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SPECIAL FEATURE

New insights into mechanism and evolution of mast flowering: feedback between theory and experiment

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Challenges in studies on flowering time: interfaces between phenological research and the molecular network of flowering genes

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Abstract Flowering time is a well-studied subject in ecology, evolution and molecular biology. Long-term phenological studies have shown relationships between flowering time and environmental and endogenous factors in many species. In contrast, molecular studies using model plants have revealed a complex regulatory network of flowering. We propose that flowering would be a model trait for the integrated study of ecology, evolution and molecular biology. We introduce briefly the flowering regulatory pathways of *Arabidopsis thaliana*, followed by molecular techniques such as transgenic manipulation, quantitative real-time PCR and detection of differentially expressed genes that could facilitate the study of 'nonmodel' species of ecological interest but with little available genome information. Application of the flowering gene network to wild species will be illustrated by two examples: modeling and prediction of the expression of flowering genes in *Arabidopsis halleri*, and the latitudinal cline of bud set and cessation in *Populus*. Finally, we discuss the challenges in integrating knowledge of the regulatory network on flowering into ecologically unique flowering phenomena such as synchronous intermittent mass flowering—the topic of this special issue.

Keywords Phenology · Flowering time · Mass flowering · General flowering · Dipterocarpaceae

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Introduction

Timing of flowering is critical to plant reproductive success. Flowering at suboptimal periods could sometimes expose plants to severe environmental conditions (Stinson 2004), low visitation by pollinators (O'Neil 1999; Aizen 2003; Morinaga et al. 2003) and high risks of herbivory (Kawagoe and Kudoh 2010). Therefore, plants have complex mechanisms that enable them to flower at appropriate times in response to both external and internal cues.

Phenological studies employing quantitative and long-term climatic data have revealed links between flowering time and factors such as weather and resources in many species (see, for example, Vasek and Sauer 1971; Opler et al. 1976; Fischer and Turner 1978; Rees et al. 2002). However, it is often difficult to identify the environmental triggers of flowering and to predict plant phenology (Chuine et al. 2003). This is because morphological signs of flowering are qualitative (flowered or not), and are visible only after complex signals have already induced flowering. It is also because the direct measurement of the internal developmental state of plants during floral initiation is lacking (Aikawa et al. 2010).

Recently, studies using molecular biology and genetics have identified the physiological and developmental mechanisms underlying flowering in the model plant *Arabidopsis thaliana* (reviewed in Michaels 2009; Amasino 2010; Fornara et al. 2010). These studies have revealed that flowering is regulated by orchestrated changes in the activities of a number of flowering genes. Internal states such as expression patterns of genes are changed dramatically immediately after the perception of flowering cues, although few morphological changes are visible externally (Balasubramanian et al. 2006). This suggests that the measurement of internal states would provide essential parameters useful in predicting when plants flower.

Although knowledge of the genes underlying flowering should be accumulated in diverse plant taxa, until

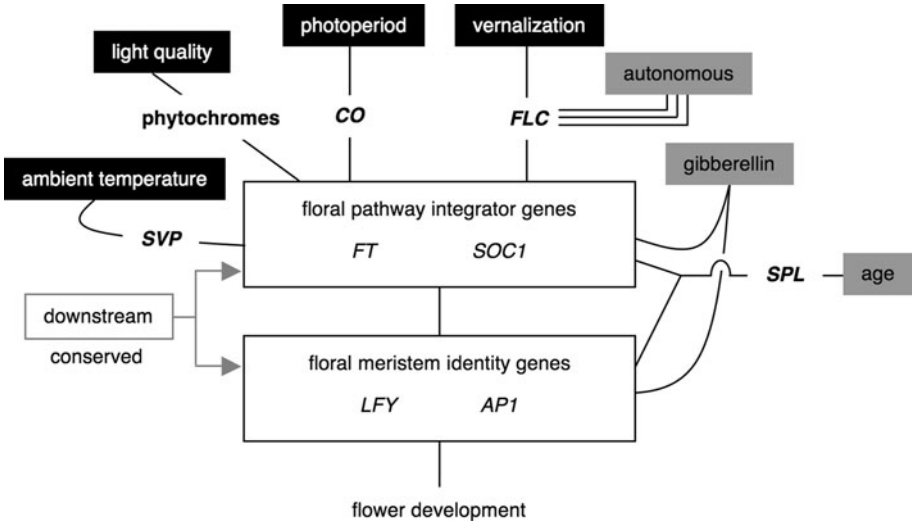

Fig. 1 Simplified scheme of flowering time control in *A. thaliana*. *Lines* indicate flowering pathways. *Black* and *gray boxes* indicate environmental and endogenous signals to flower, respectively. Flowering pathways converge on floral pathway integrator genes, and the signal is then transmitted to floral meristem identity genes further downstream. Functionally conserved orthologues of floral pathway integrator and floral meristem identity genes have been observed in other diverse plant species. The genes written in *bold* that lie upstream of floral pathway integrators are defined as upstream genes, and the others as downstream genes (see text)

recently the identification of genes involved in flowering time control was limited to only a few model plants (Meinke et al. 1998; Shimamoto and Kyozuka 2002). One of the major reasons is that a large amount of genetic and genomic resources is required to identify gene function. However, as the number of identified genes in model plants has increased, techniques in molecular biology, such as plant transformation and measurement of gene expression levels, have enabled knowledge of these genes to be applied to other species growing in natural environments (Böhlenius et al. 2006; Aikawa et al. 2010).

This review focuses on the potential application of knowledge of the flowering gene network to understand flowering events of wild species in natural environments. To this end, we first summarize our current understanding of flowering time regulation in model plants. Then, we illustrate a few examples in which information on the flowering network was utilized to understand ecological traits. Finally, we discuss the challenge of how to apply knowledge of the flowering regulatory network to the understanding of ecologically unique flowering phenomena, such as mass flowering.

Regulatory network of flowering in *A. thaliana*

Arabidopsis thaliana is a widespread annual weed in open places in temperate regions and is native to Europe, central Asia and north Africa (Mitchell-Olds and Schmitt 2006). In most of its distribution range, it flowers in spring in response to various environmental cues to initiate flowering, such as change in day length (photoperiod), prolonged cold temperature in winter (vernalization), light quality and ambient temperature (Michaels 2009; Amasino 2010; Fornara et al. 2010). Furthermore, endogenous information, such as the level of the plant hormone gibberellin and the age of the plant, is also utilized as a signal to flower (Michaels 2009; Amasino 2010; Fornara et al. 2010). These routes for sensing flowering cues are called flowering pathways (Fig. 1; Appendix 1).

The molecular and genetic basis of flowering has been studied extensively using mutants of *A. thaliana*. The first step in molecular genetic studies is to screen for mutants. So-called wild-type plants, which were collected originally under natural conditions and are maintained as inbred lines in laboratories, are subjected to mutagens such as chemicals and radiation in order to induce mutations. Mutants defective in floral promoters and repressors show late and early flowering phenotypes, respectively (Fig. 2). These studies have revealed that ~180 genes are involved in flowering time control (Fornara et al. 2010). Combined with physiological data, researchers have shown how these genes act in flowering pathways. Multiple flowering pathways converge to a small number of genes referred to as "floral pathway integrators", such as the *FLOWERING LOCUS T* (*FT*) gene and the *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) gene (Kardailsky et al. 1999; Kobayashi et al. 1999; Blázquez and Weigel 2000; Lee et al. 2000; Samach et al. 2000). The *FT* and *SOC1* genes receive inputs from multiple pathways, and then activate further downstream "floral meristem identity genes" such as *LEAFY* (*LFY*) and *APETALA 1* (*AP1*) (Lee et al. 2000; Samach et al. 2000; Abe et al. 2005; Wigge et al. 2005). Floral meristem identity genes are required in the commitment to flower. A brief overview of the genetic network of flowering pathways is summarized in Appendix 1.

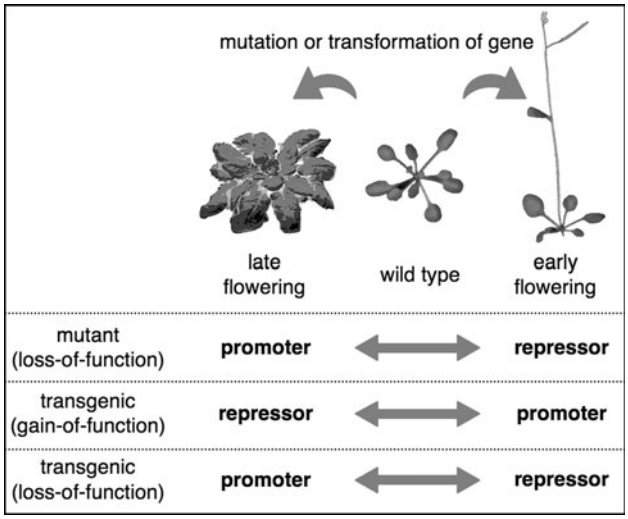

Fig. 2 The effects of mutation and transformation of floral promoter genes and repressor genes. Promoter and repressor in *bold* indicate gene activities for flowering. The column headings (wild type, or late or early flowering) indicate phenotypes caused by mutation or transformation of floral promoter or repressor genes. Mutants with defects in floral promoter genes and repressor genes show late and early flowering, respectively. When floral repressor and promoter genes are overexpressed in transgenic plants, transgenic plants show late and early flowering, respectively. This type of experiment is classified as a gain-of-function analysis. On the other hand, loss-of-function transgenic plants in which those genes are downregulated show phenotypes opposite to those of gain-of-function analyses

Conservation and divergence of the flowering regulatory network

Recently, in addition to *A. thaliana*, many flowering genes have been identified, mainly in cereal crops such as rice (*Oryza sativa*), wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) that are distantly related to *A. thaliana* in angiosperm phylogeny (reviewed in Dennis and Peacock 2009; Distelfeld et al. 2009; Greenup et al. 2009). The emerging picture is that downstream genes in flowering pathways are highly conserved but upstream genes are relatively diversified. Here, for the convenience of explanation, the genes that lie upstream of floral pathway integrators are defined as upstream genes, and the others as downstream genes (Fig. 1).

First, we discuss well-conserved downstream genes such as floral pathway integrator genes and floral meristem identity genes, orthologues of which have been found in cereal genomes (Higgins et al. 2010). Orthologues are defined as genes in two separate species that derive from the same ancestral gene of the common ancestor. Transgenic and/or mutant analyses of many of these orthologues have indicated that their functions in flowering regulation are conserved (see, for example, Kojima et al. 2002; Murai et al. 2003; Lee et al. 2004; Yan et al. 2006; Shitsukawa et al. 2007; Rao et al. 2008). Furthermore, in wheat, the regulatory network that activates expression of the *AP1* gene by a protein complex containing FT was found to be functionally conserved (Li and Dubcovsky 2008). These downstream genes are conserved not only in herbal monocotyledonous cereals but also in woody plants such as *Populus*, *Citrus*, *Malus*, etc. (see, for example, Peña et al. 2001; Böhlenius et al. 2006; Hättasch et al. 2008; Nishikawa et al. 2009, 2010; Kotoda et al. 2010).

Compared with downstream genes, upstream genes appear to be relatively diversified. Although components and mechanisms affecting photoperiod (Greenup et al. 2009) and age pathways (Chuck et al. 2007) are widely conserved in angiosperms, major differences were found in the mechanisms affecting vernalization and autonomous pathways, as well as the effects of gibberellins. In *A. thaliana*, both the vernalization and autonomous pathways are regulated by the floral repressor gene, *FLOWERING LOCUS C* (*FLC*) (Michaels and Amasino 1999; Sheldon et al. 1999). In contrast, no apparent *FLC* orthologue has been found in cereals (Greenup et al. 2009) although vernalization promotes flowering in some temperate cereals such as wheat and barley (Dennis and Peacock 2009; Distelfeld et al. 2009; Greenup et al. 2009). Although *FLC* orthologues in the Brassicaceae are clearly functionally related to *A. thaliana FLC*, conservation of the *FLC*-mediated vernalization pathway outside the Brassicaceae is controversial (Reeves et al. 2007; Locascio et al. 2009). Furthermore, the role of gibberellins in flowering seems to be species specific. In *A. thaliana*, gibberellins contribute to the promotion of flowering. In contrast, in many perennial plants, they are known to inhibit flowering (Mutasa-Göttgens and Hedden 2009).

Beyond the model plant

How useful is knowledge obtained from model plants in understanding the phenomena observed in non-model plants grown in natural environments? In this section, we discuss methodological tools and two examples.

Useful methods in molecular biology

First, we discuss some useful methods used in molecular biology to apply knowledge of genes involved in flowering time regulation to other species grown in natural environments.

One of these methods is the transgenic technique (Lloyd et al. 1986). This technique is used to confirm the function of the target flowering gene. If the function of the gene in flowering regulation is conserved, flowering time will change in transgenic plants. The analyses using transgenic plants can be classified into two types. In a gain-of-function analysis, a target gene is overexpressed (Ow et al. 1986). Therefore, the function of the target gene is enhanced in transgenic plants. The overexpression of promoter and repressor genes of flowering would show early and late flowering phenotypes, respectively

(Fig. 2). In a loss-of-function analysis, the expression of a target gene is downregulated (Chuang and Meyerowitz 2000; Schwab et al. 2006). Therefore, the function of the target gene is impaired in the transgenic plants. If the genes function as flowering promoters, the transgenic plants show late flowering (Fig. 2). On the other hand, plants transgenic for floral repressors show early flowering. In particular, the transgenic technique has been applied enthusiastically to horticultural trees such as apple and citrus, for which little genomic information is available (Peña et al. 2001; Kotoda et al. 2010; Nishikawa et al. 2010). In these trees, transgenic plants that show early flowering are expected to be valuable and powerful tools for efficient breeding as well as tools for understanding the function of flowering genes. A more comprehensive coverage of the physiological and molecular biological aspects of flowering time regulation in horticultural trees was reviewed by Wilkie et al. (2008).

It is important to note the limitations of transgenic techniques. The method of transformation is highly species specific and has been developed only in a limited number of species. To alleviate this limitation, gain-of-function transgenic *A. thaliana* plants with target genes from a different species may be used as an alternative method of demonstrating the conservation of gene function (for example, Reeves et al. 2007; Locascio et al. 2009). However, transgenic *A. thaliana* is not generally useful to test gene functions that are not observed in *A. thaliana*. When species-specific functions are analyzed, transgenic plants of the study species are required. This topic is discussed further in Example 2 below.

Another essential technique is quantitative real-time PCR (reviewed in Bustin 2000), which is used to quantify the expression level of target genes. Accurate and rapid quantification is possible by monitoring the amplification of PCR products in real time. This technique can be applied even to plant species with little genomic information because only sequence information of the target flowering genes and an internal control gene, whose expression level should not fluctuate, is required. Guidelines for practical use of real-time PCR are described in detail by Bustin et al. (2009).

However, acquisition of sequence information of flowering genes from nonmodel plants may still require some efforts. Recently, many genes that have sequence similarities to the *A. thaliana* flowering gene have been identified in strawberry (*Fragaria vesca* L.) by sequencing genes that are expressed differentially before and after floral initiation (Mouhu et al. 2009). Similar analyses would allow one to find the candidate genes responsible for flowering time regulation in other plant species. Recent advances in sequencing technologies using high-throughput sequencers are providing further opportunities to obtain a large amount of sequence data in nonmodel organisms rapidly and accurately (see, e.g., Toth et al. 2007; Vera et al. 2008). We summarize the recent progress and possibilities of the application of transcriptome profiling for the identification of differentially expressed genes in response to several biological processes in Appendix 2.

Example 1: reception of naturally fluctuating temperature as a seasonal cue for flowering in Arabidopsis halleri

In phenological modeling to predict flowering time, temperatures from an arbitrary date to the date of the flowering event are often considered (reviewed in Chuine et al. 2003). However, within this period, there are many biochemical and physiological steps with few morphological changes, such as reception of flowering signal, acquisition of competence to flower and duration of actual flower bud formation, but it is not obvious which step is determined by temperature. The lack of direct measurement of the internal developmental states often makes it difficult to predict flowering time in modeling (Chuine et al. 2003). In this example, we introduce our recent study to illustrate that data of expression levels of genes dissects the processes leading toward flowering, and that it is utilized as indicators to represent internal developmental states.

Many plants utilize cold temperature in winter (vernalization) as a cue to promote flowering (Reeves et al. 2007; Dennis and Peacock 2009; Locascio et al. 2009; Amasino 2010). Although temperature has seasonal trends, it shows day/night, day-by-day and weekly fluctuations, and these fluctuations are often inconsistent with seasonal trends. In the phenological modeling of plants, a concept known as a chilling unit is commonly used (reviewed in Chuine et al. 2003). However, the molecular and/or physiological basis of a chilling unit is still unclear, and little is known about how plants utilize temperature as a reliable informant regarding the season. To address this question, we studied the perennial *Arabidopsis halleri*, which is a relative species of *A. thaliana* that requires vernalization to flower in spring (Aikawa et al. 2010).

We focused on the floral repressor *FLC* gene in *A. halleri* because it is known to be a key component of the vernalization pathway in *A. thaliana* (“Appendix 1”) (Michaels and Amasino 1999; Sheldon et al. 1999). First, the *FLC* homologue in *A. halleri* was identified by sequence similarity, and then the functional conservation of the *FLC* gene was tested by generating transgenic *A. thaliana* in which *A. halleri FLC* was overexpressed. This transgenic plant showed a late flowering phenotype, indicating that *A. halleri FLC* functioned as a floral repressor similar to *A. thaliana FLC*. Next, we checked the expression level of *FLC* in leaves of *A. halleri* grown in a natural habitat for 2 years at ~1-week intervals by real-time PCR quantification. *FLC* expression showed a clear seasonal pattern. A gradual decrease in *FLC* gene expression was observed in winter, while a gradual increase was observed following an increase in temperature. Statistical analyses using a chilling-unit model showed that the changes in expression of *FLC* did not appear to correspond to temporal fluctuations in

temperatures, but rather to the temperatures over the past 6 weeks as if they were memorized. Furthermore, the model predictions were confirmed by transplant experiments under controlled temperature conditions. Thus, direct measurement of the gradual repression of the *FLC* gene corresponding to the temperature over the previous 6 weeks provided the mechanistic explanation as to how fluctuating temperatures in winter are utilized as reliable information regarding the seasons and as a signal to flower.

The basic idea we used in this example is simple, and similar to the concept used in traditional phenological research. Phenological research into flowering has revealed relationships between flowering and weather conditions through the association between long-term time-series data of weather and two qualitative states of plants: flowering or not flowering. On the other hand, our example replaced the visible qualitative phenotypes with internal states (i.e., levels of gene expression). In traditional phenological studies, little information was available during the long periods of nonflowering seasons. However, internal states such as gene expression change dramatically even when there are few visible morphological changes. This example shows that direct measurement of internal states enabled the dissection of processes leading toward flowering caused by cold winters, and contributed to the understanding of flowering in a fluctuating, natural environment. The idea of measuring changing internal states quantitatively in addition to examining qualitative phenotypes can be applied to many other phenological studies.

Example 2: a latitudinal cline in the timing of bud set and cessation in Populus

In *Populus* trees, growth cessation and bud set are induced in the fall, and the dormant state then starts. A latitudinal cline in the timing of bud set has been reported (Pauley and Perry 1954). Trees from northern populations show earlier bud set during fall compared with those from southern populations. Dormancy renders the trees cold tolerant in winter. Thus, the cline suggests that the difference in the timing of dormancy in different populations may be a result of local adaptation to avoid the risk of frost damage. Growth cessation and bud set are induced by short-day conditions and are under strong genetic control (Howe et al. 1996; Frewen et al. 2000). However, it is unclear how the different timing of growth cessation in different populations is controlled.

This question was addressed in the process of analyzing the *FT* gene, which is a flowering promoter gene that is a major downstream in the photoperiod pathway ("Appendix 1"). Böhlenius et al. (2006) isolated the *FT* orthologue from *Populus trichocarpa*, *PtFT1*. The function of *PtFT1* is conserved as a floral promoter, and both transgenic *A. thaliana* and transgenic *Populus tremula* × *tremuloides* that overexpress *PtFT1* showed early flowering phenotypes. In addition, transgenic *Populus* suppressed short-day-induced growth cessation and bud set. It is notable that *A. thaliana* is an annual plant without growth cessation or bud set, and thus transgenic *A. thaliana* was not useful for testing the function of *PtFT1* in such phenotypes. Furthermore, the above authors generated transgenic *Populus* in which the level of *PtFT1* was downregulated, and tested the function of *PtFT1* in growth cessation and bud set. The transgenic *Populus* was more sensitive to short-day conditions for inducing growth cessation and bud set. These authors also showed that expression of *PtFT1* was downregulated in response to a long-day to short-day shift. These results suggested that *PtFT1* expression is a suppressor of short-day-induced growth cessation and bud set.

In *A. thaliana*, the *CONSTANS* (*CO*) gene is upstream of *FT* in the photoperiod pathway and upregulates the *FT* gene only when expressed during the day ("Appendix 1") (reviewed in Kobayashi and Weigel 2007). Böhlenius et al. (2006) focused on this regulation, and then showed that the *CO*/*FT* module is conserved in *Populus*. The expression of *PtCO* showed a circadian pattern similar to that of *Arabidopsis CO*, and transgenic *Populus* showed that *PtCO* was also involved in the regulation of timing of both growth cessation and bud set. Transplant experiments under controlled light conditions showed that trees originating from four different latitudes had different timings of the peak of *PtCO* expression (Fig. 3). These peaks appeared earlier in

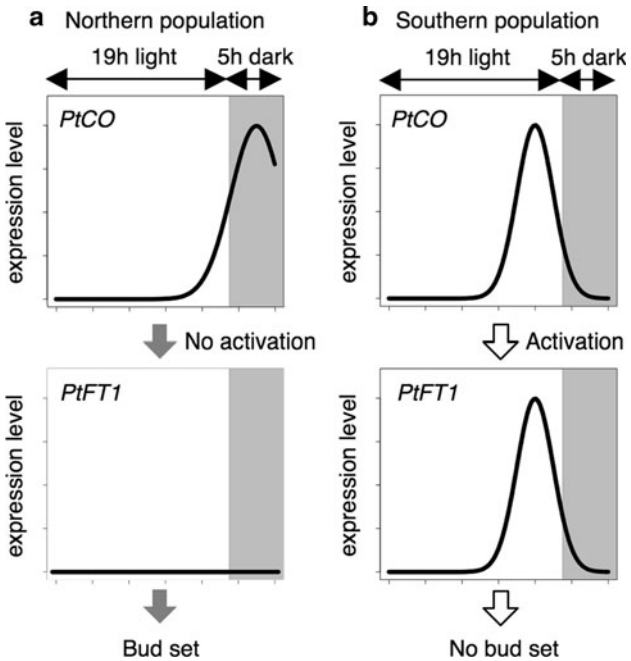

Fig. 3 Illustration of the relationship between bud set and *PtCO*/*PtFT1* expression patterns in *Populus* trees from **a** northern and **b** southern populations in 19-h day length. Each *box* shows the expression level of *PtCO* or *PtFT1*. *White* and *black parts* in each *box* indicate light and dark conditions, respectively. This figure is modified from Böhlenius et al. (2006)

southern populations. When trees were grown under a 19-h photoperiod, the peaks of *PtCO* expression of the two northernmost populations appeared in the dark, and *PtCO* did not induce *PtFT1* (Fig. 3a). These trees displayed growth cessation and bud set. On the other hand, the peaks of *PtCO* expression of the two southernmost populations appeared in the light, and then induced *PtFT1* expression. In the southernmost populations, bud set and growth cessation did not occur (Fig. 3b). This suggests that variations in the expression of *PtCO* in different populations explain the differential regulation of *PtFT1* and subsequent growth cessation and bud set.

In this example, the flowering regulatory network studied in the model species *A. thaliana* was applied to the study of a latitudinal cline in the timing of growth cessation and bud set that cannot be studied in annual species such as *A. thaliana*. Although the very mutation that was responsible for the latitudinal cline is yet to be identified, this example illustrates how the application of knowledge of the gene network, phenotypic observation of transgenic plants and measurement of the expression levels of the corresponding genes could reveal the mechanisms underlying ecologically important phenomena.

The challenge of applying knowledge of flowering genes to ecologically unique flowering phenomena through the example of synchronous intermittent flowering

In the previous section, we reviewed examples in which our understanding of conserved pathways was exploited to study ecologically relevant phenomena. In this final section, we would like to discuss the possibility of applying knowledge of flowering genes to a more complex phenomenon, intermittent synchronous mass flowering, which is the theme of this special issue.

In spring, *A. thaliana* flowers in response to annually occurring environmental signals such as photoperiod and vernalization. On the other hand, some species show supra-annual synchronous flowering within populations or communities at irregular intervals (for example, Kelly 1994; Koenig and Knops 2000; Kelly and Sork 2002; Masaki et al. 2008; Crone et al. 2009). This phenomenon is unique compared with annual flowering in terms of both supra-annual occurrence and synchronization, even with irregular cycles. Given that this phenomenon has supra-annual cycles, the triggers of this phenomenon will also have supra-annual patterns if any. This may indicate that species that show mass flowering at irregular intervals have different flowering cues and/or different mechanisms to receive the cues compared with *A. thaliana*. Therefore, application of knowledge of flowering genes in this phenomenon is expected to be more difficult than in the examples of *A. halleri FLC* and the *Populus FT*/*CO* module.

Long-term phenological studies have suggested the possibility that weather and resources are involved in mass flowering in some species (Kelly and Sork 2002). For example, synchronous matching of both the amount of resources and January temperatures over a threshold value was observed in *Chionochloa pallens* before synchronous intermittent heavy flowering (Rees et al. 2002). In addition, prolonged drought and/or low nighttime temperatures are currently accepted as the most plausible cue(s) for the synchronized intermittent flowering events in Southeast Asian tropical rainforests referred to as "general flowering" (Ashton et al. 1988; Sakai et al. 1999, 2006; Yasuda et al. 1999; Numata et al. 2003; Brearley et al. 2007). These examples indicate that individual phenomena are governed by different cues and mechanisms even though they show similar synchronous intermittent mass flowering phenotypes. Therefore, we focus on and discuss general flowering as one of the well-known supra-annual synchronous flowering events.

General flowering is a community-level synchronized flowering event that occurs at irregular intervals of less than 1 year to several years in the aseasonal Southeast Asian tropics (Ashton et al. 1988; Sakai et al. 1999, 2006; Yasuda et al. 1999; Numata et al. 2003; Brearley et al. 2007). The canopy and emergent strata of lowland rainforests in this region are dominated by the family Dipterocarpaceae. During general flowering, almost all the dipterocarp species flower together with the species of other families.

Prolonged drought and/or low nighttime temperatures associated with the El Niño Southern Oscillation are currently accepted as the most plausible cue(s) for general flowering in dipterocarps. However, it is not clear whether these factors could affect the flowering time of *A. thaliana*. Therefore, direct application of the flowering regulatory network in *A. thaliana* to dipterocarps may not be appropriate. However, as discussed above, the downstream genes in the regulatory network of flowering are well conserved in angiosperms, although flowering cues and upstream genes are diversified. Thus, downstream genes such as floral integrators and floral meristem identity genes are possibly conserved, even in dipterocarps. These genes function as promoters of flowering and their expression is increased gradually following flowering signals in *A. thaliana*. Supposing that functions of these orthologues are conserved in dipterocarps, they could be used as useful markers for flowering initiation. Furthermore, analyses of differentially expressed genes before and after the activation of marker genes, similar to the example of strawberry discussed above, will allow us to find candidate upstream genes in dipterocarps.

As discussed in the example of *A. halleri*, traditional phenological studies using qualitative final output—flowering or not flowering—have limits when analyzing the relationship between flowering and weather conditions. Plants respond to their surrounding environments and change gene expression dramatically even if morphological changes are not observed (Balasubramanian et al. 2006). In the Southeast Asian tropics, dry conditions and low temperatures occur repeatedly even in

nonflowering seasons, although the extents of these factors may not be sufficient to act as a flowering cue. Therefore, if data on the levels of gene expression can be compared with changes in weather even in nonflowering seasons, a much greater amount of information is obtained. Supposing that expression levels of upstream genes in dipterocarps change quantitatively in correspondence with the extent of drought and/or low temperature similarly to the cold-responding *FLC*, the relationships between these weather factors and general flowering can be revealed in more detail. For example, an accurate threshold of the extent to which drought and/or low temperature can act as a flowering cue is not yet clear (Ashton et al. 1988; Sakai et al. 2006; Brearley et al. 2007). Analyses of the expression of these marker genes may help to reveal the threshold and the accurate timing of the reception of the flowering cue.

Why do dipterocarps in Southeast Asia show general flowering? Data from flowering genes and analyses of their expression in dipterocarps may serve to reveal the origin of intermittent synchronous flowering in dipterocarps in Southeast Asia. Taxonomic and biogeographic evidence suggests that dipterocarps in aseasonal tropical rainforests originated in the seasonal tropics (Ashton et al. 1988). In seasonal tropical areas such as India, dipterocarps flower annually during the dry season (Singh and Kushwaha 2005, 2006). These dry seasons are accompanied by low temperatures. It is interesting that induction of flowering in dipterocarps occurs in dry and low temperature conditions regardless of whether they grow in seasonal or aseasonal tropical rainforests. This may suggest that general flowering in the aseasonal tropical rainforests has evolved as a result of developmental constraints in dipterocarps that arose in the seasonal tropics (Ashton et al. 1988). Through comparisons of flowering genes and their expression patterns between dipterocarps grown in seasonal and aseasonal regions, answers to this question may be revealed, and data from these related species will help us to understand general flowering.

Conclusion

The genetic network of flowering time control is one of the most well-known regulatory networks in *A. thaliana*, and knowledge of other model plants has enabled comparison of the regulatory network of flowering among diverse species. Downstream genes of the flowering network, such as floral pathway integrators and floral meristem identity genes, are found to be highly conserved among angiosperms.

Many plant species grown in natural environments show similar responses to environmental and endogenous signals to *A. thaliana* when they flower. In those cases, application of knowledge about the regulatory network of flowering in model plants serves to help understand the phenomena observed in wild plants. The mechanism using fluctuating temperature as seasonal information in *A. halleri* and the molecular mechanism underlying the latitudinal cline in the timing of bud set in *Populus* are revealed by applying information of the regulatory genes underlying the corresponding phenomena observed in *A. thaliana*.

However, model plants also have limitations. No matter how intensively studied in model plants, the mechanisms underlying phenomena such as synchronized intermittent flowering that are not observed in model plants may never be revealed. Therefore, studies of ecologically unique flowering events at the molecular level are still challenging, but hold the possibility of finding new gene regulatory networks. Conserved downstream genes in the flowering network will be clues for understanding such unique flowering events.

Application of genetic knowledge to wild plants provides new insights into the understanding of these ecologically important phenomena. On the other hand, studies of ecologically unique phenomena at the molecular level are expected to aid in our understanding of novel mechanisms of genetic regulation of flowering. As the number of such studies in both ecology and molecular biology increases, flowering becomes a more useful model trait for the integrated study of ecology, evolution and molecular biology.

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Appendix 1

Photoperiod pathway

In the photoperiod pathway, flowering is promoted by exposure to inductive photoperiod. Plants sense night length and use it as a flowering cue (reviewed in Imaizumi and Kay 2006; Kobayashi and Weigel 2007). *Arabidopsis thaliana* is classified as a quantitative long-day plant, meaning that it can flower more rapidly in long-day conditions, while flowering is delayed in short-day conditions. The genes involved in the photoperiod pathway were isolated using mutants defective in day-length-specific acceleration of flowering (Koornneef et al. 1991). In other words, mutants in the photoperiod pathway flower similarly under long-day and short-day conditions. The key components of the photoperiod pathway are the *CONSTANS* (*CO*) gene (Putterill et al. 1995) and the *FLOWERING LOCUS T* (*FT*) gene. Expression of the *CO* gene is regulated by circadian clock genes and shows a circadian pattern (Suárez-López et al. 2001; Yanovsky and Kay 2002). In addition,

activity of the CO protein is also regulated in a light-dependent manner through the functions of photoreceptors, and thus can induce expression of the downstream gene, *FT*, only in long-day conditions (Valverde et al. 2004). This provides the crucial mechanism of day length measurement. Expression of the *FT* gene occurs in leaves, but FT protein moves to the shoot apical meristem (SAM) in which flower development occurs, acting as a 'florigen' (Corbesier et al. 2007; Tamaki et al. 2007). Once FT protein arrives at the SAM, it interacts with another protein, FD, and activates downstream floral meristem identity genes such as *APETALA 1* (*AP1*) (Abe et al. 2005; Wigge et al. 2005), and other floral promoters such as *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) (Michaels et al. 2005; Yoo et al. 2005).

Vernalization pathway

Many individuals of *A. thaliana* grown in northern Europe need prolonged exposure to the cold of winter to render them competent to flower (Nordborg and Bergelson 1999). This process is called vernalization and ensures that they flower in favorable spring conditions. The vernalization requirement in *A. thaliana* is conferred by a floral repressor gene, *FLOWERING LOCUS C* (*FLC*) (Michaels and Amasino 1999; Sheldon et al. 1999). Without vernalization, highly expressed *FLC* represses flowering by suppressing the expression of the flowering activators *FT*, *FD* and *SOC1* (Hepworth et al. 2002; Helliwell et al. 2006; Searle et al. 2006). However, as the plants are exposed to prolonged cold, *FLC* expression is gradually decreased, and the plants become ready to flower (Michaels and Amasino 1999; Sheldon et al. 1999).

Light quality pathway

Along with the duration of light (photoperiod), light quality is also an important flowering cue. Plants recognize their growing conditions from the light quality, for example, under canopy shade conditions or in the proximity of other plants, and then show acceleration of flowering. This process is mediated by photoreceptors called phytochromes, which function upstream of the *FT* gene and regulate flowering time (Cerdán and Chory 2003; Halliday et al. 2003).

Thermosensory pathway

Another important cue is ambient temperature. Small changes in ambient temperature regulate flowering time through *FT* in a thermosensory pathway involving the floral repressor gene, *SHORT VEGETATIVE PHASE* (*SVP*) (Lee et al. 2007).

Autonomous pathway

The autonomous pathway is composed of genes that cause late flowering in both long-day and short-day conditions when they are mutated. Therefore, the term "autonomous pathway" is generally used as the pathway that is independent of environmental cues, especially photoperiod. As expected from this definition, the autonomous pathway is not a linear pathway. Rather, it is comprised of subpathways of genes with different biochemical functions (Chan and Struhl 1997; Macknight et al. 1997; Schomburg et al. 2001; He et al. 2003; Simpson et al. 2003; Ausin et al. 2004; Lim et al. 2004; Noh et al. 2004). However, these genes have a common feature in that they control flowering time by mediating the expression level of the floral repressor *FLC* (Michaels and Amasino 2001).

Gibberellin pathway

Gibberellins (GAs) are a class of plant hormones. In *A. thaliana*, GAs promote flowering (Wilson et al. 1992; Reeves and Coupland 2001; Willige et al. 2007). GA_4 is the most active form of GA in floral induction, and the endogenous levels of GA_4 in the SAM are reported to increase up to nearly 100-fold preceding floral initiation (Eriksson et al. 2006). This physiological change in the endogenous GA levels functions through the upregulation of genes such as *LEAFY* (*LFY*) and *SOC1* (Blázquez and Weigel 2000; Moon et al. 2003).

Age pathway

Plants also sense their age. Plants experience a gradual transition from the juvenile to adult phase as they grow, and this phase transition renders them competent to flower. The abundance of SQUAMOSA PROMOTER BINDING-LIKE (SPL) proteins acts as a key factor in this developmental pathway. A gradual increase in these proteins following the phase transition regulates genes such as *FT*, *SOC1*, *LFY* and *AP1* in a direct or indirect manner (Wang et al. 2009a; Wu et al. 2009; Yamaguchi et al. 2009).

Appendix 2

Application of transcriptome profiling to the identification of differentially expressed genes

A central interest in molecular biology has been to identify differentially expressed genes through comparisons of transcription levels before and after specific biological processes. Microarrays have been used widely for this purpose. Microarray platforms provide a reliable, rapid and cost-effective technology with which to

analyze genome-wide gene expression patterns by quantifying cDNA by hybridization to probes. This technique has facilitated large-scale transcriptome analysis in many organisms (for example, Schmid et al. 2003; Zeller et al. 2009). However, it has been limited mostly to model organisms with known genome sequences because gene sequences are required to design probes on microarrays.

On the other hand, in nonmodel organisms, expressed sequence tag (EST) sequencing with traditional Sanger capillary sequencers has been used successfully to analyze the transcriptome (for example, Ma et al. 2009; Pirooznia et al. 2010). The EST is a short subsequence of a transcribed cDNA sequence from a known tissue source. The example of strawberry discussed in the main text is one example of EST sequencing (Mouhu et al. 2009). However, this technique is time consuming and costly.

Alternative methods for transcriptomics using high-throughput sequencers have appeared recently (reviewed in Matsumura et al. 2008; Wang et al. 2009b; Wilhelm and Landry 2009). One of the major approaches is RNA-Seq. RNA-Seq provides both the sequence information of transcripts and their expression levels simultaneously. The millions of short reads generated by high-throughput sequencers are either aligned to a reference genome or assembled de novo to infer from which genes they were transcribed. The expression level of each gene can then be measured by counting the number of reads. RNA-Seq has the advantages of higher sensitivity and greater dynamic range of gene expression than microarrays if enough sequencing depth is achieved. Furthermore, it is more rapid and cost-effective than EST sequencing. Owing to advances in methods of de novo assembly, it can be applied to nonmodel organisms with little prior knowledge of their genome sequence (see, e.g., Barakat et al. 2009; Birzele et al. 2010; Ekblom et al. 2010; Rodriguez et al. 2010). RNA-Seq has already been applied to some nonmodel plant species to identify genes expressed differentially in response to several phenomena. For example, several candidate genes responsible for resistance to fungus infections have been revealed through the analyses of differentially expressed genes between fungus-sensitive American chestnut (*Castanea dentata*) and fungus-resistant Chinese chestnut (*Castanea mollissima*) (Barakat et al. 2009). As another example, differentially expressed genes in the resurrection plant *Craterostigma plantagineum* have been characterized during dehydration and rehydration (Rodriguez et al. 2010).

Although transcriptomics using high-throughput sequencers provide useful tools, the high run cost restricts the analysis to a small number of samples. When compared with high-throughput sequencers at the same resolution, microarrays still have an advantage in terms of cost. Therefore, a combination of both microarrays and high-throughput sequencers has been attempted for transcriptomics in nonmodel organisms. Once custom microarrays based on the sequence data generated by high-throughput sequencers have been designed, the microarray serves as a cost-effective tool with which to analyze a large number of samples. There are now some examples of the successful utilization of this system to identify differentially expressed genes (e.g., Bellin et al. 2009; Schmid et al. 2010).

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