

A fly in a tube: Macroevolutionary expectations for integrated phenotypes

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Phenotypic integration and modularity are ubiquitous features of complex organisms, describing the magnitude and pattern of relationships among biological traits. A key prediction is that these relationships, reflecting genetic, developmental, and functional interactions, shape evolutionary processes by governing evolvability and constraint. Over the last 60 years, a rich literature of research has quantified patterns of integration and modularity across a variety of clades and systems. Only recently has it become possible to contextualize these findings in a phylogenetic framework to understand how trait integration interacts with evolutionary tempo and mode. Here, we review the state of macroevolutionary studies of integration and modularity, synthesizing empirical and theoretical work into a conceptual framework for predicting the effects of integration on evolutionary rate and disparity: a fly in a tube. While magnitude of integration is expected to influence the potential for phenotypic variation to be produced and maintained, thus defining the shape and size of a tube in morphospace, evolutionary rate, or the speed at which a fly moves around the tube, is not necessarily controlled by trait interactions. Finally, we demonstrate this reduced disparity relative to the Brownian expectation for a given rate of evolution with an empirical example: the avian cranium.

KEY WORDS: Constraints, disparity, evolutionary rates, macroevolution, modularity, phenotypic integration.

"Now it is evident that the sole general principle one can apply is given by the position, the relations, and the dependencies of the parts, that is to say, by what I name and include under the term of connections."

—Étienne Geoffroy Saint-Hilaire (1818)

Biological organisms are composed of parts with varying degrees of interdependence and independence. These relationships, as it pertains to morphological structures, are termed phenotypic integration and have a long history of explicit study (Olson and Miller 1958) and an even longer history of implicit study, as this concept is embedded in every analysis that parcelates a whole organism into different components. Integration and the related topic of modularity, whereby the relationships among traits describe regions or units with strong correlations within themselves but relatively weak or no integration across regions, are fundamental biological concepts. These properties span disparate scales of biology from genes to communities and are identifiable at every scale of biological organisation. Within the specific system of the phenotype, the study of phenotypic

integration and modularity offers an almost unique power to bridge the genetic, development, and functional associations among traits with their morphological products, especially adult morphology. This aspect is of particular interest, because, for the many rare or wholly extinct taxa that represent the vast majority of organismal variation, this approach offers the only opportunity for accessing any information on the genetic and developmental architecture underlying the evolution of form.

This architecture is of interest not just for tracing the roots of organismal form, but also for understanding its evolution. It has long been hypothesized that the relationships among traits shape their variation, and thus ultimately their evolutionary trajectories, either by constraining evolution to limited directions of shape change or by facilitating the evolution of coordinated traits (Fig. 1; Klingenberg 2005; Wagner and Zhang 2011; Goswami et al. 2014). Both of these effects can significantly shape morphological diversity (disparity) on macroevolutionary time scales, and thus phenotypic integration and modularity have often been described as fundamental influences on

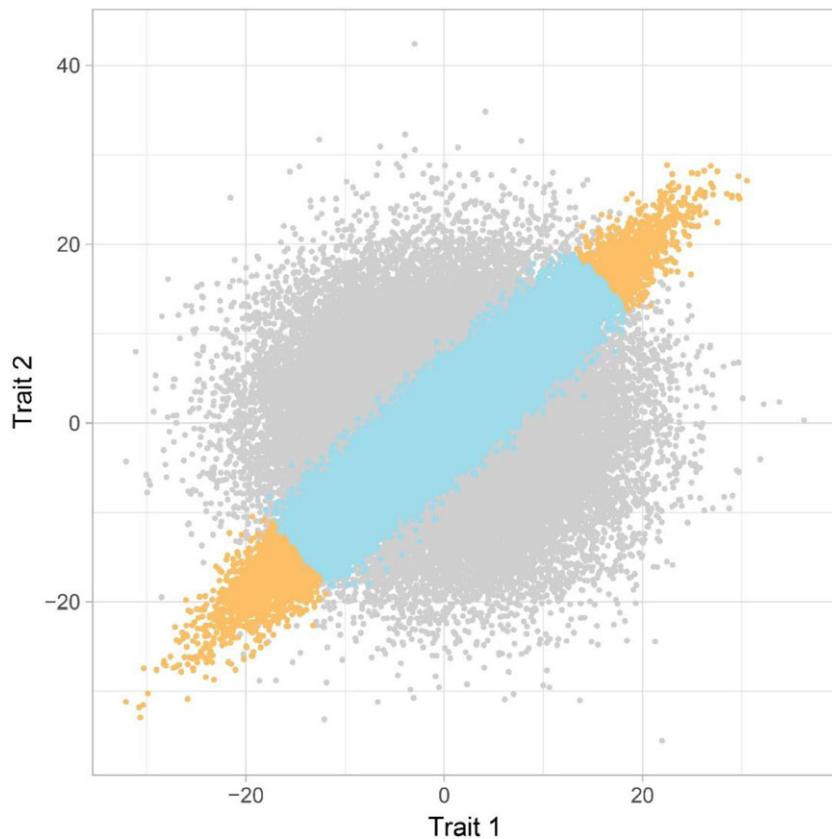


Figure 1. Simulated evolution of a bivariate trait under Brownian motion (BM). Gray points represent uncorrelated evolution between traits 1 and 2. Colored points represent high integration between traits 1 and 2. Orange points exceed the disparity expected by uncorrelated BM and would evolve when selection aligns with the major axis of variation, representing integration facilitating evolutionary change. Blue points represent an example of integration constraining disparity, as they exhibit less variation than the neutral model.

morphological evolution and the generation of biological diversity. More specifically, it is suggested that the fragmentation of trait relationships into semi-autonomous units, or modules, promotes evolvability by releasing sets of traits with divergent selection pressures from the constraints of their covariation, imposed by genetic pleiotropy or developmental canalization (Wagner and Altenberg 1996). Assessing the accuracy of this hypothesis and understanding how prevalent these various potential effects of trait integration are in the natural world requires comparative data across taxa to reconstruct the patterns of trait relationships, how they change, and how they relate to macroevolutionary patterns in organismal form and disparity (Conner et al. 2014; Goswami et al. 2015). While a rich literature exists for examining genetic and developmental associations and their relationship to phenotypic integration at a microevolutionary scale, there is yet relatively little broad comparative data for phenotypic integration and even less incorporation of this field within the study of morphological evolution at the macroevolutionary scale.

The field of macroevolution originated over 70 years ago with George Gaylord Simpson's ground-breaking work on the

concepts of evolutionary tempo and mode (Simpson 1944). Since then, thousands of studies have used a wide range of data and increasingly sophisticated quantitative methods to reconstruct the evolution of biological diversity by identifying the patterns and processes underlying trait evolution across the tree of life and through deep time (Simpson 1944; Gould and Eldredge 1977). Variation in tempo (rate) and mode (process) of phenotypic evolution through time and across lineages define adaptive radiations, periods of stasis, diversity crises, and global biodiversity gradients (Gould and Eldredge 1977; Ricklefs 2004; Landis and Schraiber 2017). Most studies assessing these patterns with deep-time data, either large comparative datasets or directly sampling the fossil record, focus on extrinsic factors, such as environment or species interactions, rather than intrinsic factors of the species themselves (Ruta et al. 2006; Harmon et al. 2010; Halliday and Goswami 2016). Where they are incorporated, species attributes are usually limited to ecological traits (Jones et al. 2015; Michaud et al. 2018), which are those that mediate the biotic and abiotic interactions for an individual, but which act on existing variation rather than shaping potential variation, as genetic and developmental interactions are thought to do. Consideration of those latter factors relies

largely on extrapolation from well-studied extant taxa (Bennett and Goswami 2013), but quantifying trait relationships allows for the estimation and comparison of genetic and developmental associations across taxa for which only adult morphology is available (Goswami et al. 2014). Moreover, the majority of trait macroevolution studies focus on univariate trait, such as body size (Slater et al. 2010; Venditti et al. 2011), or discrete characters (Clarke and Middleton 2008; Halliday and Goswami 2016). Univariate analyses prevent examination of trait interactions for obvious reasons. Studies of trait interactions with discrete data have been conducted, for example to identify suites of correlated characters and their effects on morphology-based reconstructions of phylogenetic relationships (e.g., O'Keefe and Wagner 2001; Beaulieu et al. 2013) or divergence dating (e.g., Lee 2016), which often incorrectly assume character independence. These approaches have great potential to elucidate macro-scale patterns of trait integration, particularly as large matrices of discrete data spanning diverse taxa are readily available. However, because many, if not most, discrete characters are binary, their distributions and interactions may oversimplify or overlook the subtle biological covariation that more accurately reflects the complex hierarchy of genetic and developmental relationships among traits (Goswami and Polly 2010a). Combining these approaches, for example by informing models of discrete character evolution from analyses of trait integration from continuous morphometric data, is a promising path forward. As a wealth of phenotypic data accumulates, alongside improved understanding of the processes underlying morphological evolution, it is increasingly possible to combine these different factors into unified analyses to produce a comprehensive understanding of origins, maintenance, and destruction of biological diversity. One such measure of biological diversity is morphological disparity, which quantifies the breadth of organismal form and can be used to understand phenomena such as niche occupation, evolutionary divergence, and constraint. Morphological disparity has been quantified in numerous ways, including range, morphospace volume, and pairwise distances between taxa (reviewed in Foote 1997), and with data types ranging from discrete characters to multidimensional morphometrics. With geometric morphometric data, which are the data most commonly used in recent studies of phenotypic integration and modularity, disparity is often measured as Procrustes variance, the trace of the variance-covariance matrix of Procrustes-aligned coordinate data. Here, we describe the expectations for evolution of integrated phenotypes and discuss the current advances, opportunities, and challenges in incorporating these expectations in macroevolutionary studies. We present a framework for macroevolutionary expectations of the relationship between evolutionary tempo and morphological disparity given trait integration and provide an empirical example using a recently published high-density morphometric dataset for the avian cranium.

THE EVOLUTION OF INTEGRATION AND MODULARITY, AND WHY IT MATTERS

Integration (i.e., the correlated variation or evolution of traits) is one of the main intrinsic shape characteristics that may both constrain and promote morphological evolution. It is intuitive that when traits change, they likely do so in conjunction with other traits with which share a common function or common genetic and developmental origins (Olson and Miller 1958). These relationships can be identified through quantitative analysis of phenotypic trait variation and covariation and potentially linked to the ultimate drivers of those relationships, to provide a comprehensive understanding of how intrinsic factors shape macroevolutionary patterns. For example, extensive work in the realm of quantitative genetics has been concerned with understanding how genetic variance and covariance, quantified as the G-matrix, determine the potential for evolutionary change (e.g., Cheverud et al. 1984; Schlüter 1996; Hansen et al. 2003). A major revelation of this field of research is the notion of “genetic lines of least resistance” (Stebbins 1974; Futuyma et al. 1993; Schlüter 1996) in which greater evolutionary change occurs when the direction of multivariate selection is aligned with the vector summarizing the direction of highest genetic variation (Lande 1979; Arnold 1992; Hansen and Houle 2008). Phenotypic disparity is thus the result of the interaction between selection and intrinsic constraints on variation, the latter of which is reflected in trait integration.

Patterns of phenotypic trait integration and modularity have been examined in diverse organisms at the microevolutionary and macroevolutionary scales to quantify and compare patterns across taxa, assess the genetic, and developmental roots of observed patterns in phenotype, and, more recently, ascertain whether changes in either are associated with shifts in evolutionary rate or disparity. Integration and modularity have been most frequently studied in mammals, with several studies across various parts of the skeleton (e.g., Cheverud 1995; Marroig and Cheverud 2001; Klingenberg et al. 2003; Goswami 2006b; Young 2006; Porto et al. 2008; Zelditch et al. 2008; Bennett and Goswami 2011; Kelly and Sears 2011) allowing for some determination of macroevolutionary patterns in trait relationships. Many of these studies have supported a complex six-module organisation for the mammalian cranium (Cheverud 1982; Goswami 2006a; Marroig et al. 2009; Goswami et al. 2014), with this pattern of cranial modularity being generally conserved across marsupials and placentals, although monotremes appear to have a less integrated organisation to their skulls (Goswami 2006a; Goswami et al. 2014). In contrast, postcranial modularity varies across the three clades of mammals, with placentals displaying strong integration of the fore- and hind limbs (Young and Hallgrímsson 2005) and marsupials dissociating the fore- and hind limbs (Bennett and Goswami 2011; Kelly and Sears 2011), likely related to the delayed ossification of the hind limb (Weisbecker et al. 2008;

Goswami et al. 2009). Monotremes show integration across serial homologues of the limb skeleton (e.g., femur and humerus), but not within limbs (Bennett and Goswami 2011; Kelly and Sears 2011) possibly reflecting their unusual distal to proximal limb ossification pattern (Weisbecker 2011). While patterns of cranial modularity are difficult to assign to specific developmental or functional origins (Hallgrímsson et al. 2009), limb modularity appears to more clearly reflect developmental differences among the major mammal clades. Studies of full skeletal integration are at present limited to cats, demonstrating that the presacral vertebral column is composed of five discrete modules that don't map simply onto standard anatomical regions (cervical, thoracic, lumbar; Randau and Goswami 2017a), but do reflect the developmental origins of vertebral structures (Randau and Goswami 2017b). Moreover, these studies demonstrate that the vertebral column as a whole is relatively independent of the cranial and appendicular skeletons (Randau and Goswami 2018). At present, this observed variation across extant mammals does not allow for determination of the polarity of shifts in the pattern of modularity in either the cranial or postcranial skeleton of mammals; specifically, it is unclear whether the changes in trait relationships observed across the major mammalian clades represent an increase or a decrease in integration, if either, without data outside of crown Mammalia.

Unfortunately, there is a paucity of studies of phenotypic integration and modularity in other clades (Kulemeyer et al. 2009; Sanger et al. 2011; Webster and Zelditch 2011; Adams and Felice 2014; Andjelković et al. 2017; Felice and Goswami 2018; Larouche et al. 2018), and few of these can be directly compared. For example, across nonmammalian vertebrates, there is a series of studies of external morphology of fishes, through ontogeny (Zelditch and Fink 1995; Fischer-Rousseau et al. 2009) and across taxa (Larouche et al. 2018). These studies demonstrate the modularity of the fins in particular (Larouche et al. 2018) and suggest the pattern and magnitude of integration is volatile through ontogeny. For birds, some studies have supported a highly integrated skull (Klingenberg and Marugán-Lobón 2013) while others have supported a more modular organisation (Felice and Goswami 2018). Similarly, some of these studies have suggested that allometry is the principal factor influencing bird skull shape and integration (Bright et al. 2016), whereas others have suggested that pattern and magnitude of integration reflects the developmental complexity of cranial regions (Felice and Goswami 2018). Thus there is yet little consensus on patterns of integration and modularity or their underlying roots, although there is clear evidence that developmental interactions are one important factor driving phenotypic trait relationships in vertebrates.

Across invertebrates, there have been studies in fossil clades, such as crinoids (Gerber 2013) and trilobites (Webster and Zelditch 2011), with the latter showing rapid changes in patterns of integration early in clade history, perhaps reflecting a lack

of developmental canalization during the early radiation of this group. Among living invertebrates, a number of studies have examined changes in integration of *Drosophila* wings, finding that developmental and genetic correlations shape phenotypic patterns (Klingenberg and Zaklan 2000; Hansen and Houle 2008; Houle et al. 2017). Studies of other invertebrates are limited, but one study examined differences in modularity associated with segmentation in mantis shrimps (Anderson et al. 2016). There is a long history of studies of trait organisation in plants as well (Berg 1960), with many studies demonstrating strong dissociation between flowering and vegetative structures (Armbruster et al. 2014; Diggle 2014), but again little incorporation of this work into macroevolutionary studies of plant diversification.

Thus, despite an expanding dataset for phenotypic integration and modularity, there is yet little understanding of their evolution across the tree of life. In particular, comparing results across many of these studies to ascertain shifts in integration and modularity and identify any trends in these attributes through evolutionary time is hindered by the variation in methods across studies. Morphometric data collection techniques applied in these studies range from linear metrics (e.g., Cheverud 1995; Porto et al. 2008) to 2D and 3D geometric morphometrics (e.g., Klingenberg and Zaklan 2000; Goswami 2006b; Zelditch et al. 2008; Gerber and Hopkins 2011; Sanger et al. 2011; Parsons et al. 2012), including more recent incorporation of sliding semilandmarks (e.g., Bright et al. 2016; Dumont et al. 2016; Parr et al. 2016; Fabre et al. 2018; Felice and Goswami 2018). Treatment of geometric morphometric data also varies, in particular in whether simultaneous-fit or separate-fit approaches are applied to structures composed of multiple hypothesized modules (Klingenberg 2009; Baab 2013), with the former allowing incorporation of information on the relative positions of hypothesized modules and direct comparison of alternative hypotheses of modularity, while the latter reduces the integrating effect of uniform scaling.

From this variation in quantification of morphometric data, there is additional variation in analytical approaches. A common method of summarizing trait covariances is principal component analysis (PCA), an ordination method that finds linear combinations of covarying traits (Sokal and Rohlf 2009). If most traits covary strongly, this would be reflected in relatively few eigenvectors (orthogonal, statistically independent axes) that together explain most of the variation in the original sample, i.e., the higher the integration, the fewer significant eigenvectors exist. This increased concentration of variation in few dimensions (major axes) leads to augmented disparity between the eigenvalues, which in itself can be used as a measure of trait integration (i.e., the relative eigenvalue standard deviation; Pavlicev et al. 2009). Beyond magnitude of integration, methods for analysing patterns of integration and modularity have evolved from cluster-based approaches and Euclidean distance matrix analysis (Cheverud 1982) to more

robust hypothesis-testing methods, e.g. 2-B PLS (Bookstein et al. 2003), relative eigenanalysis (Bookstein and Mitteroecker 2014), RV coefficient analysis (Klingenberg 2009) and its offshoot Covariance Ratio analysis (Adams 2016), and further to methods that can directly compare support for different hypotheses of modular organisation, such as subspace analysis (Márquez 2008) and EMMLi (Goswami and Finarelli 2016).

Extensive debate continues on the pros and cons of all of these approaches, from data collection to superimposition methods to identification of modules. Certainly, each approach has strengths and weaknesses, and it is unclear if these methods produce the same results. For example, one change that may be occurring due to data types is the incorporation of semilandmark data (Gunz and Mitteroecker 2013) to studies of integration and modularity. Type I and II geometric morphometric landmarks, those most commonly used in analyses of integration and modularity to date, are typically located at the boundaries of regions, e.g., sutures of bones, which will inevitably translate into estimates of higher integration among those bones. Semilandmarks, or more specifically sliding semilandmarks of curves and surfaces of structures, sample more morphology than these boundary-based landmarks and thus are likely to support more modular structures (Parr et al. 2016; Felice and Goswami 2018). This result is perhaps counterintuitive given that the positions of semilandmarks, especially sliding semilandmarks, are inherently relative to one another, but they also more accurately represent the complex shape, and complex relationships, of biological structures, rather than simply the few points in space where structures contact one another.

Differences in the results of some studies (e.g., Klingenberg and Marugán-Lobón 2013; Felice and Goswami 2018) may also reflect differences in the purposes of analytical approaches. For example, 2B-PLS was developed specifically for identifying integration among parts, rather than testing for modular structure. Thus while studies of PLS can identify if structures are significantly integrated, it does not test whether each structure is more strongly integrated internally, that is whether it can be described as a module. Nonetheless, at least for studies of mammal skull modularity, there are clear consistencies in the results produced from EDMA analysis of linear data (Cheverud 1982), cluster analysis of 3D geometric morphometric data using landmark-based (where x, y, and z coordinates are pooled) correlations (Goswami 2006a,b), RV coefficient analysis of 3D geometric morphometric data using coordinate-based (where x, y, z coordinates are treated separately) covariances, and maximum-likelihood analysis of both landmark- and coordinate-based correlations (Goswami and Finarelli 2016).

Given these consistencies in results for studies of mammalian skull modularity across vast differences in data collection and analytical approaches, the larger hurdle in comparing across the many studies of integration and modularity may well be that many studies do not compare similar hypotheses of modular

organisation. Moreover, many only test a single model (e.g., the neurocranial/facial two-module hypothesis for vertebrate skulls (Kulemeyer et al. 2009; Klingenberg and Marugán-Lobón 2013; Bright et al. 2016) that makes it virtually impossible to compare results across studies that assess more complex hypotheses (e.g., six cranial modules) and also hinders identification of trends in modularity through time. Analysing alternative, and more complex, models of modularity is essential to progress in this field, as there are many possible drivers of trait integration and thus many possible hypotheses of modular structures that can and should be tested (Hallgrímsson et al. 2009). It is also impractical that every possible driver of trait integration, whether genetic, developmental, or functional, will be identified *a priori* across a comparative sample, but identifying patterns of modularity within and across taxa will provide testable hypotheses that can focus future studies in the molecular or biomechanical mechanisms generating the observed patterns.

Identifying these patterns and trends in patterns of integration and modularity is also central to accurately reconstructing the factors shaping morphological evolution. As in the concept of genetic lines of least resistance, phenotypic integration may act as a constraint when the covariances among traits are strong in magnitude and the main axis of variation (i.e., the first eigenvector) is perpendicular to the direction of selection, with little variation on the remaining other axes of variation (Kirkpatrick and Lofsvold 1992; Bjorklund 1996; Schlüter 1996; Klingenberg 2005; Goswami et al. 2014). With little variation in the direction of the nearest evolutionary peak, changes toward this optimum may need to take longer trajectories, passing through less optimal states, or they can be prevented from responding to selection entirely. The same scenario may occur if traits that are highly integrated are under differential selection pressures (e.g., directional and stabilising selection). Here, phenotypic changes toward any of these optima may be hindered, perhaps until covariances change and previously integrated traits are partitioned, allowing each trait or new module to respond more independently to divergent selection pressures. In this scenario of constraint, high integration among traits may cause both stasis and homoplasy (convergence) when integration acts as a constraint to shape disparification. By either hindering shape change or allowing it to happen only on narrow trajectories, preferential regions of the morphospace are iteratively occupied whereas others are not possible, resulting in a reduction in disparity over time (Lande 1986; Eble 2004).

Alternatively, integration is expected to facilitate change in functionally linked traits. Whereas, in the absence of high covariation, increased variation in all traits linked to a specific function may hinder optimum adaptation (i.e., by generating a great number of possible trait combinations), integration among such traits may promote change in the direction of the adaptive peak by limiting variation in all other directions (Wagner 1988a;

Burger 1986). Further, by concentrating variation in a few directions, integration may facilitate an increase in disparity if these axes are parallel to selection vectors (i.e., the lines of least resistance; Schlüter 1996; Goswami et al. 2014; see below).

To date, empirical analyses have not shown a consistent relationship between high levels of integration and the effects of morphological disparity. Whereas empirical studies in invertebrates and vertebrates have demonstrated instances when high integration has been associated with lower disparity (Goswami and Polly 2010b; Claverie and Patek 2013; Felice and Goswami 2018), the opposite is also true (e.g., high integration promoting high vertebral shape disparification in felids; Randau and Goswami 2017b). Other studies have found no relationship at all, such as in a study of fossil crinoids (Gerber 2013). However, the bulk of empirical studies to date have suggested that high integration may constrain morphological disparity. Similarly, some studies have demonstrated that increasing modularity, in particular the dissociation of traits that may be subject to differential constraints or selection pressures, promotes morphological diversification and specialisation, such as in the fins of actinopterygian fishes (Larouche et al. 2018) and the appendages of mantis shrimps (Claverie et al. 2010), but there is yet little systematic study of this effect.

While there are fewer studies explicitly considering the impact of phenotypic integration on evolutionary rates, no relationship between magnitude of integration and rate was found in carnivorans (Goswami et al. 2014), although a negative relationship was identified in birds (Felice and Goswami 2018). Although a positive correlation between high integration and rates may be expected when high disparity is promoted due to similarity in direction of selection and the major axis of variation, the opposite might be expected, but is not strictly necessary, when integration constrains the level of disparification. Here, although the portion of the morphospace that is occupied is restricted (i.e., low disparity), and therefore convergences in shape are expected to occur, how fast these smaller changes in shape can happen is not affected by integration. In other words, the response to selection is shaped by the covariances among traits, but not necessarily the speed at which that response occurs.

Taken together, these factors serve to shape the available range of phenotypes that can be achieved given a trait covariance structure, but this does not necessarily also limit the rate at which that phenotype space is explored. We can visualize this relationship by envisioning a fly in a tube (Fig. 2). The tube represents the constraints imposed by trait correlations, biomechanical limitations, ecological opportunities, and other constraints, and the fly represents the position of the population mean in morphospace at a given time. The fly can traverse the inside of the tube (available/easily accessible morphospace) at any speed (evolutionary rate) but will be bounded by the walls of the tube (constraints). This model is analogous to morphospace saturation, the idea that



Figure 2. The “fly in a tube” model of evolutionary rates and disparity. The fly represents the mean trait value of a population or lineage moving through morphospace or an adaptive landscape. The shape and size of the tube represents the phenotypic disparity that is possible, which is determined by constraints including trait integration. The evolutionary rate at which the lineage traverses this potential space is uncoupled the size of the space.

intrinsic or extrinsic constraints can limit morphospace expansion even without changing rates of morphological change (Foote 1994). Phenotypic integration thus provides an expectation not only for the evolution of disparity, but also for the relationship between morphological disparity and evolutionary rate.

MACROEVOLUTIONARY EXPECTATIONS FOR AN INTEGRATED PHENOTYPE: A THEORETICAL FRAMEWORK

Building on these concepts, we can develop a theoretical framework for predicting how disparity evolves in response to different evolutionary rates and scenarios. Figure 3 illustrates this framework. Along the diagonal is the null expectation of the disparity/rate relationship according to Brownian motion. An explicit prediction of Brownian motion, the de facto null model of trait evolution, is that variance is determined by the rate of evolution. Brownian motion describes a random walk with rate = σ^2 . The variance of the trait at time t is proportional to σ multiplied by elapsed time (Felsenstein 1985; Ricklefs 2006). As such, given the same evolutionary time, higher rates of evolution will result in higher variance (Fig. 4). Thus under a neutral evolutionary process and with no correlations among traits, there will be a strict linear relationship between the rate of evolution and the variance generated (Fig. 3). Integrated phenotypes and alternative

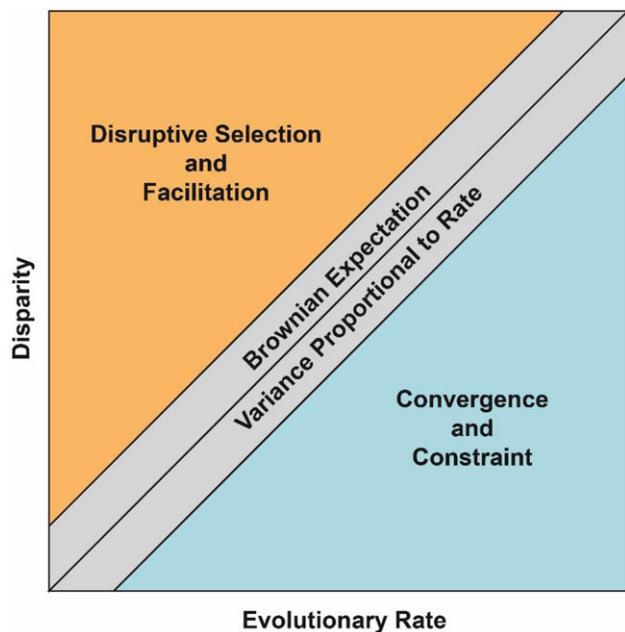


Figure 3. Theoretical framework for the relationship between evolutionary rates and trait variation. Under Brownian motion, disparity is expected to increase as rate increases. With ecological or developmental constraints or high levels of homoplasy, disparity will be lower than that expectation for a given rate (lower triangle). If the correlations among traits are aligned with the multivariate direction of selection, high disparity for a given rate can be facilitated (upper triangle). High disparity relative to rate might also be a consequence of strong disruptive selection.

evolutionary scenarios will result in more or less phenotypic variation for a given rate of evolution.

When constraints limit disparity, data points (taxa or traits) will fall into the lower triangle, describing lower disparity relative to rate. This is also a characteristic of a high occurrence of convergent evolution (Sidlauskas 2008), where the same areas of morphospace are being visited repeatedly. As discussed above, such a situation could be a result of high integration when the major axis of variation does not coincide with the direction of the selection vector. By limiting variation on dimensions other than the major eigenvector (i.e., creating the walls of the tube that define the available space), and therefore forcing the repeated occupation of similar spaces, morphological convergence is pressed. This may be the expected scenario for biomechanical and ecological traits that are integrated due to a common function, for whole structures for which variation is mainly driven by allometry (i.e., due to allometry being a strong driver of morphological integration; Klingenberg 2008; Goswami and Polly 2010b; Klingenberg and Marugán-Lobón 2013; Bright et al. 2016) or when developmental interactions or timing drives integration in traits and thus limits their potential variation (e.g., Bennett and Goswami 2011; Sears et al. 2013). In addition, biomechanical or physiological

factors can impose absolute constraints on the potential disparity that can evolve in some traits (Walker 2007). For example, trade-offs related to tracheal volume and oxygen exchange limit maximum body size in insects (Kaiser et al. 2007). This type of hard limit on trait ranges can be modeled with bounded Brownian motion (BBM, Boucher and Démery 2016). Similarly, stabilizing selection can constrain the maintenance of phenotypic variation. BBM relates to intrinsic constraints, whereas stabilizing selection concerns the relationship between the environment, phenotype, and fitness. This latter evolutionary scenario can be estimated using the Orstein-Uhlenbeck (OU) model, an extension of Brownian motion that modifies the random walk by adding a parameters that specify an optimal trait value and the strength of pull toward that optimum (Hansen 1997; Uyeda and Harmon 2014).

The upper triangle of Figure 3 describes the opposite scenario, in which high disparity is achieved with low evolutionary rates. We expect this to be rare: as others have noted, it is uncommon to find traits that exceed the expectation of the neutral model (Lynch 1990; Hansen and Houle 2004). Nonetheless, we hypothesize several scenarios that could generate this condition. First, in the presence of extinction that is not selective with regard to the traits in question, the sampled contemporaneous taxa may be highly disparate relative to one another, but have arrived at that condition via constant low rates of evolution (Foote 1993b). Second, disruptive selection, or selection against intermediate phenotypes, has the potential to create high disparity by creating sexual dimorphisms or phenotypic plasticity, or by expanding individual niche breadth (Foote 1993a; Rueffler et al. 2006). Finally, when phenotypic integration acts to facilitate, rather than constrain, phenotypic change, disparity should be higher than predicted by the neutral expectation. This effect would occur when the direction of selection is aligned with the major axis of variation in an integrated phenotype (Merilä and Björklund 1999; Goswami et al. 2014).

The variable relationship between evolutionary rate and phenotypic disparity is supported by empirical evidence. In some clades, such as plethodontid salamanders and muroid rodents, shape disparity and the rate of shape evolution exhibit a significant positive relationship (Adams et al. 2009; Alhajeri and Steppan 2018), as do a number of paleontological studies across invertebrates and vertebrates (Wagner 1995; Ruta et al. 2006; Brusatte et al. 2008). This result contrasts with findings in terrestrial (Goswami et al. 2014; Michaud et al. 2018) and aquatic carnivorans (Jones et al. 2015), as well as trilobites (Cotton 2001) and nonmammalian synapsids (Sidor and Hopson 1998; Ruta et al. 2013), which show no relationship between rate and disparity. This lack of relationship is hypothesized to be related to the presence of multiple selective regimes with unique tempo and mode within each clade (Jones et al. 2015; Michaud et al. 2018). Whereas these studies demonstrate the mutability of the

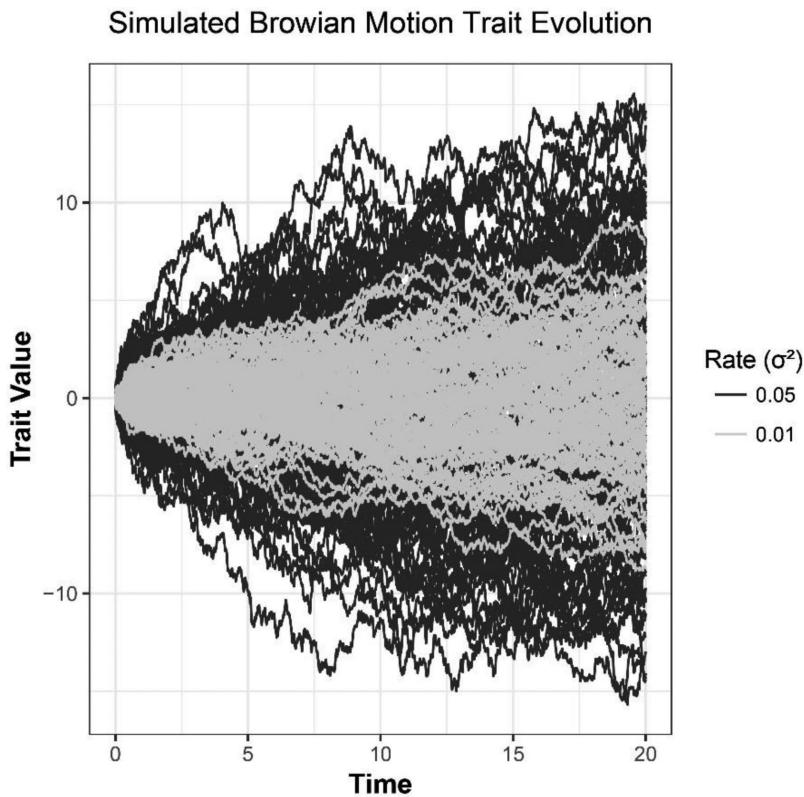


Figure 4. Simulated Brownian motion over 20 time units with low (gray) and high (black) evolutionary rates. For each evolutionary rate, 100 simulations were carried out. Brownian motion predicts that trait variance is proportional to evolutionary rate.

relationship between disparity and evolutionary rate, little is known about the specific effects of integration on this relationship. There is some evidence that high integration constrains disparity and morphospace occupation but not evolutionary rates in the carnivoran skull (Goswami et al. 2014). In contrast, high integration is associated with both low rates and low disparity in the avian skull (Felice and Goswami 2018). Further investigation of the interface between integration, disparity and rate is needed across a broader variety of taxa to test the generality of this theoretical framework.

EMPIRICAL EXAMPLE: VARIATION VERSUS RATE IN THE AVIAN SKULL

To demonstrate how interactions among organismal traits can influence the relationship between evolutionary rate and disparity in real macroevolutionary scenarios, and how we can leverage recent gains in phenomic-scale data capture and analysis to address these fundamental questions, we interrogate these characteristics in the avian skull using high-dimensional morphometric data. We utilized a recently published dataset composed of surface geometric morphometric data (757 3D landmarks and sliding semilandmarks, with semilandmark points slid to minimize bending energy) across 352 avian species (Felice and Goswami 2018) and calculated disparity for each landmark by summing the

variance of the Procrustes-aligned x , y , and z coordinate values for that landmark. This is equivalent to calculating Procrustes variance for each landmark (Adams and Otárola-Castillo 2013). Evolutionary rate for each landmark was similarly calculated as the sum of the sigma values for each coordinate of that landmark (Revell et al. 2008; Adams 2013), using a recent time-calibrated composite phylogeny of Neornithes (Jetz et al. 2012; Prum et al. 2015; Cooney et al. 2017; Felice and Goswami 2018).

Although individual landmarks are not wholly independent due to superimposition and sliding procedures, and thus results are specific to this configuration as a whole, parsing these data in a per-landmark framework provides a more detailed demonstration of the distribution of variation observed in evolutionary rates and disparity across the avian skull (Fig. 5). It is a necessary caveat that Procrustes superimposition will redistribute variance across a configuration, and this homogenizing effect is exacerbated if regions are unevenly sampled. For this reason, it is important to try to use a consistent density of morphometric data across cranial regions, an endeavour that is made easier through the use of surface semilandmarks, as we have done here. There is clear spatial heterogeneity in the distribution of variance across this configuration, with rate and variance are highest on the rostrum, anterior to the naris, and lowest on the basisphenoid. Rate and variance patterns are similar across the

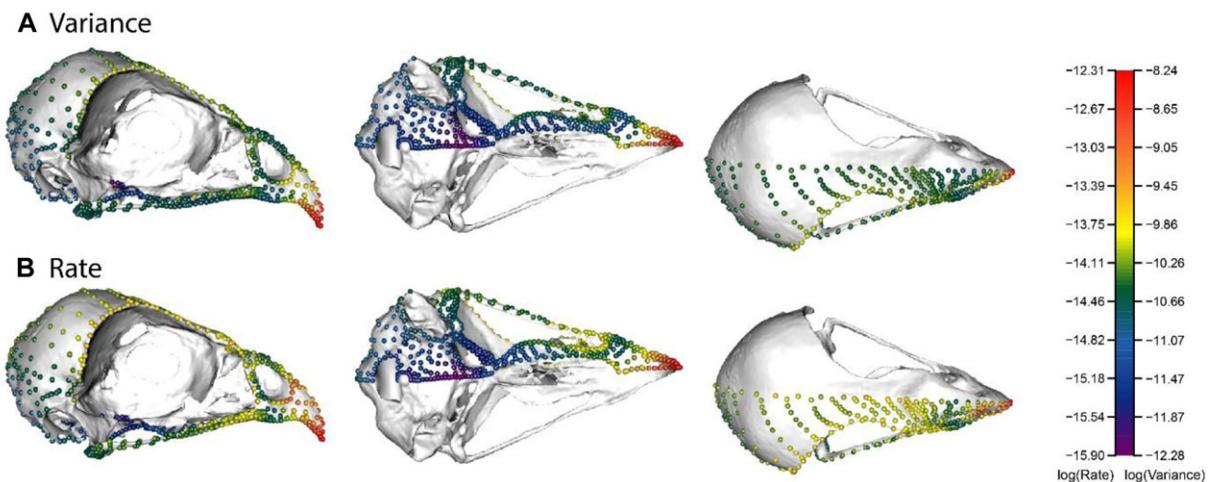


Figure 5. Per-landmark variance and rate across the avian skull. Rate and variance are heterogeneous across the skull and are highest in the anterior rostrum and cranial vault.

skull, yet there are some notable differences. For example, variance (but not rate) is high in the landmarks on the anterolateral margin of the palate and the anterior part of the cranial vault. This distribution illustrates how in empirical data, evolutionary rate does not perfectly predict disparity. These patterns of per-landmark rate and variance also reflect recently described patterns of cranial modularity in birds (Felice and Goswami 2018), which found that the avian skull is composed of seven anatomical modules. Modules corresponding to anterior mandibular cranial neural crest cell derivatives (e.g., palate, rostrum, cranial vault) exhibited high evolutionary rates and Procrustes variance, as well as low within-module correlations. Modules derived from other embryonic cell populations (e.g., occipital, basisphenoid, pterygoid/quadrat) appear to evolve more slowly, exhibit less disparity, and have high within-module correlation (Felice and Goswami 2018). Using the per-landmark data calculated here, we can further evaluate how these known patterns fit into our proposed theoretical framework of the disparity-rate relationship.

We compared the observed variance-rate relationship in each module to the null expectation under Brownian Motion with uncorrelated evolution among traits. Using the same phylogenetic hypothesis, we simulated trait evolution using the diagonal of the empirically derived sigma matrix as the evolutionary rate for each trait. This analysis was carried out using the ‘geiger’ package in R and repeated for 100 simulations (Harmon et al. 2008). We calculated the mean variance across these 100 simulations for each rate and then fit a linear regression to the simulated variance-rate relationship. The slope of each module was compared to the mean slope of all 100 simulations using standardized major axis regressions (Warton et al. 2012). Finally, we calculate a 95% prediction interval for this regression, producing the expected range of variances for each given evolutionary rate.

Landmarks corresponding to cranial modules that have previously been reported to have low variance and rates fall below the line describing the neutral expectation (Fig. 6) and have significantly lower slope (Table 1). The landmarks with the highest variance relative to their rate belong to the palate and rostrum modules, and these adhere closely to the neutral model. The slope of the regression for the rostrum is not significantly different from the null model, whereas there is marginal support for significant difference between the slope of the palate landmarks and the expectation under Brownian motion. It might be expected that structures with high variance are exceeding expectations because their potential to vary is being enhanced in some way, perhaps through adaptive evolution. However, the results reported here indicate that the most variable structures are following a simple Brownian motion process, whereas less variable traits are the ones deviating from the null model. Landmarks below the BM line (Fig. 6) have lower variance than expected for their rate of evolution, characteristic of evolutionary constraint or high homoplasy.

Previous work has demonstrated that the avian skull is unlikely to evolve under a Brownian motion model. Analysis of the principal components of this dataset recovered a lambda tree transformation to be a more likely model for each module (Felice and Goswami 2018). However, no method currently exists to precisely and accurately compare likelihoods across evolutionary models with high-dimensional data such as the raw landmark data utilized here (Adams and Collyer 2018). In addition, lambda is not the preferred statistic for quantifying phylogenetic signal in these types of data (Adams 2014). As such, we have elected to utilize Brownian motion to quantify evolutionary rates and simulate trait variance with as few assumptions as possible. However, we can consider what the effects of phylogenetic signal (lambda) would be on the variance-rate relationship. When lambda is close

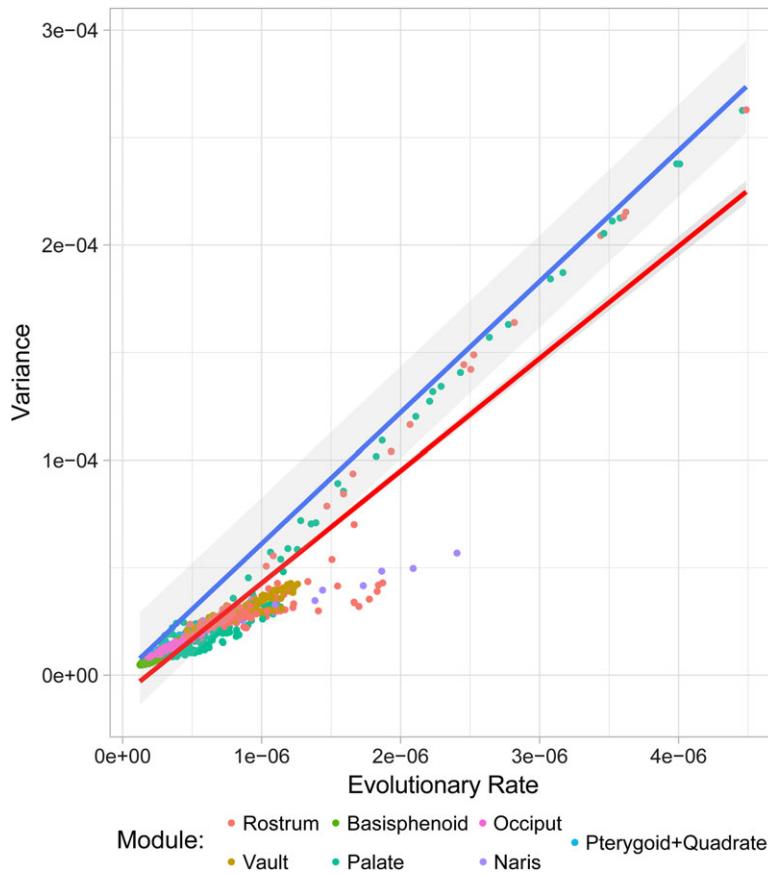


Figure 6. Variance vs rate of landmarks in the avian skull. All landmarks in the basisphenoid, palate, naris, and pterygoid + quadrate modules fall below the Brownian motion prediction (blue line), suggesting that they are under developmental or ecological constraint. Some landmarks from the rostrum and palate modules adhere closely to the Brownian expectation. Gray area: 95% prediction interval of linear regression of Brownian expectation. Red line: linear regression of observed rate-variance relationship.

Table 1. Major axis test comparing observed variance/rate slope to slope predicted under Brownian motion.

Module	Estimated slope	Lower limit	Upper limit	P-value
Naris	17.90	16.08	19.93	2.22E-16
Occiput	31.96	30.80	33.16	2.22E-16
Palate	63.60	61.82	65.44	0.026315
Pterygoid-Quadrate	40.18	39.28	41.09	2.22E-16
Rostrum	61.13	57.84	64.61	0.79469
Basisphenoid	38.79	35.79	42.03	2.22E-16
Vault	28.29	26.41	30.30	2.22E-16

to 1, trait values adhere close to the Brownian expectation and closely related taxa resemble one another. When lambda is low ($\lambda < 1$), taxa resemble each other less than would be expected solely based on phylogenetic relatedness. In this case, high levels of homoplasy would be expected (bottom triangle), as distantly related taxa display similar trait values. Although lambda is not typically defined > 1 , higher values of phylogenetic signal are possible with other metrics (e.g., Blomberg's K). In this case, trait values would be even more similar in closely related taxa

than expected under Brownian motion (Ackerly 2009; Polly et al. 2017), suggesting strong directional selection, potentially coupled with strong facilitation of coordinated trait evolution, pushing points into the upper triangle of Figure 3.

Together, these results demonstrate the complex relationship between evolutionary rate and phenotypic disparity and how each may interact with magnitude of phenotypic integration. Within a single structure, the amount of disparity achieved for a given rate can vary. In this example, phenotypic integration is constraining

the production or maintenance of trait variation but not necessarily influencing the rate of evolution. This supports previous empirical and theoretical studies that have suggested that trait correlations do not affect rates of trait evolution (Goswami et al. 2014; Michaud et al. 2018) and reinforces the “fly in a tube” model of evolutionary constraints. A necessary next step will be to analyse other clades and phenotypes in a similar framework to understand the generality of these findings and to uncover cases where amount of variation per rate exceeds the Brownian expectation.

Discussion

Phenotypic modularity and integration are fundamental features of complex organisms. The strength and patterns of trait covariation reflect the genetic, developmental, and functional associations among traits. A key assumption in studying evolutionary integration is that associations among traits have influenced the evolution of morphological disparity through time, and that reorganization of modularity patterns can facilitate or constrain macroevolutionary tempo and mode. The few studies that have considered the links between trait covariance, rate, and disparity have failed to find a consistent relationship between these factors (Adams et al. 2009; Goswami et al. 2014; Jones et al. 2015; Felice and Goswami 2018; Michaud et al. 2018). The framework we have proposed here aims to unite these findings under a common understanding of the effects of trait integration on macroevolution with testable predictions.

Testing of these hypotheses is made possible, in part, by recent advances in quantification of phenotypic variation. Semi- and fully automated techniques for collecting geometric morphometric data at the phenomic scale allow for quantifying shape data with unprecedented detail (Pomidor et al. 2016; Gao et al. 2017; Hsiang et al. 2017; Schlager 2017; Claes et al. 2018). With this type of high-dimensional morphometric data, it is possible to test more complex hypotheses of modularity and integration and to quantify patterns of within-module integration, rate and disparity than with traditional low-dimensional landmark data (Parr et al. 2016; Felice and Goswami 2018). Similarly, contrast-enhanced imaging techniques provide an opportunity to observe and quantify new aspects of anatomical variation (Gignac et al. 2016). For example, this technique has been used to compare variation in the growth of individual brain regions across marsupials to test hypotheses of developmental constraints on ontogenetic trajectories (Carlisle et al. 2017). In addition, new approaches have made phenotypic data more accessible and open than ever, meaning that it is possible to compile massive comparative datasets. These improvements include both crowd-sourcing data collection, which facilitates rapid quantification of shape data from datasets of enormous breadth (Chang and Alfaro 2016; Cooney et al. 2017), and open-access data and resource sharing with online databases such

as Phenome10k.org and MorphoSource.org, encouraging reproducibility of research (Davies et al. 2017)

However, our ability to fit evolutionary models to phenotypic data has not kept pace with our ability to quantify phenotypes. Although methods for fitting models to multivariate data exist, they are computationally intractable with more than a small handful of trait dimensions (Adams and Collyer 2018). It is possible to compare patterns of integration, evolutionary rates, and disparity among clades, but these tests are restricted to Brownian motion models alone (Adams and Collyer 2009). As a result, researchers have resorted to reducing dimensionality of phenomic datasets using principal components analysis before fitting evolutionary models (Cooney et al. 2017; Felice and Goswami 2018). Development of methods for overcoming these shortcomings and fully harnessing the power of high-dimensional data is the next step in understanding the macroevolution of integrated phenotypes.

In the sixty years since Olson and Miller formalized the study of phenotypic integration there have been astounding advances in understanding the genetic, developmental, and evolutionary factors influencing trait covariation. These studies guide the conceptual framework proposed here: integration structures the axes of phenotypic variation that are available, and the rate of evolution describes the speed at which the lineage explores potential phenotype space. In this way, the evolutionary expectation for integrated phenotypes resembles a fly in a tube, with trait covariation and other intrinsic and extrinsic factors determining the shape and size of the “tube.” Using phenomic data and phylogenetic comparative methods, there is now a path forward for investigating how integration and evolutionary rates interact to generate disparity through deep time and to uncover the evolutionary scenarios that facilitate or constrain phenotypic variation.

AUTHOR CONTRIBUTIONS

A.G. conceived of the study. A.G. and R.N.F. designed the analyses. A.G., M.R., and R.N.F. carried out analyses and wrote the manuscript.

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DATA ARCHIVING

3D scans of avian crania are available on <https://www.Phenome10k.org>.

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