming segments may be less heritable and statistically noisier because of their inflated variance, but their mean values are not necessarily poorer reflections of evolutionary history than the mean values of earlier-forming segments. This perspective, and the higher evolvability values in M_3 of some taxa (Fig. 2) may explain why the M_3 has been considered so taxonomically useful in certain rodents [66,67], despite their general characterization as a noisy, non-ideal choice for taxonomic study [7,68].

In limbs, the macroevolutionary pattern of higher evolutionary rates in later-forming segments is attributed to function, where more distal, downstream segments have more direct interaction with substrates and other sources of selective pressure [5]. This selection pressure may make their potential more easily observed, in contrast to the molar module where function is

often conserved down the tooth row and treated as a single functional complex along with other cheek teeth [69]. Investigating the macroevolution of taxa that differ in their degree of functional variation down the molar row may provide an important comparison to patterns in the limb, filling in a remaining gap in our understanding of how microevolutionary and macroevolutionary diversification align.

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Figures and Tables with Captions

Table 1. Summary statistics for molar size traits.

Trait	Tooth	PME	C vs. LP x̄ p-value	LP vs. C % Reduced	CV C	CV LP	C vs. LP CV p-value
Length	M_1	0.58	**$< 1 * 10^{-5}$**	5.53	2.76	3.04	1
	M_2	0.34	**$< 1 * 10^{-5}$**	8.81	3.88	3.39	1
	M_3	0.36	**$< 1 * 10^{-5}$**	6.67	4.75	5.88	**0.0216**
Width	M_1	1.29	**$< 1 * 10^{-5}$**	9.56	3.3	3.84	0.4008
	M_2	0.89	**$< 1 * 10^{-5}$**	7.4	3.58	4.32	0.6102
	M_3	0.38	**$< 1 * 10^{-5}$**	11.74	4.83	5.25	1

Significance values reported post-Bonferroni correction. Significant p-values are in bold.

Abbreviations: C, control group; CV, coefficient of variation; LP, low protein group; p, p-value; PME, percent measurement error; x̄, mean trait value

Table 2. P-values assessing significance of differences between pairs of traits. Low-protein CVs were used for between-trait comparisons.

	Length vs. Width			Length		Width	
	% Reduced	CV		% Reduced	CV	% Reduced	CV
M_1	< 0.0003	0.0051	M_1 vs. M_2	0.0003	0.036	1	1
M_2	1	1	M_2 vs. M_3	1	0.0021	0.0003	0.0003
M_3	< 0.0003	1	M_1 vs. M_3	1	0.0003	0.0003	0.1437

Significance values reported post-Bonferroni correction. Abbreviations: CV, coefficient of variation.

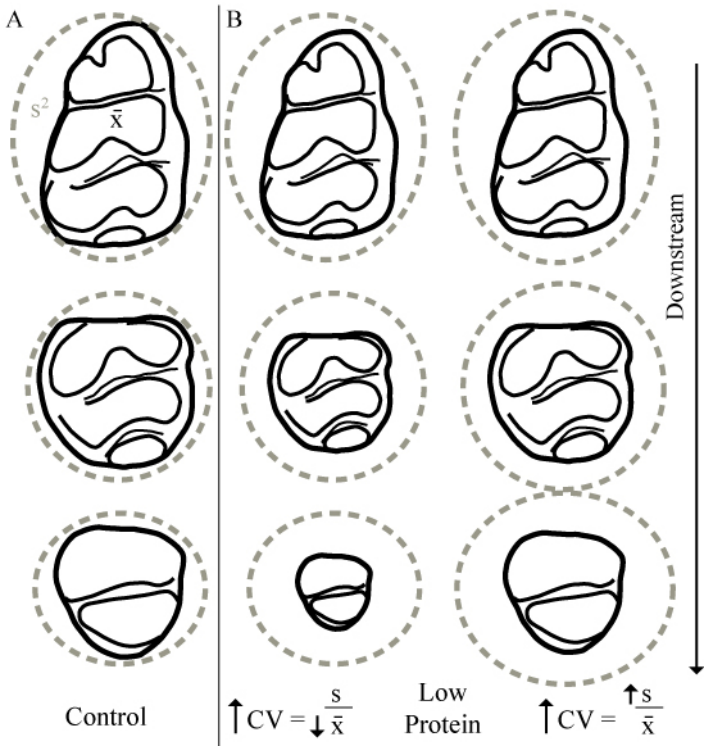

Figure 1. Conceptual diagram of two different ways in which a low-protein diet, a kind of environmental perturbation, could generate a pattern of increasing coefficients of variation (CV) in downstream tooth sizes, matching previously published patterns of variation in tooth size. A. Control group, in which each tooth sample has a mean size (black solid outline) and variance (gray dashed ellipse). B., Low Protein group, in which all tooth sizes are reduced, but either (left) downstream teeth are more strongly reduced in size than upstream teeth, resulting in an increase in CV because of a more strongly reduced denominator in the CV equation, or (right) downstream teeth have more strongly increased variance than upstream teeth, resulting in an increase in CV because of a more strongly increased numerator in the same equation. Abbreviations: CV, coefficient of variation; s, standard deviation; x□, sample mean.

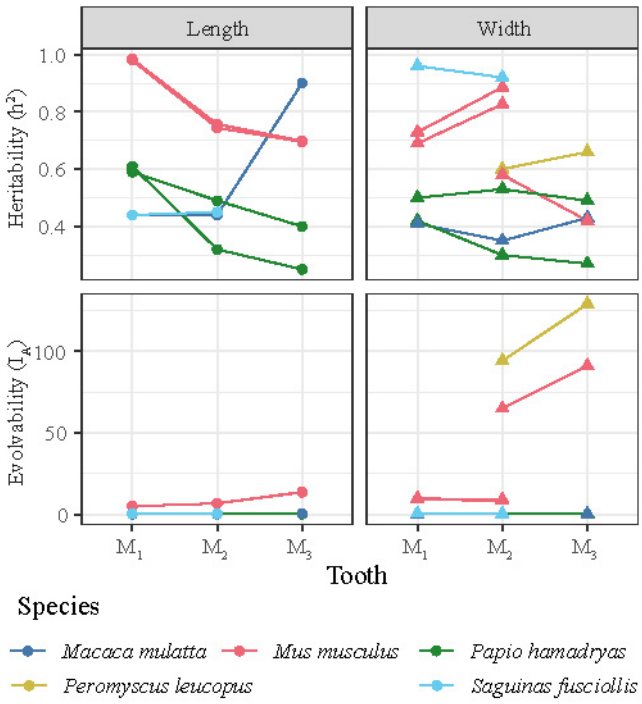

Figure 2. Evolvability (I_A) and heritability (h^2) calculated from the same set of published samples. Lines connect traits measured from the same sample.

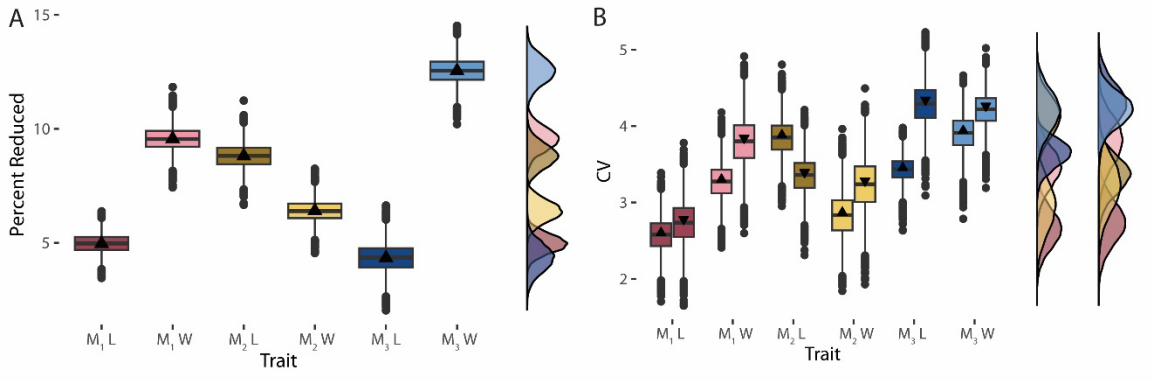

Figure 3. Effect of low-protein nutrition on the mean and variance of molar size traits, standardized by mean trait size. Box and whisker plots show median (thick bar) and mean (triangle). Corresponding density plots are to the right. Note that density plots height may not match box plot median because of the different way each approach summarizes the distribution.

A. Percent that low-protein group is reduced compared to control group. B. Coefficient of variation. Boxes with upward-facing triangles show the control group. Boxes with downward-facing triangles show the low-protein group. The pair of density plots show control (left) and low-protein (right) groups. Abbreviations: CV, coefficient of variation; L, length; W, width.

SI Table 1. Results of each sign test for consistent downstream decreases in heritability (h^2) and evolvability (I_A) in the molar tooth row of previously published samples.

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Keywords: evolvability, phenotypic plasticity, nutrition, growth, molar, Mammalia

Data accessibility statement: Data and code sufficient to reproduce results in this study are contained in a Dryad publicly accessible digital repository [*private reviewer link: XXXXXX*]