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Flower opening and closure: a review

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Abstract

Flower opening and closure are traits of a reproductive syndrome, as it allows pollen removal and/or pollination. Various types of opening can be distinguished such as nocturnal and diurnal and single or repetitive. Opening is generally due to cell expansion. Osmotic solute levels increase by the conversion of polysaccharides (starch or fructan) to monosaccharides, and/or the uptake of sugars from the apoplast. Repeated opening and closure movements are often brought about by differential elongation. In tulip petals, for example, the upper and lower sides of the mesophyll exhibit a 10 °C difference in optimum temperature for elongation growth, resulting in opening in the morning and closure in the evening. Opening and closure in several other species is regulated by changes in light intensity and, in some species with nocturnal opening, by an increase in relative humidity. A minimum duration of darkness and light are usually required for opening and closure, respectively, in flowers that open during the day. Both phytochrome and a blue light receptor seem involved in light perception. In some species, opening and closure are regulated by an endogenous rhythm, which, in all cases investigated, can be reset by changes from dark to light and/or light to dark. So far, *Arabidopsis* mutants have not been used to investigate the timing of flower opening and closure. As its flowers open and close in a circadian fashion, several mutants that are involved in the circadian clock and its light input may help to provide an insight into this type of flower opening. The co-ordination of processes culminating in synchronized flower opening is, in many species, highly intricate. The complex control by endogenous and exogenous factors sets flower opening and closure apart from most other growth processes.

Key words: Carbohydrate metabolism, circadian rhythm, differential growth, elongation growth, endogenous rhythm, fructans, hormonal regulation, osmotic potential, water relations.

Introduction

The time of flower opening marks the onset of a period in which pollinators will be attracted, leading to pollen removal in male and bisexual flowers and to pollination, fertilization and seed set in female and bisexual flowers. In many species the flowers are open permanently, whereby the opening period is terminated by a closure movement, or is terminated by petal withering or abscission. In other species, periods of opening are alternated by periods of closure.

Flower opening is often rapid. In *Oenothera biennis*, for example, full opening takes less than 20 min (Sigmond, 1930b) and in *Hedera helix* it occurs in about 5 min (Sigmond, 1929b). Flower opening is therefore interesting from a physiological point of view as, in many species, it is accompanied by a high rate of cell expansion, rather impressive movements, and complex regulation by external and internal factors. Time lapse photography shows that petal unfolding, depending on the species, shows spectacular outward spiralling, popping open, and various local movements in the petals whose final position often differs considerably from their position in the bud. It may be noted, in this scientific context, that flower opening has inspired many artists and seems of special emotional value to people.

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No comprehensive review has been published previously on floral opening and closure, as far as is known. Therefore, the most interesting and thorough publications, including those that date from rather long ago, have been selected for this review. There is no attempt to give an historical perspective, but an effort is made to assign a first observation to the right author.

This review examines the classifications of flower opening and closure, the underlying mechanical processes, the role of environmental cues such as humidity, light and temperature, and that of endogenous rhythms. Some attention is also given to hormonal regulation, carbohydrate requirements and water relations. The paper concludes by discussing hypotheses about the selective advantages of the various strategies of floral opening and closure.

Several interesting papers on flower opening and closure were written in French, and some of the finest publications have been published in German. These papers are described here in somewhat more detail than the reports written in English. As a number of the publications mentioned date from some time ago, the plant nomenclature may have changed in the meantime. The names as given in the original reports have been used here.

Varieties of flower opening and closure

The reproductive structures of the Angiosperms differ vastly and it is therefore not surprising that the mechanism of flower opening also varies considerably. Several categories may be distinguished on the basis of the mechanics of flower opening (Sigmond, 1929*a*, *b*, 1930*a*, *b*; Reid and Evans, 1986).

Firstly, depending on the species, opening may relate to the development of tissues adjacent to the flower, for example, opening may require growth of the pedicel, as in several species in the Iridaceae; or it may require forced separation or abscission of covering parts, such as bracts or sepals. In some flowers, for example in *Oenothera* spp., the sepals are connected by a zipper-like mechanism whereby cells at the sepal margins are tangled. Due to the force of the growing petals this entanglement is broken and the petals are suddenly released. In several other species the sepal margins are tightly held together by ridges, which also delay opening until the growing petals overcome their resistance. This also results in rapid opening (Sigmond 1929*a*, 1930*a*). At least three types of abscission of adjacent tissues can be distinguished. (1) The abscission zone is at the base of a bract or sepal. (2) The abscission of a cover, horizontally, along its entire circumference. The cover may be soft, sepal-like tissue, as in some species in the Papaveraceae, or more woody as in *Eucalyptus*. (3) Cell separation that results in a vertical slit of a sepalous covering structure, as in several Papaveraceae.

Secondly, opening depends on petal movements, of which at least four types can be distinguished. (1) Opening is due to reversible ion accumulation, and independent of elongation growth; (2) it depends on cellular death in a specific area of the petal; (3) it is due to loss of water during the day and refilling during the night; and (4) it depends on differential growth.

These four types of flower opening will be discussed further. The characteristics of opening that have been mentioned are not mutually exclusive. Abscission of covering parts, or pedicel growth, for example, often occurs in combination with differential petal growth.

Flower closure can be due to differential growth or to reversible turgor changes, but it may also occur because of turgor loss due to senescence. In the latter case, the closure will be permanent. The petals usually fall after senescence, but the time span between senescence and abscission varies considerably, depending on the species. In several species the flowers never close, as the petals abscise when the flower is still open.

Mechanism of opening and closure

in most of the species studied, petal movements are due to a difference in growth rate of the two sides. In species that open in the morning and mainly respond to temperature, for example, the inner surface of the petal rapidly grows in length when the temperature is raised, whereas the outer surface does not. Cooling results in a more rapid growth of the outer surface (crocus: Wiedersheim, 1904; tulip: Pfeffer, 1873; Bünning, 1929; Böhner, 1934). In tulip petals, the mesophyll cells are mainly responsible for growth; the epidermal cells only follow mesophyll expansion. The optimum temperature for growth of the outer mesophyll cells was about 10 °C lower than that of the cells at the inner side (Wood, 1953). Other examples of petal movements due to unequal growth on both sides of the petals are: *Anemone* sp., *Calendula* sp., *Colchium autumnale*, *Doronicum* sp., *Gentiana* sp., *Helleborus niger* (Kerner von Marilaun, 1891), and *Taraxacum albidum* (Tanaka *et al.*, 1987).

In *Ipomoea* (syn. *Pharbitis*), flower opening and closure are due to movements of the midrib rather than the petal lamina. Differences in cell expansion on both sides drive the movements (Kaihara and Takimoto, 1981*a*). The difference in cell expansion was due, in part, to turgor loss in a group of inner epidermal cells of the midrib. For 2 d prior to anthesis, the ultrastructure of these cells underwent rapid changes that are indicative of cell death. Other cells of the rib did not show these changes (Phillips and Kende, 1980).

Silene saxifraga flowers close during the day and open during the night for about 5 d. The petals roll up during the day. The inrolling (closing) movement was due to net water loss. Outrolling (opening) was apparently due to

refilling of the cells with water. The flowers remain open longer on dry days if the soil is wet, and do not close at all on wet days (Halket, 1931). Several other *Silene* species show the same mechanism (Kraus, 1879; Kerner von Marilaun, 1891; Lindman, 1897).

Opening and closure may be due to reversible expansion and contraction of cells. To date this has only been convincingly demonstrated in *Gentiana kochiana*. The petals in this species are about 5 cm long and are fused at the basal end. Petals contain a 1–2 cm long zone that expands and contracts at 1–3 cm beneath the petal tips. Detailed measurements on both sides of the petal showed that the epidermis cells on the inner side expanded during the day and contracted at night. The outer epidermis, by contrast, did not show a change in length. It was concluded that the movements are due to turgor changes (Claus, 1926). A similar mechanism has been suggested for *Kalanchoë blossfeldiana*. As in *Gentiana*, the lowermost halves of the petals are fused and show no movements, and the zone responsible for movement is located where the petals separate. Detailed measurements on cell length are lacking, but differences in petal fresh weight occurred in parallel with the movements (Becker, 1953; zur Lippe, 1957), which is an argument in favour of reversible movements. The osmolarity of the upper epidermis was twice as high during the day than during the night, which also suggests reversible ion uptake (Schrempf, 1977). Abscisic acid dampened the rhythm of opening and closure, and diminished the rhythmic change in osmotic potential of the cells involved (Schrempf, 1980). In younger *Kalanchoë* flowers (in which the petals were still growing), opening and closing movements were stronger than in older flowers, in which elongation growth was no longer detectable (Karvé *et al.*, 1961). This suggests that differential growth may also be involved in the movements, at least in young flowers.

In the following four sections the physiology of the growth reactions will be reviewed briefly, and only insofar as the data are specific for flower opening.

Carbohydrate metabolism

In most species, the mobilization of storage carbohydrates and/or the import of sucrose accompanies flower opening. Young petal cells of many species contain considerable amounts of starch which, shortly before opening, is rapidly converted to glucose and fructose (Ho and Nichols, 1977; Hammond, 1982). Young petals that reportedly contain high starch concentrations include *Alstroemeria peregrina* (Collier, 1997), *Lilium* (Bieleski *et al.*, 2000*b*), *Magnolia grandiflora* (Griesel, 1954), *Rosa* (Ho and Nichols, 1977), *Tradescantia reflexa* (Horie, 1961), and *Turnera ulmifolia* (Ball, 1933). The important role of starch breakdown in

lily petal growth was borne out by the use of degradation inhibitors (Bieleski *et al.*, 2000*b*).

Petals of unopened daylily (*Hemerocallis* sp.) flowers, in contrast, contained no starch but a high concentration of fructan, an oligosaccharide with a high number of fructose monomers. Upon flower opening, fructan was rapidly degraded (Bieleski, 1993). Fructan also accumulated in the petals of *Phippsia algida* (Solhaug and Aares, 1994) and *Campanula rapunculoides* (Vergauwen *et al.*, 2000), prior to opening.

Some petals contain both starch and fructan. In chrysanthemum petals, for example, both polysaccharides were degraded during petal expansion (Trusty and Miller, 1991). Finally, some flowers reportedly contain neither fructan nor starch (or only very low starch concentrations) just prior to opening. An example is *Sandersonia* (Eason *et al.*, 1997; J Eason, personal communication, 2002). The petals of these species, nonetheless, do show increased contents of glucose and fructose, apparently as a result of sucrose uptake from the apoplast.

Flower opening may be due to a combination of sugar uptake and degradation of various polysaccharides. In gladiolus florets, where starch was a source of soluble carbohydrate, the increase in sugar content was 7–8 times higher than the decrease in starch content, which is indicative of sugar uptake (Yamane *et al.*, 1991). Similarly, in freesia florets the increase in perianth sugars was more than 10 times higher than the decrease in starch content (van Meeteren *et al.*, 1995). The sugar content and dry weight of opening freesia florets was dramatically reduced when stems were reduced in length, indicating that the stem was a major source of carbohydrate.

Competition for carbohydrates has been reported between petals of one flower, and among the opening flowers and flower buds. In cut Madelon roses, removal of the outer petals directly after harvest resulted in an increase in the surface area and in the sugar content of the innermost petals once they had fully developed (Marissen, 1991). Competition between buds follows from standard horticultural practice, where flower buds are removed in order to produce larger sized single flowers. Competition may be aggravated under conditions of restrained carbohydrate availability. In lily plants grown at low light intensity, for example, removal of young florets resulted in the increased dry weight of the remaining florets (van Meeteren *et al.*, 2001).

Growing buds and opening flowers can hasten the time to senescence in flowers that are already open. Removal of floral buds in cut lily inflorescences clearly increased the longevity of the open flowers that remained attached (van der Meulen-Muisers *et al.*, 1995). The life span of open lily flowers was also longer when they were detached, shortly after opening, from the inflorescence. A sharp decrease occurred in total carbohydrate content (starch, glucose, fructose, sucrose, and glycerol glucoside) in the petals of

lily flowers that remained attached to the inflorescence, but a much smaller decrease was observed in detached flowers (van der Meulen-Muisers, 2000). These data indicate carbon transfer from senescing flowers to opening ones, at least in cut inflorescences held at low light intensities, in which sugars rapidly become limiting for bud growth and flower opening.

Cell wall expansion

Cell wall changes during flower growth and opening have thus far received little attention (Winkenbach, 1971; Wiemken-Gehrig *et al.*, 1974; de Vetten and Huber, 1990; O'Donoghue *et al.*, 2002*a*, *b*). Growing carnation petals showed high activities of cellulase and pectin esterase (Panavas *et al.*, 1998). Brummel *et al.* (1999) demonstrated the expression of expansins, which are important in acid-induced cell wall loosening, in tomato flowers. However, acid-induced growth did not seem to control corolla expansion in *Ipomoea nil*. Applied fusicoccin (a proton efflux promoter) and sodium ortho-vanadate (an inhibitor) had no significant effect on the growth of young corollas. Buffers over a wide pH range also had no effect on the expansion (Raab and Koning, 1987).

Water relations

Cell elongation is usually very sensitive to a drop in water potential. Indeed, flower opening in cut rose flowers is often inhibited as a result of a blockage in the basal stem part, which results in a low water potential in the flower (van Doorn, 1997*a*). However, in some flowers, the leaves may be completely wilted but their flowers open normally. In cotton, for example, flower petals continued to expand when all leaves on the plant had wilted and the expansion of young leaves had ceased. The petals apparently removed water from other plant parts (Trolinder *et al.*, 1993). Similarly, in cut chrysanthemum flowers, the leaves often wilt, due to a blockage for water transport in the xylem of the basal stem part. Nonetheless, the flowers remain turgid for a much longer period. The relative independence on water availability may be an adaptation to an environment where regular drought occurs. Once the flowers are formed in these species, reproduction apparently gets priority over the maintenance of vegetative tissue.

Hormonal regulation

Regulation by endogenous hormones, except for the role of ethylene, is as yet unclear. Gibberellin application promoted opening in several flowers, including *Ipomoea nil* (Raab and Koning, 1987), *Gaillardia grandiflora* (Koning, 1984) and statice (Steinitz and Cohen, 1982). Growth of isolated petals of Madelon roses, placed in water, depended on the position of the petal on the flower, as shown by Kuiper *et al.* (1991). About 15 of the outermost petals, if placed in water, reached the same area and fresh weight irrespective of sugar feeding. Area and FW were the same as in petals that remained attached to the intact plant. The second group reached full size only if sucrose was added to the water, and the third group (the innermost whorls) only fully expanded when placed in a solution containing GA_3 and sucrose. The petals seem, therefore, to be dependent first on gibberellin and external carbohydrates, and then gradually to lose this dependence, starting with the hormone requirement. However, rigorous tests in which gibberellin synthesis or action was blocked have apparently not been reported.

Ethylene promoted or inhibited flower opening depending on the species. In roses, these differences even depended on the cultivar (Reid *et al.*, 1989). A role of endogenous ethylene in opening followed from inhibitor studies in potted rose plants (Cushman *et al.*, 1994; Tjosvold *et al.*, 1995), cut rose flowers (Yamamoto *et al.*, 1994) and cut gladiolus inflorescences (Serek *et al.*, 1994).

Opening in *Ipomoea nil* flowers was promoted by ABA. Koning (1986) concluded that the effect of ABA in *Ipomoea nil* was due to increased ethylene production. The effect of ABA could be eliminated by the ethylene biosynthesis inhibitors aminoethoxyvinyl glycine and cobalt ions. *Ipomoea* flower opening was inhibited by IAA (Kaihara and Takimoto, 1983). As IAA can promote or inhibit ethylene production, depending on the concentration and the tissue, the effects of IAA in *Ipomoea* may also be mediated by ethylene.

In *Arabidopsis*, a mutant defective in anther dehiscence and flower opening was identified. The defect could be rectified by an exogenous application of jasmonic acid or linolenic acid. The mutated gene was a phospholipase, apparently involved in jasmonic acid synthesis. In some species, therefore, jasmonic acid may also be involved in flower opening (Ishiguro *et al.*, 2001).

Regulation of opening and closure by external cues

Floral opening in several species is apparently independent of specific external regulation as it occurs at any time of the day. Floral opening in other species, belonging to a large number of families, show a relationship with the time of day. Flower closure may similarly be independent or dependent of the time of the day, depending on the species. This dependence on the time of the day may be regulated by external cues such as temperature, humidity, and light and/or by an internal rhythm.

Is flower opening due to changes in humidity, temperature or light?

Opening in many species occurs in the morning, correlated with an increase in temperature and light intensity, and

with a decrease in ambient humidity. In species that open in the afternoon or at night, the movement is correlated with decreasing temperature and light intensity, and with an increase in humidity. Are these environmental changes, alone or in combination, adequate for opening?

Floral opening in most species tested did not react to changes in relative humidity (RH). Examples are *Ipomoea purpureum*, *Taraxacum taraxacum* (Hensel, 1905), *Helianthemum guttatum* (Davy de Virville and Obaton, 1922*a*), *Turnera ulmifolia* (Ball, 1933), *Kalanchoë* (Bünsow, 1953*a*), and *Portulaca* (Ichimura and Suto, 1998). Some exceptions are found in the Caryophyllaceae and Onagraceae. High humidity hastened opening of *Silene saxifraga* (Halket, 1931) and *Lychnis dioica* (Davy de Virville and Obaton, 1922*b*, 1923, 1924). According to Sigmond (1930*a*, *b*), opening in *Oenothera biennis* and *O. lamarckiana* was also advanced by placing flowers at high RH. Arnold (1959), in contrast, showed that opening of *Oenothera berteriana* and *O. campylocalyx* was not much influenced by RH. All species in which opening was advanced by high humidity are nocturnal. Only if the flower opens in the late afternoon or at night may the stimulating effect of high RH on opening have a biological role. Apparently, a positive effect on opening of a decrease in RH has not been reported, indicating that opening in the morning is generally independent of RH.

Both temperature and light clearly affect opening in several species. When *Gentiana rhaetica* plants, of which the flowers had closed during the night, were exposed to an increase in outside temperature from 7°C to 42°C, the flowers opened within 3 min. So in this species an increase in temperature was sufficient for opening (Kerner von Marilaun, 1891). Similarly, in *Portulaca* plants held at 20 °C, a rise in temperature was sufficient for rapid opening, although light intensified the response. Exposure to light without a temperature change did not result in full opening (Ichimura and Suto, 1998). Effects of light, however, must be interpreted with caution. In such experiments the flower temperature must be determined and if possible rigorously controlled. Depending on petal pigmentation and anatomy, light may increase petal temperature even at constant air temperature (McKee and Richards, 1998).

The opening of several flowers that bloom in spring also depends mainly on temperature. A small temperature rise in the morning was adequate for full opening of *Ficaria*, *Galanthus*, *Tulipa*, and *Crocus*. A detectable opening movement occurred by a rise of 0.2 °C in *Crocus*, whereas the minimum in *Tulipa* was 1 °C (Pfeffer, 1873; Andrews, 1929). Other species required a larger temperature difference. In order of decreasing sensitivity, Pfeffer (1904, page 494) mentioned *Adonis vernalis*, *Ornithogalum umbellatum*, *Colchicum autumnale*, *Ranunculus ficaria*, *Anemone nemorosa*, and *Malope tifida*. The latter species responded to a change of 5–10 °C. Temperature effects on

opening may be due to an influence on a rapid growth process (as in the spring-blooming flowers already mentioned), or a stimulation of the basal growth rate. Hensel (1905) showed that high temperature promoted opening in *Ipomoea purpurea*, *Linum usitatissimum*, and *Oxalis stricta*, but the treatment had to last for several h and therefore apparently affected the basal growth rate.

Several other flowers respond mainly to light. Examples are *Oxalis rosea*, *Nymphaea alba*, and several species in the Asteraceae such as *Leontodon* spp. (Pfeffer, 1904, p. 494), *Tragopogon brevirostre*, and *Bellis perennis* (Oltmanns, 1895).

Two examples, showing the rather complex interplay of temperature and light on opening in some flowers, will be given. The capitulum of *Taraxacum albidum* opened in response to a rise in temperature, both in light and in darkness. Capitula also opened upon a change from darkness to light. Both temperature and light had an effect only at 18 °C or higher (Tanaka *et al.*, 1987, 1988).

In *Oxalis martiana* a rise in temperature was sufficient for the opening of flowers on plants held in darkness. The required increase in temperature depended on the temperature at which the plants were held. Flowers on plants held continuously at 20 °C, for example, would be almost fully open within 3 h if the temperature rise was 10 °C (Tanaka *et al.*, 1989). These effects may be explained by a 'temperature sum' as the degree of opening 3 h after a 5 °C increase at 20 °C was similar to that of 10 °C increase at 15 °C.

If the *Oxalis* plants were exposed to temperatures as described, but the light (about 12 W m^{-2}) was also switched on, opening was hastened. In plants continuously held in darkness at 20 °C or 25 °C, light alone was sufficient for opening. Light had no effect in plants held at 15 °C or lower (Tanaka *et al.*, 1989). These data suggest that the ambient temperature determined whether flower opening in this species depends on an increase in temperature, light or both.

Pfeffer (1873) observed that the effect of a rise in temperature, in temperature-sensitive day-blooming flowers, was much higher in the morning than in the evening. This time-dependence was also found in flowers that responded mainly to light. These results indicate that the effect of environmental factors on opening depend on the time since the last closure. As a rule, the reaction (to temperature or light) was greater the longer the flowers had been closed. This was corroborated by Oltmanns (1895) and by Stoppel and Kniep (1910).

Light also regulates the opening of some night-blooming species. In *Oenothera lamarkiana* light inhibited opening. Light was perceived by the lower third of the green sepals. Only wavelengths between 400 nm and 510 nm were effective, that is, the blue and green region of the spectrum (Saito and Yamaki, 1967). Blue light receptors are specifically effective at these wavelengths,

thus may be involved in the inhibition of opening by light (Cashmore *et al.*, 1999).

Effects of temperature and light on flower closure

In most species, flower closure responds to external factors in a way similar to opening. In some species, however, closure is apparently due to an endogenous rhythm even if opening is not. In still other species closure is due to senescence.

When senescence determines closure, it can be delayed by cool weather. Several ephemeral flowers can last for more than one day (or even several days) if the temperature is low (Burgerstein, 1901; Hensel, 1905; Davy de Virville and Obaton, 1922*a*). *Hemerocallis* (daylily), for example, which normally lasts one day, was open for two days in September and for three days by the end of October (Kerner von Marilaun, 1891).

Oltmanns (1895) showed, after detailed measurements, that the delay of closure in *Lactuca* sp. on overcast days was an effect of light, not of temperature. Field observations on some other species in the Asteraceae indicated the same effect of light. Flowers of *Tragopogon brevirostre*, for example, opened early in the morning and closed between 09.00 h and 10.00 h if the light intensity was relatively high, but they closed considerably later when the light intensity was lower. Burgerstein (1901) noted that flower closure during summer occurred earlier during the day, in several Asteraceae species, compared with closure during spring or autumn. Furthermore, experiments with *Calendula arvensis* showed closure following an increase in light intensity (Stoppel, 1910). The closing effect of high light intensity may not be limited to the Asteraceae, as a similar response was observed in *Kalanchoë* (Crassulaceae; Karvé *et al.*, 1961).

Tulip and crocus are examples of flowers that close due to a temperature drop. The minimum decrease in temperature was about equal to the temperature rise required for opening (Pfeffer, 1873; Andrews, 1929).

Some *Taraxacum* spp. and *Oxalis martiana* are representatives of species where flower closure seems to be regulated by an endogenous rhythm (Tanaka *et al.*, 1987, 1988, 1989). Although opening in three *Taraxacum* species depended on temperature and light, the time of capitulum closure was apparently independent of environmental conditions. Flowers of *T. albidum* and *T. japonicum*, for example, closed 8–11 h after the beginning of opening, both under natural conditions and under constant light and temperature (Tanaka *et al.*, 1987, 1988).

These results show that opening and closure depend mainly on light and temperature, and that the effect of humidity seems restricted to some nocturnal species. The results also show that, in some species, a rather complicated interaction exists between the influence of light and temperature.

Regulation of opening and closure by duration of darkness and light

Several authors concluded that a minimum period of darkness was required for opening of day-bloomers in the light, and that a minimum period of light was necessary for their closure in darkness. Examples are *Bellis perennis* (Pfeffer, 1873), *Tragopogon brevirostre* (Oltmanns, 1895), and *Kalanchoë* (Bünsow, 1953*a*, *b*). Opening of *Calendula arvensis*, for example, required 3 h of darkness (Stoppel, 1910), whereas in *Hedera helix* the dark requirement was at least 5.5 h (Sigmond, 1929*b*). Artificial lighting during the night prevented flower opening in *Turnera ulmifolia* which required a few h of darkness prior to opening (Ball, 1933). These experiments are reminiscent of an effect of phytochrome. Indeed, in *Ipomoea nil*, flower opening was promoted by red light, and the effect of red light was reversed by a subsequent exposure to far-red light. The petals were found to be the sites of photoperception (Kaihara and Takimoto, 1980, 1981*a*, *b*). Perception of the duration of light or darkness may involve a relatively slow phytochrome reaction (Lumsden, 1991).

Reopening of *Oxalis* flowers exhibited a darkness and low temperature requirement. In flowers that had closed under continuous light, a minimum period of 1 h of darkness at 5 °C was required for full and rapid reopening in the light at 25 °C. However, this darkness/cold treatment was only effective if given more than 5 h after the flowers had fully closed in the light (Tanaka *et al.*, 1989).

Hybrid tea roses open slowly—and only once—and terminate their life by petal abscission. When held at constant temperature and a 12 h light and dark cycle, full opening took a few days. Petal growth (and opening movement) was confined to about 5 h early in the morning. Growth started shortly before the lights were switched on. Placing rose flowers in continuous light or darkness immediately resulted in a steady slow growth rate. The results indicate that cyclic light and darkness was required for the periodicity of growth and opening (Evans and Reid, 1986, 1988). The onset of growth in rose flowers may require a minimum duration of darkness. Similarly, opening in Asiatic lily flowers (*Lilium* hybrid) started after about 7 h of darkness. Flowers held in continuous darkness showed irregular opening, indicating that one change of light to darkness (and possibly a minimum duration of darkness) was necessary for the timing of opening (Bieleski *et al.*, 2000*a*).

The requirement of a period of darkness for opening and a period of light for closure may be part of an endogenous rhythm. In *Hedera helix*, for example, opening and closure depended on changes from light to dark and vice versa. Under natural conditions this leads to a 24 h rhythm, whereby opening occurs about 12 h after the onset of darkness. Despite this rhythm, opening also occurred after shorter periods of darkness, but at least about 6 h of

darkness were required, after 12 h of light (Sigmond, 1929*b*).

Repeated opening and closure, role of endogenous rhythms

The presence of an endogenous rhythm of opening and closure is usually tested by placement in constant darkness or constant light. In all species investigated, the rhythm, if it existed, continued for some time under constant light or darkness, but it was also much affected by changes from light to darkness or vice versa.

Examples of species with an endogenous rhythm are the day-bloomers *Calendula arvensis*, *Bellis perennis* (Stoppel, 1910; Stoppel and Kniep, 1910), *Hedera helix* (Sigmond, 1929*a*), and *Kalanchoë blossfeldiana* (Bünsow, 1953*a*, *b*). It was also found in the night-bloomers *Cestrum nocturnum* (Overland, 1960), *Cereus grandifolius* (Schmucker, 1928) and several species in the genus *Oenothera* (Arnold, 1959; Takimoto, 1986; Sigmond, 1930*a*, *b*). *Calendula arvensis* is taken as an example and briefly compared with some of the other species.

When grown outdoors, the flowers of *C. arvensis* open in the morning and close in the afternoon, for about 5 d prior to petal wilting. Temperature changes had no effect on opening or closure. Under continuous darkness, opening and closure showed a 24 h rhythm. When the leaves were subjected to a different day/night cycle from the flowers, the opening and closing rhythm followed that of the flowers. The 24 h rhythm in continuous darkness also occurred in plants that had previously been subjected to a day/night cycle of 6 h rather than 12 h, in plants that had been held under continuous light, and in plants previously subjected to irregular periods of light and darkness. The flowers thus show a rhythm based on a clock that apparently resides in the flower, and which is unaffected by the light/dark regime prior to continuous darkness.

The time of the onset of darkness, however, had a large effect on the rhythm. In plants grown in the field and brought into 12 h darkness, starting at 12 noon, the flowers from then on showed a 24 rhythm whereby they followed the new cycle. Normally all flowers on a plant opened and closed at the same time. However, if buds on the same plant were placed in darkness at various times, they opened and closed at different times, set by the time of the onset of continuous darkness. This effect was also observed if closed buds were placed in darkness, so the endogenous rhythm can be set at a rather early stage of development. By contrast, in flowers that had already opened and assumed a specific rhythm, it was almost impossible to change the phase.

In light/dark (LD) cycles of 6 h or 9 h, flower opening followed the imposed cycle (i.e. they were 6 h open and 6 h closed, etc). Interestingly, if the light and dark periods were made as short as 2 h, the flowers again showed a 24 h rhythm. Within limits, therefore, the endogenous rhythm can become entrained by shorter light/dark cycling. Entrainment is defined as the process whereby the plant, exposed to repeated periods of light and darkness with a period P, changes its rhythm to the same period.

Similar results were obtained with *Bellis perennis*, which showed an endogenous rhythm only in continuous light (Stoppel, 1910) and in *Kalanchoë blossfeldiana*, where the rhythm occurred both in continuous light and continuous darkness. When the latter was subjected to light and dark periods of 10 h, 8 h and 6 h, the rhythm followed the newly imposed cycle. When the light and dark periods were as short as 4 h or 2 h, the flowers again showed a 24 h cycle (Bünsow, 1953*a*, *b*). The time of transfer to continuous darkness or continuous light would set the phase of the endogenous rhythm (Karvé *et al.*, 1961; Zimmer, 1962).

When plants of *Cereus grandifolius* (a night-bloomer) were kept in darkness during the day and exposed to light at night, the normal periodicity was retained for some days but gradually became weaker, and then assumed a new phase in which opening occurred at the end of the light period (Schmucker, 1928). Similar observations were made in *Oenothera berteriana*, *O. campylocalyx* (Arnold, 1959), and *O. lamarckiana* (Takimoto, 1986).

Circadian rhythms in flower opening and closure are thus highly dependent on changes from light to darkness (and/or vice versa). In the species tested, light was invariably the entrainment stimulus. Work on rhythms in other plant parts shows that temperature can also be such a stimulus (Bünning, 1973; Hayama and Coupland, 2003). A temperature-dependent endogenous rhythm may therefore also exist in flower opening and closure, but if it does it seems to be rare.

Control mechanisms: the molecular basis

The molecular control of opening and closure, at specific times of the day, is currently unknown. This is true both for single opening and closure, for repeated movements for which no evidence of an endogenous rhythm exists, and for repeated movements in which such a rhythm is apparently involved. Nonetheless, the circadian clock of plants is currently being dissected and this evidence may be helpful for hypothesis formation.

The advantages of *Arabidopsis* mutants have not, as yet, been employed to study the timing mechanism of floral opening and closure. In the laboratory, flowers of *A. thaliana* open in the morning, shortly after the lights are switched on, and they close at about midday. These movements are repeated for 2–3 d, until the petals are shed almost turgid. Mutants with another pattern of floral opening and closure have apparently not been selected (G Angenent, personal communication 2003). Circadian

leaf movements have been studied in some detail in *Arabidopsis* mutants (Michaels and Amasino, 1999; Swarup *et al.*, 1999; Yanovsky *et al.*, 2001; Staiger *et al.*, 2003). One out-of-phase mutant has been characterized in which the peaks of leaf movement occurred 3.6 h earlier than normal (Salomé *et al.*, 2002). The mechanism and control of these leaf movements may be similar to that of petal movements involved in flower opening and closure. However, the relevance of the data on leaf movements for floral opening and closure has yet to be established.

In *Arabidopsis,* the expression of several genes depends on the time of day. A 6 h interval microarray, involving more than 7000 genes, showed that about 2% cycled with a circadian rhythm (Schaffer *et al.*, 2001). In *Arabidopsis* and other plants, several genes with a circadian expression pattern have been identified, for example, a catalase (Kwon and An, 2001), cysteine protease (Ueda *et al.*, 2000), a putative β-amylase (Chandler *et al.*, 2001), nitrate reductase (Tucker *et al.*, 1998), sucrose phosphate synthase (Rufty *et al.*, 1983), and chlorophyll *a/b* binding proteins (*Cab*) of photosystems I and II (Riesselmann and Piechulla, 1990; McClung, 2000). Interestingly, Thain *et al.* (2000) showed that the circadian rhythm of *Cab* gene expression can be set to different phases in various parts of the plants, and even within the same leaf, using localized dark/light treatments. The clock in each cell apparently functions independently of that of neighbouring cells. This agrees with the results on flower opening in *Calendula arvensis*, described above: buds on the same plant, placed in darkness at various times, opened and closed at different times, set by the time of the onset of continuous darkness (Stoppel, 1910; Stoppel and Kniep, 1910).

The expression pattern of *Cab* genes in tomato is surprisingly similar to the characteristic of the endogenous rhythm of flower opening (especially that of *Calendula, Bellis,* and *Kalanchoë*). Under a 12 h LD cycle *Cab* was increasingly expressed towards the end of the dark period. The peak of expression could be entrained to LD cycles of various lengths, even to one as short as 6 h light and 6 h darkness. Under constant light, the rhythm levelled off and its phases increased, but 3 h of darkness and subsequent light brought it back to a 24 h cycle with large amplitudes. A new phase was then observed, which depended on the time of switching the lights on (Piechulla, 1989; Riesselmann and Piechulla, 1990). A deletion analysis of the promotor of the *Cab* genes (more recently designated light-harvesting-complex genes) showed a sequence of 47 nucleotides that is necessary for conferring circadian oscillations. Part of this sequence overlaps with a known sequence for phytochrome-mediated gene expression (Piechulla *et al.*, 1998).

Circadian fluctuations in hormone production have also been observed. In cotton cotyledons, for example, ethylene production rose at the end of the darkness period, under a 12 h LD cycle. Production reached a maximum early during the next light period. The results indicated that the fluctuation in ethylene release was not due to a rhythm in stomatal opening (Rikin *et al.*, 1984). In sorghum seedlings an ethylene rhythm was related to circadian expression of the mRNA for ACC oxidase (Finlayson *et al.*, 1999). A similar rhythm was observed in gibberellin biosynthesis in sorghum (Foster and Morgan, 1995). The floral stem of *Arabidopsis* showed a circadian pattern of elongation growth, related to a circadian oscillation of IAA concentrations. Auxin transport inhibitors abolished the growth oscillation (Jouve *et al.*, 1999). Such circadian fluctuations in hormone levels have not, as yet, been investigated in relation to flower opening and closure.

A few questions still remain largely unanswered: what is the nature of the circadian oscillator and the light sensor, and what is the signal transduction pathway? Two closely related (MYB-type) DNA-binding proteins (designated CCA1 and LHY) have been identified in *Arabidopsis*, which regulate the expression of light-harvesting-complex genes and are connected to circadian leaf movements. Both genes showed a circadian expression pattern. Mutant genes were expressed at a constant high level, which suggested that they were part of a feedback circuit that regulates its own expression. The two genes are therefore either part of the central oscillator of the circadian clock, or are closely associated with it. Another protein, designated TOC1, seems also part of the circadian oscillator. Overexpression of TOC1 completely abolished circadian rhythms in the expression of a number of genes (Yanovsky and Kay, 2001; Hayama and Coupland, 2003).

At least one of these proteins (CCA1) bound to a region of the *Arabidopsis* light-harvesting-complex promoter that is necessary for its phytochrome responsiveness. Phytochrome also affected the expression of the CCA1 gene itself: expression was affected by light signals known to reset the clock (Wang and Tobin, 1998; Schaffer *et al.*, 1998). Several reports now point to phytochromes A, B, D, and E as sensors for setting circadian rhythms in *Arabidopsis* (Yanovsky and Kay, 2001; Wang and Deng, 2003). In addition, specific blue light receptors (Cryptochromes 1 and 2) are involved in the input of blue light into the circadian rhythms in *Arabidopsis* (Cashmore *et al.*, 1999; Yanovsky and Kay, 2001).

As *Arabidopsis* flowers open and close in a circadian fashion, the numerous mutants that have been used to investigate the circadian clock, and the input of light and temperature to the clock, may prove useful for the further study of flower opening and closure.

Strategies of flower opening and closure

It is not clear, from an ecological and evolutionary point of view, why some flowers last as long as they do (Ashman and Schoen, 1994; van Doorn, 1997*b*), nor is it obvious

why they open and close as they do. Opening and closure show a wide range of strategies. Presumably, these strategies have been selected to optimize reproductive success at a minimal (metabolic) cost. Relatively easy to explain from a selective point of view it seems, is the existence of night-bloomers that are pollinated by a small group of nocturnal animals only. The time that pollinators are active may obviously exert selective pressure. An initial simulation model dealt with the efficiencies of pollen removal and pollen deposition, estimated from field data on *Lonicera japonica*. The flower opens in the evening and stays open for several nights and days. According to the model, anthesis at dusk rather than in the morning was favoured in this species, as long as pollen deposition efficiency of nocturnal pollinators was higher than that of day pollinators, even when day pollinators transferred more pollen (Miyake and Yahara, 1999).

Several flowers remain open day and night, although they are pollinated only during the day or night. This may, at least in some species, relate to a circadian pattern of scent production. In *Petunia*, for example, the flower scents only during the night, which may explain, at least partially, why it is usually only pollinated by moths. Not all open flowers smell. Perhaps, at least in some species, circadian floral opening and closure may be an adaptation that avoids the cost of scent production?

Flowers occur in a vast range of ecological situations. It is unsurprising, therefore, to find different ambient factors which regulate opening and closure, but these have not been categorized to their ecological niches, nor have they been explained in terms of selective advantages. May, for example, the tendency of early-spring flowering species such as tulip and crocus to close after a drop in temperature relate to protection of the reproductive parts from being covered in snow, or from being damaged by hail or rain, at a time when pollinator activity is low?

Among the flowers that show repeated opening and closure, some open irregularly, only if the temperature is high and/or the sun is shining (such as dandelion), whereas others open and close at regular intervals. In general, the advantage of regular flower closure is not very clear. So far, some hypotheses have been put forward but these have not been tested. Closure, for example, may aid in pollination as it traps pollinating insects (e.g. in fig species and *Nymphaea* spp.). Kerner von Marilaun (1891) thought that flower closure during the night may avoid pollen wetting. His idea may be extended to flowers of species such as dandelion, which open only if the weather is fine. Pfeffer (1904, p.481) suggested that intermittent flower closure may help to exclude insects, may protect against cold or avoid damage by high light intensity.

In addition, regular closure may limit water loss and limit the entry of potentially harmful micro-organisms.

Limitation of flower opening to periods of pollinator activity may reduce the risk of entry of airborne pathogens and avoid the risk of pathogens being delivered by non-pollinating insects. Bacteria and fungi usually do not grow on dry petal tissues, but the presence of water (for example dew) on the flowers, or high ambient humidity, expedites fungal and bacterial growth. Flowers that avoid wetting by rain or dew, or avoid opening at times of very high humidity, seem therefore better adapted. This may explain why several flowers open when the morning dew has already disappeared, and why some nocturnal flowers close before the dew occurs.

Conclusions

In most species, flower opening is due to (local) elongation growth or to local ion accumulation that is not accompanied by growth. The elongation growth of petals, leading to opening, does not seem different from that in other plant parts, as it requires a source of energy and cell wall loosening and expansion.

Cell elongation during flower opening stands out, firstly, by its high rate, and, secondly, by its rather specific regulation. The timing of opening is regulated by factors such as temperature, the quality and quantity of light, and the duration of both light and darkness. Flower closure, if it occurs, may be related to senescence or an active process. In the latter case, it is often regulated in a way similar to that of opening.

In a few species the requirements for repeated opening and closure, and the role of an endogenous rhythm, have been elucidated. The phase of the endogenous rhythms can be altered by a single switch from light to darkness (or vice versa). Thus far, only a few reports hint at the nature of the sensor of the light stimuli. Results in only one species (*Ipomoea nil*) suggest a role of phytochrome, and perhaps a blue light receptor is involved in the inhibition, during the day, of opening in a nocturnal species (*Oenothera lamarkiana*).

The co-ordination of processes culminating in synchronized flower opening is, in many species, highly intricate. This complex control by endogenous and exogenous factors sets flower opening and closure apart from most other growth processes. Although some of the interplay between the various environmental and internal factors is now known, mainly by observing opening and closure as a phenotype, little is known of the cellular and molecular mechanisms behind it. At least in *Arabidopsis* this may rapidly change. Its flowers open and close for a few days according to a circadian rhythm. There are now many *Arabidopsis* mutants that have been related to the circadian clock and its light input. It is suggested that these be used for the further study of circadian flower opening and closure.

Acknowledgement

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