Phenotypic integration in plants

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Abstract

The interrelationships among phenotypic traits of plants has been of interest to plant evolutionary biologists for almost a century. Broadly defined, phenotypic integration refers to the correlations among traits within functional units (such as a flower). In this review, the classic research of Berg (1960) on the relationships among traits within floral modules, the dissociation of the floral module from the vegetative module are reviewed and advances in our understanding of these relationships is explored. Recent data on changes in integration of traits during development or across environments, as well as phylogenetic comparisons of integration, are highlighted. Common statistical approaches used to address these issues are briefly reviewed. In summary, it is argued that our understanding of the evolution of phenotypic integration will continue to advance as studies simultaneously examine the developmental, physiological and genetic mechanisms behind the genotype to phenotype map, and consider how selection acts on complex functional phenotypes.

Keywords: genotype-phenotype map, module, phenotypic correlations, phenotypic integration, phenotypic plasticity.

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Introduction

The evolution of multivariate phenotypic character correlations has been of interest to plant ecologists and evolutionists for many decades (Clausen & Hiesey 1958). Proper functioning of complex phenotypes requires that numerous traits simultaneously work together (Gould & Lewontin 1979; Cheverud 1982). Olson and Miller (1958) were the first to study multivariate trait interactions under the rubric of morphological integration. They were interested in the relationships among multiple traits as quantified by correlation coefficients and as qualitatively described by their biological function or development, and they were particularly interested in the overlap between the quantitative and qualitative patterns. Through time, Olson and Miller's concept of morphological integration was expanded to include life history, physiological and anatomical characters and the broader term 'phenotypic integration' was applied to encompass this range of characters (Cheverud 1982). Broadly, phenotypic integration is defined as the phenotypic correlation structure of multiple characters (e.g. Zelditch 1988; Schlichting 1989a).

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Complex character units, such as a flower, can be defined as a phenotypic module (Wagner 1996). Such a module performs a specific plant function, in this case reproduction. Plants with strong relationships (high correlations) between the component traits of the module such as anther, stigma, petals, sepals, etc. are hypothesized to most efficiently perform the functions of dispersing pollen, attracting pollinators and developing fruit (Berg 1960; Armbruster *et al*. 1999). Selection may favor plants with these specific relationships among traits, thus may act on integration (Olson & Miller 1958; Berg 1960). The patterns of selection will result in changes or maintenance of phenotypes in future generations if genetic variation for the processes underlying integration is present (Falconer & Mackay 1996). Clausen & Hiesey (1958) were among the first to examine the extent of phenotypic integration among sets of traits in plants. Their investigations were motivated by the question of how suites of morphological characters used to distinguish species were maintained as units by selection. In *Potentilla glandulosa*, phenotypic integration was pervasive, yet the patterns of correlations differed among crosses of distinct pairs of ecotypes (Clausen & Hiesey 1958; Grant 1979). Their data demonstrated that ecotypes were differentiated in their patterns of phenotypic integration, suggesting that not only single traits, but their patterns of correlations also

could evolve (Clausen & Hiesey 1958; Schlichting & Pigliucci 1998).

The construction or maintenance of a phenotypic module occurs through a combination of three general processes: (1) shared or closely linked genetic components (linkage or pleiotropy), (2) shared development or hormonal regulation, or (3) trade-offs resulting from differential patterns of allocation, for example, to biomass or to reproductive output in a long-lived perennial (Berg 1960; Cheverud *et al.* 1989; Schlichting 1989a). These three processes are nonexclusive, especially as development and allocation patterns can be under genetic control. Assuming that phenotypic integration has an underlying genetic basis, albeit complex, the potential evolutionary trajectories of traits within a phenotypic module will be influenced by other traits in the module. Positive phenotypic correlations between quantitative traits suggest that they will simultaneously increase (or decrease) in size, shape, and/or number (Falconer & Mackay 1996). A negative correlation indicates that as one trait increases the other decreases; this can be considered a trade-off or a genetic constraint (de Jong 1993; Falconer & Mackay 1996; Nicotra *et al.* 1997). Alternatively, pairs of characters that lack significant correlations are generally dissociable. Consequently, the sign (positive, negative or non-significant) of the correlations among traits has important implications for the evolution of the entire organism or complex phenotype of interest. We are just beginning to understand the details of the molecular genetic aspects of integration particularly for plants (Chernoff & Magwene 1999; Murren & Kover, in press); therefore in the following discussion, the phenotypic aspects of integration and changes in patterns of correlation structure across clades and environments will be explored.

While lineages may exhibit consistent differences in their patterns of phenotypic integration, phenotypic correlation matrices themselves can be dynamic among environments and taxa. Recently, a number of studies have examined how patterns of correlations change across environments (e.g. Waitt & Levin 1993; Nicotra *et al.* 1997; Olson & Antonovics 2000). Just as clones of the same genotype may differ in quantitative trait values between environments (i.e. a trait may be plastic), so may the relationships among traits also change (i.e. trait correlations may be plastic—Schlichting 1989a,b). Yet, there have been few attempts to elucidate the relative importance of shared ecology or shared ancestry on the evolution of phenotypic integration in plants (Antonovics 1976; Murren *et al.* 2002). Among the few studies, a strong signal for either ecology or phylogenetic history in the evolution of patterns of phenotypic integration has been difficult to find (e.g. Cheverud 1989; Callahan & Waller 2000). This lack of signal may be in part due to changes in the expression of and interactions between genetic components, develop-

mental patterns, or selection pressures across environments and among clades—all of these can potentially influence the observed pattern of phenotypic integration.

The complex interactions among the genetics, development and allocation processes that result in phenotypically integrated traits can be considered as a single process, the genotype to phenotype mapping function (G→P map). The pattern of how the genetic information is translated through development, in conjunction with physiology, into a whole organism, defines the G→P map. Here, it is argued that in order to advance our understanding of the evolution of phenotypic integration we must take a combined approach, which simultaneously examines the developmental, physiological and genetic mechanisms behind the G→P map, and how selection acts on the complex functional phenotype. Current research regarding the understanding of patterns of correlation, the mechanisms behind these patterns and how patterns of integration change across environments and across clades will be assessed.

Functional hypotheses

The study of phenotypic integration has largely focused on ascertaining whether there are strong correlations among traits considered to be parts of a single functional unit (module) and identifying whether correlations exist between modules. For example shoot and root systems could be considered two different modules, therefore research to understand their functional integration would examine correlations within shoots, within roots and the independence of shoots and roots. The present review will begin by summarizing common statistical approaches taken to address the question of phenotypic integration. Then, the limited number of functional hypotheses that have been tested to date will be examined, and how these serve as both a biological and statistical springboard from which we can develop new functional hypotheses based on our current understanding of physiological ecology, development and genetics will be described.

Statistical approaches

Addressing phenotypic integration from a quantitative perspective began by close examination of phenotypic correlation matrices (Berg 1960; Grant 1979) and as graphical representations of these Pearson correlation coefficients (e.g. Clausen & Hiesey 1958). The figures are useful in visualizing patterns of correlations within and between modules. Typically, the figures are star shaped with the points representing the measured characters and lines between pairs of characters indicating correlations exceeding a certain threshold below a specified *P*-value, or following a sequential Bonferroni correction of the *P*-

value: a matrix-wide correction for the number of multiple tests (Rice 1989). Functionally integrated complex characters were considered present if there were a number of strong (high magnitude) correlations within a priori described groups of functionally related traits, and a lack of correlations with other functional units (Berg 1960). These diagrams remain useful for exploratory data analyses and visualization of patterns of integration (e.g. Schlichting 1989a, b; Murren *et al.* 2002).

Beyond this graphical representation of correlation matrices, a number of methods have been implemented specifically to test hypotheses concerning the membership of traits within a hypothesized phenotypic module or to test differences among environments or taxa. As significance of correlation coefficients is largely dependent on sample size, and subsets of the same matrix are not independent, re-sampling techniques to derive boundaries around the correlation coefficient have been advocated (e.g. Conner & Sterling 1995, 1996). A bootstrap re-sampling procedure randomly selects individuals, which are likely independent (Efron 1982), and from these samples correlations are calculated. The *z*-transformed correlation coefficients (to improve normality; see Sokal & Rohlf 1995 for additional details and limitations) can be used in standard parametric statistics, such as analysis of variance. Conner and Sterling (1995) repeated this bootstrap procedure 200 times, and considered a single correlation as significantly different between two groups if the 200 *t* statistics ± 2 SD did not overlap zero. Re-sampling methods have been widely implemented in investigations of specific relationships among pairs of traits in different populations.

To examine the difference between pairs of entire matrices instead of single correlations, a randomization approach that has been widely used is the Mantel test (e.g. Cheverud *et al.* 1989; Venable & Búrquez 1990; Waitt & Levin 1993; Schlichting & Pigliucci 1995; Murren *et al.* 2002). This approach, advocated by Cheverud (1989; Cheverud *et al.* 1989), tests the similarity between a theoretical matrix (developed from the researcher's hypothesis of integration) and an empiric matrix. Significance of the correlation between the theoretical and empiric matrices can be assessed by using several distributions or by using Monte Carlo simulation. Sample sizes should be considered with care as small samples have relatively lower repeatability (Cheverud 1989; Sokal & Rohlf 1995). The Mantel test is a dynamic tool as many hypotheses of integration can be considered and can offer an understanding of how whole matrices change between species or environments. A combined examination of changes in specific single correlations and matrix-wide changes across environments or species has led to greater understanding of the patterns of phenotypic integration (e.g. Schlichting & Pigliucci 1995; Callahan & Waller 2000).

Multiple alternative hypotheses can also be considered in cases where specific relationships between independent variables (e.g. vegetative traits) and a dependent variable (fitness related traits) can be defined in a regression framework. Using regression, it is possible to estimate selection on an individual trait while simultaneously accounting for multiple traits that may influence fitness (Lande & Arnold 1983; Endler 1986). Path analysis, a specific model of regression, tests explicit hypotheses of development or functional relationships (Ariyo *et al.* 1987; Callahan & Waller 2000). Lechowicz and Blais (1988) used regression to estimate selection and combined this with an analysis of the correlation structure of traits with respect to reproductive output in several environments. They argued that the patterns of phenotypic correlations between emergence time, leaf and stem biomass and reproductive success illustrated the adaptive significance of these traits in the cocklebur (*Xanthium strumarium*).

Numerous other statistical approaches have been implemented to test if suites of characters can evolve independently, including principal component analyses (Armbruster *et al.* 1999), common principal components (Camara *et al.* 2000; but see Houle *et al.* 2002), discriminate function analysis (Schlichting & Pigliucci 1995), set correlation analysis (Murren *et al.* 2002); other methods continue to be developed. In particular, there is promise in recently implemented graphical modeling methods. For example, Magwene (2001) discussed using these methods to specifically address questions of phenotypic integration and modularity. This approach is advantageous as it allows the exploration of overall patterns of integration as well as tests of specific hypotheses of trait relationships. The plurality of available methodologies suggests that a consensus has not been reached on which techniques are most appropriate for testing functional hypotheses. Here, it is suggested that the use of multiple complementary tests will offer much insight, and aid us in gaining a greater understanding of both the patterns of phenotypic integration and the appropriateness of particular statistical methods.

Functional hypotheses

The classic study of phenotypic integration in plants examined patterns of trait correlations of two functional modules, floral traits and vegetative traits (Berg 1960). Berg's widely cited study was the first to clearly present a functional hypothesis for the existence of relationships among many traits. She examined specialist and generalist-pollinated plants and hypothesized that selection for trait correlations within flowers would be stronger for specialist-pollinated plants than for generalist-pollinated plants. She suggested that this would insure precise place-

ment of pollen on the pollinator's body in the specialist-pollinated plants. Berg also examined whether there are distinct floral and vegetative modules (i.e. whether the magnitude of the correlation between the two types of traits is low). She hypothesized that vegetative trait correlations might exhibit wider phenotypic variance depending on the environment (an early formulation of the concept of plasticity of trait correlations). But because of strong pollinator-mediated selection, floral traits would be more canalized and less susceptible to change in size based on environmental conditions. Berg (1960) surmised that selection would operate to maintain distinct floral and vegetative 'correlation pleiades' (trait clusters or modules: correlations within trait groups but not across trait groups) for specialist pollinated taxa. Generalist pollinated taxa, in contrast might show weaker correlations among floral traits, as the pollinators would have a variety of forms and thus exert selection pressures from multiple directions, and Berg suggested that the hypothesis for decoupling of floral and vegetative characters would not necessarily hold.

Berg (1960) addressed these hypotheses by examining the Pearson correlations of a number of vegetative and reproductive characters in each of 19 herbaceous plant taxa. The circumscription of correlation pleiades was based on the strength (magnitude) of the correlations within the a priori described groups of functionally related traits. The data supported the idea of two correlation pleiades (one vegetative and one floral) for specialist pollinated plants. However, the vegetative and the floral traits of the generalist pollinated plants showed no evidence for separation into distinct groups. Her findings suggested that the floral characters (and correlations among them) of specialist-pollinated species were canalized.

The functional hypotheses laid down by Berg, and which of her data generally support these hypotheses, have formed the foundation for further investigations of phenotypic integration of plant reproductive and vegetative modules. Conner & Sterling (1995, 1996) revisited Berg's hypotheses using updated statistical techniques. They focused on plants with specialized insect pollinators and again tested the hypothesis that there would be limited correlation between vegetative and floral characters. Six floral traits and two to six vegetative traits were measured in each of five species of temperate herbaceous plants (four in the Brassicaceae; one in the Polemoniaceae), only one of the species was shared with Berg's study. In all cases they found that the mean correlation within functional units (floral or vegetative traits) was much stronger (i.e. the Pearson correlation coefficient was significantly larger) than the mean across functional units. They offered several interpretations for this pattern: (1) relationships could be, in part, due to shared ancestry of the taxa chosen, as four taxa were from the same plant family; (2) relationships could have derived from changes coincident with changes in overall plant size; and (3) relationships possibly were a result of unknown developmental or functional relationships. Across all these specialist-pollinated taxa, flower size and vegetative traits may have evolved independently, as vegetative and floral suites of traits appeared dissociable.

To examine if the patterns found in temperate species were paralleled in tropical species, Armbruster *et al.* (1999) investigated patterns of phenotypic correlations of floral and vegetative traits of several distantly related taxa in Costa Rica. In addition to the examination of the functional hypotheses, the authors proposed the use of the coefficient of variation to explore whether the variation within traits differs between taxa visited by specialist and generalist pollinators. Broadly, the coefficients of variation for floral traits were less than that of vegetative traits and specialists had lower variation than generalist species. Generally, their conclusions were in agreement with Berg's hypotheses: significant correlations within and reduced correlations between modules for specialist-pollinated species. Yet, they noted that correlations within modules were remarkably weak, overall patterns were complicated and patterns of integration appeared more species-specific than in Berg's temperate species.

Quantitative genetics theory suggests that selection should produce a positive genetic correlation among functionally related traits (Hartl & Clark 1997). Negative correlations, in contrast, constrain evolution and maintain genetic variation for fitness (Burd 1999; Olson & Antonovics 2000). Within a flower, there may be trade-offs (negative correlations) for male and female characters, or alternatively, there may be strong positive correlations among traits within the shared developmental unit (the entire flower). Positive correlations within either the male or female module were the rule for *Campanula rapunculoides* (Vogler *et al.* 1999; see also Schlichting & Devlin 1989). However, only two correlations were significant across all three light environments: a trade-off between male traits and ovule number and a positive correlation between corolla length and pollen number per flower. Negative phenotypic and genetic correlations existed for fruit and stamen production in the subdioecious herb *Astilbe biternata* (Olson & Antonovics 2000). Burd (1999) examined possible trade-offs between flower number and ovule or pollen production: in four of 10 species there were significant indications of trade-offs between flower number and other floral characters. Examinations of the mechanisms behind both positive and negative correlations, and within and across environments (e.g. Tucic & Avramov 1996) may lead to a better understanding of floral evolution.

The majority of rigorous studies of phenotypic integration in plants have focused on the differentiation between

floral and vegetative modules. In part, differing patterns of integration among taxa may be due to the investigator's classification of a character and interpretation of which vegetative or floral characters are important to the hypothesis at hand (Wagner 2001). For example, Berg (1960) included inflorescence size and floret number as vegetative traits, although these traits could arguably be considered floral/reproductive traits. Developing meaningful hypotheses of functional relationships among traits may be one of the greatest challenges for the study of phenotypic integration in plants. Informed by the physiological ecology of the study species, Nicotra *et al.* (1997) developed new functional hypotheses for vegetative traits associated with photosynthesis. Similarly, independent research on development and physiology can inform testable hypotheses for the evolution of plant modules and phenotypic integration (as currently being employed in several labs, Pigliucci, pers. comm.; Ackerly pers. comm.). The interactions among fields will indeed further our understanding of multivariate phenotypic correlations. Developing novel functional hypotheses from independent data sets (Wagner 2001) may offer alternative biological explanations when established hypotheses (e.g. Berg's correlation pleiades) are not supported.

The role of development

Central to the concept of morphological integration popularized by Olson & Miller (1958) are the combined roles of trait development and function. Phenotypic integration may be influenced by selection on both accurate function and on a synchronized developmental progression. Conceptualization of how trait correlations change through development has been investigated largely by animal biologists (e.g. Cheverud 1988; 1995; 1996; Zelditch 1988; Fink & Zelditch 1996; Smits *et al.* 1996). Cheverud (1996) identified developmental integration as a special case of phenotypic integration: 'morphological elements that interact during formation directed by a common external source' (e.g. hormones). In plants, we lack a precise definition of a developmental module. However, there are possible means to tackle this question. Plant meristems offer an excellent system to follow the differentiation of specific cell lines into particular organs and to study cell to cell interactions during development. In addition, an examination of the differentiation and timing of organ development in conjunction with the regulatory function of hormones may further establish the role of development in our understanding of the evolution of phenotypic integration (Purugganan 1998). Although there are a limited number of studies that explicitly examine the role of development in the evolution of phenotypic integration in plants, there are a few informative examples.

Runions & Geber (2000) formally tested ideas of developmental integration for flowers of two subspecies of *Clarkia*, by examining developmental changes associated with the evolution of selfing. They hypothesized that similar patterns of trait covariance within and between species would suggest that developmental integration has limited the independent evolution of floral traits in these taxa. Alternatively, different patterns of trait correlations between the two species would be evidence that selection acted on developmental processes to produce two types of integrated flowers. For all traits examined, the subspecies differed substantially in both morphology and development, evident in the independent clustering of outcrossing and selfing populations in principal component space. The sign of the relationship between developmental timing and developmental rate were opposite for the two subspecies, indicating that these two processes could be uncoupled. The authors concluded that the observed difference of developmental duration and rate of these two subspecies contributed, in part, to the evolutionary flexibility of development of floral parts, which may have allowed, or been due to, differential selection on developmental traits. These results underscore the importance of examining different components of developmental timing to understand how phenotypic modules are constructed, how integration within modules is maintained and how modules may change through evolutionary time.

Genetic and hormonal regulation play central roles in the unfolding of the genetic information into an organism's phenotype, the G → P map. At this time, data collection on endogenous hormone levels is difficult to obtain for large numbers of individuals in a short period of time. Nonetheless, regulation of phenotypes by hormones may be one of the most exciting frontiers in the understanding of integration in plants. Interconnection among spatially isolated meristems is regulated by cytokinins, auxins and gibberellins as well as other hormones (Steeves & Sussex 1989) and, in turn, these can regulate integration among plant organs. Hormone type and quantity as well as the interactions between hormones and the external environment may dramatically influence patterns of integration, as demonstrated by Amzallag (1999) in *Sorghum bicolor*. Independent addition of gibberellins (GA) and cytokinins (CK) resulted in increased phenotypic integration among the few measured traits (shoot weight, adventitious root weight, seminal root weight and total root weight). Intermediate levels of cytokinins (10 µm) had the highest amount of integration (mean z {transformed correlation coefficient} = 1.56). However, there was a linear increase in integration with increased amounts of GA and also with joint application of GA and CK. When plants were grown in a salt medium, there were dramatic changes in character correlations as well as patterns of integration. In this new

environment, increased CK resulted in higher-level integration (mean $z = 1.48$ at 100 µm), whereas integration was much reduced for all GA treatments. Interestingly, joint application of GA and CK at both 1 µm and 100 µm had higher levels of integration ($z = 1.47$, 1.65, respectively) than the intermediate level (10 µm) of both hormones or GA alone at the highest amount.

Results from the sorghum studies suggested four possible impacts of changes in hormonal levels: (1) changes in physiology, (2) direct influence on the shoot meristem and indirect influence on root meristem, (3) or vice versa, and (4) changes in the sensitivity of the meristem to the hormonal environment. All of these may have interacted to change the relationships among phenotypic traits. This study highlights both the importance of the regulatory role of hormones, which affect levels of integration, as well as the interaction between internal (hormone levels) and external environmental conditions on the pattern of phenotypic integration and the evolution of the genotype to phenotype map. Although this study focused on a limited number of traits, it offers a promising research direction.

Plasticity of integration: The environment

As our understanding of phenotypic plasticity has increased (Schlichting 1986; Scheiner 1993; Sultan 1995; Via *et al*. 1995), a natural extension has been to examine whether trait correlations change across environments (Schlichting 1989a, b). Plants which lack any response to environmental change across traits and have a canalized multivariate phenotype may be restricted to a narrow environmental range (Schlichting 1989a). Because of this, the plasticity of correlations can be adaptive. As the responses of individual traits are not always the same to different environments, patterns of phenotypic integration can vary dramatically among environments (Schlichting 1986; Waitt & Levin 1993). At the same time, phenotypically plastic responses to environmental heterogeneity must be coordinated in order to produce a properly functioning organism. A number of different approaches used to study the plasticity of correlations will also be examined.

Using three closely related species of *Phlox* grown in six different greenhouse treatments (see Table 1), Schlichting (1989b) showed decisively that plasticity of univariate traits often results in plasticity of correlations among traits. Patterns of correlations differed among the three species and among environments, which translated into significant heterogeneity among treatments in the relationships between the traits and flower production. For *Phlox drummondii* the number of changes in significance in correlation structure was four times that expected by chance. Examination of variation among *Phlox drummon-*

dii populations in their correlation matrices showed that strong correlations within functional groups were consistent across environments, suggesting that integration was consistent for some populations despite plasticity of individual traits (Waitt & Levin 1993). These results emphasized that as correlation patterns changed across environments, the multivariate phenotypes that selection could act upon also varied, and such changes could significantly alter the potential evolutionary directions of species across environments.

Chapin (1991) suggested that a generalized response syndrome to 'stressful' environments might exist, which would elicit a common phenotypic response among species. Schlichting & Pigliucci (1995) tested Chapin's hypothesis against the alternative that plastic responses are specific to the particular form of stress. In *Phlox drummondii*, they found that phenetic relationships among populations are environment specific. The maintenance of fitness across environments suggests that the plants deal effectively with environmental change via allometric plastic responses to the environment. They rejected the generalized stress response hypothesis and suggested that differential changes across environments are likely to confer appropriate phenotypic changes among functionally related traits, enabling the maintenance of fitness across environments.

Under specific sets of environmental conditions, groups of traits that perform a specific function may be canalized for the task. However, we know little about the prevalence of plasticity of correlations within different kinds of functional modules. Nicotra *et al*. (1997) examined patterns of phenotypic integration for biomass and growth traits versus leaf level photosynthetic traits of two species of *Piper*. Using principal components analysis, they sought to test whether a pioneer (*Piper sancti-felicis*) and shade tolerant species (*Piper arieianum*) differed in their vegetative responses to high and low light environments. They found that the first principal component (PC1, with root to shoot ratio and photosynthetic maximum having the highest eigenvectors) clearly separated the light treatments, whereas PC2 (with conductance and chlorophyll per unit area having the highest loadings) clearly differentiated between species. Similar patterns of differentiation of environmental treatment and species were found for two *Lobelia* species grown under high and low nutrients (Pigliucci *et al*. 1997; Schlichting & Pigliucci 1998). Despite differences in the habitats of the two *Piper* species, correlations within environments were similar and correlations for both species supported the hypothesis of high intra-module integration and generally low correlations between modules (multiple distinct correlation pleiades). These data supported the idea that integration could allow flexibility yet coordination to changing environments. Although the two species of *Piper* differed in pattern of

Table 1 A cross study comparison of integration when the entire phenotypic correlation matrices are available.

Taxa	Ecology	N	Integration statistic/correlation coefficient/> 0.7				Integration statistic/correlation coefficient/> 0.5			
			Inter-module	Intra-module 1	Intra-module 2	Total matrix	Inter-module	Intra-module 1	Intra-module 2	Total matrix
Calathea crotalifera[1]	Specialist	15	0.07	1	0.14	0.29	0.36	1	0.24	0.45
Canna glauca[1]	Specialist	20	0	0.1	0	0.2	0	0.6	0.13	0.15
Echinodorus sp.[1]	Generalist	16	0	0.5	0	0.16	0.1	1	0.33	0.44
Eichhornia crassipes.[1]	Specialist	9	0	0.2	0	0.06	0.5	0.5	0.16	0.19
Cyperus sp.[1]	Wind	11	0	0.33	0	0.04	0	0.33	0.3	0.14
Poaceae[1]	Wind	10	0	0.2	0	0.10	0.1	0.4	0	0.24
Croton sp.[1]	Generalist	15	0.20	0.7	0.27	0.31	0.5	0.9	0.4	0.54
Muntingia calabura[1]	Generalist	10	0.03	0.1	0.13	0.07	0.2	0.2	0.27	0.22
Wedelia sp.[1]	Generalist	10	0.03	0.6	0.43	0.24	0.11	0.7	0.67	0.38
Trillium erectum[5]	Diptera pollinated	90	0.53	0.8	0.5	0.55	0.8	1	0.79	0.82
Trillium grandiflorum[5]	Hymenoptera pollinated	100	0.53	0.8	0.57	0.58	0.88	1	0.79	0.86
Piper arieianum[2]	High light	24	0	0.27	0.14	0.09	0.07	0.47	0.19	0.18
Piper arieianum[2]	Low light	24	0	0.13	0.19	0.08	0.12	0.2	0.29	0.18
Piper sancti-felicis[2]	High light	18	0	0.33	0.23	0.13	0.02	0.6	0.33	0.22
Piper sancti-felicis[2]	Low light	18	0.02	0.07	0.24	0.09	0.12	0.27	0.38	0.22
Campanula rapunculoides[6]	Hot environment	34		0		0.03		0.1		0.14
Campanula rapunculoides[6]	Cool environment	27		0		0.03		0.2		0.25
Campanula rapunculoides[6]	Potbound	30		0		0		0.1		0.14
Phlox drummondii[3] (Elgin)	Low fertilizer	120	0.04	0	0	0.02	0.16	0.4	0.17	0.24
Phlox drummondii[3] (Elgin)	Medium fertilizer	120	0.13	0.07	0	0.09	0.17	0.27	0	0.18
Phlox drummondii[3] (Elgin)	High fertilizer	120	0.13	0.07	0	0.09	0.17	0.4	0.17	0.24
Phlox drummondii[3] (Lexington)	Low fertilizer	120	0.13	0.33	0	0.18	0.17	0.4	0	0.22
Phlox drummondii[3] (Lexington)	Medium fertilizer	120	0.08	0	0	0.04	0.17	0.47	0.17	0.27
Phlox drummondii[3] (Lexington)	High fertilizer	120	0	0	0	0	0.13	0.4	0	0.2
Phlox drummondii[4]	Control	20	0	0.07	0	0.02	0.08	0.33	0.13	0.15
Phlox drummondii[4]	Low water	20	0.19	0.13	0.13	0.17	0.31	0.40	0.33	0.36
Phlox drummondii[4]	Low nutrients	20	0	0.07	0.07	0.03	0.03	0.33	0.20	0.14
Phlox drummondii[4]	Leaf removal	20	0.03	0	0	0.02	0.06	0.20	0.27	0.14
Phlox drummondii[4]	Meristem clip	20	0.04	0.17	0.20	0.11	0.17	0.17	0.33	0.22
Phlox drummondii[4]	Small pots	20	0.03	0.07	0.07	0.05	0.08	0.20	0.13	0.12
Phlox cuspidata[4]	Control	20	0.06	0.20	0.07	0.09	0.19	0.40	0.27	0.26
Phlox cuspidata[4]	Low water	20	0.08	0.13	0	0.08	0.19	0.33	0.27	0.24
Phlox cuspidata[4]	Low nutrients	20	0.03	0	0.13	0.05	0.31	0.40	0.40	0.35
Phlox cuspidata[4]	Leaf removal	20	0.08	0.07	0.07	0.08	0.22	0.40	0.13	0.24
Phlox cuspidata[4]	Meristem clip	20	0.04	0.17	0	0.04	0.17	0.33	0.33	0.24
Phlox cuspidata[4]	Small pots	20	0.13	0.13	0.13	0.09	0.11	0.33	0.27	0.20
Phlox roemeriana[4]	Control	20	0.06	0.07	0.07	0.06	0.28	0.60	0.13	0.32
Phlox roemeriana[4]	Low water	20	0.06	0.07	0.07	0.06	0.19	0.27	0.13	0.20
Phlox roemeriana[4]	Low nutrients	20	0.06	0	0.07	0.05	0.22	0.20	0.33	0.24
Phlox roemeriana[4]	Leaf removal	20	0.11	0.07	0.07	0.09	0.19	0.27	0.33	0.24
Phlox roemeriana[4]	Meristem clip	20	0.04	0	0.13	0.07	0.21	0.50	0.27	0.27
Phlox roemeriana[4]	Small pots	20	0	0.07	0.07	0.03	0.22	0.27	0.20	0.23

[1]Armbruster *et al.* (1999); [2]Nicotra *et al.* (1997); [3]Waitt & Levin; [4]Schlichting (1989b); [5]Irwin (2000) [6]Vogler *et al.* (2000). The metric of integration is presented for each species (and population), ecologic characteristic or treatment contrasted in the study. A metric of phenotypic integration is calculated by dividing the number of correlations meeting or exceeding a magnitude threshold for the correlation coefficient (0.5 or 0.7) or divided by the total number of correlations. Intra-modular compares only the correlations within a module. For example Module 1 represents vegetative traits and module 2 represents floral traits for the first 11 species in the table. In both *Piper* species, module 1 represents photosynthesis related traits and module 2 represents growth related traits. I defined vegetative and floral modules for *Phlox*[4], *Campanula*, and *Trillium*. Inter-module is defined as correlations between traits belonging to the first and second module. Total matrix is the integration metric calculated for the entire phenotypic correlation matrix

plasticity and characters which exhibit plasticity, they were similar in number of significant correlations among modules and shared the majority of significant correlations in the high light treatment, suggesting that evolutionary patterns of integration in plants may be due to shared ancestry rather than distinctions in ecology.

Over the last decade, the numbers of studies that have reported correlation matrices allow us to look for common patterns of integration within and between modules across taxa and between environments. If we examine a simple metric of integration [(number of correlations exceeding a particular threshold, e.g. 0.5 or 0.7)/total number of measured correlations] we can assess the pervasiveness of intramodule integration versus intermodule dissociation across several plant taxa (Table 1). Overall, levels of matrix-wide integration range from 0 to 100% depending on species, environment and significance level considered (average integration ranging from 0.11 to 0.27 depending on which significance cutoff was used). Using a cutoff of a correlation coefficient of 0.7, results in lower integration values than a cutoff of 0.5.

The proportion of integration can be higher when evaluated within modules (average across studies 0.11–0.42) than across modules (average 0.08–0.21), and floral modules appear more integrated than vegetative modules (range of average floral 0.19–0.42 *vs* average vegetative 0.11–0.26). Several studies found no evidence for significant integration within the hypothesized module. This comparison among studies demonstrates the importance of considering appropriate significance levels supporting the presence of modules, as well as the pervasiveness of integration among clearly defined functional or developmental modules.

Using another metric allows us to examine how patterns of integration change across environments: (number of correlations that differ in magnitude greater than or equal to 0.5 across environments/total number of calculated correlations; Nicotra *et al.* 1997). In *Piper arieianum* 18% of the correlations changed by greater than 0.50 across two light environments and 21% of correlations changed in *Piper sancti-felicis* (Nicotra *et al.* 1997). This proportion of correlations approximates the level of integration within a single environment (Table 1) and these species' abilities to change correlation structure may reflect the chance of these species of encountering heterogeneous light environments. For traits related to heterophylly in the plant genus *Proserpinaca*, integration of genotypes in both flooded and draw-down conditions were similar: Integration within environments was 28–31% (Wells 2001), but no correlations across environments met the criteria for the Nicotra *et al.* (1997) metric. These results suggest that selection may favor constancy of integration in taxa native to highly heterogeneous environments, as is the case in temperate ponds.

Phylogenetic patterns of phenotypic integration

Recently, studies have begun to ask the question of how patterns of integration evolve across a clade (Cheverud 1989; Steppan 1997a; b; Ackerly 1999). Murren *et al.* (2002) formally tested the relative importance of shared ecology and shared ancestry among six species of *Brassica* and *Raphanus sativus*. We found that patterns of correlation were independent of both ecology and ancestry; instead hybrid taxa were shown to be most highly integrated. We concluded that in this clade a finer scale quantitative examination of ecologic characteristics might be warranted, as there were only subtle differences among species in ecologic classifications used in our analyses. Although patterns of correlation structure among populations may be indicative of time since divergence or differential selection among populations, multitrait correlation matrices rarely reflect phylogenetic information (Cheverud 1996; Callahan & Waller 2000; Murren *et al.* 2002). The lack of congruence between ecology and phylogeny may be due to our treatment methodologies or, because in many studies populations from only a single ecologic gradient are collected, which may not reflect the complexity of the selective environments across sites or across geographic areas.

New phylogenetic techniques should contribute to our understanding of how phenotypic correlations reflect patterns of correlated evolutionary change, a question that piqued the interest of Clausen & Hiesey (1958) a half-century ago. Ackerly & Donoghue (1998) are at the forefront of the current research involved in examining the evolution of phenotypic correlations across a phylogeny using a modified version of phylogenetic independent contrasts (Harvey & Pagel 1991; Ackerly 2000). Using a molecular phylogeny of North American and Japanese members of the Aceraceae, they examined the evolution of Corner's rules, that is that twig thickness, leaf size, and inflorescence size are positively intercorrelated and each is inversely related to density of crown branching (Corner 1949). Their analysis differentiated two functional units, traits associated with Corner's rules (first principal component) and traits associated with sapling allometry (second principal component). Their results suggested high levels of convergence among traits across this phylogeny, yet they found no support for trait conservation (Ackerly & Donoghue 1998). This pioneering work lays a foundation for expansion of these tests to other plant clades, environments and modules.

Evolution of the study of phenotypic integration: Bridging the gap from G → P new frontiers

The present paper has broadly defined modules as phenotypic units of functionally related traits within which we expect tight correlations. Yet, these modules could

alternatively be viewed as dynamic entities, with fluid patterns of integration. Definitions of modularity vary among authors from interacting organs during development (Raff 1996), to partially independent components of the genome that guide cellular organization (Kirschner & Gerhart 1998), to pleiotropic or epistatic interactions among components of a morphological functional unit (Wagner & Altenberg 1996). These definitions share elements and highlight the importance of the interactions among functional, developmental and genetic regulation in the building of a properly functioning phenotype (Schlichting & Murren in press). Mezey *et al.* (2000) tackle the question of modularity of the genotype to phenotype map using quantitative trait loci (QTL) analysis to identify portions of the genome that are associated with quantitative phenotypic characters. They define modularity statistically as interrelationships among genetic components (QTL) through pleiotropy and contrast it to parcelation (a reduction in observed pleiotropy in comparison to other distinct functional groups). The approach of Mezey *et al.* (2000) found support for the modularity of one of the two hypothesized functional units in the mouse jaw. Similar combinations of molecular and statistical analyses could be employed in plant systems to test the genetic basis of morphological modules (see also Murren & Kover, in press).

Building functional hypotheses based on natural history and physiological relationships will both broaden our concept of the plant module and offer new insights into the relative importance of mechanisms behind the maintenance of intramodule correlations. Future studies may build upon the foundation of work on floral and vegetative modules (Herrera *et al.* 2002), in particular to compare across broader types of pollination systems (wind, water, bird *vs* insect). These data could be used to identify the impacts of differential selection pressures on multivariate correlations. The plasticity of trait correlations across environments reminds us that the nature of trait relationships is dynamic and that selection may favor different relationships among traits in different environments. Our understanding of the mechanisms and patterns of integration will continue to grow as alternative statistical approaches are developed and evaluated.

Although our understanding of the genotype to phenotype map, which underlies observed patterns of phenotypic integration, is currently limited, new techniques show promise for shedding light on this issue. Quantitative trait loci analyses can be used as a first step towards identifying genetic components involved in polygenic phenotypic traits and the pleiotropic and epistatic relationships among these genes (e.g. Juenger *et al.* 2000; Viera *et al.* 2000). Microarray techniques are used to examine timing and expression of quantitative traits (Kehoe *et al.* 1999). Both of these methods have limitations, but also

have already suggested the importance of multiple structural and regulatory elements responsible for building traits and functional units. When we combine analyses of development, genetics, physiology and the resultant phenotypic correlation matrix, these examinations will lead to clarifications of the evolution of functional relationships among morphological traits and shed light on the complex G → P map.

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