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Epigenetic Interactions

THE DEVELOPMENTAL ROUTE TO FUNCTIONAL INTEGRATION

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CONTENTS

Epigenetic interactions are obviously necessary for normal development—without them there would be no primary embryonic induction, no epithelial–mesenchymal interactions, and no interactions between differentiated tissues such as muscles and bones. These interactions are necessary not only for normal development but also for normal function, if only because they produce the structures that carry out function. The ability of jawed vertebrates to eat typically requires having a jaw, and without epithelial–mesenchymal interactions there would be no jaw. However, eating requires more than just having a jaw, and epigenetic interactions do more than just produce it. Epigenetic interactions also integrate developmentally heterogeneous tissues into a coherent functional whole, coordinating the development of bones with that of the skeletal muscles that move the bones and with that of the teeth, not to mention the tongue, nerves, and blood vessels. All of these, taken together, comprise a single integrated whole—the feeding system. In the more general case, bones and muscles can be regarded as a single functional system because bones provide skeletal struts and levers that are moved by

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the forces supplied by muscles (Herring, 1994). Obviously, the system does not work in the absence of its parts; but even if all the parts are there, the system does not work very well when they are disproportionate relative to each other. Excessively strong muscles coupled to a small, slight mandible could generate forces capable of yanking the mandible out of its joint; conversely, excessively weak muscles inserting on a massive jaw could generate forces too weak to open the jaw at all. Even modest disproportions can imperil function, such as when they lead to a misalignment of the jaws and teeth; in the case of a rodent's ever-growing incisors, malocclusion means that the incisors can grow through the roof of the mouth, which is usually lethal.

That epigenetic interactions benefit individuals by integrating functionally interacting parts seems obvious, but that benefit does not mean that epigenetic interactions evolved to serve that particular biological role. Whether epigenetic interactions, or morphological integration more generally, are adaptations is a topic addressed by Hansen in this volume (see Chapter 20); and as is clear from his chapter, this adaptive scenario can be challenged on several grounds. One alternative hypothesis is that integration is an intrinsic feature of developmentally modular systems, a hypothesis that has an important and novel implication because it means that the developmental basis of integration may be critical for understanding its evolutionary origin. Another alternative theory is that integration is not an adaptation in its own right but, rather, a correlated effect of some other adaptation, another hypothesis that emphasizes the theoretical significance of the developmental basis of integration. As we argue herein, the developmental basis of integration may be just as crucial to the evolution of integration as it is to the development of an individual.

We begin by briefly reviewing the concept of morphological integration and then focus more specifically on the distinction between developmental and functional integration to provide the context for analyzing the developmental basis of functional integration. In that section, we highlight the distinctive features of epigenetic pleiotropy and why those features might make them distinctive for the evolution of integration in general and for functional integration in particular. Finally, we turn to one classic model system for studies of integration, the mammalian mandible, one that has been regarded as a paradigm for the theory that integration is an adaptation but one which also may exemplify the two alternative theories and that clearly reveals some of the complications of testing theories about integration.

AN OVERVIEW OF MORPHOLOGICAL INTEGRATION

ORGANISMS AS INTEGRATED SYSTEMS

The idea of morphological integration is grounded in the perception that organisms are not collections of isolated parts but, rather, coherent systems. Consequently, fitness depends on the *relationships* among traits rather than on their individual values. Taking the simplest possible case of two traits, fitness depends on their relationship when the adaptive value of one trait is conditional on the phenotype of the other. In the case of a lever, for example, the force that it transmits depends on the ratio between the in- and out-lever arms (mechanical advantage). This ratio gives the ratio of output to input force. It is this ratio, not the lengths of the individual lever arms, that matters to force transmission, so unless the length of one arm is fixed, there is no optimal value for either of them. Rather, the optimal value for one depends on the length of the other (and on the optimal value for the output force generated by the system). Most biological levers are more complex than this simple case, if only because something has to move the lever and that something else is part of the system for generating the force. Additionally, most biological levers confront more than one functional challenge. For the mandible, one of those other challenges is to open the jaw wide enough to engulf the food; that matters for optimizing the form of

the lever because adaptations that increase gape (such as lengthening the jaw) diminish mechanical advantage. Consequently, animals must compromise force or gape, meaning that they must either eat less resistant or smaller foods or else acquire specializations that enable them to accommodate both functions (Herring and Herring, 1974; Dumont and Herrel, 2003; Taylor and Vinyard, 2009; Williams et al., 2009). This is a fairly simple case compared to others such as the python muzzle, which comprises many more components that must all work together so that changing any one of them can alter the way the whole works (Frazzetta, 1975).

One outcome of this integration among parts is that the whole is very literally greater than the sum of its parts. Owing to the interdependence among traits, fitness of traits is not additive. In the contrasting case, when fitness actually is additive, we can calculate the net effect of improving each part by summing the improvements of each individual part. For example, increasing performance of one part by five units and of another by three would give a net improvement of eight units; but as Emerson and colleagues (1990) demonstrate in the case of flying frogs, improvements in performance for individual traits do not sum. They estimated the improvement in performance of those frogs due to the acquisition of several traits (enlarged hands and feet, full webbing between fingers and toes, lateral skin flaps, and elongation of the body) and found that the net improvement is more than the sum. As they put it, there is a synergy between traits.

Development, like function, requires interactions; and therefore, development, like function, is an integrated system. That may be most obvious in the case of an inductive interaction because the competence of a tissue to respond to an inductive signal matters as much as the signal itself. For example, one of the intercellular signaling molecules that is involved in patterning the jaw is endothelin-1, expressed in the branchial arch epithelium. Endothelin affects jaw patterning by binding to its cognate receptor, the endothelin-A receptor, found in the mesenchymal cells of the arches (Clouthier et al., 1998). Knocking out the receptor produces multiple defects, including, in the first arch, a homeotic transformation of lower jaw structures into structures resembling the upper jaw (Abe et al., 2007). The skeletal defects are accompanied by neuromuscular transformations (Sato et al., 2008). These defects arise, in part, from mispatterning of neural crest cells—the domain of maxilla-like gene expression is expanded and that of mandible-specific gene expression is lost (Ozeki et al., 2004; Ruest et al., 2004). In the absence of endothelin between embryonic day (E)8.5 and E9.0 of the mouse, expression of two genes of the distal-less gene family, *Dlx5* and *Dlx6*, is not induced (Ruest et al., 2005; Ruest and Clouthier, 2009). Those genes specify intra-arch (maxillary vs. mandibular) identity, so in the absence of endothelin, there is a homeotic transformation of the mandible into a maxilla-like structure (Depew et al., 1999, 2002; Beverdam et al., 2002). After E9.5, endothelin is dispensable and *Dlx* expression depends on fibroblast growth factor signals (Trumpp et al., 1999; Tucker et al., 1999; Fukuhara et al., 2004). This case exemplifies the significance of signal–receptor interactions, of epithelial–mesenchymal interactions, and of temporal interactions from up- to downstream along a signaling cascade, all of which are forms of developmental integration.

To this point, we have treated integration as an inarguable property of organisms, we have treated function and development as both conceptually and biologically distinct, and we have treated integration as if it were a property of individual organisms. However, even though integration is an inarguable property of functional systems, organisms are not fully integrated systems. The optimal jaw morphology does not depend on the proportions of the hands and feet, for example. If organisms actually were fully integrated systems, it would not be possible to improve jaw function without also altering the hands and feet; and if they were fully integrated developmental systems, any modification of jaw development would affect the development of

the hands and feet. That developmental connection between jaws, hands, and feet is not an absurd idea. Although not mentioned above, *Dlx5* and *Dlx6* also regulate limb chondrogenesis and chondrocyte hypertrophy (Hsu et al., 2006), so the jaw is, in fact, developmentally integrated with the limbs to a degree. That integration between them is even anticipated on the grounds that both jaws and limbs can be viewed as appendages.

The relationship between developmental and functional integration is complex because development and function may be conceptually distinct but they are not causally distinct. We will return to this issue of the relationship between developmental and functional integration later because it has particularly important consequences for morphological integration via epigenetic interactions. That relationship between developmental and functional integration matters not only to individuals, the ones who develop and function, but also possibly to populations.

INTEGRATION AT THE POPULATION LEVEL

Morphologists had long recognized that organisms are integrated systems, but integration at the population level nonetheless proved difficult to recognize owing to the methods commonly used by quantitative morphologists. Those morphologists lacked both the techniques and technology to measure morphologies as integrated systems even when they wanted to. Whether individual morphologists favored holistic or reductionist approaches to morphology, they were forced to extreme reductionism by their methods because, with the major exception of studies of allometry, there was no useful method for analyzing relationships among traits. That quantitative morphologists were well aware of integration and sought to analyze it is evident in the reception of Olson and Miller's (1958) pioneering work *Morphological Integration*, which offered a method for analyzing integration. Reviews of the book acknowledge the importance of the subject, although many objected to the method on technical grounds (e.g., Howells, 1958; Imbrie, 1958; Buettner-Janusch, 1959; Bock, 1960; Van Valen, 1965). Such methods remain an area of active investigation (e.g., Klingenberg et al., 2001a, 2003; Monteiro et al., 2005; Richtsmeier et al., 2005; Mitteroecker and Bookstein, 2007; Klingenberg, 2008, 2009; Magwene, 2008; Monteiro and Nogueira, 2009). However, even though there are still outstanding questions about methods, we no longer face the major problem confronting early workers. That problem was mentioned by several reviewers of *Morphological Integration*: The study of integration was once remarkably labor-intensive. Buettner-Janusch (1959) characterized that labor as "monumental" (because it required calculating correlation and partial coefficients between many measurements, then clustering them). Simpson (1958) even called that amount of labor "appalling." Not surprisingly, integration was not regularly studied quantitatively until a desk calculator was no longer a rare luxury.

What made the extraordinary labor seem worthwhile was the discovery that statistical correlations make biological sense. That was the major point of Olson and Miller's book, aside from their method. The reason that correlations make biological sense is because developmentally and functionally integrated traits tend to be highly correlated with each other but not with developmentally or functionally independent traits (e.g., Terentjev, 1931; Olson and Miller, 1958; Berg, 1960; Van Valen, 1962; Long and Kamensky, 1967; Long and Frank, 1968; Gould and Garwood, 1969; Leamy, 1975; Long and Captain, 1977). At present, that sensible structure may seem so obvious that it goes without saying, but explaining why correlations are organized that way requires making two substantial conceptual leaps.

The first conceptual leap is from individuals to populations. Integration at the individual level need not translate into statistical correlations at the population level. As emphasized by Hallgrímsson and colleagues (2009), covariation requires variation; in the absence of variation, there is integration at the individual level but no

covariation at the population level, as demonstrated by the integration of clones. As individuals, clones are integrated neither less than nor differently from individuals within a heterogeneous population; but clones do not vary, so statistical correlations among traits in a population of invariant clones will not reveal the integration seen within individuals (Hallgrimsson et al., 2009). Thus, to explain integration at the population level, we need to explain how *variation* is produced and limited as well as how it is structured. Theories about integration at the population level thus explain the structure of (co)variation, including how evolutionary processes can match the structure of (co)variation to functional and developmental dependencies among traits. Statistical correlations measure (or, more precisely, phenotypic correlations approximate) the average effect of segregating alleles in a population; the question is why those correlations should have any predictable structure at all, much less one predicted by function and development.

The second conceptual leap lies in the mechanism of correlation. Functionally correlated traits are statistically correlated, but function, by itself, supplies no mechanism to produce the correlation. In contrast, development does produce correlations because development generates the phenotype and variation in development generates variation in phenotypes. Thus, the finding that functionally correlated traits are statistically correlated posed a major question: How does the (co)variation generated by development come to match that predicted by function? The early answers to that question are most succinctly summarized by Van Valen (1965): There must be selection specifically for developmental patterns that affect functional complexes as wholes. That does not necessarily mean that selection builds up integration, starting with independent traits. It could mean that selection dismantles correlations, producing independence among whole complexes. That dismantling was proposed by Schmalhausen (1949) and his student Berg (1960), who hypothesized that stabilizing selection would lead to the internalization of regulatory systems, producing adaptive plasticity, canalization, and integration. Integration was regarded as a form of canalization in that it arose by reducing the sensitivity of tissues to the internal environment created by other tissues. A decrease in that sensitivity would lead to the formation of correlation *pleiades*—sets of highly correlated traits that are independent of other such sets. Another hypothesis, formulated by Reidl (1977, 1978), was that the genetic organization of development would evolve to copy the functional dependencies among traits, leading not only to the correlation among coadapted traits but also to their coevolution.

Cheverud (1982, 1984) formalized the hypothesis of genetic systems evolving to match functional dependencies among traits, using Lande's (1980) model for the evolution of pleiotropy. According to this theory, patterns of pleiotropy should resemble what would be expected from developmental and functional theory because (1) traits that must interact in development or function are likely to interact in fitness, (2) the form of the fitness interaction determines the optimal correlation between traits, and (3) under stabilizing selection, the joint distribution of the additive genetic values evolves to match the shape of the fitness topography (Cheverud, 1982, 1984). Presented more graphically, Figure 17.1 shows four topographies that differ in two respects—the rate at which fitness decreases away from the optimum and the degree to which fitness depends on the relationship between the two traits. Figure 17.1A shows a case in which fairly large deviations from the optimum (at the center of the white circle) are tolerated without reducing fitness, and the traits are adaptively independent. In this case, it does not much matter what values an individual has for either x or y or what value they have for x for a given value of y. Figure 17.1B shows a case in which deviations from the mean have a larger impact on fitness, causing it to fall off more rapidly as an organism deviates from the optimum. However, just like the case shown in Figure 17.1A, the two traits are adaptively independent.

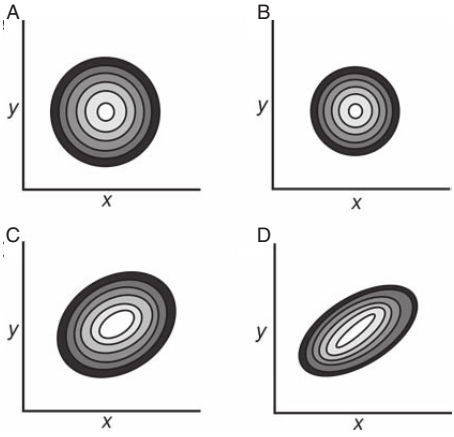

FIGURE 17.1 Four adaptive topographies for two traits, *x* and *y* (after Cheverud, 1982). The optimum is located at the center of the central (white) circle. Deviations from the optimum that are equal in fitness are shown as isoclines; regions of equal fitness have the same tone. (A) Large deviations from the optimum do not much reduce fitness; *x* and *y* are adaptively independent. (B) Deviations from the mean have a larger impact on fitness; *x* and *y* are adaptively independent. (C) Large deviations from the optimum have modest effects on fitness; *x* and *y* are moderately interdependent. (D) Because the adaptive value of each trait depends heavily on the phenotype of the other, the impact of a deviation from the optimum on fitness depends on its direction.

In Figure 17.1C, like in Figure 17.1A, deviations from the mean have a relatively modest effect on fitness but the two traits are now moderately interdependent—the fitness of an individual with a given value for *x* depends (slightly) on its value for *y*. Figure 17.1D shows a case in which the two traits are more strongly coupled. In this case, the fitness of an individual with a given value of *x* depends heavily on its value for *y*. The tightness of their coupling is evident in the shape of the ellipse—the disparity between the lengths of the major and minor axes is far greater than in the other three cases.

Formally, the shape of the adaptive topography is represented in the fitness matrix W, whose elements measure the spread of fitness around the optimal values. In this matrix, large values signify that traits are under weak stabilizing selection. The adaptive interdependencies thus shape the pattern of correlational stabilizing selection (represented in W), which directs the evolution of the genetic covariances (represented in the genetic covariance matrix, G). Consequently, G comes to match the pattern of stabilizing selection (Lande, 1980; Cheverud, 1982, 1996a). Mutational effects contribute to the pattern of genetic integration, but they too are hypothesized to evolve to match the pattern of stabilizing selection.

A limitation of this theory is that it explains how integration could evolve to correlate adaptively interdependent, genetically independent traits but it does not explain the dismantling (or parcellation; see Wagner, 1996) of integrated complexes. That dismantling is a more complex process because it requires reducing some correlations while others are maintained or even augmented (Wagner, 1996). General models for this combination of outcomes, based on realistic assumptions, remain the subject of active theoretical investigation (see, e.g., Wagner et al., 2005, 2007). Although much work remains to be done on this important issue, we will leave it to focus on two others that bear more directly on the relationship between developmental and functional integration.

The first of these concerns the expectation of a correspondence between developmental and functional integration. That expectation was subtly altered by the formalization of the theory which no longer specifically predicts that developmental integration will evolve to match

functional integration. Rather, it predicts that genetic integration will evolve to match the adaptive topography. The second issue is raised by modern emphasis on pleiotropy, in contrast to earlier theories that emphasized developmental process. Here, the question is whether the developmental origin of pleiotropy matters to the evolution of integration, specifically, to the ability of developmental integration to adapt to functional integration.

DEVELOPMENTAL "AND/OR" FUNCTIONAL INTEGRATION?

In the formal theory for the evolution of integration, developmental and functional integration play the same theoretical role—both shape the adaptive topography and it is the shape of that topography, not the specific cause of its shape, that directs the evolution of pleiotropy. Given that development and function play the same theoretical role and that the distinction is immaterial, it may seem unnecessary to distinguish them. Nonetheless, we will argue that there are good reasons for sharpening the distinction, reasons that lie in the distinctive question posed by functional integration.

WHY BLUR THE DISTINCTION BETWEEN DEVELOPMENTAL AND FUNCTIONAL INTEGRATION?

There are at least four good reasons for intentionally blurring the distinction between developmental and functional integration. The first is that function, like form, is a dynamic feature of organisms. Function is often studied as if it were static because functional morphologists have traditionally focused on adults. However, clearly function is not peculiar to adults because organisms must be capable of feeding and breathing (at the very least) long before they are adults. If they cannot perform such basic functions, they will not survive to become adults. Several studies examine ontogenetic changes in how functional systems work, including analyses of the changes in muscle activity, orientation of muscles, movements resulting from muscular activity, and the neural coordination of those movements (e.g., Lakars and Herring, 1980; Langenbach et al., 1992, 2001; Huang et al., 1994). Others analyze ontogenetic changes in performance, such as bite force, which depends on several morphological features that change over the course of ontogeny such as body size, muscle mass, and craniofacial geometry (e.g., Erickson et al., 2003; Thompson et al., 2003; Herrel and O'Reilly, 2004; Herrel et al., 2006). If function is viewed in terms of traits that develop, the coupling between developing functional attributes can be regarded as both functional and developmental. Discriminating the developmental coupling of functional traits from the functional coupling of developing traits seems so arbitrary that the distinction makes little sense.

The second reason for intentionally blurring the distinction between development and function is that development can be viewed in functional terms. This functional approach to development can be seen in a general model for transcriptional regulatory networks that proposes subdividing these systems into subnetworks of interconnected genes. Each gene in the network performs a particular developmental function at a specific time and place, such as specification of regional identity or induction of cell division or the specialized functions of particular cell types, like fusion of myoblasts (Busser et al., 2008; Davidson and Levine, 2008; Oliveri et al., 2008). That functional analysis of development produces a general model based on the functional demands imposed on developmental systems (e.g., the need to specify regional identity or to maintain the registration among interacting parts), yielding general models for how such systems work, such as through a combinatoric code of nested transcription factors (e.g., Depew et al., 1999, 2005). Because developmental processes serve functions, the integration between components of developmental systems are as much functional as they are developmental. That entirely erases any distinction between functional and developmental integration.

The third reason for intentionally blurring the distinction between development and function is that function can be morphogenetic. The morphogenetic consequences of functions such as mastication and locomotion have been a major focus of studies on the impact of mechanical factors on the development of bones (e.g., Lanyon, 1980; Herring and Lakars, 1981; Hall and Herring, 1990; Carter and Orr, 1992; Herring et al., 2005). The general idea is that function loads bones and cartilages, thereby deforming them. That deformation is measured by strain, the ratio between the deformed and original lengths of the structure; and strain directs the growth of bones and cartilages. Such basic principles have been used to predict how abnormal muscle and joint forces lead to abnormal skeletal phenotypes, such as the abnormal hip-reaction forces of children with cerebral palsy (Shefelbine and Carter, 2004). During normal development the angle between the neck of the femur and an axis extending between the two femoral condyles (i.e., anteversion angle) decreases from about 30° (at birth) to 15° by skeletal maturity. In children with cerebral palsy, that angle does not decrease but may even increase. Biomechanical models for the hip joint–reaction forces show that the predicted force at the hip of children with cerebral palsy is more anterolaterally directed than it is in normal children. Applying the predicted loads to a finite element model of the femur (the resultant stresses and deformations at the growth front), Shefelbine and Carter calculated the growth rate and direction and the model predicted the observed decrease of the anteversion angle in children under normal loading conditions versus the slight increase in that angle in children under the loading conditions altered by cerebral palsy. Given that function can be viewed as a morphogenetic process, there is arguably no meaningful distinction between function and development.

The fourth and final reason for blurring the distinction between development and function arguably motivates sharpening it. This is the argument that functional integration is achieved through developmental integration (Cheverud, 1996a; Rollian and Willmore, 2009). This attainment of functional integration via development might seem so obvious as to be a truism because correlations must be achieved through development—without variation in development there is no phenotypic variation and, therefore, no phenotypic covariation. However, the question is whether developmental integration evolved so as to achieve functional integration. A second question arises from the consequences of achieving developmental integration—once achieved, unless those developmental interactions occupy a peripheral position in the developmental system, they may become indispensable to development and persist for that reason even when the morphological traits they produce become functionally decoupled. Once acquired, and therefore incorporated in G, that correlation can become a developmental determinant of W.

WHY SHARPEN THE DISTINCTION BETWEEN DEVELOPMENTAL AND FUNCTIONAL INTEGRATION?

There are two main reasons for distinguishing developmental from functional integration, one of which paradoxically arises from the reasons for blurring them. Both reasons follow from the hypothesis so succinctly stated by Van Valen (1965), which is that developmental integration evolves to match functional integration. Van Valen did not contend that developmental integration always evolves to match functional integration, but we will focus on the hypothesis that it does. This hypothesis differs subtly from the one formalized in quantitative genetic terms by Cheverud (1982), which states that genetic integration (G) evolves to match the adaptive topography (W) as determined by a combination of developmental and functional integration. The two hypotheses predict a match between two patterns of integration, but they differ about what matches what. The distinction may be clarified by defining two other matrices, D and F, for developmental and functional integration, respectively. The first hypothesis, which

predicts that developmental integration evolves to match functional integration, predicts that D evolves to match F, from which it would also follow that G will match both D and F. The other hypothesis, as formalized by Cheverud, predicts that G will evolve to match W, with W resembling either D or F or neither, being instead some (weighted) combination of the two.

One important difference between the two hypotheses is that the first explicitly connects the evolution of D to that of F. The other is not about the evolution of either D or F but rather that of G, given D and F. The first explains how functional integration could be manifest in G when function, by itself, cannot produce correlations among traits except by way of development. The theory predicts that developmental integration will evolve so as to correlate functionally interdependent traits, and this will lead to correlations at the population level (G). In contrast, the second focuses on the origin of G, given that D and F exist. D determines the structure of W because developmental interactions already exist and disrupting them will impair fitness. Developmental interactions, such as embryonic induction, are necessary for normal development, so we would anticipate that disrupting them would reduce fitness. However, given that they already exist, we are not predicting how D will evolve; rather, the theories derive W from what is known about D, as it exists. A priori, in the absence of any knowledge about development, we might not predict that embryonic induction would evolve, although Reidl (1977) did. Thus, the theory that predicts G will evolve to match W does not say how D should evolve. That does not mean that it is straightforward to predict G given what we know about D; predicting G from D is not at all straightforward in light of the complexity of development (Hallgrímsson et al., 2007a, 2007b, 2009). The important point is that we are not predicting the origin of pleiotropy on an individual level. Rather, we are predicting the origin of pleiotropy on a population level from what we know about pleiotropy on an individual level. We are explaining how pleiotropy on an individual level can predict pleiotropy at the population level, an explanation predicated on the impact that D has on W.

In contrast, the theory that D evolves to match F is about the evolution of D. The logic of this hypothesis is very different because F represents the *optimal* pattern of functional integration, the one predicted from functional theory, not what we already know to exist. In this case, we are predicting what ought to exist at the individual level. This theory thus explains the origin of pleiotropy at the individual level in terms of the optimal patterns of functional coupling. Real organisms will not fit the predictions made by F unless development produces the expected correlations. Thus, for that optimal pattern of functional integration to be manifest in G, it must first determine the structure of D (as it is only D that can produce phenotypic [co] variation). Development (an individual-level process) is thus expected to evolve so as to integrate functionally coupled structures, and there is no reason to expect that this match would ever arise unless (1) D evolves to match F or (2) the match is purely incidental, i.e., D just happens to match F. One main reason for distinguishing between developmental and functional integration is that the hypothesis that developmental integration evolves to match functional integration cannot be tested unless we can separate them.

The second major reason for distinguishing between developmental and functional integration is that no one can doubt the impact of development on fitness, but it is not so clear that functional integration has a large impact on fitness. No one would deny that embryonic induction, developmental patterning processes, epithelial–mesenchymal interactions, and hormonal regulation of growth all have significant consequences for fitness. However, there are real questions that can be asked about the impact of functional integration on fitness. Deviations from normal development can dramatically impair fitness, so we would expect that W is structured largely by developmental integration. In that case, G might then be

shaped primarily by developmental interactions. Functional integration would then have a smaller impact on W and, thus, on G. The idea that G (or the phenotypic covariance matrix, P) is shaped largely by developmental architecture has been suggested by some of the few studies that explicitly test for a match to F (e.g., Herrera, 2001; Herrera et al., 2002). That D and F even make different predictions is, by itself, a rebuttal to the idea that D evolves to match F. Conversely, that D and F often make highly similar predictions, as documented by some of the few other studies that explicitly test for developmental and functional integration (e.g., Kingsolver and Wiernasz, 1991), is by itself support for the hypothesis that D evolves to match F.

Whether functional integration has an important impact on W remains an open question even if we grant that function does have an impact on fitness. That is because, as emphasized by many morphologists, morphology (and function) matters to fitness by way of its impact on performance (Arnold, 1983; Koehl, 1996; Alfaro et al., 2005). Most measures of performance, such as bite force or suction index, are complex functions involving multiple traits. That is important because multiple morphologies can produce the same value on an index of performance, making the relationship between morphology and performance many to one (Alfaro et al., 2004; Collar and Wainwright, 2006; Wainwright, 2007; Young et al., 2007). If fitness depends solely on an individual's value on the index, e.g., on its bite force, and multiple morphologies produce the same bite force, then individuals may be functionally equivalent despite their different morphologies. Under these conditions, fitness may be tied weakly to morphological details of individual traits and even more weakly to the relationships among traits. For example, if all that matters is bite force, selection might not care about the ratio between the in- and out-lever arms because that is just one determinant of bite force. Massive jaw muscles could overcome any disadvantage due to a low mechanical advantage, as can muscles that are oriented to take the best advantage of leverage, as can large head or body size. If it does not matter how a given bite force is achieved, integration might have little impact on fitness.

It is when relationships among traits do matter that integration is anticipated to have an impact on fitness. Should body size be under stabilizing selection, for example, it would not enhance fitness to increase bite force by increasing body size. Similarly, should the optimal muscle mass depend on the ability of the jaw to resist the forces produced by muscles, then increasing muscle mass beyond the jaw's ability to resist the forces would not enhance fitness even if that too increases bite force. Bite force itself may be under stabilizing selection if there is a disadvantage in building a jaw more powerful than needed by food, so the various determinants of bite force might be balanced to maintain the optimal value of that trait. When proportions matter, there may be fewer effective degrees of freedom with respect to fitness than anticipated from the mathematical many-to-one relationship between morphology and performance.

If (and only if) proportions do matter so that the balance among traits must be maintained despite variation in them individually would we anticipate functional integration. Only then would we expect developmental integration to evolve to match it. It is not presently known whether this balance among the parts of a complex system actually is characteristic of natural functional systems. That makes the significance of functional integration for fitness a still open question. That is another powerful rationale for treating developmental and functional integration separately because combining D and F into a model for W does not allow us to ask whether functional integration structures W. If we can predict G from F, we can demonstrate not only that F determines W but also that D matches F.

Biomechanical theory offers the obvious source of hypotheses regarding functional integration even if the models are sometimes unrealistically simple. Modeling the jaw as a lever is an obvious oversimplification, yet, as Spencer

(1998) points out, despite that, numerous studies have shown that the model's predictions are correct. Deriving a model for mandibular integration from that simple lever model, we would predict that the most highly correlated muscle moment and resistance arms would be for the muscles that make the largest contribution to feeding performance. Consequently, we would predict a higher correlation between the in- and out-lever arms for the superficial masseter than for the temporalis muscles in rodents because the mechanical advantage of the superficial masseter makes a far greater contribution to both gnawing and chewing performance. As anticipated, based on a sample of eastern fox squirrels (*Sciurus niger*) collected over a winter in western Michigan, the correlation between superficial masseter lever arms is a statistically highly significant 0.536 whereas that between temporalis lever arms is a statistically nonsignificant 0.198.

The fact that those two correlations are predictable on functional grounds does not mean that developmental integration evolved so as to correlate the lever arms of the eastern fox squirrel jaw. Developmental integration, or at least the mechanism correlating the lever arms, could have evolved under natural selection but not selection *for* integration; alternatively, it might not be an evolved property at all, being instead an intrinsic feature of the mandibular developmental system. Because nonadaptive theories depend strongly on the developmental origin of correlations, we consider these and their consequences for integration, focusing most specifically on functional integration.

THE DEVELOPMENTAL ORIGINS OF INTEGRATION

One classical representation of the genotype–phenotype map (Figure 17.2) represents pleiotropy by connecting genes (G1–G6) to phenotypic traits (T1–T7). Like all schematics, this one is highly abstract, representing only the general features that matter most to the theory. It therefore omits all theoretically unimportant details along with those that would make the diagram system-specific. Schematics like this are interesting not only for what they do show—the features most critical to the theory—but also for what they do not show—the features deemed theoretically trivial or too system-specific to have the generality needed for a useful theory. An interesting feature of this particular representation of the genotype–phenotype map is that it shows functionally associated traits to be the ones that are linked by many pleiotropic effects. Traits within the same functional unit, F1 or F2, are the ones jointly affected by the vast majority of pleiotropic effects. This figure thus portrays the definition of an evolutionary module that is given verbally, that an "evolutionary module" is a complex of characters that (1) collectively serve a primary functional role, (2) are tightly integrated by strong pleiotropic effects of genetic variation, and (3) are relatively independent from other such units.

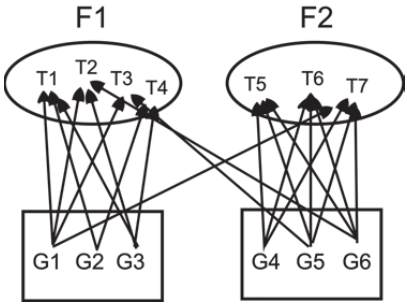

FIGURE 17.2 A classic representation of the genotype–phenotype map (after Wagner, 1996; Wagner and Altenberg, 1996). The boxes, below, enclose genes (G) that tend to affect groups of traits (T) that belong to the same functional complex (F).

What makes this a distinctive representation of the genotype–phenotype map and what distinguishes this definition of an evolutionary module is that the modules comprise characters that collectively serve a primary functional role. The figure thus depicts functional integration, and the definition of an evolutionary module even makes functional integration a defining criterion of the concept. The figure does not

display the complex architecture of evolutionary modules that is discussed in the text (Wagner and Altenberg, 1996, 972), which refers to more hierarchical structures, gradations, and overlapping modules. Those are omitted but seen as part of the quantitative characterization of modules that the authors are encouraging by the research program that they outline. It is not that those details are regarded as immaterial so much as that they remain to be characterized. However, one critical detail left out of the figure is the developmental route by which the pleiotropic effects are produced. Although those developmental details, like the anatomy of the traits, are system-specific, the diagram does not recognize classes of developmental causes as fundamental to the theory. Were they fundamental, they too would have been illustrated.

The incorporation of those developmental details as fundamental to theory is at the heart of models in developmental quantitative genetics (Atchley, 1984, 1987; Cowley and Atchley, 1990, 1992; Atchley and Hall, 1991). One critical distinction made by developmental quantitative-genetic models is between two classes of processes whereby genes affect the phenotype. The first is designated "intrinsic genetic" and the second "extrinsic epigenetic," largely following the classification by van Limborgh (1970, 1972). According to this classification, genes can affect the cells in which they are expressed (*intrinsic* genetic effects) or they can affect cells in which they are not expressed (*extrinsic* epigenetic effects). In the case of intrinsic genetic effects, pleiotropy occurs when the same gene is expressed in different places or different times; pleiotropy is thus due to the temporally or spatially iterated expression of the same gene. For example, Goosecoid (*Gsc*) encodes a homeodomain-containing protein and is involved in multiple developmental processes, including at gastrulation and during organogenesis (Yamada et al., 1995). To determine whether *Gsc* affects the cells in which it is expressed, chimeras containing *Gsc*-null cells were marked with beta-galactosidase and *Gsc* cells with a lacZ allele that enables visualization of *Gsc*-expressing cells. It was found that the chimeras have defects similar to *Gsc*-null mice and that the effects are proportional to the proportion of *Gsc*-null cells in the region (Rivera-Perez et al., 1999). Thus, the effects of *Gsc* are intrinsic genetic (i.e., cell-autonomous), and *Gsc* affects multiple craniofacial tissues including part of the mandible, components of the middle ear (malleus, tympanic ring), external auditory meatus, musculature (including tongue), and nasal cavity and pits (Yamada et al., 1995).

Epigenetic effects occur when the phenotype of one cell population conditions the genes expressed by another. Such effects can be subdivided into two general categories, according to whether the interaction is spatial or temporal. Epithelial–mesenchymal interactions are a paradigm case of the spatial class of epigenetic interactions, as are muscle–bone interactions (e.g., Herring and Lakars, 1981; Hall and Herring, 1990; Habib et al., 2005; Rot-Nikcevic et al., 2006, 2007). Epigenetic cascades are a paradigm case of the temporal class of epigenetic interactions—a mutation that acts early in development can have cascading effects through the system owing to the fact that each developmental process depends on what happened before it. Perhaps the most obvious example of this is secondary or tertiary embryonic induction. Primary embryonic induction refers to the induction of the neural tube by the chordamesoderm. Secondary embryonic induction occurs when the optic vesicle (an outcome of primary embryonic induction) induces the lens from the overlying ectoderm. Tertiary induction occurs when the lens induces the cornea from the overlying ectoderm. Any variation introduced into the first step would affect the second, which would then affect the third.

Pleiotropy can result from a complex combination of intrinsic genetic, spatial, and temporal epigenetic effects (for purposes of brevity, we will omit "extrinsic" from now on and simply refer to these as "epigenetic" effects). For example, a gene may be expressed at multiple times and places, leading to intrinsic genetic pleiotropy, and that gene product may be the

epigenetic signal that initiates a cascade. For example, members of the transforming growth factor- (Tgf-) family mediate many developmental processes, including the stimulation of chondrocyte proliferation and the inhibition of their terminal differentiation (Moses and Serra, 1996; Chai et al., 2003). Mice that lack a receptor for Tgf- (Tgfbr2) have fewer chondrocytes; thus, they have unusually small mandibles, reduced coronoids, narrowed condylar processes, and no angular processes (Oka et al., 2007). This is a case of intrinsic genetic pleiotropy as the effects can all be traced to the reduced number of cells within the condensations that give rise to either Meckel cartilage or the secondary cartilages of the three mandibular processes (Oka et al., 2007). Inhibition of Tgf- signaling also leads to epigenetic pleiotropy, as in the case of its effects on Scleraxis (*Scx*), a member of the basic helix–loop–helix superfamily (Anthwal et al., 2008) involved in tendon development (Liu et al., 1996; Murchison et al., 2007). Inhibition of Tgf- signaling interrupts *Scx* expression in muscle attachment sites, such as the angular process of the mandible. That, in turn, deprives the angular process of the mechanical stimulation necessary for maintaining secondary cartilage (Anthwal et al., 2008). Thus, any variation in a gene encoding Tgf- affects not only all structures in which that gene is expressed (e.g., chondrocytes of the mandible) but also any structure in which the gene is not expressed but whose development depends on the gene product and all subsequent steps that depend on the phenotype of the prior stage.

Intrinsic genetic and extrinsic epigenetic effects may both be involved in generating correlations between any two traits, but the two kinds of pleiotropy are nonetheless distinct: Intrinsic genetic effects have no inherent directionality, but extrinsic epigenetic effects do—there is an earlier or later event (temporal directionality) or a signaler or responder (spatial directionality). These are inherently asymmetrical relationships. An important consequence of this directionality is that heritable variation can be passed from one tissue to another, inducing heritable variation in traits that are otherwise genetically invariant (Cowley and Atchley, 1992). Because of the fundamental distinction between intrinsic and extrinsic genetic effects, two distinct classes of pleiotropy were also recognized, intrinsic genetic and epigenetic.

This general characterization of *intrinsic genetic* and *epigenetic* was recently updated, generalized, and depicted by Lieberman and Hall (2007). This picture, Figure 17.3, shows development, not pleiotropy per se; but it highlights the distinction between genetic and epigenetic and puts it in terms of a general developmental model, following Carroll and colleagues (2001), although the details of the model were regarded as perhaps most suitable for invertebrate development. Because those system-specific details lie primarily in the labels for the genes, for their expression domains (body axes, fields), and for the origins of the epigenetic effects (regional, mechanical), they can be modified as needed. What the picture shows is the hierarchical structure of development, both the linear hierarchy in time and the branching hierarchy in space—parts of the organism are sequentially subdivided over time. The picture also shows that the genetic and epigenetic hierarchies are different but concurrent—the genetic hierarchy is visible in the links from gene to gene (above) and the epigenetic hierarchy is shown by the curly brackets linking levels (below). Epigenetic interactions are shown as connecting temporally distinct modules of gene expression, but the interactions are defined as much spatially as temporally as is evident in the distinction between local tissue interactions, regional interactions, and systemic interactions. Because this diagram is in such a different graphical language from the classic depiction of the genotype–phenotype map (Figure 17.2), it is difficult to compare the two directly.

To make the two mappings more directly comparable to each other, we can use yet another representation of the genotype–phenotype map, Figure 17.4 (after Klingenberg, 2008). This was drawn to be directly comparable to the classic one but also to fill in the missing

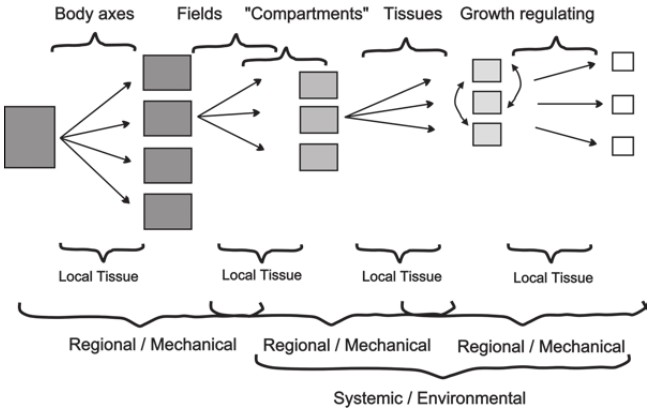

FIGURE 17.3 Genetic and epigenetic developmental hierarchies (after Lieberman and Hall, 2007), in terms of the developmental model of Carroll and colleagues (2001). The genetic hierarchy is represented by links from gene to gene (above), and the epigenetic hierarchy is shown by the curly brackets linking levels (below).

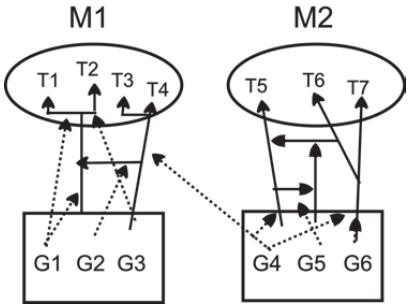

FIGURE 17.4 A developmentally explicit version of the genotype–phenotype map (after Klingenberg, 2008). The boxes, below, enclose genes (G) that tend to affect groups of traits (T) that belong to the same developmental module (M). Developmental pathways are shown as solid lines. The impact of a gene on a pathway is represented as a dotted line intersecting it; a direct interaction between pathways is represented as an intersection between solid lines.

mapping function. This representation is more abstract than Lieberman and Hall's diagram, but interpreting it may require a less abstract one, such as Lieberman and Hall's. As in the classic depiction of the genotype–phenotype map, genes are placed at the bottom of the figure and traits at the top. However, this one shows that genes affect developmental pathways, not traits; and it can therefore show two developmentally distinct routes to pleiotropy. Unlike the Lieberman and Hall diagram, this one does not draw intrinsic genetic and epigenetic interactions as if they were distinctly different hierarchies; rather, a developmental pathway is shown as a solid line and the impact of a gene on it is represented as a dotted line that intersects it. Interactions between the pathways, such as between a signal and receptor, are shown as intersections of the solid lines. This diagram also differs from the classic one in that traits are grouped into developmental modules (M1, M2) rather than functional modules.

Klingenberg contrasts two routes to pleiotropy that largely overlap Atchley and Hall's distinctions between intrinsic genetic and extrinsic genetic pleiotropy. However, the extent to which they overlap depends partly on one's understanding of the concept of "pathway" because this is a key concept in this particular representation of the genotype–phenotype map. The first route to a correlation is by parallel variation, which closely resembles intrinsic genetic pleiotropy. *Parallel variation* arises when the same gene is expressed at two times or places; the correlation results from the fact that the same gene affects two otherwise independent pathways. The two pathways may comprise the

same genes, expressed in the same temporal sequence and tissues, but they are nonetheless considered independent and arranged in parallel because the genes are expressed either at different times or in different places (within different developmental modules). The other route to pleiotropy is by direct interactions, which closely resembles epigenetic pleiotropy. Direct interactions involve signaling interactions or other mechanisms that transmit variation from up- to downstream, from earlier to later, and/or from one pathway to another.

Like the distinction between intrinsic genetic and epigenetic pleiotropy, that between parallel variation and direct interactions concerns the contrast between variation arising from the expression of the same gene at two times or places—the phenotypic covariation arises from the variation of the gene itself versus the case when phenotypic variation arises from the spatial or temporal interactions between developmental pathways (Klingenberg et al., 2001a, 2003; Klingenberg, 2004, 2005, 2008). Klingenberg, however, sharpens the distinction between them by highlighting another contrast: The two routes to pleiotropy differ in the relationship between the source of the variation and the cause of the correlation. In the case of parallel variation, the source of variation is itself the cause of the correlation, whereas in the case of direct interactions, it is not. In the direct interactions, the source of the variation can be any genetic (or environmental) factor upstream of the interaction; the source of the variation is even immaterial to the correlation because it does not produce the correlation. Rather, the correlation requires only that there be a mechanism that regularly associates the development of the two traits—the mechanism that transmits the variation from one pathway to another is the cause of the correlation, and it can be entirely unrelated to the source of the variation itself. Thus, pleiotropy can result from parallel variation or from direct interactions.

There is a clear distinction between intrinsic genetic and epigenetic pleiotropy (corresponding nearly to the distinction between parallel variation and direct interactions). We can thus define epigenetic interactions by this distinctive property: In epigenetic interactions, the genes expressed in one cell population depend on the phenotype of another. There are thus two distinctive features of epigenetic interactions. First, the developmental response to the interaction is conditional on genes not expressed within the responding cells. It is, instead, conditional on the cell's environment. Second, that developmental response is conditional on the phenotype, rather than genotype, that generates the cell's environment. The response depends on the chemical and/or physical environment of the cell.

THE ROLE OF EPIGENETIC INTERACTIONS IN THE EVOLUTION OF INTEGRATION

It is still largely unknown how, or whether, the distinctive features of epigenetic interactions matter to the evolution of integration. Few models for the evolution of integration have noted the distinction; most often, that distinction has figured in theories of phenotypic evolution, which concern the evolution of the average phenotype (Atchley, 1984, 1987; Cowley and Atchley, 1990, 1992; Atchley and Hall, 1991; Atchley et al., 1991, 1994; Atchley and Zhu, 1997; Lieberman and Hall, 2007). When epigenetic interactions have been considered in the context of the evolution of variational properties, the term *epigenetic* has been defined more broadly than it is here. However, there are two models for the evolution of integration and/or modularity that both emphasize the distinctive role of epigenetic interactions. An interesting feature of both is that they propose a nonadaptive model; neither postulates selection specifically for integration (or modularity). However, the two models differ profoundly in the role that natural selection plays in the evolution of integration and modularity and in what precisely makes epigenetic interactions special.

One model, the "erosional model," for the origin of modularity accounts for the preferential

reduction of integration between some traits; that is, integration is lessened between some traits even as it is maintained or augmented between others (Wagner and Mezey, 2004; Wagner et al., 2007). This can be viewed as a model for the evolution of modular variation rather than for integration, but such a model is needed because, in general, integration is more likely to be restructured than to continually increase or decrease. To explain how integration can be restructured rather than how correlations could uniformly increase or decrease, we need to understand how some correlations can increase while others decrease. According to this model, natural selection does play a major role in the evolution of modularity, but there is no selection for modularity. Rather, it is selection for *robustness* (the ability to produce the same phenotypic outcome in the face of environmental and/or genetic variation). Modularity spontaneously appears as a side effect. The crux of the theory is that selection for robustness preferentially eliminates epigenetic effects, thereby decoupling modules that were integrated by those effects (Wagner and Mezey, 2004; Wagner et al., 2007). Because epigenetic effects are reduced, variability also is; and because those are a cause of integration between modules, the boundary between modules is sharpened. The two reasons that epigenetic effects are thought to be distinctive and relevant to this theory are (1) epigenetic interactions must be mediated through processes that depend on cell–cell interactions as well as complex molecular cascades, making them a large target for mutation, and (2) the effects of a gene on the cell in which it is expressed should be difficult to eliminate because the gene product serves a necessary function. One prediction of the hypothesis is that modules of trait variation will come to coincide with developmental modules.

This general outline of the theory closely resembles Berg's (1960) in that she also postulated that correlations between groups of traits would be dismantled, forming two modules out of one by the process of decreasing the sensitivity of tissues to neighboring tissues. However, the erosional theory was not based on hers; rather, it was based on a model for the evolution of RNA structure toward a target secondary structure. Simulations of that process resulted in selection against phenotypic variation (because average fitness decreases with the number of structures into which it can fold), which led to canalization against genetic variation and spontaneously increased modularity (Ancel and Fontana, 2000). Reasoning by analogy, intrinsic genetic and epigenetic effects were construed in terms of the two ways in which a base substitution can affect the RNA phenotype (Wagner and Mezey, 2004; Wagner et al., 2007). In the case of RNA secondary structure, a base substitution can alter the secondary structure of which that base is a physical part, or it can affect another part of the secondary structure of which it is not a physical part. The first was interpreted as equivalent to the focal effect of a gene (intrinsic genetic effects), the second to epigenetic effects. Although the two lines of reasoning led to a very similar conclusion, i.e., that modularity can evolve by eliminating epigenetic effects, Berg did not see modularity as a side effect of selection for canalization. Rather, she saw modularity as a target of selection because modularity makes functionally unrelated traits developmentally independent of each other. In contrast, the erosional theory makes modularity a side effect of selection for robustness. This contrast between seeing modularity as a target of selection and seeing it as an indirect effect of selection on something else does not lie in the mechanistic evolutionary theory for how modularity evolves. Rather, it lies in the classification of variational properties. Berg saw integration by epigenetic effects as a form of maladaptive plasticity (at least when those interactions occur between functionally heterogeneous tissues), whereas Wagner and Mezey treat modularity and integration as a kind of variational property distinct from robustness.

One other model is more strictly nonadaptive in that integration and modularity are not

even side effects of selection for something else. Rather, they are not evolved properties at all. Instead, they are intrinsic to a developmentally modular system (Klingenberg et al., 2003; Klingenberg, 2004, 2005, 2008). The crux of this argument is that integration due to direct interactions is hypothesized to be exceptionally conservative, whereas that due to parallel variation is not (Klingenberg, 2004, 2005). Klingenberg reasons that pleiotropy due to parallel variation should evolve quite readily because the pleiotropic effects of each locus can change independently of any other and variation in those pleiotropic effects is highly likely. As an example of the process, he considers the evolution of pleiotropic effects for a gene with two *cis*-regulatory elements. Pleiotropy can arise from allelic variation in the coding region because that will affect both pathways along which the gene is expressed. However, allelic variation in one regulatory region will alter the expression of the gene along only one pathway. When the regulation of each gene can evolve independently, pleiotropy should evolve readily. However, altering integration due to direct interactions should be far more difficult because that must occur by a change in the interactions themselves, such as by a change in the inductive signaling process. Variation in the structure of direct interactions is hypothesized to be rare, largely because any variation arising upstream has the same effect downstream—the variation arising upstream of the interaction is structured by the downstream interaction because it is the interaction that transmits and therefore structures the phenotypic effect of that variation. Any change in the structure of the direct interactions is likely to be dramatic because that will affect the patterns of pleiotropy of all loci *upstream* of the interaction whose effects are structured by the interaction. Klingenberg hypothesizes that this will change the modular structure of development, which would probably have substantial (and typically deleterious) effects. Thus, if integration is due primarily to direct interactions, it should be highly conserved.

EPIGENETIC INTERACTIONS AND THE EVOLUTION OF FUNCTIONAL INTEGRATION

We suggest that integration due to epigenetic interactions evolves as an adaptation to produce integration between functionally integrated traits, turning Berg's scenario on its head. She considered the case in which epigenetic interactions take place between functionally unrelated traits, so development of one tissue is keyed to signals transmitted from the other when the tissues are adaptively decoupled. However, epigenetic interactions between functionally integrated traits are a form of adaptive plasticity when (1) the optimal phenotype for one tissue is conditional on the phenotype of another and (2) development toward that optimum depends on signals transmitted from the tissue that determines the optimum. This differs from the conventional form of adaptive plasticity only in the sense that the "environment" is internal to the organism. Both the environment that determines optimal phenotype and the one that provides the signals keying development toward that optimum are other tissues within the same organism. An adaptive response to those signals is thus a form of adaptive plasticity even if the environment contains genes.

This is not an unusual view of the muscle–bone relationship, which has long been a paradigm case of epigenetic interaction and of adaptive plasticity. Bone's ability to respond to muscle loading and other sources of strain via physiological processes is called *bone adaptation* (e.g., Forwood and Turner, 1995; Judex et al., 1997; Turner, 1998; LaMothe et al., 2005; Kesavan et al., 2006) even though no evolutionary process or outcome is implied. No doubt, bone adaptation is adaptive in the sense of enhancing fitness because there is an advantage in having bones capable of withstanding the forces loading them. It is also adaptive to have bones that can respond to unloading by resorption. If bones could not resorb, not only would they waste calcium but they could not grow by resorbing bone in one place while depositing it in another. That would mean that foramina could

not enlarge to accommodate growing nerves and that bone marrow cavities could not enlarge to encompass blood-forming cells. Blindness and severe anemia are among the consequences of mutations that deprive bone-resorbing cells (osteoclasts) of their ability to resorb bone (Gruneberg, 1936; Gerritsen et al., 1994; Heaney et al., 1998). All that said, we do not know if bone adaptation qualifies as an adaptation in the historical sense of having arisen under natural selection for its current function: adjusting the bone's phenotype to loading.

The example of bone adaptation may seem unfortunate because it is so often viewed as case of adaptive plasticity in the traditional sense, meaning that it is nongenetic. Epigenetic pleiotropy is obviously genetic, but the logic of the argument does not depend on whether variation in the signal (loading) is heritable or not. Bone does not care whether variation in loading is determined by the muscle's genotype or its external environment. Mutations or gene knockouts that affect muscle development have pleiotropic effects on bone (Herring and Lakars, 1981; e.g., Hall and Herring, 1990; Rot-Nikcevic et al., 2006, 2007; Jones et al., 2007). Those effects are predictable from general biomechanical principles (Herring and Lakars, 1981), not from principles specific to the genetic pathways. What matters to the response is the signal itself, i.e., bone strain. Thus, this example is useful because it puts the emphasis on the characteristics of the signal. It may also moderate the perception that direct interactions are highly conserved. The structure of the pathway by which bone responds to muscle may be highly conserved, as it assumed to be when using mouse models to understand the pathways of bone adaptation in humans (e.g., Srivastava et al., 2005; Xing et al., 2005; Zhong et al., 2005; Reeves et al., 2007; Robling et al., 2007). However, the spatial distribution of that signal depends on muscle mass and the orientations of muscle fibers and, in the case of function-induced strains, on the mechanics of the function (e.g., mastication, gnawing, or jumping). Thus, the spatial structure of direct interactions may be no more conservative than the spatial organization of the signals.

The most obvious value of this example is that it is a familiar case of both adaptive plasticity and an epigenetic interaction, making the conceptual and mechanistic link between them intuitively obvious. That could also be a disadvantage of this example because the link between adaptive plasticity and epigenetic pleiotropy might not extend to cases in which the signaling tissue determines the optimum for the responding one. Yet, a general advantage of epigenetic pleiotropy for functionally integrated structures is that their integration is built into the developmental system. It is interesting that Reidl's (1977) examples of developmental integration correlating functionally interdependent parts were classic examples of epigenetic interactions, embryonic induction, and epigenetic cascades and that many empirical studies of integration in vertebrate skulls similarly predict integration from epigenetic pleiotropy—correlations resulting from the transduction of mechanical signals transmitted by the soft tissue of a functional unit (e.g., Cheverud, 1982, 1988, 1995, 1996b; Gonzalez-Jose et al., 2004; Ackermann, 2005; Goswami, 2006, 2007). Transduction of mechanical signals may play a larger role in the origin of integration than it does in the development of an organism because the processes that play a larger role in shaping the organism's body plan (e.g., developmental patterning) are likely under far stronger stabilizing selection. Processes that contribute most to morphological integration are the ones in which variation is tolerated so long as proportions are maintained. In the case of parts that interact by way of mechanical signals and whose optimal form is determined, in part, by those signals, epigenetic pleiotropy builds their integration into the developmental system.

THE MAMMALIAN MANDIBLE IS A PARADIGM, BUT FOR WHAT?

The mammalian mandible has been characterized as a paradigm for the adaptive theory of integration because the mandible's functionally

integrated parts are also genetically integrated and its functionally independent parts are modular in the genotype–phenotype map (Cheverud et al., 1997, 2004; Mezey et al., 2000; Ehrich et al., 2003; Cheverud, 2004). The mandible has also been used as support for the theory that direct interactions (and parallel variation) build developmental integration into functional systems (Zelditch et al., 2008, 2009). Also, the mandible has been viewed as providing equivocal support for both the theory that modularity is an adaptively evolved property and for the theory that modularity is an automatic outcome of the developmental system (Klingenberg, 2005). The mandible has received so much attention partly because it has long served as a classic model system for studies of complex morphologies, meaning those that arise from developmentally heterogeneous parts but form a structural and functional whole (e.g., Atchley, 1983; Bailey, 1985, 1986; Atchley and Hall, 1991; Cheverud et al., 1991; Atchley et al., 1992; Klingenberg et al., 2001b, 2003; Monteiro et al., 2005; Marquez, 2008; Zelditch et al., 2008, 2009). Its status as a paradigm for the adaptive theory of integration is important because it makes studies of the mandible especially valuable for testing specific predictions of the theory, such as that pleiotropic loci typically integrate traits within the same functional complex (rather than reflecting the balance between positive and negative pleiotropy). However, at present, there is no consensus regarding the evolutionary origin or even the structure of mandibular integration.

One major problem is that there is also no consensus on either the functional organization of the mandible or its modular developmental structure. The dominant theory of mandibular function in the quantitative-genetic literature, shown in Figure 17.5, is that the mandible serves two functions: (1) bearing muscles and (2) bearing teeth. The mandible is thus divided into two functional modules, and that conception of mandibular function is the basis for the expected architecture of genetic integration (Cheverud et al., 1997, 2004; Mezey et al., 2000; Ehrich et al., 2003; Klingenberg et al., 2003; Roseman et al., 2009). As recognized by the advocates of the two-module theory, the incisor root extends into the muscle-bearing region and the attachments of many muscles (lateral masseter, anterior zygomaticomandibularis, digastric, mylohyoid, genioglossus, and geniohyoid) are partly or wholly within the tooth-bearing region. Nevertheless, the mandible is divided into those two distinct functional modules. One of the alternative functional theories claims that muscle insertion sites are functionally integrated units, with the remainder of the mandible being functionally nonintegrated, and that is the model for the expected architecture of modularity (Badyaev and Foresman, 2004; Badyaev et al., 2005). A third theory claims that the mandible serves three primary functions, (1) gnawing, (2) chewing, and (3) ingestion; but because these functional units overlap anatomically, the mandible should not be a modular structure (Zelditch et al., 2008, 2009). This lack of consensus makes it difficult to argue one way or another about the relationship between functional organization, developmental modularity, and integration.

The discrepancies between developmental theories of mandibular modularity are equally

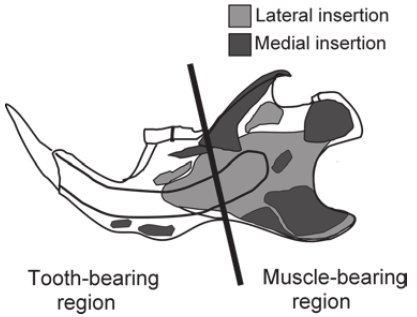

FIGURE 17.5 The dominant quantitative-genetic model for the developmental and functional integration of the mandible (after Atchley et al., 1985; Cheverud et al., 1991). Muscle attachment areas are shown by the shaded regions, distinguishing medially from laterally inserting muscles. The line crossing the mandible at the level of the posterior molar alveolus represents the boundary between the muscle-bearing and tooth-bearing developmental and functional modules.

profound. One view is that there are two developmental modules (corresponding to the two functional units discussed above): one for the part of the jaw that interacts epigenetically with muscles, another for the part that interacts epigenetically with teeth (Cheverud et al., 1997, 2004; Mezey et al., 2000; Ehrich et al., 2003; Roseman et al., 2009). The major alternative, shown in Figure 17.6 is that mandibular modules correspond to mesenchymal condensations (Atchley and Hall, 1991; Hall, 2003; Zelditch et al., 2008, 2009; Monteiro and Nogueira, 2009). These two developmental theories yield different predictions for two different reasons. First, the two-module theory predicts high integration between parts that the condensation model considers distinct modules. Second, and perhaps more important, the two-module view not only combines condensations but also splits one of them, assigning different regions of the ramus to the "tooth-bearing" and "muscle-bearing" modules. Other theories of developmental modularity could be derived from recent studies of developmental patterning (e.g., Mina, 2001; Mina et al., 2002; Cobourne and Sharpe, 2003; Haworth et al., 2004; Tucker and Sharpe, 2004; Dobreva et al., 2006; Depew and Compagnucci, 2008; Caton and Tucker, 2009). Such theories might also postulate two broad modules but ones that correspond to the signaling domains of fibroblast growth factor-8 and bone morphogenetic protein-4 signaling domains, each of which could be subdivided into expression domains of the transcription factors induced by the epithelial signal.

These discrepancies between views of functional integration, like those between views of developmental modularity, have important implications for assessing the theory that mandibular genetic integration evolves to match its functional integration or even that the functional integration and modularity just coincide with one another. The discrepancies have equally important implications for assessing the theory that mandibular integration is an intrinsic feature of its developmental modularity. The most obvious consequence of these

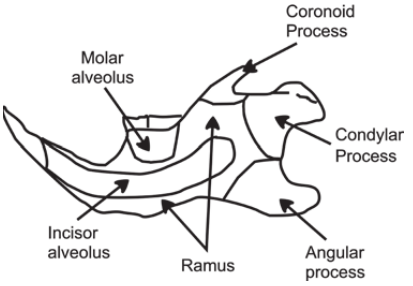

FIGURE 17.6 An alternative model for the developmental modularity of the mandible (after Atchley and Hall 1991). Each labeled region arises from a separate mesenchymal condensation.

discrepancies is that the evidence in favor of either hypothesis depends as much on the expectations as it does on the data. If we expect that natural selection will produce two modules of variation, one corresponding to the muscle-bearing region and another corresponding to the tooth-bearing region, finding that the model fits the data is taken as support for the theory. However, finding that developmental modularity is also structured like that makes the support for the evolutionary theory more equivocal because that pattern is equally consistent with the idea that the functional architecture of variation is an automatic consequence of developmental modularity. That is the crux of one dispute (see especially Klingenberg et al., 2003; Klingenberg, 2005). However, finding that the data support the developmental two-module structure would actually argue against the adaptive model for the origin of pleiotropy should the functions of the mandible be interpreted as gnawing and mastication (or as serving as the attachment site for independent muscles).

Our analyses, conducted with our collaborator Aaron Wood, suggest that the mandible is not modular in its variation, that developmental patterning modules are not always coherent units of variation, that the regions loaded by reaction forces are coupled to those loaded by muscle-generated forces, and that these correlations are as evident, or even more so, in the structure of direct interactions as in the

structure of phenotypic integration (Zelditch et al., 2008, 2009). Comparisons between species also suggest that rodents differ in structure of integration in a way compatible with their different functional demands due to differences in food consistency. Whether differences in consistency of food actually eaten by the animals are consequential for the structure of integration is not yet clear, but that obviously bears on the issue of whether function itself (e.g., gnawing, biting) can induce integration between functionally integrated parts. If so, then epigenetic pleiotropy and epigenetic plasticity may cooperate in building developmental integration into functional systems.

CONCLUSION

Epigenetic and intrinsic genetic pleiotropy are distinct developmental mechanisms of integration, and we have argued that this warrants treating them as theoretically distinct, whether a theory aims to explain developmental or evolutionary origins of integration. Distinguishing these two forms of pleiotropy will no doubt complicate evolutionary theories for integration because, in some respects, epigenetic pleiotropy is more akin to adaptive plasticity than it is to intrinsic genetic pleiotropy; but epigenetic pleiotropy is also distinct from adaptive plasticity in one crucial respect—the internal environment of the organism contains its genes. It may thus seem that epigenetic pleiotropy ought to be viewed as an interaction between genes in that the impact of variation in alleles at one locus is contingent on those at another, but that would miss what makes epigenetic pleiotropy like adaptive plasticity: The tissue doing the signaling not only conditions the expression of genes within the responding tissue but also determines the optimal phenotype for it. That is why epigenetic pleiotropy matters most for functionally interdependent traits that express few genes in common and that interact primarily via signaling interactions, such as muscles and bones. It is through their signaling interactions that they maintain the internal coherence of a functional module.

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