Review

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Pleiotropy and the evolution of floral integration

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Summary

Floral traits often show correlated variation, both within and across species. One explanation for this pattern of floral integration is that different elements of floral phenotypes are controlled by the same genes, that is, that the genetic architecture is pleiotropic. Recent studies from a range of model systems suggest that the pleiotropy is common among the loci responsible for floral divergence. Moreover, the effects of allelic substitutions at these loci are overwhelmingly aligned with direction of divergence, suggesting that the nature of the pleiotropic effects was adaptive. Molecular genetic studies have revealed the functional basis for some of these effects, although much remains to be discovered with respect to the molecular, biochemical and developmental mechanisms underlying most quantitative trait loci (QTL) responsible for floral differences. Developing a detailed understanding of the nature of pleiotropic mutations and their phenotypic consequences is crucial for modeling how the genetic architecture of trait variation influences the tempo and trajectory of floral evolution.

I. Introduction

Traits that interact as part of a functional unit often show correlated variation, a pattern commonly referred to as phenotypic integration (Armbruster *et al.*, 2014). Flowers have provided an important model for studying the evolution of integration because interactions among their parts are closely tied to their function. For example, the position of the reproductive organs with respect to the perianth parts influences the precision and accuracy of pollination (Armbruster *et al.*, 2009), and the relative lengths of the male and female parts mediates the level of outcrossing (Motten & Stone, 2000). Given these functional interactions, transitions among mating systems or pollination modes often involve changes in multiple aspects of floral morphology and physiology (Culley *et al.*, 2002; Fenster *et al.*, 2004). At a macroevolutionary scale, coordinated changes in floral traits are manifest as correlated shifts across phylogenies (Ree, 2005; Ornelas *et al.*, 2007). Similar patterns of floral trait co-variation are also abundant within species (Conner & Via, 1993; Perez-Barrales *et al.*, 2007). The prevalence of correlated floral variation above and below the species level raises the question – could such concerted changes happen through one or a few mutations, or do the transitions require a large number of mutations in many different loci?

The answer to this question depends on the genetic architecture of floral variation. Although most floral traits are controlled by multiple loci, many of the underlying genes are pleiotropic, creating the potential for a single mutation to simultaneously affect multiple traits (Fig. 1). Indeed, pleiotropy is thought to be one of the principal drivers of morphological integration (Klingenberg, 2008) and plays a key role in generating correlated

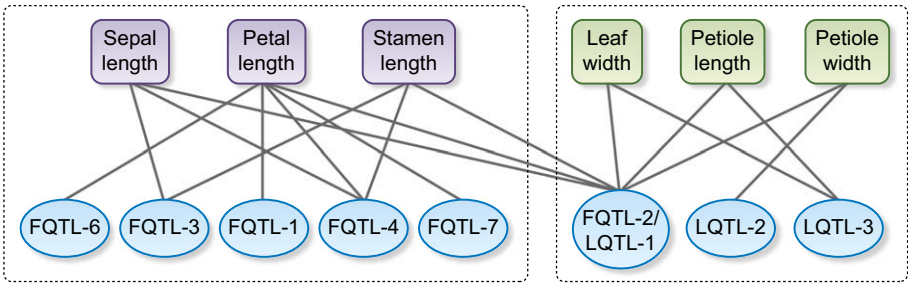

Fig. 1 Schematic depiction of genetic architecture of quantitative floral and vegetative traits in *Arabidopsis thaliana*. Lines indicate a significant additive effect of a quantitative trail locus (QTL; oval) on a trait (rectangle). All of the traits are polygenic (affected by multiple QTL). Although many of the loci are pleiotropic (i.e. affect multiple traits), the overall structure shows modularity (dashed boxes) in that floral traits tend to share QTL and the same is true for leaf traits. Data from Juenger *et al.* (2005); only a subset of the traits measured are depicted but all QTL detected for those traits are shown.

floral trait variation (Conner, 2002). This pleiotropic architecture could facilitate rapid floral evolution if the resulting covariation of floral traits is aligned with the direction of selection, allowing evolution to move along genetic lines of least resistance (Schluter, 1996). On the whole, however, pleiotropy has been most commonly considered to act as a constraint on evolution because mutations that move one trait toward its optimum may move others away from their optima (reviewed in Wagner & Zhang, 2011). This theoretical prediction is highly dependent on, among other things, the presumed nature of pleiotropy – that is, the number of traits controlled by pleiotropic genes, and the magnitude and directionality of these effects (Hansen, 2003; Pavlicev & Hansen, 2011).

With advances in statistical and molecular genetics, studies of pleiotropy and its impact on evolution have begun to move from theoretical to empirical. For example, genome-wide reverse genetic screens in yeast, mice and nematodes suggest that pleiotropy is extensive but far from universal (Wang *et al.*, 2010, but see Hill & Zhang, 2012). Although a handful of genes appear to affect many traits, most affect a relatively small number of traits, allowing for substantial modularity in genetic architecture (Wagner *et al.*, 2007). These results are consistent with patterns that have long been observed from quantitative trait locus (QTL) mapping (Juenger *et al.*, 2005; Fig. 1), and they suggest that mutations at pleiotropic loci may not wreak havoc across the organism, but instead result in localized changes in a set of traits or tissues. In addition, fine-scale investigations have begun to dissect the mechanistic basis for pleiotropy in natural populations (Scarcelli *et al.*, 2007; Paaby *et al.*, 2014). Although theory has focused almost entirely on antagonistic pleiotropy, such empirical studies have also uncovered cases of adaptive (positive) pleiotropy, where mutations have multiple positive effects on fitness (Ostrowski *et al.*, 2005; Lovell *et al.*, 2013).

This paper aims to survey the extent and nature of pleiotropy in the genetic architecture of floral-trait variation and consider the potential mechanisms responsible for these pleiotropic effects. Pleiotropic relationships will directly shape the range of segregating floral phenotypes as well as the range of phenotypes likely to arise by new mutations. Depending on the alignment of this variation with the direction of selection, pleiotropy could either impede evolutionary transitions (Otto, 2004) or facilitate them (Lovell *et al.*, 2013). Thus, estimating the degree of pleiotropy in the genetic architecture of floral traits has important implications for predicting the trajectory and pace of floral evolution.

II. Extent and nature of pleiotropy in floral trait architecture

The principal approach used to estimate the genetic architecture of trait variation is QTL mapping. This method seeks to find statistical associations between segregating phenotypic variation and genomic markers linked to loci (QTL) that control that variation (Mackay, 2001). QTL mapping has been widely employed in plants to identify regions of the genome with potential for crop improvement and has been instrumental in natural systems for understanding the number, distribution and effect sizes of loci that control differences between populations and species. In the context of floral integration, QTL mapping can be used to identify genomic regions that affect multiple traits and the degree to which those alleles at those loci are adaptively pleiotropic or antagonistic.

QTL studies from a diversity of angiosperm groups demonstrate that floral trait differences between species can arise through changes in a few major effect QTL, many of which are pleiotropic. Bradshaw *et al.* (1998) defined a major QTL as one that explains 25% or more of the variation, and they found at least one such locus for each of the floral traits they measured. Most of these regions affected multiple floral traits. For example, the *YUP* locus on linkage group D_c explains over 80% of the variation in carotenoid production, and that region is also associated with differences in petal width and the degree of petal reflexing (Bradshaw *et al.*, 1998). Subsequent multi-trait mapping studies in other systems have uncovered QTL of similarly large effect sizes, particularly for flower color (Wessinger *et al.*, 2014, but see Nakazato *et al.*, 2013) as well as significant pleiotropy (Hall *et al.*, 2006; see also Fig. 2 and Supporting Information Table S1). Consistent with having shared genetic bases, floral traits often show extensive correlated variation in mapping populations, with coefficients above 0.8 for some pairs of traits (Hall *et al.*, 2006; Wessinger *et al.*, 2014). Some of these pleiotropic relationships are not surprising, for example, loci that affect multiple aspects of flower size, which might encode genes for shared growth regulators (Brock *et al.*, 2012). For other traits, such as flower shape and color, the extent of pleiotropy would have been hard to predict given what we currently know about the genes involved in the floral development (Specht & Howarth, 2015).

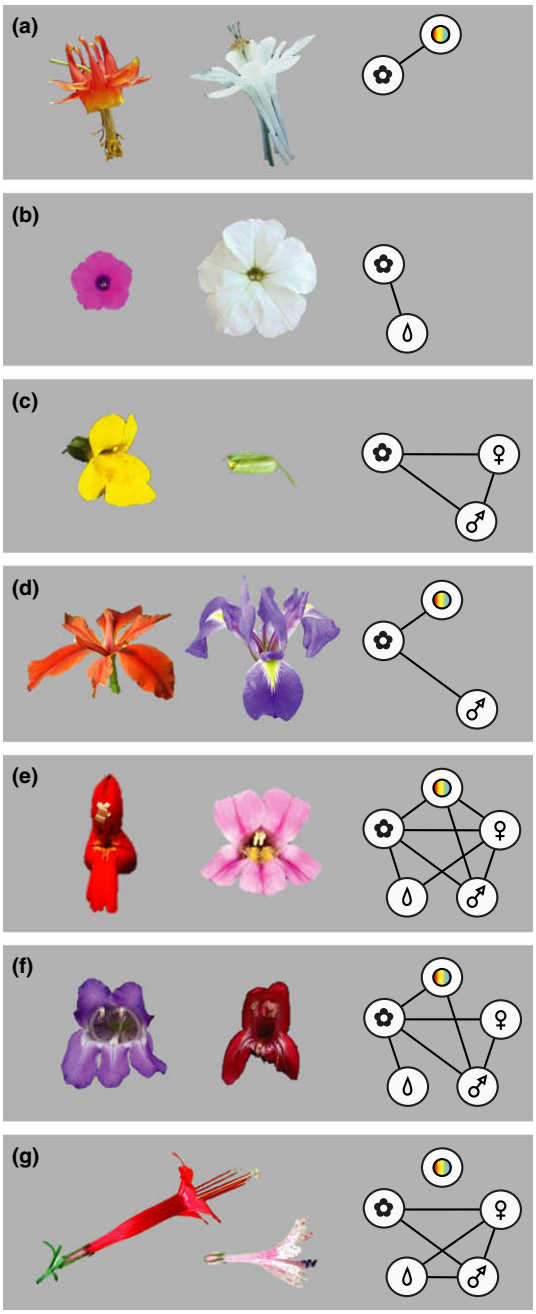

Fig. 2 Pleiotropic relationships among floral traits in model systems for floral divergence. Floral traits are grouped into five categories (depicted in a pentagon, clockwise from top): color, male traits, female traits, reward, and perianth morphology. Traits measured in a given study are shown for each pair of species (e.g. only color and morphology were measured in (a)), and traits that share one or more quantitative trait locus (QTL) are connected by solid lines. Species pairs used as parents in QTL studies are arranged (top to bottom) by the number of traits measured: (a) *Aquilegia formosa* and *A. pubescens*; (b) *Petunia integrifolia* and *P. axillaris*; (c) *Mimulus guttatus* and *M. nasutus;* (d) *Iris hexagona* and *I. fulva*; (e) *M. cardinalis* and *M. lewisii*; (f) *Penstemon barbatus* and *P. neomexicanus*; and (g) *Ipomopsis guttata* and *I. tenuifolia*. Images adapted from original studies (listed in Supporting Information Table S1), wikimedia commons or nsf.gov.

What is particularly notable about these QTL is the nature of their pleiotropic effects, which is nearly always aligned in the direction of the parental phenotypes (Table S1). For example, a QTL on linkage group 4 in *Penstemon* affects both petal reflexing and nectar concentration, and the additive effect of substituting the *P. barbatus* allele (the parent with more reflexed petals and more dilute nectar) is an increase in reflexing and decrease in nectar concentration (Wessinger *et al.*, 2014). This is consistent with the possibility that an allelic substitution at a single locus could have simultaneously moved both petal position and nectar concentration from the ancestral *P. neomexicanus*-like state toward the derived *P. barbatus*-like phenotype (Fig. 2). Moreover, the fixation of this allele in the hummingbird-pollinated *P. barbatus* was likely adaptive as the combination of reflexed petals and dilute nectar has evolved independently in several *Penstemon* lineages that are pollinated by birds (Wilson *et al.*, 2004). Assuming that the floral differences in other species pairs (Fig. 2) evolved due to natural selection, the pleiotropy of the underlying QTL (Table S1) may have been adaptive in these cases as well. Such adaptive pleiotropy (Fig. 3) has received little attention in theoretical studies relative to its counterpart, antagonistic pleiotropy, but presumably when such variation arises in natural populations, it would quickly be fixed if indeed the suite of resultant changes is favored by selection (Lovell *et al.*, 2013).

Although QTL studies have been instrumental in providing an initial genome-wide assessment of pleiotropy, it is important to note that co-localization of traits to the same QTL does not provide definitive evidence that particular genes or the mutations within them are pleiotropic. Depending on the resolution of the study, a single QTL may span a large number of genes, and thus the observed effect of that QTL on multiple traits could be due to a series of linked genes, which individually are not pleiotropic. Moving from QTL affecting variation to particular genes and individual mutations remains challenging (Mackay *et al.*, 2009), although the process may be accelerated if QTL contain well-characterized candidate genes (Juenger *et al.*, 2000). Indeed, such a combination of fine-scale mapping and identification of candidate genes was used to dissect a QTL controlling an entire suite of floral traits in *Petunia*, including color, scent, stamen length and pistil length (Hermann *et al.*, 2013). Detailed analysis of introgression lines revealed that one 0.8-cM region contained a string of genes, including three MYB transcription factors, which independently regulate distinct aspects of floral phenotype. Thus, what first appeared to be a highly pleiotropic floral QTL is, in fact, a tightly linked cluster of genes that individually are minimally pleiotropic.

Although it is possible that similar clusters of linked genes could be found to comprise previously documented floral QTL (Table S1), several lines of evidence suggest that truly pleiotropic genes may often be found to underlie pleiotropic QTL. First, in at least one case, the tight correlations among floral traits showed no decline despite many rounds of enforced random mating (Conner, 2002), which points to pleiotropy as opposed to linkage as the underlying cause. Second, genes with pleiotropic effects on multiple floral traits are well known from molecular and developmental studies of mutant lines in *Arabidopsis* and other model systems (Aida *et al.*, 1997; Krizek *et al.*, 2006). Of course, one could argue that the knock-out mutants studied in model systems are not likely to mirror the types of pleiotropic alleles that could contribute to adaptation in nature. However, genetic studies increasingly

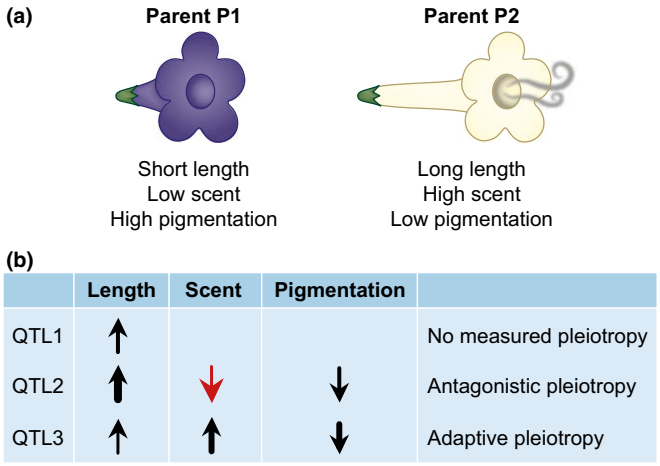

Fig. 3 Types of pleiotropic quantitative trail loci (QTL) detected in crosses between a hypothetical species pair. (a) The two parental lines (P1 and P2) show fixed differences in three floral traits: corolla length, scent production and pigmentation. (b) The table shows the magnitude (arrow thickness) and direction (increase/decrease) of substituting an allele from the P2 parent at each of the three detected QTL. For example, adding a P2 allele at QTL1 increases corolla tube length, consistent with the directionality of the difference between the parents (P2 has the longer corolla). This QTL (QTL1) affects just one trait and thus does not contribute to correlated floral variation. Alternately, QTL could affect more than one trait, and either move the affected traits in opposing directions (black and red arrows, QTL2) or consistently in the direction of the parental difference (all black arrows, QTL3). The adaptive pleiotropy of QTL3 assumes that the trait combinations in the parents were fixed due to adaptation. Substitution of a P2 allele could also move one or all traits in the direction opposite to what would be predicted (that is, all red arrows); this is not depicted, but might be expected under past fluctuating selection or drift.

support a role for loss-of-function (LOF) mutations in the evolution of interspecific floral differences (Wessinger & Rausher, 2012). Moreover, a recent study in *Brassica* is consistent with adaptive pleiotropy of such mutations. Zhang *et al.* (2015) discovered that a transposon-mediated LOF mutation in a carotenoid cleavage dioxygenase (CCD) simultaneously causes a shift from white scented flowers to yellow unscented flowers in *B. napus*. CCD is responsible for converting carotenoids into apocarotenoid volatiles and thus the LOF leads directly to the accumulation of yellow carotenoids. Although the adaptive significance was not assessed in the *B. napus* case, transitions between colored flowers without scent and pale flowers with scent are commonly associated with shifts from diurnal to nocturnal pollinators (Fenster *et al.*, 2004, but see White *et al.*, 1994). Biochemical connections between color and scent compounds are also known from the phenylpropanoid and terpenoid pathways (Bar-Akiva *et al.*, 2010), and segregating variation in shared regulatory genes (Ben Zvi *et al.*, 2012) could explain the co-variation of color and scent often seen in natural populations (Majetic *et al.*, 2007).

III. The evolution of pleiotropy

By identifying genetic variation that contributes to differences between present-day populations or species, quantitative and molecular genetic studies capture a snapshot of the extent and nature of pleiotropy in a single evolutionary timeslice. These snapshots differ among taxa for the same sets of traits (Fig. 2), which reflects that fact that pleiotropy itself is evolving. Thus, an important challenge for evolutionary biologists is understanding how pleiotropy arises and how quickly it changes over evolutionary timescales (Arnold *et al.*, 2008; Guillaume & Otto, 2012). One possible explanation for the predominance of adaptive pleiotropy in floral trait QTL (Table S1) is that, although the spectrum of mutations includes all types of pleiotropy (Fig. 3), those which are adaptively pleiotropic have a higher probability of fixation (Wessinger *et al.*, 2014). Alternatively, evolution may act to break genotype–phenotype relationships that result in antagonistic pleiotropy, making that class of variants rare relative to those that result in minimal or adaptive pleiotropy. For example, changes in gene regulation, either through mutations in *cis*-regulatory elements or *trans*-acting factors, could reduce antagonistic pleiotropy by erasing links in the gene regulatory network. Gene duplication is another well-studied mechanism that reduces pleiotropy through subfunctionalization or specialization of gene copies (Des Marais & Rausher, 2008). In addition, modifiers may evolve that act to modulate the level and possibly the type of pleiotropy (reviewed in Paaby & Rockman, 2013). Quantitative genetic methods for detecting such loci (relationship QTL or rQTL) have thus far only been applied to animals (Pavlicev *et al.*, 2008), although selection experiments hint at their existence in plant systems (Delph *et al.*, 2011).

IV. Pleiotropy as an impediment or facilitator of phenotypic evolution

Pleiotropy has commonly been viewed as a factor that constrains or impedes evolution (reviewed in Wagner & Zhang, 2011). This conclusion largely relies on assumptions about the nature of pleiotropy – that is, that many of the effects of pleiotropic mutations will have negative consequences for fitness. However, some mutations may result in selectively advantageous changes in multiple traits (Lovell *et al.*, 2013), and over time, the genetic basis for pleiotropy may evolve to minimize antagonistic effects, making multivariate adaptive states more robust to gene flow and recombination. Indeed, as reviewed above, most loci involved in floral divergence appear to exhibit adaptive pleiotropy, with effects aligned with the fixed differences between species. This pattern suggests that pleiotropy does not constrain floral evolution, but instead may play an important role in achieving the coordinated evolution of sets of interacting floral traits. Nonetheless, we still know little about the molecular basis for pleiotropy in any of the model systems for floral divergence (Fig. 2). Identifying the genes that underlie pleiotropic QTL and characterizing their mode of action in a wide range of taxa will be key to modeling how pleiotropy and the resulting trait–covariance structure evolve. Building such a mechanistic framework will provide new opportunities for understanding how the genetic architecture of floral variation shapes the rate and direction of floral evolution over short and long evolutionary timescales (Conner *et al.*, 2011; Bolstad *et al.*, 2014).

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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Level of pleiotropy for selected floral traits from QTL mapping studies

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