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Pleiotropy in developmental regulation by flowering-pathway genes: is it an evolutionary constraint?

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Summary

Pleiotropy occurs when one gene influences more than one trait, contributing to genetic correlations among traits. Consequently, it is considered a constraint on the evolution of adaptive phenotypes because of potential antagonistic selection on correlated traits, or, alternatively, preservation of functional trait combinations. Such evolutionary constraints may be mitigated by the evolution of different functions of pleiotropic genes in their regulation of different traits. *Arabidopsis thaliana* flowering-time genes, and the pathways they operate in, are among the most thoroughly studied regarding molecular functions, phenotypic effects, and adaptive significance. Many of them show strong pleiotropic effects. Here, we review examples of pleiotropy of flowering-time genes and highlight those that also influence seed germination. Some genes appear to operate in the same genetic pathways when regulating both traits, whereas others show diversity of function in their regulation, either interacting with the same genetic partners but in different ways, or potentially interacting with different partners. We discuss how functional diversification of pleiotropic genes in the regulation of different traits across the life-cycle may mitigate evolutionary constraints of pleiotropy, permitting traits to respond more independently to environmental cues, and how it may even contribute to the evolutionary divergence of gene function across taxa.

Key words: Divergence, Dormancy, Flowering, Genetic pathway, Germination

I. Introduction

Pleiotropy is defined as one gene influencing more than one trait. Pleiotropy, together with linkage disequilibrium due to physical linkage or population structure, causes genetic correlations among traits. Of these contributors to genetic correlations, pleiotropy is most long-lasting, because linkage disequilibrium diminishes with recombination, whereas the strength of pleiotropy diminishes only through the evolution of the function of the pleiotropic gene or of the pathways in which it operates (Cheverud, 1996; Cheverud *et al.*, 2004; Pavlicev & Wagner, 2012; Guillaume & Otto, 2012; Pavličev & Cheverud, 2015; Chebib & Guillaume, 2017).

By contributing to correlations among traits, pleiotropy influences patterns of selection on those traits and their evolutionary responses to selection. Correlated traits are subjected to both direct selection acting on the first trait, and indirect selection that acts on correlated traits (Lande, 1979; Lande & Arnold, 1983). Total selection on a trait is the sum of direct selection and indirect selection acting through all correlated traits, which may reinforce or oppose the direction of direct selection. Therefore, pleiotropy may facilitate the evolution of coordinated responses of multiple functionally related phenotypes, but it also may prevent optimum phenotypes from evolving for any single trait (Fisher, 1930; Atchley, 1984; Wagner, 1988; Barton, 1990; Wagner and Altenberg, 1996; Crespi, 2000; Orr, 2000; Griswold & Whitlock, 2003; Brakefield, 2006; Hansen and Houle, 2008; Wagner *et al.*, 2008; Walsh & Blows, 2009). Pleiotropy, often referred to as a genetic trade-off, is frequently considered to be one of the most plausible explanations for sub-optimal or even maladaptive phenotypes.

Although pleiotropy and genetic correlations are thoroughly integrated into theoretical and empirical treatments of evolutionary outcomes using a quantitative-genetic framework, molecular biologists have less enthusiastically embraced the phenomenon of pleiotropy, focusing instead on less "noisy" genes when investigating the genetic pathways that regulate traits of interest. It is challenging enough to identify genetic loci that have clear phenotypic effects on traits under precise environmental conditions, without being hindered by issues of incomplete penetrance, compromised performance because of other "side effects," or uncertain functional significance because of diffuse effects on traits other than the trait of interest. It is doubtful that geneticists would have had such success in inferring the complex genetic pathways whereby traits are regulated over the course of development and in response to specific environmental stimuli had they not narrowed their focus to specific traits in specific controlled environments.

Although such an approach has had enormous success in characterizing genes and genetic pathways that regulate important traits, inferences about the functional significance of these genes are far more challenging. This is because, although effects of these genes were detected on one trait, that gene may regulate other traits that were not measured (Pavlicev & Wagner, 2012); those unmeasured traits may be subjected to selection, perhaps even more strongly than the original trait of interest.

Here, we discuss examples of pleiotropy in one of the best characterized genetic pathways in plants: those that regulate flowering time in *Arabidopsis thaliana*. We first briefly describe the genetic pathway of flowering-time regulation. Using that as a reference, we review studies that have shown pleiotropic effects of flowering-time genes on other functionally significant traits, especially germination and dormancy. After discussing examples of genetic pleiotropy, we describe a form of pleiotropy that we refer to as "environmentally-induced pleiotropy", which appears to be commonly manifest in genes that control the environmental responses of developmental transitions. We next query how concordant the genetic pathways are, whereby a single gene regulates more than one trait; that is, are entire segments of pathways shared in the regulation of multiple traits, or do pleiotropic genes have different interactions with partners, have different interacting partners, or operate in completely different pathways in their regulation of multiple traits? Finally, we discuss the potential of pleiotropy to contribute to the divergence of gene function across taxa.

In the examples reviewed below, genetic pleiotropy has been confirmed (as opposed to close linkage) based on genetic studies of mutations and functional genetic studies that directly manipulate gene activity or functionality. The detailed molecular basis of that pleiotropy is rarely known, however, preventing classification into distinct types of pleiotropy with respect to their molecular mechanism (e. g. Hodgkins, 1998; Wagner and Zhang, 2011; Paaby and Rockman, 2014). From the perspective of evolutionary outcomes via correlated selection, however, such distinctions are not important except insofar as the mechanism alters the strength of genetic correlations among characters (Wright, 1968; Paaby and Rockman, 2014). Moreover, this review does not attempt to quantify the pleiotropy of specific genes in terms of the total number of traits it may affect or the total strength of pleiotropy across all traits (Wagner and Zhang 2011, Hill and Zhang 2011). While quantifications of genome-wide pleiotropy are germane to understanding evolvability, costs of complexity (Fisher 1930), and genetic load (Poon and Otto, 2000), in this review we focus discussion on the pleiotropic regulation of specific traits identified *a priori* as ecologically important. With this closer focus, we aim to gain insight into the mechanisms whereby one gene regulates more than one trait, and the possible mechanisms whereby the evolutionary constraints imposed by pleiotropy may be mitigated (Cheverud *et al*., 2004; Pavlicev *et al*., 2008).

II. The case study of flowering time in *Arabidopsis thaliana*

In *Arabidopsis thaliana*, flowering time has been used as a convenient and precise phenotype for analysing the function of environmental detection pathways in plants. In many plants, the seasonal timing of flowering is regulated by several environmental cues that vary over the course of the year. For instance, photoperiod is a reliable cue of time of year, especially when combined with temperature cues, such as duration of chilling (a.k.a. vernalization), which indicates the passage of winter. Ambient temperature itself varies seasonally, as do light cues, since seasonal canopies emerge and then senesce, and nutrient pulses, as rain cycles mobilize nutrients. Such cues are sensed, and their signals integrated, to regulate the seasonal timing of reproduction. This phenological trait has known fitness consequences in many plants (e.g. Hall & Willis, 2006; Korves *et al.*, 2007; Anderson & Mitchell□Olds, 2011; Wadgymar *et al.*, 2018), as it determines the availability of resources and the duration of time for seed set, as well as the availability of pollinators for plants that require them. It has been implicated in responses to climate change, such that adjusting flowering time can mitigate the probability of local extinction (Willis *et al.*, 2008, 2010; Wolkovich *et al.*, 2013). For these reasons, flowering time has been a classic phenotype of environmentally regulated development that has clear ecological significance.

Flowering time lends itself to precise environmental and genetic manipulation. Especially in controlled conditions, it is straightforward to manipulate individual environmental cues, including photoperiod, temperature, vernalization, light quality, nutrition or other experimental variables. By using carefully chosen experimental conditions, strong phenotypes can be observed for loss-of-function alleles of specific genes, exposing their role in flowering-time regulation. This approach has led to the characterization of multiple intersecting pathways of flowering-time regulation in *A. thaliana* (Simpson & Dean, 2002; Fig. 1A). The vernalization pathway senses prolonged chilling, such that flowering is repressed by the central flowering regulator, *FLOWERING LOCUS C* (*FLC*), until *FLC* is repressed by exposure to prolonged chilling. This pathway has become a model for understanding the epigenetic regulation of development in response to an environmental cue (Michaels & Amasino, 1999; Sheldon *et al.*, 2000; Michaels *et al.*, 2003; reviewed by Ream *et al.*, 2012). The genes formally-known as the autonomous pathway also repress *FLC* via processing of the anti-sense transcript, and mediate flowering responses to ambient temperature, nutritional status, and plant age (Lee & Amasino, 1995, 2013; Reeves & Coupland, 2000; Rouse *et al.*, 2002; Blázquez *et al.*, 2003; Lim *et al.*, 2004; Samach &

Wigge, 2005; Bäurle & Dean, 2008; Huijser & Schmid, 2011; Lin & Tsay, 2017; Weber & Burow, 2018). Immediate targets of *FLC*, the floral integrator genes *FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*), are co-regulated by the photoperiod pathway which induces flowering in long days (Samach *et al.*, 2000; Andrés & Coupland, 2012). The gibberellin signalling pathway (GA pathway) also regulates *SOC1* and *LEAFY* (*LFY*) expression to promote flowering (Conti, 2017).

Genes in each pathway have effects on flowering in response to controlled conditions. More challenging has been to show that these genes have strong effects on flowering and fitness in real-world conditions (Song *et al.*, 2018). Many experiments have provided convincing evidence that genes involved in the regulation of flowering are under selection in *Arabidopsis*, both in *A. thaliana* and *A. arenosa* (Baduel *et al.*, 2018). Much of this evidence is in the form of molecular signatures of selection, such as reduced variation within the region of these genes, consistent patterns of introgression of these genes, or outlier analysis that show that these loci are more divergent or less divergent among populations than random loci. More directly, one study showed that *FLC* haplotype variation was strongly associated with variation in seed yield in different environments, such that slow vernalising haplotypes yielded more seeds when sown in fall, but rapid vernalisers yielded more seeds when sown in spring or summer (Li *et al.*, 2014b). Genome-wide association studies (GWAS) using populations collected from across the geographic range of *A. thaliana* showed that flowering-time genes interact with local climatic conditions to predict fruit production (Fournier-Level *et al.*, 2011). Other studies using experimental populations of *A. thaliana* showed that loci associated with accelerated flowering were strongly favoured under stressful conditions and short growing seasons (Fournier□Level *et al.*, 2013; Taylor *et al.*, 2017). In populations of *A. arenosa* that colonized highly disturbed railway habitats, the *CONSTANS* (*CO*) locus and *FLC* exhibited pronounced allelic divergence from populations found in less disturbed habitats (Baduel *et al.*, 2018). These results suggest that disturbed habitats select for rapid-cycling behaviour, whereby shorter generation time, facilitated in part by rapid flowering, is favorable. In ruderal species such as *A. thaliana* and *A. arenosa*, disturbance and short growing season is likely a significant selective influence on the evolution of their life history (Levin, 1974).

However, it is not always clear whether time to flowering itself is the phenotype under strongest selection, or whether the target of selection may be other traits that are pleiotropically co-regulated by the same gene network. Field studies of *A. thaliana* have failed to detect strong effects on flowering time of known flowering-time genes, when contrasting genotypes were planted under field conditions (e.g. Wilczek *et al.*, 2009; Chiang *et al.*, 2009). Major flowering-time mutants, including mutants of *CO*, *FT*, *FRIGIDA* (*FRI*) and *VERNALIZATION INSENSITIVE 3* (*VIN3*), which in the laboratory show strong phenotypes, flowered only a few days later than the wild type in many locations under multiple sowing times (Wilczek *et al.*, 2009). Major differences in the time of flowering were expressed only when seeds were sowed during a specific two-week window of time in the autumn, indicating that allelic effects of these genes are highly sensitive to other aspects of life-cycle phenology, such as germination time. In such cases it is not clear whether variation at these individual loci is maintained by apparently subtle effects on flowering time alone, or whether selection occurs through pleiotropic effects on other traits.

III. Examples of genetic pleiotropy of flowering-time genes in *Arabidopsis thaliana*

Flowering-time genes in *A. thaliana* have diverse roles in plant physiology and development beyond regulating the number of days to flowering. They have been implicated in traits as diverse as stomatal conductance and water use, to pathogen resistance. They have also been implicated in the timing of other environmentally regulated developmental transitions, such as bud break and germination, collectively termed phenology.

1. Pleiotropic effects of flowering-time genes on non-phenological traits:

Major genes that regulate responses to vernalization, *FRIGIDA (FRI)* and *FLC,* pleiotropically influence non-phenological traits. Functional *FRI* alleles, in addition to delaying flowering, have been correlated with drought resistance, such that variation in *FRI* functionality can produce either slow-flowering plants that can withstand drought, or rapid flowering plants that escape drought (Lovell *et al.*, 2013). This effect is mediated by *FRI* through the activation of proline synthesis in response to water stress and depends on *FLC* (Chen *et al.*, 2018). This example shows that flowering time may evolve in concert with physiological tolerances to stresses associated with seasonally variable environments, and that such coordination is the result, at least in part, of pleiotropy.

Several floral integrators are highly pleiotropic. Floral integrators are expressed in stomata and influence stomatal aperture. For instance, early flowering lines have larger apertures, and this correlates with *FT* gene expression levels (Kinoshita *et al.*, 2011; Ando *et al.*, 2013). Vernalization also increases stomatal aperture (Kimura *et al.*, 2015), and this is associated with an increase in *SOC1* and *FT* gene expression. Furthermore, overexpression of *SOC1* alone is sufficient to increase stomatal opening (Kimura *et al.*, 2015). Whether these effects, observable under controlled conditions in the laboratory, have any influence in the field is yet to be tested.

Genes in the autonomous-pathway have also been implicated in traits associated with drought response or the balance of water use and photosynthetic efficiency, such as chlorophyll accumulation, leaf shape, and inflorescence shape (Martínez□Zapater *et al.*, 1995; Henderson *et al.*, 2005). In addition, they are involved in defence against fungal pathogens, response to cold stress, circadian clock regulation, and general vigor (Koornneef *et al.*, 1998; Meier *et al.*, 2001; Kim *et al.*, 2004; Salathia *et al.*, 2006; Veley & Michaels, 2008; Lyons *et al.*, 2015). Because the function of these proteins in RNA processing is highly general, however, it is not always clear whether these effects are mediated by the influence of the autonomous-pathway genes on *FLC* gene expression levels. Nonetheless, the fact that variation at these genes affects so many traits, some of which are associated with seasonally variable stressors such as drought, cold, and pathogen load, raises the possibility of the correlated regulation of seasonal phenology and tolerance to seasonal environmental factors.

Other genes involved in the regulation of flowering-time have much wider pleiotropic effects, such as those involved in hormone signalling, photomorphogenic responses and circadian clock (Table 1). Table 1 lists a select sample of these genes and provides references that give details on their mechanisms of action.

2. Pleiotropic effects of flowering-time genes on other phenological traits:

The seasonal timing of developmental transitions such as flowering, germination, or budbreak can have strong fitness consequences, because different life stages have different environmental tolerances or optima. For instance, young seedlings may be vulnerable to drought because of inadequate root establishment, whereas later life stage may be more resistant; likewise, vegetative tissues may be more cold-tolerant than developing reproductive tissue. For this reason, it is beneficial for plants to time the transition from one life stage to another so that each life stage is matched to the environment that it can tolerate. To maintain

coordination with external environmental cues, plant developmental transitions are regulated by internal and external environmental signals, and the signaling pathways involved have pleiotropic effects on developmental transitions across multiple life stages.

The seasonal regulation of flowering time requires halting development until some stimulus alleviates that repression. The broad phenomenon of developmental arrest pertains to many life-stage transitions besides flowering, such as bud and seed dormancy, and some of these involve flowering-time genes. For instance, flowering and growth cessation in poplar is regulated by a *CO/FT* regulatory module (Böhlenius *et al.*, 2006), and induction of poplar bud dormancy requires the transcriptional regulation by ABA of an orthologue of *SHORT VEGETATIVE PHASE* (SVP; Singh *et al.*, 2019). Orthologues of SVP have been implicated in seasonal control of growth arrest in apple and kiwifruit (Wu *et al.*, 2012, 2017). Multiple *FT* homologues, as well as *FLD, LFY* and *AP1* homologues, have been identified and associated with bud dormancy in several temperate tree species (Cooke *et al.*, 2012; Lloret *et al.*, 2018). Winter bud dormancy also mediates iteroparity in perennial species, and homologs of *A. thaliana* flowering-time genes have been shown to regulate perenniality in its relatives. Specifically, *PERENNIAL FLOWERING 1* (*PEP1*) in *Arabis alpina* is a homolog of *A. thaliana's FLC;* it, too, suppresses the development of meristems into reproductive structures (Albani *et al.*, 2012). Unlike the irreversible suppression of weak *FLC* haplotypes in *A. thaliana*, which enables all remaining meristems to proceed through development, *PEP1* is reversibly repressed, such that only some meristems develop into reproductive structures whereas others are later suppressed into quiescence, remaining available for allocation to reproduction in subsequent years. As such, *PEP1* is essential for the expression of a perennial and iteroparous life history.

Another critical point of developmental arrest in plants is seed dormancy. Dormancy prevents seeds from germinating at inappropriate times of year even though ambient environmental conditions may be temporarily favourable. Dormancy is induced at the later stages of seed maturation, and it is also maintained in seeds after imbibition under certain conditions. Flowering-time genes alter dormancy and germination in *A. thaliana* (Table 2), including the central regulator of flowering time, *FLC*, (Chiang *et al.*, 2009; Blair *et al.*, 2017), genes in the vernalization pathway (Liu *et al.*, 2011; Auge *et al.*, 2017), the autonomous pathway (Jiang *et al.*, 2012; Cyrek *et al.*, 2016; Auge *et al.*, 2018), the photoperiod pathway including the phytochromes (Casal & Sánchez, 1998; Cadman *et al.*, 2006; Penfield & Hall, 2009; Chen *et al.*, 2014), the independent temperature-sensing gene

SVP (Penfield & Hall, 2009; Chen *et al.*, 2014) and microRNAs *MIR156* and *MIR172* (Huo *et al.*, 2016). Floral integrator genes, including *SOC1*, *FT*, and *AP1* (Penfield & Hall, 2009; Chiang *et al.*, 2009; Chen *et al.*, 2014), are also involved in the regulation of germination. *TEMPRANILLO1 and TEMPRANILLO2* (*TEM1* and *TEM2*) were associated with flowering time by regulating the major floral integrator *FT* (Castillejo & Pelaz, 2008). Interestingly, *TEM1* and *TEM2* are both strongly expressed in secondarily dormant *A. thaliana* seeds (Cadman *et al.*, 2006), suggesting a role for these genes in dormancy regulation. Thus, many genes in multiple flowering pathways also have pleiotropic effects on germination and dormancy. In fact, for some of these genes, their effect on germination was more pronounced than their effect on flowering time under field conditions (Chiang *et al.*, 2009).

Because primary seed dormancy is induced during the late stages of seed maturation, the seasonal conditions at the time of reproduction and seed maturation have strong effects on seed dormancy and germination. Moreover, the seed coat, derived from maternal tissue, strongly mediates germination behaviour. Seed-coat thickness and seed-coat colour (determined by tannin levels) are strongly influenced by maternal photoperiod, temperature, and altitude in diverse species (Gutterman, 1978, 2002; Fenner, 1991; Toorop *et al.*, 2012). In *A. thaliana*, temperature has strong effects on seed dormancy induction, with cool maternal temperatures inducing strong dormancy (Donohue *et al.*, 2008; Chen *et al.*, 2014; Burghardt *et al.*, 2015; Springthorpe & Penfield, 2015).

These maternal environmental effects on seed dormancy are regulated by signalling pathways that either operate wholly within maternal tissues, or that begin with signal perception in maternal tissues followed by the transmission of those signals to zygotic tissues, either via mobile signalling factors or epigenetic inheritance (Penfield and MacGregor, 2017). Flowering-time genes are involved in these maternal environmental effects. For example, phytochromes, which regulate many developmental processes in plants, including flowering (Franklin & Whitelam, 2004), contribute to maternal temperature effects on germination, with active *PHYD* being required specifically for germination of seeds matured under cool conditions but not warm conditions (Donohue *et al.*, 2008). Flowering-time genes also affect properties of the tissues surrounding the embryo, including seed tannin content, suberin deposition and seed permeability, and consequently also alter seed dormancy and germination (Chen *et al.*, 2014; MacGregor *et al.*, 2015).

Environmental signals transmitted to seeds can alter germination responses not only to maternal environmental conditions but also responses to conditions experienced by seeds themselves. For example, the light quality during seed maturation can alter germination responses of seeds to their own light environment (Leverett *et al.*, 2016), and seed-maturation temperature alters the temperature at which germination can proceed (Burghardt *et al.*, 2016). By influencing the environmentally mediated induction of seed dormancy, flowering-time genes thereby also may alter germination responses to post-dispersal environments.

3. Pleiotropic effects of dormancy genes on flowering-time

Just as several flowering-time genes influence seed germination, so too major seed-dormancy genes are implicated in flowering-time regulation. In particular, a major regulator of seed dormancy, *DELAY OF GERMINATION1* (*DOG1*; Bentsink *et al.*, 2006) has been associated in genome-wide association analyses with variation in flowering time (Atwell *et al.*, 2010). In the Col-0 accession, however, no flowering-time phenotype has been described for *dog1* mutants, suggesting that *DOG1* may not regulate flowering time in this accession. In contrast, loss of *DOG1* expression in lettuce promoted early flowering, which was accompanied by changes in *MIR156* and *MIR172*, two miRNAs that regulate the length of the vegetative phase in *A. thaliana* (Figure 1; Huo *et al.*, 2016). Direct manipulation of *MIR156* and *MIR172* in *A. thaliana* altered not only flowering time but also germination, suggesting that *DOG1* influences dormancy by regulating microRNA metabolism. Given that *DOG1* is strongly expressed in seeds in *A. thaliana*, it is not clear whether seed-specific *DOG1* expression or other flowering-time genes influence flowering time by regulating the starting levels of *MIR156*, or whether in lettuce *DOG1* is also expressed in leaves. In *A. thaliana* embryos, *DOG1* is expressed mainly in phloem (Nakabayashi *et al.*, 2012), which suggests that *DOG1* may act genetically upstream of *FT* in controlling flowering time. Such results strongly suggest that dormancy genes may play a more prominent role in flowering-time regulation in some species other than in *A. thaliana,* or even in some of its accessions. One possibility is that developmental checkpoints after the floral transition, but before bolting, exist, similar to those frequently described in perennial plants in which regulation of floral bud break is the key determinant of flowering time.

The above examples show that genes involved in the regulation of the transition to flowering are also frequently involved in other developmental transitions (and *vice versa*). In the case of seed dormancy and germination, the response of germination to maternal environmental factors also involves flowering-time genes. Some of these genes alter qualities of the maternal tissue that surrounds seeds and thereby influence germination; others are known to be diffusible (e.g. *FT*) and possibly transmitted via provisioning to seeds. In this manner, flowering-time genes can regulate the responses of seed germination to environmental factors, whether experienced by mothers or even by themselves.

IV. Environmentally induced pleiotropy

An additional mechanism whereby flowering-time genes can express pleiotropic effects on other traits is that flowering time itself determines environmental conditions experienced by traits expressed subsequently, and those environmental conditions in turn alter phenotypic expression of those traits (Figure 1b). This phenomenon is likely to be especially important under natural, seasonally variable conditions. Such environmentally-induced pleiotropy is presumably common for genes that regulate environmentally cued developmental transitions, such as flowering, bud-break, shoot emergence, and seed germination. When a gene regulates a trait, which in turn influences a second trait, the dynamic is described as "vertical" pleiotropy (Paagy and Rockman 2013). Such vertical pleiotropy also contributes to genetic correlations and thereby influences evolutionary outcomes (Wright 1968).

Many of the examples discussed above demonstrated the effects of flowering-time genes on other developmental traits even when plants were grown under precisely controlled and constant environments. However, many of those examples also showed that environmental conditions during seed maturation can strongly alter the depth of seed dormancy and thereby germination timing. Even very small changes in the seed-maturation conditions can dramatically affect the dormancy of *A. thaliana* seeds (Springthorpe & Penfield, 2015). Under natural seasonally variable conditions, the timing of flowering can determine the photoperiod, temperature, or canopy coverage that plants experience during seed maturation, and these environmental factors in turn can induce different levels of seed dormancy. Under field conditions, such a dynamic would be manifest as pleiotropy, whereby a gene that alters flowering time would also alter seed germination.

The same dynamic applies to other developmental transitions. Counter-intuitively, genes that affect seed dormancy in the lab have larger effects on flowering date under natural conditions than do genes known to affect flowering time in the lab, reflecting the importance of germination date in determining the vegetative environment and thereby the time to flower (Huang *et al.*, 2010; Chiang *et al.*, 2011). In this example, allelic variation of the dormancy gene, *DOG1*, altered the season of seed germination, the rate of flowering, and determined the overall life history that was expressed, with less dormant alleles expressing a winter-annual life history but more dormant alleles being spring annuals (Chiang *et al.*, 2013).

Such pleiotropy may also indirectly influence non-phenological traits, and indeed any trait that exhibits plasticity to seasonal environments. For instance, aspects of leaf development, including leaf area, thickness, cuticle thickness and other features associated with increased stress tolerance are sensitive to photoperiod or temperature during leaf development (Armstrong *et al.*, 2006); leaves are less likely to require evaporative cooling under cooler temperatures (Crawford *et al.*, 2012), which could potentially influence stomatal density (Beerling & Chaloner, 1993; Luomala *et al.*, 2005). Moreover, plants that flower later may be more likely to encounter pathogens attempting to gain entry to leaves through stomata (Underwood *et al.*, 2007). Thus, one mechanism whereby genes that regulate developmental timing can also influence leaf traits is by determining the seasonal environmental conditions under which leaves develop.

While genetic pleiotropy can be detected under highly controlled laboratory conditions, environmentally induced pleiotropy may be completely invisible unless organisms are permitted to develop under natural seasonally variable conditions. Consequently, pleiotropy is likely much more commonly expressed in the wild than would be predicted from controlled genetic analyses in the lab. Inferences on the functional significance of genes studied under controlled laboratory conditions should be made with that in mind. Because of the genetic pleiotropy of flowering-time genes on germination, and because flowering time influences the environmental conditions during seed set and thereby seed dormancy, selection on flowering-time genes may operate through their effects on germination, pathogen resistance or seed yield even more strongly than through their effects on flowering time *per se*. Likewise, because dormancy determines the season of seed germination, which determines the exposure of seedlings to major flowering cues such as photoperiod or vernalization, dormancy genes may be selected through their effects on flowering time as well as

germination time. Incorrect inferences on gene function are likely when indirect mechanisms of pleiotropy, operating through environmental pathways, are not considered.

V. Diversity of gene function across the life cycle

For adaptive life cycles to be expressed, the timing of developmental transitions across the life cycle needs to be coordinated with the changes in seasonal environmental conditions. By providing an integrated mechanism for environmental responses, pleiotropy may contribute to that coordination. For instance, when high levels of *FLC* simultaneously promotes germination and represses flowering in the autumn (Chiang *et al.*, 2009), it imposes the winter-annual life history typical of the species. However, to express adaptive life cycles, different life stages may need to respond independently to environmental cues--responding to the same cue in a different manner or responding to different cues entirely. After all, different developmental transitions necessarily need to occur at different times of year and therefore under different seasonal conditions. How do such independent responses occur when the same genes regulate more than one developmental transition?

Evolutionary constraints of pleiotropy can be mitigated and result in more independent regulation of traits, despite sharing components of genetic pathways. Pleiotropy is likely to be most constraining when entire genetic pathways are shared among traits (Figure 2a). In this case, an environmental cue affects a gene, which transmits the environmental signal down the shared pathway, thereby regulating two (or more) different traits with the shared signal. More independent environmental responses of traits may occur if one gene responds differently to the same cue to regulate different traits (Figure 2b); for example, if high temperature up-regulates the gene at one life stage but down-regulates it at another. Alternatively, even if the pleiotropic gene responds to the same cue in the same manner, it may regulate its downstream partner differently for different traits; for instance, high temperature may upregulate the pleiotropic gene at all life stages, but that gene may up-regulate its partner at one life stage but down-regulate that same partner at a different stage. The ability of a pleiotropic gene to respond differently to the environment, or to interact with its partner differently, when regulating different traits would permit distinct responses of different traits to the same environmental cue, despite sharing components of genetic pathways. In this manner, potentially detrimental effects of pleiotropy can be mitigated. Finally, pleiotropy could be least constraining when genetic pathways diverge soon after the

pleiotropic gene (for example, the pleiotropic gene has more than one interacting partner), such that different downstream genes regulate different traits, each according to their own environmental responses (Figure 2c). The more elements in a pathway that regulate only one trait (as opposed to both traits), the more opportunity for the independent regulation of those traits by the environment. To understand how constraining pleiotropy is likely to be for adaptive evolution, we need to know the degree to which pleiotropic genes operate in the same manner and in the same genetic pathways at multiple life stages. Exploring the degree of concordance of the pathways that sense and transduce environmental cues at different developmental stages may elucidate how pleiotropic genes are able to regulate adaptive life cycles. Further analysis of the pathways whereby flowering-time genes regulate germination shows evidence of concordance of some genetic pathways across life stages, but functional divergence of gene function across life stages in other pathways.

The autonomous pathway appears to show a high degree of concordance in the pathways whereby it regulates germination and flowering (Figure 2a). The genes in the autonomous pathway down-regulate *FLC* expression via epigenetic interactions, and by doing so allow flowering to proceed (Simpson, 2004; Cheng *et al.*, 2017). The genes *FY*, *FLK*, *FCA*, *FPA* and *FVE* also regulate seed germination, and they do so in a manner that is consistent with conservation of their regulation of *FLC* (Auge *et al.*, 2018). Specifically, disruption of autonomous-pathway genes increases germination, which is consistent with their role as repressors of *FLC*—a promoter of germination. Furthermore, different combinations of double mutants of the autonomous pathway genes show responses suggesting conservation of genetic interactions among flowering and germination. However, the molecular mechanisms by which they regulate germination might also differ from that displayed in the regulation of flowering. *FY* regulates germination by increasing sensitivity to ABA, and this is independent of protein domains required for FY-FCA protein interaction and regulation of flowering time (Jiang *et al.*, 2012). Furthermore, *FY* is required for proper RNA 3' processing of the proximally polyadenylated short *DELAY OF GERMINATION1* (*DOG1*) transcript (Cyrek *et al.*, 2016), the key regulator of dormancy in *A. thaliana* seeds (Alonso-Blanco *et al.*, 2003; Bentsink *et al.*, 2006). Therefore, evidence suggests that autonomous-pathway genes largely operate through *FLC* in their regulation of both germination and flowering, but some of those genes may also have different functions elsewhere in the genetic pathway that regulates germination.

In contrast to the autonomous pathway, several genes in the vernalization pathway appear to have different functions when regulating flowering time versus germination, even with respect to how they interact with *FLC*. The vernalization pathway includes inducers of *FLC*—*FRIGIDA* (*FRI*) and *VERNALIZATION INDEPENDENCE3* (*VIP3*)—and repressors of *FLC*--*VERNALIZATION2* (*VRN2*) and *VERNALIZATION INSENSITIVE3* (*VIN3*). All these genes also influence germination (Auge *et al.*, 2017). *FRI* and *VIN3* regulate germination in a manner that is consistent with conservation of their function as regulators of *FLC*, while *VIP3* and *VRN2* regulate germination either independently of *FLC* or by regulating *FLC* in a manner that differs from their function in flowering-time regulation. Disruption of *FRI* also altered germination even when *FLC* was not functional (albeit weakly), indicating that *FRI* operates in a germination pathway that is independent of *FLC*. In addition, *VIP4*, *VIP5* and *VIP6*, all genes known to be inducers of *FLC* at the pre-reproductive stage and therefore repressors of flowering (Oh *et al.*, 2004), enhance seed dormancy (Liu *et al.*, 2011). This effect on dormancy is inconsistent with their role as *FLC* repressors, since a lower *FLC* level is expected to increase rather than decrease germination. These genes also increase in expression during seed maturation (Liu *et al.*, 2011), yet *FLC* is also up-regulated at late stages of seed maturation (Chiang *et al.*, 2009). Combined these results suggest that *VIP* genes (including *VIP3*) might regulate germination independently of *FLC* (Figure 2c) or by up-regulating, rather than down-regulating, *FLC* during seed maturation (Figure 2b).

Downstream of *FLC*, the floral integrators *FT*, *FD* and *SOC1* integrate temporal and spatial information to regulate flowering (Wigge *et al.*, 2005; Lee & Lee, 2010; Wellmer & Riechmann, 2010). Some concordance of function has been demonstrated in how these downstream integrators regulate germination. Specifically, mutant seeds of *SOC1* have a higher germination propensity than its reference wild type accession, and mutant seeds of the meristem identity gene *AP1*, which is positively regulated (directly or indirectly) by *FT* and *SOC1*, also show a response consistent with functional conservation farther downstream in the flowering pathway during the regulation of germination (Chiang *et al.*, 2009; Wellmer & Riechmann, 2010).

In contrast, some functional differences have been characterized in floral integrator genes for their regulation of germination responses to seed-maturation temperature (Chen *et al.*, 2014). In particular, *FT* is required to reduce dormancy when maternal plants are grown in warm temperatures; *ft* mutants matured in warm conditions fail to repress the synthesis of

proanthocyanidins, which decrease coat permeability and thereby decrease germination (Chen *et al.*, 2014; MacGregor *et al.*, 2015). This effect appears to be mediated by regulation of *FLC* by *FT* (Chen & Penfield, 2018). As such, it appears that, instead of *FLC* repressing *FT* as it does in flowering-time regulation, *FT* can regulate *FLC* expression during its regulation of seed germination (Chen & Penfield, 2018). The ability of both *FLC* and *FT* to affect seed germination independently may explain why in some assays *FLC* acts a germination repressor (Chen & Penfield, 2018), and sometimes as a germination inducer (Chiang *et al.*, 2009). Thus, there may be more than one mechanism by which flowering time genes affect seed dormancy.

In summary, autonomous-pathway genes and some downstream integrators appear to have more concordant function across the life cycle than genes in the vernalization pathway. It is interesting to note that the autonomous pathway is cued by many internal cues (age, nutritional status) and only partially by external cues, unlike the vernalization pathway; whether this difference contributes to its functional concordance across development is not known. Functional diversity of pleiotropic genes across the life cycle certainly occurs, however. Such diversity appears to occur through multiple mechanisms, from potentially regulating the same interacting partners differently at different life stages, to acting independently of them. Pleiotropic constraints, therefore, can evolve through the evolution of how genes within a pathway interact.

VI. Can pleiotropy be a precursor to divergence in gene function across taxa?

Pleiotropic genes can have different functions in their regulation of different traits, as discussed above. Can this functional diversity within a single individual in any way contribute to divergence of gene function among taxa? Although no examples illustrate this evolutionary scenario, to our knowledge, certain key components of that scenario have been documented.

First, pleiotropic genes exhibit functional diversity in their regulation of different traits. Although some genes exhibit concordant function when regulating different traits (e.g. autonomous-pathway genes regulating flowering and germination), several genes regulate flowering through different mechanisms than they use to regulate germination. Some appear to operate through different partners—for example *FRI* influencing germination

independently of *FLC* (Blair *et al.*, 2017; Auge *et al.*, 2017), or *FY* regulating flowering and germination through different protein domains (Jiang *et al.*, 2011). Others may retain partners but interact with them differently when regulating different traits. For instance, a gene may up-regulate a partner at one life stage but down-regulate it in another (e.g. the *VIP* genes, potentially; Oh *et al.*, 2004; Liu *et al.*, 2011).

Second, many genes originally identified in the *A. thaliana* flowering time pathway are conserved across taxa, suggesting strong selection for maintaining seasonal detection systems in plants (Ream *et al.*, 2012; Shrestha *et al.*, 2014). Even lineages as evolutionarily distant as the grasses have homologs of major *A. thaliana* flowering-time genes. Some of these genes retain similar function, even over such evolutionary distances. For instance, in *Brachipodium distachyon*, the homolog of *AtFT* (*BdFT1*) has a similar function in both taxa of promoting flowering after its repression is released (Woods *et al.*, 2017). Some genes also show evidence of conserved pleiotropy, such as barley homologs of the *A. thaliana* floral integrator *SOC1* (*HvSOC1-like1* and *HvSOC1-like2)*, which are highly expressed during seed development and likely have a role in dormancy regulation and pre-harvest sprouting in this crop species (Papaefthimiou *et al.*, 2012).

Third, while genes are conserved across taxa, the genetic pathways in which these genes function can vary, even when they regulate the same trait of flowering time. Sometimes homologous genes act in the same pathway, but the order of those genes differs, and sometimes they differ in the immediate interacting partners. For instance, the homolog of *AtCO* in *Brachypodium* (*BdVRN2*) and wheat (*TaVRN2*), and the orthologues of *AtFT* in wheat and barley, *VRN3*, have the function that *AtFLC* has in the *A. thaliana* flowering pathway (Yan *et al.*, 2004, 2006; Sharma *et al.*, 2017). Similarly, the homolog of *AtAP1* in *Brachypodium* (*BdVRN1*) has the function that *AtVRN1* has in *A. thaliana* (Feng *et al.*, 2017). In other words, as these flowering-time genes have diverged between these taxa, they acquired new molecular functions and new interacting partners that caused them to be in different positions within the flowering-time pathway (Donohue, 2017).

Fourth, homologous genes have diverged across taxa such that they may even regulate different traits. *MOTHER OF FT AND TFL1* (*MFT*) is an inducer of flowering in *A. thaliana* and acts redundantly with *FT* (Yoo *et al.*, 2004). *MFT* is also an inducer of germination in *A. thaliana*, mediating GA, ABA and BR crosstalk (Xi *et al.*, 2010; Xi & Yu, 2010). Homologs of this gene, however, are expressed in seeds in several species, and they act primarily as a

germination regulator (Nishikawa *et al.*, 2008; Li *et al.*, 2014a; Tao *et al.*, 2014). For example, the wheat homolog of *MFT* shows high expression in dormant grains, but the gene does not affect flowering (Nakamura *et al.*, 2011).

One recent example shows that pleiotropy itself can diverge across taxa (Hughes *et al.*, 2019). In *Arabis alpina*, the homolog of *A. thaliana's FLC*, *PEP1*, is a repressor of flowering (as is *FLC* in *A. thaliana*). In *A. thaliana*, *FLC* promotes seed germination (as discussed above), but in *A. alpina PEP1* promotes seed dormancy. In both species, the pleiotropic effects of those genes appear to impose the life-cycle typical of the species: in winter-annual *A. thaliana*, *FLC* could promote germination in the autumn and represses flowering of those seedlings until after winter; in spring-germinating perennial *A. alpina*, *PEP1* could postpone germination until spring and enforce a perennial life cycle via the stage-specific expression of *PEP1*, as discussed above.

These results show that the specific ways in which taxa have diverged in gene function and corresponding genetic pathways may be similar to ways in which the functions of pleiotropic genes differ in their regulation of different traits within a species. Once diversity of function in pleiotropic genes evolves within a species, divergence in function across taxa can occur by modifying components of each pleiotropic pathway, or by the atrophy of one of those pathways through mutation. Mutations may disrupt one function but not others if they occur in a specific domain that regulates one trait but not the other, or in a specific *cis*-regulatory region that affects only one trait. In short, an ancestral pleiotropic gene that regulates more than one trait (Figure 3a) may evolve functional diversity in the regulation of those traits (Figure 3b). Subsequent loss of function of components of one pathway within one taxon, and loss of different components in the other pathway in another taxon (Figure 3c-f), could cause the gene to have two qualitatively different functions in the two taxa, in a sort of sub-functionalization.

Some evidence that this mechanism of divergence in gene function can occur comes in the form of genetic variation in gene function within *A. thaliana* itself. *MFT* expression in *A. thaliana* seeds shows some degree of natural variation, and it is regulated by soil temperature during dormancy cycling and correlated with germinability in seed banks (Footitt *et al.*, 2011, 2013, 2014). Genetic variation exists in the strength of the contribution of *MFT* to the regulation of germination. Also, the transcription factor *SPATULA* (*SPT*) regulates *MFT* expression, but whether it up- or down-regulates it differs between accessions (Vaistij *et al.*,

2013), such that a knockout of *SPT* in one accession increases germination, while a knockout in the other accession decreases germination. This surprising result can be explained by the fact that *SPT* operates in two different pathways: one that promotes germination and the other that represses it (Vaistij *et al.*, 2013). In one background, the promotive pathway is stronger whereas in the other background the repressive pathway is the dominant one. Consequently, the effect of disrupting *SPT* function is the opposite in the two backgrounds. This example shows that a gene that operates in more than one pathway may quickly evolve to have opposite functions across accessions within a species (Figure 3d; Trait 2a and 2b). Can that same process of divergence occur across taxa? Intriguingly, this example of *SPT*, in which disruption of a gene operating in more than one pathway has opposite phenotypic effects in two backgrounds of the same species, is not unlike the example of the divergence of gene function across species, in which *PEP1* had the opposite effect on germination in *A. alpina* compared to *FLC* in *A. thaliana*.

VII. Summary and conclusions

The flowering-time pathway in *Arabidopsis thaliana* is one of the best characterized genetic pathways in plants, yet new pleiotropic functions of its genes are being discovered rapidly. Even though patterns of population-genetic variation reveal evidence for natural selection on these genes, interpretations of their functional and adaptive significance will need revision to accommodate the different pathways of direct and indirect selection acting through the multiple traits that a single gene may regulate. Organisms developing in the wild, moreover, may express even more pleiotropy than can be detected under controlled conditions in the lab, since indirect pathways of pleiotropy operate through environmental interactions, whereby one trait may alter the environment experienced by subsequent life stages, which in turn alters the expression of plastic traits expressed at later stages. Whether pleiotropy coordinates responses to environments across the life cycle or constrains the expression of optimal life cycles remains to be tested in the field. However, evolutionary constraints of pleiotropy can potentially be mitigated, such that different life stages or traits can respond independently to environmental cues, when different molecular functions evolve for the regulation of different traits. Such functional diversity has been observed across the life cycle of a single species, as shown in the example of pleiotropic regulation of flowering and germination in *A. thaliana*. Divergence of gene function also occurs across taxa. It is possible that pleiotropy itself, when genes have different functions across traits, may contribute to the

divergence of gene function across taxa, but comparative-genetic studies that investigate evolutionary changes in gene function and in pleiotropy itself would need to test that possibility.

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Table 1 Genes with wide pleiotropic effects in plant growth and development: we highlight those that regulate germination and flowering, along with other traits.

Gene	Effects on development	References
Gibberellins (GA)		
AtRGA	Counteracts GA promotion of flowering	Yu *et al*. 2004
	Modulates floral development	Tyler *et al*. 2004
	Enhances RGL2 function on germination repression	Cao *et al*. 2005
AtGAI	Enhances RGL2 function on germination repression	Cao *et al*. 2005
AtRGL1	Modulates floral development	Tyler *et al*. 2004
	Enhances RGL2 function on germination repression	Cao *et al*. 2005
AtRGL2	Major repressor of germination	Lee *et al*. 2002; Tyler *et al*. 2004
	Counteracts GA promotion of flowering	Yu *et al*. 2004
	Modulates floral development	Tyler *et al*. 2004
Abscisic Acid (ABA)		
AtABI5	Acts downstream of ABI3 to determine a post-germination developmental checkpoint and arrest seedling growth	Lopez-Molina *et al*. 2001; Lopez-Molina *et al*. 2002
	Delays flowering by direct transactivation of *FLC*	Wang *et al*. 2013
AtABH1	Negatively regulates ABA signaling during germination via interaction with ABI4	Hugouvieux *et al*. 2001; Daszkowska-Golec *et al*. 2012
	Delays flowering time by regulating mRNA processing of *CO*, *FLC* and *FLM*	Kuhn *et al*. 2007
Photomorphogenesis		

AtCRY1	Promotes flowering in response to BL (redundantly with CRY2 and PHYA)	Mockler *et al*. 2003
HvCRY1	Inhibits germination in response to BL by inducing *HvNCED1* and *AtNCED9*, and repressing *ABA8'OH-1*	Barrero *et al*. 2014
AtCRY2	Promotes flowering in response to BL (redundantly with CRY1 and PHYA)	Mockler *et al*. 2003
AtPHYB	Temperature-dependent control of flowering time via *FT* regulation	Halliday *et al*. 2003
	Induces germination; regulates seed responsiveness to GA (increases sensitivity)	Arana *et al*. 2014; Sánchez-Lamas *et al*. 2016
	Strongly represses flowering; required for photoperiodic response	Sánchez-Lamas *et al*. 2016
AtPHYA	Promotes flowering in response to BL (redundantly with CRY1 and CRY2) and to FR	Mockler *et al*. 2003
	Induces germination under low R:FR; regulates seed responsiveness to GA (increases sensitivity)	Arana *et al*. 2014; Sánchez-Lamas *et al*. 2016
AtPHYC	Negatively regulates germination under light	Arana *et al*. 2014
	Regulates seed responsiveness to GA (decreases sensitivity)	Sánchez-Lamas *et al*. 2016
	Required for photoperiodic response and regulation of flowering	Sánchez-Lamas *et al*. 2016
AtPHYD	Regulates seed responsiveness to GA (decreases sensitivity)	Arana *et al*. 2014; Sánchez-Lamas *et al*. 2016
	Promotes phyA-induction of germination in low R:FR	Arana *et al*. 2014
	Required for cycling out of seed secondary dormant state induced by hot stratification	Martel *et al*. 2018

AtPHYE	Promotes phyA-induction of germination in low R:FR	Arana *et al*. 2014
	Regulates seed responsiveness to GA (decreases sensitivity)	Arana *et al*. 2014; Sánchez-Lamas *et al*. 2016
	Strongly represses flowering	Sánchez-Lamas *et al*. 2016
Clock genes		
AtGI	Regulates flowering response to photoperiod by direct activation of *FT*	Fowler *et al*. 1999; Park *et al*. 1999; Zhang *et al*. 2007
	Required for promotion of germination by phyA in response to FR and for response of seeds to dormancy breaking treatments and hormones	Oliverio *et al*. 2007; Penfield and Hall 2009
SlGI	Correlated with phyA-mediated inhibition of germination in response to prolonged FR irradiation	Auge *et al*. 2009

Table 2 Genes in specific flowering-time pathways that exhibit pleiotropic effects on germination/dormancy (and vice versa).

Gene	Effects on development	References
Vernalization		
AtFLC	Represses flowering by repressing expression of floral integrators	Michaels & Amasino 1999; Sheldon *et al*. 2000
	Promotes germination and requires a functional *FRI* to exert its action	Chiang *et al*. 2009; Blair *et al*. 2017; Auge *et al*. 2017
AtFRI	Represses flowering through regulation of *FLC*	Johanson *et al*. 2000; Michaels and Amasino 2001
	Promotes germination likely through regulation of *FLC*; negatively influences germination when combined with a non-functional *FLC*	Blair *et al*. 2017; Auge *et al*. 2017
	Enhances drought resistance by activating proline synthesis in an *FLC*-dependent way	Lovell *et al*. 2013; Chen *et al*. 2018
AtVIP3	Represses flowering through regulation of *FLC*	Zhang *et al*. 2003
	Negatively influences germination, likely independently of *FLC*	Auge *et al*. 2017
AtVIN3	Involved in the epigenetic silencing of *FLC* to induce flowering by vernalization	Sung and Amasino 2004
	Negatively regulates germination, likely in an *FLC*-dependent manner	Auge *et al*. 2017
AtVRN2	Involved in the epigenetic silencing of *FLC* to induce flowering by vernalization	Bastow *et al*. 2004
	Positively influences germination, likely independently of *FLC*	Auge *et al*. 2017

AtVIP4 *AtVIP5* *AtVIP6/ELF8*	Represses flowering through regulation of *FLC*	Zhang and van Nocker 2002; Oh *et al*. 2004
	Enhance seed dormancy	Liu *et al*. 2011
Autonomous pathway		
AtFY	Enhances seed dormancy; increases sensitivity to ABA; required for RNA processing of *DOG1*	Jiang *et al*. 2012; Cyrek *et al* 2016; Auge *et al*. 2018
AtFLK	Enhances seed dormancy	Auge *et al*. 2018
AtFCA	Enhances seed dormancy	Auge *et al*. 2018
AtFPA	Enhances seed dormancy	Auge *et al*. 2018
AtFVE	Enhances seed dormancy	Auge *et al*. 2018
Floral integrators and meristem identity genes		
AtFT	Integrates environmental information from the flowering signaling pathways	Wigge *et al*. 2005; Lee and Lee 2010; Wellmer and Reichmann 2010
	Required to induce germination when seed maturation occurs in warm temperatures; regulates coat permeability through regulation of proanthocyanidins; regulates *FLC* expression during germination through *COOLAIR*	Chen *et al*. 2014; Chen *et al*. 2018
AtFD	Integrates environmental information from the flowering signaling pathways	Wigge *et al*. 2005; Lee and Lee 2010; Wellmer and Reichmann 2010
AtSOC1	Integrates environmental information from the flowering signaling pathways	Wigge *et al*. 2005; Lee and Lee 2010; Wellmer and Reichmann

		2010
	Negative regulator of germination	Chiang *et al*. 2009
HvSOC1-like1 *HvSOC1-like2*	Likely role in dormancy regulation and pre-harvest sprouting	Papaefthimiou *et al*. 2012
AtAP1	Required for determine floral organ identity	Madel *et al*. 1992
	Negative regulator of germination	Chiang *et al*. 2009
Other flowering time genes		
AtMFT	Induces flowering, acting redundantly with *FT*	Yoo *et al*. 2004
	Regulates germination; associated with germinability of seeds banks	Xi *et al*. 2008; Li *et al*. 2014; Footitt *et al*. 2011, 2013, 2014
TaMFT	Correlated with dormancy	Nakamura *et al*. 2011
Dormancy-related genes		
AtDOG1	Major regulator of seed dormancy	Alonso-Blanco *et al*. 2003; Bentsink *et al*. 2006
	Regulates flowering time by influencing miR156 and miR172 levels	Huo *et al*. 2016
	Regulates RNA processing of *FY*	Cyrek *et al*. 2016

Figure Legends

Figure 1. Major flowering pathways in *Arabidopsis thaliana* and the potential pathways of pleiotropy. **(a)** Flowering time is regulated by diverse pathways of environmental inputs, which are integrated to regulate the transition to reproduction. **(b)** Direct pleiotropy, or genetic pleiotropy, occurs when one gene is involved in genetic pathways that regulate more than one trait. Environmentally-induced pleiotropy occurs when a gene regulates seasonal developmental timing, which determines seasonal conditions experienced subsequently, which in turn influences the expression of later traits. An example is that of a gene that regulates the timing of seed germination; germination time determines the seasonal environmental conditions experienced after germination (e.g. exposure to chilling), which in turn influences flowering time. Both direct genetic pleiotropy and environmentally induced pleiotropy may contribute to genetic correlations among traits.

Figure 2. Mechanisms of mitigation of pleiotropic constraints. **(a)** Concordant pathways: Strong genetic correlations between two traits result when many components of genetic pathways regulate more than one trait (strong pleiotropy). The pleiotropic gene and its associated pathway can be said to be concordant in function across the two traits. Such concordance may impede independent responses of the two traits to environmental cues, impairing the expression of adaptive phenotypes. **(b)** Divergence in regulation of genes within a shared pathway: Left, constraints of pleiotropy can be mitigated if the pleiotropic gene is regulated differently by the same environmental cue at different life stages or in different traits. Right, it can also be mitigated if the pleiotropic gene regulates the same downstream partner differently at different life stages or in different traits. This diversification of function has the potential to allow independent responses to the environment by different traits and the potential for achieving optimal phenotypes in different life stages. **(c)** Divergence in pathways: Constraints of pleiotropy can be mitigated if the pleiotropic gene acts in two independent pathways to regulate two traits.

Figure 3: How pleiotropy may contribute to divergence of gene function across taxa. In Ancestral Taxon 1 **(a)**, a gene is pleiotropic, and its function is highly concordant in its regulation of two traits. In Taxon 2 **(b)**, the gene has evolved different functions in its regulation of two traits, and it also evolved a third function--a pathway that antagonistically regulates the second trait (e.g. similar to *SPT* and its antagonistic regulation of

germination/dormancy). After taxonomic divergence (divergence of ecotypes within species, or speciation), the gene may lose one or more functions through loss-of-function mutations. Loss of function mutations may disrupt one function but not the others if they occur in a specific domain or in a specific *cis*-regulatory region that affects only one trait. For example, Taxon A **(c)** lost function for Trait 2a and 2b (germination/dormancy); Taxon B **(d)** lost function for Trait 1 (flowering); Taxon C **(e)** lost function for Trait 2b (dormancy), and Taxon D **(f)** lost function for Trait 2a (germination). In this manner, the gene regulates different traits in the derived taxa (Taxon A vs B), or it regulates the same trait in opposite directions (Taxon C vs D; similar to *FLC* and its homolog *PEP1*).

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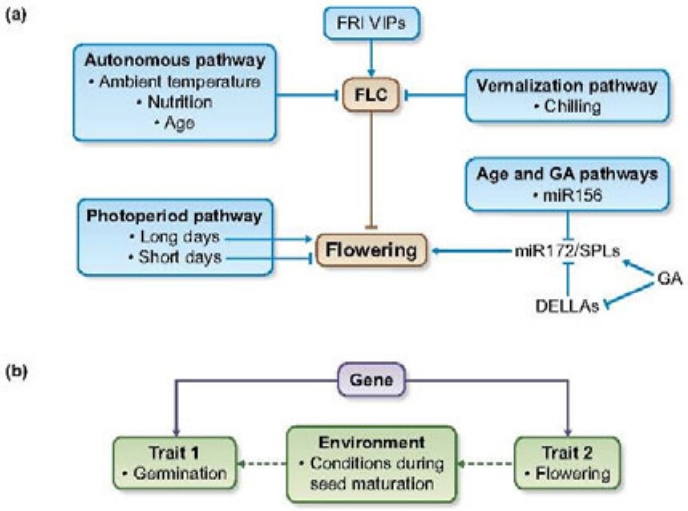

Figure 1

Tansley Review 29239

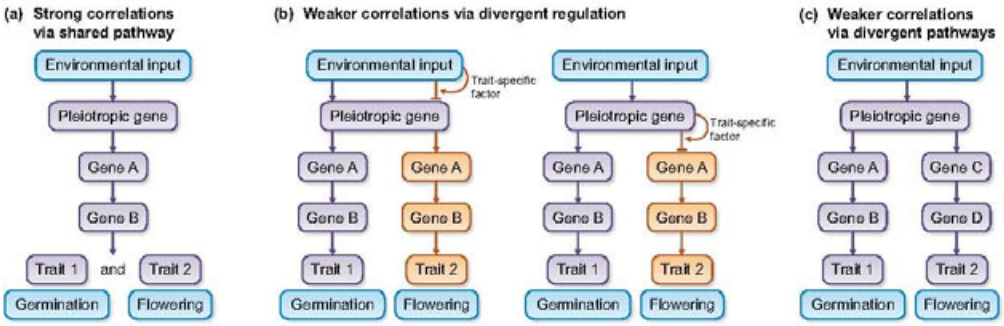

Figure 2

Tansley Review 29239

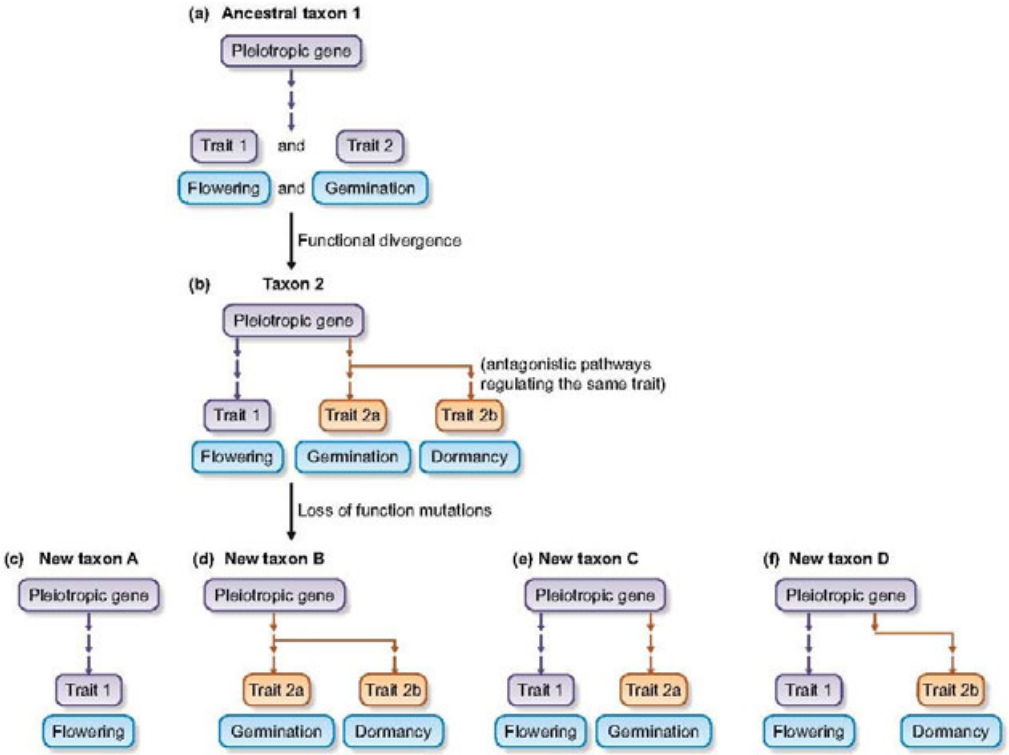

Figure 3

Tansley Review 29239