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

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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 a systematic review of published mammalian molar sizes, then directly measure 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 developmental instability as an influence separate from general environmental effects on the developing embryo. When evaluated in terms of evolvability, 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 (Young *et al.*, 2015). 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 (Kavanagh *et al.*, 2007; Böhmer *et al.*, 2015).

In mammalian teeth as an example, divergent 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 such as the third molars (M_3) are considered to have greater evolutionary potential than early-developing, upstream segments such as the first or second molars (M_1, M_2). Support comes from observations of faster evolutionary rates downstream ($M_3 > M_2 > M_1$) sometimes documented directly (Sofaer *et al.*, 1971; Mongle *et al.*, 2022; Amaral *et al.*, 2024), sometimes implied by greater taxonomic utility of the M_3 as a more sensitive indicator of species differences, especially in rodents and suoids (Hershkovitz, 1967; Barnosky, 1987; Van der Made, 1996; Polly, 2003). These observations translate into higher macroevolutionary diversity, or variance, in M_3s vs. earlier-developing segments (Billet & Bardin, 2021).

In microevolutionary contexts, where phenotype likely has both inherited genetic basis and non-inherited plastic basis, molars usually, but not always, share the same predicted pattern of higher phenotypic variation in later-forming segments (ex., (Schuman & Brace, 1954; Bader & Lehmann, 1965; Gingerich, 1974; Cuozzo, 2008) but see (Emery-Wetherell & Davis, 2018) for a counter-example). 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 (Alvesalo & Tigerstedt, 1974; Hlusko *et al.*, 2011). Second, successive molars have higher levels of fluctuating asymmetry, or non-inherited phenotypic

variation usually attributed to developmental instability (Sofaer *et al.*, 1971; Alibert *et al.*, 1994). In microevolutionary contexts, the higher variance has been attributed to longer exposure to or greater phenotypically plastic sensitivity to environmental influences (Butler, 1983; Townsend *et al.*, 2009), or "diminished genetic control" (Harris, 2003). The emerging interpretation is that greater phenotypic variation between individuals 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. Similar findings and discrepancies in interpretation have been found in vertebrate limbs, another segmented structure (Hallgrímsson *et al.*, 2002; Stepanova & Womack, 2020; Rothier *et al.*, 2023). The largely separated nature of macroevolutionary and microevolutionary inquiry (Rolland *et al.*, 2023) may explain why this discrepancy has not been noticed before, to our knowledge. It highlights the need for studies that bridge the two scales.

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 use two complementary approaches to test two complementary explanations for the microevolutionary interpretation of reduced evolutionary potential in successive segments (hereafter: Reduced Potential model) using the mammalian molar tooth module as a study system. Both explanations focus on how decreasing heritability is partitioned among causes. The first could be that reduced heritability could be caused by reduced levels of additive genetic

variation in successive molars, indicating a stronger discordance between microevolution and macroevolution. The second explanation is that reduced heritability could be caused by increased levels of environmentally induced variation, which may mask concordance between the two levels of evolution.

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 (Futuyma & Kirkpatrick, 2017). To address this interpretation, we conducted a systematic review of patterns of heritability down the molar row, then used these results to calculate evolvability. 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 (Houle, 1992; Hansen & Wagner, 2023). 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) (Houle, 1992). 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 (Hansen *et al.*, 2011). 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 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 (West-Eberhard, 1989). Environments may refer to climatic conditions, such temperature inducing trait change (Gupta & Lewontin, 1982), as well as other external conditions, such as nutrition availability (Sciulli *et al.*, 1979), as well as highly local conditions, such as slight differences in the developmental microenvironment surrounding symmetric structures, known as developmental instability (Leamy & Klingenberg, 2005). Resulting structures share genotypes and should be phenotypically perfectly symmetrical, but often carry a small amount of fluctuating asymmetry (Van Valen, 1962).

Increasing downstream phenotypic plasticity could refer to at least two different patterns. It is important to differentiate between them to better link patterns to processes. In this case, differentiation allows us to study types of developmental processes that form molar teeth. We assembled 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 (Holloway *et al.*, 1961; Sciulli *et al.*, 1979; Blankenship-Sefczek *et al.*, 2024).

Specifically, trait means may not respond to the environment in the same way as trait variances (Fig. 1). An environmental condition that induces consistent changes in developmental pathways can cause a change in trait means in a sample. For example, reduced growth rates

could affect trait means, but would not necessarily affect variance (Miller & German, 1999; Schwab & Moczek, 2016). 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 (Polly, 1998). We refer to a situation where a single environmental change has an increasingly strong effect on downstream segments as one of decreasing environmental canalization (Hallgrímsson *et al.*, 2002).

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 (Palmer & Strobeck, 1986; Hallgrímsson *et al.*, 2002). In the case of poor nutrition, increased developmental instability could be caused by decoherence, or a loss of co-regulation of traits driven by disruptions to gene regulatory networks when homeostasis is lost (Lea *et al.*, 2019). Therefore, in this study we assess our expectations through both means and variances.

Second, more specific 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 (Hlusko *et al.*, 2006). Molars also reach their maximum length earlier in development, before they reach maximum width (Christensen *et al.*, 2023), 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 (Miller & German, 1999; Lui & Baron, 2011), 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 (Thesleff & Sharpe, 1997; Young *et al.*, 2015). 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 (Kavanagh *et al.*, 2007). 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 (Hallgrímsson *et al.*, 2002).

Alternatively, effects may not compound. Although there is evidence of compounding in processes producing activation/inhibition ratios that permit enamel knot formation (Kavanagh *et al.*, 2007), that system does not require compounding in other processes like a general growth mechanism which might also affect molar sizes in conjunction with enamel knot dynamics. This hypothesis of multiple influences on size, some compounding and some not, is consistent with prior findings. Molar size phenotypes are incompletely described by a single model of compounding developmental process (Roseman & Delezene, 2019; Vitek *et al.*, 2020). 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 (Hlusko *et al.*, 2011; Hardin, 2020). 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 (Butler, 1983; Townsend *et al.*, 2009). Either model could explain previously observed patterns of decreasing heritability and increasing phenotypic variance down the tooth row (Kavanagh *et al.*, 2007; Riga *et al.*, 2014; Denes *et al.*, 2018), 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 conducted a systematic literature review (Supplementary Methods (Page *et al.*, 2021)). The objective of this systematic review was (A) to assess the consistency of the evidence for the common perception that heritability decreases down the molar row, and (B) to use reported parameter values to calculate evolvability and evaluate whether it has any consistent pattern down the molar row. Trait heritability values and their standard errors were collated for analysis. To meet minimum criteria for calculating evolvability, publications needed to report the following for buccolingual width or mesiodistal length for at least two lower 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

202 = $h^2 * V_P / m^2$. Error in evolvability was estimated by resampling: a simulated sample of 1,000
203 additive variance estimates was calculated by sampling a normal distribution with the mean and
204 standard error of a given sample from the literature. The resulting simulated estimates were then
205 summarized as a standard deviation, which was plotted around each estimate. Given the range of
206 study designs, we did not conduct a meta-analysis of patterns across successive teeth. Instead, we
207 tabulated the number of datasets that reported higher, lower, or ambiguous levels of heritability
208 or evolvability in downstream molars. Some datasets are right and left sides from the same
209 individuals, which we visualized separately for clarity but for which we included only one side,
210 the left, in the tabulation.

211 *Trait Plasticity*

212 To test Predictions 2 and 3, we leveraged data collected from a previously conducted
213 controlled experiment originally intended to study the effect of maternal malnutrition on insulin
214 action and adipose tissue in male offspring of Wistar Han rats (*Rattus norvegicus*; Crl:WI[Han]
215 strain). This type of laboratory study used inbred lines to control for genetic variation between
216 individuals, allowing us to assume that all phenotypic variation was due to some component of
217 environment. Thus, by comparing phenotypic patterns between traits and between experimental
218 groups we could study the impact of environment on traits. In this case, the main environmental
219 variable that differed between control and experimental groups was the quality of the maternal
220 diet throughout gestation and suckling (8% low-protein experimental diet group vs. 20% protein
221 control diet group), and we measured its effect on offspring phenotype (Martin-Gronert *et al.*,
222 2016; Ward *et al.*, 2021). Nutritional variation is known to influence dental phenotypes,
223 providing a high likelihood of observing environmental impacts in our study (Holloway *et al.*,
224 1961; Miller & German, 1999; Calsa *et al.*, 2022). 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 (Larson & Bader, 1976). 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 E16-17 (Larson & Bader, 1976; Christensen *et al.*, 2023). 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 (Larson & Bader, 1976; Kavanagh *et al.*, 2007). 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 (Christensen *et al.*, 2023), 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 (Martin-Gronert *et al.*, 2016; Ward *et al.*, 2021). 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 (Cignoni *et al.*, 2008). 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 (Polly, 1998), we calculated percent measurement error for each trait (Yezerinac *et al.*, 1992).

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 resampling approach (N replicates = 10,000) to test the hypothesis that one trait was more variable (larger CV) than another trait compared to what would be expected by chance. We specifically tested hypotheses that CV should increase between successive widths (one-tailed), between successive lengths (one-tailed), that it should differ between length and width (two-tailed), and whether it differed between control and experimental groups (two-tailed). Bootstrap resamples of low-protein CVs were used to generate the null hypothesis distribution 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) (Houle, 1992). To evaluate whether effect size differences were significant, we used a bootstrapping resampling approach. 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 (Holm, 1979). All analyses and visualizations were conducted in R version 4.4.1 (R Core Team, 2015) using packages `dplyr`, `reshape2`, `ggplot2`, `ggthemes`, and `patchwork` (Wickham, 2007, 2009; Wickham *et al.*, 2023; Arnold, 2024; Pedersen, 2024).

Results

Evolvability

Eight reports of 536 items recovered in literature searches reported length or width heritability for multiple molars, including two records found by searching the citations of six studies recovered from database searches (Dempsey & Townsend, 2001; Hlusko *et al.*, 2011) (SI Fig. 1). Seven samples from five of those eight reports met all three criteria for calculating evolvability (Bader, 1965; Leamy & Bader, 1968; Leamy & Touchberry, 1974; Hlusko *et al.*, 2011; Hardin, 2019). Evolvability (I_A) patterns were generally ambiguous or increasing along the molar row for both lengths and widths (Fig. 2). Heritability (h^2) patterns were generally ambiguous or decreasing along the molar row (SI Table 1-2).

Trait Plasticity

Measurement error was low across traits (<2%, Table 1). All six 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 phenotypic variances (Townsend *et al.*, 2009). Phenotypic variances are higher in the lengths of later-forming teeth. However, a more systematic review and comparison of published heritability values does not support a consistent link between higher downstream variances and lower downstream heritability or evolvability. The largely ambiguous patterns for widths in both variance-based measures appears to be more common than has been previously recognized. 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. Our results are not attributable to measurement error, as has potentially been an issue in other studies of variation (Polly, 1998).

Phenotypic Plasticity in Serially Segmented Structures

Multiple processes are potentially mixed together in the characterization of later-forming molars as more sensitive to environmental influence. The discordant patterns of phenotypic plasticity in trait means vs. trait variances allows us to distinguish between two different types of

environmentally related processes and their potentially different relationship to serial segments. This finding is robust to the limitations of our study. We acknowledge that because the experimental design did not include observations of how long each molar took to form, we cannot test the alternative hypothesis of environmental canalization scaled by exposure time. With a single sample of one species included in our study of plasticity, it is also possible that we did not capture a stronger effect in later-forming molar traits that may be present in other species or genotypes (Holloway *et al.*, 1961; Tonge & McCance, 1973). However, none of these limits to generalizability contradict the point that the phenotypically plastic responses of trait means and variances are decoupled in later-forming molars. Different developmental mechanisms either respond differently to the environment or respond to different components of the environment.

Instead of a single mechanism underlying a Reduced Potential model and impacting both trait means and variances, we hypothesize that at least two separate types of environmentally sensitive processes impact molar size traits. The first process we hypothesize is an effect of nutritional environment on general organismal growth, which affects molars to the degree that they are environmentally canalized, or developmentally independent of overall organismal growth. The developmental non-independence from general growth across molars need not compound down the molar row, allowing for nonlinear patterns like those we observed in our study, especially for width measurements (Fig. 3A, Table 1), with no requirement for a shared pattern across structures like teeth, limbs, and vertebral segments with very different developmental underpinnings.

The second process we hypothesize is 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 (Sofaer *et al.*, 1971; Gingerich,

1974; Hlusko *et al.*, 2011). Our data collection protocol did not permit us to evaluate fluctuating asymmetry, a more standard statistic for inferring developmental instability (Palmer & Strobeck, 1986; Hallgrímsson *et al.*, 2002), but prior studies have found a consistent pattern of downstream increase, consistent with our explanation (Sofaer *et al.*, 1971; Alibert *et al.*, 1994). The other phenomenon that would produce such an effect, antisymmetry, is not a feature of mammalian tooth sizes (Van Valen, 1962; Leamy *et al.*, 2005).

Unlike environmental canalization, developmental instability might compound more generally across serial structures if it were caused by iterative expression of a gene regulatory network. Notably, molar length fits expected patterns of this model (Table 2), given that length is more conceptually closely tied to a model of compounding activation/inhibition dynamics than molar width (Kavanagh *et al.*, 2007). The lack of significant difference in CV between the two experimental groups (Table 1) suggests that this compounding instability is not related to nutrition, but instead may be a more general feature of certain developmental mechanisms (Hallgrímsson *et al.*, 2002). When successive segments interact with each other through expression of one gene regulatory network, any stochastic developmental instability in the network 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 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.

In sum, our results contradict the commonly held hypothesis that increasing developmental instability is mechanistically related to increasing sensitivity to environment and decreasing environmental canalization (Hallgrímsson *et al.*, 2002). Although compounding developmental instability can potentially explain patterns of variance-related statistics, it cannot explain either the patterns of environmental canalization or of evolvability. If this model holds, then any set of serial segments that share a single gene regulatory network may show this pattern of increasing variance, not necessarily tethered to increasing sensitivity to the environment.

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 (Hlusko *et al.*, 2006). 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 (Hlusko *et al.*, 2016). 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 (Hlusko *et al.*, 2011). 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 (Hlusko *et al.*, 2016).

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 (Hallgrímsson *et al.*, 2002)) 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 (Brown *et al.*, 2017; Oyanagi *et al.*, 2019; Christensen *et al.*, 2023). 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 (Tagliabracci *et al.*, 2015; Chrudinová *et al.*, 2024). 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 (Estrella *et al.*, 2024). 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 (Christensen *et al.*, 2023).

Reconciling Macro- and Microevolutionary Interpretations

Overall, we found equal or increasing evolutionary potential down the molar row in microevolutionary contexts. This interpretation reconciles microevolutionary and macroevolutionary interpretations of molars and segment patterns, with implications for other serially segmented structures (Mongle *et al.*, 2022). 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. To the contrary, none of the samples had lower evolvabilities in the M_3 than in upstream molars. Instead, the M_3 either had equal evolvability, suggesting a role for relaxed selection on the M_3 compared to upstream molars (Gingerich & Winkler, 1979; Mongle *et al.*, 2022), or had increased evolvability, suggesting that there is also comparatively more additive

genetic variation for selection to act on in these teeth (Fig. 2). This perspective may explain why the M_3 has been considered so taxonomically useful in certain rodents (Barnosky, 1993; Polly, 2003), despite their general characterization as a noisy, non-ideal choice for taxonomic study (Gingerich, 1974; Vezzosi & Kerber, 2018). Mammalian molars appear to be another case where heritability is not as informative of evolutionary potential as evolvability (Hansen *et al.*, 2011).

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 (Rothier *et al.*, 2023). 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 (Ungar, 2010). 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 $\bar{x}$ 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; $\bar{x}$, 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.

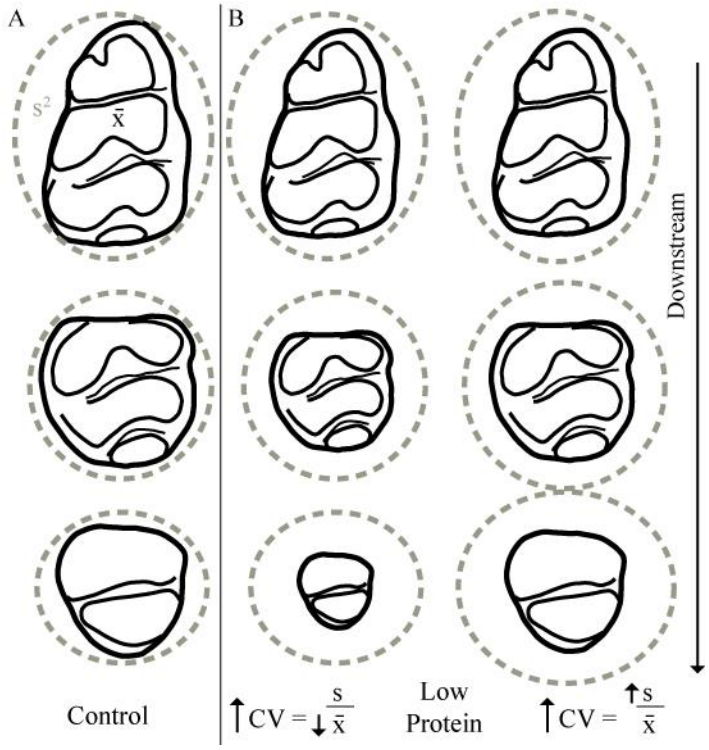
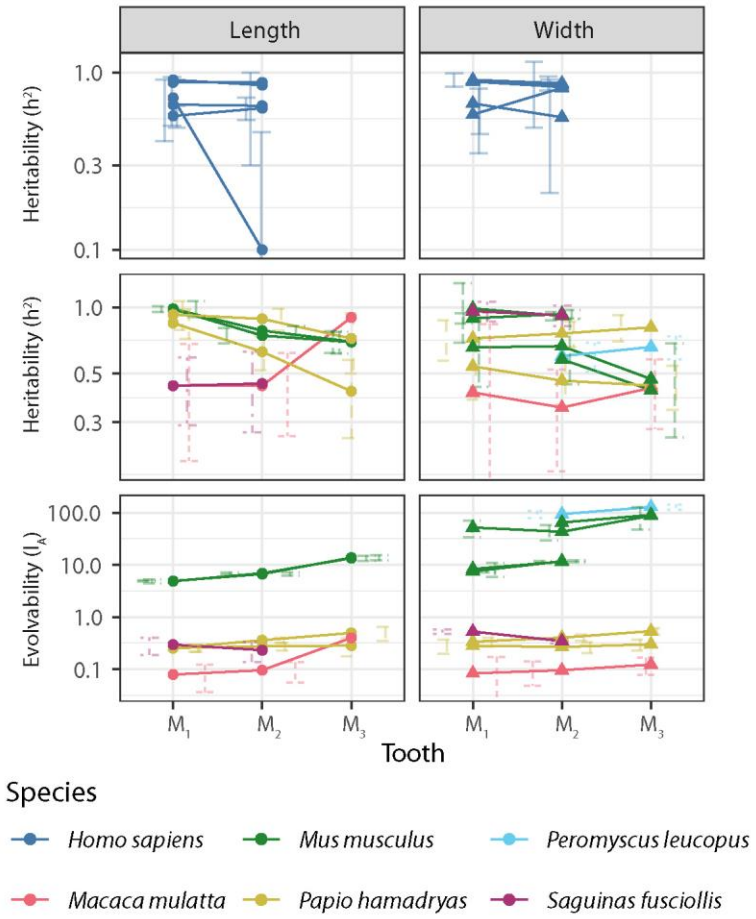


Figure 1. Conceptual diagram of two different ways in which a low-protein diet, a kind of environmental perturbation, could generate a pattern of increasing coefficients of variation (CV) in downstream tooth sizes, matching previously published patterns of variation in tooth size. A. Control group, in 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; $\bar{x}$, sample mean.



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655 Figure 2. Evolvability (I_A) and heritability (h^2) calculated from a systematic review of published
656 samples. Lines connect traits measured from the same sample. Top panel shows values from
657 studies that did not report enough information to calculate evolvability. Middle panel shows
658 samples with paired evolvability estimates (bottom panel). Bars indicate standard error, jittered
659 to better show overlap. Note that the y-axis is on a logarithmic scale to better display the range of
660 evolvability estimates.

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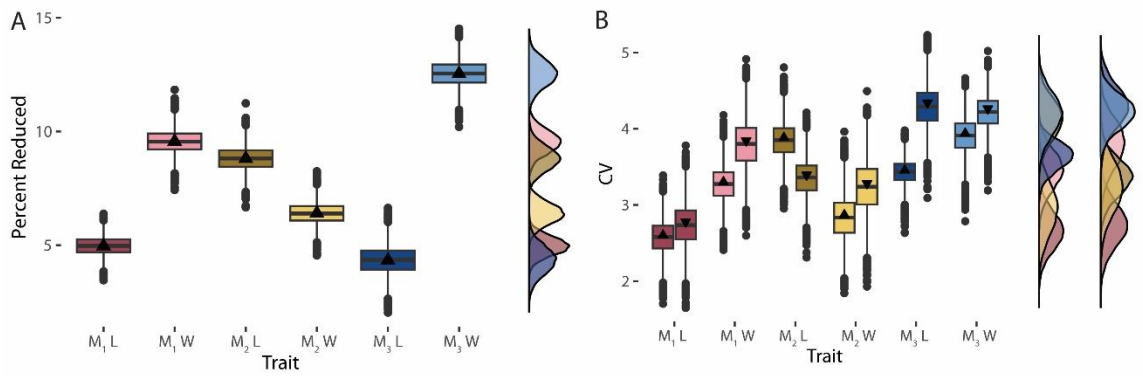

Figure 3. Effect of low-protein nutrition on the mean and variance of molar size traits, standardized by mean trait size. Box and whisker plots show median (thick bar) and mean (triangle). Corresponding density plots are to the right. Note that density plots height may not match box plot median because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

Supplementary Figure 1. PRISMA 2020 flow diagram of report retrieval.

Supplementary Table 1. Characteristics of studies recovered from the systematic literature search.

Supplementary Table 2. 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.