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SYMPOSIUM

Integration and the Developmental Genetics of Allometry

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Synopsis Allometry refers to the ways in which organismal shape is associated with size. It is a special case of integration, or the tendency for traits to covary, in that variation in size is ubiquitous and evolutionarily important. Allometric variation is so commonly observed that it is routinely removed from morphometric analyses or invoked as an explanation for evolutionary change. In this case, familiarity is mistaken for understanding because rarely do we know the mechanisms by which shape correlates with size or understand their significance. As with other forms of integration, allometric variation is generated by variation in developmental processes that affect multiple traits, resulting in patterns of covariation. Given this perspective, we can dissect the genetic and developmental determinants of allometric variation. Our work on the developmental and genetic basis for allometric variation in craniofacial shape in mice and humans has revealed that allometric variation is highly polygenic. Different measures of size are associated with distinct but overlapping patterns of allometric variation. These patterns converge in part on a common genetic basis. Finally, environmental modulation of size often generates variation along allometric trajectories, but the timing of genetic and environmental perturbations can produce deviations from allometric patterns when traits are differentially sensitive over developmental time. These results question the validity of viewing allometry as a singular phenomenon distinct from morphological integration more generally.

Introduction

Most morphological and physiological traits covary with organismal size (Huxley 1932; Gould 1966; Schmidt-Nielsen 1984). For morphological traits, variation that correlates with size is usually important, commonly accounting for a large proportion of the total variance (Mosimann 1970) and often invoked as an explanation for evolutionary trends or constraints (Gould 1966; Lande 1979). For this reason, allometric variation is familiar and commonly analyzed. Despite this familiarity, the developmental and genetic determinants of allometric variation are mostly unknown. This matters because understanding these determinants is necessary to make sense of allometry either as a source of variation within species or as a constraint on evolutionary trajectories.

To dissect the developmental-genetics of allometry, it is useful to view allometry as a form of integration. It is special, not in the sense of being qualitatively different from other determinants of covariation patterns, but because organismal size is so fundamentally important. Integration is the tendency for traits to covary as a consequence of variation in developmental processes (Hallgrimsson et al. 2009). Patterns of covariation arise when processes that influence multiple traits vary. There are many such processes and they act at different scales, times, and anatomical locations in development. The covariance structure for a set of traits is determined by multiple such processes that may interact, reinforce, or erase each other's effects. This is the palimpsest model of integration (Fig. 1)

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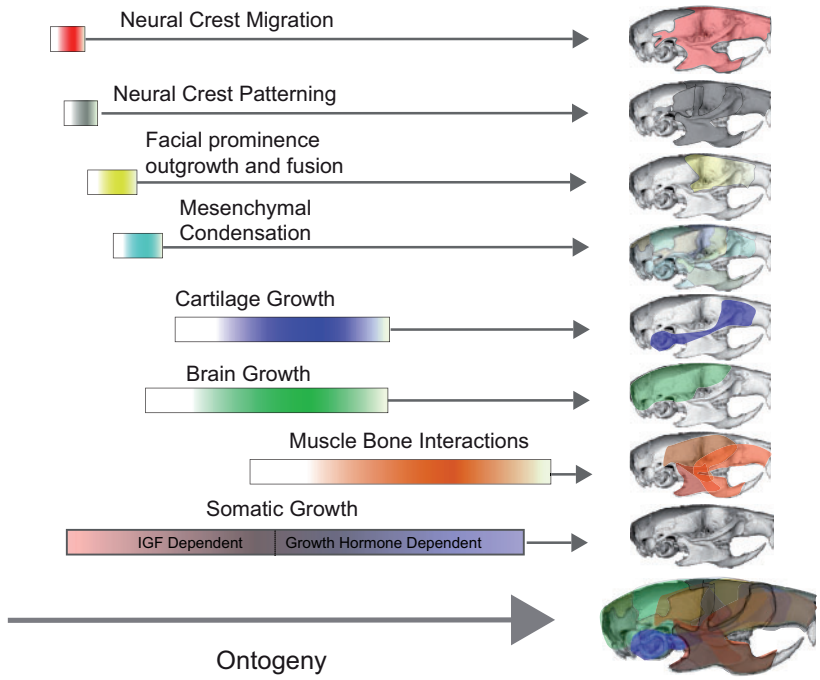

Fig. 1 The palimpsest model for the generation of covariation structure. The tissue-level processes listed are not exhaustive and intended only to illustrate the concept. Processes acting at different times and scales of development drive covariation among traits that may be transient or persist to influence the adult covariation structure.

(Hallgrimsson et al. 2009). Allometry can be defined either as covariation in traits due to variation in size or as the correlation of size and shape (Klingenberg 2016). These definitions of allometry differ in important ways (Klingenberg 2016), but they agree that allometric variation is a form of covariation – either among traits or in shape due to underlying variation in size. Viewed as a special case of integration, allometry is, therefore, the tendency for covariation to arise as a consequence of developmental processes that influence size (Fig. 1).

Allometric variation is generally divided into ontogenetic, static, and evolutionary levels (Cheverud 1982). Ontogenetic allometry refers to the tendency for shape or proportions to change across development while static allometry refers to the tendency for covariation of shape and size (or trait proportions with variation in size) for individuals at the same stage or age of development. Evolutionary allometry is the tendency for interspecific phenotypic differences to align with allometric variation; the general expectation is that evolutionary allometry aligns with static allometry within related species, although this is not always the case (Voje et al. 2014).

Allometry is occasionally discussed as a form of integration (Shingleton et al. 2007). More commonly, though, allometric variation is presented without reference to integration (Smith 1980; Stevens 2009; Gayon 2015) or as a special form of covariation that must be disentangled in studies of integration (Klingenberg 2016). This is probably due to the separate conceptual histories of allometry and integration. While morphological integration, formalized by Olson and Miller (1958), can be traced to Darwin's "laws of correlated growth" (Darwin 1859), allometry comes from the work of Julian Huxley (Huxley 1924). Huxley (1932) related his work to D'Arcy Thompson's ideas about the role of differential growth (Thompson 1917). However, his focus was on the description and functional consequences of scaling – or the change in proportion of a morphological or physiological trait with variation in size. Consequently, the literature on allometry has dealt mainly with the description and functional consequences of the scaling of various aspects of organismal form and function with variation in size.

Defining allometry as a form of integration reveals the key questions necessary to understand its developmental and genetic basis. First among these is what are the developmental processes that drive variation in size and, secondly, how do these processes produce the correlated effects on shape or sets of traits that manifest as allometric variation? To address this question, however, we first need to arrive at some understanding of what is meant by size. In this review, we consider these three questions and use examples from mouse and human craniofacial variation as illustrations. We argue that allometry

is complex. By this we mean that what counts as allometric variation consists of partly overlapping patterns of covariation that are driven by multiple underlying processes. These processes are related via common influences on organismal size.

What is an appropriate measure of size?

This obvious question is often missed in studies of allometry—particularly in those dealing with morphological allometry. In Schmidt-Nielsen's (1984) treatise on scaling, size refers to organismal size, usually measured as body mass. Similarly, for Pélabon et al. (2014), allometry refers to the covariation among traits that results from variation in body size, generally measured as mass. However, there are multiple ways to quantify organismal size. Surface area, volume, overall length or height, skeletal mass, among others, are all relevant measures of organismal size. Furthermore, geometric morphometric studies of allometry very often use the centroid size (or log centroid size) of the structure quantified in a particular study (Monteiro 1999; Mitteroecker et al. 2004; Gonzalez et al. 2013; Klingenberg 2016). Similarly, approaches that, following Mosimann (Mosimann 1970; Cheverud 1982), use the first Principal Component (PC) of variation to quantify allometry are also using the size of the structure itself rather than body size. The implicit assumption here is either that the size of the structure, as measured by centroid size, is an appropriate proxy for organismal size or that the size of the structure being measured is inherently meaningful as a measure of size.

These assumptions are rarely stated. This criticism applies to analyses in earlier work from our group (Hallgrimsson et al. 2007). Clearly, it is not always the case that the size of a structure is a good proxy for body mass. Variation in the size of a skull, metacarpal, or fin may be determined by processes that are distinct from those that determine variation in overall body size. In many studies, the covariance of the size of the structure with body size is unknown. The assumption that the size of the structure is inherently meaningful as a measure of size is equally problematic. Some vertebrates have skulls with relatively large calvaria and small faces while others have very large jaws and very small calvaria. Skull centroid size is different in these extremes as on one hand, brain size and its developmental determinants is an important determinant of skull centroid size while on the other it may be the developmental determinants of jaw size. It is likely, therefore, that craniofacial allometry, as determined by the regression of shape on cranial centroid size will be different in those two extremes, although this question has not been examined systematically. Within mammals, species with larger skulls appear to have relatively longer jaws (Cardini and Polly 2013; Cardini 2019) and smaller mammals have relatively larger brains (Lande 1979; Schmidt-Nielsen 1984). There are likely functional and developmental reasons for why jaws and brains scale allometrically to body mass, but these relationships are confounded when the proxy for body mass is the size of the skull.

A similar part/whole problem actually occurs for body mass as well. Among mammal species, variation relative to size for body mass increases with size because the proportion of mass devoted to more variable body mass components tends to increase with mass (Hallgrímsson and Maiorana 2000). Specifically, as species mass increases, less mass tends to be devoted to viscera, cardiovascular, and neurological structures and more to fat and skeletal structures; on average, the proportion of body mass accounted for by the former varies less than the latter (Calder 1996). These body mass components have different developmental determinants and they differ in their tendency to vary within populations and species. This also means that body mass is not precisely homologous across the full range of body sizes in mammalian species.

The part/whole problem crops up in analyses of many other structures. In analyses of insect wings, allometric variation is very often measured by the covariance of wing shape and wing centroid size (Debat et al. 2003). In a recent example, Dellicour et al. (2017) analyze wing size and shape in three species of bees. Their conclusions deal with the purported lack of a common body-size wing shape allometry and yet body size is never measured. Instead, wing centroid size is assumed to be proxy for body size. To justify this assumption, they cite Outomuro and Johansson (2011). However, that study, which deals with damselflies, does not measure body mass either, also making the assumption that wing size is a proxy for body size. They justify this assumption based on separate, unpublished data, in which body length has a correlation with wing centroid size of $r = 0.741$. In other words, the fact that 55% (r^2) of the variation in centroid size is explained by variation in body length in damselflies is taken as validation for the use of wing centroid size as a measure of body mass in bees. This is obviously problematic. It raises the question, among others, of the significance of the 45% of variation in centroid size that is unrelated to body size. In *Drosophila*, classic work has shown that the ratios for wing size to measures of

body size vary among individuals in *Drosophila* and these ratios respond to selection (Robertson 1962). The covariance of wing size (and other traits) and body size is likely to vary among populations within species and among species of insects (Shingleton et al. 2009). As with the example of the face and skull, variation in wing size, body mass, body length, etc, will be driven by processes that are partially overlapping. Uncritical assumptions about the relationship between the size of a structure and overall organismal size will obscure these relationships and confound studies of allometry.

Understanding allometry within the broader context of morphological integration, however, can sharpen the focus on the complex underpinnings of size related variation in morphology. If we take seriously the claim that allometry is a form of integration, then what must be argued or reasonably proposed is that the measure of size is meaningful with respect to the developmental processes that influence the relationship between size and shape or form. For centroid size in shape studies, this is likely to be true in many instances simply because size and shape are measured for the same configuration. Yet simultaneously, complex and species-specific relationships between local and global size measures highlight why defaulting to centroid size is likely to be insufficient to the objective of understanding allometry itself.

Is allometry "a thing"?

Is allometry a distinct biological phenomenon? Viewed as a special case of integration, this question becomes whether the patterns captured as allometric variation are driven by a shared set of underlying processes. If this is the case, different measures of size should converge on a common allometric pattern of variation. The allometric signal recovered via different measures of size should also converge on a common genetic basis. Finally, variation in size produced via genetic or environmental perturbations should also converge on a common set of allometric effects. Here, we examine each of these contentions via examples from our studies of craniofacial variation in mice and humans.

Do different measures of size converge on a common axis of allometry?

Recently, we examined the question of whether different measures of size converge on a common pattern of covariation in human faces (Larson et al. 2018). Human facial shape, like most morphological features, varies with size (Gonzalez et al. 2011b; Mitteroecker et al. 2013; Freidline et al. 2015; Larson et al. 2018). Our study is based on 7173 Tanzanian and North American children aged 2–18 years. We asked whether the facial shape covariation associated with body mass, stature, centroid size, head circumference, and age converged on a common pattern. Facial shape correlated significantly with all of these variables in our sample and the effects are highly co-linear. This is expected if they converge on a common pattern. Figure 2A shows the facial variation associated with each of these factors when considered independently. Broadly, with the exception of head circumference, the effects are fairly similar. However, closer inspection of the shape vectors associated with each factor reveals distinctions among them in magnitude and direction (Fig. 2B). Analysis of the shape covariation associated with centroid size and body mass in Diversity Outcross mouse skulls (see below) shows a similar pattern (Fig. 3A). While the axes of shape covariation associated with these two size measures are grossly similar, they are by no means identical. The answer to the first question, at least in the human face and mouse skull, is thus somewhere in between the two alternatives. Different size measures are associated not only with overlapping but also distinct patterns of covariation.

Does allometric variation converge on a common genetic basis?

The second prediction of the hypothesis that allometry is a distinct biological phenomenon implies that patterns of allometry converge on a shared genetic basis. A fair bit is known about developmental perturbations that can alter allometry or modulate change along allometric trajectories in various animal models. Systemic endocrine factors such as insulin-like growth factor (IGF), ecdysone, growth hormone, or insulin influence overall size and, when perturbed, can modulate allometric variation as well as size (Stern and Emlen 1999; Nijhout 2003; Shingleton et al. 2008; Gonzalez et al. 2013). However, this does not mean that those same mechanisms are responsible for population- or species-level variation in allometric trajectories or in trait proportions. Instead, allometric variation, like many other aspects of morphology is highly polygenic and may be associated with genetic variants of uncertain relation to growth or known mechanisms that regulate body size.

In our recent work on the genetics of human facial shape, the pattern of shape covariation with facial centroid size is among the most heritable aspects of facial shape variation (Cole et al. 2017). Facial shape allometry—in this case the covariance of facial

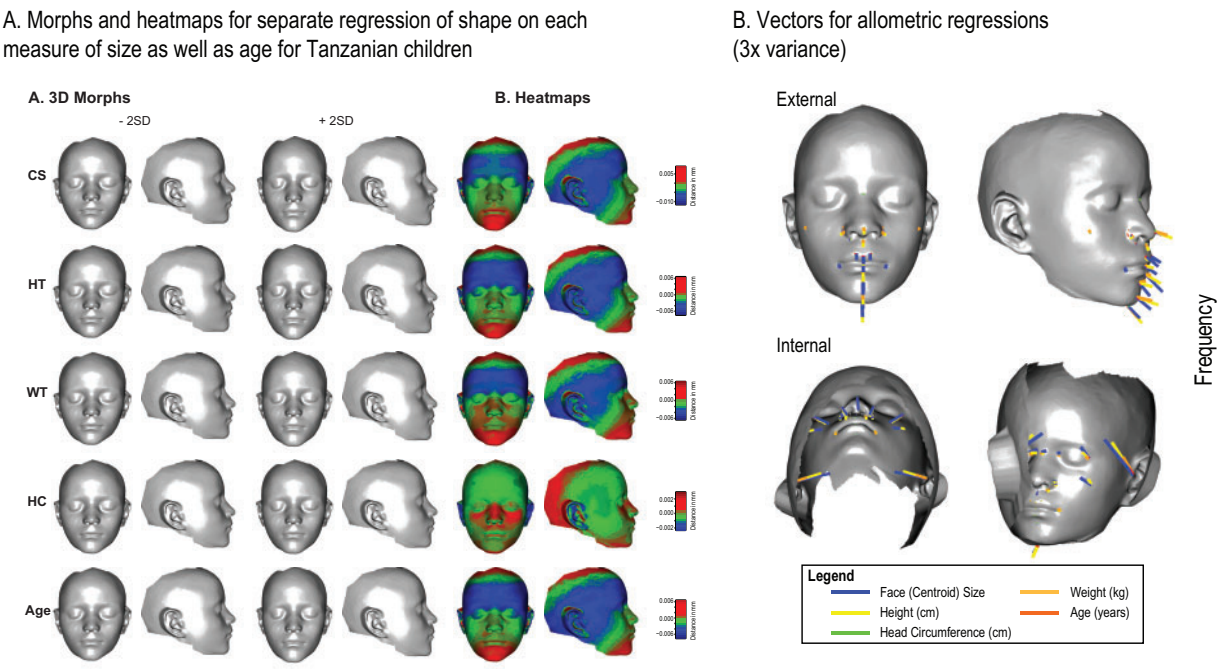

Fig. 2 The covariation of facial shape with different measures of size in children. (**A**) shows the covariation of human facial shape with different measures of size. (**B**) shows the directions of change associated with each of the regressions of facial shape on a measure of size. Panel A is taken from Fig. 6 in Larson et al. (2018) and panel B is Fig. 8A in the same paper.

shape and size—is associated with variation in PDE8A (Cole et al. 2016). PDE8A is a cyclic nucleotide phosphodiesterase that, while expressed in the face, has no known mechanistic connection to modulation of cranial size.

In mouse limbs from the Lg/Sm intercross, Pavlicev et al. (2008) identified relationship QTL (rQTL), loci producing variation in the relationships among traits, for limb element size and body mass. Of the 11 rQTL, 8 are also associated with variation in the traits themselves. They determined that the 11rQTL effects that reached genome-wide significance were explained primarily by differential epistasis at 40 other loci. In a related study, Pavlicev et al. (2013) showed that rQTL for hind versus forelimb proportions tend to involve regulatory rather than coding regions of the genome. These studies suggest that variation in allometry is likely to be polygenic with a complex architecture involving multiple gene interaction effects.

In a sample ($N = 997$) of Diversity Outbred (DO) mice (Churchill et al. 2012), we considered whether the "allometric" variation associated with body mass on the one hand and cranial centroid size on the other converge on a common genetic basis. The DO mice are derived from long-term outbreeding of crosses among eight founder mouse strains. These founders are the same strains used in the Collaborative Cross project (Churchill et al. 2004; Iraqi et al. 2012). We modified the multivariate-genotype phenotype (MGP) method of Mitteroecker et al. (2016), designed for a two-way cross, to the eight-founder DO design (Aponte JD, et al., manuscript in preparation). We use regularized partial-least squares to compute latent variables that maximize the covariation between the full genomic and phenotypic variance–covariance matrices. Individual single nucleotide polymorphism (SNP) effects can be quantified as loadings on to these latent variables. We quantified skull shape using 68 3D landmarks from microCT scans as in earlier work (Percival et al. 2016, 2018).

To determine whether body mass and centroid size recovered similar patterns of genetic effects, we regressed shape on centroid size and body mass using ProcD Allometry in Geomorph (Adams et al. 2014). We then obtained and compared the individual SNP loadings from an MGP analysis of the resulting two sets of allometry regression scores. We limited the analysis to the 3197 SNPs (out of 48k) with non-zero loadings (defined as coefficient $>1 \times 10^{-6}$) for both body mass (BM) and centroid size (CS). We make no claim here that the individual SNPs in this set reach genome-wide significance for association with either BM or CS. SNPs for BM and CS allometry are positively correlated ($r = 0.45$) (Fig. 3B). When we plot these SNPs by whether they are associated with BM, CS, or both, we see

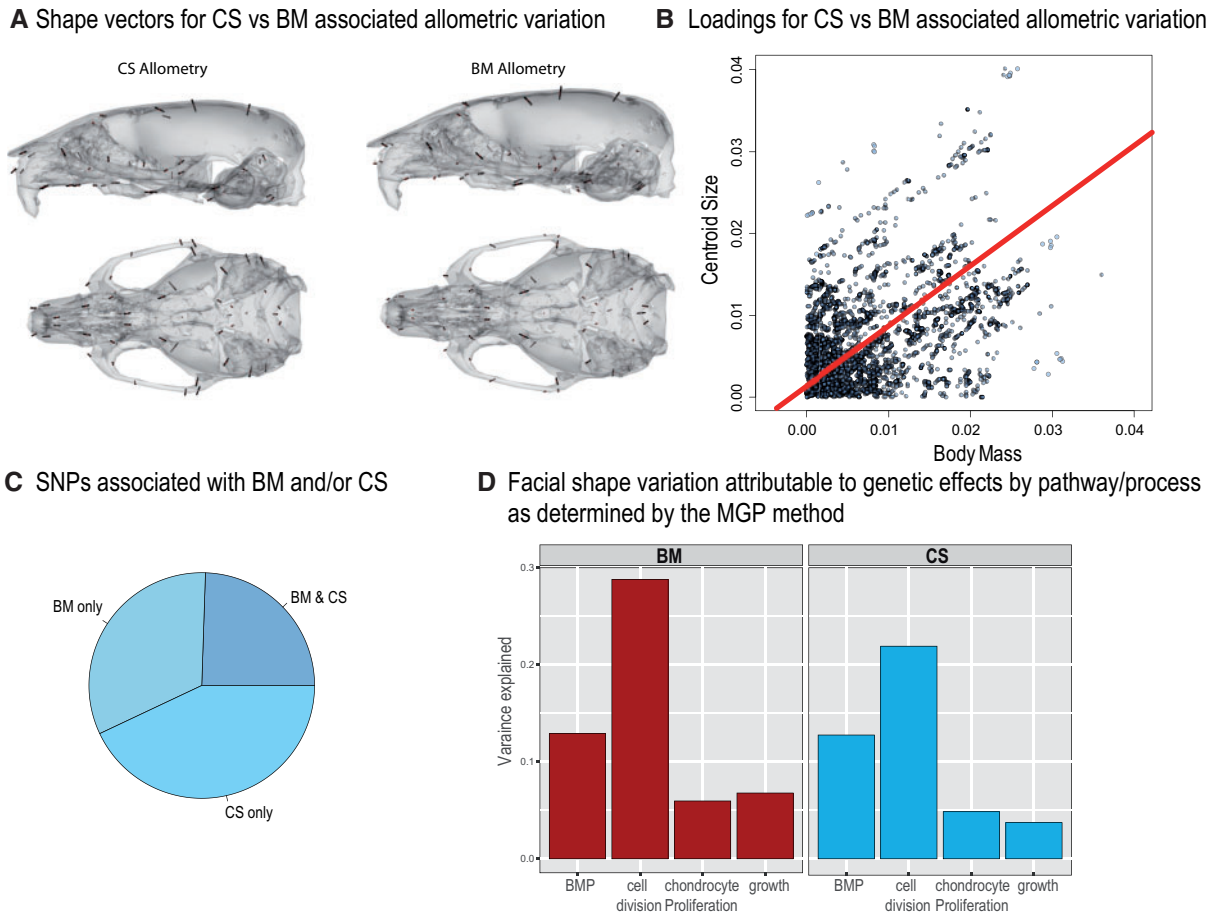

Fig. 3 The genetic basis for "allometric" variation as quantified using cranial centroid size and body mass. (**A**) shows the shape vectors associated with the regressions of craniofacial shape on cranial CS and BM. (**B**) shows absolute loadings for variation at SNP and allometric variation as quantified using CS and BM. (**C**) shows the distribution of SNPs with effects on allometric variation as quantified using CS, BM or both. This shows that a large proportion of SNPs affect only or the other form of allometric variation. (**D**) shows the proportion of variance explained by MGP analyses of SNPs annotated to be associated with the BMP pathway, cell division, chondrocyte proliferation and growth. (**E**) shows QTL scan results for allometric variation as determined by regressions of facial shape on cranial centroid size and body mass.

that they divide roughly evenly across these three groups (Fig. 3C). Thus, there is some support for a common genetic basis for BM and CS allometry, and also evidence for unique genetic determinants of BM and CS specific allometric patterns of covariation.

To examine this further, we used the MGP approach to associate variation in SNPs in genes associated with BMP signaling, chondrocyte proliferation, cell cycle, and overall somatic growth with the allometric variation associated with CS or BM. These results, shown in Fig. 3D, show that these processes explain similar amounts of variation in CS or BM-related allometric variation. In Fig. 3E, we show QTL scan data for allometric variation as determined by regressions of facial shape on cranial centroid size and body mass (Broman et al. 2019).

Here, the variable mapped is the common allometric component allometric regression score as quantified using ProcD.lm (Adams et al. 2014). This plot shows nicely the overall concordance of genetic signals for allometric variation as determined by these two measures of size. Overall, these results support the contention that the developmental-genetic basis for allometric variation is largely (but not entirely) common to different characterizations of size.

Do environmental and genetic perturbations to growth modulate a common axis of allometric variation?

In a classic study, Cheverud (1988) showed that genetic (G) and phenotypic (P) variance–covariance matrices for morphological features are surprisingly similar when effective sample sizes were sufficient to estimate G with precision and accuracy. This observation that genetic and phenotypic covariances matrices are very similar has been repeated in a variety of contexts (Roff 2012) although it is not universally supported (Willis et al. 1991). At the heart of these putative associations between G, P, and E (environmental) matrices is the "Cheverud Conjecture" (Roff 1995), which states that P will be similar enough to G such that P may be substituted for G when performing evolutionary analyses. This implies that variation in genetic and environmental influences have similar relationships with phenotypic variation. Cheverud (1984) made the observation that we expect covariation from these different sources to be similar because the same developmental processes structure both the environmental and genetic influences on morphology. Our hypothesis about genetic versus environmental influences on allometry, are based on his observation.

We have tested this hypothesis in human facial shape in a large sample ($N = 5844$) of Tanzanian children (Cole et al. 2018). These children were enrolled in schools in the Mwanza region of Northwestern Tanzania. These schools vary significantly in socioeconomic status, and the children vary in growth outcomes as determined by growth attainment relative to age by World Health Organization standards (Fig. 4A). As we had full genome data for (Illumina 2.5 M+Exome SNP Array) for 3605 individuals, we were able to calculate genetic and environmental variance–covariance matrices using Genome-Wide Complex Trait Analysis (Yang et al. 2011). The environmental and genetic covariances matrices are significantly correlated at 0.584 ($P < 0.001$, Mantel's test with 1000 permutations) (Fig. 4B) in spite of the fact that there is a negative bias inherent to the estimation of this correlation (Cheverud 1995). This negative bias arises from the tendency for estimation errors to be negatively associated as a result of the fact that P is the sum of G and E. Growth faltering and growth attainment relative to age are mostly due to environmental variance and strongly correlated with PCs 1 and 3 of the environmental variance–covariance matrix (Fig. 4C–E). Growth faltering also correlates significantly with the first genetic PC. Growth faltering correlates with both static and ontogenetic allometry, but in opposite directions (Fig. 4F). Children who are small relative to age due to growth faltering have faces that correspond to those expected in taller and younger children.

These growth faltering results support the hypothesis that environmental and genetic perturbations can modulate allometric variation via a common developmental basis. This is consistent with other studies of environmental and genetic modulation of growth. In a variety of systems and organisms, such studies have shown that modulation of growth, whether due to genetic or environmental perturbation, tends to alter morphology along trajectories predicted from either ontogenetic or static allometry (Shingleton et al. 2007; Mirth et al. 2016).

Nutritional stress and relative brain size

For allometric variation in craniofacial morphology, an additional complication is the differential effect of nutritional stress on brain growth. Maternal and early childhood nutritional stress models have shown that nutritional stress affects brain size less than height or weight (Kramer et al. 1989; Fang 2005; Samuelsen et al. 2007; Baker et al. 2010; van den Broek et al. 2010). This phenomenon, termed "brain sparing" has also been shown in animal models of nutritional stress (Reichling and German 2000; Gonzalez et al. 2016). Nutritional stress also alters craniofacial shape in rat models (Lobe et al. 2006; Gonzalez et al. 2011a,c). In a study of the effect of maternal low protein nutritional stress, Gonzalez et al. (2016) showed that nutritionally stressed neonates have relatively larger brains and smaller faces. In other words, the craniofacial effects of nutritional stress are, at least in part, due to its differential effects on brain growth compared to other aspects of the craniofacial complex.

Interestingly, the possibility that brain size and body mass may generally scale allometrically (non-isometrically) has not been seriously considered within the biomedical literature on brain sparing. Instead, the assumption is that brain and body mass should scale isometrically at birth (or in preterm) infants. Therefore, the observation that small

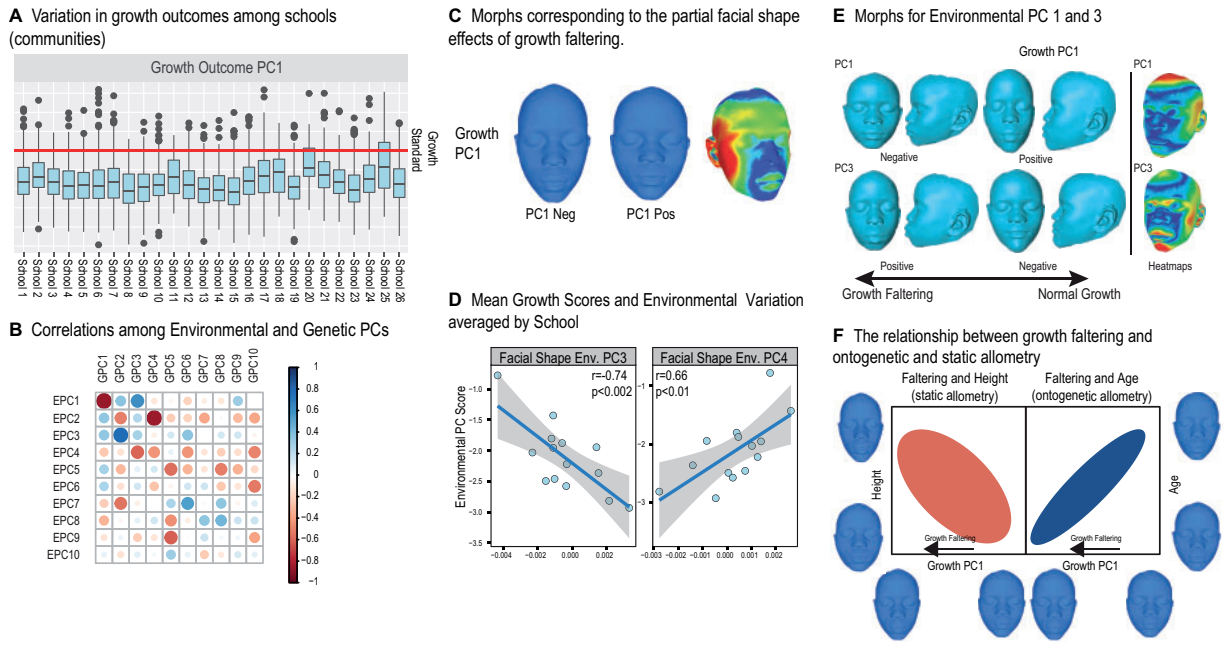

Fig. 4 Growth faltering and facial shape in Tanzanian children. (**A**) plots growth outcomes by school for the entire sample. Growth faltering is the first PC of all growth attainment variables relative to age. (**B**) visualizes the correlation matrix for the first 10 PCs of the genetic and environmental variance covariance matrices. (**C**) shows the variation in facial shape associated with growth faltering. (**D**) plots school average values for growth attainment and environmental PCs 1 and 3. (**E**) shows the facial shape variation associated with environmental PCs 1 and 3 as morphs and heatmaps. (**F**) summarizes the relationship between growth faltering and the partial regressions of facial shape on height and age. These figures are taken from Figs 3, 7 and 8 in Cole et al. (2018).

neonates have proportionately larger brains has required special explanations such as the preferential shunting of blood to the head as an adaptation to nutritional stress (Cahill et al. 2014). Intrauterine growth restriction (IUGR) is defined on the basis of birth weight or gestational size relative to age and the commonly applied standard is the 10th percentile relative to a reference population (Geraci et al. 2018; Wollmann 1998). Presumably this means that, regardless of population health-level indicators, 10% of the population is born with IUGR. Within this group, infants are designated as having "symmetric" or "asymmetrical" growth retardation based on whether their head circumferences are larger than expected relative to body mass. Clinically, this is often diagnosed on the basis of an absolute threshold, the ratio of head circumference to abdominal circumference, or to body mass (Platz and Newman 2008). These ratios are good predictors of poor health outcomes in low birth weight infants (Platz and Newman 2008; Suhag and Berghella 2013).

From an evolutionary perspective, the clinical literature on brain sparing and IUGR is difficult to understand. Do children with very low birth weight have relatively larger brains just because brain size does not scale isometrically with body mass? If so, is brain sparing a real phenomenon and, if it is not, then are we missing the bigger picture by focusing on causes and consequences of asymmetric growth retardation? Clearly, this is an area that would benefit from applying the theory that has been developed around the study of allometric variation in evolutionary biology and evolutionary developmental biology. A recent study of a very large sample (150,000) of pre-term infants shows that the relationship between head circumference and body mass is nonlinear, with the slope declining above 1000 g (Geraci et al. 2018). There is little evidence in these data for meaningful thresholds or use of constant ratios for diagnosis. Instead, as the authors argue, these measures can only be understood when considered jointly and when allometric scaling is taken into account (Geraci et al. 2018).

Craniofacial allometry and the role of differential timing

Parts of organisms grow at different rates over developmental time. It is not surprising, therefore, that timing has loomed large in studies of allometry. Differential timing, was central to much of the literature on allometry prior to the advent of mechanistic developmental biology (Huxley 1924; 1932; Gould

1977). In insects, the timing of hormonal events that regulate body size and the differential sensitivity of developing structures to these events is central to both the control of body size and its allometric consequences (Davidowitz et al. 2004; Shingleton et al. 2007). The relationship of brain to skull growth also illustrates both the importance of timing for allometric variation and the sensitivity to developmental drivers of allometric variation to both genetic and environmental effects.

We have investigated the influence of modulation of growth hormone on craniofacial morphology and skeletal growth (Kristensen et al. 2010; Gonzalez et al. 2013). In these studies, we used mice with a spontaneous null mutation in the growth hormone releasing hormone receptor (*Ghrhr*). These mice grow normally during prenatal development, but exhibit a dramatic reduction in postnatal somatic growth due to dramatic reduction in secreted growth hormone (Godfrey et al. 1993). Homozygous mutant mice were treated with growth hormone at either postnatal Day 21 or Day 35 and compared to untreated mutant homozygotes and heterozygous littermates, which have normal growth hormone synthesis (Fig. 5A). We tested the hypothesis that the timing of growth hormone treatment affects not only the amount of recovery on somatic growth but also the degree and direction of change in craniofacial shape.

Growth hormone treatment produced a partial recovery in body mass for *Ghrhr* null mice (Kristensen et al. 2010) and a roughly proportional recovery in cranial centroid size (Gonzalez et al. 2013). However, craniofacial shape differed significantly among the treatment groups (Fig. 5). This appears to be largely due to differential responsiveness to growth hormone treatment by the brain and the rest of the skull. As shown in Fig. 5B, size-recovery is most marked in the face. In comparison, the basicranium shows less responsiveness to growth hormone while the cranial vault does not appear to change at all. As seen in Fig. 5C, brain growth is affected by the *Ghrhr* mutation but it is not affected by the growth hormone treatment. These results make sense in light of the timing of growth of components of the craniofacial complex. Brain growth occurs relatively early and is virtually complete (95%) by Day 21 (Orr et al. 2016). On the other hand, basicranial elements and, to an even greater extent, the bones of the face, have a larger amount of growth remaining at Day 21 (Maga 2016; Vora et al. 2015). The GH treatment, at Day 21, comes too late to influence brain growth but it does influence facial and craniofacial growth. This produces an overall change in craniofacial shape which is not along the same trajectory as ontogenetic allometry. Despite this, much of the change in shape, produced by the growth hormone treatment produces change in the same direction as allometric effects. This is seen in the between groups PCA shown in Fig. 5D. Focusing in on the face using a longitudinal approach (manuscript in preparation), we see that growth hormone treatment pulls individual growth trajectories closer to the growth hormone sufficient controls (Fig. 5E). This last result is intriguing as it suggests evidence for robustness or canalization of an underlying allometric pattern of growth.

Taken together, these results show that delayed delivery of growth hormone can alter craniofacial morphology by altering the ratio of brain to skull growth. However, the parts of the skull that are able to respond to growth hormone at the time of treatment grow in a manner that converges back to the allometric shape trajectories of growth hormone sufficient mice. The mechanisms by which the face, as it recovers in size, converges on to the shape expected relative to size are not known.

Conclusion

Integration, or the tendency for traits to covary, is a fundamental property of developing individuals in populations. Allometry is the subset of this variation that is generated by the processes that regulate size. Here, we have examined evidence for the proposition that there is a single underlying axis of allometric variation for morphology with a focus on the mouse and human craniofacial complex. Specifically, we asked whether allometric variation relates to a single underlying developmental determinant of covariation or whether such patterns are specific to alternative measures of size. We find that the answer to this question lies somewhere in between these alternatives. The allometric variation associated with different measures of size converge only partly on a common pattern. Furthermore, the genetic bases of such patterns in the mouse skull overlap extensively but are also somewhat distinct. While there is much evidence that environmental modulation of size produces changes in shape that are predicated from patterns of static and ontogenetic allometry, the timing of genetic or environmental perturbations can disrupt such patterns when tissues or traits are differentially sensitive to perturbations over an ontogenetic trajectory.

These findings support the hypothesis that variation in organismal and trait size tends to be associated with common axes of allometric variation. However, they also show that allometric variation

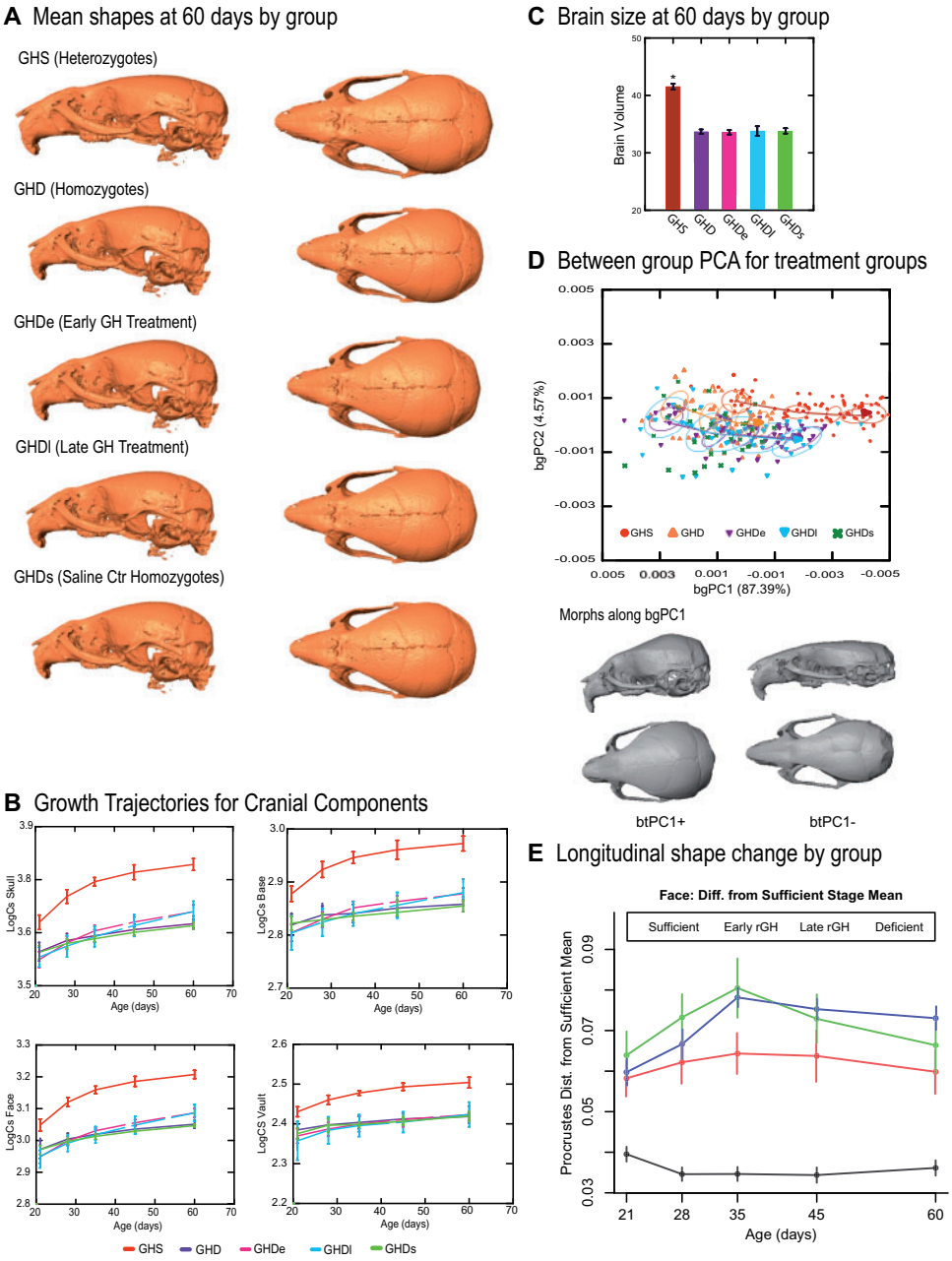

Fig. 5 Rescue of growth by growth hormone in growth hormone deficient mice. (**A**) shows mean craniofacial shapes at 60 days for all treatment groups. (**B**) shows the growth trajectories for the whole skull, the basicranium, face and cranial vault. (**C**) shows average brain size by treatment groups. The error bars are standard errors of the mean. (**D**) shows the result of a between group PCA for all treatment groups. The best fit lines show ontogenetic progression with age within each group. (**E**) shows the analysis of longitudinal shape change from one age group to the next for the face only. All panels are taken from Gonzalez et al. (2013).

is not reducible to simple genetic determinants and suggest that allometric patterns of variation may also be driven by multiple processes in development. We are only beginning to understand the developmental underpinnings of such patterns in a few developmental systems. This is an important goal because allometric patterns of variation are so pervasive in nature, often accounting for surprising magnitudes of variation both within populations and among species. Addressing the developmental basis for allometric variation is, therefore, a significant step toward explaining variation in complex traits, more generally, as well as the myriad ways development interacts with evolutionary processes to produce organismal diversity.

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