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Deciphering the Palimpsest: Studying the Relationship Between Morphological Integration and Phenotypic Covariation

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Abstract

Organisms represent a complex arrangement of anatomical structures and individuated parts that must maintain functional associations through development. This integration of variation between functionally related body parts and the modular organization of development are fundamental determinants of their evolvability. This is because integration results in the expression of coordinated variation that can create preferred directions for evolutionary change, while modularity enables variation in a group of traits or regions to accumulate without deleterious effects on other aspects of the organism. Using our own work on both model systems (e.g., lab mice, avians) and natural populations of rodents and primates, we explore in this paper the relationship between patterns of phenotypic covariation and the developmental determinants of integration that those patterns are assumed to reflect. We show that integration cannot be reliably studied through phenotypic covariance patterns alone and argue that the relationship between phenotypic covariation and integration is obscured in two ways. One is the superimposition of multiple determinants of covariance in complex systems and the other is the dependence of covariation structure on variances in covariance-generating processes. As a consequence, we argue that the direct study of the developmental determinants of integration in model systems is necessary to fully interpret patterns of covariation in natural populations, to link covariation patterns to the processes that generate them, and to understand their significance for evolutionary explanation.

Keywords

Morphological integration; Developmental constraints; Covariation; Morphometrics; Evolvability; Mice; Rodents; Primates

Introduction

Development is relevant to evolution because it structures the expression of phenotypic variation on which natural selection acts (Alberch 1982; Raff and Kaufman 1983; Hall 1999). Integration and modularity are two related ways by which this occurs (Cheverud 1996; Wagner 1996), and so are key determinants of evolvability (Raff and Sly 2000; Griswold 2006; Jones et al. 2007; Wagner et al. 2007; Hansen and Houle 2008). Morphological integration refers to the coordinated variation of functionally and developmentally related features of organisms (Olson and Miller 1958), while modularity refers to the division of developmental systems into partially dissociated components that are themselves integrated. Integration biases the direction of evolution via the correlated

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effects of mutations on phenotypic variation and modularity affects the evolvability of complex systems by limiting the effects of mutations to sets of functionally or developmentally related traits (Raff 1996; Raff and Sly 2000; Wagner and Mezey 2004; Wagner et al. 2007), and thus the potentially deleterious effects of these mutations on fitness. Integration and modularity, like canalization, novelty, and constraint, represent fundamental ways in which development is relevant to evolutionary explanation and thus are central to evolutionary developmental biology (i.e., "evo-devo") (Hendrikse et al. 2007).

That said, a conceptual difficulty with the study of integration and modularity is the conflation of pattern with process. This stems in part from confusion between what is being measured and the phenomena that those measurements are intended to capture (Hendrikse et al. 2007; Willmore et al. 2007; Boughner and Hallgrímsson 2008). Thus, while modularity and integration are almost exclusively investigated through measures of phenotypic covariation or correlation, often too little thought is given to whether observed covariation and integration represent the same thing and, if they are not, then to how they relate to each other.

This problem can be illustrated by defining the two concepts. Modularity is based on the idea of a map of connections or interactions among the components of a system in which some areas have denser internal versus external connections (Wagner 1996; Schlosser and Wagner 2003; Wagner et al. 2007). Similarly, integration is based on the idea that selection for shared function between traits favours pleiotropy for those traits, leading to shared developmental effects among them. Shared developmental effects, in turn, lead to coordinated variation and, thus, evolutionary bias (Fig. 1). The opposite of integration would be the absence of interactions or parcellation (Wagner 1996). A modular organization is one in which selection for integration and parcellation has created a developmental architecture (i.e., the interactions that connect genetic to phenotypic variation through development) in which some areas are more tightly connected to each other than they are to others. Modularity and integration are, therefore, features of this developmental architecture that arise through the action of natural selection or genetic drift (Müller and Wagner 1996; Wagner et al. 2007). Like canalization, integration and modularity are dispositional concepts (Wagner et al. 1997), by which we mean that they refer to general propensities or potentials rather than actual states. They thus refer to the *tendency* of a system to produce variation that is modularized and/or integrated rather than a pattern and/or magnitude of integration and modularity per se.

To see why these are different, imagine a sample of clones raised under identical conditions and absolutely devoid of variation. Without variation, these clones would exhibit no covariation structure. However, their developmental architecture is still just as modularized or integrated as the parent population since the absence of variation does not change the nature of the developmental interactions. An invariant population thus has integrated and modularized developmental systems in the sense that the integrated/modularized covariation structure would emerge from the properties of the developmental architecture once variation is introduced, but we would not be able to detect (measure) it via covariation. If variation were reintroduced into such a population, then the developmental determinants of integration would structure that variation and thus influence evolvability. The concepts of integration and modularity thus refer to intrinsic characteristics of organisms of which the observed presence of phenotypic covariation or correlation is simply a variance-dependent proxy.

This distinction is important, but it creates both theoretical and practical problems. The theoretical problem is that quantitative-genetic parameterizations of integration use the additive genetic variance-covariance matrix (i.e., the **G** matrix) or similar proxies (Burger

1986; Jones et al. 2007; Hansen and Houle 2008). The dependence on variance for the definition/detection of integration and modularity is therefore built into population-genetic measures. Alternative parameterizations of integration that do not depend on variance are possible but have not been proposed (Hansen personal communication). The second problem is more practical but no less important. Our ability to detect integration and modularity depends on the presence or absence of particular patterns of variation in the sample that one is studying. The problems this variance-dependence creates at both a theoretical and practical level are explored in this paper.

Integration and Covariation

What is integration in developmental terms? In our view, it refers to the tendency of a developmental system to produce covariation. This definition contrasts with the more common conceptualization of integration in the literature. Current definitions of integration tend to focus on the observed correlations. That is, traits are considered integrated when they covary (Klingenberg 2008, 2009). This is in accord with the original definition articulated by Olson and Miller (1958). As argued above, the difficulty with this integration concept is that it depends critically on the presence of variation. Our definition, in contrast, mirrors Wagner et al.'s (1997) definition of variability in that it is dispositional. Integration is to covariation as variability is to variation and as solubility is to solution, to borrow the example used by Wagner et al. (1997). Integration thus exists independently of variation as a property of developmental systems. Perhaps the term “covariability” would better capture this dispositional nature.

But what sort of property is it? Is it a process or a pattern? If integration is a process, then it could be called upon to explain observations about phenotypic variation. For instance, the statement that the hind and forelimb covary because they are integrated should have some explanatory value. In fact, this seemingly tautological statement does have some explanatory value. It implies that there is some form of developmental connection or interaction between the hind and forelimb such that in the presence of the appropriate variation, covariation is produced. Integration here refers to the connections between the developmental mechanisms of the limbs that have the *potential* to generate covariation. These connections are an abstraction above the level of actual developmental mechanisms. In that sense, it is more appropriate to think of integration as features of developmental architecture. It is the set of potential connections or interactions between developmental components that, in the presence of variation, produce covariation structure. In the case of the hind and forelimb, integration refers to the shared developmental influences between the limbs and limb elements that shape limb covariation structure. When these mechanisms change so as to alter the tendency of the system to produce covariation, there has been an evolutionary change in integration. The dramatic changes in covariation structure seen with divergence of hind and forelimb divergence shape and size is probably an example of this (Young and Hallgrímsson 2005; Rolian 2009). Many changes in covariation structure, however, can occur without alterating the underlying pattern of developmental interactions that produce covariation (Hallgrímsson et al. 2007b).

To determine whether covariation structure can change independently of the developmental patterning underlying integration, Hallgrímsson et al. (2007b) compared covariation structure among mutant and wildtype mice for which the developmental processes affected by mutation are known to varying degrees. All of the analyses discussed in this section are based on the framework of geometric morphometrics (Bookstein 1991; Dryden and Mardia 1998) and so they deal with the analysis of shape variation in which size has been removed through the Procrustes superimposition step (Gower 1975; Rohlf and Slice 1990).

Brachymorph mice have a null mutation in the Papps-2 (3′-phosphoadenosine 5′-phosphosulfate synthetase 2) gene that codes for an enzyme involved in sulfation of sulfated glycosaminoglycans. In these mutants, the undersulfation of the glycosaminoglycans in the cartilage in these mutants is linked to a significant reduction of cartilage growth as measured by cell proliferation rates within the cranial synchondroses or long-bone growth plates (Ford-Hutchinson et al. 2005; Cortes et al. 2009). Sulfated proteoglycans appear to be necessary for normal Indian hedgehog (Ihh) signaling in the growth plate (Cortes et al. 2009), where Ihh plays a critical role in regulating cell proliferation within the growth plate (Maeda et al. 2007). The phenotypic variance in craniofacial shape is increased in mutants compared to wildtype littermates (Hallgrímsson et al. 2006). This increase is particularly marked in the chondrocranium, which implies that the variance of cell proliferation within the synchondroses is increased. However, this has not been assessed directly. Sulfation of proteoglycans in cartilage is significantly reduced in brachymorph mice compared to the wildtype, while the variance of sulfation appears to be similar (Cortes et al. 2009). The untested implication is that there may be a nonlinear relationship between proteoglycan sulfation and cartilage growth such that the mutants are more sensitive to the degree of proteoglycan sulfation compared to wildtype mice. The same range of sulfation variation may thus translate into an increased range of phenotypic variation in terms of chondrocranial size and cranial shape (Hallgrímsson et al. 2006). If true, then this would be a case in which variation in canalization is produced by a nonlinear relationship between developmental factors as hypothesized by Klingenberg and Nijhout (1999) (Fig. 2).

In brachymorph mice, the increased variance of chondrocranial size and, presumably, chondrocranial growth, appears to result in an increase in morphological integration (Hallgrímsson et al. 2006) as measured by the scaled variance of eigenvalues (Wagner 1989). These results are summarized in Fig. 3. The likely explanation is that the increased variance in chondrocranial growth drives a set of correlated responses in the rest of skull. As the variance of chondrocranial growth increases, the magnitude of this correlated response increases. If this response is large relative to other determinants of covariation, then the result is an increase in the magnitude of morphological integration as measured by the scaled variance of eigenvalues. More importantly, the covariance structure in the brachymorph mice is completely different compared to that of their wildtype littermates. The matrix correlation between the covariance matrices is only 0.33 which is much lower than one would expect between strains of inbred mice and lower also than matrix correlations for craniofacial landmark data between related species of rodents (Jamniczky and Hallgrímsson 2009).

Brachymorph mice differ from their wildtype littermates only in that they are homozygous for the Papps-2 null mutation. Both samples are inbred and thus have negligible genetic variance. However, there is significant phenotypic variation in both groups, and this phenotypic variation is structured by developmental processes just as it would be in outbred samples. It is thus remarkable that this dramatic increase in phenotypic variance and integration as well as change in the phenotypic covariance structure is driven by a single mutation.

A second mutant, *Trsp*$^{fl/fl}$*Col2a1-Cre* mice have an osteo-chondroprogenitor-cell specific deletion of the selenocysteine tRNA gene (Downey et al. 2009). Cartilage growth in this cartilage specific knockout model is reduced via a completely different mechanism than in the brachymorph mice. Here, the mechanism involves selenoprotein deficiency and a consequent reduced ability to defend chondrocytes and osteoblasts against damage caused by oxidative stress (Downey et al. 2009). Remarkably, the phenotypic effects of the mutation are very similar to those seen in the brachymorph, although they are more extreme. While our sample is insufficient for detailed comparisons of covariance structure,

phenotypic variance is clearly increased in this mutant. There is also a strong tendency for the variation in chondrocranial size to produce a highly integrated set of changes elsewhere in the skull (Fig. 3).

Finally, our work with the megencephaly (Mceph) mutant tells a similar story about covariance and integration. In this case, the brain growth is increased significantly (roughly 25%) as the result of a mutation in the Kcna1 gene (Donahue et al. 1996; Petersson et al. 1999; Diez et al. 2003; Petersson et al. 2003). The increase is not confined to particular cell types and results in a larger but normally shaped brain. Our morphometric analysis revealed that the mutation produces an increase in the variance of brain size. This variation in brain size drives a correlated set of responses in the rest of the skull. In fact, the response of the mouse skull to changes in brain size is characteristic and holds widely across mutants and strains (Hallgrímsson et al. 2007a; Hallgrímsson and Lieberman 2008; Lieberman et al. 2008). The increase in brain size variance also produces an increase in morphological integration as measured by the scaled variance of eigenvalues (Fig. 4). Most importantly, as with the brachymorph mutation, the mceph mutation also dramatically alters covariance structure. The matrix correlation between the phenotypic variance covariance matrices for mceph/mceph mice and C57BL6/J mice is only 0.22. This is far outside the repeatabilities of these variance covariance matrices (the distribution of matrix correlations between each matrix and its resampled self) and so indicates that the covariance structure is very different in the mutant compared to the wildtype.

In the megencephaly and brachymorph mice, and probably in the cartilage-selenoprotein deficient mouse, the mutation produces an increase in the scaled variance of eigenvalues which is the usual measure of the magnitude of morphological integration (Pavlicev et al. 2009). This measure of integration captures the extent to which variation is unequally distributed across principal components in a principal components analysis. Integration increases because a greater proportion of the total variation is concentrated along fewer principal components. A mutation that increases the variance of some developmental process that produces a set of correlated responses would be expected to increase the variance of eigenvalues under two conditions. The first is that the increase in variance is large and the correlated responses to that variance are high. The second is that the mutation increases the variance of a process that was already a major determinant of covariation structure. We infer that these mutations are increasing morphological integration in one of these ways. Similar arguments could be made about the increases in variance and integration in other mouse mutants that we have worked with such as the cartilage specific knockout of Pten (Pten $Cre^{fl/fl}$ or the Crf4 (short faced) mouse (Hallgrímsson et al. 2007a; Boughner et al. 2008).

If the scenario is accurate, then one can imagine the opposite situation in which a mutation might act to decrease the integration measured in this way. This could occur if a mutation increased the variance of a developmental factor that is not a significant determinant of covariation in the wildtype, but not sufficiently to make it a primary element of covariation. In this case, the mutation would be increasing the eigenvalue of a principal component that would otherwise be very small. Alternatively, the mutation could produce a suite of effects that are very poorly correlated. In both of those cases, a mutation would decrease integration.

There are good examples of this in the mouse mutants that we have worked with. One of these is a mouse model that is heterozygous for a gene-trap insertion in the *Nipbl* gene, and generated as a model for Cornelia de Lange Syndrome (Kawauchi et al. 2009). The *Nipbl* or “Nipped-B-like” gene codes for a protein that interacts with cohesin to contribute to chromatin cohesion (Hagstrom and Meyer 2003). These mice exhibit a range of

physiological and morphological changes. The craniofacial phenotype is quite characteristic. It is associated with reduced overall size, reduced brain size and an upturned nasal region (Kawauchi et al. 2009). The mutation affects chromosomal integrity and not the function of any particular gene. It is not surprising, therefore, that microarray analysis reveals small but numerous changes in gene expression in genes with widely different functions (Kawauchi et al. 2009). One might also expect that the variance of gene expression is increased but this was not tested in this study. The phenotypic variance for craniofacial shape, however, is significantly increased (Fig. 5). The fact that there is a highly characteristic phenotype associated with both this mutation in mice and also with Cornelia de Lange syndrome in humans, would lead one to predict that this increased variance would be associated with increased integration. Variation along a penetrance spectrum for this phenotype would be expected to manifest as a highly correlated set of changes. Instead, integration is not altered as the variance of eigenvalues is only slightly decreased (Fig. 8b). *Nipbl* haploinsufficiency may thus increase the variance of processes that contribute little to the wildtype integration pattern. Alternatively, the effects of the *Nipbl* mutation may vary both in kind and in magnitude among individuals, producing a "disintegrating" influence. In this case, the increased variance produced by the mutation is not associated with a large change in covariance structure. The matrix correlation between the $Nipbl^{+/-}$ sample and the wildtype, at 0.61 is only slightly outside the repeatabilities of the two matrices.

Another case which illustrates the same point is the A/WySn mouse. A/WySn mice, along with related A strains, exhibit clefting of the primary palate with incomplete penetrance and variable expression. Clefting in these mice is caused by the interaction of at least two genetic factors, *clf1* and *clf2* (Juriloff et al. 2001). *Clf1* is now known to be an IAP retrotransposon insertion that interferes with expression of *Wnt9b* (Juriloff et al. 2006). The primary palate forms when the maxillary prominences fuse to the medial and lateral nasal processes of the frontonasal prominence. Our morphometric analysis of embryos spanning primary palate formation from A/WySn and related strains shows that the midface is reduced in size during this period and that there is a particular reduction in the outgrowth of the maxillary prominence (Young et al. 2007; Parsons et al. 2008) (Fig. 6). During primary palate formation in A/WySn mice phenotypic variance for facial shape is also significantly increased (Parsons et al. 2008). In this case, however, this increased variance is associated with a decrease in integration (Fig. 6d). Interestingly, the phenotypic variance is not significantly increased in adults. Although, integration is not significantly decreased in the sample of 90-day-old mice used for this analysis, it is in 30-day-old mice (Hallgrímsson et al. 2004a). Further, the adult covariance structure is altered significantly by the mutation. The matrix correlation between A/WySn mice and Balb/C, the closest related strains that does not carry the *clf1* mutation is only 0.45. The matrix correlation between A/WySn mice carrying the *clf1* mutation and a strain in which *clf1* and *clf2* have been replaced with C57Bl/6J equivalents and backcrossed for seven generations is also only 0.42. The mutation may influence covariance structure by increasing the variance of a process (facial prominence outgrowth) that may normally contribute little to the adult covariance structure. The same process likely results in the significant alterations in covariance structure.

These results show that in inbred mice that have little or no genetic variance, single mutations can radically alter covariance structure. Depending on how they influence development, single mutations can also significantly increase or decrease the overall magnitude of covariation, as defined by measures of morphological integration. These results are consistent with the finding that integration can increase in certain disease-related phenotypes such as in the relationship between the brain and skull in craniosynostosis (Richtsmeier et al. 2006; Richtsmeier and Deleon 2009). It is unlikely that any of the mutations discussed here (with the possible exception of the *Nipbl* mutation) actually change the developmental interactions and processes that generate covariation. The mceph

mutation does not influence the potential of the growth of the brain to affect the rest of the—it just makes the brain larger and more variable in size. Similarly, the chondrocranial mutants do not influence how variation in chondrocranial growth affects the overall shape of the skull. They change the amount and variance of chondrocranial growth. Thus, covariation structure is changed without affecting how development integrates the expression of phenotypic variation.

In natural populations, however, covariation structure tends to be much more stable (Steppan 1997; Ackermann and Cheverud 2000; Marroig and Cheverud 2001; Jamniczky and Hallgrímsson 2009) (Fig. 7). That said, there are interesting exceptions to this including significant changes in cranial covariance structure during ontogeny (Zelditch et al. 2006). Given that covariance structure is so highly labile in inbred mice, it is unlikely that the stability seen in natural species and populations actually strongly reflects the underlying pattern of developmental integration and modularity. This view is supported by the lack of consistent relationships seen between phylogenetic or morphological distance and covariance distance (Steppan 1997; Ackermann and Cheverud 2000; Marroig and Cheverud 2001; Jamniczky and Hallgrímsson 2009). We have argued recently that this discrepancy between the observed stability of covariance in natural rodent populations compared to inbred mice implies that microevolutionary forces such a stabilizing selection are acting to maintain covariance structure (Jamniczky and Hallgrímsson 2009).

The examples discussed here illustrate the difficulty that stems from not making a conceptual distinction between covariance structure and integration or pattern and process. Phenotypic covariance is the form of data that is almost always used to study developmental integration and modularity. These concepts, however, refer to properties of development that are deeper than observed phenotypic correlations. The developmental systems of a population of invariant clones should theoretically be no less integrated than those of an outbred population of the same species with a high genetic and phenotypic variance. In both situations, development would have the same tendency to produce covariance. They differ only in what variation happens to be present. We have shown that both the magnitude of integration and covariance structure can be altered significantly by single mutations.

Mitteroecker and Bookstein have pointed out a related and equally important issue (Mitteroecker and Bookstein 2007; Mitteroecker 2009) which is that multiple configurations of developmental determinants of covariance can lead to the same covariance structure. Underlying developmental modularity and integration may thus have complex relationships to observed patterns of phenotypic covariation. Integration and modularity are aspects of developmental architecture that influence evolvability by structuring the translation of genetic variation into the phenotypic variation on which natural selection acts (Hallgrímsson et al. 2002; Hendrikse et al. 2007; Wagner et al. 2007). They are not equivalent in any sense to observed patterns of covariation. It is a significant problem for the study of integration that measures of integration and modularity can change without actual changes in the underlying developmental determinants of the tendency for covariation. Clearly better conceptual and experimental tools are needed to decipher the patterns of phenotypic variation and covariation in order to get at the underlying developmental basis for these important phenomena.

The Relationship Between Phenotypic Variance and Measures of Morphological Integration

In the mouse mutant examples discussed above, integration as measured by the scaled variance of eigenvalues could either increase or decrease as the result of single mutations which were also associated with increased variance of some aspect of development. This

observation begs the question of whether there is a more general relationship between the phenotypic variance and integration as measured by the scaled variance of eigenvalues (SVE). In this measure of integration, the variance of eigenvalues based on a variance/covariance matrix is scaled to the mean eigenvalue so that one can compare integration among samples that differ in the magnitude of variance.

We compared SVE among 25 samples of mice where each sample is an inbred strain, a cross between strains or a mutant strain on an isogenic background. These results, shown in Fig. 8, show an interesting pattern. Firstly, the mutant samples have significantly higher phenotypic variances than the other strains (Fig. 8b). The average trace of the variance covariance matrices for mutants is 0.002 compared to 0.0009 for wildtype strains. These matrices are based on Procrustes superimposed landmarks so size has been removed. There is thus a twofold difference on average between the multivariate phenotypic variances of mutant and wildtype samples for inbred strains of mice. This is consistent with the common observation that mutations with significant phenotypic effects often increase variance in addition to shifting the mean (Waddington 1942; Scharloo 1964, 1991). In our data, these increases in variance are most often but not always accompanied by increases in integration. This trend results in an among-strain correlation of 0.85 between integration (measured by the scaled variance of eigenvalues) and the phenotypic variance (measured by the trace of the variance covariance matrix [TVC] of Procrustes superimposed coordinates). This correlation is not happening simply as a consequence of the variance covariance matrices are scaling up with the variance because we are using the scaled variance of eigenvalues (Wagner 1989). The scaled variance of eigenvalues is divided by the mean eigenvalue. Like the trace, the mean eigenvalue is a multivariate measure of variance and the mean eigenvalue is simply the trace divided by the number of variables. Instead, this effect is occurring because the variance that is there tends to be distributed on the first few principal components in those strains with high variance and more evenly among those with lower variance (Fig. 8d). This concentration of variance on fewer components does not happen automatically as the variance increases but instead is happening because of the way the developmental systems of these mutants are structuring the increased variance associated with the mutations.

Interestingly, even after correcting for differences in sample variance by scaling the variance of eigenvalues to the mean eigenvalue, there is still an artifactual relationship between SVE and variance. This artifact occurs whether one uses SVE or VE based on the correlation matrix. As we have shown elsewhere using simulation, the sampling errors for VE and variance are correlated and the magnitude of this correlation depends on the covariance structure of the traits (Young et al., submitted). This artifact is also present in our samples. For resamples with replacement from our original sample, we obtain a correlation of 0.37 between SVE and TVC across samples. To show that the among-strain correlation is not due to this artifact, we plot the entire resampled data in Fig. 8c. This plot shows that even though there are correlations between SVC and TVC within the resampled distributions for each strain, the overall trend in the data is due to the among-strain correlation between integration and variance.

The two samples with the highest variance and integration are the Crf4 mutant and the Pten collagen specific knockout. The Crf4 mutation produces a complex phenotype that involves reduced brain size, reduced basicranial length and a shortened face (Boughner et al. 2008). The mutation exhibits variable expressivity, but this variation is highly structured such that the different effects of the mutation are highly correlated. Although adult Crf4 mice have short faces, embryos from this strain have advanced and prognathic faces relative to stage (Boughner et al. 2008). We have hypothesized that this relates to a slower rate of brain growth during face formation, which may be the underlying driver behind this highly integrated but variable phenotype.

The Pten mutation is a cartilage specific knockout of the Pten gene driven by collagen II. Since Pten is a mitosis inhibitor, we hypothesized initially that this mouse would exhibit increased chondrocanial growth. This provides an interesting contrast to other mutations such as the Brachymorph and *Trsp*$^{fl/fl}$ *Col2a1-Cre* mouse which have reduced chondrocanial growth. Indeed, the Pten mouse does exhibit increased cartilage growth and this does influence cranial shape as well as long-bone length (Ford-Hutchinson et al. 2007; Hallgrímsson et al. 2007a). A complicating factor, however, is that the basicranial synchondroses in this mouse exhibit abnormalities that include bony spurs that can cause premature cessation of growth (Ford-Hutchinson et al. 2007). The increased variance in this mouse may be due in part to this apparently stochastic process. Even though different synchondroses are affected in different individuals at different ages, the resulting variation may be quite highly integrated because it involves variation in basicranial growth.

In most cases, the variance and integration of specific mutants are both increased compared to that of their wildtype control samples. This is true for brachymorphs versus C57BL/6J, for example, or the Megencephaly mutant versus their wildtype littermates as well as a few others in this analysis. In other cases, the opposite pattern is seen such as in the *Nipbl* heterozygote or the A/WySn examples discussed in the previous section. In all cases, however, variance and integration appear to vary in a coordinated manner.

Developmental Determinants of Covariation Structure

Covariation or correlation among traits results from variation in developmental processes (Hallgrímsson et al. 2007b; Mitteroecker and Bookstein 2007; Mitteroecker 2009). When processes vary, they produce correlated variation in the structures that they influence. This simple realization that covariation or correlation among traits results from variation in developmental processes is often overlooked, at least implicitly, in studies of integration. A common oversight, for instance, is to fail to take this into account when predicting changes in integration in either some natural or experimental context. One would expect integration to change when the variance of some underlying developmental determinant is altered, either in absolute terms or relative to the variances of other such determinants. In this section, we discuss two developmental contexts—the growth of the appendicular skeleton and the formation of the vertebrate face. We present these cases to illustrate how covariation and correlation may arise in those systems and how understanding the developmental basis for these patterns improves our understanding of the developmental basis and evolutionary significance of morphological integration.

The Growth of the Vertebrate Appendicular Skeleton

Tetrapod fore- and hind limbs show patterns of covariation produced by pleiotropic effects of genes involved in limb development (Wright 1932; Magwene 2001; Young and Hallgrímsson 2005; Rolian 2009). These plieotropic effects, in turn, reflect the serial homology of elements of the two limbs (Ruvinsky and Gibson-Brown 2000). The vertebrate limb develops through several stages, each of which could potentially contribute to patterns of integration. Initially, each limb buds out from the ventral body wall. Once the limb bud forms, outgrowth is controlled through signals emanating from the Apical Ectodermal Ridge (AER). Most of the proliferation within the limb bud happens in the zone immediately deep to the AER (Hinchliffe and Johnson 1980). Within the limb bud, the skeletal elements form initially as mesenchymal condensations. These then differentiate into cartilaginous rudiments that take on the basic shape of the eventual bony element. Primary ossification centers form in the diaphyses of the bones and at each end the cartilage is reorganized into growth plates (Fig. 9). Subsequent bone lengthening and to some extent widening happens at these growth plates. Other dimensions of growth and shape change involve modeling and remodeling through bone apposition and resorption, mainly on the periosteal and endosteal

surfaces. All of these gross level processes have the potential to influence to covariation structure of the limb. However, as limb element length is largely determined by the activity of growth plates, we'll focus on that as the source of variation and covariation in patterns of limb element lengths.

Among mammals, limb element lengths vary dramatically in absolute terms, in terms of the proportion of each limb represented by different elements and in terms of the proportion of the hind limb and forelimb. Interestingly, forelimb proportions vary more than hind limb proportions among mammalian species, possibly because of reduced mechanical constraints on forelimb function (Schmidt and Fischer 2009). Within species, individual limb elements, which form through the same basic process, can vary dramatically in size (e.g., human foot phalanges versus the femur). At the same time, different limb element sizes within species tend to be highly correlated (Wright 1932). Clearly, this pattern of correlated variation comes about through the integrating effects of developmental processes. While much is known about the developmental mechanisms operating within the growth plate, little is known about how these mechanisms are modulated to produce this combination of great potential for divergence and high degree of integration in the expression of variation within species.

To address this question, Rolian (2008) compared cellular growth parameters for different limb elements between mice and gerbils, two rodent species that show important differences in intra- and interspecific limb bone lengths. Three cellular parameters, the number of cells in the proliferating zone, the frequency with which those cells divide and their degree of hypertrophy as they enter the hypertrophic zone appear to account for the vast majority of variation in the growth that occurs at the growth plate of different bones within species and the same bone among species (Kember 1993; Kirkwood and Kember 1993; Ballock and O'Keefe 2003). Interestingly, he found that within the two species, the varying growth of long bones is explained best by variation in the size of the proliferating chondrocyte pool early in bone growth as well as the sizes of the terminal hypertrophic cells (Rolian 2008). Rolian hypothesized that these differences among elements within each species in the size of the proliferating chondrocyte pool were related to the size of the mesenchymal condensation and/or cartilage Anlage before the formation of the growth plate. In contrast, the differences between the two species—which mostly relate to absolute differences in body size between mice and gerbils—were best explained by differences in the rate of chondrocyte proliferation within the proliferating zone (Rolian 2008). This finding is consistent with other examples such as the finding that the bat wing exhibits increased proliferation in the metatarsus growth plates, associated with increased expression of *Bmp2* (Sears et al. 2006).

Although it is difficult to generalize from these two rodent species to other mammals, these results are interesting because they highlight how there can be “divisions of labour” among different mechanisms in generating variation and covariation structure in limb bone lengths. Although Rolian's study dealt specifically with the developmental determinants of variation and not covariation in limb lengths, these results do suggest how different mechanisms and different aspects of the regulation of the growth plate could act to generate different components of covariation. Variation in overall growth clearly contributes to correlated variation in limb element length. This variation might be driven by several different mechanisms including systemic regulation of Growth Hormone, systemic IGF-1, insulin, thyroid hormone, estradiol and testosterone (Savendahl 2005). These systemic factors act on all parts of the growth plate, but probably most effectively on chondrocyte proliferation rate. Using the scheme of Hallgrímsson et al. (2002) and Young and Hallgrímsson (2005) for understanding the developmental basis for limb variation, these sorts of factors would influence covariation among all limb elements (Fig. 10). Other factors acting more locally would likely underlie differences in growth rates among limb elements. Such factors, such

as locally produced IGF-1, Indian hedgehog protein, Parathyroid hormone-related protein (PTHrP), as well as receptors or factors that influence responsiveness to systemic factors would produce differences among limb elements. Depending on how these factors are distributed, they would produce covariation among serially homologous elements as well as limb-specific variation (Fig. 10). Finally, there may well be factors acting at the level of the hind versus forelimb. Such factors are known in early development (Fig. 10). Limb identity appears to be established early in development, with differential expression of genes such as Tbx 4 and 5 (Gibson-Brown et al. 1996). Later acting differences likely exist as well, but it is also possible that some processes acting early in development translate to postanatal patterns of morphological integration (Wagner 2005).

The potential processes discussed above and the pattern of their effects across the appendicular skeleton represent the developmental basis for integration in the vertebrate limb. These processes determine how genetic variation is translated into a pattern of phenotypic covariation. When factors influencing size are invariant, they will not contribute to limb covariation. Similarly, when the variance of factors influencing one of the integration components in Fig. 10 increases in proportion to others, the covariation structure that is produced will change. Phenotypic covariation structure in the appendicular skeleton thus results from integration as an element of developmental architecture combined with the genetic and environmental variation that happens to be present in a particular population.

Shh Signalling and the Formation of the Vertebrate Midface

The vertebrate face forms from two paired outgrowths or prominences that form within the first branchial arch combined with a single midline prominence that forms ventral to the oropharynx. The lower jaw forms from the fusion of the two mandibular prominences. The primary palate and midface form as the maxillary prominence fuses with two projections from the midline prominence, the medial and lateral nasal processes (Cox 2004) (Fig. 11). As these processes grow and fuse to form the face, they interact in several ways with the developing brain (Marcucio et al. 2005). Some of this interaction is mechanical or epigenetic in that the processes must grow at a sufficient rate to overcome the simultaneous expansion of the head that is driven by the growth of the forebrain (Wang and Diewert 1992; Diewert and Lozanoff 1993). This interaction also involves molecular signaling that emanates from the brain and regulates cell proliferation in the face (Marcucio et al. 2005; Hu and Marcucio 2009a). To understand how processes acting at the level of face formation might drive morphological integration in the skull, we'll consider the role of Sonic hedgehog signaling in the brain and the early growth of the midface. This illustrates nicely how phenotypic variation and covariation in a complex morphological trait may be driven by developmental processes.

Work by Marcucio and colleagues has uncovered the existence of a signaling center in the stomodeal ectoderm, which they named the Frontonasal Ectodermal Zone or FEZ (Hu et al. 2003; Hu and Marcucio 2009a). *Shh* signaling in the brain is required for the differentiation of the Ectoderm to form the FEZ (Marcucio et al. 2005; Eberhart et al. 2006). Once formed, the FEZ regulates outgrowth of the midface through production of SHH which diffuses into the underlying mesenchyme and acts through the *Shh* receptor *Ptc* and the *Gli* transcription-factors to regulate cell proliferation (Fig. 10). While the FEZ is conserved between mammalian and avian embryos, the spatial organization of gene expression patterns is different in these animals (Hu and Marcucio 2009b). The unique organization appears to underlie phenotypic differences in the faces of these animals, Molecular changes in the FEZ can be produced by altering signaling by the SHH pathway in the brain. For instance, blocking SHH signaling in the brain led to malformations in the forebrain, an absence of FEZ activity, and a narrowing and truncation of the upper jaw (Marcucio et al. 2005). In contrast, activating the SHH pathway in the brain of avian embryos altered properties within

the basal forebrain and transformed the avian FEZ, and the subsequent growth patterns of the upper jaw, to resemble those of a mouse (Hu and Marcucio 2009a). These results suggest that alterations of *Shh* expression in the forebrain modulate proliferation and outgrowth of the midface and produce a continuous axis of covarying shape change that involved the width of the face, the width of the brain and the entire shape of the face. In this case, variation in the amount of SHH protein expressed in the brain could produce an integrated pattern of shape change in the face. SHH signaling in the brain regulates morphogenesis of the brain and controls FEZ formation. Therefore SHH signaling would contribute to regulation of the size and shape of brain, the position and size of the eyes, the width of the face, the shape and angulation of the maxillary prominences, and the degree of prognathism of the midface. There are many ways in this developmental system could be modulated including several pathways that are becoming better understood. It is likely, though, that many of these alterations would produce variation that converges on this or a similar integrated axis of shape variation. That remains to be explored in future work. It is also not known to what extent this integrated axis of variation relates to variation in the shape of the human face, although it is strikingly similar to the pattern of phenotypic variation observed in the range of disease phenotypes that include the holoprosencephaly spectrum on one end and Greig Cephalopolysyndactyly at the other and include a range of normal variation in between the two extremes.

In this case, as in the others discussed in this paper, covariation is produced through variation in an underlying developmental process. The development of the face has the potential to produce this pattern of covariation when faced with variation in *Shh* expression and would likely respond in a similar way to variation in other elements of that signaling pathway and perhaps even to related pathways such as FGF signaling in the FEZ. The tendency of the system to respond to *Shh* signaling by varying along an integrated shape axis is an example of morphological integration. The covariation structure produced is a result of this tendency combined with the variation that happens to be present in the system.

The Palimpsest Model of Covariation Structure

We have argued elsewhere that covariation structure comes about through variation in covariance-generating developmental processes (Hallgrímsson et al. 2007b). In this paper, we've discussed several developmental contexts in which this occurs. The difficulty in analyzing phenotypic covariation structure in complex morphological forms, however, is that there are often many developmental processes that vary in such a way as to influence covariation. These processes may act at different times or overlap in time and space. Either way, each covariance generating process will blur or obscure the effects of the others on the overall covariance structure. We've reviewed elsewhere some of the processes that may act to generate covariation structure in the mammalian skull (Hallgrímsson et al. 2007b). These developmental determinants of covariation layer and overlap in ways that make the eventual covariation structure very difficult to decipher (Fischer-Rousseau et al. 2009). The combined effect of these processes thus make covariance a bit like a palimpsest—in Medieval times, a reused velum scroll on which the shadows of the various texts accumulate over time—in that the underlying determinants of integration and modularity may not be decipherable from phenotypic covariance or correlation data (Fig. 12).

It is not surprising, therefore, that establishing patterns of modularity in complex structures like the skull is quite difficult based on phenotypic data. Insight can often be gained using *a priori* hypotheses about developmental determinants of modularity (Cheverud 1982; Lieberman et al. 2000; Hallgrímsson et al. 2004b; Bastir et al. 2006; Willmore et al. 2006; Hansen et al. 2007; Porto et al. 2009) and new methods such as singular warps analysis have improved our ability to test hypotheses about integration patterns (Bookstein et al. 2003;

Monteiro et al. 2005; Gunz and Harvati 2007). However, it is usually very difficult to determine solely from phenotypic data how to dissect a complex morphological structure to reveal underlying determinants of integration.

In the context of morphology, modules can be defined as regions that share more connections internally than they do externally to other such regions (Klingenberg 2009) or share some particular mechanistic determinant such as the influence of a particular gene (Raff and Sly 2000; Wagner et al. 2007). These are variational modules as defined by Wagner et al. (Wagner and Mezey 2004; Wagner et al. 2007). Such regions don't have to be spatially contiguous. Serially homologous limb elements can be considered a module, for instance (Hallgrímsson et al. 2002). Spatial contiguity, however, is a condition in some statistical conceptualizations of morphological modularity (Klingenberg 2009). Klingenberg (2009) has developed a statistical method for comparing the strength of internal versus external covariance in order to dissect out spatially contiguous modules from phenotypic covariance data. This method compares an a priori division of a structure into modules to randomly drawn partitions of that structure in terms of the strength of association within and outside the module. We applied this technique to 3D landmark data for a pooled dataset of 300 mice from 12 inbred wildtype strains and used it to test for the existence of five hypothetical “modules.” Our hypothetical modules in this case follow the literature in this area and are based on well-accepted developmental criteria. As shown in Fig. 13, none of these modules actually exist in our sample in the sense defined by Klingenberg, although the division of the skull into regions derived from neural crest and somatic mesoderm comes close. This doesn't mean that that skull lacks a modular and integrated developmental architecture. It simply means that the ways in which developmental processes produce covariation structure in the skull are sufficiently complex that they do not translate into a simple schema that allows us to divide the skull into definitive morphological modules (Roseman et al. 2009).

If covariation structure is the combined result of the integrating effects of developmental architecture and the variances of covariance-generating processes, then how does covariation structure evolve in developmental terms? One obvious way would be through changes in the relative variances of covariance-generating processes. This is illustrated through the changes in covariance structure that we see in the mutations on isogenic backgrounds in our mouse samples. In these cases, minimal genetic variance creates a condition in which a change in the variance of some developmental process is sufficient to alter covariation structure. This is probably rare in natural populations. If there is abundant genetic variance for the developmental processes that generate covariance structure, then the changes in the variance of one process relative to others have to be very large to produce changes in covariance structure. This is probably why covariation structure tends to be stable in natural populations but is highly labile in inbred mouse strains (Jamniczky and Hallgrímsson 2009). Another major way for covariance structure to evolve is via changes in how developmental mechanisms generate integration. For example, if *Shh* signaling in the brain no longer induced the FEZ, then the integration of brain and face growth would likely be changed during the formation of the upper jaw. Finally, the appearance of novel integrating mechanisms or the disappearance of mechanisms would also change the covariation pattern produced. An example of that would be if gene duplication resulted in the appearance and subsequent divergence of multiple versions of a factor that differentially regulates growth in some structure. The divergence of function in duplicated versions would be associated with novel integrative influences. This sort of scenario has probably played out on the macro-evolutionary scale.

The tendency for stability in covariance structure due to the presence of variation is also the reason that studies of integration in natural populations do “work” in some sense. This is

despite, as we have argued here, the complex relationship between developmental integration and phenotypic covariance structure. Analyses of covariance structure stand up to predictions about large-scale evolutionary patterns such as the relationship between hind and forelimb divergence in morphology and the covariance of hind and forelimb structures (Young and Hallgrímsson 2005). However, understanding how covariation comes about is relevant to interpreting the results of studies like that. For example, if the among-limb covariation is reduced in species with divergent hind and forelimbs, then this likely implies a change in the relative importance of developmental processes that act jointly on the limbs compared to those that act on the limbs individually. This might occur through changes in the variances of developmental processes. Much more likely, though, is that it occurs through changes in how common versus individual developmental factors are influencing the growth and development of the limbs.

This kind of understanding is also relevant to making predictions about patterns of integration in experimental or microevolutionary contexts. If one wishes to study the role of diet and mastication in the integration of the upper and lower jaw, for instance, one needs an experimental design that creates changes in the variance of the factors that are hypothesized to influence covariation. One could make similar arguments about studies that make predictions about changes in integration as the result of various kinds of genetic or environmental perturbations.

Perhaps the most significant implication of the Palimpsest Model is that attempts to dissect phenotypic correlation or variance covariance matrices to search for signatures of underlying developmental determinants is unlikely to be productive in most cases. The relationship between the developmental determinants of integration and phenotypic covariance is simply too complicated for that to work well in all but the simplest instances. Alternative approaches that take into account this complexity are necessary. It is possible, however, to make predictions about changes in covariance structure that are based on well-grounded developmental hypotheses. Here the assumption is not that we can fully understand the processes that generate the pattern of covariation. Rather, one assumes that it is possible to make reasonable predictions about how a covariance structure might respond to some hypothetical perturbation or change. An alternative approach that we advocate is to combine the analysis of natural populations with model organisms in which the source of covariance is known to some extent or can be controlled. The mouse mutants that we have worked with inform how specific developmental effects produced coordinated changes throughout the skull. One could use this information to look for similar patterns within and among species of rodents or even primates to test hypotheses about the developmental basis for evolutionary change (Hallgrímsson and Lieberman 2008). Most importantly, the palimpsest view of integration highlights the need to understand the developmental mechanisms that structure the expression of phenotypic variation.

Conclusion

Covariation structure is a crucial determinant of evolvability because it determines the extent to which selection on a trait produces correlated responses in other traits (Cheverud 1982; Cheverud 1984; Muller and Wagner 1996; Wagner et al. 2007). To the extent that the correlated responses influence fitness, the pattern of covariation can either slow or speed up the rate of morphological change in response to selection. Integration and the covariation structure that results from it can determine whether complex morphological transitions occur as the result of a few or many underlying developmental changes (Lieberman et al. 2002). In a complex structure like the human skull, for example, developmentally based patterns of covariation are crucial determinants of evolvability (Martínez-Abadías et al. 2009).

We have argued here that integration, like canalization or developmental stability, is a dispositional concept that refers to the tendency of developmental systems to produce correlated variation. We have argued for a clear distinction between pattern and process. Covariation structure and the measures of integratedness such as the variance of eigenvalues are *patterns* of variation. Integration, however, as a property of developmental architecture, exists at the level of developmental mechanism. It refers to the ways in which developmental processes structure variation so as to produce covariation. Therefore, covariation structure is the result of integration at the level of developmental mechanisms combined with the variation that happens to be present in a particular sample. In complex morphological structures, this is complicated by the fact that the developmental determinants of covariance can be myriad, can overlap and can erase or obscure each other's effects. This view of integration, which we call the Palimpsest Model, should not be seen as a criticism of the study of morphological integration. This is a crucial issue in evolutionary developmental biology because it speaks to how development structures the expression of phenotypic variation. Integration, when combined with variation, produces covariation and is thus a principal developmental determinant of evolvability. The developmental determinants of evolvability, in turn, is the central question of evolutionary developmental biology (Hendrikse et al. 2007). Like Love (2006), we view the study of morphological variation as central to the theoretical framework of evolutionary-developmental biology. However, this view of integration speaks to the need to conduct analyses of integration in ways that are informed by and closely tied to our growing understanding of the mechanistic basis for development. Increased use of model organisms and experimental biological techniques in studies of phenotypic variation will support that goal. In this we underscore the argument of Müller and Newman (2005) that the theoretical framework of evolutionary developmental biology needs to overtly incorporate knowledge about the properties of developmental systems.

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References

Ackermann RR, Cheverud JM. Phenotypic covariance structure in tamarins (genus Saguinus): A comparison of variation patterns using matrix correlation and common principal component analysis. American Journal of Physical Anthropology. 2000; 111(4):489–501. [PubMed: 10727968]

Alberch, P. Developmental constraints in evolutionary processes. In: Bonner, JT., editor. Development in evolution. Springer; Berlin and New York: 1982. p. 313-332.

Ballock RT, O'Keefe RJ. The biology of the growth plate. Journal of Bone and Joint Surgery—American Volume. 2003; 85-A(4):715–726.

Bastir M, Rosas A, O'Higgins P. Craniofacial levels and the morphological maturation of the human skull. Journal of Anatomy. 2006; 209(5):637–654. [PubMed: 17062021]

Blechschmidt, IE. The stages of human development before birth. An introduction to human embryology. Karger Medical Publishers; Basel: 1961.

Bookstein, FL. Morphometric tools for landmark data. Cambridge University Press; Cambridge: 1991.

Bookstein FL, Gunz P, Mitteroecker P, Prossinger H, Schaefer K, Seidler H. Cranial integration in Homo: Singular warps analysis of the midsagittal plane in ontogeny and evolution. Journal of Human Evolution. 2003; 44(2):167–187. [PubMed: 12662941]

Boughner JC, Hallgrímsson B. Biological spacetime and the temporal integration of functional modules: A case study of dento-gnathic developmental timing. Developmental Dynamics. 2008; 237(1):1–17. [PubMed: 18058914]

Boughner JC, Wat S, Diewert VM, Young NM, Browder LW, Hallgrímsson B. Short-faced mice and developmental interactions between the brain and the face. Journal of Anatomy. 2008; 213(6):646–662. [PubMed: 19094181]

Burger R. Evolutionary dynamics of functionally constrained phenotypic characters. IMA Journal of Mathematics Applied in Medicine and Biology. 1986; 3(4):265–287. [PubMed: 3453840]

Capdevila J, Izpisua Belmonte JC. Patterning mechanisms controlling vertebrate limb development. Annual Review of Cell and Developmental Biology. 2001; 17:87–132.

Cheverud JM. Phenotypic, genetic, and environmental integration in the cranium. Evolution. 1982; 36:499–516.

Cheverud JM. Quantitative genetics and developmental constraints on evolution by selection. Journal of Theoretical Biology. 1984; 110:155–171. [PubMed: 6492829]

Cheverud JM. Developmental integration and the evolution of pleiotropy. American Zoologist. 1996; 36:44–50.

Cortes M, Baria AT, Schwartz NB. Sulfation of chondroitin sulfate proteoglycans is necessary for proper Indian hedgehog signaling in the developing growth plate. Development. 2009; 136(10): 1697–1706. [PubMed: 19369399]

Cox TC. Taking it to the max: The genetic and developmental mechanisms coordinating midfacial morphogenesis and dysmorphology. Clinical Genetics. 2004; 65(3):163–176. [PubMed: 14756664]

Diewert VM, Lozanoff S. Growth and morphogenesis of the human embryonic midface during primary palate formation analyzed in frontal sections. Journal of Craniofacial Genetics and Developmental Biology. 1993; 13(3):162–183. [PubMed: 8227289]

Diez M, Schweinhardt P, Petersson S, Wang FH, Lavebratt C, Schalling M, et al. MRI and in situ hybridization reveal early disturbances in brain size and gene expression in the megencephalic (mceph/mceph) mouse. European Journal of Neuroscience. 2003; 18(12):3218–3230. [PubMed: 14686896]

Donahue LR, Cook SA, Johnson KR, Bronson RT, Davisson MT. Megencephaly: A new mouse mutation on chromosome 6 that causes hypertrophy of the brain. Mammalian Genome. 1996; 7(12):871–876. [PubMed: 8995755]

Downey CM, Horton CR, Carlson BA, Parsons TE, Hatfield DL, Hallgrímsson B, et al. Osteo-chondroprogenitor-specific deletion of the selenocysteine tRNA gene, Trsp, leads to chondronecrosis and abnormal skeletal development: A putative model for Kashin-Beck disease. PLoS Genetics. 2009; 5(8):e1000616. [PubMed: 19696890]

Dryden, IL.; Mardia, KV. Statistical shape analysis. Wiley; Chichester: 1998.

Eberhart JK, Swartz ME, Crump JG, Kimmel CB. Early Hedgehog signaling from neural to oral epithelium organizes anterior craniofacial development. Development. 2006; 133(6):1069–1077. [PubMed: 16481351]

Fischer-Rousseau L, Cloutier R, Zelditch ML. Morphological integration and developmental progress during fish ontogeny in two contrasting habitats. Evolution and Development. 2009; 11(6):740–753. [PubMed: 19878295]

Ford-Hutchinson AF, Ali Z, Lines SE, Hallgrímsson B, Boyd SK, Jirik FR. Inactivation of Pten in osteo-chondroprogenitor cells leads to epiphyseal growth plate abnormalities and skeletal overgrowth. Journal of Bone and Mineral Research. 2007; 22(8):1245–1259. [PubMed: 17456009]

Ford-Hutchinson AF, Ali Z, Seerattan RA, Cooper DM, Hallgrímsson B, Salo PT, et al. Degenerative knee joint disease in mice lacking 3′-phosphoadenosine 5′-phosphosulfate synthetase 2 (Papss2) activity: A putative model of human PAPSS2 deficiency-associated arthrosis. Osteoarthritis and Cartilage. 2005; 13(5):418–425. [PubMed: 15882565]

Gibson-Brown JJ, Agulnik SI, Chapman DL, Alexiou M, Garvey N, Silver LM, et al. Evidence of a role for T-box genes in the evolution of limb morphogenesis and the specification of forelimb/hindlimb identity. Mechanisms of Development. 1996; 56:93–101. [PubMed: 8798150]

Gower JC. Generalized procrustes analysis. Psychometrika. 1975; 40(1):33–51.

Griswold CK. Pleiotropic mutation, modularity and evolvability. Evolution and Development. 2006; 8:81–93. [PubMed: 16409385]

Gunz P, Harvati K. The Neanderthal "chignon": Variation, integration, and homology. Journal of Human Evolution. 2007; 52(3):262–274. [PubMed: 17097133]

Hagstrom KA, Meyer BJ. Condensin and cohesin: More than chromosome compactor and glue. Nature Reviews. Genetics. 2003; 4(7):520–534.

Hall, BK. Evolutionary developmental biology. Kluwer; Dordrecht: 1999.

Hallgrímsson B, Brown JJY, Ford-Hutchinson AF, Sheets HD, Zelditch ML, Jirik FR. The brachymorph mouse and the developmental-genetic basis for canalization and morphological integration. Evolution & Development. 2006; 8(1):61–73. [PubMed: 16409383]

Hallgrímsson B, Dorval CJ, Zelditch ML, German RZ. Craniofacial variability and morphological integration in mice susceptible to cleft lip and palate. Journal of Anatomy. 2004a; 205(6):501–517.

Hallgrímsson B, Lieberman DE. Mouse models and the evolutionary developmental biology of the skull. Integrative and Comparative Biology. 2008; 48(3):373–384. [PubMed: 21669799]

Hallgrímsson B, Lieberman DE, Liu W, Ford-Hutchinson AF, Jirik FR. Epigenetic interactions and the structure of phenotypic variation in the cranium. Evolution and Development. 2007a; 9(1):76–91.

Hallgrímsson B, Lieberman DE, Young NM, Parsons T, Wat S. Evolution of covariance in the mammalian skull. Novartis Foundation Symposium. 2007b; 284:164–185. discussion 185–190.

Hallgrímsson B, Willmore K, Dorval C, Cooper DM. Craniofacial variability and modularity in macaques and mice. Journal of Experimental Zoology. Part B, Molecular and Developmental Evolution. 2004b; 302(3):207–225.

Hallgrímsson B, Willmore K, Hall BK. Canalization, developmental stability, and morphological integration in primate limbs. Yearbook of Physical Anthropology. 2002; 45:131–158.

Hansen TF, Houle D. Measuring and comparing evolvability and constraint in multivariate characters. Journal of Evolutionary Biology. 2008; 21:1201–1219. [PubMed: 18662244]

Hansen T, Pélabon C, Armbruster W. Comparing variational properties of homologous floral and vegetative characters in Dalechampia scandens: Testing the berg hypothesis. Evolutionary Biology. 2007; 34(1):86–98.

Hendrikse JL, Parsons TE, Hallgrímsson B. Evolvability as the proper focus of evolutionary developmental biology. Evolution and Development. 2007; 9(4):393–401. [PubMed: 17651363]

Hinchliffe, JR.; Johnson, DR. The development of the vertebrate limb. Oxford University Press; New York and London: 1980.

Hu D, Marcucio RS. A SHH-responsive signaling center in the forebrain regulates craniofacial morphogenesis via the facial ectoderm. Development. 2009a; 136(1):107–116. [PubMed: 19036802]

Hu D, Marcucio RS. Unique organization of the frontonasal ectodermal zone in birds and mammals. Developmental Biology. 2009b; 325(1):200–210. [PubMed: 19013147]

Hu D, Marcucio RS, Helms JA. A zone of frontonasal ectoderm regulates patterning and growth in the face. Development. 2003; 130(9):1749–1758. [PubMed: 12642481]

Jamniczky HA, Hallgrímsson B. A comparison of covariance structure in wild and laboratory muroid crania. Evolution. 2009; 63(6):1540–1556. [PubMed: 19210537]

Jones AG, Arnold SJ, Burger R. The mutation matrix and the evolution of evolvability. Evolution: International Journal of Organic Evolution. 2007; 61(4):727–745. [PubMed: 17439608]

Juriloff DM, Harris MJ, Brown CJ. Unravelling the complex genetics of cleft lip in the mouse model. Mammalian Genome. 2001; 12(6):426–435. [PubMed: 11353389]

Juriloff DM, Harris MJ, McMahon AP, Carroll TJ, Lidral AC. Wnt9b is the mutated gene involved in multifacial nonsyndromic cleft lip with or without cleft palate in A/WySn mice, as confirmed by a genetic complementation test. Birth Defects Research (Part A): Clinical and Molecular Teratology. 2006; 76:574–579.

Kawauchi S, Calof AL, Santos R, Lopez-Burks ME, Young CM, Hoang MP, et al. Multiple organ system defects and transcriptional dysregulation in the Nipbl(±) mouse, a model of Cornelia de Lange Syndrome. PLoS Genetics. 2009; 5(9):e1000650. [PubMed: 19763162]

Kember NF. Cell kinetics and the control of bone growth. Acta Paediatrica. 1993; 82(Suppl 391):61–65. [PubMed: 8219479]

Kirkwood J, Kember N. Comparative quantitative histology of mammalian growth plates. Journal of Zoology. 1993; 231:543–562.

Klingenberg CP. Morphological integration and developmental modularity. Annual Review of Ecology and Systematics. 2008; 39:115–132.

Klingenberg CP. Morphometric integration and modularity in configurations of landmarks: Tools for evaluating a priori hypotheses. Evolution & Development. 2009; 11(4):405–421. [PubMed: 19601974]

Klingenberg CP, Nijhout HF. Genetics of fluctuating asymmetry: A developmental model of developmental instability. Evolution. 1999; 53(2):358–375.

Kristensen E, Parsons TE, Hallgrímsson B, Boyd SK. A novel 3-D image-based morphological method for phenotypic analysis. IEEE Transactions on Biomedical Engineering. 2008; 55(12): 2826–2831. [PubMed: 19126464]

Lieberman DE, Hallgrímsson B, Liu W, Parsons TE, Jamniczky HA. Spatial packing, cranial base angulation, and craniofacial shape variation in the mammalian skull: Testing a new model using mice. Journal of Anatomy. 2008; 212(6):720–735. [PubMed: 18510502]

Lieberman DE, McBratney BM, Krovitz G. The evolution and development of cranial form in Homo Sapiens. PNAS. 2002; 99(3):1134–1139. [PubMed: 11805284]

Lieberman DE, Pearson OM, Mowbray KM. Basicranial influence on overall cranial shape. Journal of Human Evolution. 2000; 38(2):291–315. [PubMed: 10656780]

Love A. Evolutionary morphology and Evo-devo: Hierarchy and novelty. Theory in Biosciences. 2006; 124(3):317–333. [PubMed: 17046363]

Maeda Y, Nakamura E, Nguyen MT, Suva LJ, Swain FL, Razzaque MS, et al. Indian Hedgehog produced by postnatal chondrocytes is essential for maintaining a growth plate and trabecular bone. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104(15):6382–6387. [PubMed: 17409191]

Magwene PM. New tools for studying integration and modularity. Evolution. 2001; 55(9):1734–1745. [PubMed: 11681729]

Marcucio RS, Cordero DR, Hu D, Helms JA. Molecular interactions coordinating the development of the forebrain and face. Developmental Biology. 2005; 284(1):48–61. [PubMed: 15979605]

Marroig G, Cheverud JM. A comparison of phenotypic variation and covariation patterns and the role of phylogeny, ecology, and ontogeny during cranial evolution of new world monkeys. Evolution. 2001; 55(12):2576–2600. [PubMed: 11831671]

Martínez-Abadías N, Esparza M, Sjøvold T, González-José R, Santos M, Hernández M. Heritability of human cranial dimensions: Comparing the evolvability of different cranial regions. Journal of Anatomy. 2009; 214:19–35. [PubMed: 19166470]

Mitteroecker P. The developmental basis of variational modularity: Insights from quantitative genetics, morphometrics, and developmental biology. Evolutionary Biology. 2009 doi:10.1007/s11692-009-9075-6.

Mitteroecker P, Bookstein F. The conceptual and statistical relationship between modularity and morphological integration. Systematic Biology. 2007; 56(5):818–836. [PubMed: 17934997]

Monteiro LR, Bonato V, Dos Reis SF. Evolutionary integration and morphological diversification in complex morphological structures: Mandible shape divergence in spiny rats (Rodentia, Echimyidae). Evolution & Development. 2005; 7(5):429–439. [PubMed: 16174036]

Müller GB, Newman SA. The innovation triad: An EvoDevo agenda. Journal of Experimental Zoology. Part B, Molecular and Developmental Evolution. 2005; 304(6):487–503.

Muller GB, Wagner GP. Homology, Hox genes, and developmental integration. American Zoologist. 1996; 36:4–13.

Olson, EC.; Miller, RA. Morphological integration. University of Chicago Press; Chicago: 1958.

Parsons TE, Kristensen E, Hornung L, Diewert VM, Boyd SK, German RZ, et al. Phenotypic variability and craniofacial dysmorphology: Increased shape variance in a mouse model for cleft lip. Journal of Anatomy. 2008; 212(2):135–143. [PubMed: 18093101]

Pavlicev M, Cheverud J, Wagner GP. Measuring morphological integration using eigenvalue variance. Evolutionary Biology. 2009; 36:157–170.

Petersson S, Persson AS, Johansen JE, Ingvar M, Nilsson J, Klement G, et al. Truncation of the Shaker-like voltagegated potassium channel, Kv1.1, causes megencephaly. European Journal of Neuroscience. 2003; 18(12):3231–3240. [PubMed: 14686897]

Petersson S, Sandberg Nordqvist A, Schalling M, Lavebratt C. The megencephaly mouse has disturbances in the insulin-like growth factor (IGF) system. Brain Research. Molecular Brain Research. 1999; 72(1):80–88. [PubMed: 10521601]

Porto A, de Oliveira F, Shirai L, De Conto V, Marroig G. The evolution of modularity in the mammalian skull I: Morphological integration patterns and magnitudes. Evolutionary Biology. 2009; 36(1):118–135.

Raff, RA. The shape of life. The University of Chicago Press; Chicago: 1996.

Raff, RA.; Kaufman, TC. Embryos, genes, and evolution. MacMillan Publishers; New York: 1983.

Raff RA, Sly BJ. Modularity and dissociation in the evolution of gene expression territories in development. Evolution & Development. 2000; 2(2):102–113. [PubMed: 11258388]

Richtsmeier JT, Aldridge K, DeLeon VB, Panchal J, Kane AA, Marsh JL, et al. Phenotypic integration of neurocranium and brain. Journal of Experimental Zoology. Part B, Molecular and Developmental Evolution. 2006; 306(4):360–378.

Richtsmeier JT, Deleon VB. Morphological integration of the skull in craniofacial anomalies. Orthodontics & Cranio-facial Research. 2009; 12(3):149–158.

Rohlf FJ, Slice DE. Extensions of the Procrustes method for the optical superimposition of landmarks. Systematic Zoology. 1990; 39(1):40–59.

Rolian C. Developmental basis of limb length in rodents: Evidence for multiple divisions of labor in mechanisms of endochondral bone growth. Evolution & Development. 2008; 10(1):15–28. [PubMed: 18184354]

Rolian C. Integration and evolvability in primate hands and feet. Evolutionary Biology. 2009; 36(1): 100–117.

Roseman C, Kenny-Hunt J, Cheverud J. Phenotypic integration without modularity: Testing hypotheses about the distribution of pleiotropic quantitative trait loci in a continuous space. Evolutionary Biology. 2009; 36(3):282–291.

Ruvinsky I, Gibson-Brown JJ. Genetic and developmental bases of serial homology in vertebrate limb evolution. Development. 2000; 127(24):5233–5244. [PubMed: 11076746]

Savendahl L. Hormonal regulation of growth plate cartilage. Hormone Research. 2005; 64(Suppl 2): 94–97. [PubMed: 16286781]

Scharloo W. Mutant expression and canalization. Nature. 1964; 203:1095–1096. [PubMed: 14223104]

Scharloo W. Canalization: Genetic and developmental aspects. Annual Review of Ecology and Systematics. 1991; 22:65–93.

Schlosser, G.; Wagner, GP. Introduction: The modularity concept in developmental and evolutionary biology. In: Schlosser, G.; Wagner, GP., editors. Modularity in development and evolution. The University of Chicago Press; Chicago and London: 2003. p. 1-11.

Schmidt M, Fischer MS. Morphological integration in mammalian limb proportions: Dissociation between function and development. Evolution. 2009; 63(3):749–766. [PubMed: 19087186]

Sears KE, Behringer RR, Rasweiler JJ, Niswander LA. Development of bat flight: Morphologic and molecular evolution of bat wing digits. Proceedings of the National Academy of Sciences of the United States of America. 2006; 103(17):6581–6586. [PubMed: 16618938]

Steppan SJ. Phylogenetic analysis of phenotypic covariance structure I. Contrasting results from matrix correlation and common principal component analysis. Evolution. 1997; 51:571–586.

Waddington CH. The canalisation of development and the inheritance of acquired characters. Nature. 1942; 150:563.

Wagner GP. A comparative study of morphological integration in *Apis mellifera* (Insecta, Hymenoptera). Z. zool. Syst. Evolut. - forsch. 1989; 28:48–61.

Wagner GP. Homologues, natural kinds and the evolution of modularity. American Zoologist. 1996; 36:36–43.

Wagner GP. The developmental evolution of avian digit homology: An update. Theory in Biosciences. 2005; 124(2):165–183. [PubMed: 17046354]

Wagner GP, Booth G, Bagheri-Chaichian H. A population genetic theory of canalization. Evolution. 1997; 51(2):329–347.

Wagner, GP.; Mezey, JG. The role of genetic architecture constraints in the origin of variational modularity. In: Schlosser, G.; Wanger, GP., editors. Modularity in development and evolution. 2004. p. 338-358.Chicago and London: The University of Chicago Press

Wagner GP, Pavlicev M, Cheverud J. The road to modularity. Nature Genetics. 2007; 8:921–931.

Wang K-Y, Diewert VM. A morphometric analysis of craniofacial growth in cleft lip and noncleft mice. Journal of Craniofacial Genetics and Developmental Biology. 1992; 12(3):141–154. [PubMed: 1517393]

Willmore KE, Leamy L, Hallgrímsson B. Effects of developmental and functional interactions on mouse cranial variability through late ontogeny. Evolution and Development. 2006; 8(6):550–567. [PubMed: 17073938]

Willmore KE, Young NM, Richtsmeier JT. Phenotypic variability: Its components, measurement and underlying developmental processes. Evolutionary Biology. 2007; 34(3–4):99–120.

Wright S. General, group and special size factors. Genetics. 1932; 17:603–619. [PubMed: 17246669]

Young NM, Wagner GP, Hallgrímsson B. Long legs and short arms: Development and evolvability of human limbs. submitted.

Young NM, Hallgrímsson B. Serial homology and the evolution of mammalian limb covariation structure. Evolution. 2005; 59(12):2691–2704. [PubMed: 16526515]

Young NM, Wat S, Diewert VM, Browder LW, Hallgrímsson B. Comparative morphometrics of embryonic facial morphogenesis: Implications for cleft-lip etiology. The Anatomical Record (Hoboken). 2007; 290(1):123–139.

Zelditch ML, Lundrigan BL, Garland T. Developmental regulation of skull morphology. I. Ontogenetic dynamics of variance. Evolution & Development. 2004; 6(3):194–206. [PubMed: 15099307]

Zelditch ML, Mezey J, Sheets HD, Lundrigan BL, Garland T Jr. Developmental regulation of skull morphology II: Ontogenetic dynamics of covariance. Evolution & Development. 2006; 8(1):46–60. [PubMed: 16409382]

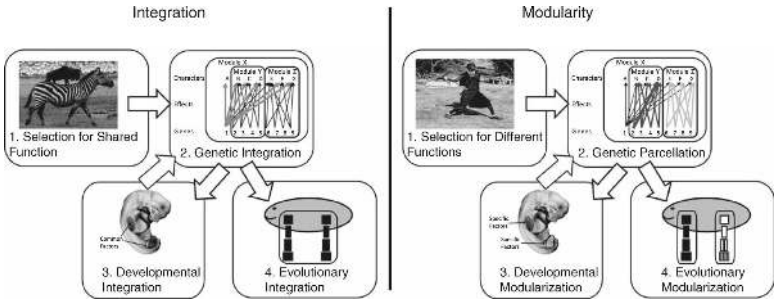

Fig. 1.

Schematic showing the evolution of integration and modularity based on Cheverud (1996) and Wagner (1996). The example illustrated is shared versus divergent limb function. The embryo shown is modified from Blechschmidt (1961)

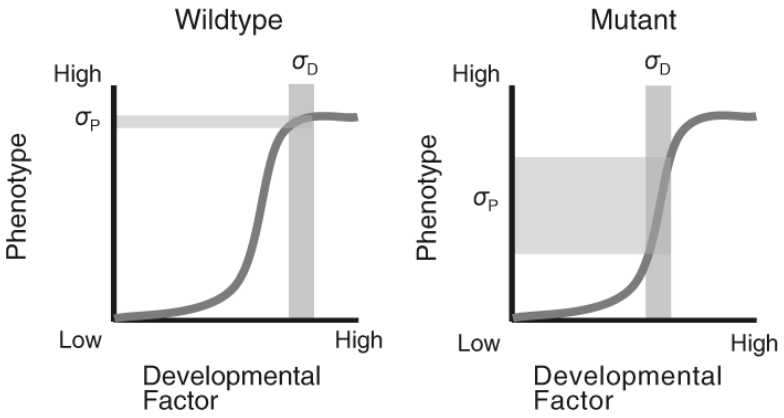

Fig. 2.

Hypothetical example showing how differences in phenotypic variance could be generated by nonlinear relationships between developmental determinants and phenotypic outcomes. The grey areas represent the variances of the developmental factor and the phenotypic result. In both cases, the variance of the developmental factor is the same. The phenotypic variance, however depends on the mean because of the underlying nonlinearity

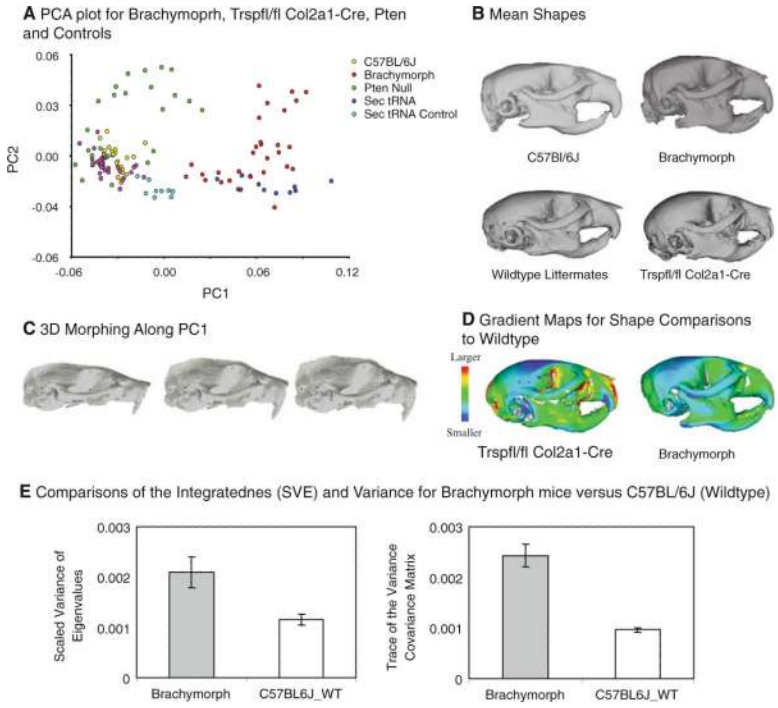

Fig. 3.

Analysis of Brachymorph and *Trsp*$^{fl/fl}$*Col2a1-Cre* Mice. **a** PCA plot for samples of both mutants with controls as well as the collagen specific knockout of the Pten gene. PC1 captures the variation in chondrocranial growth among the samples. **b** Average shapes obtained by superimposition and averaging of the individuals included in the analysis shown in A. **c** 3D morphing of PC1 showing the pattern of covariation in overall craniofacial shape that corresponds to chondrocranial growth. **d** Volumetric shape comparisons using the method of Kristensen et al. (2008). **e** The scaled variances of eigenvalues and multivariate variances for the Brachymorph samples and C5B7BL/6J wildtype mice. The error bars shown are standard deviations for both variables obtained through resampling the original datasets with replacement 1000 times. This also reveals that the differences are highly significant ($P < 0.001$)

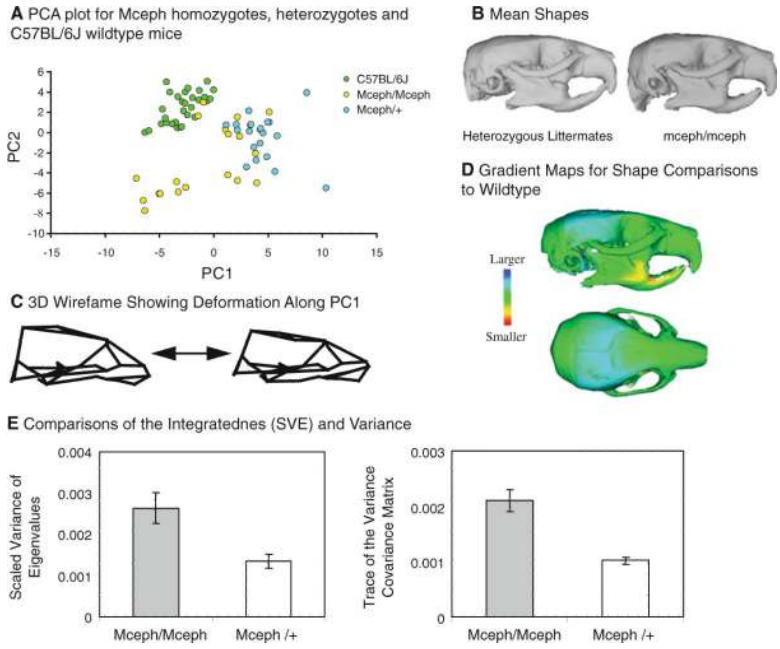

Fig. 4.

Morphometric analysis of the megencephaly mutant. **a** PCA plot of mceph homozyotes, heterozygote littermate controls and C57BL/6J mice. **b** Average shapes obtained by superimposition and averaging of the individuals included in the analysis shown in A. **c** Wireframe deformation showing the shape variation along PC1. This variation is distributed throughout the skull, particularly the basicranium. **d** Volumetric shape comparisons showing that the largest differences are in the neurocranium. **e** The scaled variances of eigenvalues and multivariate variances. The error bars shown are standard deviations for both variables obtained through resampling the original datasets with replacement 1000 times. This also reveals that the differences are highly significant ($P < 0.001$)

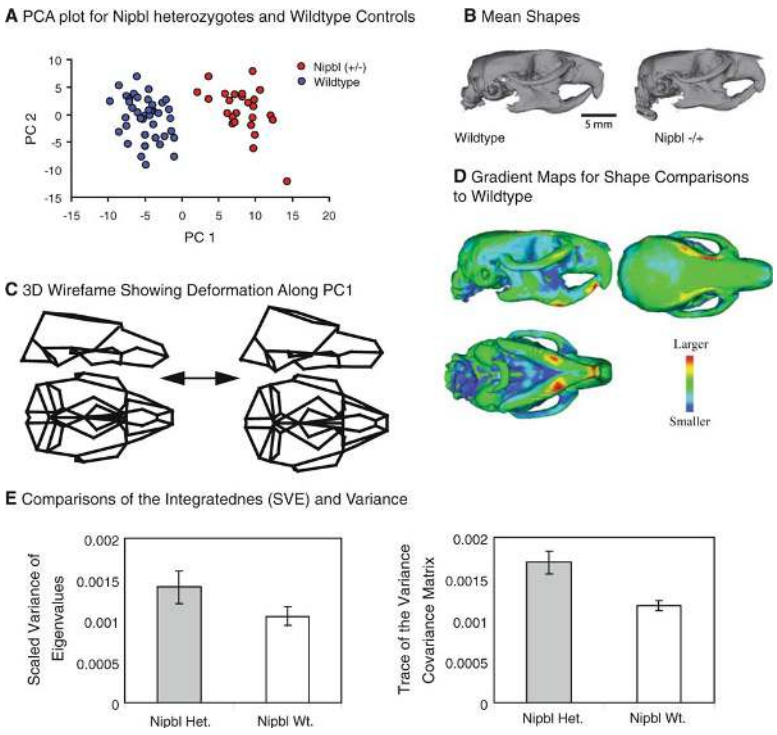

Fig. 5.

Morphometric analysis of the Nipbl mutant. **a** PCA plot for Nipbl heterozygotes and wildtype controls. **b** Average shapes obtained by superimposition and averaging of the individuals included in the analysis shown in **a**. **c** Wireframe deformation showing the shape variation along PC1. **d** Volumetric shape comparisons showing that the largest differences are in the face and basicranium. **e** The scaled variances of eigenvalues and multivariate variances. The error bars shown are standard deviations for both variables obtained through resampling the original datasets with replacement 1000 times. This reveals that the differences are significant ($P < 0.001$) for variance but not for SVE

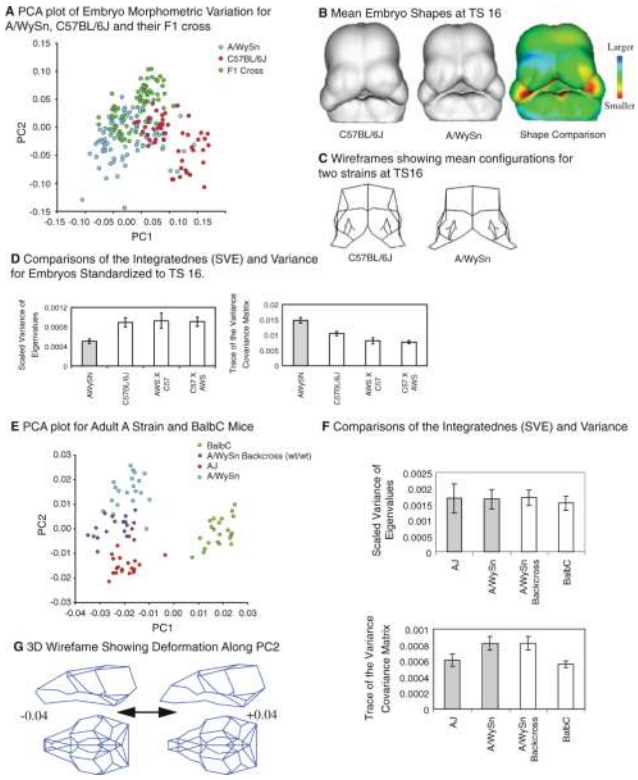

Fig. 6.

Morphometric analysis of the A/WySn mutant. **a** Shows a PCA of embryonic craniofacial morphology. These data were standardized to TS 16 and to centroid size so as to remove shape variation associated with developmental stage and size. The reason for the double standardization is that A/WySn embryos tend to be smaller and delayed relative to stage. **b** Shows the mean shape at TS16. In this case as well as a gradient map showing the shape comparison of these two averages. The largest difference is in the magnitude of maxillary prominence outgrowth. **c** Wireframes depicting the mean shapes in data standardized to centroid size and TS at TS16. **d** The scaled variances of eigenvalues and multivariate variances for the embryonic sample. The error bars shown are standard deviations for both variables obtained through resampling the original datasets with replacement 1000 times. The SVE difference between A/WySn and the other strains is significant using both the Procrustes Distance based ANOVA (Zelditch et al. 2004) and resampling of SVE ($P < 0.001$). The variances are also significantly different between A/WySn and the other groups based on resampling ($P < 0.001$). **e** PCA plot based on Procrustes superimposed landmark data from adult (90 day) samples. **f** The scaled variances of eigenvalues and multivariate variances. The error bars shown are standard deviations for both variables obtained through resampling the original datasets with replacement 1000 times. Adult A/WySn mice do not differ significantly from the others in either measure. **g** 3D wireframe showing the variation in shape among A/WySn, AJ and A/WySn backcross mice that have C57BL/6J alleles for *clf1* and *clf2* Facial length varies along this axis with the longest faces in A/WySn mice

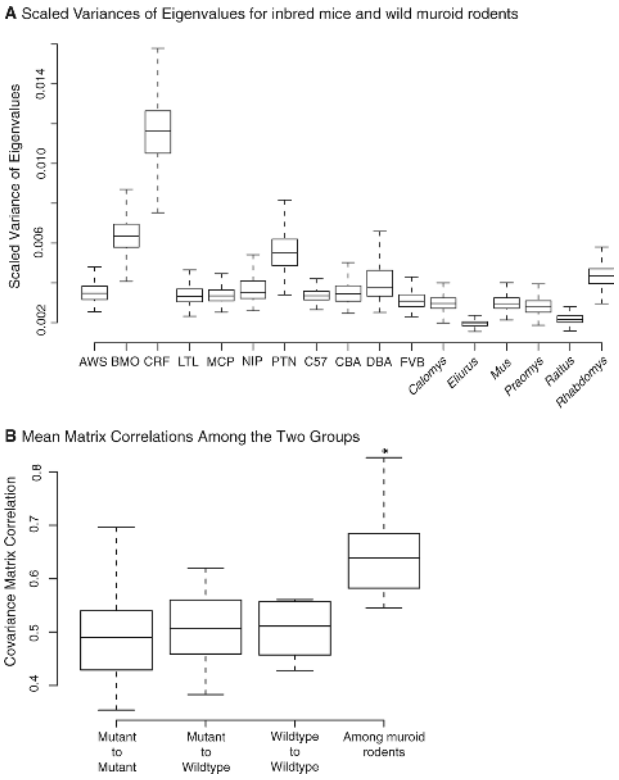

Fig. 7.

a Comparison of the variance scaled variances of eigenvalues (SVE) across inbred mouse strains and wild muroid rodents. This graphs shows the greater variation in SVE among inbred mutant and wildtype strains. **b** Shows the mean matrix correlations among these groups. This shows the significantly greater stability of covariance structure in the wild muroid sample

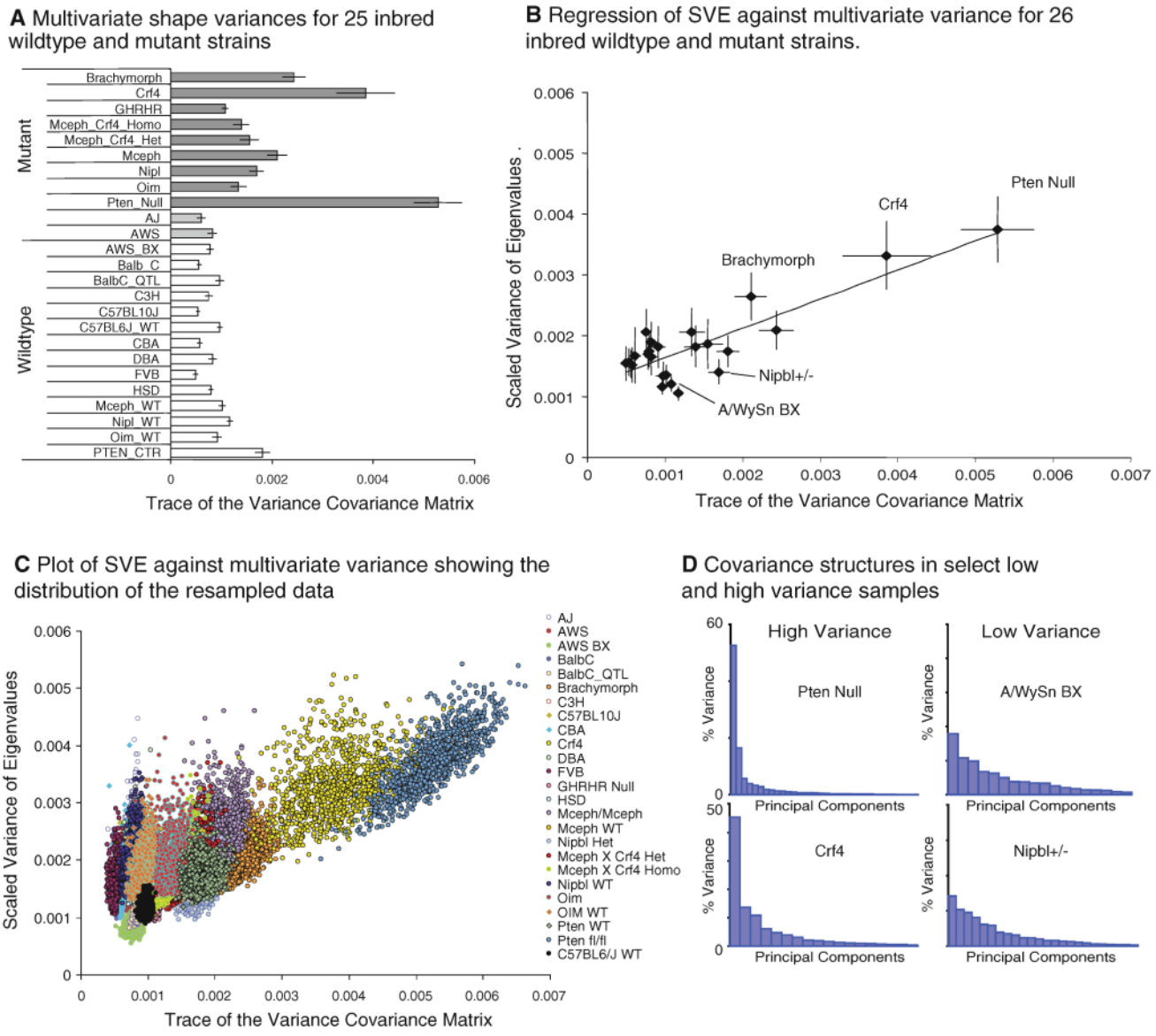

Fig. 8.

The relationship between variance and integratedness in inbred mice. **a** Shape variances for wildtype strains and mutants showing the higher variances in mutant strains. All strains are inbred with the exception of BlabCQTL and HSD. **b** Regression of SVE on variance ($r = 0.85$, $P < 0.01$). Error bars are standard deviations obtained by resampling the original data with replacement. **c** Plot of the resampled data. This plot shows that while there are some artifactual correlations between SVE and variance within samples, the pattern across samples is much more distinct. **d** Typical eigenvalue distributions for high and low variance samples. SVE is increasing with variance because of how that variance is distributed across principal components and not simply as an artifact of variance

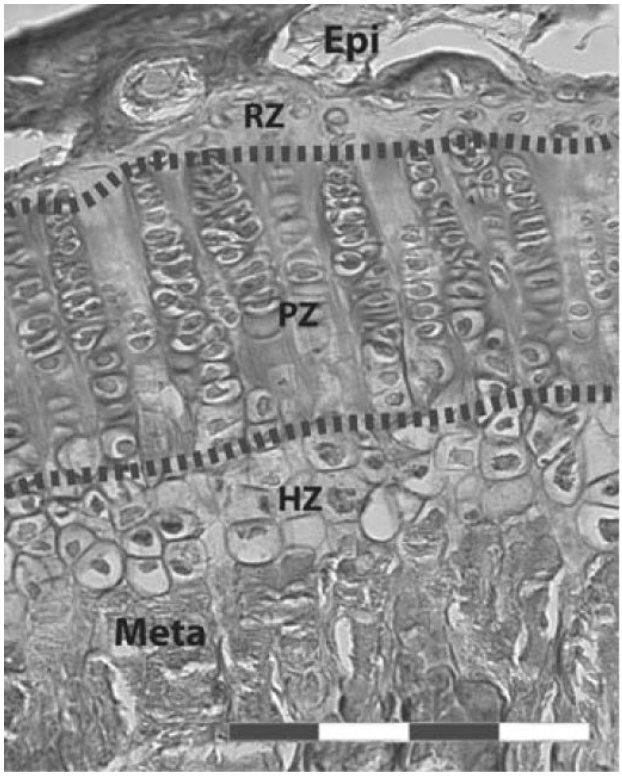

Fig. 9.

Longitudinal section through the proximal tibial growth plate of a nine-week old Mongolian gerbil (*Meriones unguiculatus*), illustrating the process of endochondral bone growth. In the growth plate, chondrocytes are organized into columns oriented parallel to the direction of longitudinal growth. Initially dormant chondrocytes in the resting zone (RZ), adjacent to the epiphysis (Epi), eventually undergo a highly orchestrated life cycle of cell proliferation (proliferating zone, PZ), hypertrophy (hypertrophic zone, HZ), and apoptosis near the metaphysis (Meta). During the latter phase the cells are resorbed by chondroclasts, leaving behind a cartilaginous "scaffold" for osteoid deposition. This life cycle is the same in the growth plates of all vertebrate long bones, providing a limited number of developmental mechanisms to generate variation in limb bone length within/among species. Scale bar = 200 μm

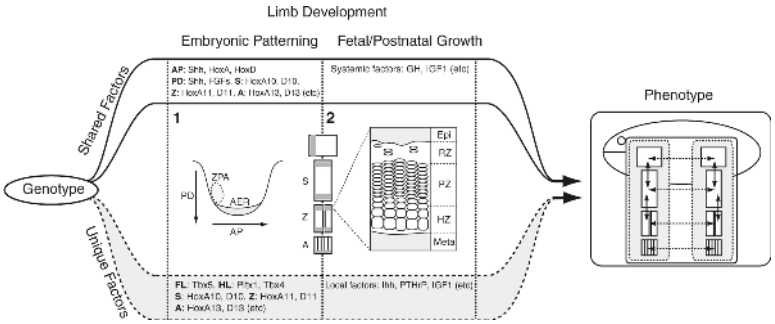

Fig. 10.

Diagram showing the relationship between genotype, organismal development and adult covariation structure in vertebrate limbs. Vertebrate limb development consists of two major phases, an embryonic patterning phase during which limb identity is specified and the mesenchymal precursors of the future limb bones are established with the proper three-dimensional (e.g., proximodistal (PD), anteroposterior (AP)) patterning (1), and a fetal and postnatal growth phase in which growth occurs chiefly via endochondral bone growth (2). Fore-(FL) and hindlimbs (HL) share a common genetic architecture. Accordingly, variance in shared developmental processes (*upper solid arrow*) is expected to increase covariation between limbs overall and between homologous elements. Conversely, a few early and late limb patterning and developmental processes are known to be uniquely expressed in the fore- and hindlimb, and in individual elements (*lower dashed arrow*) (Ruvinsky and Gibson-Brown 2000; Capdevila and Izpisua Belmonte 2001). Variance in these processes may reduce covariation between the limbs, and/or between neighboring elements within a limb, and thus increase independently selectable variation among individual limb bones. The adult limb phenotype represents the cumulative and superimposed effects of these developmental processes on covariance structure. Note that processes specific to homologous elements may increase covariation between them (*dashed horizontal arrows*) even though they may reduce covariation with neighboring elements within a limb (*solid vertical arrows*). Other abbreviations:, *AER* apical ectodermal ridge, *ZPA* zone of polarizing activity, *S* stylopod, *Z* zeugopod, *A* autopod. Growth plate abbreviations as in Fig. 9

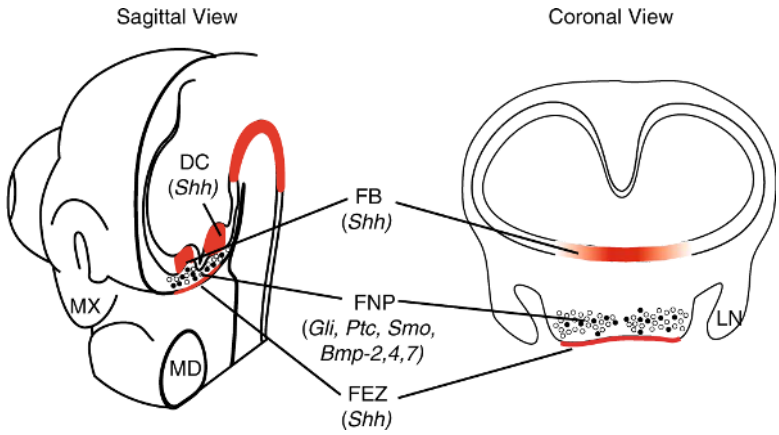

Fig. 11.

Schematic showing the spatial relationship of the FEZ and regions of the brain that express *Shh* during midfacial formation and outgrowth. *Shh* expression in the brain (DC: Diencephalon; TE: Telencephalon) initiates mesenchymal proliferation and the formation of the FEZ. *Shh* expression in the FEZ then continues to regulate outgrowth through proliferation within the frontonasal prominence (FNP). *LN* Lateral nasal process; *MX* Maxillary process; *MD* Mandibular process)

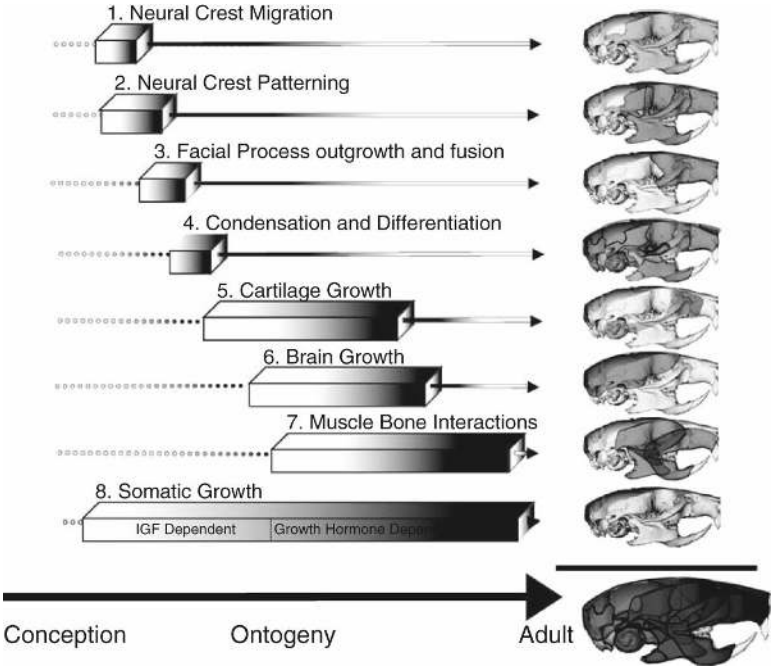

Fig. 12.

Schematic illustration of the Palimpsest Model as applied to the mouse skull. Multiple developmental processes acting at different times and influencing overlapping anatomical regions each leave a covariation imprint that adds up to a very complex covariation structure

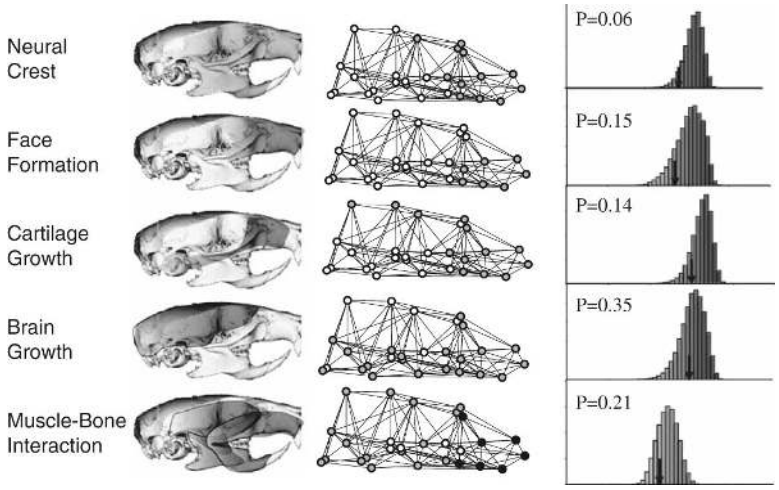

Fig. 13.

Tests of modularity using the method of Klingenberg (2009) based on 3D landmark data for 300 mice from 12 wildtype inbred strains. The anatomical regions tested are shown both as outlines and in terms of the division of landmarks into hypothetical modules. The histograms show the distribution of RV coefficients obtained from permutation of all possible combinations of contiguous landmarks. The RV coefficient is a measure of the strength of internal (within-module) covariance relative to external covariance. These results show that cranial covariation structure tends not to conform to simple hypotheses about modularity in this sample