The Integrated Phenotype

Courtney J. Murren

Department of Biology, College of Charleston, 66 George Street, Charleston, SC 29424, USA

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1E-mail: murrenc@cofc.edu

Synopsis

Proper functioning of complex phenotypes requires that multiple traits work together. Examination of relationships among traits within and between complex characters and how they interact to function as a whole organism is critical to advancing our understanding of evolutionary developmental plasticity. Phenotypic integration refers to the relationships among multiple characters of a complex phenotype, and their relationships with other functional units (modules) in an organism. In this review, I summarize a brief history of the concept of phenotypic integration in plant and animal biology. Following an introduction of concepts, including modularity, I use an empirical case-study approach to highlight recent advance in clarifying the developmental and genomic basis of integration. I end by highlighting some novel approaches to genomic and epigenetic perturbations that offer promise in further addressing the role of phenotypic integration in evolutionary diversification. In the age of the phenotype, studies that examine the genomic and developmental changes in relationships of traits across environments will shape the next chapter in our quest for understanding the evolution of complex characters.

Introduction

The rapid expansion of publicly available genomic data over the last decade has provided ample genetic information about model and nonmodel organisms, as well as about replicate populations within species (e.g., 1000 genomes project in humans and 1001 genomes project in Arabidopsis thaliana). The expansion of these data will continue as costs continue to decrease and efficiency of methods increase (Lister et al. 2009). In addition, information from these data sets is contributing to advances in broad areas of evolutionary biology, from species’ phylogenetic relationships to the evolution of genomes (Alfoldi et al. 2011; Rutter et al. 2012). Whereas genetic and genomic data have become abundant in the last decade, synthesis of accompanying phenotypic data lag behind.

The era of the phenotype or phenomics (Houle et al. 2010; Furman and Tester 2011; Sozzani and Benfey 2011). The rapid expansion of genomic data in the last decade was accompanied by conceptual and analytical growth in bioinformatics. An analogous conceptual and methodological set of advances is needed to pull together vast historic and contemporary phenotypic data sets and to interpret these large phenotypic data sets on their own, through development, and in light of genomic data. A general question in evolutionary biology is the relationship between variation at the genomic level and in variation in complex phenotypes. One place to begin to build an understanding of complex phenotypes and the influences of genomic, developmental, and environmental variation is through the examination of phenotypic integration.

History and origins

Phenotypic integration refers to the biological relationships among multiple characters of a complex phenotype, and their relationships with other
functional units (or modules) in the organism (Schlichting 1989; Pigliucci and Preston 2004). Proper functioning of complex phenotypes requires that traits work together within a module. Modularity describes the relative independence of complex traits. The interactions among characters may change through development and across environments (Murren 2002). Formalization of the concept of phenotypic integration began more than half a century ago, inspired by research dating back to descriptive work in the early 1900s by Thompson (1917) (Pigliucci and Preston 2004). Olson and Miller (1958) defined the term morphological integration as the interdependence of morphological traits to produce an organized functional organism. Their book emphasized the role of development in trait function, and argued the importance of using statistical approaches to study morphological integration. Their perspectives on complex morphological phenotypes inspired much subsequent research on morphology and evolution of form (see Afterword by Chernoff and Magwene 1999 in Olson and Millers 1958; also see Cheverud 1982, 1996; Klingenberg 2010). From the early formal discussions of phenotypic integration, the joint importance of development, environment, and differentiation in patterns of integration among species or populations has been apparent. The history of the concept has shaped the modern study of integration such that the study of ecological, genetic, and developmental influences on complex traits are woven together.

About the time that Olson and Miller were examining animal morphologies, Clausen et al. (1940) and Clausen and Heisey (1958, 1960) were conducting long-term investigations on a suite of plant species planted across an altitudinal gradient in the Sierra Nevada. These botanists and cytogeneticists hypothesized that species’ differences are largely due to differences in patterns of what they termed character coherence (patterns in the relationships among traits). Their work emphasized the role of the environment in both the maintenance of character coherence and the plasticity of phenotypes (although it was not termed plasticity at that time). These studies of patterns of variation of traits’ relationships stimulated a shift in research focus in plant biology toward investigations that examined the influence of the environment on traits and their functional relationships on integration. Berg (1960) developed and tested hypotheses of whether patterns of traits’ relationships (that she termed correlation pleiades) differed between taxa pollinated by a specialized or generalized pollinator systems. She found evidence for greater integration within flowers for plants with species-specific pollination systems than for those with generalist pollination systems. Tests of Berg’s hypotheses in various species and communities are now numerous, yet have mixed support (Armbruster et al. 1999; Hansen et al. 2007; Edwards and Weinig 2011).

A small but active group of researchers worked on aspects of integration in the 1980s and 1990s building evidence of the importance of development and environmental variation (Cheverud 1982; Schlichting 1989; Cheverud 1996; Wagner and Altenberg 1996; Callahan and Waller 2000). Following publication of the volume Phenotypic Integration edited by Pigliucci and Preston (2004), the conceptual framework of phenotypic integration moved into mainstream evolutionary biology. At the time of preparation of this article, over 100 citations with the key word phenotypic integration were indexed in Web of Science (ISI) since 2004; with plants and vertebrate animals the most widely examined organisms. Differing perspectives on the evolution of complex traits and phenotypic integration from these two major lineages of eukaryotes is, in part, due to differences in body plans, and to the influence of the research questions framed by Berg and colleagues on the plant side and Olson and Miller on the animal side.

In the first section of this article, I provide a brief overview to the concepts of modules, modularity, and morphological integration—a specific aspect of phenotypic integration. Exhaustive coverage of modularity is beyond the scope of this review; rather, the goal of this section of the article is to provide an entrée into the rapidly growing literature and to provide a foundation from which to discuss new empirical advances and future challenges. In the second half of this article, I use empirical case studies to highlight recent work on phenotypic integration that offer promise in our quest for a synthetic understanding of complex characters. Specifically, I draw attention to recent studies examining (1) how environmental perturbation influences development and morphological integration, (2) the influence of maternal effects on integration within and between generations, (3) approaches to investigate modules, and (4) genomic approaches to studying the genetic basis of integration. I end by highlighting new empirical approaches that hold promise for linking genetics, development, and environment in future
research on integration. Throughout this review, I emphasize aspects of inquiry that have support in both animal and plant systems. I also emphasize perspectives unique to plant or animal research in an effort to encourage cross-fertilization between approaches applied in these distinct clades of multicellular eukaryotic organisms.

**Phenotypic integration and modularity**

A module is a term applied broadly to describe an aspect of an organism’s phenotype when there are tight associations among traits within the complex character, but the links with other character complexes are not as strong (see also Wagner 1996; Schlichting and Pigliucci 1998; Murren 2002; Hallgrímsson and Hall 2005; Klingenberg 2010). This pattern of associations of traits leads to a semi-autonomous nature (termed modularity) in which certain traits may interact with other modules depending on the function involved. Although semi-independent, different modules of an organism must at some level work together as a functional whole organism.

The construction or maintenance of a phenotypic module occurs through a combination of nonexclusive processes: shared or closely linked developmental or genetic components (Schlichting 1989; Conner 2002; Roseman et al. 2009), or trade-offs resulting from differential patterns of allocation (Tonsor and Scheiner 2007). Suites of characters that work together as a particular functional unit such as a flower (Conner and Sterling 1996), or a mandible (Cheverud 1982) can be defined as functional modules. These modules may be composed of additional submodules with specific subfunctions (Murren 2002; Bissell and Diggle 2008). Organisms with strong relationships (high correlations) between the traits within the module are hypothesized to have the most efficient functional performance (Brock and Weinig 2007; Pélabon et al. 2011). Selection may act on trait relationships, thus integration can be adaptive (Ordano et al. 2008). Assuming that phenotypic integration has an underlying heritable basis, albeit complex, the potential evolutionary trajectories of traits within a phenotypic module will be influenced by other traits in the module and by the selective environment.

Modules may have enhanced function when correlations among traits are high; thus selection may act on integration. In addition, the extent of evolutionary flexibility of a complex character may be determined by whether variation is modular or integrated with other aspects of the organism’s phenotype (Wagner and Altenberg 1996; Roseman et al. 2009; Drake and Klingenberg 2010). Modularity may enhance potential responses to selection, as semi-autonomous sets of characters that perform different functions may respond independently to selection (Bissell and Diggle 2010). Alternatively, strong integration among functional units may limit evolutionary change if selection acts in opposing directions on different aspects of a complex phenotype with strong internal correlations (Wagner and Altenberg 1996). Selection on phenotypic interaction is, therefore, considered to favor modular genetic and developmental systems (Hallgrímsson et al. 2009), including hormonal regulation (Ketterson et al. 2009). Although I have described modules from a largely phenotypic perspective, modules and integration may be considered from other biological viewpoints, including developmental, functional, and genetic perspectives. In practice, how we define functional modules when the limits of a functional module occur will influence our interpretations of their evolution (see approaches to the study of modules below and Fornoni et al. 2009).
A synthetic theory of phenotypic integration and modularity has yet to emerge (Arnold 2005). From a quantitative genetics perspective, evaluation of the appropriateness of the G-matrix has formed much of the theoretical basis of integration. These investigations were inspired in large part by Cheverud (1982). Quantitative genetic models have been instrumental in developing hypotheses of multivariate selection on suites of characters, and new tools for matrix comparison may direct future work from this perspective (Arnold 2005; Marroig et al. 2011). However, other aspects of theory of integration are not as well developed. As eloquently described by Moczek, a synthetic theory of developmental evolution is yet to emerge. Development and epigenetic approaches are at the heart of much of the contemporary work on phenotypic integration (Hallgrimsson et al. 2009). The conceptual model of $G \to P$ map advocated by Wagner (1996), Wagner and Altenberg (1996), and Pigliucci (2010) describes links between sets of genes and sets of traits. This conceptual framework is particularly valuable in light of the rapidly expanding genomic data (e.g., see discussion of quantitative trait loci (QTL) approaches below). As an extension of this model, Klingenberg (2003) included development as an additional conceptual component. He added that the developmental system (as well as environmental system) may modulate the path between sets of genes and phenotypic modules. Empirical investigations of integration currently are rapidly accumulating in the literature, thus the majority of this review emphasizes empirical work.

**Genetics of phenotypic integration**

Phenotypic modules may also have associated underlying genetic modularity, primarily through pleiotropy or genetic linkage (Cheverud 1982; Wagner 1996; Cheverud et al. 1997; Juenger et al. 2000; Cheverud 2004; Murren and Kover 2004). Genetic basis of covariance or of correlation patterns among phenotypic traits is attributed to aspects of genetic architecture such as pleiotropy (or pleiotropic QTL), close linkage, or linkage disequilibrium (LD) among physically unlinked genes (Cheverud 2004; Murren and Kover 2004; Roseman et al. 2009). A single pleiotropic gene or QTL region simultaneously influences the variation of several traits that can act through developmental processes (Hallgrimsson et al. 2009). Pleiotropy, therefore, can be an important mechanism in shaping or constraining patterns of multitrait evolution. Alternatively, LD among physically unlinked genes may arise through natural selection of particular combinations of alleles that enhance functional efficiency (Conner and Sterling 1995).

**Morphological integration**

A strength of research on integration in animal biology has been the study of morphological shape-based phenotypes (Raff 1996; Klingenberg 2010). The emphasis on morphological integration follows from the tradition of Olson and Miller (1958). Studies of morphological integration largely rely on data collected on landmark positions—precise locations of particular morphological features that can be identified in all organisms examined in a study (Fig. 1) (Cooper et al. 2011). To examine the evolution of form, these landmark data sets are often analyzed through Procrustes superimposition—a method that employs the landmark information to extract variation in shape, standardized for size and orientation (for more detailed information, see Klingenberg 2010; Viscosi and Cardini 2011). These tools have been widely employed to examine morphologies of terrestrial invertebrates (Klingenberg et al. 2010) and vertebrates (Young and Badyaev 2010). Recently, this technique has been implemented to study the shape of a compound leaf (Klingenberg et al. 2012), suggesting that certain aspects of plant form, such as flowers (for methodology, see van der Niet et al. 2010; Ordano et al. 2008; Benitez-Vieyra et al. 2009), may be well suited for landmark analyses.

**Development and modularity**

Development has been considered a key component of the study of integration since the formalization of the concept (Olson and Miller 1958). Environmental or genetic perturbations can shift developmental pathways, often resulting in phenotypic changes. Divergence in timing of floral development between two species of *Clarkia*—one selfing and one outcrossing—that occupy discrete habitats that vary in moisture availability suggests that selection acting on developmental processes contributes to distinct patterns of phenotypic integration and potentially to diversification (Runions and Geber 2000). Whether separate aspects such as the timing and the rate of
development can be uncoupled (Runions and Geber 2000) will influence the evolutionary flexibility of developmental traits and the resulting patterns of integration. Often development and function (Olson and Miller 1958) are discussed in tandem, as these biological mechanisms are indistinguishable in many studies. Two major approaches have attempted to disentangle development from function. First, studies of developmental instability treat the variation produced through random events during development by examining the covariation of the patterns of asymmetry among traits within individuals. An influence of shared development can be concluded when high covariances among developmental instabilities are detected, as the environment (generally) and genomics are held constant. Second, disruption of genes known to influence development across the phenotype of the organism may uncover shared developmental processes when covariance among multiple traits change with introduction of variation into the system. Examinations of environmental perturbations on developmental plasticity draw attention to the influence of epigenetics on phenotypic integration (Hallgrimsson et al. 2007, 2009).

Approaches to the study of modules

A large and growing literature exists on modularity (Schlosser and Wagner 2004; Hallgrimsson and Hall 2005; Hallgrimsson et al. 2009; Klingenberg 2010). Investigations into how complex characters are partitioned into modules apply one of two main approaches: examinations of modules identified through data sets (data-defined) and those described a priori (researcher defined).

Modules that are “data-defined” are identified through a variety of statistical approaches that examine patterns of covariation of traits. Numerous statistical methodologies for delineating membership of traits in modules as well as patterns of integration within and between modules have been applied to phenotypic data. These methods rely on measurable variation in patterns of covariation and are particularly well described in the literature on morphological integration using landmarks (for example, but not limited to Mitteroecker and Bookstein 2007; Marquez 2008; Bissell and Diggle 2008; Magwene 2009; Bissell and Diggle 2010). These data-driven approaches are valuable in uncovering modules with specialized functions within a larger complex of characters. For example, two character suites with distinct functions (one pollinator attraction, the other pollen transfer) within the flower were detected in two closely related species: *Nicotiana alata* and *Nicotiana forgetiana* (Bissell and Diggle 2010). Combined phenotypic and quantitative genetic analyses indicated that these modules are stable through evolutionary time, yet the connections between modules are evolutionarily labile. Co-expression networks built on vast microarray phenotypes (particularly for crop and other model organisms) have been built to elucidate putative gene modules (Ficklin et al. 2010). These data-driven approaches have likewise been informative in highlighting a mechanistic basis for integration, including shared development, and shared genetics when observable differences in patterns of co-variation are observed (see below). Hallgrimsson et al. (2009) caution that unless phenotypic variance exists in the sample, many developmental modules may not be uncovered via analyses of patterns of phenotypic (e.g., morphological) covariance data.

“Researcher-defined” modules rely on prior research and knowledge of an organism to describe phenotypic modules. Investigations of phenotypic integration that define functional modules a priori often address particular hypotheses (e.g., Berg’s hypothesis described above regarding greater correlation among traits in floral vs vegetative modules and associated with the pollinator-mediated selective environment) (Fig. 2) (Armbruster et al. 2004; Ordano et al. 2008; Pélabon et al. 2011), examine changes through development or across environments in strength of integration or in phenotypic composition of modules (Murren et al. 2002; Bossdorf and Pigliucci 2009; Pélabon et al. 2011), or assess differential functional roles of adjacent parts (Klingenberg et al. 2010). Pélabon et al. (2011) examined Berg’s hypothesis of decoupling of floral and vegetative traits in two populations of *Dalechampia scandens* (a neotropical vine) across gradients of nutrients and of availability of light. *Dalechampia* blossoms are in fact inflorescences that include both a male and a female subinflorescence (Fig. 2). The showy portion of the blossom is an involucral bract (a modified leaf). Examination of developmentally proximate structures, or of homologous structures with different functions, enables contributions of function and development to be separated. Greater variation was found in traits not associated with pollination than in those involved in pollination. This pattern held
across different environments, and their data were consistent with Berg’s hypothesis of patterns of variation within and between in pollination traits in comparison with vegetative traits. Patterns of trait variation of homologous leaf and bract structures in *Dalachampia* suggest that functional differences between vegetative and pollination-related characters are of greater importance than is proximity of aspects of the phenotype or shared elements of a developmental pathway. Thus, any potential developmental limits were likely broken by natural selection.

A well-studied system employed to test hypotheses of phenotypic integration is the mouse mandible. The mandible has been described to have two functional regions: an alveolar region and an ascending ramus region (Fig. 1) (Cheverud et al. 1997; Mezey et al. 2000). The alveolar region is the area that includes the teeth. The ascending remus is the area of muscle attachment and connection to the rest of the skull (Cheverud et al. 1997; Roseman et al. 2009). Roseman et al. (2009) challenged the notion that this pair of phenotypic regions forms two discrete modules, as various investigations have failed to find patterns of integration and modularity that consistently fall into one or the other of these two regions. Employing the perspective that a shared genetic basis defines modules (in this case shared QTL and pleiotropy), Roseman et al. (2009) defined two classes of pleiotropic QTL: local and global. If QTL effects are distributed in a more narrow phenotypic region (or among those traits that are highly correlated) than expected by chance, these are considered locally pleiotropic. If QTL effects are more widely distributed throughout the entire phenotype examined, these are considered globally pleiotropic. Using a “data-defined” approach to examine the coherence of two “researcher-defined” modules, two classes of QTL emerged from the mouse-mandible data set. The first class included a large proportion of pleiotropic QTL that are more highly correlated than due to chance, and a second set distributed as if by

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**Fig. 2** From Pelabon et al. Line drawings of *Dalechampia scadens* in a study of integration within floral and vegetative structures and homologous structures. (A) The leaf and blossom. (B) Adaxial leaf and stipules (outgrowths underneath the leaf). (C) Blossom. (D) Abaxial leaf and stipules. Bracts of blossom are modified leaves, and central portion of the blossom includes male and female flowers making up an inflorescence.
chance. The distribution of QTL effects in their data set provides evidence favoring a conception of modularity based on physical proximity of traits along a continuum both for phenotypic correlation of traits and for QTL expression data sets.

Determining the relative merit of a “researcher-defined” or “data-defined” approach to the study of modules, in part, depends on the particular hypotheses to be tested. The “data-defined” approach may be instrumental in identifying QTL that vary in importance through ontogeny (see also Hallgrimsson and Lieberman 2008), identifying ecological and evolutionarily important functional modules previously undefined (Bissell and Diggle 2008, 2010) or in identifying traits that change functional roles during development and resulting in traits that vary in the extent they overlap in function at different developmental stages and resulting in a continuum in variation of modularity, such as in the mouse mandible (Roseman et al. 2009). Bissell and Diggle (2010) suggested that studies of broad functional modules (such as the flower), defined a priori, may obfuscate patterns of integration and may have led to the mixed support for Berg’s hypotheses across systems. Roseman et al. (2009) concluded that modules defined a priori restrict one’s ability to detect nuances in patterns of the relationships of traits and the effects of pleiotropic QTL that may vary from patchy to continuous. However, “researcher-defined” modules are appropriate when particular hypotheses of locomotion, floral function, or physiology are addressed specifically.

Structural equation modeling (SEM) together with model selection approaches, combine aspects of both researcher-defined and data-driven perspectives (Tonsor and Scheiner 2007; Bissell and Diggle 2010; Santos and Cannatella 2011) and may aid in the assessment of putative functional modules. This regression-based approach allows the testing of hypotheses of causal structure as well as discovery of previously unknown relationships, and the potential to compare patterns across environmental treatments or classes of mutant (Tonsor and Scheiner 2007). Applying SEM to aposomatic, diet, growth rate, and size traits in dendrobatid frogs revealed two phenotypic networks: one associated with aposematism and the other associated with scale (Santos and Cannatella 2011). Initial SEM models were built based on a priori knowledge of aposematism and body size and then compared against alternative models that included different traits. Using a novel phylogenetically informed analysis and ancestral reconstruction in this clade, Santos and Cannatella (2011) were able to narrow down the alternative hypotheses for the evolution of the complex trait for avoiding becoming prey to two. Phylogenetic approaches, such as those presented by Santos and Cannatella (2011) (see also Kolbe et al. 2011), offer a hope for gaining an understanding of the evolutionary steps that result in complex traits with behavioral, physiological, ecological, and size components. Combining the strengths of “researcher-defined” and “data-defined” modules in analyses may be particularly important as we move beyond model systems in evo–devo studies, and aim to evaluate whether patterns of phenotypic covariance, and their developmental and genetic bases, are species-specific.

Developmental plasticity and integration

Phenotypic plasticity is a term typically used to describe different phenotypes of a single trait, expressed in response to the environment (Pigliucci and Marlow 2001). Multiple traits may respond simultaneously to an environmental gradient; thus, patterns of integration themselves may be plastic, uncovering different suites of phenotypic variation (Schlichting and Pigliucci 1998). Developmental processes may contribute to these differential patterns of plasticity and ultimately to diversification (Plenig et al. 2010). Here I focus on two fresh perspectives that link development and environment: (1) the effect of short-term environmental perturbation during development and (2) the effect of longer-term maternal effects on patterns of integration.

Environmental perturbations during development

Environmental perturbations in early development can influence future development and ultimately adult form. During pregnancy, an environmental perturbation of reduced nutrient quality in the diet influenced the patterns of integration of the rat skull through ontogeny (Gonzalez et al. 2011a). These results emphasize how plastic responses may come about through alternative developmental pathways and compensatory mechanisms. In a companion study, phenotypic integration through development was examined following reduction in nutrients at several points in development (Gonzalez et al. 2011b). In rats, the timing and duration of the
nutritional environmental perturbation impacts the changes in covariance structure. Although all three main structures of the skull were reduced in size in the low-nutrient treatment, whether the vault, face, and base had the greatest change depended on the time during development at which the environmental perturbation occurred. In both studies, environmental perturbation influenced variance in cranial morphology, lending evidence to developmental plasticity as a critical influence on patterns of phenotypic integration.

Maternal effects
Maternal effects influence patterns of integration of traits across generations. In a study of a polymorphic lizard species, *Uta stansburiana*, Lancaster et al. (2010) provided evidence that maternal effects influence phenotypic integration of behavior with coloration of throat and dorsum, egg size, and shape of the body. Moreover, female lizards influence offspring's traits through levels of hormones adjusted through environmental social cues of the throat colors of neighbors and mate and level of stress. The result is an appropriate pairing of dorsal and throat colors, egg size, and anti-predator behavioral and patterning traits in the offspring. Adaptive maternal effects in this system maintain phenotypic integration as beneficial combinations of traits would otherwise be broken by recombination.

In a plant system, Galloway and Burgess (2009) experimentally manipulated the timing of flowering in *Campanulastrum americana* to examine the influence of phenology on reproductive integration and life-history characters in the offspring generation. A shift in a set of reproductive traits (including flowering, maturation of fruit, and dispersal) occurred together regardless of whether flowering was experimentally shifted earlier or later. However, timing of maternal reproduction influenced the frequency of offspring expressing an annual life history. Although plastic, the tight integration of reproductive characters suggests that responses of flowering to climatic change would include shifts in the entire suite of reproductive characters, and substantially impact development and life history in the offspring generation.

Genomic analyses via QTL approaches
QTL studies in both plants and animals point to pleiotropy (or close linkage) as the primary component of the genetic basis of integration (Cheverud 2004). Co-localization of QTL for different traits is a metric for identifying genetic modules. In the agricultural crop, oil rapeseed (*Brassica rapa*), analyses of floral and vegetative traits in a set of RILs (recombinant inbred lines) revealed high levels of integration within the floral module and little differentiation between floral and vegetative modules (Edwards and Weinig 2011). Edwards and Weinig (2011) suggested pleiotropy or close physical linkage as the likely genetic mechanisms behind these patterns of integration, given two lines of evidence. Recombination across a large collection of RILs reduces LD among physically distant genes. Colocalization of QTL for vegetative and floral traits is consistent with pleiotropy or close linkage, two genetic mechanisms that are indistinguishable when using a QTL approach. Cooper et al. (2011) presented evidence for co-localized QTL for traits with shared feeding function and skull shape in cichlids. In addition, there was evidence for QTL for correlations among traits themselves. Two alternative perspectives have been used to describe the evolutionary influence of pleiotropy. Pleiotropy may constrain the independent evolution of highly correlated traits (Edwards and Weinig 2011), or contribute to adaptive diversifications via phenotypic integration (Cooper et al. 2011).

Genomic approaches to examining the genetic basis of phenotypic integration over the past decade have largely (although not entirely) focused on QTL analyses. Future studies that include fine mapping (Murren and Kover 2004; Edwards and Weinig 2011) will help to disentangle the relative importance of close linkage and pleiotropy. Distinguishing these two effects are not possible with current QTL methods. In combination with the information from whole-genome projects in model organisms and agricultural organisms (including crops, stock fish, livestock, and pest species), combined QTL and fine-mapping studies may lead to identification of candidate genes that open doors to investigation of function and of timing of specific genes and gene combinations.

A path ahead: Genomic, ecological, and developmental perturbations
New tools on the horizon and novel application of classical tools offer potentially novel avenues for examining genome-wide, ecological, and developmental
influences on patterns of phenotypic integration. For example, using classical phenotypic approaches, hybrid species provide a view into the influence of broad changes in genomic composition on the evolution of integration, through the reshuffling of naturally occurring variation and through changes in large components of the genome. In systems where the parental and hybrids are extant, the contrast of the genomic combinations can paint an evolutionary picture of integration. In a study of seven species of \textit{Brassica} and closely related \textit{Raphanus} that included hybrids and their diploid relatives, hybrids were more integrated both within and among modules than was either of their parental species (Murren et al. 2002). Yet, neither cytogenetics nor ecological similarity explained differences among species in patterns of phenotypic correlations of morphological and life-history traits. In part, these differences between parental species and hybrids may reflect the long stable history of this hybrid complex. At the other end of the hybrid spectrum, cichlid fishes in East African rift lakes are known for their phylogenetic diversity and their propensity to hybridize in ecological timescales (Parsons et al. 2011). Two species of cichlid fish from Lake Malawi that differ in foraging mode and jaw width were experimentally hybridized to examine the capacity of this genomic perturbation for altering patterns of integration (Parsons et al. 2011). Analyses of head shape (from lateral and ventral views) uncovered greatly reduced patterns of integration in hybrids compared with either parent. Some hybrid individuals showed evidence of transgressive segregation—extreme phenotypes in the hybrids in comparison with parental species. Those individuals that showed transgressive segregation had further reductions in phenotypic integration. These patterns offer suggestion of how hybridization can promote evolution of novel phenotypes, break up patterns of integration, and contribute to diversification in a rapidly evolving group (Parsons et al. 2011). Further studies of hybrids are needed to elucidate the contributions of introduction of novel phenotypic variation compared with the selective environment. These studies will be particularly fruitful in groups such as \textit{Brassica} and cichlids for which genomic and ecological data are plentiful and growing, and for which experimental manipulations are possible.

Another successful tool in the study of phenotypic integration is the introduction of novel phenotypic variation by way of new mutations that disrupt developmental pathways. One such approach is EMS mutagenesis—a method that induces new mutations in an experimentally tractable time frame, introduces novel genetic variation, and potentially alters trait means and their patterns of phenotypic integration. Camara et al. (2000) demonstrated that EMS mutagenesis in \textit{A. thaliana} altered patterns of integration by changing an array of particular pair-wise correlations of traits. Interestingly, an especially strong and well-studied correlation between flowering time and number of rosette leaves was less labile in the mutagenized lines than were other relationships of traits. Other more recent approaches to genetic perturbations, such as the introduction of introgression of mutations that influence wing shape in various genetic backgrounds of \textit{Drosophila melanogaster} (Dworkin et al. 2009), and comparison of mutants in \textit{Mus musculus} known to influence aspects of the complex craniofacial phenotype with wild-type \textit{M. musculus} and its close relatives (Jamniczky and Hallgrimsson 2009), may offer insights into the influence of gene–gene interactions into patterns of phenotypic integration.

Model multicellular organisms such as \textit{Arabidopsis}, \textit{Drosophila}, and \textit{Mus} are especially well suited for examination of the effects of genomic perturbation on patterns of phenotypic integration, as many genomic data are publicly available, and genetic lines are available at stock centers (e.g., ABRC, Bloomington and DSSC Drosophila species stock center). One such resource includes the T-DNA knockouts in \textit{A. thaliana} (e.g., SALK T-DNA) (O’Malley and Ecker 2010). This set of early 17,000 T-DNA knockout lines includes single-gene knockouts all in the same genetic background. Although the genomic resource is available, phenotypic data are rare, largely lacking, or rarely detected (Kuromori et al. 2006; Hanada et al. 2009; see Hillenmeyer et al. 2008 for a combined data set of phenotypic and genomic data in yeast). unPAK (undergraduates phenotyping \textit{Arabidopsis} knockouts www.arabidopsisunpak.org) is a network of laboratories investigating the influence of single-gene knockouts on complex phenotypic characters such as germination success, size of rosettes and inflorescences, fruit production, overall plant biomass, and ultimately patterns of integration. Phenotyping with the ultimate goal of full genome coverage is intensive both of time and labor. The unPAK approach is much like a randomized complete block design (Gotelli and Ellison 2004) with plants grown and
phenotyped through time, across experimentally implemented treatments, and across multiple laboratories. Numerous undergraduate researchers at each institution employ shared protocols, deposit data into a shared database, and contribute novel insights and observations. In this age of the phenotype, collecting phenotypic data such as those planned by unPAK, is the next frontier in investigation of questions of phenotypic integration and of the role of developmental plasticity.

Evaluating epigenetics as a contributor to heritable phenotypic diversity (West-Eberhard 2003; Richards et al. 2010), and trans-generational plasticity are key to translating environmental influences on integration. Advances in detection of differential patterns of DNA methylation among genotypes (Lister et al. 2009; Becker et al. 2011) and experimental alteration of DNA methylation (Bossdorf et al. 2010) put within reach a greater understanding of the influence of heritable epigenetic polymorphisms on patterns of phenotypic integration.

It must be emphasized that additional environmental (Pélabon et al. 2011) and developmental perturbation studies (Gonzalez et al. 2011a, 2011b) are much needed across diverse systems. These types of studies aid in the understanding of these influences on changes in patterns of integration and modular structure and would allow synthesis across multicellular eukaryotes. Short- and long-term perturbation studies are complementary and would provide data on buffering and changes in developmental modularity (sensu Klingenberg 2003). Investigation of nonmorphological modules, such as defense traits (Huang et al. 2010), is also needed to evaluate whether common themes exist across classes of phenotypes. Comparisons between existing pairs of strains, for example, wild types and agricultural lineages specifically selected for defense traits (e.g., transgenic plants such as Bt corn), provide additional systems that have been perturbed genetically, uncovering variation, and appropriate for phenotypic approaches. Model approaches (such as SEM) will aid in the assessment of module composition. Further joining theoretical advances that formally include developmental, genomic, and environmental variation into our empirical understanding of modules and integration will be an important next step.

The age of genomics made possible the tools to experimentally introduce genetic variation in myriad ways and, more recently, to modify epigenetics through changes in DNA methylation. In the age of the phenotype, characterizing phenotypes across environments and through development, while employing tools that perturb genomic, ecological, or developmental systems will shape the next chapter in the quest for understanding how complex characters and their relationships are built and maintained through evolutionary time.

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