Modularity and intra-floral integration in metameric organisms: plants are more than the sum of their parts

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Abstract

Within-individual variation in virtually every conceivable morphological and functional feature of reiterated structures is a pervasive feature of plant phenotypes. In particular, architectural effects, regular, repeatable patterns of intra-individual variation in form and function that are associated with position are nearly ubiquitous. Yet, flowers also are predicted to be highly integrated. For animal-pollinated plants, the coordination of multiple organs within each flower is required to achieve the complex functions of pollinator attraction and orientation, pollen donation and pollen receipt. To the extent that pollinators may select for multiple independent functions, phenotypic integration within flowers may also be modular. That is, subsets of floral structures may be integrated but vary independently of other subsets of structures that are themselves integrated. How can phenotypic integration and modularity be understood within the context of architectural effects? This essay reviews recent research on patterns of floral integration and modularity and explores the potential for spatial and temporal changes in the selective environment of individual flowers to result in positional variation in patterns of morphological integration.

1. Intra-individual variation among metamers is common

Most land plants develop indeterminately. Although the basic body plan is established during embryogenesis, plant form unfolds throughout ontogeny via the activity of apical meristems and continual organogenesis. Shoot apical meristems constantly generate new and fundamentally similar units, commonly referred to as metamers. As a result of the indeterminate nature of plant ontogenies, plant phenotypes are both spatially and temporally dynamic. This distinctive feature of development presents a significant challenge for understanding how phenotypes evolve, what aspects of the phenotype might be integrated, and the role of natural selection in shaping patterns and magnitudes of integration. What exactly does it mean for an indeterminately growing organism to be phenotypically integrated? In this essay, I will discuss recent research on patterns of intra-individual variation in integration and modularity and the implications of this work for understanding phenotypic evolution.

Because of the repetitive nature of shoot development, plant biologists have tended to focus their phenotypic analyses on individual metamers, or parts of metamers—the shapes of leaves, the sizes of petals, etc.—and assume that these units are inherently similar. Yet, within-individual variation in virtually every conceivable morphological and functional feature of metamers is a pervasive feature of plant phenotypes (comprehensively reviewed in [1]). While some of this variation may reflect random deviations in developmental outcome or fluctuations in the immediate environment, much intra-individual variation occurs in regular, predictable, species-specific patterns. For example, successive leaves along the length of a shoot commonly differ in such features as size, shape, thickness, tissue types, trichome density and more (multiple examples in [2,3]). Such regular patterns of intra-individual variation among vegetative metamers are well-known features of ontogeny, and this phenomenon is
Table 1. Patterns of intra-inflorescence variation in reproductive structures attributable to position alone. Each study tabulated below incorporated explicit controls for architectural variation as distinct from resource re-allocation or other aspects of phenotypic plasticity. The table summarizes information from 94 taxa in 81 species and 43 families (see the electronic supplementary material for data and individual citations). The individual studies examined different sets of characters in different ways. In order to summarize the patterns of variation, the characters were categorized as follows: ‘corolla size’ includes any measurement of corolla or petal length, width or mass; ‘gynoecium size’ includes length or mass of the ovary or gynoecium as a whole; ‘ovule no.’ is ovule number; ‘androecium size’ includes stamen number, length or mass; ‘pollen no.’ includes pollen number or mass; ‘fruit size’ includes fruit length, mass, seed number per fruit or total seed mass; ‘maturation’ includes fruit or seed maturation. The numerical entries are the number of studies that examined positional variation and the number of studies in which positional variation was detected.

<table>
<thead>
<tr>
<th>character</th>
<th>number of studies</th>
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<tr>
<td>corolla size</td>
<td>21</td>
<td>19</td>
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<tr>
<td>gynoecium size and/or ovule no.</td>
<td>56</td>
<td>51</td>
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<tr>
<td>androecium size and/or pollen no.</td>
<td>29</td>
<td>22</td>
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<td>fruit size</td>
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<td>fruit maturation</td>
<td>46</td>
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- Such experiments require comparison of flowers and fruits at contrasting locations within the architecture of the inflorescence while holding other sources of variation constant, and comparing flowers and fruits at the same location on plants under different conditions. The most common manipulation is pollination and fruit set. See [8,10] and the electronic supplementary material, figure S1 for a complete explanation.

Table 2. Summary of studies examining morphological integration of floral and fruit characters. The table was compiled from the data and citations provided in table 1.

2. Intra-floral integration and modularity

Morphological integration refers to the coordinated variation of functionally, historically and/or developmentally related features of organisms, while modularity refers to the decoupling of such features into partially dissociated components that are themselves integrated [25–28]. Although typically assessed through analyses of patterns of covariation among mature traits, integration is understood to reflect co-regulation of the developmental processes that generate those traits ([29], ch. 7; [28,30]). Modularity and integration...
are fundamental aspects of biological organization and are key determinants of evolvability [31,32]. While integration may enhance functionality, complete integration of component parts, if this reflects underlying genetic structure (strong linkage or pleiotropy), may limit evolutionary change [33]. Modularity, by contrast, is thought to enhance evolvability by maintaining relationships among developmentally and functionally related characters (within modules) while allowing for independent evolution of other sets of integrated characters (of other modules) that do not share common development or function [26,28,34–36].

Animal-pollinated flowers are predicted to be highly integrated ([37,38]; reviewed in [39]): the coordination of multiple organs within each flower is required to achieve the complex functions of pollinator attraction and orientation, pollen donation and pollen receipt. As a consequence, functional, developmental and genetic correlations among floral traits are likely to evolve in response to pollinator-mediated selection [38,40–44]. Beginning with Berg’s [38] seminal work demonstrating that correlation among characters of flowers with specialized insect pollinators were strong and independent of correlations among vegetative characters, the question of floral integration has been examined in numerous species and from a variety of perspectives (reviewed in [39]). Empirical support for the hypothesis of floral integration has been mixed, and the magnitude of integration differs widely among taxa [39]. In general, however, flowers are more highly integrated than expected based on random trait correlations [45]. One potential explanation for the marked heterogeneity among taxa in levels of integration, and the unexpectedly weak integration observed in the flowers of some taxa, is that flowers are not single fully integrated units. Rather, the multiple functions of the flower are each performed by distinct suites of integrated characters that share a common function but are independent of sets of structures with alternative functions, that is, floral variation/covariation may be modular [46–49]. Independence among functional modules would reduce measures of overall integration for the flower as a whole.

The notion of modularity in relation to function encompasses both intra-floral integration of subsets of structures (as opposed to flower-wide integration), and relative independence among those integrated subsets. Numerous studies provide evidence of the first criterion, intra-floral integration. Because integration is expected to result from selection by pollinators, these analyses typically begin by predicting integration of particular floral structures based on hypotheses of shared function (the ‘a priori’, or researcher-defined approach, sensu [50]) and ask whether correlations among those structures are stronger than correlations among other floral structures. For example, organs (or parts of organs) involved in placement of pollen on pollinators and subsequent receipt of pollen by stigmas are predicted to be highly integrated. The specific traits involved often include some region of the corolla (e.g. corolla tube) that directs or limits pollinator entry, portions of the stamens that affect anther placement, and the part of the gynoecium that determines the height of the stigma. Integration among these characters occurs in a broad range of taxa. Several studies found that phenotypic and, where studied, genetic correlations between corolla tube and stamen lengths far exceed correlations among other floral features [46,51]. Strong correlations among corolla tube, stamen and gynoecium measures have been detected in many other studies [42,52–65]. The prevalence of correlations among the traits involved in pollen deposition and receipt led Rosas-Guerrero et al. [61] to refer to them as ‘efficiency function traits’ because their integration is assumed to lead to more efficient transfer of pollen.

This a priori approach also has been used to examine other aspects of intra-floral integration related to function. For example, among species of *Schizanthus* (Solanaceae), transitions from bee pollination to hawkmoth or hummingbird pollination are associated with changes in the function of the individual corolla lobes [66]. Only those lobes that are involved in providing a landing platform and determining pollen deposition are integrated. Similarly, for *Lavandula* (Lamiaceae) the upper and lower corolla lips, which are involved in pollinator handling, are more highly correlated with each other than they are with the floral tube [67]. In self-pollinating species, for which transfer of pollen by pollinators is likely less important than ensuring deposition of self-pollen, stamen and gynoecium lengths are correlated but these organs are not correlated with corolla tube length [68,69].

Intra-floral integration also has been detected by inspection of data collected without regard to particular hypotheses of function. Strong phenotypic correlations among sets of traits involved in pollinator attraction have been discovered in several taxa via this data-driven approach: *Agilegia formosa* and *A. pubescens* (Ranunculaceae; [70]); *Arabidopsis* (Brassicaceae; [71]); *Iris gracilipes* (Iridaceae; [58]); *Prunus mahaleb* (Rosaceae; [39]), *Silene virginica* (Caryophyllaceae; [44]); *Mimulus luteus* (Phrymaceae; [72]); *Brassica rapa* (Brassicaceae; [73]); *Primula* (Primulaceae; [64]) and *Iris germanica* (Iridaceae; [74]).

While these analyses of covariation among sets of traits that share a common function provide compelling evidence for intra-floral integration, they do not necessarily provide evidence of modularity. Studies of intra-floral integration typically show that correlation among the focal traits is much greater than their correlation with other traits, or greater than correlation among other traits, but such comparisons do not address the second critically important aspect of modularity, the independence of the set of focal traits from other sets of traits that are themselves integrated. It is the capacity for independent variation among sets of integrated traits that is thought to facilitate morphological and functional evolution [34].

A small number of studies explicitly examine independence among sets of integrated floral traits, usually by means of principal components analysis (PCA). For two closely related species of *Nicotiana* (Solanaceae), PCA and structural equation modelling of multiple floral measurements identified two distinct suites of floral characters that are phenotypically and genetically highly correlated with each other but relatively independent of the other set of characters [48,62]. One group of integrated traits included the lengths of the floral tube, stamens and gynoecium, the same set predicted a priori as efficiency function traits, as described above. The second set of integrated traits detected in the flowers of *Nicotiana* was associated with the corolla limb (the flared portion of the corolla) and corolla tube aperture. This second set of flower features likely functions in pollinator attraction [39,66,67,72,75]. For *Nicotiana*, the two sets of traits are each putatively related to function and are
also phenotypically and genetically integrated but each set is independent of the other. Hence, the flowers are modular and the two sets of traits could be considered as an efficiency function module and an attraction module. Although not specifically a study of modularity, analyses of flower measurements of several species of *Primula* also found that anther position, stigma position and corolla tube length loaded primarily on one principal component, whereas corolla mouth diameter, corolla limb length and corolla width loaded onto the second [64], suggesting that variation of these two sets of traits is independent. Moreover, the flower traits of *Primula* associated with each of the two principal components are consistent with the efficiency and attraction functions as described for *Nicotiana*. For *P. malaclem*, PCA showed association among attraction traits that was independent of traits related to herkogamy [39] suggesting some modularity of these flowers. Significantly, for each of these taxa, the identity of the traits encompassed in the individual modules is concordant with presumed function.

The results of other studies using PCA have been less clear about modularity. For flowers of *I. pumila*, Tucic et al. [74] identify two modules based on PCA. Yet, all floral measures (dry mass) loaded strongly on PC1 with the exception of stamen mass. While this analysis shows independence of variation in stamen mass, this one measure does not constitute a module [26,27].

Herrera et al. [49] took a different approach to the question of modularity. They examined intra-floral modularity for *Helleborus foetidus* (Ranunculaceae) by comparing the observed correlation structure with alternative patterns predicted by hypotheses of complete integration versus independence of sets of characters. Their analysis supported the correlation within and independence among traits involved in pollinator access versus those of the nectararies. Carvallo & Medel [72] used conditional independence to identify four putative modules in flowers of *M. luteus*. Comparative approaches using an explicit phylogeny also can suggest intra-floral modularity. For 20 species of *Ipomea* (Convolvulaceae), traits associated with efficiency function are correlated, and traits associated with attraction are correlated (albeit less strongly), but independence between these sets of traits was not explicitly analysed. Yet, taking phylogenetic structure into account, comparison between self-incompatible species and self-compatible species showed that integration of efficiency function traits differed between these groups while there was no difference in integration of attractive traits. This analysis suggests that traits associated with pollen transfer evolve independently of those involved in attraction and such independence may reflect an underlying modularity.

Although the number of studies that explicitly examine independence of sets of floral traits is small, it is noteworthy that all have detected independence. Consideration of these few studies in combination with the strong evidence for intra-floral integration of particular sets of floral features that share a common function suggests that intra-floral modularity may be common among animal-pollinated taxa. Nevertheless, while flowers perform multiple functions and pollinators are expected to select on these separate functions, we do not know whether this generally has led to the evolution of multiple, independent, intra-floral modules. The issue remains unresolved for lack of investigations that address both intra-floral integration in relation to function and independence of sets of multiple integrated traits.

Understanding the extent of intra-floral integration and modularity is critical for understanding both morphological evolution and developmental regulation. Integration may preserve functionality in the face of genetic and environmental perturbation while modularity limits the consequences of those perturbations to subsets of the phenotype and allows functionally related sets of traits to evolve independently [26,28,34]. For example, intra-floral modularity may have facilitated rapid morphological evolution in association with pollinator shifts for the two *Nicotiana* species described above. Floral morphology differs dramatically between these close relatives and corresponds to differences in their predominant pollinator [76]. Although flowers of both species have a corolla tube and flared limb, *Nicotiana alata* flowers are white with a long narrow tube and are hawkmoth pollinated, while *N. forstiana* flowers are red with a much shorter corolla tube and are hummingbird pollinated. Integration among all of the traits associated with corolla length combined with their relative independence from the traits associated with the limb may have facilitated coordinated change of corolla tube, stamen and style length in relation to the mouthparts of the pollinator (hawkmoth versus hummingbird) while leaving the limb characters involved in attraction unchanged ([62]; note that the ancestral state is not known in this case). Corolla tube and limb sizes vary tremendously among the 95 species of the genus *Nicotiana* [77], suggesting that independent evolution of these characters may have occurred throughout the clade. Analysis of integration and modularity of multiple species within a phylogenetic context would provide important evidence for evaluating the evolutionary implications of modularity.

A second potential example of independent evolution of floral modules comes from species of the *Mimulus guttatus* (Phrymaceae) complex where self-fertilization appears to have evolved numerous times from outcrossing. Crosses between an outcrossing and selfing species show that corolla size characters are highly genetically correlated and quantitative trait loci (QTL) analysis points to a pleiotropic basis for these associations. Similarly, the characters that determine stigma–anther separation are correlated and, moreover, are associated with QTL that do not control corolla characters [57]. The independent genetic control of these two sets of traits for *M. guttatus* and related species may have allowed the evolution of selfing via changes in stigma anther separation without affecting other floral traits [57], thus allowing selfing without negative effects on outcross pollen donation (pollen discounting; [78–80]).

Studies of modularity also inform developmental genetic analyses. To the extent that integration of morphological characters is due to underlying developmental genetic regulation, studies of modularity can suggest which structures may share regulatory pathways. Similarly, analyses of modularity highlight those features that develop independently. For example, regions of the corollas of *Nicotiana* show three contrasting patterns of variation and covariation [48]. The corolla tube above the point of stamen attachment forms part of the efficiency module and covaries with the stamen and gynoecium while the flared region of the same corolla is included in the attraction module and varies independently of the tube. The region of the tube below the stamen attachment varies independently of the other two regions. Although all three regions are part of the same organ whorl and must share many aspects of development, morphogenesis of
these regions of the corolla must be regulated autonomously to some extent.

3. Architectural effects on integration and modularity?

Given that (i) integration (and perhaps modularity) of sets of reproductive characters is a common feature of flower form and function and (ii) inflorescence architecture is a significant source of intra-individual variation, the question arises: are phenotypic integration and modularity maintained over flower positions along a plant axis? That is, are whole-plant reproductive phenotypes integrated? The coordinated development and genetic co-regulation that underlies patterns of intra-floral character correlation may maintain integration of floral parts within the multiple flowers of individuals despite among-flower variation (architectural effects). Conversely, if individual floral traits vary independently with respect to architecture, then this will disrupt integration and patterns of covariation may differ among the flowers of the same plant.

In *Nicotiana alata* and *N. forgetiana*, the species described in the preceding section as having distinct and independent efficiency and attraction modules, there are significant architectural effects on overall flower size: flower size declines with position within an inflorescence and among successive inflorescences [48]. Despite this size variation, the identity of the particular traits included in the intra-floral modules (efficiency module and attraction module), integration of traits within each module, and independence between the modules, is maintained. The two modules, however, show contrasting patterns of variation with respect to flower position. All of the traits associated with the attraction module (measures of corolla limb and mouth) decline in size with flower position, while the sizes of traits associated with the efficiency module (lengths of corolla tube, stamens and gynoecium) are invariant with flower position (figure 1). Thus, despite an overall decline in flower size with position, the presumed ‘fit’ of the flower to the pollinator is maintained constant over time and space. Perhaps closer inspection of the many other taxa with positional variation in corolla size (table 1) would also show stability in that part of the corolla that affects pollen placement.

Ishii & Harder [81] also found significant intra-inflorescence variation in the size of *Delphinium glaucum* (Ranunculaceae) flowers that was associated with flower position, and examined the effect of this size variation on integration. Individual petal size and correlations among petal traits were invariant with position, whereas sepal size declined and correlation among the sepals was weaker in more distal flowers. Modularity was not examined explicitly; however, the independent responses of these two sets of traits to flower position suggest that integration in these flowers may be modular. Moreover, in *Delphinium*, the small non-showy petals interact physically with pollinators and may comprise an (invariant) efficiency module, while the sepals are large and showy and may constitute an attraction module.
The contrasting patterns of intra-inflorescence variation in integration of traits involved in attraction versus efficiency for both Nicotiana and Delphinium suggest that there is not simply an overall spatial or temporal (e.g. due to aging) decline in the capacity of development to produce a functional flower (no decline in flower-wide canalization or developmental stability). Rather, the contrasting patterns of change in integration between the attractive traits and the efficiency traits may reflect differential intensity and timing of selection by pollinators. Ishii & Harder [81] suggest that for Delphinium, the requirement for direct physical interaction of the petals with pollinators may lead to strong stabilizing selection for integration of petal traits of all flowers regardless of their position within an inflorescence or time of anthesis. By contrast, while the showy sepals function in attraction, they do so in conjunction with all of the open flowers on the plant. As the season progresses, and more flowers are open on the inflorescence, the functional requirements of the sepals are relaxed. Individual plants with decreased sepal size and integration of later produced flowers would not be at a disadvantage in terms of pollinator attraction. The Nicotiana species also showed stability of the efficiency module over position, and decline in sizes (but not of integration) of attractive traits, a pattern consistent with this suggestion. Alternatively, the contrasting patterns of positional variation of the two modules could be related to predictable changes in the internal and external environment under which individual flowers develop. If resources available for flower development decline over a season (and with position), e.g. due to commitment of resources to maturation of earlier initiated fruit or to changes in water or nutrient availability, integration of the floral parts involved in pollen transfer may be more critical for continued reproductive function of distal flowers and may be maintained at the expense of size and integration of attractive traits.

Other patterns of intra-inflorescence variation, although not addressing character integration and modularity, are consistent with stability of ‘efficiency function’ traits and variation in attractive traits with flower position. Corolla lip length of the orchid Dactylorhiza declines with flower position but spur length does not [82]. In flowers of Aquilegia canadensis, nectar spur, gynoecium and stamen lengths are invariant with position while the size of the showy sepals declines [83]. Stabilizing selection on floral morphology has long been expected to reduce among-individual variation; it may well reduce intra-individual variation of particular sets of morphological traits as well.

In contrast to the stability of integration demonstrated for efficiency function traits of Nicotiana and Delphinium, Zhao et al. [84] found that both the strength of correlations and the number of traits that were correlated declined with flower position in Aconitum gymnantrum (Ranunculaceae). Because only one of the traits was a size measure (the others were dry masses), the potential relationship of the traits to functions such as pollen transfer and pollinator attraction cannot be inferred. These results do confirm, however, that the extent of developmental coordination varies among flowers within inflorescences and suggest that conclusions about intra-floral integration will differ depending on what aspects of the phenotype are assessed.

Are phenotypic integration and modularity maintained over flower positions along a plant axis? There are too few studies available to answer this question, yet it is a critical aspect of the evolution of metameric organisms. Flowers are clearly not more-or-less identical repeated units. Flowers and floral structures show complex and non-random patterns of spatial and temporal intra-individual variation. While we can measure multiple flowers per individual, along an inflorescence or over the course of a season, and derive some description that includes means and variances, this does not incorporate the critical non-random aspect of morphological variation encompassed by the notion of ‘architectural effect’ nor does it adequately describe the phenotype that actually interacts with pollinators. It is not the ‘average flower morphology’ that evolves.

4. Evolution of phenotypic integration and modularity in metameric organisms

Intra-floral integration has been related, at least conceptually, to pollinator-mediated selection for particular functions, and modularity is expected to result when subsets of floral traits perform different functions. Phenotypic integration and modularity, although necessarily assessed by measures of multiple flowers, are inherently properties of individual flower development that result in a particular functional conformation of structures. By contrast, fitness is a property of an individual. Individual fitness is, in part, the sum of the reproductive success of each flower, which results from its interactions with pollinators. However, as noted by Ishii & Harder [81], the context in which the interactions of flowers and pollinators occur can change dramatically over the course of a season. Some of the changes are external: for example, the number of potential mates or intra- and interspecific competitors, the prevalence of pollinators and multiple aspects of the abiotic environment. Some of the changes are due to the dynamics of indeterminate ontogeny: the number of flowers that are open simultaneously, the number of maturing fruits competing with flowers for resources. To the extent that integration and modularity are the result of selection by pollinators, such temporal changes mean that selection for intra-floral integration and modularity also may change within a single reproductive season. Because of the positional information that is inherent in plant axes, any predictable, directional change in the selective environment of the individual flowers produced by a plant potentially may be matched by positional variation in flower morphology, integration and modularity. Perhaps the current paradigm of selection on individual flowers (or on some aspect of mean flower morphology) can be augmented by assessing the relative success of particular patterns of intra-individual variation in morphology. The result could be a far more comprehensive view of the evolution of plants as whole organisms rather than a collection of parts.

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Endnote

1The term ‘module’ also has been used to refer to plant metamers. I do not use ‘module’ or ‘modularity’ in this sense.


