Chapter

24 The Control of Flowering

MOST PEOPLE LOOK FORWARD to the spring season and the profusion of flowers it brings. Many vacationers carefully time their travels to coincide with specific blooming seasons: *Citrus* along Blossom Trail in southern California, tulips in Holland. In Washington, D.C., and throughout Japan, the cherry blossoms are received with spirited ceremonies. As spring progresses into summer, summer into fall, and fall into winter, wildflowers bloom at their appointed times.

Although the strong correlation between flowering and seasons is common knowledge, the phenomenon poses fundamental questions that will be addressed in this chapter:

- How do plants keep track of the seasons of the year and the time of day?
- Which environmental signals control flowering, and how are those signals perceived?
- How are environmental signals transduced to bring about the developmental changes associated with flowering?

In Chapter 16 we discussed the role of the root and shoot apical meristems in vegetative growth and development. The transition to flowering involves major changes in the pattern of morphogenesis and cell differentiation at the shoot apical meristem. Ultimately this process leads to the production of the floral organs—sepals, petals, stamens, and carpels (see Figure 1.2.A in **Web Topic 1.2**).

Specialized cells in the anther undergo meiosis to produce four haploid microspores that develop into pollen grains. Similarly, a cell within the ovule divides meiotically to produce four haploid megaspores, one of which survives and undergoes three mitotic divisions to produce the cells of the embryo sac (see Figure 1.2.B in **Web Topic 1.2**). The embryo sac represents the mature female gametophyte. The pollen grain, with its germinating pollen tube, is the mature male gametophyte generation. The two gametophytic structures produce the gametes (egg and sperm

cells), which fuse to form the diploid zygote, the first stage of the new sporophyte generation.

Clearly, flowers represent a complex array of functionally specialized structures that differ substantially from the vegetative plant body in form and cell types. The transition to flowering therefore entails radical changes in cell fate within the shoot apical meristem. In the first part of this chapter we will discuss these changes, which are manifested as *floral development*. Recently genes have been identified that play crucial roles in the formation of the floral organs. Such studies have shed new light on the genetic control of plant reproductive development.

The events occurring in the shoot apex that specifically commit the apical meristem to produce *flowers* are collectively referred to as **floral evocation**. In the second part of this chapter we will discuss the events leading to floral evocation. The developmental signals that bring about floral evocation include endogenous factors, such as *circadian rhythms*, *phase change*, and *hormones*, and external factors, such as day length (*photoperiod*) and temperature (*vernalization*). In the case of photoperiodism, transmissible signals from the leaves, collectively referred to as the **floral stimulus**, are translocated to the shoot apical meristem. The interactions of these endogenous and external factors enable plants to synchronize their reproductive development with the environment.

FLORAL MERISTEMS AND FLORAL ORGAN DEVELOPMENT

Floral meristems usually can be distinguished from vegetative meristems, even in the early stages of reproductive development, by their larger size. The transition from vegetative to reproductive development is marked by an increase in the frequency of cell divisions within the central zone of the shoot apical meristem. In the vegetative meristem, the cells of the central zone complete their division cycles slowly. As reproductive development commences, the increase in the size of the meristem is largely a result of the increased division rate of these central cells. Recently, genetic and molecular studies have identified a network of genes that control floral morphogenesis in *Arabidopsis*, snapdragon (*Antirrhinum*), and other species.

In this section we will focus on floral development in *Arabidopsis*, which has been studied extensively (Figure 24.1). First we will outline the basic morphological changes that occur during the transition from the vegetative to the reproductive phase. Next we will consider the arrangement of the floral organs in four whorls on the meristem, and the types of genes that govern the normal pattern of floral development. According to the widely accepted ABC model (which is described in Figure 24.6), the specific locations of floral organs in the flower are regulated by the overlapping expression of three types of floral organ identity genes.

The Characteristics of Shoot Meristems in *Arabidopsis* Change with Development

During the vegetative phase of growth, the *Arabidopsis* vegetative apical meristem produces phytomeres with very short internodes, resulting in a basal rosette of leaves (see Figure 24.1A). (Recall from Chapter 16 that a phytomere consists of a leaf, the node to which the leaf is attached, the axillary bud, and the internode below the node.)

As plants initiate reproductive development, the vegetative meristem is transformed into an indeterminate **primary inflorescence meristem** that produces floral meristems on its flanks (Figure 24.2). The lateral buds of the

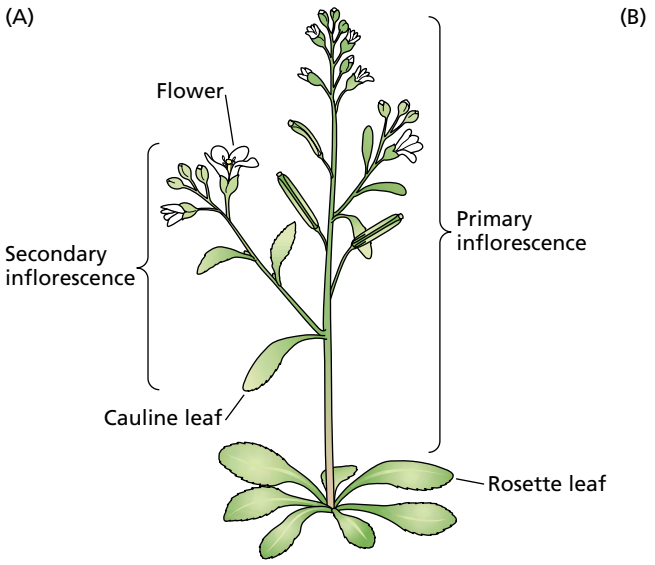


FIGURE 24.1 (A) The shoot apical meristem in *Arabidopsis thaliana* generates different organs at different stages of development. Early in development the shoot apical meristem forms a rosette of basal leaves. When the plant makes the transition to flowering, the shoot apical meristem is transformed into a primary inflorescence meristem that ultimately produces an elongated stem bearing flowers. Leaf primordia initiated prior to the floral transition become cauline leaves, and secondary inflorescences develop in the axils of the cauline leaves. (B) Photograph of an *Arabidopsis* plant. (Photo courtesy of Richard Amasino.)

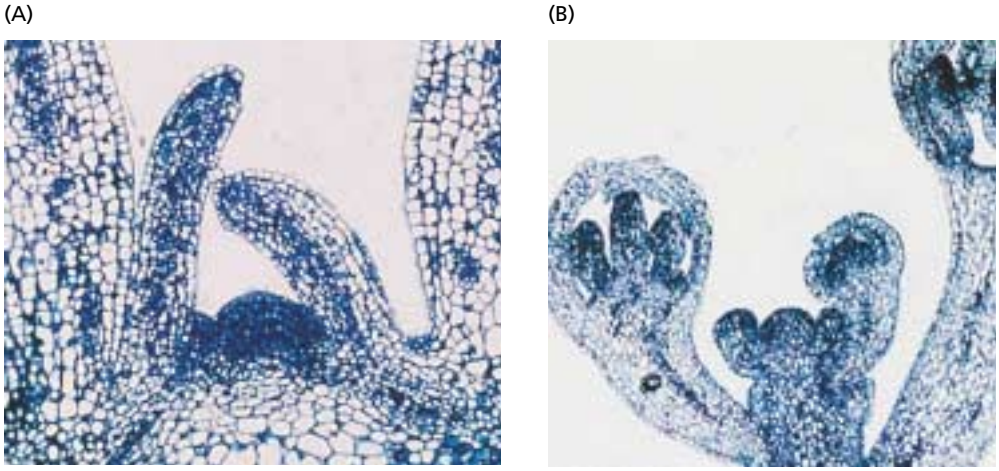


FIGURE 24.2 Longitudinal sections through a vegetative (A) and a reproductive (B) shoot apical region of *Arabidopsis*. (Photos courtesy of V. Grbic´ and M. Nelson, and assembled and labeled by E. Himelblau.)

cauline leaves (inflorescence leaves) develop into **secondary inflorescence meristems**, and their activity repeats the pattern of development of the primary inflorescence meristem, as shown in Figure 24.1A.

The Four Different Types of Floral Organs Are Initiated as Separate Whorls

Floral meristems initiate four different types of floral organs: sepals, petals, stamens, and carpels (Coen and Carpenter 1993). These sets of organs are initiated in concentric rings, called **whorls**, around the flanks of the meristem (Figure 24.3). The initiation of the innermost organs, the carpels, consumes all of the meristematic cells in the apical dome, and only the floral organ primordia are present as the floral bud develops. In the wild-type *Arabidopsis* flower, the whorls are arranged as follows:

- The first (outermost) whorl consists of four sepals, which are green at maturity.

The second whorl is composed of four petals, which are white at maturity.

- The third whorl contains six stamens, two of which are shorter than the other four.
- The fourth whorl is a single complex organ, the gynoecium or pistil, which is composed of an ovary with two fused carpels, each containing numerous ovules, and a short style capped with a stigma (Figure 24.4).

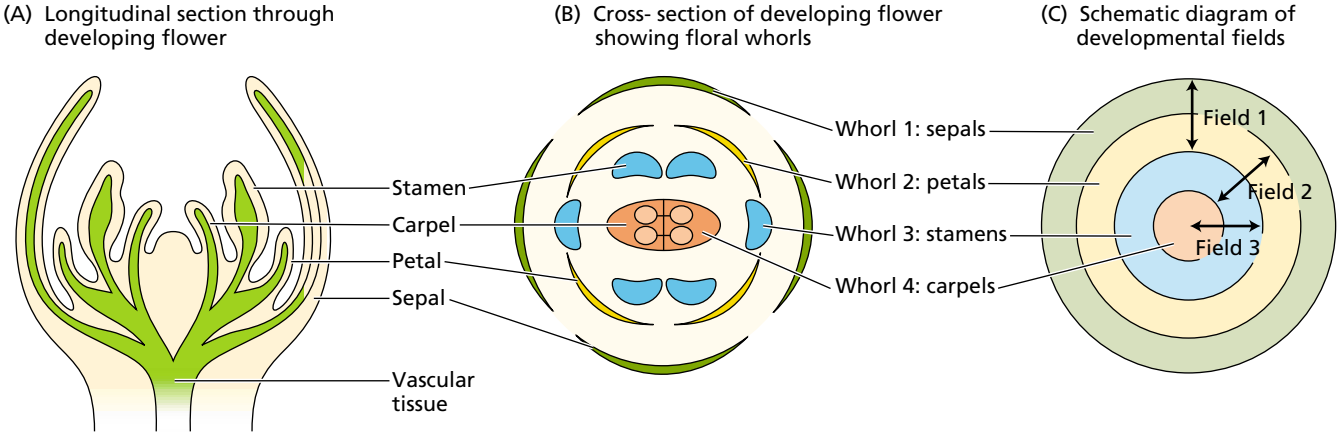


FIGURE 24.3 The floral organs are initiated sequentially by the floral meristem of *Arabidopsis*. (A and B) The floral organs are produced as successive whorls (concentric circles), starting with the sepals and progressing inward. (C) According to the combinatorial model, the functions of each whorl are determined by overlapping developmental fields. These fields correspond to the expression patterns of specific floral organ identity genes. (From Bewley et al. 2000.)

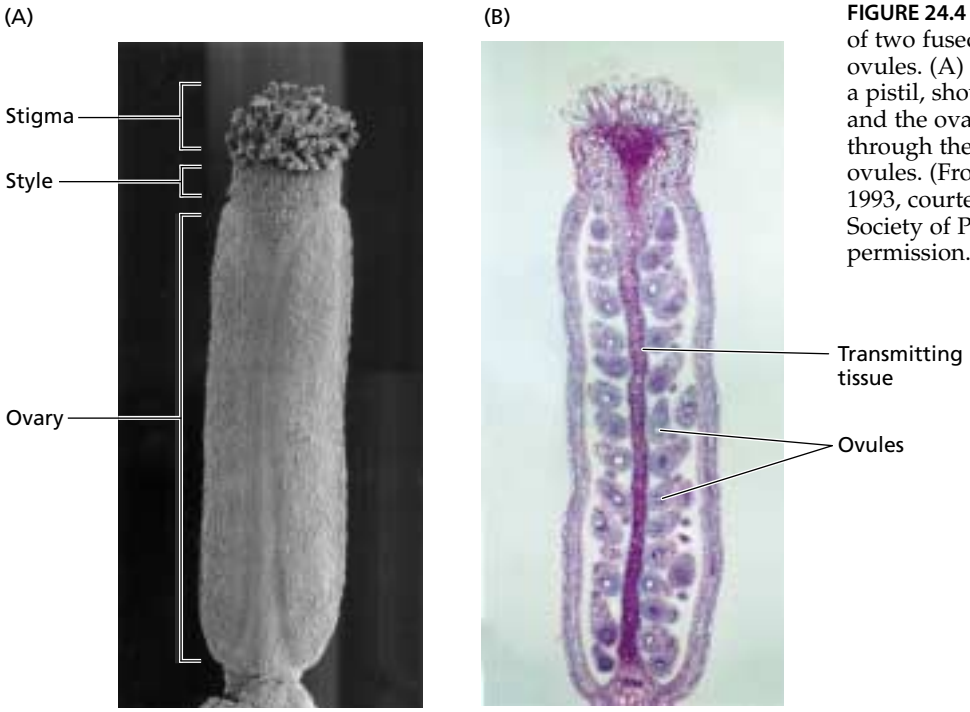


FIGURE 24.4 The *Arabidopsis* pistil consists of two fused carpels, each containing many ovules. (A) Scanning electron micrograph of a pistil, showing the stigma, a short style, and the ovary. (B) Longitudinal section through the pistil, showing the many ovules. (From Gasser and Robinson-Beers 1993, courtesy of C. S. Gasser, © American Society of Plant Biologists, reprinted with permission.)

Three Types of Genes Regulate Floral Development

Mutations have identified three classes of genes that regulate floral development: floral organ identity genes, cadastral genes, and meristem identity genes.

1. **Floral organ identity genes** directly control floral identity. The proteins encoded by these genes are transcription factors that likely control the expression of other genes whose products are involved in the formation and/or function of *floral* organs.
2. **Cadastral genes** act as spatial regulators of the floral organ identity genes by setting boundaries for their expression. (The word *cadastre* refers to a map or survey showing property boundaries for taxation purposes.)
3. **Meristem identity genes** are necessary for the initial induction of the organ identity genes. These genes are the positive regulators of floral organ identity.

Meristem Identity Genes Regulate Meristem Function

Meristem identity genes must be active for the primordia formed at the flanks of the apical meristem to become floral meristems. (Recall that an apical meristem that is forming floral meristems on its flanks is known as an inflorescence meristem.) For example, mutants of *Antirrhinum* (snapdragon) that have a defect in the meristem identity gene *FLORICAULA* develop an inflorescence that does not produce flowers. Instead of causing floral meristems to form in the axils of the bracts, the mutant *floricaula* gene results in the development of additional inflorescence meristems at the bract axils. The wild-type *floricaula* (*FLO*) gene controls the determination step in which floral meristem identity is established.

In *Arabidopsis*, *AGAMOUS-LIKE 20*[1] (*AGL20*), *APETALA1* (*AP1*), and *LEAFY* (*LFY*) are all critical genes in the genetic pathway that must be activated to establish floral meristem identity. *LFY* is the *Arabidopsis* version of the snapdragon *FLO* gene. *AGL20* plays a central role in floral evocation by integrating signals from several different pathways involving both environmental and internal cues (Borner et al. 2000). *AGL20* thus appears to serve as a master switch initiating floral development.

Once activated, *AGL20* triggers the expression of *LFY*, and *LFY* turns on the expression of *AP1* (Simon et al. 1996). In *Arabidopsis*, *LFY* and *AP1* are involved in a positive feedback loop; that is, *AP1* expression also stimulates the expression of *LFY*.

Homeotic Mutations Led to the Identification of Floral Organ Identity Genes

The genes that determine floral organ identity were discovered as **floral homeotic mutants** (see Chapter 14 on the

[1] Also known as *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS* 1 (*SOC1*).

web site). As discussed in Chapter 14, mutations in the fruit fly, *Drosophila*, led to the identification of a set of homeotic genes encoding transcription factors that determine the locations at which specific structures develop. Such genes act as major developmental switches that activate the entire genetic program for a particular structure. The expression of homeotic genes thus gives organs their identity.

As we have seen already in this chapter, dicot flowers consist of successive whorls of organs that form as a result of the activity of floral meristems: sepals, petals, stamens, and carpels. These organs are produced when and where they are because of the orderly, patterned expression and interactions of a small group of homeotic genes that specify floral organ identity.

The floral organ identity genes were identified through homeotic mutations that altered floral organ identity so that some of the floral organs appeared in the wrong place. For example, *Arabidopsis* plants with mutations in the *APETALA2* (*AP2*) gene produce flowers with carpels where sepals should be, and stamens where petals normally appear.

The homeotic genes that have been cloned so far encode transcription factors—proteins that control the expression of other genes. Most plant homeotic genes belong to a class of related sequences known as **MADS box genes**, whereas animal homeotic genes contain sequences called homeoboxes (see Chapter 14 on the web site).

Many of the genes that determine floral organ identity are MADS box genes, including the *DEFICIENS* gene of snapdragon and the *AGAMOUS*, *PISTILLATA1*, and *APETALA3* genes of *Arabidopsis*. The MADS box genes share a characteristic, conserved nucleotide sequence known as a *MADS box*, which encodes a protein structure known as the *MADS domain*. The MADS domain enables these transcription factors to bind to DNA that has a specific nucleotide sequence.

Not all genes containing the MADS box domain are homeotic genes. For example, *AGL20* is a MADS box gene, but it functions as a meristem identity gene.

Three Types of Homeotic Genes Control Floral Organ Identity

Five different genes are known to specify floral organ identity in *Arabidopsis*: *APETALA1* (*AP1*), *APETALA2* (*AP2*), *APETALA3* (*AP3*), *PISTILLATA* (*PI*), and *AGAMOUS* (*AG*) (Bowman et al. 1989; Weigel and Meyerowitz 1994). The organ identity genes initially were identified through mutations that dramatically alter the structure and thus the identity of the floral organs produced in two adjacent whorls (Figure 24.5). For example, plants with the *ap2* mutation lack sepals and petals (see Figure 24.5B). Plants bearing *ap3* or *pi* mutations produce sepals instead of petals in the second whorl, and carpels instead of stamens in the third whorl (see Figure 24.5C). And plants homozygous for the *ag* mutation lack both stamens and carpels (see Figure 24.5D).

Because mutations in these genes change floral organ identity without affecting the initiation of flowers, they are homeotic genes. These homeotic genes fall into three classes—types A, B, and C—defining three different kinds of activities (Figure 24.6):

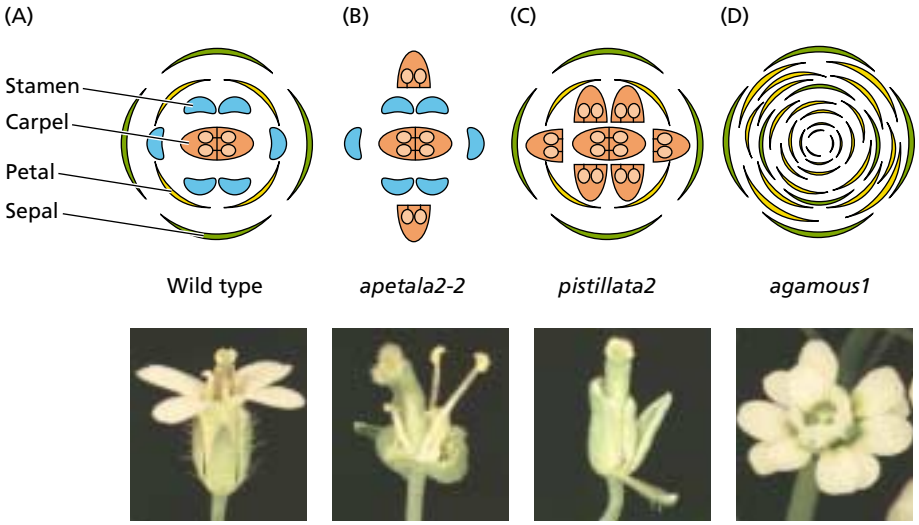


FIGURE 24.5 Mutations in the floral organ identity genes dramatically alter the structure of the flower. (A) Wild type; (B) *apetala2-2* mutants lack sepals and petals; (C) *pistillata2* mutants lack petals and stamens; (D) *agamous1* mutants lack both stamens and carpels. (From Bewley et al. 2000.)

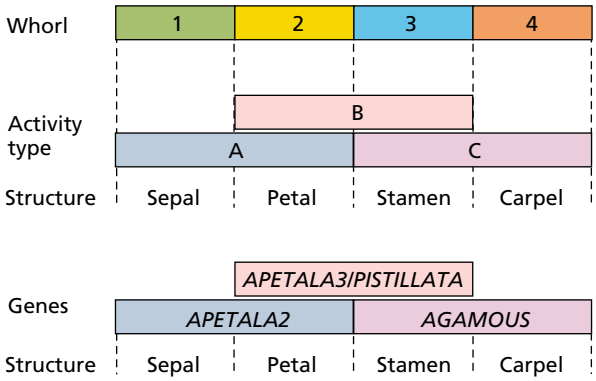


FIGURE 24.6 The ABC model for the acquisition of floral organ identity is based on the interactions of three different types of activities of floral homeotic genes: A, B, and C. In the first whorl, expression of type A (*AP2*) alone results in the formation of sepals. In the second whorl, expression of both type A (*AP2*) and type B (*AP3/PI*) results in the formation of petals. In the third whorl, the expression of B (*AP3/PI*) and C (*AG*) causes the formation of stamens. In the fourth whorl, activity C (*AG*) alone specifies carpels. In addition, activity A (*AP2*) represses activity C (*AG*) in whorls 1 and 2, while C represses A in whorls 3 and 4.

1. Type A activity, encoded by *AP1* and *AP2*, controls organ identity in the first and second whorls. Loss of type A activity results in the formation of carpels instead of sepals in the first whorl, and of stamens instead of petals in the second whorl.
2. Type B activity, encoded by *AP3* and *PI*, controls organ determination in the second and third whorls. Loss of type B activity results in the formation of sepals instead of petals in the second whorl, and of carpels instead of stamens in the third whorl.
3. Type C activity, encoded by *AG*, controls events in the third and fourth whorls. Loss of type C activity results in the formation of petals instead of stamens in the third whorl, and replacement of the fourth whorl by a new flower such that the fourth whorl of the *ag* mutant flower is occupied by sepals.

The control of organ identity by type A, B, and C homeotic genes (the ABC model) is described in more detail in the next section.

FIGURE 24.7 A quadruple mutant (*api1, ap2, ap3/pi, ag*) results in the production of leaf-like structures in place of floral organs. (Courtesy of John Bowman.)

The role of the organ identity genes in floral development is dramatically illustrated by experiments in which two or three activities are eliminated by loss-of-function mutations (Figure 24.7). Quadruple-mutant plants (*ap1, ap2, ap3/pi,* and *ag*) produce floral meristems that develop as pseudoflowers; all the floral organs are replaced with green leaflike structures, although these organs are produced with a whorled phyllotaxy. Evolutionary biologists, beginning with the eighteenth-century German poet, philosopher, and natural scientist Johann Wolfgang von Goethe (1749–1832), have speculated that floral organs are highly modified leaves, and this experiment gives direct support to these ideas.

The ABC Model Explains the Determination of Floral Organ Identity

In 1991 the **ABC model** was proposed to explain how homeotic genes control organ identity. The ABC model postulates that organ identity in each whorl is determined by a unique combination of the three organ identity gene activities (see Figure 24.6):

- Activity of type A alone specifies sepals.
- Activities of both A and B are required for the formation of petals.
- Activities of B and C form stamens.
- Activity of C alone specifies carpels.

The model further proposes that activities A and C mutually repress each other (see Figure 24.6); that is, both A- and C-type genes have cadastral function in addition to their function in determining organ identity.

The patterns of organ formation in the wild type and most of the mutant phenotypes are predicted and explained by this model (Figure 24.8). The challenge now is to understand how the expression pattern of these organ identity genes is controlled by cadastral genes; how organ identity genes, which encode transcription factors, alter the pattern of other genes expressed in the developing organ; and finally how this altered pattern of gene expression results in the development of a specific floral organ.

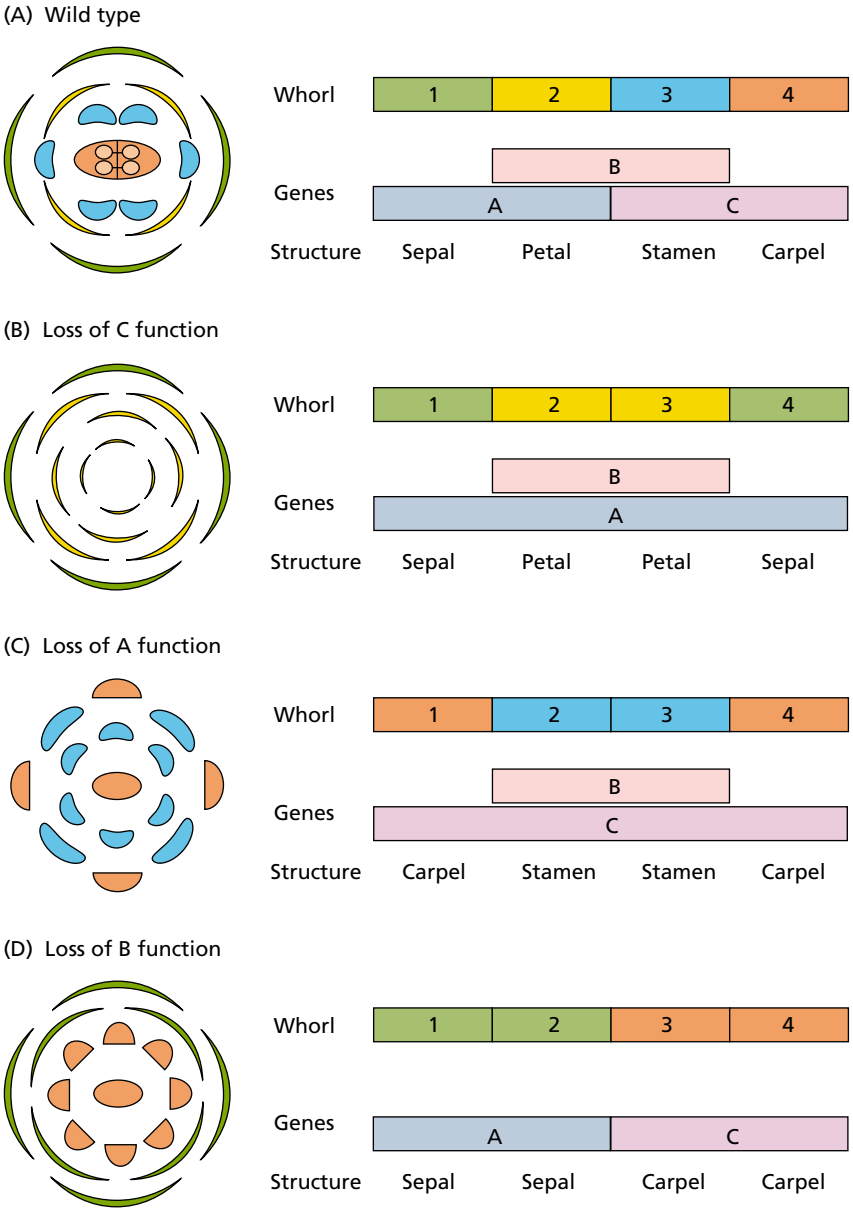


FIGURE 24.8 Interpretation of the phenotypes of floral homeotic mutants based on the ABC model. (A) Wild type. (B) Loss of C function results in expansion of the A function throughout the floral meristem. (C) Loss of A function results in the spread of C function throughout the meristem. (D) Loss of B function results in the expression of only A and C functions.

FLORAL EVOCATION: INTERNAL AND EXTERNAL CUES

A plant may flower within a few weeks after germinating, as in annual plants such as groundsel (*Senecio vulgaris*). Alternatively, some perennial plants, such as many forest trees, may grow for 20 or more years before they begin to produce flowers. Different species flower at widely different ages, indicating that the age, or perhaps the size, of the plant is an *internal* factor controlling the switch to reproductive development. The case in which flowering occurs strictly in response to internal developmental factors and does not depend on any particular environmental conditions is referred to as *autonomous regulation*.

In contrast to plants that flower entirely through an autonomous pathway, some plants exhibit an absolute requirement for the proper environmental cues in order to flower. This condition is termed an *obligate* or *qualitative* response to an environmental cue. In other plant species, flowering is promoted by certain environmental cues but will eventually occur in the absence of such cues. This is called a *facultative* or *quantitative* response to an environmental cue. The flowering of this latter group of plants, which includes *Arabidopsis*, thus relies on both environmental and autonomous flowering systems.

Photoperiodism and vernalization are two of the most important mechanisms underlying seasonal responses. *Photoperiodism* is a response to the length of day; *vernaliza-*

tion is the promotion of flowering—at subsequent higher temperatures—brought about by exposure to cold. Other signals, such as total light radiation and water availability, can also be important external cues.

The evolution of both internal (autonomous) and external (environment-sensing) control systems enables plants to carefully regulate flowering at the optimal time for reproductive success. For example, in many populations of a particular species, flowering is synchronized. This synchrony favors crossbreeding and allows seeds to be produced in favorable environments, particularly with respect to water and temperature.

THE SHOOT APEX AND PHASE CHANGES

All multicellular organisms pass through a series of more or less defined developmental stages, each with its characteristic features. In humans, infancy, childhood, adolescence, and adulthood represent four general stages of development, and puberty is the dividing line between the nonreproductive and the reproductive phases. Higher plants likewise pass through developmental stages, but whereas in animals these changes take place throughout the entire organism, in higher plants they occur in a single, dynamic region, the **shoot apical meristem**.

Shoot Apical Meristems Have Three Developmental Phases

During postembryonic development, the shoot apical meristem passes through three more or less well-defined developmental stages in sequence:

1. The juvenile phase
2. The adult vegetative phase
3. The adult reproductive phase

The transition from one phase to another is called **phase change**.

The primary distinction between the juvenile and the adult vegetative phases is that the latter has the ability to form reproductive structures: flowers in angiosperms, cones in gymnosperms. However, actual expression of the reproductive competence of the adult phase (i.e., flowering) often depends on specific environmental and developmental signals. Thus the absence of flowering itself is not a reliable indicator of juvenility.

The transition from juvenile to adult is frequently accompanied by changes in vegetative characteristics, such as leaf morphology, phyllotaxy (the arrangement of leaves on the stem), thorniness, rooting capacity, and leaf retention in deciduous plants (Figure 24.9; see also **Web Topic 24.1**). Such changes are most evident in woody perennials, but they are apparent in many herbaceous species as well. Unlike the abrupt transition from the adult vegetative phase to the reproductive phase, the transition from juvenile to vegetative adult is usually gradual, involving intermediate forms.

FIGURE 24.9 Juvenile and adult forms of ivy (*Hedera helix*). The juvenile form has lobed palmate leaves arranged alternately, a climbing growth habit, and no flowers. The adult form (projecting out to the right) has entire ovate leaves arranged in spirals, an upright growth habit, and flowers. (Photo by L. Taiz.)

Sometimes the transition can be observed in a single leaf. A dramatic example of this is the progressive transformation of juvenile leaves of the leguminous tree *Acacia heterophylla* into phyllodes, a phenomenon noted by Goethe. Whereas the juvenile pinnately compound leaves consist of rachis (stalk) and leaflets, adult phyllodes are specialized structures representing flattened petioles (Figure 24.10).

Intermediate structures also form during the transition from aquatic to aerial leaf types of aquatic plants such as *Hippuris vulgaris* (common marestail). As in the case of *A. heterophylla*, these intermediate forms possess distinct regions with different developmental patterns. To account for intermediate forms during the transition from juvenile to adult in maize (see **Web Topic 24.2**), a **combinatorial model** has been proposed (Figure 24.11). According to this model, shoot development can be described as a series of independently regulated, *overlapping* programs (juvenile, adult, and reproductive) that modulate the expression of a common set of developmental processes.

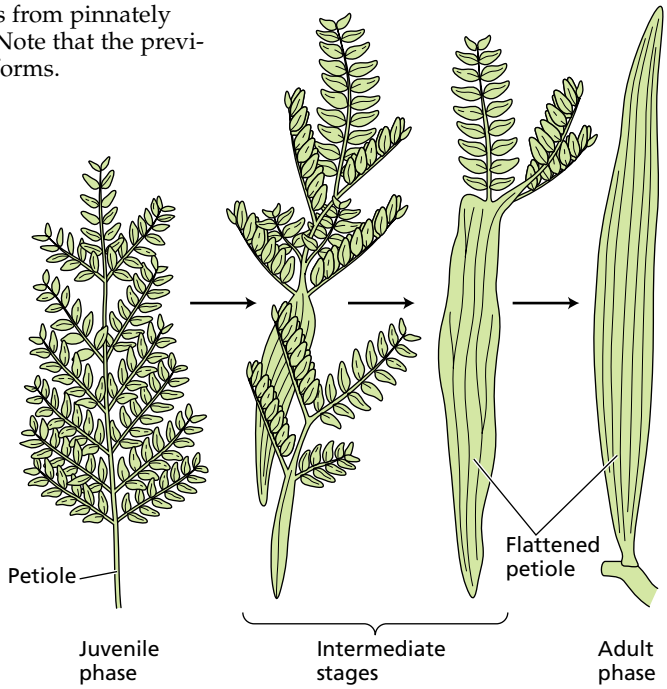


FIGURE 24.10 Leaves of *Acacia heterophylla*, showing transitions from pinnately compound leaves (juvenile phase) to phyllodes (adult phase). Note that the previous phase is retained at the top of the leaf in the intermediate forms.

In the transition from juvenile to adult leaves, the intermediate forms indicate that different regions of the same leaf can express different developmental programs. Thus the cells at the tip of the leaf remain committed to the juvenile program, while the cells at the base of the leaf become committed to the adult program. The developmental fates of the two sets of cells in the same leaf are quite different.

Juvenile Tissues Are Produced First and Are Located at the Base of the Shoot

The sequence in time of the three developmental phases results in a spatial gradient of juvenility along the shoot axis. Because growth in height is restricted to the apical meristem, the juvenile tissues and organs, which form first, are located at the base of the shoot. In rapidly flowering herbaceous species, the juvenile phase may last only a few days, and few juvenile structures are produced. In contrast, woody species have a more prolonged juvenile phase, in some cases lasting 30 to 40 years (Table 24.1). In these cases the juvenile structures can account for a significant portion of the mature plant.

Once the meristem has switched over to the adult phase, only adult vegetative structures are produced, culminating in floral evocation. The adult and reproductive phases are therefore located in the upper and peripheral regions of the shoot.

Attainment of a sufficiently large size appears to be more important than the plant's chronological age in determining the transition to the adult phase. Conditions that retard growth, such as mineral deficiencies, low light, water stress, defoliation, and low temperature tend to prolong the juvenile phase or even cause **rejuvenation** (reversion to juvenility) of adult shoots. In contrast, conditions that promote vigorous growth accelerate the transition to the adult phase. When growth is accelerated, exposure to the correct flower-inducing treatment can result in flowering.

Although plant size seems to be the most important factor, it is not always clear which specific component associated with size is critical. In some *Nicotiana* species, it appears that plants must produce a certain number of leaves to transmit a sufficient amount of the floral stimulus to the apex.

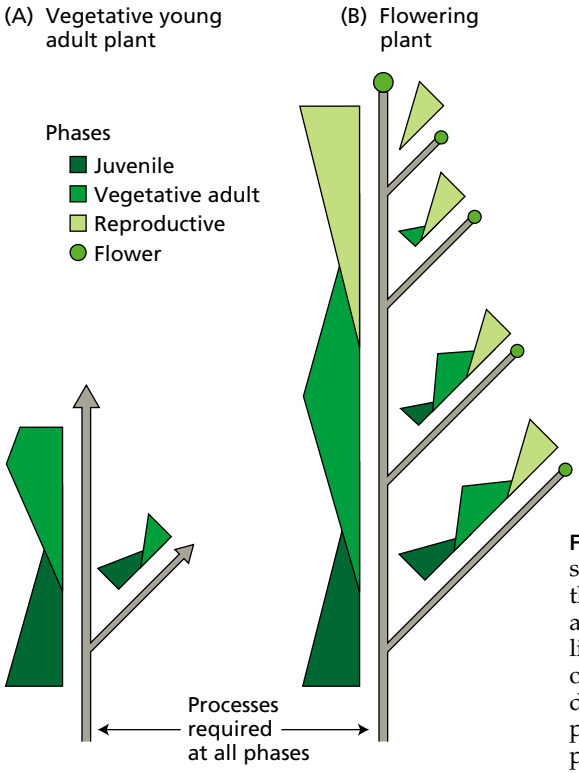


FIGURE 24.11 Schematic representation of the combinatorial model of shoot development in maize. Overlapping gradients of expression of the juvenile, vegetative adult, and reproductive phases are indicated along the length of the main axis and branches. The continuous black line represents processes that are required during all phases of development. Each of the three phases may be regulated by separated developmental programs, with intermediate phases arising when the programs overlap. (A) Vegetative young adult plant. (B) Flowering plant. (After Poethig 1990.)

TABLE 24.1
Length of juvenile period in some woody plant species

Species	Length of juvenile period
Rose (*Rosa* [hybrid tea])	20–30 days
Grape (*Vitis* spp.)	1 year
Apple (*Malus* spp.)	4–8 years
Citrus spp.	5–8 years
English ivy (*Hedera helix*)	5–10 years
Redwood (*Sequoia sempervirens*)	5–15 years
Sycamore maple (*Acer pseudoplatanus*)	15–20 years
English oak (*Quercus robur*)	25–30 years
European beech (*Fagus sylvatica*)	30–40 years

Source: Clark 1983.

Once the adult phase has been attained, it is relatively stable, and it is maintained during vegetative propagation or grafting. For example, in mature plants of English ivy (*Hedera helix*), cuttings taken from the basal region develop into juvenile plants, while those from the tip develop into adult plants. When scions were taken from the base of the flowering tree silver birch (*Betula verrucosa*) and grafted onto seedling rootstocks, there were no flowers on the grafts within the first 2 years. In contrast, the grafts flowered freely when scions were taken from the top of the flowering tree.

In some species, the juvenile meristem appears to be capable of flowering but does not receive sufficient floral stimulus until the plant becomes large enough. In mango (*Mangifera indica*), for example, juvenile seedlings can be induced to flower when grafted to a mature tree. In many other woody species, however, grafting to an adult flowering plant does not induce flowering.

Phase Changes Can Be Influenced by Nutrients, Gibberellins, and Other Chemical Signals

The transition at the shoot apex from the juvenile to the adult phase can be affected by transmissible factors from the rest of the plant. In many plants, exposure to low-light conditions prolongs juvenility or causes reversion to juvenility. A major consequence of the low-light regime is a reduction in the supply of carbohydrates to the apex; thus carbohydrate supply, especially sucrose, may play a role in the transition between juvenility and maturity. Carbohydrate supply as a source of energy and raw material can affect the size of the apex. For example, in the florist's chrysanthemum (*Chrysanthemum morifolium*), flower primordia are not initiated until a minimum apex size has been reached.

The apex receives a variety of hormonal and other factors from the rest of the plant in addition to carbohydrates and other nutrients. Experimental evidence shows that the application of gibberellins causes reproductive structures to form in young, juvenile plants of several conifer families. The involvement of *endogenous* GAs in the control of reproduction is also indicated by the fact that other treatments that accelerate cone production in pines (e.g., root removal, water stress, and nitrogen starvation) often also result in a buildup of GAs in the plant.

On the other hand, although gibberellins promote the attainment of reproductive maturity in conifers and many herbaceous angiosperms as well, GA_3 causes rejuvenation in *Hedera* and in several other woody angiosperms. The role of gibberellins in the control of phase change is thus complex, varies among species, and probably involves interactions with other factors.

Competence and Determination Are Two Stages in Floral Evocation

The term *juvenility* has different meanings for herbaceous and woody species. Whereas juvenile herbaceous meristems flower readily when grafted onto flowering adult plants (see **Web Topic 24.3**), juvenile woody meristems generally do not. What is the difference between the two?

Extensive studies in tobacco have demonstrated that floral evocation requires the apical bud to pass through two developmental stages (Figure 24.12) (McDaniel et al. 1992). One stage is the acquisition of competence. A bud is said to be **competent** if it is able to flower when given the appropriate developmental signal.

For example, if a vegetative shoot (scion) is grafted onto a flowering stock and the scion flowers immediately, it is demonstrably capable of responding to the level of floral stimulus present in the stock and is therefore competent. Failure of the scion to flower would indicate that the shoot apical meristem has not yet attained competence. Thus the juvenile meristems of herbaceous plants are competent to flower, but those of woody species are not.

The next stage that a competent vegetative bud goes through is determination. A bud is said to be **determined** if it progresses to the next developmental stage (flowering) even after being removed from its normal context. Thus a florally determined bud will produce flowers even if it is grafted onto a vegetative plant that is not producing any floral stimulus.

In a day-neutral tobacco, for example, plants typically flower after producing about 41 leaves or nodes. In an experiment to measure the floral determination of the axillary buds, flowering tobacco plants were decapitated just above the thirty-fourth leaf (from the bottom). Released from apical dominance, the axillary bud of the thirty-fourth leaf grew out, and after producing 7 more leaves (for a total of 41), it flowered (Figure 24.13A) (McDaniel 1996). However, if the thirty-fourth bud was excised from the plant and either rooted or grafted onto a stock without leaves near the base, it produced a complete set of leaves (41) before flowering. This result shows that the thirty-fourth bud was not yet florally determined.

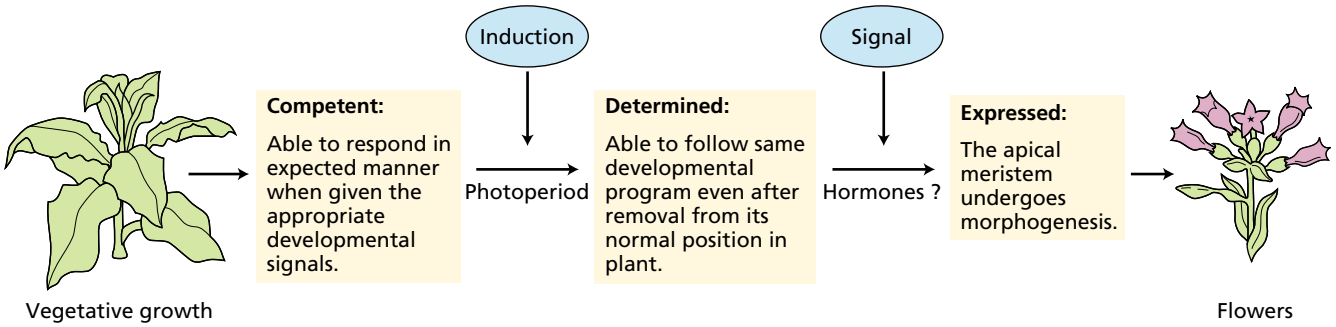


FIGURE 24.12 A simplified model for floral evocation at the shoot apex in which the cells of the vegetative meristem acquire new developmental fates. To initiate floral development, the cells of the meristem must first become competent. A competent vegetative meristem is one that can respond to a floral stimulus (induction) by becoming florally determined (committed to producing a flower). The determined state is usually expressed, but this may require an additional signal. (After McDaniel et al. 1992.)

In another experiment, the donor plant was decapitated above the thirty-seventh leaf. This time the thirty-seventh axillary bud flowered after producing four leaves *in all three situations* (see Figure 24.13B). This result demonstrates that the terminal bud became florally determined after initiating 37 leaves.

Extensive grafting of shoot tips among tobacco varieties has established that the number of nodes a meristem produces before flowering is a function of two factors: (1) the strength of the floral stimulus from the leaves and (2) the competence of the meristem to respond to the signal (McDaniel et al. 1996).

In some cases the **expression** of flowering may be delayed or arrested even after the apex becomes determined, unless it receives a second developmental signal that stimulates expression (see Figure 24.12). For example, intact *Lolium temulentum* (darnel ryegrass) plants become committed to flowering after a single exposure to a long day. If the *Lolium* shoot apical meristem is excised 28 hours after the beginning of the long day and cultured in vitro, it will produce normal inflorescences in culture, but only if the hormone gibberellic acid (GA) is present in the medium. Because apices cultured from plants grown exclusively in short days never flower, even in the presence of

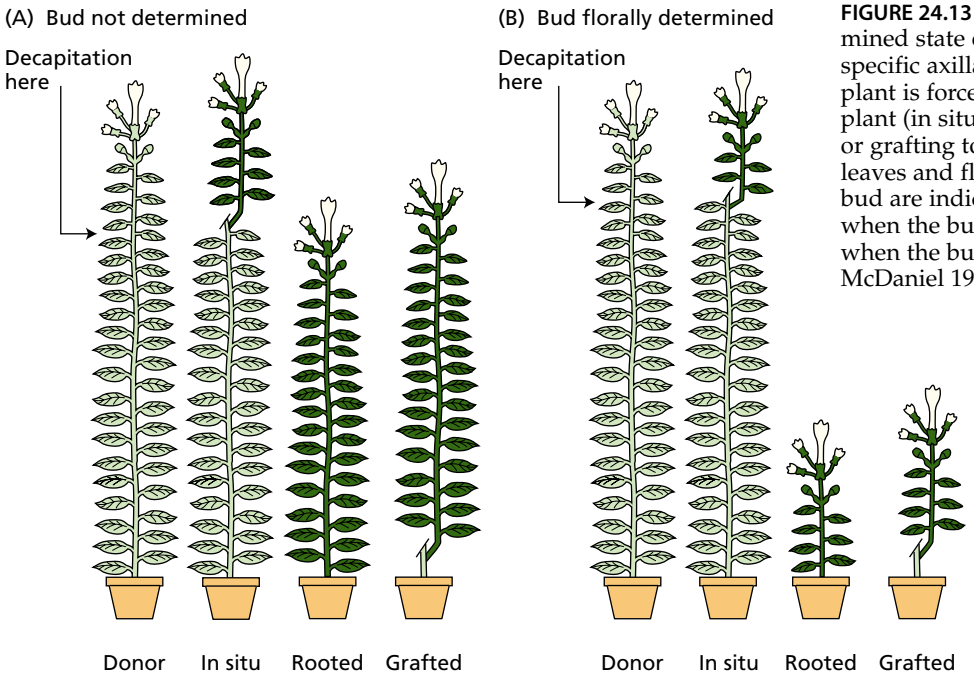


FIGURE 24.13 Demonstration of the determined state of axillary buds in tobacco. A specific axillary bud of a flowering donor plant is forced to grow, either directly on the plant (in situ) by decapitation, or by rooting or grafting to the base of the plant. The new leaves and flowers produced by the axillary bud are indicated by shading. (A) Result when the bud is not determined. (B) Result when the bud is florally determined. (After McDaniel 1996.)

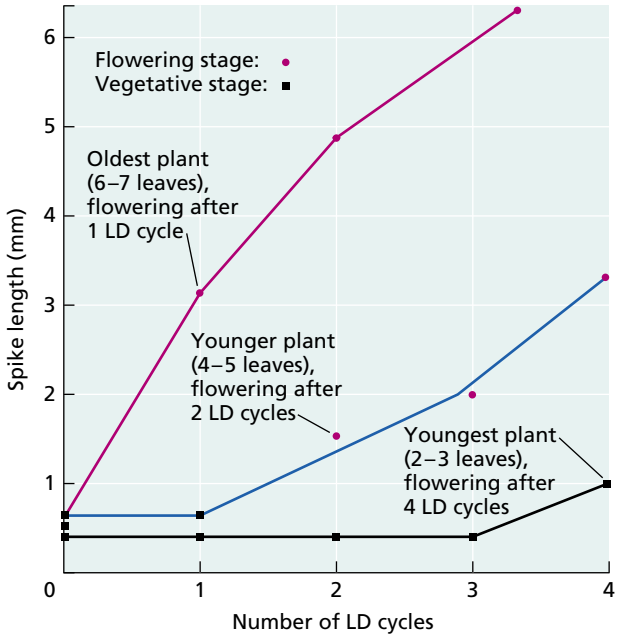


FIGURE 24.14 Effect of plant age on the number of long-day (LD) inductive cycles required for flowering in the long-day plant *Lolium temulentum* (darnel ryegrass). An inductive long-day cycle consisted of 8 hours of sunlight followed by 16 hours of low-intensity incandescent light. The older the plant is, the fewer photoinductive cycles are needed to produce flowering.

GA, we can conclude that long days are required for determination in *Lolium*, whereas GA is required for *expression* of the determined state.

In general, once a meristem has become competent, it exhibits an increasing tendency to flower with age (leaf number). For example, in plants controlled by day length, the number of short-day or long-day cycles necessary to achieve flowering is often fewer in older plants (Figure 24.14). As will be discussed later in the chapter, this increasing tendency to flower with age has its physiological basis in the greater capacity of the leaves to produce a floral stimulus.

Before discussing how plants perceive day length, however, we will lay the foundation by examining how organisms measure time in general. This topic is known as **chronobiology**, or the study of **biological clocks**. The best-understood biological clock is the circadian rhythm.

CIRCADIAN RHYTHMS: THE CLOCK WITHIN

Organisms are normally subjected to daily cycles of light and darkness, and both plants and animals often exhibit rhythmic behavior in association with these changes. Examples of such rhythms include leaf and petal movements (day and night positions), stomatal opening and closing, growth and sporulation patterns in fungi (e.g., *Pilobolus* and *Neurospora*), time of day of pupal emergence (the fruit fly *Drosophila*), and activity cycles in rodents, as well as metabolic processes such as photosynthetic capacity and respiration rate.

When organisms are transferred from daily light–dark cycles to continuous darkness (or continuous dim light), many of these rhythms continue to be expressed, at least for several days. Under such uniform conditions the period of the rhythm is then close to 24 hours, and consequently the term circadian rhythm is applied (see Chapter 17). Because they continue in a constant light or dark environment, these circadian rhythms cannot be direct responses to the presence or absence of light but must be based on an internal pacemaker, often called an endogenous oscillator. A molecular model for a plant endogenous oscillator was described in Chapter 17.

The endogenous oscillator is coupled to a variety of physiological processes, such as leaf movement or photosynthesis, and it maintains the rhythm. For this reason the endogenous oscillator can be considered the clock mechanism, and the physiological functions that are being regulated, such as leaf movements or photosynthesis, are sometimes referred to as the hands of the clock.

Circadian Rhythms Exhibit Characteristic Features

Circadian rhythms arise from cyclic phenomena that are defined by three parameters:

1. **Period**, the time between comparable points in the repeating cycle. Typically the period is measured as the time between consecutive maxima (peaks) or minima (troughs) (Figure 24.15A).
2. **Phase**[2], any point in the cycle that is recognizable by its relationship to the rest of the cycle. The most obvious phase points are the peak and trough positions.
3. **Amplitude**, usually considered to be the distance between peak and trough. The amplitude of a biological rhythm can often vary while the period remains unchanged (as, for example, in Figure 24.15C).

In constant light or darkness, rhythms depart from an exact 24-hour period. The rhythms then drift in relation to solar time, either gaining or losing time depending on whether the period is shorter or longer than 24 hours. Under natural conditions, the endogenous oscillator is

[2] The term *phase* should not be confused with the term *phase change* in meristem development, discussed earlier.

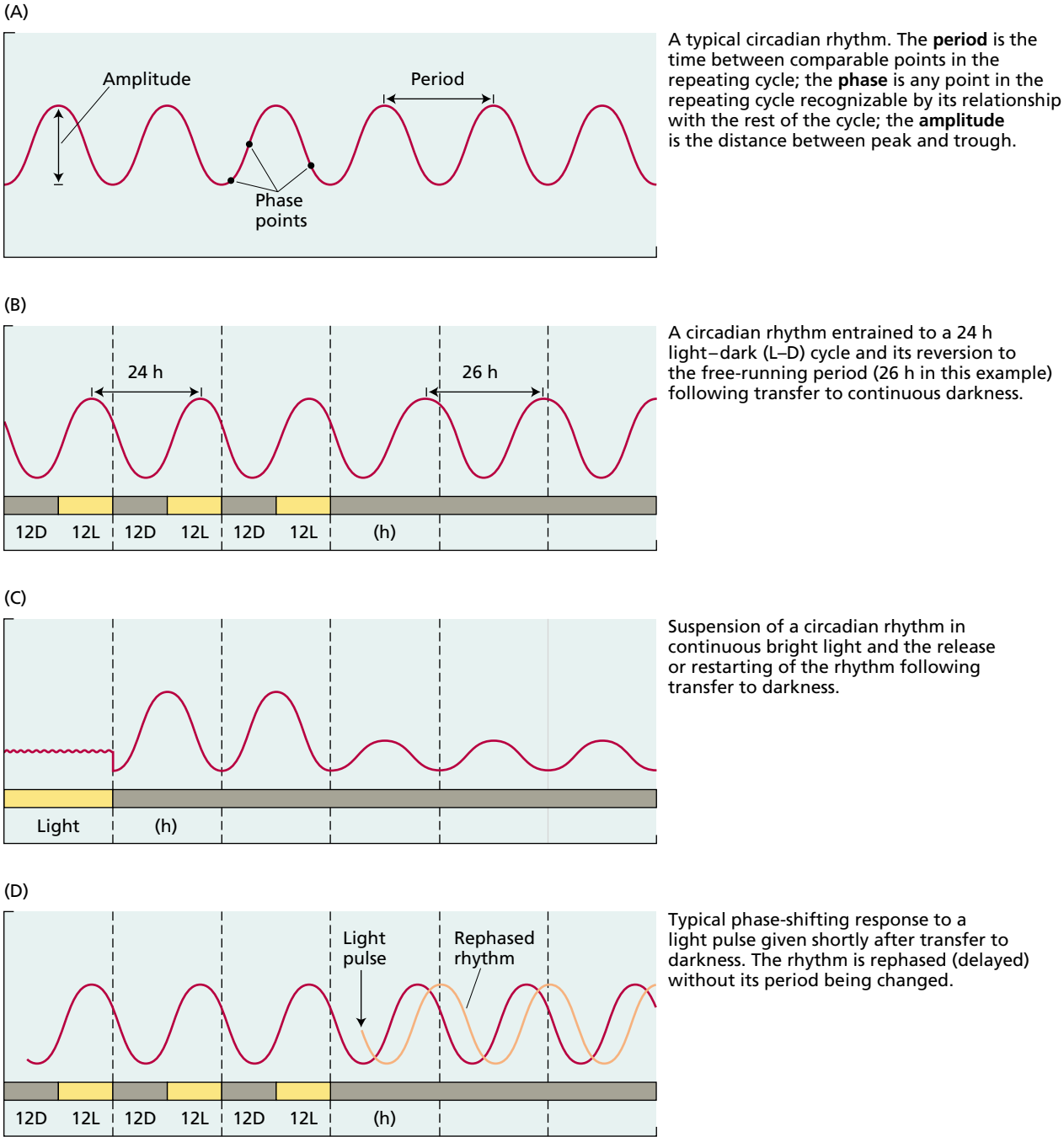


FIGURE 24.15 Some characteristics of circadian rhythms.

entrained (synchronized) to a true 24-hour period by environmental signals, the most important of which are the light-to-dark transition at dusk and the dark-to-light transition at dawn (see Figure 24.15B).

Such environmental signals are termed **zeitgebers** (German for "time givers"). When such signals are removed—for example, by transfer to continuous darkness—the rhythm is said to be **free-running,** and it reverts to the circadian period that is characteristic of the particular organism (see Figure 24.15B).

Although the rhythms are generated internally, they normally require an environmental signal, such as exposure to light or a change in temperature, to initiate their expression. In addition, many rhythms damp out (i.e., the

amplitude decreases) when the organism is in a constant environment for some time and then require an environmental zeitgeber, such as a transfer from light to dark or a change in temperature, to be restarted (see Figure 24.15C). Note that the clock itself does not damp out; only the coupling between the molecular clock (endogenous oscillator) and the physiological function is affected.

The circadian clock would be of no value to the organism if it could not keep accurate time under the fluctuating temperatures experienced in natural conditions. Indeed, temperature has little or no effect on the period of the free-running rhythm. The feature that enables the clock to keep time at different temperatures is called **temperature compensation**. Although all of the biochemical steps in the pathway are temperature-sensitive, their temperature responses probably cancel each other. For example, changes in the rates of synthesis of intermediates could be compensated for by parallel changes in their rates of degradation. In this way, the steady-state levels of clock regulators would remain constant at different temperatures.

Phase Shifting Adjusts Circadian Rhythms to Different Day–Night Cycles

In circadian rhythms, the operation of the endogenous oscillator sets a response to occur at a particular time of day. A single oscillator can be coupled to multiple circadian rhythms, which may even be out of phase with each other. How do such responses remain on time when the daily durations of light and darkness change with the seasons? The answer to this question lies in the fact that the phase of the rhythm can be changed if the whole cycle is moved forward or backward in time without its period being altered.

Investigators test the response of the endogenous oscillator usually by placing the organism in continuous darkness and examining the response to a short pulse of light (usually less than 1 hour) given at different phase points in the free-running rhythm. When an organism is entrained to a cycle of 12 hours light and 12 hours dark and then allowed to free-run in darkness, the phase of the rhythm that coincides with the light period of the previous entraining cycle is called the **subjective day**, and the phase that coincides with the dark period is called the **subjective night**.

If a light pulse is given during the first few hours of the subjective night, the rhythm is delayed; the organism interprets the light pulse as the end of the previous day (see Figure 24.15D). In contrast, a light pulse given toward the end of the subjective night advances the phase of the rhythm; now the organism interprets the light pulse as the beginning of the following day.

As already pointed out, this is precisely the pattern of response that would be expected if the rhythm is to stay on local time. Therefore, these phase-shifting responses enable the rhythm to be entrained to approximately 24-hour cycles with different durations of light and darkness, and they demonstrate that the rhythm will run differently under different natural conditions of day length.

Phytochromes and Cryptochromes Entrain the Clock

The molecular mechanism whereby a light signal causes phase shifting is not yet known, but studies in *Arabidopsis* have identified some of the key elements of the circadian oscillator and its inputs and outputs (see Chapter 17). The low levels and specific wavelengths of light that can induce phase shifting indicate that the light response must be mediated by specific photoreceptors rather than rates of photosynthesis. For example, the red-light entrainment of rhythmic nyctonastic leaf movements in *Samanea*, a semitropical leguminous tree, is a low-fluence response mediated by phytochrome (see Chapter 17).

Arabidopsis has five phytochromes, and all but one of them (phytochrome C) have been implicated in clock entrainment. Each phytochrome acts as a specific photoreceptor for red, far-red, or blue light. In addition, the CRY1 and CRY2 proteins participate in blue-light entrainment of the clock, as they do in insects and mammals (Devlin and Kay 2000). Surprisingly, CRY proteins also appear to be required for normal entrainment by red light. Since these proteins do not absorb red light, this requirement suggests that CRY1 and CRY2 may act as intermediates in phytochrome signaling during entrainment of the clock (Yanovsky and Kay 2001).

In *Drosophila*, CRY proteins interact physically with clock components and thus constitute part of the oscillator mechanism (Devlin and Kay 2000). However, this does not appear to be the case in *Arabidopsis*, in which *cry1/cry2* double mutants have normal circadian rhythms. Precisely how *Arabidopsis* CRY proteins interact with the endogenous oscillator mechanism to induce phase shifting remains to be elucidated (Yanovsky et al. 2001).

PHOTOPERIODISM: MONITORING DAY LENGTH

As we have seen, the circadian clock enables organisms to determine the time of *day* at which a particular molecular or biochemical event occurs. **Photoperiodism**, or the ability of an organism to detect day length, makes it possible for an event to occur at a particular time of *year*, thus allowing for a *seasonal* response. Circadian rhythms and photoperiodism have the common property of responding to cycles of light and darkness.

Precisely at the equator, day length and night length are equal and constant throughout the year. As one moves away from the equator toward the poles, the days become longer in summer and shorter in winter (Figure 24.16). Not surprisingly, plant species have evolved to detect these seasonal changes in day length, and their specific photoperiodic responses are strongly influenced by the latitude from which they originated.

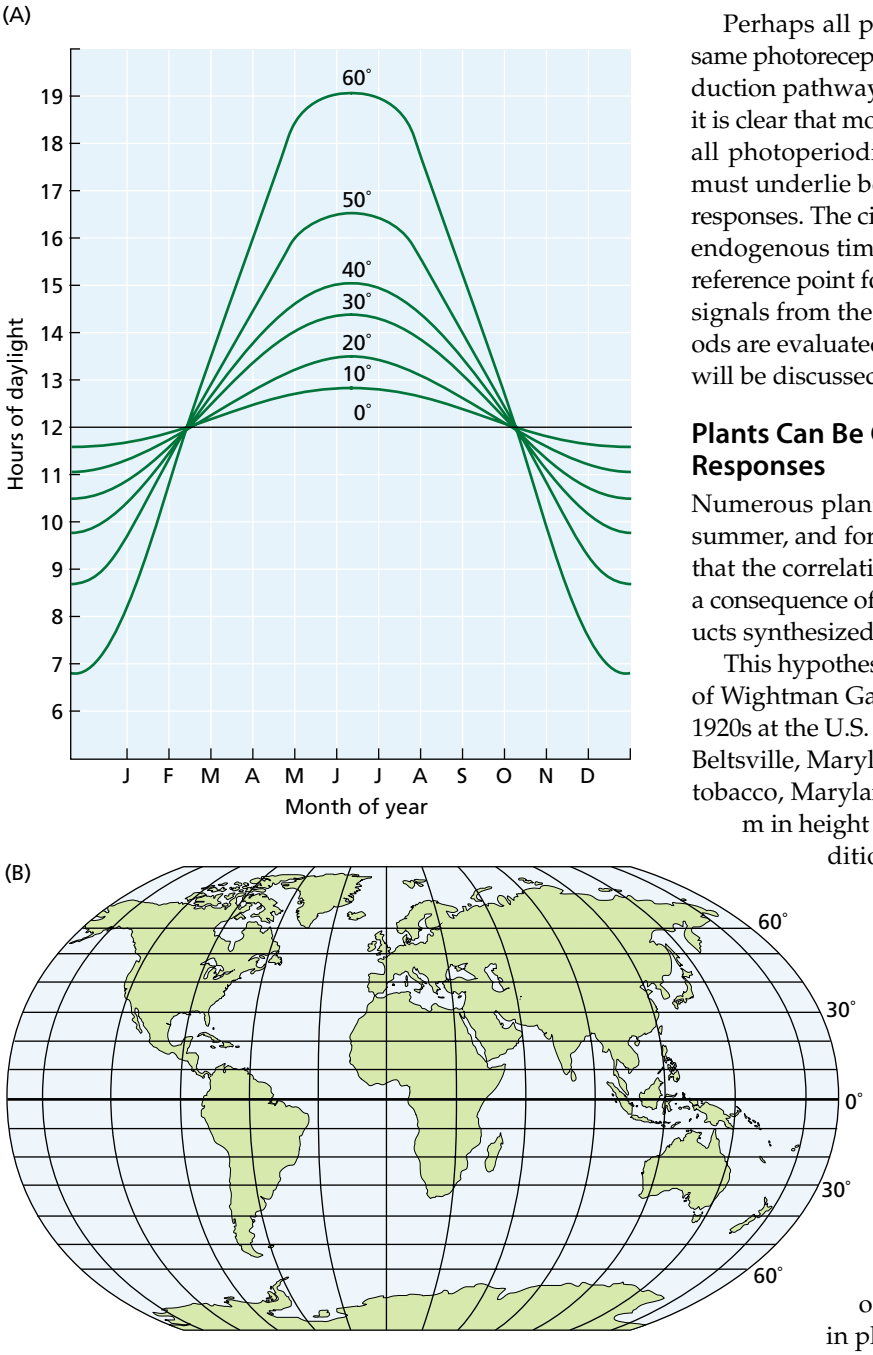


FIGURE 24.16 (A) The effect of latitude on day length at different times of the year. Day length was measured on the twentieth of each month. (B) Global map showing longitudes and latitudes.

Photoperiodic phenomena are found in both animals and plants. In the animal kingdom, day length controls such seasonal activities as hibernation, development of summer or winter coats, and reproductive activity. Plant responses controlled by day length are numerous, including the initiation of flowering, asexual reproduction, the formation of storage organs, and the onset of dormancy.

Perhaps all plant photoperiodic responses utilize the same photoreceptors, with subsequent specific signal transduction pathways regulating different responses. Because it is clear that monitoring the passage of time is essential to all photoperiodic responses, a timekeeping mechanism must underlie both the time-of-year and the time-of-day responses. The circadian oscillator is thought to provide an endogenous time-measuring mechanism that serves as a reference point for the response to incoming light (or dark) signals from the environment. How changing photoperiods are evaluated against the circadian oscillator reference will be discussed shortly.

Plants Can Be Classified by Their Photoperiodic Responses

Numerous plant species flower during the long days of summer, and for many years plant physiologists believed that the correlation between long days and flowering was a consequence of the accumulation of photosynthetic products synthesized during long days.

This hypothesis was shown to be incorrect by the work of Wightman Garner and Henry Allard, conducted in the 1920s at the U.S. Department of Agriculture laboratories in Beltsville, Maryland. They found that a mutant variety of tobacco, Maryland Mammoth, grew profusely to about 5 m in height but failed to flower in the prevailing conditions of summer (Figure 24.17). However, the plants flowered in the greenhouse during the winter under natural light conditions.

These results ultimately led Garner and Allard to test the effect of artificially providing short days by covering plants grown during the long days of summer with a light-tight tent from late in the afternoon until the following morning. These artificial short days also caused the plants to flower. This requirement for short days was difficult to reconcile with the idea that longer periods of radiation and the resulting increase in photosynthesis promote flowering in general. Garner and Allard concluded that the length of the day was the determining factor in flowering and were able to confirm this hypothesis in many different species and conditions. This work laid the foundations for the extensive subsequent research on photoperiodic responses.

The classification of plants according to their photoperiodic responses is usually based on flowering, even though many other aspects of plants' development may also be affected by day length. The two main photoperiodic response categories are short-day plants and long-day plants:

1. **Short-day plants** (**SDPs**) flower only in short days (*qualitative* SDPs), or their flowering is accelerated by short days (*quantitative* SDPs).

FIGURE 24.17 Maryland Mammoth mutant of tobacco (right) compared to wild-type tobacco (left). Both plants were grown during summer in the greenhouse. (University of Wisconsin graduate students used for scale.) (Photo courtesy of R. Amasino.)

2. **Long-day plants** (**LDPs**) flower only in long days (*qualitative* LDPs), or their flowering is accelerated by long days (*quantitative* LDPs).

The essential distinction between long-day and short-day plants is that flowering in LDPs is promoted only when the day length *exceeds* a certain duration, called the **critical day length**, in every 24-hour cycle, whereas promotion of flowering in SDPs requires a day length that is *less than* the critical day length. The absolute value of the critical day length varies widely among species, and only when flowering is examined for a range of day lengths can the correct photoperiodic classification be established (Figure 24.18).

Long-day plants can effectively measure the lengthening days of spring or early summer and delay flowering until the critical day length is reached. Many varieties of wheat (*Triticum aestivum*) behave in this way. SDPs often flower in fall, when the days shorten below the critical day length, as in many varieties of *Chrysanthemum morifolium.* However, day length alone is an ambiguous signal because it cannot distinguish between spring and fall.

Plants exhibit several adaptations for avoiding the ambiguity of day length signal. One is the coupling of a temperature requirement to a photoperiodic response. Certain plant species, such as winter wheat, do not respond to photoperiod until after a cold period (vernalization or overwintering) has occurred. (We will discuss vernalization a little later in the chapter.)

Other plants avoid seasonal ambiguity by distinguishing between *shortening* and *lengthening* days. Such "dual–day length plants" fall into two categories:

1. **Long-short-day plants** (**LSDPs**) flower only after a sequence of long days followed by short days. LSDPs, such as *Bryophyllum*, *Kalanchoe*, and *Cestrum nocturnum* (night-blooming jasmine), flower in the late summer and fall, when the days are shortening.
2. **Short-long-day plants** (**SLDPs**) flower only after a sequence of short days followed by long days. SLDPs, such as *Trifolium repens* (white clover), *Campanula medium* (Canterbury bells), and *Echeveria harmsii* (echeveria), flower in the early spring in response to lengthening days.

Finally, species that flower under any photoperiodic condition are referred to as *day-neutral plants*. **Day-neutral plants** (**DNPs**) are insensitive to day length. Flowering in DNPs is typically under autonomous regulation—that is, internal developmental control. Some day-neutral species,

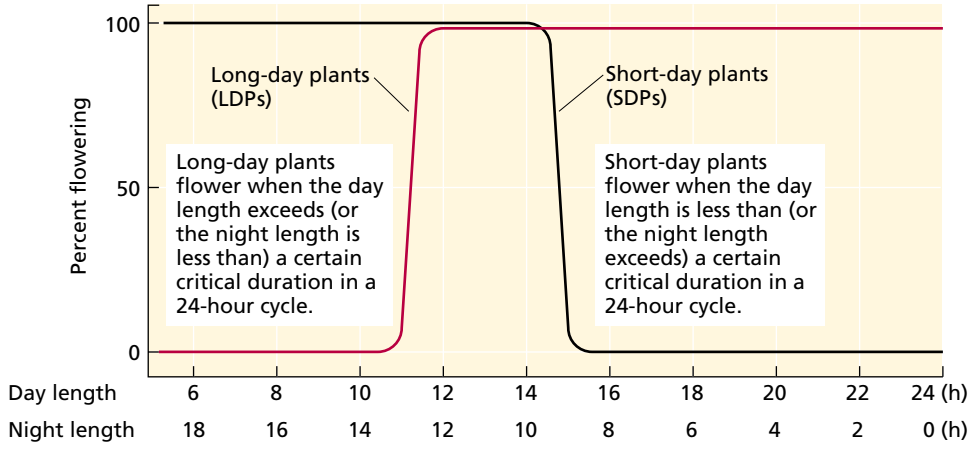


FIGURE 24.18 The photoperiodic response in long- and short-day plants. The critical duration varies between species: In this example, both the SDPs and the LDPs would flower in photoperiods between 12 and 14 h long.

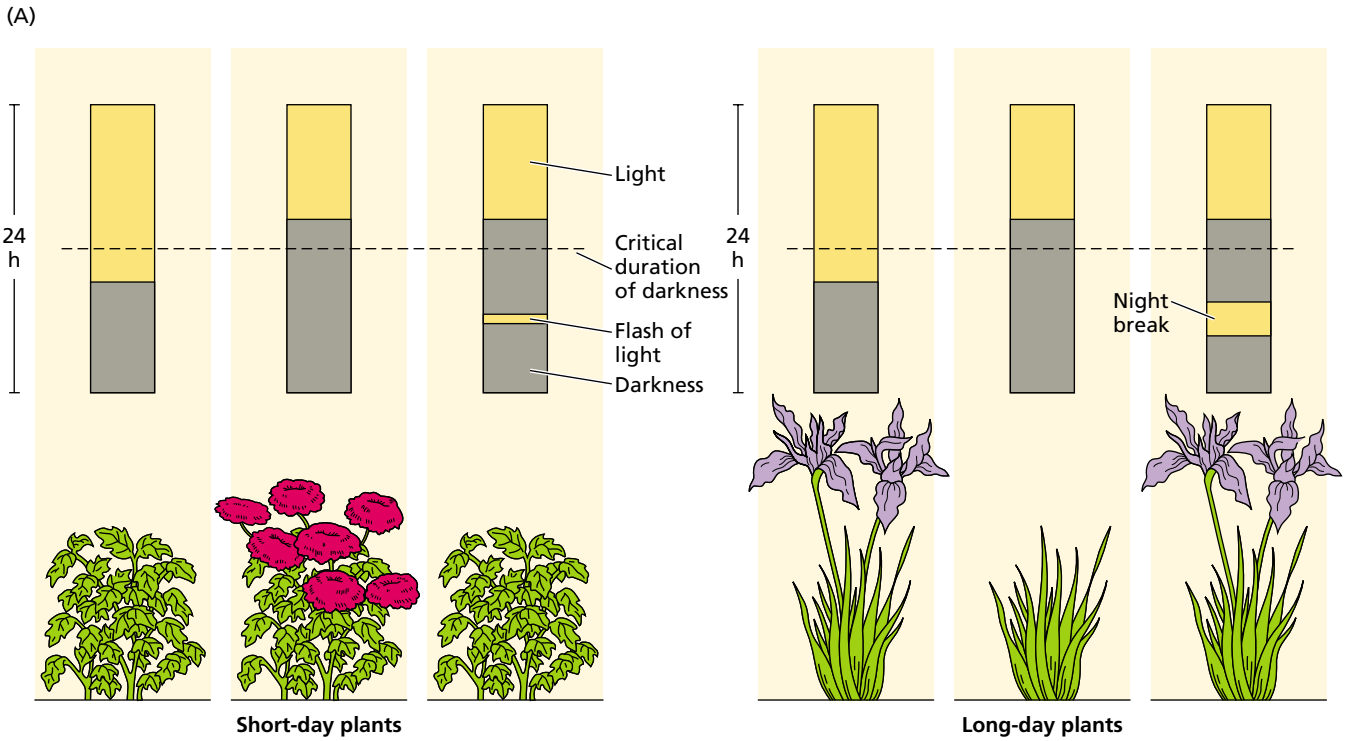


FIGURE 24.19 The photoperiodic regulation of flowering. (A) Effects on SDPs and LDPs. (B) Effects of the duration of the dark period on flowering. Treating short- and long-day plants with different photoperiods clearly shows that the critical variable is the length of the dark period.

such as *Phaseolus vulgaris* (kidney bean) evolved near the equator where the daylength is constant throughout the year. Many desert annuals, such as *Castilleja chromosa* (desert paintbrush) and *Abronia villosa* (desert sand verbena), evolved to germinate, grow, and flower quickly whenever sufficient water is available. These are also DNPs.

Plants Monitor Day Length by Measuring the Length of the Night

Under natural conditions, day and night lengths configure a 24-hour cycle of light and darkness. In principle, a plant could perceive a critical day length by measuring the duration of either light or darkness. Much experimental work in the early studies of photoperiodism was devoted to establishing which part of the light–dark cycle is the controlling factor in flowering. Results showed that flowering of SDPs is determined primarily by the duration of darkness (Figure 24.19A). It was possible to induce flowering in SDPs with light periods longer than the critical value, provided that these were followed by sufficiently long nights (Figure 24.19B). Similarly, SDPs did not flower when short days were followed by short nights.

More detailed experiments demonstrated that photoperiodic timekeeping in SDPs is a matter of measuring the duration of darkness. For example, flowering occurred only when the dark period exceeded 8.5 hours in cocklebur

(*Xanthium strumarium*) or 10 hours in soybean (*Glycine max*). The duration of darkness was also shown to be important in LDPs (see Figure 24.19). These plants were found to flower in short days, provided that the accompanying night length was also short; however, a regime of long days followed by long nights was ineffective.

Night Breaks Can Cancel the Effect of the Dark Period

A feature that underscores the importance of the dark period is that it can be made ineffective by interruption with a short exposure to light, called a **night break** (see Figure 24.19A). In contrast, interrupting a long day with a brief dark period does not cancel the effect of the long day (see Figure 24.19B). Night-break treatments of only a few minutes are effective in *preventing* flowering in many SDPs, including *Xanthium* and *Pharbitis*, but much longer exposures are often required to *promote* flowering in LDPs.

In addition, the effect of a night break varies greatly according to the time when it is given. For both LDPs and SDPs, a night break was found to be most effective when given near the middle of a dark period of 16 hours (Figure 24.20).

The discovery of the night-break effect, and its time dependence, had several important consequences. It established the central role of the dark period and provided a valuable probe for studying photoperiodic timekeeping. Because only small amounts of light are needed, it became possible to study the action and identity of the photoreceptor without the interfering effects of photosynthesis and other nonphotoperiodic phenomena. This discovery has also led to the development of commercial methods for regulating the time of flowering in horticultural species, such as *Kalanchoe*, chrysanthemum, and poinsettia (*Euphorbia pulcherrima*).

The Circadian Clock Is Involved in Photoperiodic Timekeeping

The decisive effect of night length on flowering indicates that measuring the passage of time in darkness is central to photoperiodic timekeeping. Most of the available evidence favors a mechanism based on a circadian rhythm (Bünning 1960). According to the **clock hypothesis**, photoperiodic timekeeping depends on an endogenous circadian oscillator of the type involved in the daily rhythms described in Chapter 17 in relation to phytochrome. The central oscillator is coupled to various physiological processes that involve gene expression, including flowering in photoperiodic species.

Measurements of the effect of a night break on flowering can be used to investigate the role of circadian rhythms in photoperiodic timekeeping. For example, when soybean

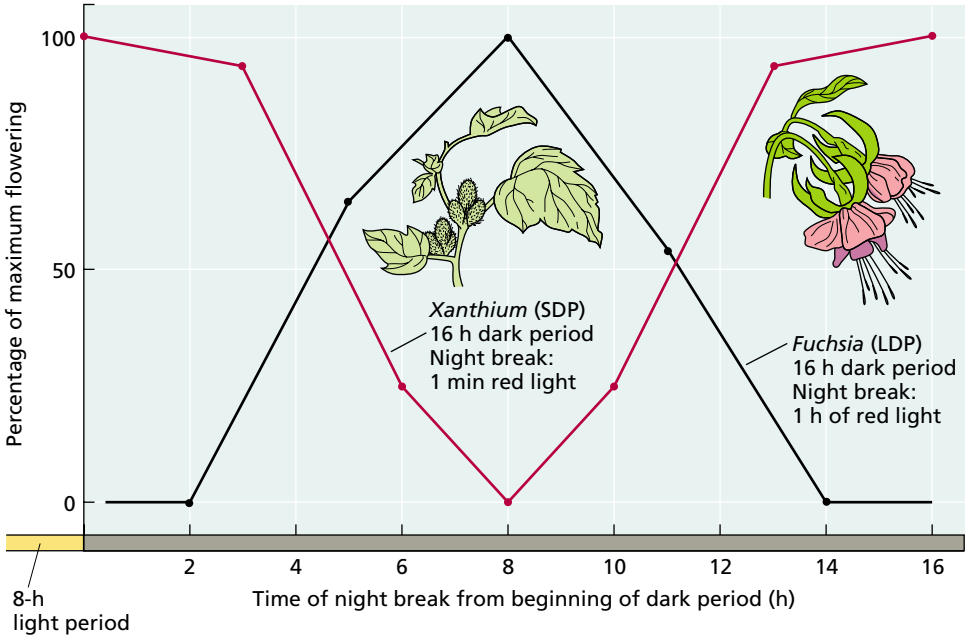


FIGURE 24.20 The time when a night break is given determines the flowering response. When given during a long dark period, a night break promotes flowering in LDPs and inhibits flowering in SDPs. In both cases, the greatest effect on flowering occurs when the night break is given near the middle of the 16-hour dark period. The LDP *Fuchsia* was given a 1-hour exposure to red light in a 16-hour dark period. *Xanthium* was exposed to red light for 1 minute in a 16-hour dark period. (Data for *Fuchsia* from Vince-Prue 1975; data for *Xanthium* from Salisbury 1963 and Papenfuss and Salisbury 1967.)

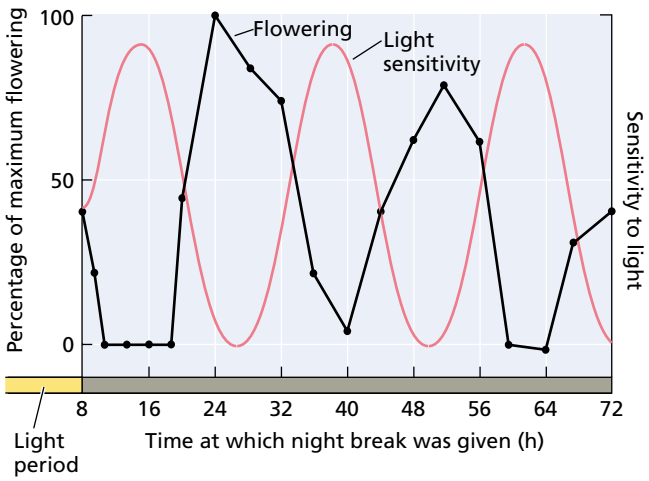


FIGURE 24.21 Rhythmic flowering in response to night breaks. In this experiment, the SDP soybean (*Glycine max*) received cycles of an 8-hour light period followed by a 64-hour dark period. A 4-hour night break was given at various times during the long inductive dark period. The flowering response, plotted as the percentage of the maximum, was then plotted for each night break given. Note that a night break given at 26 hours induced maximum flowering, while no flowering was obtained when the night break was given at 40 hours. Moreover, this experiment demonstrates that the sensitivity to the effect of the night break shows a circadian rhythm. These data support a model in which flowering in SDPs is induced only when dawn (or a night break) occurs after the completion of the light-sensitive phase. In LDPs the light break must coincide with the light-sensitive phase for flowering to occur. (Data from Coulter and Hamner 1964.)

plants, which are SDPs, are transferred from an 8-hour light period to an extended 64-hour dark period, the flowering response to night breaks shows a circadian rhythm (Figure 24.21).

This type of experiment provides strong support for the clock hypothesis. If this SDP were simply measuring the length of night by the accumulation of a particular intermediate in the dark, any dark period greater than the critical night length should cause flowering. Yet long dark periods are not inductive for flowering if the light break is given at a time that does not properly coincide with a certain phase of the endogenous circadian oscillator. This finding demonstrates that flowering in SDPs requires both a dark period of sufficient duration and a dawn signal at an appropriate time in the circadian cycle (see Figure 24.15).

Further evidence for the role of a circadian oscillator in photoperiod measurement is the observation that the photoperiodic response can be phase-shifted by light treatments (see **Web Topic 24.4**).

The Coincidence Model Is Based on Oscillating Phases of Light Sensitivity

The involvement of a circadian oscillator in photoperiodism poses an important question: How does an oscillation with a 24-hour period measure a critical duration of darkness of, say, 8 to 9 hours, as in the SDP *Xanthium*? Erwin Bünning proposed in 1936 that the control of flowering by photoperiodism is achieved by an oscillation of phases with different sensitivities to light. This proposal has evolved into a **coincidence model** (Bünning 1960), in which the circadian oscillator controls the timing of light-sensitive and light-insensitive phases.

The ability of light either to promote or to inhibit flowering depends on the phase in which the light is given. When a light signal is administered during the light-sensitive phase of the rhythm, the effect is either to *promote* flowering in LDPs or to *prevent* flowering in SDPs. As shown in Figure 24.21, the phases of sensitivity and insensitivity to light continue to oscillate in darkness in SDPs. Flowering in SDPs is induced only when exposure to light from a night break or from dawn occurs after completion of the light-sensitive phase of the rhythm. In other words, *flowering is induced when the light exposure is coincident with the appropriate phase of the rhythm*. This continued oscillation of sensitive and insensitive phases in the absence of dawn and dusk light signals is characteristic of a variety of processes controlled by the circadian oscillator.

The Leaf Is the Site of Perception of the Photoperiodic Stimulus

The photoperiodic stimulus in both LDPs and SDPs is perceived by the leaves. For example, treatment of a single leaf of the SDP *Xanthium* with short photoperiods is sufficient to cause the formation of flowers, even when the rest of the plant is exposed to long days. Thus, in response to photoperiod the leaf transmits a signal that regulates the transition to flowering at the shoot apex. The photoperiod-regulated processes that occur in the leaves resulting in the transmission of a floral stimulus to the shoot apex are referred to collectively as **photoperiodic induction**.

Photoperiodic induction can take place in a leaf that has been separated from the plant. For example, in the SDP *Perilla crispa*, an excised leaf exposed to short days can cause flowering when subsequently grafted to a noninduced plant maintained in long days (Zeevaart and Boyer 1987). This result indicates that photoperiodic induction depends on events that take place exclusively in the leaf.

Grafting experiments, which have contributed greatly to our understanding of the floral stimulus, will be discussed in more detail later in the chapter.

The Floral Stimulus Is Transported via the Phloem

Once produced, the flowering stimulus appears to be transported to the meristem via the phloem, and it appears to be

chemical rather than physical in nature. Treatments that block phloem transport, such as girdling or localized heat-killing (see Chapter 10), prevent movement of the floral signal.

It is possible to measure rates of movement of the flowering stimulus by removing a leaf at different times after induction, and comparing the time it takes for the signal to reach two buds located at different distances from the induced leaf. The rationale for this type of measurement is that a threshold amount of the signaling compound has reached the bud when flowering takes place, despite the removal of the leaf.

Studies using this method have shown that the rate of transport of the flowering signal is comparable to, or somewhat slower than, the rate of translocation of sugars in the phloem (see Chapter 10). For example, export of the floral stimulus from adult leaves of the SDP *Chenopodium* is complete within 22.5 hours from the beginning of the long night period. In the LDP *Sinapis*, movement of the floral stimulus out of the leaf is complete by as early as 16 hours after the start of the long-day treatment. These rates are consistent with a floral stimulus that moves in the phloem (Zeevaart 1976).

Because the floral stimulus is translocated along with sugars in the phloem, it is subject to source–sink relations. An induced leaf positioned close to the shoot apex is more likely to cause flowering than an induced leaf at the base of a stem, which normally feeds the roots. Similarly, noninduced leaves positioned between the induced leaf and the apical bud will tend to inhibit flowering by serving as the preferred source leaves for the bud, thus preventing the floral stimulus from the more distal induced leaf from reaching its target. This inhibition also explains why a minimum amount of photosynthesis is required by the induced leaf to drive translocation.

Phytochrome Is the Primary Photoreceptor in Photoperiodism

Night-break experiments are well suited for studying the nature of the photoreceptors involved in the reception of light signals during the photoperiodic response. The inhibition of flowering in SDPs by night breaks was one of the first physiological processes shown to be under the control of phytochrome (Figure 24.22).

In many SDPs, a night break becomes effective only when the supplied dose of light is sufficient to saturate the photoconversion of Pr (phytochrome that absorbs red light) to Pfr (phytochrome that absorbs far-red light) (see Chapter 17). A subsequent exposure to far-red light, which photoconverts the pigment back to the physiologically inactive Pr form, restores the flowering response.

In some LDPs, red and far-red reversibility has also been demonstrated. In these plants, a night break of red light promoted flowering, and a subsequent exposure to far-red light prevented this response.

Action spectra for the inhibition and restoration of the flowering response in SDPs are shown in Figure 24.23. A peak at 660 nm, the absorption maximum of Pr (see Chapter 17), is obtained when dark-grown *Pharbitis* seedlings are

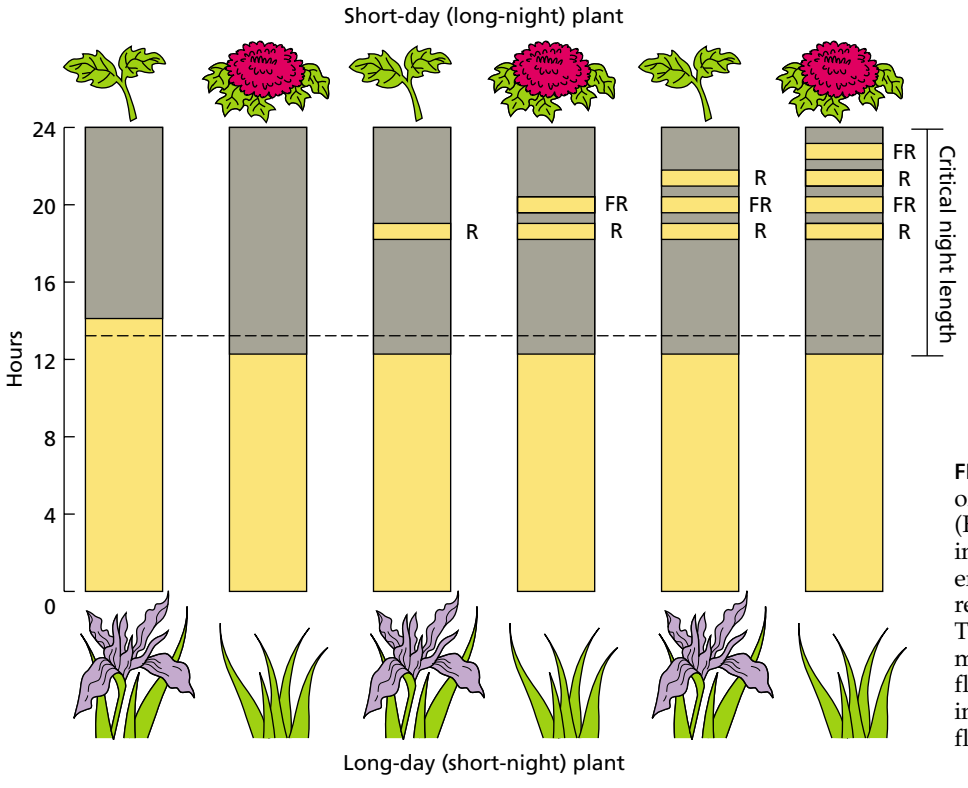


FIGURE 24.22 Phytochrome control of flowering by red (R) and far-red (FR) light. A flash of red light during the dark period induces flowering in an LDP, and the effect is reversed by a flash of far-red light. This response indicates the involvement of phytochrome. In SDPs, a flash of red light prevents flowering, and the effect is reversed by a flash of far-red light.

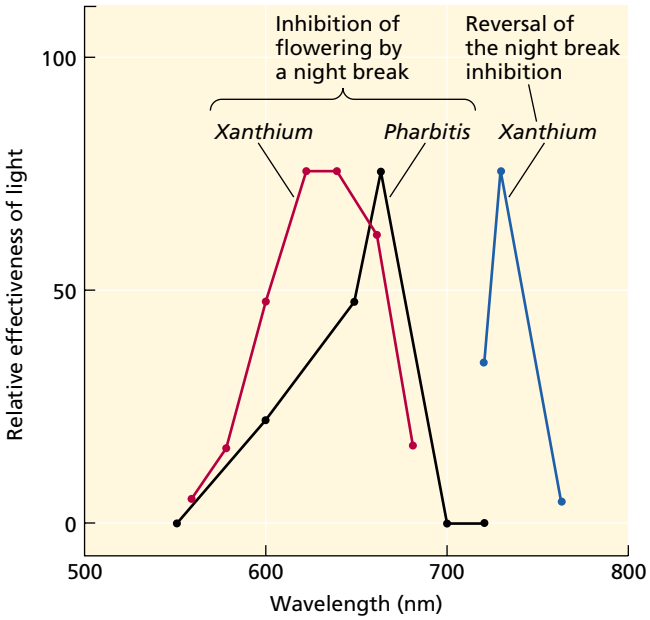


FIGURE 24.23 Action spectra for the control of flowering by night breaks implicates phytochrome. Flowering in SDPs is inhibited by a short light treatment (night break) given in an otherwise inductive period. In the SDP *Xanthium strumarium*, red-light night breaks of 620 to 640 nm are the most effective. Reversal of the red-light effect is maximal at 725 nm. In the dark-grown SDP *Pharbitis nil*, which is devoid of chlorophyll and its interference with light absorption, night breaks of 660 nm are the most effective. This 660 nm maximum coincides with the absorption maximum of phytochrome. (Data for *Xanthium* from Hendricks and Siegelman 1967; data for *Pharbitis* from Saji et al. 1983.)

used to avoid interference from chlorophyll. In contrast, the spectra for *Xanthium* provide an example of the response in green plants, in which the presence of chlorophyll can cause some discrepancy between the action spectrum and the absorption spectrum of Pr. These action spectra and the reversibility between red light and far-red light confirm the role of phytochrome as the photoreceptor that is involved in photoperiod measurement in SDPs.

In LDPs the role of phytochrome is more complex, and a blue-light photoreceptor (which will be discussed shortly) also plays a role in controlling flowering.

Far-Red Light Modifies Flowering in Some LDPs

Circadian rhythms have also been found in LDPs. A circadian rhythm in the promotion of flowering by far-red light has been observed in barley (*Hordeum vulgare*) and *Arabidopsis* (Deitzer 1984), as well as in darnel ryegrass (*Lolium temulentum*) (Figure 24.24). The response is proportional to the irradiance and duration of far-red light and is therefore a high-irradiance response (HIR). Like other HIRs, PHYA is the phytochrome that mediates the response to far-red light (see Chapter 17). In both cases, when the plant is exposed to far-red light for 4 to 6 hours, flowering is promoted compared with plants maintained under continuous white or red light—a response mediated by PHYB. The rhythm continues to run in the light.

In SDPs, on the other hand, a characteristic feature of the timing mechanism is that the rhythm of the response to far-red light damps out after a few hours in continuous light and is restarted upon transfer to darkness.

The response to far-red light is not the only rhythmic feature in LDPs. Although relatively insensitive to a night break of only a few minutes, many LDPs can be induced to flower with a longer night break, usually of at least 1 hour. A circadian oscillation in the flowering response to such a long night break has been observed in LDPs, showing that a rhythm of responsiveness to light continues to run in darkness.

Thus, circadian rhythms that modify the flowering response in LDPs have been shown to run both in the light (promotion by far-red light) and in the dark (promotion by red or white light). However, we do not yet know how the circadian rhythm is coupled to the photoperiodic response.

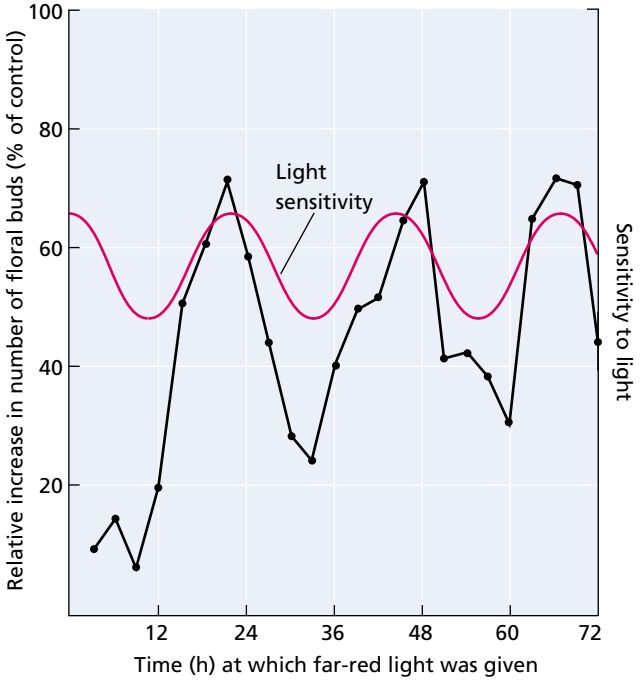


FIGURE 24.24 Effect of far-red light on floral induction in *Arabidopsis*. Four hours of far-red light was added at the indicated times during a continuous 72-hour daylight period. Data points in the graph are plotted at the centers of the 6-hour treatments. The data show a circadian rhythm of sensitivity to the far-red promotion of flowering (red line). This supports a model in which flowering in LDPs is promoted when the light treatment (in this case far-red light) coincides with the peak of light sensitivity. (After Deitzer 1984.)

A Blue-Light Photoreceptor Also Regulates Flowering

In some LDPs, such as *Arabidopsis*, blue light can promote flowering, suggesting the possible participation of a blue-light photoreceptor in the control of flowering. The role of blue light in flowering and its relationship to circadian rhythms have been investigated by use of the luciferase reporter gene construct mentioned in **Web Topic 24.6**. In continuous white light, the cyclic luminescence has a period of 24.7 hours, but in constant darkness the period lengthens to 30 to 36 hours. Either red or blue light, given individually, shortens the period to 25 hours.

To distinguish between the effects of phytochrome and a blue-light photoreceptor, researchers transformed phytochrome-deficient *hy1* mutants, which are defective in chromophore synthesis and are therefore deficient in *all* phytochromes (see Chapter 17), with the luciferase construct to determine the effect of the mutation on the period length (Millar et al. 1995).

Under continuous white light, the *hy1* plants had a period similar to that of the wild type, indicating that little or no phytochrome is required for white light to affect the period. Furthermore, under continuous red light, which would be perceived only by PHYB (see Chapter 17), the period of *hy1* was significantly lengthened (i.e., it became more like constant darkness), whereas the period was not lengthened by continuous blue light. These results indicate that both phytochrome and a blue-light photoreceptor are involved in period control.

The role of blue light in regulating both circadian rhythmicity and flowering is also supported by studies with an *Arabidopsis* flowering-time mutant: *elf3* (*e*arly *f*lowering *3*) (see **Web Topics 24.5 and 24.6**). Confirmation that a blue-light photoreceptor is involved in sensing inductive photoperiods in *Arabidopsis* was recently provided by experiments demonstrating that mutations in one of the cryptochrome genes, *CRY2* (see Chapter 18), caused a delay in flowering and an inability to perceive inductive photoperiods (Guo et al. 1998). As discussed in Chapter 18, *CRY1* encodes a blue-light photoreceptor controlling seedling growth in *Arabidopsis*. Thus, various *CRY* family members have, through evolution, become specialized for different functions in the plant. As noted earlier, the CRY protein has also been implicated in the entrainment of the circadian oscillator (see Chapter 17).

VERNALIZATION: PROMOTING FLOWERING WITH COLD

Vernalization is the process whereby flowering is promoted by a cold treatment given to a fully hydrated seed (i.e., a seed that has imbibed water) or to a growing plant. Dry seeds do not respond to the cold treatment. Without the cold treatment, plants that require vernalization show delayed flowering or remain vegetative. In many cases these plants grow as rosettes with no elongation of the stem (Figure 24.25).

In this section we will examine some of the characteristics of the cold requirement for flowering, including the

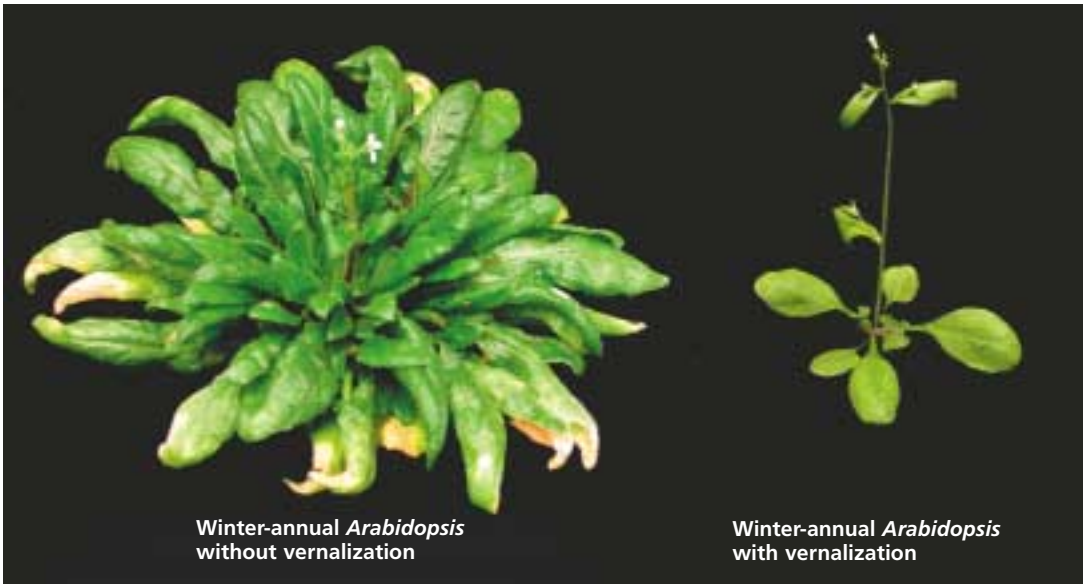


FIGURE 24.25 Vernalization induces flowering in the winter-annual types of *Arabidopsis thaliana*. The plant on the left is a winter-annual type that has not been exposed to cold. The plant on the right is a genetically identical winter-annual type that was exposed to 40 days of temperatures slightly above freezing (40°C) as a seedling. It flowered 3 weeks after the end of the cold treatment with about 9 leaves on the primary stem. (Courtesy of Colleen Bizzell.)

range and duration of the inductive temperatures, the sites of perception, the relationship to photoperiodism, and a possible molecular mechanism.

Vernalization Results in Competence to Flower at the Shoot Apical Meristem

Plants differ considerably in the age at which they become sensitive to vernalization. Winter annuals, such as the winter forms of cereals (which are sown in the fall and flower in the following summer), respond to low temperature very early in their life cycle. They can be vernalized before germination if the seeds have imbibed water and become metabolically active. Other plants, including most biennials (which grow as rosettes during the first season after sowing and flower in the following summer), must reach a minimal size before they become sensitive to low temperature for vernalization.

The effective temperature range for vernalization is from just below freezing to about 10°C, with a broad optimum usually between about 1 and 7°C (Lang 1965). The effect of cold increases with the duration of the cold treatment until the response is saturated. The response usually requires several weeks of exposure to low temperature, but the precise duration varies widely with species and variety.

Vernalization can be lost as a result of exposure to devernalizing conditions, such as high temperature (Figure 24.26), but the longer the exposure to low temperature, the more permanent the vernalization effect.

Vernalization appears to take place primarily in the shoot apical meristem. Localized cooling causes flowering when only the stem apex is chilled, and this effect appears to be largely independent of the temperature experienced by the rest of the plant. Excised shoot tips have been successfully vernalized, and where seed vernalization is possible, fragments of embryos consisting essentially of the shoot tip are sensitive to low temperature.

In developmental terms, vernalization results in the acquisition of competence of the meristem to undergo the floral transition. Yet, as discussed earlier in the chapter, competence to flower does not guarantee that flowering will occur. A vernalization requirement is often linked with a requirement for a particular photoperiod (Lang 1965). The most common combination is a requirement for cold treatment *followed* by a requirement for long days—a combination that leads to flowering in early summer at high latitudes (see **Web Topic 24.7**). Unless devernalized, the vernalized meristem can remain competent to flower for as long as 300 days in the absence of the inductive photoperiod.

Vernalization May Involve Epigenetic Changes in Gene Expression

It is important to note that for vernalization to occur, active metabolism is required during the cold treatment. Sources of energy (sugars) and oxygen are required, and temperatures below freezing at which metabolic activity is suppressed are not effective for vernalization. Furthermore, cell division and DNA replication also appear to be required.

One model for how vernalization affects competence is that there are stable changes in the pattern of gene expression in the meristem after cold treatment. Changes in gene expression that are stable even after the signal that induced the change (in this case cold) is removed are known as **epigenetic regulation**. Epigenetic changes of gene expression in many organisms, from yeast to mammals, often require cell division and DNA replication, as is the case for vernalization.

The involvement of epigenetic regulation in the vernalization process has been confirmed in the LDP *Arabidopsis*. In winter-annual ecotypes of *Arabidopsis* that require both vernalization and long days to flower, a gene that acts as a repressor of flowering has been identified: ***FLOWERING LOCUS C*** (***FLC***). *FLC* is highly expressed in nonvernalized shoot apical meristems (Michaels and Amasino 2000). After vernalization, this gene is epigenetically switched off by an unknown mechanism for the remainder of the plant's life cycle, permitting flowering in response to long days to occur (Figure 24.27). In the next generation, however, the gene is switched on again, restoring the requirement for

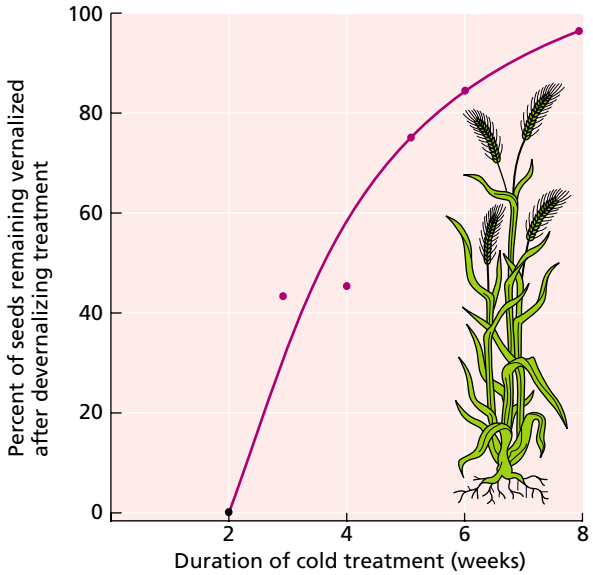


FIGURE 24.26 The duration of exposure to low temperature increases the stability of the vernalization effect. The longer that winter rye (*Secale cereale*) is exposed to a cold treatment, the greater the number of plants that remain vernalized when the cold treatment is followed by a devernalizing treatment. In this experiment, seeds of rye that had imbibed water were exposed to 5°C for different lengths of time, then immediately given a devernalizing treatment of 3 days at 35°C. (Data from Purvis and Gregory 1952.)

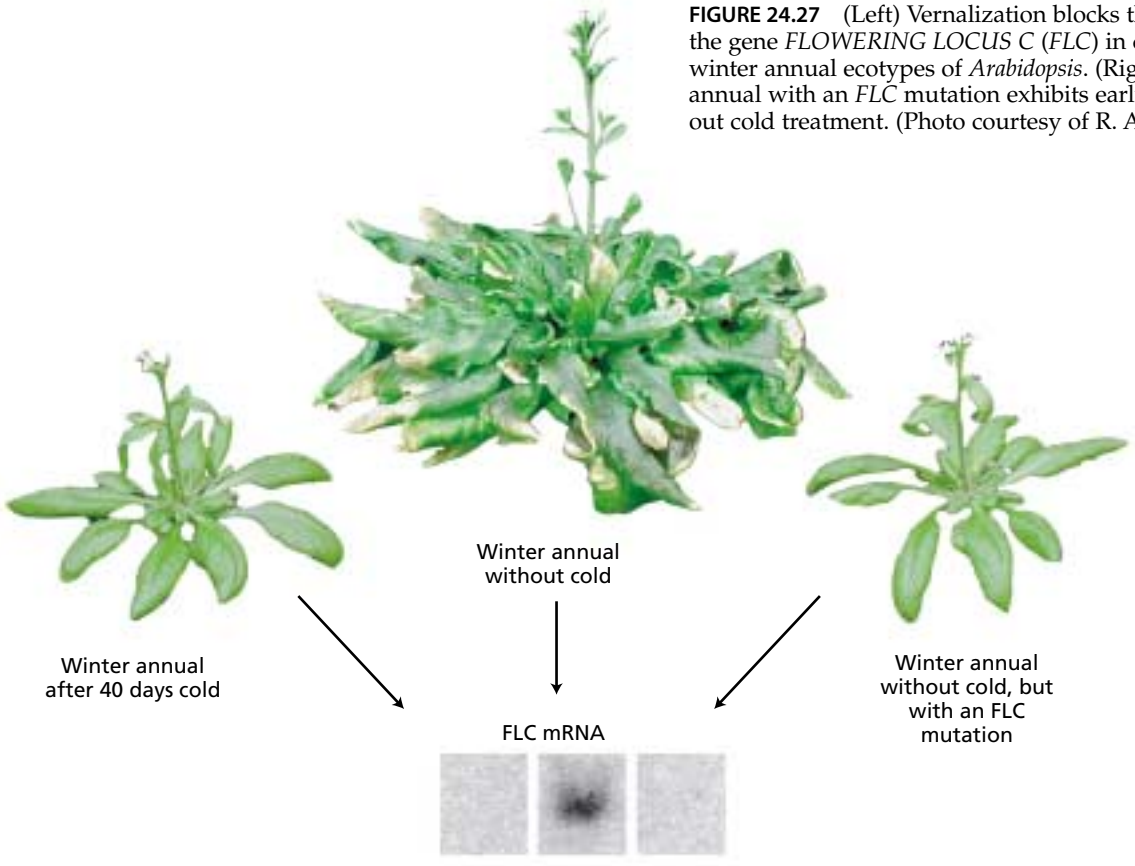


FIGURE 24.27 (Left) Vernalization blocks the expression of the gene *FLOWERING LOCUS C* (*FLC*) in cold-requiring winter annual ecotypes of *Arabidopsis*. (Right) A winter annual with an *FLC* mutation exhibits early flowering without cold treatment. (Photo courtesy of R. Amasino.)

cold. Thus in *Arabidopsis*, the state of expression of the *FLC* gene represents a major determinant of meristem competence (Michaels and Amasino 2000).

BIOCHEMICAL SIGNALING INVOLVED IN FLOWERING

In the preceding sections we examined the influence of environmental conditions (such as temperature and day length) versus that of autonomous factors (such as age) on flowering. Although floral evocation occurs at the apical meristems of the shoots, some of the events that result in floral evocation are triggered by biochemical signals arriving at the apex from other parts of the plant, especially from the leaves. Mutants have been isolated that are deficient in the floral stimulus (see **Web Topic 24.6**).

In this section we will consider the nature of the biochemical signals arriving from the leaves and other parts of the plant in response to photoperiodic stimuli. Such signals may serve either as activators or as inhibitors of flowering. After years of investigation, no single substance has been identified as the universal floral stimulus, although certain hormones, such as gibberellins and ethylene, can induce flowering in some species. Hence, most current models of the floral stimulus are based on multiple factors.

Grafting Studies Have Provided Evidence for a Transmissible Floral Stimulus

The production in photoperiodically induced leaves of a biochemical signal that is transported to a distant target tissue (the shoot apex) where it stimulates a response (flowering) satisfies an important criterion for a hormonal effect. In the 1930s, Mikhail Chailakhyan, working in Russia, postulated the existence of a universal flowering hormone, which he named **florigen**.

The evidence in support of florigen comes mainly from early grafting experiments in which noninduced receptor plants were stimulated to flower by being grafted onto a leaf or shoot from photoperiodically induced donor plants. For example, in the SDP *Perilla crispa*, a member of the mint family, grafting a leaf from a plant grown under inductive short days onto a plant grown under noninductive long days causes the latter to flower (Figure 24.28). Moreover, the floral stimulus seems to be the same in plants with different photoperiodic requirements. Thus, grafting an induced leaf from the LDP *Nicotiana sylvestris*, grown under long days, onto the SDP Maryland Mammoth tobacco caused the latter to flower under noninductive (long day) conditions.

The leaves of DNPs have also been shown to produce a graft-transmissible floral stimulus (Table 24.2). For example, grafting a single leaf of a day-neutral variety of soy-

FIGURE 24.28 Demonstration by grafting of a leaf-generated floral stimulus in the SDP *Perilla*. (Left) Grafting an induced leaf from a plant grown under short days onto a noninduced shoot causes the axillary shoots to produce flowers. The donor leaf has been trimmed to facilitate grafting, and the upper leaves have been removed from the stock to promote phloem translocation from the scion to the receptor shoots. (Right) Grafting a noninduced leaf from a plant grown under LDs results in the formation of vegetative branches only. (Photo courtesy of J. A. D. Zeevaart.)

bean, Agate, onto the short-day variety, Biloxi, caused flowering in Biloxi even when the latter was maintained in noninductive long days. Similarly, a leaf from a day-neutral variety of tobacco (*Nicotiana tabacum*, cv. Trapezond) grafted onto the LDP *Nicotiana sylvestris* induced the latter to flower under noninductive short days.

In a few cases, flowering has been induced by grafts between different genera. The SDP *Xanthium strumarium* flowered under long-day conditions when shoots of flowering *Calendula officinalis* were grafted onto a vegetative *Xanthium* stock. Similarly, grafting a shoot from the LDP *Petunia hybrida* onto a stock of the cold-requiring biennial *Hyoscyamus niger* (henbane) caused the latter to flower under long days, even though it was nonvernalized (Figure 24.29).

In *Perilla* (see Figure 24.28), the movement of the floral stimulus from a donor leaf to the stock across the graft union

FIGURE 24.29 Successful transfer of the floral stimulus between different genera: The scion (right branch) is the LDP *Petunia hybrida*, and the stock is nonvernalized *Hyoscyamus niger* (henbane). The graft combination was maintained under LDs. (Photo courtesy of J. A. D. Zeevaart.)

TABLE 24.2
Transmissible factors regulate flowering.

Donor plants maintained under flower-inducing conditions	Photoperiod type[a,b]	Vegetative receptor plant induced to flower	Photoperiod type[a,b]
Helianthus annus	DNP in LD	*H. tuberosus*	SDP in LD
Nicotiana tabacum Delcrest	DNP in SD	*N. sylvestris*	LDP in SD
Nicotiana sylvestris	LDP in LD	*N. tabacum* Maryland Mammoth	SDP in LD
Nicotiana tabacum Maryland Mammoth	SDP in SD	*N. sylvestris*	LDP in SD

Note: The successful transfer of a flowering induction signal by grafting between plants of different photoperiodic response groups shows the existence of a transmissible floral hormone that is effective.

[a]LDPs = Long-day plants; SDPs = Short- day plants; DNPs = Day-neutral plants.

[b]LD, long days; SD, short days.

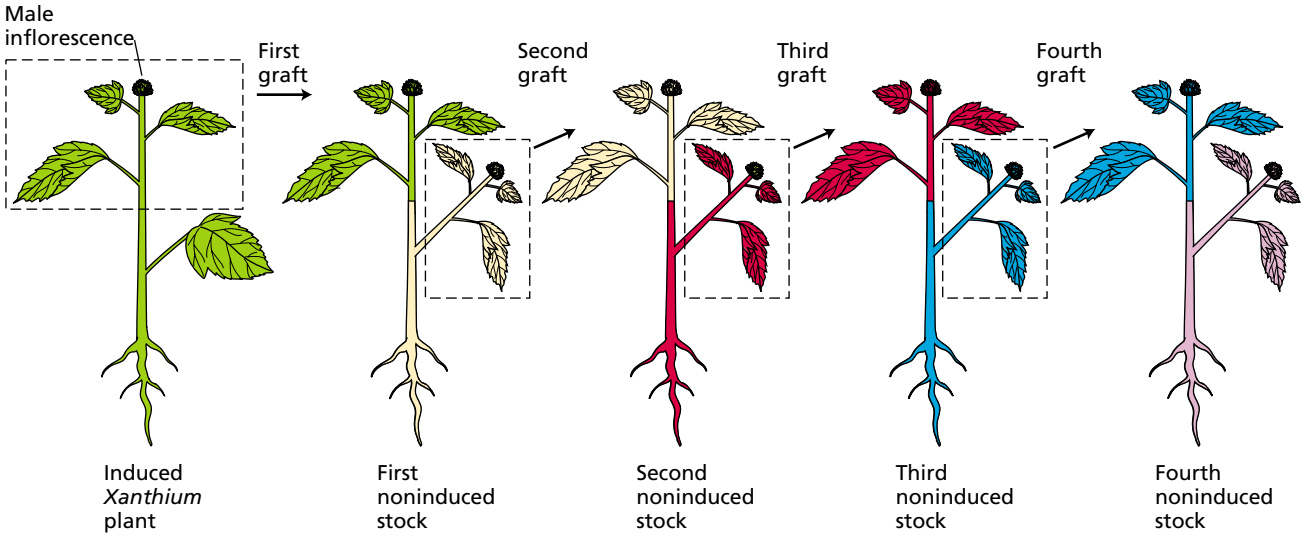
correlated closely with the translocation of ^{14}C-labeled assimilates from the donor, and this movement was dependent on the establishment of vascular continuity across the graft union (Zeevaart 1976). These results confirmed earlier girdling studies showing that the floral stimulus is translocated along with photoassimilates in the phloem.

Indirect Induction Implies That the Floral Stimulus Is Self-Propagating

In at least three cases—*Xanthium* (SDP), *Bryophyllum* (SLDP), and *Silene* (LDP)—the induced state appears to be self-propagating (Zeevaart 1976). That is, young leaves that develop on the receptor plant after it has been induced to flower by a donor leaf can themselves be used as donor leaves in subsequent grafting experiments, even though these leaves have never been subjected to an inductive photoperiod. This phenomenon is called *indirect induction*.

It is characteristic of indirect induction that the strength of the floral stimulus from the donor leaf remains constant even after serial grafting of new donors to several plants has taken place (Figure 24.30A). This suggests that the induced state is in some way propagated throughout the

(A) Indirect induction can be demonstrated in serial grafting experiments in *Xanthium*.



(B) Grafting of induced leaf to uninduced shoot causes flowering in multiple grafts in *Perilla*.

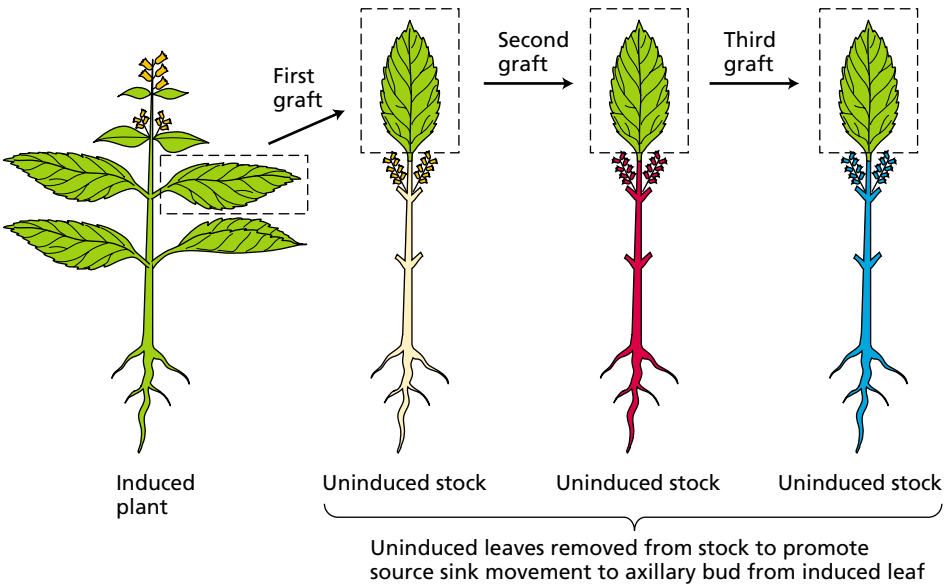


FIGURE 24.30 Different types of leaf induction in *Xanthium* and *Perilla*. (A) *Xanthium* exhibits indirect induction. Noninduced leaves from a plant induced to flower are capable of inducing other plants to flower even though they have never received an inductive photoperiod. This suggests that the floral stimulus is self-propagating. (B) In *Perilla*, only the leaf given the inductive photoperiod is capable of serving as a donor for the floral stimulus. In *Perilla* as well as *Xanthium*, one leaf can continue to induce flowering in successive grafting experiments (Lang 1965).

plant. Although this feature of the floral stimulus has sometimes been described as viruslike, it is unlikely that the floral stimulus can replicate itself like a virus. Rather, the floral stimulus is likely to be a molecule that induces its own production in a positive feedback loop. In *Xanthium* (cocklebur), removal of all buds from the shoot blocks indirect induction, indicating that meristematic tissue, or perhaps auxin, is required for propagation of the induced state.

On the other hand, indirect induction does not occur in the SDP *Perilla*. In *Perilla*, only the leaf actually given an inductive photoperiod is capable of transmitting the floral stimulus in a grafting experiment (see Figure 24.30B). Thus the floral stimulus of *Perilla* is not self-propagating as it is in *Xanthium*, *Bryophyllum*, and *Silene*. Either the mechanism for a positive feedback loop is absent in *Perilla* leaves, or translocation of the floral stimulus is restricted to the meristem so that it never enters the leaves.

Unlike *Xanthium*, which requires the presence of a bud for stable induction, *Perilla* leaves can be stably induced even when detached from the plant. Once induced, *Perilla* leaves cannot be uninduced, and the same leaf can continue to serve as a donor of the floral stimulus in successive grafting experiments without any reduction in potency (Zeevaart 1976).

Evidence for Antiflorigen Has Been Found in Some LDPs

Grafting studies have implicated transmissible inhibitors in flowering regulation as well. Such inhibitors have been called **antiflorigen**, but (like florigen) antiflorigen may consist of multiple compounds. For example, grafting an uninduced leafy shoot from the LDP *Nicotiana sylvestris* onto the day-neutral tobacco cultivar Trapezond suppressed flowering in the day-neutral plant under short days but not long-day conditions (Figure 24.31). On the other hand, when an uninduced donor from the SDP Maryland Mammoth was grafted onto Trapezond, it had no effect on flowering in either short-day or long-day conditions. This and similar results suggest that the leaves of LDPs, but not SDPs, produce flowering inhibitors under noninductive conditions.

Similar studies in peas have led to the identification of several genetic loci that regulate steps in the biosynthetic pathways of both floral activators and floral inhibitors (see **Web Topic 24.5**).

Attempts to Isolate Transmissible Floral Regulators Have Been Unsuccessful

The many attempts to isolate and characterize the floral stimulus have been largely unsuccessful. The most common approach has been to make extracts from induced leaf tissue and test for their ability to elicit flowering in noninduced plants. In other experiments, investigators have extracted and analyzed phloem sap from induced plants. In some studies, extracts from one of these sources have induced flowering in test plants, but these results have not been consistently reproduced. Most of these extractions have focused on small molecules.

FIGURE 24.31 Graft transmission of an inhibitor of flowering. Non-induced rosettes from the LDP *Nicotiana sylvestris* were grafted onto the day-neutral tobacco (*Nicotiana tabacum*, cv. Trapezond). Flowering of the day-neutral plant was suppressed under short days (left branch of plant on right), but not under long days (left branch of plant on left). Arrowheads indicate graft unions. (From Lang et al. 1977.)

Recent studies using fluorescent tracers have shown that in *Arabidopsis* there is actually a *decrease* in the movement of small molecules from the leaf-to-shoot apex via the symplast at the time of floral induction (Gisel et al. 2002). The lack of tracer movement from the leaf to the shoot apex may indicate either a reduction in overall symplastic transport to the shoot apex, or a change in the selectivity of the plasmodesmata during floral induction. There is increasing evidence that macromolecular traffic between cells via plasmodesmata plays essential roles in normal meristem development and function (see Chapter 16). Particles as large as viruses can move from cell to cell via plasmodesmata, and throughout the plant via the phloem. Phloem translocation of small RNAs has recently been implicated in the spread of a viral resistance mechanism throughout plants (Hamilton and Baulcombe 1999). It is therefore possible that the floral stimulus is a macromolecule, such as RNA or protein, that is translocated via the phloem from the leaf to the apical meristem, where it functions as a regulator of gene expression (Crawford and Zambryski 1999).

However, thus far attempts to identify such a signal have been unsuccessful.

Efforts to isolate a specific, graft-transmissible inhibitor of flowering have also been unsuccessful. Thus, despite unequivocal data from grafting experiments showing that transmissible factors regulate flowering (see Table 24.2) (Zeevaart 1976), the substances involved remain elusive.

Gibberellins and Ethylene Can Induce Flowering in Some Plants

Among the naturally occurring growth hormones, gibberellins (GAs) (see Chapter 20) can have a strong influence on flowering (see **Web Topic 24.8**). Recent studies suggest that gibberellin promotes flowering in *Arabidopsis* by activating expression of the *LFY* gene (Blazquez and Weigel 2000). Exogenous gibberellin can evoke flowering when applied either to rosette LDPs like *Arabidopsis*, or to dual–day length plants such as *Bryophyllum*, when grown under short days (Lang 1965; Zeevaart 1985).

In addition, application of GAs can evoke flowering in a few SDPs in noninductive conditions, and in cold-requiring plants that have not been vernalized. As previously discussed, cone formation can also be promoted in juvenile plants of several gymnosperm families by addition of GAs. Thus, in some plants exogenous GAs can bypass the endogenous trigger of age in autonomous flowering, as well as the primary environmental signals of day length and temperature.

As discussed in Chapter 20, plants contain many GA-like compounds. Most of these compounds are either precursors to, or inactive metabolites of, the active forms of GA. In some situations different GAs have markedly different effects on flowering and stem elongation, such as in the long-day plant *Lolium temulentum* (darnel ryegrass) (see **Web Topic 24.9**).

These observations suggest that the regulation of flowering may be associated with specific GAs, but they do not prove that GA is the hypothetical flowering hormone. In fact, a certain level of GA is likely to be required for flowering in many species, but other pathways to flowering are necessary as well. For example, a mutation in GA biosynthesis renders the quantitative LDP *Arabidopsis thaliana* unable to flower in noninductive short days but has little effect on flowering in long days, demonstrating that endogenous GA is required for flowering in specific situations (Wilson et al. 1992).

Considerable attention has been given to the effects of day length on GA metabolism in the plant (see Chapter 20). For example, in the long-day plant spinach (*Spinacia oleracea*), the levels of gibberellins are relatively low in short days, and the plants maintain a rosette form. After the plants are transferred to long days, the levels of all the gibberellins of the 13-hydroxylated pathway ($GA_{53} \rightarrow GA_{44} \rightarrow GA_{19} \rightarrow GA_{20} \rightarrow GA_1$; see Chapter 20) increase. However, the fivefold increase in the physiologically active gibberellin, GA_1, is what causes the marked stem elongation that accompanies flowering.

In addition to GAs, other growth hormones can either inhibit or promote flowering. One commercially important example is the striking promotion of flowering in pineapple (*Ananas comosus*) by ethylene and ethylene-releasing compounds—a response that appears to be restricted to members of the pineapple family (Bromeliaceae). Thus, as discussed next, the floral stimulus may be composed of many components, and these components may differ in different groups of plants.

The Transition to Flowering Involves Multiple Factors and Pathways

It is clear that the transition to flowering involves a complex system of interacting factors that include, among others, carbohydrates, gibberellins, cytokinins, and, in the bromeliads, ethylene (see **Web Topic 24.10**). Leaf-generated transmissible signals are required for determination of the shoot apex in both autonomously regulated and photoperiodic species. Determining whether these transmissible signals consist of single or multiple components is a major challenge for the future.

Recent genetic studies have established that there are four genetically distinct developmental pathways that control flowering in the LDP *Arabidopsis* (Blazquez 2000). Figure 24.32 shows a simplified version of the four pathways:

1. The *photoperiodic pathway* involves phytochromes and cryptochromes. (Note that PHYA and PHYB have contrasting effects on flowering; see **Web Topic 24.11**.) The interaction of these photoreceptors with a circadian clock initiates a pathway that eventually results in the expression of the gene *CONSTANS* (*CO*), which encodes a zinc-finger transcription factor that promotes flowering. *CO* acts through other genes to increase the expression of the floral meristem identity gene *LEAFY* (*LFY*).

2. In the dual *autonomous/vernalization pathway*, flowering occurs either in response to internal signals—the production of a fixed number of leaves—or to low temperatures. In the autonomous pathway of *Arabidopsis*, all of the genes associated with the pathway are expressed in the meristem. The autonomous pathway acts by reducing the expression of the flowering repressor gene *FLOWERING LOCUS C* (*FLC*), an inhibitor of *LFY* (Michaels and Amasino 2000). Vernalization also represses *FLC*, but perhaps by a different mechanism (an epigenetic switch). Because the *FLC* gene is a common target, the autonomous and vernalization pathways are grouped together.

3. The *carbohydrate*, or *sucrose*, *pathway* reflects the metabolic state of the plant. Sucrose stimulates flowering in *Arabidopsis* by increasing *LFY* expression, although the genetic pathway is unknown.

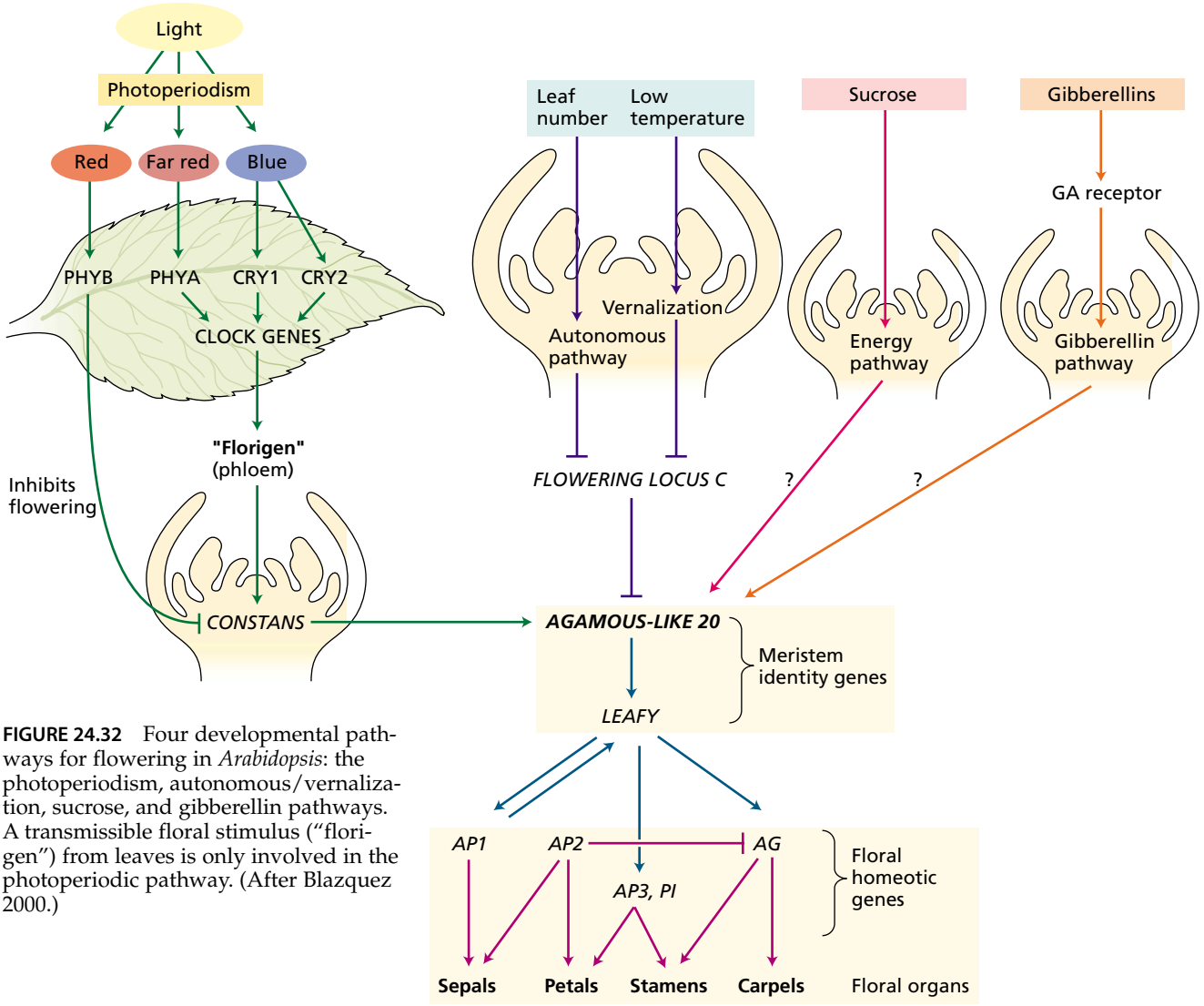


FIGURE 24.32 Four developmental pathways for flowering in *Arabidopsis*: the photoperiodism, autonomous/vernalization, sucrose, and gibberellin pathways. A transmissible floral stimulus ("florigen") from leaves is only involved in the photoperiodic pathway. (After Blazquez 2000.)

4. The *gibberellin pathway* is required for early flowering and for flowering under noninductive short days.

All four pathways converge by increasing the expression of the key floral meristem identity gene *AGAMOUS-LIKE 20* (*AGL20*). The role of *AGL20*, a MADS box–containing transcription factor, is to integrate the signals coming from all four pathways into a unitary output. Obviously the strongest output signal occurs when all four pathways are activated.

Figure 24.33 shows the level of *AGL20* gene expression in the shoot apical meristem of an *Arabidopsis* plant after shifting from noninductive short days (8-hour day length) to inductive long days (16-hour day length). Note that an increase in *AGL20* expression can be detected as early as 18 hours after the beginning of the long-day treatment (Borner et al. 2000). Thus it takes only 10 hours beyond an 8-hour short day for the meristem to begin responding to the floral stimulus from the leaves. This timing is consistent with previous measurements of the rates of export of the floral stimulus from induced leaves (discussed earlier in the chapter).

Although many pathways feed into *AGL20*, there must be some redundancy in the system because flowering is only delayed, but not completely blocked, in *agl20* mutants. Thus, one or two other genes must be able to take over the role of *AGL20* when it is mutated.

Once turned on by *AGL20*, *LFY* activates the floral homeotic genes—*APETALA1* (*AP1*), *APETALA3* (*AP3*), *PISTILLATA* (*PI*), and *AGAMOUS* (*AG*)—that are required for floral organ development. *APETALA2* (*AP2*) is expressed in both vegetative and floral meristems and is therefore not affected by *LFY*. However, as discussed earlier in the chapter, *AP2* exerts a negative effect on *AG* expression (see Figure 24.6).

Besides serving as a floral homeotic gene, *AP1* functions as a meristem identity gene in *Arabidopsis* because it is involved in a positive feedback loop with *LFY*. Conse-

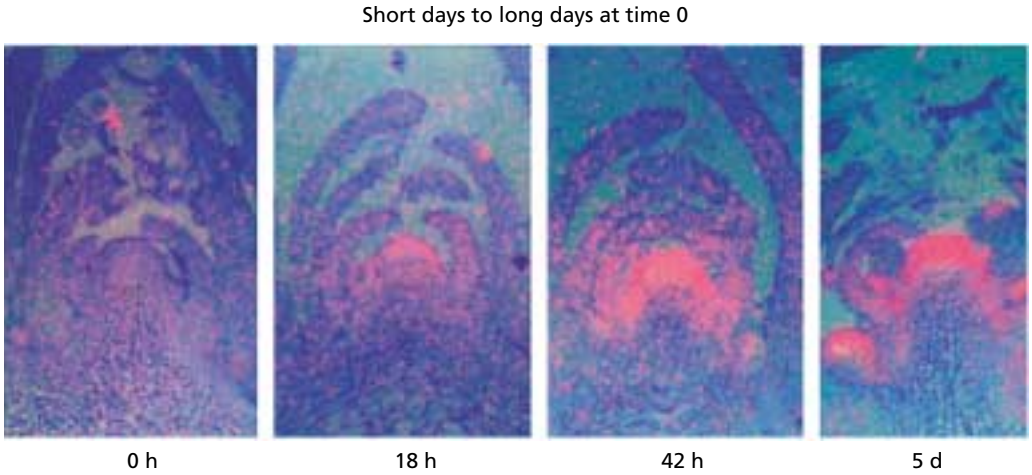


FIGURE 24.33 Increase in expression of the gene *AGAMOUS-LIKE 20* (*AGL20*) during floral evocation in the shoot apical meristem of *Arabidopsis*. The times after shifting the plants from SDs to LDs are indicated. (From Borner et al. 2000.)

quently, once the transition to flowering has reached this stage, flowering is irreversible.

The existence of multiple flowering pathways provides angiosperms with maximum reproductive flexibility, enabling them to produce seeds under a wide variety of conditions. Redundancy within the pathways ensures that reproduction, the most crucial of all physiological functions, will be relatively insensitive to mutations and evolutionarily robust.

The details of the pathways undoubtedly vary among different species. In maize, for example, at least one of the genes involved in the autonomous pathway is expressed in leaves (see **Web Topic 24.12**). Nevertheless, the presence of multiple flowering pathways is probably universal among angiosperms.

SUMMARY

Flower formation occurs at the shoot apical meristem and is a complex morphological event. The rosette plant *Arabidopsis* has been an important model for studies on floral development. The four floral organs (sepals, petals, stamens, and carpels) are initiated as successive whorls. Three classes of genes regulate floral development. The first class contains positive regulators of the floral meristem identity. *APETALA1* (*AP1*) and *LEAFY* (*LFY*) are the most important *Arabidopsis* floral meristem identity genes.

Meristem identity genes are positive regulators of another class of genes that determine floral organ identity. There are five known floral organ identity genes in *Arabidopsis*: *APETALA1* (*AP1*), *APETALA2* (*AP2*), *APETALA3* (*AP3*), *PISTILLATA* (*PI*), and *AGAMOUS* (*AG*). Cadastral genes make up the third group. Cadastral genes act as spatial regulators of the floral organ identity genes by setting boundaries for their expression.

The genes that control floral organ identity are homeotic. Most homeotic genes in plants contain the MADS box. Mutations in these genes alter the identity of the floral organs produced in two adjacent whorls. The ABC model seeks to explain how the floral homeotic genes control organ identity through the unique combinations of their products. Type A genes control organ identity in the first and second whorls. Type B activity controls organ determination in the second and third whorls. The third and fourth whorls are controlled by type C activity.

The ability to flower (i.e., to make the transition from juvenility to maturity) is attained when the plant has reached a certain age or size. In some plants, the transition to flowering then occurs independently of the environment (autonomously). Other plants require exposure to appropriate environmental conditions. The most common environmental inputs for flowering are day length and temperature.

The response to day length—photoperiodism—promotes flowering at a particular time of year, and several different categories of responses are known. The photoperiodic signal is perceived by the leaf. Exposure to low temperature—vernalization—is required for flowering in some plants, and this requirement is often coupled with a day length requirement. Vernalization occurs at the shoot apical meristem. Photoperiodism and vernalization interact in several ways.

Daily rhythms—circadian rhythms—can locate an event at a particular time of day. Timekeeping in these rhythms is based on an endogenous circadian oscillator. Keeping the rhythm on local time depends on the phase response of the rhythm to environmental signals. The most important signals are dawn and dusk.

Short-day plants flower when a critical duration of darkness is exceeded. Long-day plants flower when the length

of the dark period is less than a critical value. Light given at certain times in a dark period that is longer than the critical value—a night break—prevents the effect of the dark period. Light also acts on the circadian oscillator to entrain the photoperiodic rhythm, an effect that is important for timekeeping in the dark. The photoperiodic mechanism shows some variation in short-day and long-day responses, but both appear to involve phytochrome and a circadian oscillator.

When photoperiod-responsive plants are induced to flower by exposure to appropriate day lengths, leaves send a chemical signal to the apex to bring about flowering. This transmissible signal is able to cause flowering in plants of different photoperiodic response groups. In noninductive day lengths, a transmissible inhibitor of flowering may be produced by the leaves of LDPs.

Although physiological experiments, especially grafting, indicate the existence of a transmissible floral stimulus and, in some cases, flowering inhibitors, the chemical identity of these factors is not known. Plant growth hormones, especially the gibberellins, can modify flowering in many plants.

The transition to flowering is regulated by multiple signals and multiple pathways. In *Arabidopsis*, flowering is controlled by four pathways: the photoperiodic, autonomous/vernalization, sucrose, and GA pathways. All of these pathways converge to regulate the meristem identity genes *AGAMOUS-LIKE 20* (*AGL20*) and *LEAFY (LFY)*. *AGL20* and *LFY*, in turn, regulate the floral homeotic genes to produce the floral organs. The existence of multiple pathways for flowering provides angiosperms with the flexibility to reproduce under a variety of environmental conditions, thus increasing their evolutionary fitness.

Web Material

Web Topics

24.1 Contrasting the Characteristics of Juvenile and Adult Phases of English Ivy (*Hedera helix*) and Maize (*Zea mays*)
A table of juvenile vs. adult morphological characteristics is presented.

24.2 Regulation of Juvenility by the *TEOPOD* (*TP*) Genes in Maize
The genetic control of juvenility in maize is discussed.

24.3 Flowering of Juvenile Meristems Grafted to Adult Plants
The competence of juvenile meristems to flower can be tested in grafting experiments.

24.4 Characteristics of the Phase-Shifting Response in Circadian Rhythms
Petal movements in *Kalenchoe* have been used to study circadian rhythms.

24.5 Genes That Control Flowering Time
A discussion of genes that control different apects of flowering time is presented.

24.6 Support for the Role of Blue-Light Regulation of Circadian Rhythms
The role of ELF3 in mediating the effects of blue light on flowering time is discussed.

24.7 Regulation of Flowering in Canterbury Bell by Both Photoperiod and Vernalization
Short days acting on the leaf can substitute for vernalization at the shoot apex in Canterbury Bell.

24.8 Examples of Floral Induction by Gibberellins in Plants with Different Environmental Requirements for Flowering
A table of the effects of gibberellins on plants with different photoperiodic requirements.

24.9 The Different Effects of Two Different Gibberellins on Flowering (Spike Length) and Elongation (Stem Length)
GA_1 and GA_{32} have different effects on flowering in *Lolium*.

24.10 The Influence of Cytokinins and Polyamines on Flowering
Other growth regulators beside gibberellins may participate in the flowering response.

24.11 The Contrasting Effects of Phytochromes A and B on Flowering
A brief discussion of the effects of phyA and phyB on flowering in *Arabidopsis* and other species.

24.12 A Gene That Regulates the Floral Stimulus in Maize
The INDETERMINATE 1 gene of maize regulates the transition to flowering and is expressed in young leaves.

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