Abstract
This review is an update of a 2003 review (Journal of Experimental Botany 54,1801–1812) by the same corresponding author. Many examples of flower opening have been recorded using time-lapse photography, showing its velocity and the required elongation growth. Ethylene regulates flower opening, together with at least gibberellins and auxin. Ethylene and gibberellic acid often promote and inhibit, respectively, the expression of DELLA genes and the stability of DELLA proteins. DELLA results in growth inhibition. Both hormones also inhibited and promoted, respectively, the expression of aquaporin genes required for cell elongation. Arabidopsis miRNA319a mutants exhibited narrow and short petals, whereby miRNA319a indirectly regulates auxin effects. Flower opening in roses was controlled by a NAC transcription factor, acting through miRNA164. The regulatory role of light and temperature, in interaction with the circadian clock, has been further elucidated. The end of the life span in many flowers is determined by floral closure. In some species pollination resulted in earlier closure of turgid flowers, compared with unpollinated flowers. It is hypothesized that this pollination-induced effect is only found in flowers in which closure is regulated by ethylene.

Key words: Carbohydrates, cell wall, flower, humidity, diurnal clock, growth, hormone, miRNA, petal, temperature, tepal, sepal, water relations.

Introduction
More than 10 years ago we published a review paper on the opening and closing movements of flowers (van Doorn and van Meeteren, 2003). We classified opening types (such as nocturnal or diurnal, and single or repetitive) and discussed the physiology of the opening and closure movements, and their regulation by both external factors and the circadian clock.

It was noted that external factors, such as temperature and light, are important regulators, often setting the circadian clock. Floral movements are based on changes in osmotic pressure in cells that do not elongate, or on differential elongation growth. Both involve an increase in osmotic pressure, followed by water uptake. Prior to opening, osmotic solute levels increased, for example by the conversion of polysaccharides (starch or fructan) to monosaccharides, and/or the uptake of sugars from the apoplast.

The present review will focus on recent advances in our understanding of the biological clock, and on the physiological background of opening and closure movements, including information on carbohydrate metabolism, water flow, and hormonal regulation. The effect of pollination on flower closure will also be discussed, as well as the genetics of opening, recent mathematical modelling of petal expansion, and the possible role of floral electric fields in opening and closure.

Opening and closure: some more examples
Although most flowers open at least once, some species have flowers that do not open. This reproduction strategy is called cleistogamy or automatic self-pollination. It has been reported in at least 693 Angiosperm species from 228 genera and 50 families, including the genera Arachis (peanut) and Pisum (pea). It apparently evolved from flowers that opened normally (Lu et al., 2012).
Several species show opening and closure movements for several days in a row. In such species, the end of the floral life span can be determined by senescence, abscission, or by closure while the petals are still turgid (van Doorn, 1997; Yamada et al., 2006). Many flowers that are open during the day and closed during the night for a number of days are in the Asteraceae—thus the movements refer to ray florets on inflorescences—for example, common daisy (Bellis perennis), the field marigold (Calendula arvensis), Cupid’s dart (Catananche caerulea), Gazania sp., dandelion (Taraxacum sp.), and straw flower (Xerococcus sp.). Such opening and closure movements are also found, for example, in the Crassulaceae (Kalanchoë blossfeldiana), the Geraniaceae (geranium, Pelargonium sp.), the Liliaceae (Lilium sp.; Tulipa ×hybrida), the Nymphaeaceae (Nymphea sp.), and the Papaveraceae (California poppy, i.e. Eschscholzia californica, and bloodroot, Sanguinaria canadensis).

Flowers of these species often close during a specific time of the day, although the time varies with light and temperature. For example, Tragopogon pratensis closes already early in the morning, Sonchus arvensis and Sonchus oleraceus close at about midday, and some other species close early in the afternoon (Anagallis arvensis, Hieracium pilosella, Cichorium intybus, and Taraxacum officinale; Brauner and Rau, 1966, p. 114). All mentioned species belong to the Asteraceae, except A. arvensis (Primulaceae).

Species that are open during the night and closed during the day, for several days, are found, for example, in the Brassicaceae (night-scented stock, Matthiola longipetala), Cactaceae (Selenicereus grandiflorus=Cereus grandifolius; Peniocereus greggii), Capparidaceae (honeysuckle, Lonicer a heckrottii), the Caryophyllaceae (Silene sp.), Convolvulaceae (Ipomoea alba), Iridaceae (night gladiolus, Gladiolus tristis), Nyctaginaceae (Mirabilis jalapa), Scrophulariaceae (night phlox, Zaluzianskya capensis), Solanaceae (Petunia; Cestrum nocturnum), and Onagraceae (Oenothera). A genus can contain species that open during the day and species that open during the night (e.g. Ipomoea; Gladiolus; Hemerocallis).

Kogler (2010) studied rhythmic movements in unpollinated flowers of the orchid Bulbophyllum lobbii. The flowers opened and closed for ~9 d. The difference between opening and closure became increasingly smaller—that is, the movements became weaker—rather than opening. Most flowers opened between midnight and 02:00, whereas closure occurred between 19:00 and 23:00 of the next evening; thus the period of closure was shorter than that of opening. A 24h rhythm of the movements was observed under light/darkness conditions. The movements also continued under constant light or darkness. Changes from light to darkness or vice versa reset the circadian clock. Temperature had no influence on the rhythm, although high temperature negatively affected the speed of movement. Other species in the same genus showed similar effects (Kogler, 2010). These data are very similar to those on Calendula arvensis (Asteraceae) (Stoppel, 1910) and Kalanchoë blossfeldiana (Crassulaceae) (Bunsow, 1953a, b; Karvé et al., 1961; Zimmer, 1962), which both open during the day.

Flowers of many species close at least once. Such closure can occur in flowers that are still fully turgid, thus before the petals wilt (senescence) or fall off. In these flowers, closure determines the end of the flower life span. Closure of flowers at the end of their life, irrespective of any previous closure, has been described in members of the Aizoaceae, Asteraceae, Balsaminaceae, Campanulaceae, Cactaceae, Caryophyllaceae, Convulvulaceae, Gentianaceae, Cucurbitaceae, Malvaceae, Nyctaginaceae, Oenotheraceae, Portulacaceae, and Solanaceae in the dicots, and Cannaceae, Commelinaceae, and Iridaceae in the monocots (van Doorn, 2002; Franchi et al., 2014). Wilting of the petals or tepals, as a result of senescence, can also result in (or at least be accompanied by) flower closure, for example in Iris ×hollandica. These examples are clearly not included in the category of closure of turgid flowers.

Flower opening demonstrated by time-lapse photography


These time-lapse movies show that opening in some species is very sudden. They also demonstrate that opening can occur when most of the tepal growth has already taken place, or, at the other extreme, before the tepals grow severalfold. Additionally, the movies illustrate that opening is often related to lateral (outward) movements at the petal base, as well as a curvature towards the petal tops.

Several flowers show periodic opening movements, namely a sudden and relatively large opening movement is followed by an extended period of no opening, followed by one or more short periods of further opening. The petals in these flowers often show a slight closing movement during the period in which opening is stalled. This closing movement can vary in amplitude, depending on the species and cultivar.
Examples are *Crocosmia*, most *Dahlia* cultivars, *Nymphaea*, and some *Rosa* cultivars.

**Flower opening and closure in relation to dispersal of viable pollen**

After flower opening, pollen quality might be compromised by adverse weather. Two main groups of pollen grains have been distinguished with regard to dehydration hazards: (i) pollen with relatively low water content at the time of dispersal, which are usually desiccation resistant; and (ii) pollen with a relatively high water content, which are usually desiccation sensitive (Franchi et al., 2014). Pollen viability (ability to germinate) was tested in eight species with desiccation-sensitive pollen: *Alcea rosea* and *Althaea officinalis* (both in the Malvaceae), *Cucurbita pepo* (Cucurbitaceae), *Ipomoea bonariensis* (Convolvulaceae), *Lavatera arbo-rea* (Malvaceae), *Mirabilis jalapa* (Nyctaginaceae), *Oenothera organensis* (Onagraceae), and *Oplutia dil-enii* (Cactaceae). In all species, pollen viability was high (80–100%) when the flowers opened. After anthesis, pollen viability decreased almost linearly with time. In some species, viability was as low as 30% within 1 d. In all species floral longevity was ended by closure of turgid flowers. Flower longevity varied from 6 h (*C. pepo*) to a few days in the Malvaceae species. The authors suggested that one of the reasons for the relatively early end of the floral life span might be to prevent dispersal of pollen with reduced viability (Franchi et al., 2014). More detailed experiments are required to test this idea.

von Hase et al. (2006) studied flower closure in relation to relative humidity (RH) in some night-blooming species growing in the desert. Species were in the Asteraceae (*Argotis merxmuelleri* and *Didelta carnosa* var. tomentosa), Aizoaceae (*Lampranthus hoerleinianus*, *Delosperma cras-sum, Ruschia subpaniculata,* and three *Cephalophyllum* species), Neuradaceae (*Grielium grandiflorum*), and Oxalidaceae (*Oxalis eckloniana*). The hypothesis was tested that during the rainy season temporary floral closure would provide protection of the pollen against moisture due to rain, fog, or dew. Exposure to very high RH in the laboratory damaged the pollen of *G. grandiflorum* and most species tested in the Aizoaceae, but had no effect in the species in the Asteraceae. In the field, the percentage of open flowers increased rapidly when the air temperature became higher than ~20 °C; that is, when the RH became relatively low. It was concluded that in most species tested the results did not contradict the hypothesis. In a study on 80 spring-flowering plant species in 46 families, Mao and Huang (2009) confirmed that in many species pollen longevity was greatly reduced by wetting. Less than a quarter of the species tested had pollen with a relatively high resistance to moisture.

The data in this section do not yet provide experimental proof for the hypothesis that the time to temporary or definite flower closure is under selective pressure induced by optimization of the dispersal of viable pollen. The data do provide suggestions for further experimental work.

**Petal senescence, petal abscission, and flower closure after pollination; effects of pollen load**

Unpollinated flowers of many species, for example orchids, may remain open for a considerable period, but the petals senesce (wilt, wither) or abscise shortly after pollination. These effects of pollination are due to ethylene. Flowers of other species (often in different families) have a life span that is independent of pollination. This is found in species in which petal senescence or abscission is not affected by ethylene (van Doorn, 1997).

In other species, pollination induces early closure of turgid flowers. In most of these species the same effect was found after treatment with exogenous ethylene, suggesting that pollination acts through ethylene. Only a few species are known thus far in which flower closure was not hastened after ethylene treatment (*Hemerocallis* hybrids, *Lachenalia* sp, and *Exacum* affinis; van Doorn, 2002).

*Crepis capillaris* (Asteraceae) open early in the morning. The flower heads closed within 3 h of hand pollination (carried out early in the morning), whereas unpollinated flower heads stayed open until late afternoon. Early closure due to pollination was also found in *Crepis biennis* and *Leontodon autumnalis*, but not in *Taraxacum officinale* (all in the Asteraceae). Early closure following pollination was not always permanent. Relatively young flower heads (*C. biennis* and *L. autumnalis*) opened again the next morning, exposing fresh, previously immature florets. To check whether the measured response to pollination was just a response to mechanical stimulation, *C. biennis* flower heads were touched with flower heads of *Picris hieracioides* in a way that mimicked pollination. This had no effect on flower opening (Fründ et al., 2011). Even if flower life is ended shortly after pollination, longevity often exhibits a plastic response to pollination. In *Digitalis*, for example, Stead and Moore (1983) found that an increasing pollen load on the stigma resulted in earlier petal abscission, and thus in shorter flower life span. Flowers of *Chamerion angustifolium* (Onagraceae, syn. *Epilobium angustifolium*) close in response to pollination. Two predictions were tested: (i) flower closure is triggered by pollination, not by fertilization; and (ii) flower closure responds differently to various kinds of pollination (outcrossing or self-pollination) and amounts of pollen (none, low, or high). Flower closure was initiated 4 h after pollination, well before fertilization (25 h). Flower closure times were reduced with increasing pollen loads up to 700 pollen grains. Flower closure was slower after self-pollination than after outcrossing. The results show that flower closure is plastic in this species, and shows an adaptive response that promotes both outcrossing and seed production (Clark and Husband, 2007).

**Flower opening mutants**

Mutants in genes that identify floral parts can result in abnormal flowers, often lacking petals, having other flower organs instead of petals, or having petals instead of other organs.
Some less intrusive mutants can lead to alteration of petal development, with consequences for flower opening; for example, HOTHEAD (HTH) which prevents flower opening. The mutant gene inhibited organ separation, although it did not completely block petal emergence (Krolkowski et al., 2003; Lolle et al., 2005).

An oilseed rape (Brassica napus) mutant also lacked opening (Fig. 1). The mutated gene encodes a RINGv E3 ubiquitin ligase that causes reduced cutin biosynthesis or loading. The mutation was associated with self-pollination (Lu et al., 2012). Opposite effects (earlier flower opening) have also been described in mutants, for example in Melilotus alba (white sweetclover; Hirsch et al., 2010). Other mutants will be described in the section ‘Regulation by hormones’.

**Regulation by light, temperature, and the circadian clock**

Opening in several species responds to light and to the length of the dark period. Light sensing in plants has been reviewed recently by Jiao et al. (2007) and by Kami et al. (2010). In flowers where opening takes place before dawn, it is usually determined by the length of the dark period. This seems to be regulated by PHYTOCHROME INTERACTING FACTOR 4 (PIF4) and PIF5, which control the circadian clock (Imaizumi, 2010).

Opening in other flowers responds to changes in temperature. Temperature sensing in plants has been reviewed recently by Ruelland and Zachovski (2010), McClung and Davis (2010), and Mittler et al. (2012). It is as yet largely unknown how plants determine ambient temperature, but several hypotheses have been proposed.

The effect of a rise in temperature (in some day-blooming flowers) was larger in the morning than in the evening, and a similar time of day effect was found in the response to light of night-blooming flowers held in darkness. This suggested an interaction of these environmental factors with the circadian clock (van Doorn and van Meeteren, 2003). Recent advances in our insight into the physiology of plant circadian rhythms have been reviewed by, for example, Pruneda-Paz and Kay (2010), Imaizumi (2010), Robertson and Gutiérrez (2010), and de Montaigu et al. (2010). About 90% of the transcripts in the Arabidopsis thaliana genome are rhythmically expressed. The clock mechanism consists of interlocked regulatory feedback circuits: a 24h core loop (also called the CCA1/LHY-TOC1 loop), a morning loop, and an evening loop. The morning and evening loops seem to fine-tune rhythmicity.

Light receptors provide input signals for resetting the circadian clock. Photoreceptors can inhibit COP1 (CONSTITUTIVELY PHOTOMORPHOGENIC 1), which is an E3 ubiquitin ligase. Light regulates circadian rhythms in part by antagonizing COP1 activity (Máš and Yanovsky, 2009). Temperature also can reset the clock, whereby daily temperature oscillations as small as 4 °C are sometimes effective. Several clock components are required for such resetting (McClung and Davis, 2010). A temperature-sensitive oscillator has been identified that can be phased independently from the oscillator that is synchronized by light. A connection between the light-sensing and temperature-sensing systems might exist as photoreceptor signalling is temperature sensitive (Máš and Yanovsky, 2009).

**Regulation by hormones**

Plant growth is regulated by hormones such as ethylene, auxin, cytokinins, brassinosteroids, abscisic acid (ABA), jasmonic acid (JA), brassinosteroids, strigolactone, and a number of peptides (Hedden and Thomas, 2006; Santner et al., 2009; Wang and Irving, 2011). At least exogenous ethylene, auxin, gibberellic acid, and ABA affect petal expansion and flower opening.

Ethylene promoted flower opening in carnation (Jones and Woodson, 1997), Phalaenopsis orchids (Bui and O’Neill, 1998), and Petunia (Tang and Woodson, 1996).

Ethylene inhibited flower opening in several rose cultivars, although in some rare cultivar it promoted opening (Reid et al., 1989a, b; Macnish et al., 2010). This difference might relate to a similar response curve, whereby a relatively small dose promotes opening and a relatively high dose inhibits it, and whereby the sensitivity of the cultivar differs. Ethylene, when inhibiting opening in roses, promoted the expression of DELLA genes, a family of growth repressors. The expression of a DELLA gene (RhGAI1) was induced by ethylene treatment, via EIN3-3, a protein involved in ethylene signalling, which bound to the promoter of RhGAI1. Cell expansion was promoted in RhGAI1-silenced rose petals, whereas the expression of nine cell expansion-related genes was altered. The DELLA protein bound to the promoter of a gene involved in cell wall synthesis, thereby repressing it (Luo et al., 2013).

Ethylene hastened flower opening in rose cv. Samantha, whereas 1-methylcyclopropene (1-MCP), an ethylene receptor inhibitor, impeded opening. Ethylene promoted flower ethylene production, but, contrary to expectation, 1-MCP did not inhibit this production. While the expression of some genes involved in ethylene synthesis was up-regulated by ethylene, 1-MCP did not affect their expression. Ethylene...
also resulted in a drastic increase in transcript levels of the ethylene receptor ETR3, and also induced expression of the ethylene signal transduction elements CTR1 and CTR2, whereas 1-MCP reduced transcript levels of these three genes to below those in untreated controls (Ma et al., 2006; Xue et al., 2008). Ethylene receptor genes are negative regulators (i.e. they suppress ethylene effects when not bound to ethylene). Up-regulated transcription of ethylene receptor genes was accompanied by a large increase in protein degradation, with a final result of a decrease of receptor protein after ethylene treatment (Kevany et al., 2007). The results of Ma et al. (2006) and Xue et al. (2008) indicate that the positive effect of endogenous ethylene on flower opening in the rose cultivar studied occurs at the level of ethylene sensitivity rather than ethylene synthesis. Data published by Xue et al. (2008) additionally suggested that the gynoecia were the main site of ethylene production.

In Arabidopsis, knockout of the ethylene receptor genes ETR1 plus ERS1 (Qu et al., 2007; Hall et al., 2012) resulted in hypersensitivity to ethylene. Plants were dwarfed and flowers showed early arrested petal development. Expression of inserted wild-type ETR1 in these plants reversed the abnormal phenotypes.

In rose petals in which ethylene promoted opening, ethylene rapidly induced a gene encoding a NAC transcription factor (RhNAC100). Accumulation of its transcript was modulated via miRNA164-dependent post-transcriptional regulation. Overexpression of RhNAC100 in A. thaliana substantially reduced the petal size by repressing petal cell expansion. Silencing of RhNAC100 in rose petals significantly increased petal size. As many as 22 out of 29 cell expansion-related genes tested exhibited changes in expression in RhNAC100-silenced rose petals. Two aquaporin genes were also among the targets of RhNAC100 (Pei et al., 2013).

Flower buds of several Ipomoea (previously called Pharbitis) species open early in the morning. Flower buds that were cut from plants in the field before noon opened quite slowly the next morning, whereas buds cut in the evening of the same day opened normally. Inclusion of ABA in the water in which the flowers were stood promoted the opening of the buds that had been cut before noon. In contrast, auxin completely inhibited opening of buds cut in the evening (Kaihara and Takimoto, 1983). These data suggested the hypothesis that both ABA and auxin were endogenous regulators. In citrus flowers, Goldschmidt (1980) found an increase in petal ABA concentration and a decrease in stamen ABA level by the time of flower opening. This might also suggest a positive effect of ABA on opening.

Removal of the stamens often results in a decrease in petal growth, for example in Petunia (Weiss and Halevy, 1989) and in Mimulus (Barr and Fishman, 2011). It has been suggested that this is due to gibberellins produced in the stamens, as application of gibberellic acid substituted for the stamens (Weiss and Halevy, 1989). Gibberellic acid-deficient Arabidopsis mutant plants exhibited reduced petal elongation. As noted above, gibberellic acid promotes cell elongation by opposing the function of DELLA proteins. The Arabidopsis DELLA proteins RGA, RGL1, and RGL2 jointly repressed petal growth in gibberellic-acid-deficient plants (Cheng et al., 2004). Ethylene and gibberellic acid often antagonistically affect growth. The two hormones act antagonistically not only on DELLA gene expression but also on the stability (and the activity in at least the case of gibberellic acid) of DELLA proteins (Luo et al., 2013; Sarnowska et al., 2013).

The Arabidopsis mutant defective in anther dehiscence (dad1) shows inhibited petal elongation and delayed flower opening, accompanied by reduced JA concentrations. The defect was rescued by application of JA or linolenic acid. The DAD1 protein was apparently a chloroplastic phospholipase A1 that catalyses the initial step of JA biosynthesis. The expression of DADI was restricted to stamen filaments. It was therefore concluded that JA from the filaments regulates petal growth (Ishiguro et al., 2001).

Petal growth in A. thaliana was regulated by AUXIN RESPONSE FACTORS (ARF8). Petals of arf8 mutants were significantly larger than those of the wild type, due to increased cell number and increased cell expansion, associated with a change in expression of auxin-responsive genes (Varaud et al., 2011). The A. thaliana mutant JAGGED shows little petal growth (Sauret-Güeto et al., 2013). JAGGED encodes a zinc finger transcription factor, which has been linked to cell proliferation and cell enlargement. JAGGED directly repressed PETAL LOSS (PTL), which has been implicated in auxin effects. miRNA319a mutants in A. thaliana exhibited narrow and short petals. miRNA319a functions by regulating TCP transcription factors, including TCP3 and TCP10 (Nag et al., 2009) which both regulate auxin effects (Koyama et al., 2010; Danisman et al., 2013; Wang et al., 2013).

Genes specific for flower opening of carnation flowers were obtained using suppression subtractive hybridization. Of the 235 unique cDNA fragments, as many as 211 did not match a known nucleotide sequence of carnation genes in the databases. BLASTX search of nucleotide sequences revealed that putative functions of the translational products include transcription, signalling, cell wall modification, lipid metabolism, and transport. Genes encoding an Aux/IAA protein and an auxin influx carrier protein were differentially expressed (Harada et al., 2010).

In Petunia×hybrida and Nicotiana attenuata, transcripts of the gene EOBII (EMISSION OF BENZENOIDS) accumulated during floral bud growth, with a maximum at flower opening. Reducing transcript levels, using RNA interference (RNAi), resulted in flowers that did not open and showed premature senescence. Core phenylpropanoid pathway transcripts and cell wall modifier transcript levels were altered in these RNAi flowers. The RNAi-induced changes could be partially complemented by feeding with sucrose or gibberellic acid. Additionally, if ethylene sensitivity was blocked, the RNAi flowers opened (Colquhoun et al., 2011).

Flower opening in Iris×hollandica depends on elongation of the pedicel and ovary. This growth moves the floral bud upwards, allowing the tepals to move laterally. In Iris cv. Blue Magic, ethylene had no effect on opening in unstressed flowers, but was the cause of lack of opening in water-stressed flowers, as it inhibited elongation of the pedicel and ovary.
Trolinder grows in the Mediterranean region and

Conversely, the data confirmed that some flowers can

Whereas in species, endogenous ethylene, auxin, gibberellins, and JA are

Suggested that endogenous auxins are among the regulators

The data in this section suggest that, depending on the

Relationship between the circadian clock

Circadian factors affect hormone concentrations. For example, cytokinin, auxin, and ABA concentrations show diurnal patterns. TANDEMZINC KNUCKLE/PLU3 (TZP) affects diurnal growth patterns. Its gene expression oscillates, with a peak in the morning. TZP regulates the expression of auxin-related genes (Nováková et al., 2005).

Microarray studies showed significant overlap between transcripts controlled by the circadian clock and those related to auxin, JA, and ABA activity (Más and Yanovsky, 2009). For example, auxin sensitivity depends on the time of day because the circadian clock regulates auxin signal transduction. The magnitude of growth responses as a result of the same exogenous auxin application therefore depended on the time of day (Covington and Harmer, 2007). Conversely, there are also effects of hormones on the circadian system. Cytokinins delayed the circadian phase, auxins regulated the circadian amplitude and clock precision, whereas brassinosteroids and ABA modulated circadian periodicity. The hormone-activated ARABIDOPSIS RESPONSE REGULATOR 4 and the photoreceptor phytochrome B were elements in the input of the cytokinin signal to the circadian clock (Hanano et al., 2006).

Water relations and carbohydrate metabolism

Flower opening requires elongation growth or osmotic oscillations, thus influx of water into cells. In several species, water enters growing floral buds and opening flowers even when the water potential of the rest of the plant is low. For example,

under drought conditions, the petals of cotton continued to expand when all leaves on the plant had wilted. The water potential of the petal was consistently higher than that of the subtending leaves, both during and after anthesis (Trolinder et al., 1993). These data might be explained by maintenance of a high osmotic pressure in the petal cells.

Capparis spinosa grows in the Mediterranean region and blooms during the dry local summer. Adequate petal turgor was sustained mainly due to high osmotic pressure, with contributions of at least proline and soluble sugars. Flowers open at dusk and remain open until the next morning. On the second day of opening, the petals lost turgor at about 10:00 h, and abscised by about noon. The highest proline and sugar concentrations were found at flower opening and the lowest the next morning. Petals had higher sugar concentrations than other parts of the plant (Rhizopoulos et al., 2006). The data confirmed that some flowers can accumulate considerable concentrations of osmotically active solutes.

Aquaporins are small transmembrane proteins that are important for cell expansion, as they facilitate water diffusion across membranes. Regulation of water flow occurs through changes in aquaporin density and activity. At the whole-organ level, aquaporins modify water conductance at ‘gatekeeper’ cell layers (Chaumont and Tyerman, 2014). Examples of aquaporins are plasma membrane intrinsic proteins (PIPs) and tonoplast intrinsic proteins (TIPs). Although exogenous ethylene promoted opening in cv. Samantha roses, it reduced petal size, related to inhibited expansion of abaxial subepidermal cells. After ethylene treatment, lower expression of a PIP gene (Rh-PIP2;1) was correlated with inhibited petal expansion. Furthermore, in Rh-PIP2;1-silenced flowers, petal expansion was inhibited (Ma et al., 2008). The promoter region of Rh-PIP2;1 was most active in organs that were rapidly expanding, and increased after gibberellin acid treatment (Li et al., 2009). Expression of a TIP gene in cv. Samantha rose petals was also negatively affected by ethylene (Xue et al., 2009).

Tulip flowers held in darkness open at 20 °C and close at 5 °C. When the flowers were opening, a putative plasma membrane aquaporin became phosphorylated which was suggested to activate the water channel. The plasma membrane aquaporin became dephosphorylated during petal closure, indicating inactivation of the channel (Azad et al., 2004).

Cell walls during petal growth

The expression of four genes encoding xyloglucan endotransglucosylase/hydrolase (XTH) and of three genes encoding expansins (DeEXPA1–DeEXPA3) was studied in carnation. The results suggested that two of the XTH genes and two of the expansin genes were involved in petal growth during flower opening (Harada et al., 2011).

Galactose was the major non-cellulosic neutral sugar in Petunia petal cell walls. During the 24 h period of flower opening, the galactose content of polymers doubled, whereas by day 2 after flower opening the galactose content had
the flowers open very rapidly. Holding the outer tepals in check. When this latch gives way, the rims of the outer tepals are located in a groove in the cell wall related genes were also up-regulated in Arabidopsis plants overexpressing the NAC gene.

Genetics of the timing of opening and closure

Daylilies (Hemerocallis fulva) which open early in the morning and close in the evening were crossed with nightlilies (Hemerocallis citrina) which open in the evening and close in the morning. The hybrids were fertile. Flower opening times of F1 hybrids were highly variable. F2 hybrids, in contrast, showed a bimodal distribution, with peaks both in the morning and in the evening. The ratio did not deviate from 1:1. A two-gene model would explain the observed segregation patterns (Nitta et al., 2010).

Boikoglou (2008) investigated temperature entrainment and light/dark entrainment of the A. thaliana circadian clock. The transcription of several genes in the clock displayed a unique response. The role in entrainment of two evening-expressed genes, TOC1 and GI, was further examined using genetics. Based on crossing experiments, a model was proposed in which GI is a main gene involved in resetting of the clock by light while TOC1 is a main gene involved in clock resetting by temperature.

Mechanics of flower opening

Forterre (2013) summarized the main physical mechanisms plants use to achieve movements, emphasizing the role of turgor and water transport. It was concluded that the speed of movements is limited by the rate of water transport. Some plant structures overcome this limit by imposing a barrier to the system, which can originate from geometrical constraints, allowing elastic potential energy to be stored until the barrier is overcome, resulting in very fast movement. This is similar to the findings of Bieleski et al. (2000) in lily. In floral buds, the rims of the outer tepals are located in a groove in the dorsal part of the midrib of the underlying tepals, effectively holding the outer tepals in check. When this latch gives way, the flowers open very rapidly. Liang and Mahadevana (2011) noted that the edges of lily tepals were wrinkled as the flower opened. They suggested that flower opening depended on differential growth at the tepal edges.

Using Asiatic lilies and Eustoma grandiflorum as examples, Ijiri et al. (2010) simulated petal development during flower opening based on the observation that opening is mainly due to cell expansion. They used an elastic triangular mesh to represent a petal. Growth was simulated by developing each triangular region of the mesh. The simulation first grew each triangle independently. The data realistically showed petal growth and curvature.

Effects of opening and closure on floral electric fields

Flowers are negatively charged, while flying insects are positively charged. This has been suggested to facilitate pollen transfer between insects and flowers. A recent paper in Science indicates that the electric fields of flowers also might be pollinator cues (Clarke et al., 2013). It has not been studied yet how long the electric field lasts in relation to stigma receptivity and pollen availability. It also is not yet clear when the flowers attain the electric field, thus whether and how the field is related to flower opening or closure.

Conclusions

During the period 2003–2013 progress has been made mainly in knowledge of hormonal regulation of flower opening, and in the role of signal transduction factors, transcription factors, and miRNA. Some progress has also been made on the role of the circadian clock. Additionally, work has begun on the selective forces, other than specialization to pollinators, which control the timing of opening and closure.

For example, both ethylene and gibberellic acid can control flower opening through regulation of DELLA proteins, which inhibit growth. Ethylene inhibits opening in most rose cultivars, but at the same concentration it promoted opening in other cultivars. In the case of inhibited opening, ethylene induced EIN3-3, a component of the ethylene signal transduction pathway. EIN3-3 bound to the promoter of a DELLA gene, activating its expression. The DELLA gene bound to the promoter of at least one cell wall-related gene, and its expression was associated with increased expression of at least nine other cell expansion-related genes. Ethylene-promoted opening was correlated with increased expression of the ethylene receptor ETR3, and an element in the ethylene signal transduction pathway (CTR).

The opening effect of ethylene in roses was accompanied by a rapid increase in the mRNA levels of a gene encoding a NAC transcription factor (RhNAC100). The NAC transcript levels were regulated by miRNA164. Additionally, miRNA319a mutants in A. thaliana had narrow and short petals. miRNA319a affects the levels of TCP transcription factors, which in turn regulate auxin effects.

Both temperature and light can reset the circadian clock. A genetic study led to a model suggesting that GI is a main
gene involved in resetting the clock by light while TOCI is a main gene involved in resetting of the clock by temperature. PIF4 and PIF5 are part of the signalling pathway to the circadian clock after a change from light to dark or vice versa.

The end of the flower life span in several species is due to flower closure. In some plants, pollination advanced flower closure. Pollination might exert this effect through increased ethylene production. Only in a few species did flower closure at the end of the life span seem not to be regulated by ethylene. It was hypothesized that pollination would not hasten flower closure in these species.

Little is still known about the factors, apart from pollinator activity, that exert selective pressure on the timing of flower opening and closure. It has been suggested that preventing the pollen from becoming wet and therefore unviable might be among these factors. Other evidence suggests that early flower closure in arid environments might be selected due to pollen desiccation.

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