

# Seasonal flowering and evolution: the heritage from Charles Darwin

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**Abstract.** To survive, plants optimise their seasonal flowering time and set seed to avoid extremes of the environment including frost, heat and drought. Additionally, pollination may need to be tightly regulated in time so that it coincides with flowering of other individuals and/or with the presence of bird or insect pollinators. It is now clear that plants use seasonal changes in natural light intensity, daylight duration and temperature to achieve reproducible timing of flowering year-in-year-out. In more recent studies, genetic and molecular approaches are beginning to provide a basis for understanding heritability, an essential component of Darwin's concept of evolution.

**Additional keywords:** *Arabidopsis*, *Bromus*, daylength, *FLC*, *Fragaria*, *FRI*, *FT*, light intensity, *Pimelea*, *Poa*, speciation, temperature, *Themeda*.

## Introduction

Charles Darwin wrote in 'The Origin of Species':

*'the organs of vegetation on which their (the plants) whole life depends are of little signification compared with organs of reproduction with their product the seed of paramount importance'* (Darwin 1859, p. 414).

Control of flowering is placed centrally in the evolution of plants by Darwin's recognition that species survive best if they produce more seed. Environmental regulation of seasonality of flowering and set seed is critical for this survival as it allows seeds to develop in the most favourable conditions. However, it was not until the early part of the last century that some understanding of seasonal regulation of flowering was reported. Both daylength (photoperiod; see Garner and Allard 1920) and winter cold (vernalisation; see Gassner 1918) were shown to regulate flowering time and Bünning (1936) proposed that endogenous circadian rhythms provided the clock mechanism that was essential for accurate measurement of the length of day. Armed with the knowledge of how the extent and timing of flowering is regulated seasonally and with current evidence of its molecular and genetic regulation, Darwin would certainly have speculated on the evolutionary importance of the regulation of flowering.

A further aspect of Darwin's view of evolution related to plant migration leading to geographical isolation:

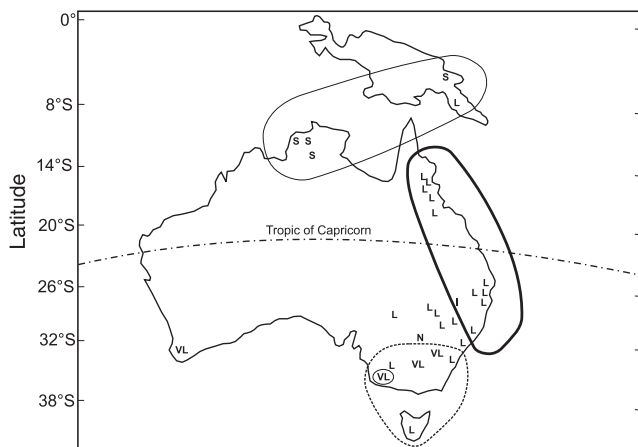
*'All the grand leading facts of geographical distribution are explicable in the theory of*

*migration together with subsequent modification and the multiplication of new forms'* (Darwin 1859, p. 408).

Thus, adaptation to different latitudinal environments should be evident in the types of seasonal inputs used by plants to control their flowering.

An early study of flowering response with 31 lines of an Australasian perennial grass, *Themeda australis* (R. Br.) Stapf, collected over a wide geographical range of origin illustrates the match between the type of response to environment and expectation for each line based on site daylength and temperature. In controlled environment studies, strains of *Themeda* from latitudes 6°S to 15°S flowered in short days (SD or day neutral plants; Fig. 1). Those from mid latitudes where daylength is longer, responded to longer daylengths (LD), while accessions from more southerly, colder sites, required prolonged exposure to winter cold (vernalisation) followed by LD. A daylength-insensitive selection was found in a desert environment where independence from normal seasonal cues would be required for rapid flowering (Evans and Knox 1969).

Darwin allowed for flexibility in the path to speciation. He accepted the possibility of species retaining similarities as in those with bipolar distribution. In contrast, he also accepted differences associated with geographical isolation and subsequent random population drift. As Darwin observed during his voyage on the Beagle, Australian species provide an excellent illustration of the effects of divergence following isolation. In some regions of Australia, 75% of the plant species are endemic and in their evolution they developed unique shapes



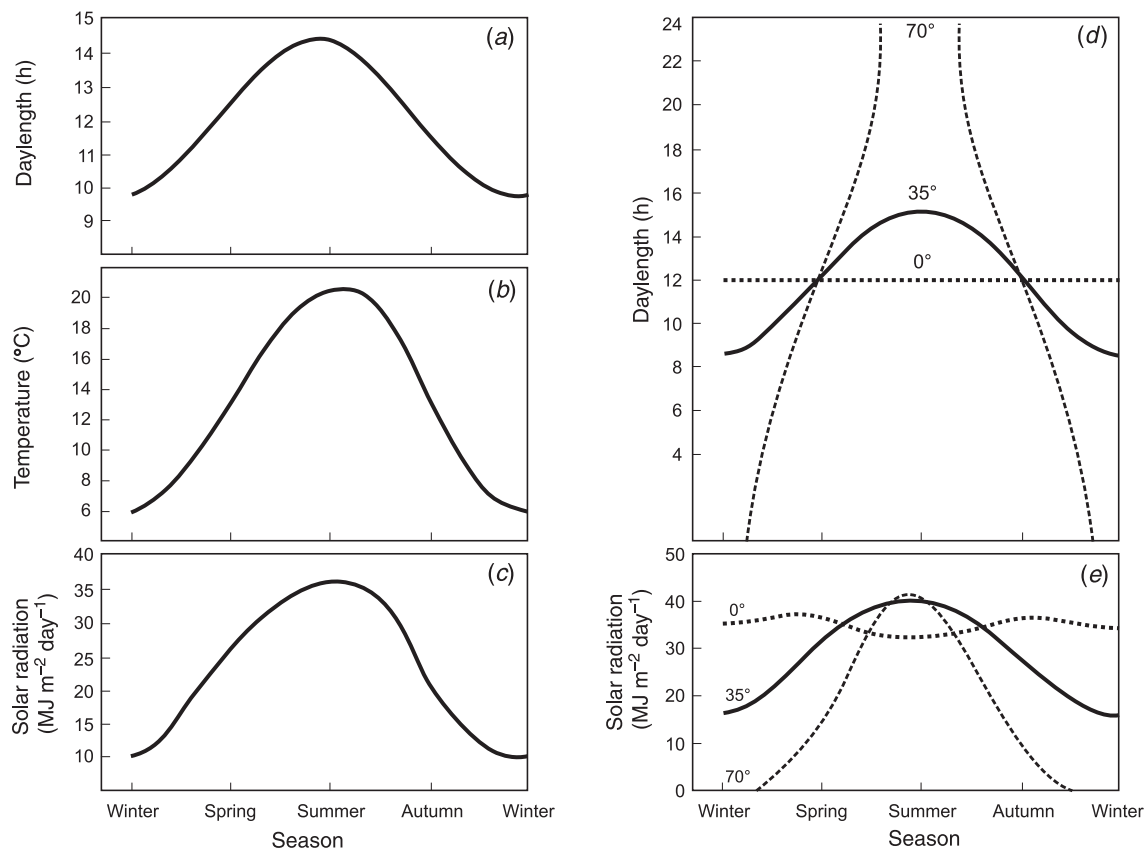
**Fig. 1.** Effect of latitude of origin on flowering of clones of *Themeda australis* exposed in controlled environments to different daylengths and to cold vernalisation. The use of different daylengths allowed the identification of short day (SD) response to decreasing hours of light per day or of a long day (LD) response to increasing hours of light per day. Response types are shown as S, short day; L, long day; N, neutral; I, intermediate; V, vernalisation. (Adapted from Evans and Knox 1969).

and forms especially of their flowers. Interestingly, despite this clear divergence in plant shape and form, Australian species and those across the globe utilise common mechanisms for regulating

seasonal flowering (see above for *Themeda*) although, as discussed below, across species, and even between individual plants within a species, there is great flexibility in the choice of environmental signals used to regulate seasonality of flowering.

### The seasonal environment and flowering

The seasonal pattern of change in daylength (Fig. 2*a, d*) is identical from year to year and many plants regulate their flowering by responding to daylengths shorter than a critical length, (short day responsive types, SDP), or to daylengths exceeding a critical length (long day plants, LDP) (see summary in Thomas and Vince Prue 1997). Summer flowering at high latitudes typically will involve a LD response (cf. Fig. 2*d*) but there are notable exceptions. For example, *Koenigia islandica* L., a tiny arctic-alpine annual plant with geographic distribution extending beyond 80°N in Spitzbergen and Greenland is day neutral across a temperature range of 6–21°C (Heide and Gauslaa 1999). Conversely, insensitivity might be expected in the tropics where there is little or no change in daylength. Nevertheless, some tropical species flower in response to SD and their time measurement is so precise that they can detect seasonal daylength changes of 1–3% (i.e. 7 to 21-min difference in the length of the day). Such sensitivity is seen in rice (*Oryza sativa* L.), a tropical SDP, where some lines respond to a SD of 11 h 50 min, while



**Fig. 2.** Seasonal changes in daylength, temperature and solar radiation. The daylength data (*a, d*) are from standard meteorological records. The data at Latitude 35.3°S for temperature and solar radiation at ground level at Canberra, Australia (*b, c*), was averaged over 30 years (R. King, unpubl. data). The radiation incident on the earth (*e*) is based on Gates (1965).

others respond to 12 h; the 10 min longer daylength delaying flowering by 30 to 50 days (Dore 1959).

For mid latitudes, despite its wide daily fluctuations, temperatures averaged over many years show consistent seasonal patterns (Fig. 2*b* for average monthly temperatures at Canberra, Australia, 35.3°S). At higher latitudes and at altitude, there is comparable seasonality but with a greater range while, over the year, temperature varies little at the equator. For many species, flower number and, hence, seed set declines almost linearly with increase in temperature above 20 to 25°C as in an Australian perennial shrub *Crowea exalata* F. Muell (King *et al.* 2008*a* and references therein for other species). Thus, flowering early in spring is beneficial for avoiding later higher temperatures but not if too early as flowers can be damaged by winter/early spring frosts. To meet these seasonal requirements, some species only flower after up to 2–3 months winter vernalisation involving exposure to cold temperatures below 10 to 12°C (see Lang 1965 and references therein). Other species may only flower when temperatures reach between 15 and 22°C although they may grow at temperatures outside this range (see references in Heide 1994; King 1998). When flowering is induced by these mild temperatures, there may be little or no effect of daylength (see King *et al.* 1996) or both mild temperature and daylength may be important (see later).

Light intensity, although highly variable from day to day, is rather constant near the equator but shows marked seasonality further away (Fig. 2*c*, *e*). As well as responding to daylength, several plant species show dramatic increases in flowering with increasing light intensity. Independence between a true photosynthetic and a true daylength response is clear when the daily light integral is equalised (see King *et al.* 2008*a* and references therein). Further, as discussed later, total radiation in combination with a thermal sum strongly correlates with reproductive output (see Rawson 1988; for wheat (*Triticum aestivum* L.) grown at different latitudes). Clearly, along with daylength, increasing light intensity in spring and summer is likely to impact strongly on flowering.

Rainfall shows seasonal consistency in tropical areas where there is a marked monsoon. However, it may be the drop in temperature associated rainfall that leads to flowering of tropical species such as mango (*Mangifera indica* L.; Wilkie *et al.* 2008). Although its significance is unclear, Stinchcombe *et al.* (2004) reported an interesting finding in a common garden experiment at one site with lines of the annual herb, *Arabidopsis thaliana* (L.), differing in the *FRIGIDA* (*FRI*) gene for vernalisation response. Flowering was associated with January rainfall at their sites of origin. Perhaps there has been selection pressure for other factors including photosynthesis and plant form, which could allow a flowering response to variable inputs including winter rainfall.

## The regulation of flowering involves multiple adaptations to local environments

### Response to daylength and vernalisation

SD and LD photoperiodic responses often predominate in annual plants, which complete their entire life cycle in a single growing season but perennials may also show simple one component responses. For example, as indicated in Fig. 1, different lines of the perennial grass *Themeda* flower in LD, in SD, in all daylengths

(day neutral plants), or only in intermediate daylengths of ~12 h. Two component responses involving exposure first to winter vernalisation and then to LD may also be important in *Themeda* (Fig. 1) as also in some herbaceous winter annuals including *Arabidopsis* and in cereals including wheat (see references in Thomas and Vince Prue 1997). However, at least for *Arabidopsis*, it can also behave as a summer annual, other of its multiple floral pathways allowing it to bypass its vernalisation requirement.

Molecular and genetic studies with *Arabidopsis* have highlighted critical roles in vernalisation for two genes, *FRI* and *FLOWERING LOCUS C* (*FLC*). Cold conditions promote flowering by causing *FLC* degradation while *FRI* delays flowering by promoting *FLC* expression. The most important genes for its LD response are *CONSTANS* (*CO*) and *FLOWERING LOCUS T* (*FT*) (reviewed in Turck *et al.* 2008).

As for some annuals, perennials often flower after exposure to winter chilling (vernalisation) followed by LD in late spring and summer (Fig. 1). Other perennials may show a dual daylength induction response and this is particularly important for perennial plants. A dual daylength requirement for sequential SD and LD, or LD and SD enables a plant to distinguish between spring and autumn when photoperiodic conditions are identical. Because the sequence of inductive conditions is crucial, dual induction photoperiodic plants will not flower in either constant SD or constant LD conditions and, hence, they are distinct from daylength neutral species (Heide 2004). Such a dual daylength response is also not to be confused with intermediate daylength responses where LD and SD inputs may be satisfied simultaneously (Runkle *et al.* 2001).

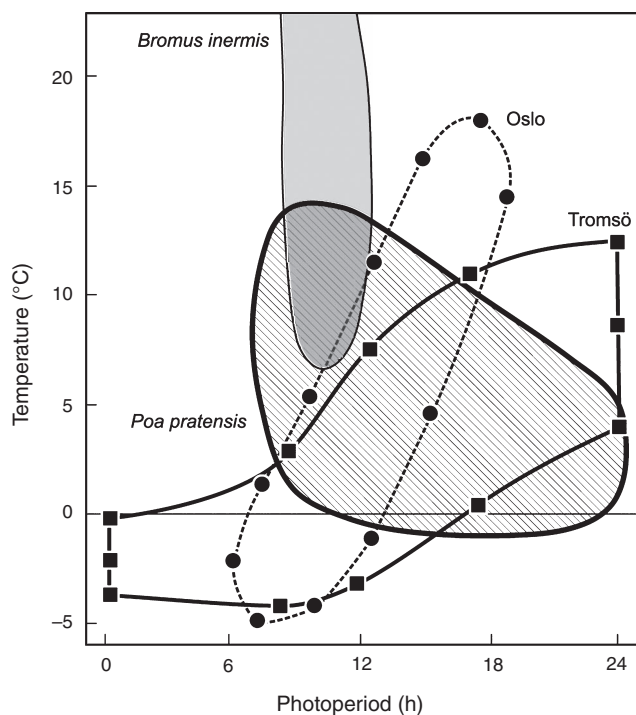
Dual daylength, short-long-day plants are found in cold temperate regions where both temperature and daylength vary greatly between seasons, and where early flowering is important for seed maturation. Some tropical and sub-tropical plants also show a dual daylength adaptation but often require the opposite sequence of daylength (long-short-day plants see references in Lang 1965; Thomas and Vince Prue 1997).

Interestingly, in some species, winter SD can substitute for the winter cold response and for many perennial plants, notably temperate perennial grasses and sedges, the two types of winter responses exist side by side and may act independently and interchangeably (Heide 1994, 1997). The two pathways are distinct, as demonstrated by differences in critical exposure times and other kinetic characteristics but, as an evolutionary mechanism, the versatility of the alternative SD/vernalisation primary induction system offers a beautiful safety mechanism with SD acting as a fall-back alternative in case of inadequate winter chill.

### Response to mild temperatures

Another two-component, dual-induction response is seen in species that flower in response to moderate/mild temperatures (>12°C) and sometimes along with simultaneous exposure to an appropriate daylength. In such species, the physical displacement between average temperature and daylength (e.g. Fig. 2*a*, *c*) can be represented by the physical photothermal envelopes shown for two locations in Norway in Fig. 3 (lines joining the symbols).

The impact of such seasonal phototherms on flowering is illustrated in Fig. 3 for the grass *Bromus inermis* Leyss., an



**Fig. 3.** Seasonal climate phototherms based on yearly site temperature and daylength for Tromsø (69°39'N) and Oslo (59°55'N), Norway. The shaded envelopes show experimentally determined limits for induction of flowering of two grasses: *Bromus inermis* (grey envelope) flowers at Oslo, and *Poa pratensis* (hashed envelope) flowers at both sites (based on Heide 1994).

American species introduced to Scandinavia over 100 years ago. It has an obligatory SD requirement for primary floral induction but the experimentally determined response window for *B. inermis* only overlaps the physical phototherm for the lower latitude (55°55'N) where it is currently found. Thus, precision in the timing of seasonal flowering of *B. inermis* places clear geographical constraints on its survival. In contrast, *Poa pratensis* L., a native arctic alpine grass species, would be successful in both environments (Fig. 3). Precision similar to that in *B. inermis* is evident in several perennial grasses where their seasonal flowering may require a combination of vernalisation and/or SD during autumn and winter, followed by increasing daylength (LD) and warmer temperatures in spring (see Heide 1994).

Recent studies with strawberry (*Fragaria* spp.) further demonstrate how combinations of daylength and temperature enhance seasonal flowering and, conversely, inappropriate experimental or natural conditions cause a reduction in flowering associated with increased runnering and vegetative growth. For example, a range of Norwegian populations of the diploid wood strawberry (*Fragaria vesca* L.) all initiated flowers in both SD and LD at 9°C, they only flowered in SD at 15 and 18°C while they did not flower at all at 21°C (Heide and Sønsteby 2007; and references therein). Conversely, leaf growth and stolon formation (runnering) were stimulated by LD and high temperature. Similar, but less extreme temperature modifications of the daylength responses are found in *F. × ananass* Duch, the common June-bearing (octoploid) cultivated strawberries

(Heide 1977; Sønsteby and Heide 2006), and are inherited from the American octoploid parental species *Fragaria chiloense* L. and *Fragaria virginiana* Duch (Sønsteby and Heide 2008, 2009 and references therein). In contrast, the ever-bearing (recurrent-flowering) strawberry cultivars are LDPs, which flower freely in LD at temperatures as high as 27°C (Sønsteby and Heide 2007). Clearly, within a single genus there is great potential for creating new and environmentally adapted types under both natural and artificial selection pressures.

The negative effects of high temperature on flower number of strawberry (Sønsteby and Heide 2007) highlight a further complexity of environmentally regulated flowering, which is dramatically illustrated by the interplay between temperature and daylength for the leguminous Australian perennial *Hardenbergia violaceae* (Schneevé) Stem. This latter species requires SD of 12 h or less for flowering but it only sets seed at an average daily temperature of 15°C, but not at 12°C or 18°C (King 1998). At 21°C, flowers form but quickly abort and, presumably, there was also abortion at 18°C. Thus, in nature, the very narrow photothermal window of *Hardenbergia* allows flowering in late autumn and early spring so that seeds develop in spring. Then with rising summer temperatures, its seasonal window for reproduction closes and the plant returns to vigorous vegetative growth and any immature seed rapidly abort (King 1998).

#### Response to light intensity

Although photoperiod may be a dominant factor of the light environment, radiation can also have large effects and especially in LD species. In the annual grass *Lolium temulentum* L., irradiance in SD for the 2 weeks before exposure to a single florally-inductive LD was highly correlated with the rate of flower development. Without the LD exposure, the same full sunlight exposure did not induce flowering (King and Evans 1991). Furthermore, when light intensity was varied during 16 h of the single LD, flowering was strongly enhanced in association with a photosynthetically-driven increase in shoot apex sugar content (King and Evans 1991).

In *Arabidopsis*, the link between photosynthesis and flowering is both compelling and even more immediate. A single photosynthetic LD upregulates expression of the gene *FT* whose protein is a transmitted florigen (reviewed in Turck *et al.* 2008; for *Arabidopsis* and other LD and SD plants). For *Arabidopsis*, within 8 h of starting a single high irradiance LD, there is a photosynthetically-driven increase in leaf and shoot apex sucrose content. At the same time, *FT* expression in the leaf blade increases and by 10 days, flower buds are evident (King *et al.* 2008b). Independently, a separate photoresponse via phytochrome causes *FT* upregulation, but as part of a low light photoperiodic LD response involving no photosynthetic input (King *et al.* 2008b). Consequently, in nature, the LD regulation of flowering via *FT* will involve both a response to the photosynthetic light integral and to a light durational component.

As an aside, it is unclear why high light intensity upregulates *FT* expression in LD but not in SD. In SD, increasing light intensity to give matched daily light integrals only slightly increased *FT* expression (King *et al.* 2008b). Perhaps, in addition to phytochrome, there is a second photoresponse in

the LD photoperiod via the CRYPTOCHROME (CRY) photoreceptor proteins, which respond to blue-rich light.

Like *L. temulentum* and *Arabidopsis*, several other LD plants show independent and additive flowering responses to light intensity (daily light integral) and daylength (King and Ben-Tal 2001 and references therein). In SD species, the daily light integral is unlikely to be as important while some species, such as the herbaceous perennial *C. exalata*, may be unresponsive to daylength but flower when their daily light receipt exceeds a third or more of full sunlight levels (King *et al.* 2008a and see references therein). Seasonally, such a light intensity regulation of flowering of *Crowea* is 'opportunistic' and somewhat atypical, but clearly, some qualification is required of Darwin's claim that there is 'little signification of organs of vegetation' (Darwin 1859, p. 414).

#### Response to rainfall and drought

Opportunistic, drought-avoidance flowering of the ephemeral desert annual, *Pectis papposa* Harvey and A. Gray, illustrates an extreme in adaptation involving insensitivity to both daylength and temperature. After rainfall, it germinates and forms as few as two or three leaves before flowering with either the terminal shoot apex or the axillary buds becoming floral. In this way, should rainfall persist, some vegetative meristems are available for further growth and reproduction (Hayashi *et al.* 1994). Adaptively, there is some similarity between the rapid flowering of *P. papposa* and that of the arctic annual *K. islandica*, which is also constrained by a brief growing season (Heide and Gauslaa 1999). Such adaptation for rapid flowering could be characterised by little or no dependence on flowering time genes.

The persistence of vegetative meristems when *P. papposa* flowers is consistent with the way perennation is achieved in *Arabis alpina* L. where the *PERPETUAL FLOWERING 1* (*PEP1*) gene blocks flowering of some branch meristems. Interestingly, like its *Arabidopsis* homologue *FLC*, expression of *PEP1* is under epigenetic control in response to temperature and seasonal perennation is reversible through loss and gain of chromatin modifications (Wang *et al.* 2009). Additional meristem modifications in other species are likely to relate to genes such as *TERMINAL FLOWER* (*TFL*) and the *MORE AXILLARY GROWTH/RAMOSUS* (*MAX/RMS*) branching genes (Beveridge 2006). For example, in citrus before flowering and fruiting, the expression of a homologue of *TFL* is elevated in juvenile trees and then declines in association with the development of a sufficient vegetative structure (Pillitteri *et al.* 2004). Similarly, for the perennial grass, *Lolium perenne* (L.), seasonally reduced expression of a *TFL* homologue may also be essential for its flowering as suggested by Jensen *et al.* (2001).

#### Species in their natural environment: processes regulating flowering show geographical adaptation

Several studies have provided broad support for adaptation of a species to its local environment. For example, in the study cited above with *Themeda*, when tested in controlled conditions, it showed latitudinal differences in daylength and vernalisation response that matched the conditions at their sites of origin (Fig. 1). Other species including both dicots and grasses with

wide latitudinal distribution also show clinal variation in their critical daylength for flowering (Cumming 1969; Heide 1994; Aamlid *et al.* 2000).

Similar geographical differences in the vernalisation requirement for flowering are illustrated from studies with ecotypes of European thistle (*Cirsium vulgare* Suvi) grown in a common nursery garden. Compared with lines originating from the Mediterranean, there was a greater requirement for vernalisation in lines from colder more northerly sites as in Scandinavia or from high altitudes (Wesselingh *et al.* 1994).

Latitude also affects the balance between perennial and annual forms. At high latitudes perennial plants predominate (Körner 1995; and references in Heide and Gauslaa 1999) and they have a clear advantage because their overwintering photosynthetic tissues can contribute to early, rapid new spring growth and, so, to early flowering (Miller and Tieszen 1972). In such cases, as discussed above, perenniality of flowering is often achieved, by the sequential action over two seasons of two components of the environment.

A further adaptation for obtaining 'pseudo' perenniality involves change to flower development. Some high arctic species form floral primordia in one year, they overwinter and then, in the following spring, they rapidly flower (Heide *et al.* 1990; Heide 1992). A similar perenniality with its requirement for renewed floral induction occurs in some species from mid latitudes when they pass through a seasonal phase of excessive summer vegetative growth as in *Fragaria* (see Heide and Sønsteby 2007) or of winter bud dormancy as in chrysanthemum (T. Hisamatsu, pers. comm.).

Adaptation does not require identical mechanisms for regulation of flowering time at the same location across species or even for local variants within a species. For example, for two herbaceous perennial species of *Lechenaultia* originating from similar latitudes in Western Australia, one flowers in response to LD, the other to SD (King *et al.* 2008a). Similarly, within accessions of *T. australis* from northerly latitudes, one flowered in LD but other lines responded to SD (Fig. 1).

A further complexity is the presence of mechanisms that control flowering but lack adaptive value. As an example, *Cerastium regelii* Ostenf., an arctic-alpine species has a strong SD primary induction requirement for flowering in subsequent LD even though it will never experience SD conditions in a non-frozen condition in its natural environment (Heide *et al.* 1990). Like many other high-latitude dual induction species, it has an alternative, low temperature (vernalisation) pathway of primary induction, which is operative also under LD conditions (see above).

#### Species in their natural environment; some lessons from domesticated plants

'Why if man can by patience select variations most useful to himself, should not nature . . . ?'  
(Darwin 1859, p. 469).

Changes in the flowering responses of crop species associated with their domestication raise interesting parallels with adaptation of flowering response to the natural environment. Consistently,

introductions of foreign species into a new climate have been associated with selection for earliness of flowering and links have been suggested with photoperiod and vernalisation responses with all major crops including wheat, maize (*Zea mays* L.), rice and soybean (*Glycine max* L.) (see review in Evans 1993). Particularly for temperate cereals, there is extensive information on the genetic changes associated with differences in vernalisation response to winter cold (summarised in Trevaskis *et al.* 2007). However, caution is required in ascribing all changes in earliness to photoperiod and vernalisation responses. For rice and soybean, juvenility rather than photoperiodic response may have been the focus of this selection by humans (Evans 1993).

Early flowering with domestication of crop species is also evident as change in the effects of field temperatures on their development rate (phenology). The importance of temperature is clear from computations involving a heat sum approach in combination with daylength information. Such computations provide reasonable assessments of flowering times of annual crops (see references in Craufurd and Wheeler 2009) but little is known of associated genetic mechanisms. Studies of flowering in natural populations could benefit from this computational approach especially for the growing literature on effects of global warming on phenology.

Another predictor of the physical environment involves the photothermal quotient, which assesses the combined response to light intensity and temperature (i.e. radiation per unit area per degree day). For wheat, this quotient approximates well to the effect of latitude on wheat yield. For example, Rawson (1988) reported a very strong positive linear relationship between wheat yield at different maritime latitudes and the photothermal quotient for the period of 4 weeks pre-anthesis ( $r^2=0.92$ ). Obviously, greater seed production in nature should confer a selective advantage so response to the photothermal quotient could be usefully incorporated into studies of adaptation, at least in annual plants.

In other cases, domestication appears to have focussed not just on the initiation of flowering but, as well, on subsequent stem elongation. For example, temperate winter cereals avoid winter cold damage by delaying flowering and stem elongation until vernalised over winter. However in assessing the response of winter wheat lines developed in the 1980s in the UK, Evans (1987) found one line, Maris Templar, which was insensitive to vernalisation. It initiated flowers in winter but was able to withstand cold because its stem did not elongate until exposed to LD of spring and early summer.

Overall, change in flowering time has been critical in domestication of crop species and may involve several environmental adaptations in addition to those for daylength and vernalisation response. However, it is uncertain whether this evidence from studies of domestication provides a template for understanding evolution of wild species.

### Has adaptation of flowering time to the environment been significant for evolution of a species?

Evidence for the adaptive nature of seasonal flowering does not address the question of evolutionary advantage and species fitness. More informative are observations of the adaptation of introduced species and of the environmental tolerance of a

species when its historical range of adaptation is examined in reciprocal transplant studies.

Adaptation of an introduced species by the establishment of latitudinal flowering ecotypes can take place in surprisingly short periods of time. Thus, for the short day annual cocklebur (*Xanthium strumarium* L.), McMillan (1973) reported the establishment of latitudinally determined ecotypes in Australia in less than 150 years after the original introduction. In the short-long-day grass *B. inermis* 100 years after its introduction to Norway, Heide (1984) found changes in the critical photoperiods for both primary and secondary induction of flowering.

The value of two way transfers to uncover historic adaptation is illustrated in studies with a native Australian perennial shrub, *Pimelea ferruginea* Labill, a maritime species that grows over a 600 km latitudinal range in Western Australia on sand dunes within 100 m of the Indian Ocean. Large batches of vegetatively propagated lines of *P. ferruginea* were generated from cuttings from single plants collected over the full range of the natural distribution of the species.

In controlled environment studies, all plants flowered 4–6 weeks after transfer from a warm growth temperature to mild temperatures matching those of early winter (i.e. 15 to 18°C). Over many years of records, temperatures at the most northerly site (28°S) consistently track 4°C higher than those at the cooler most southerly site (31°S). Most interestingly, the ecotypes collected from the latitudinal extremes (28 v. 31°S) showed a matching 3–4°C difference in their temperature optimum for flowering (King *et al.* 1996).

Simultaneously, batches of the same plants were transferred back to their natural sites of origin. They flowered normally in response to cool winter conditions at their sites of origin. However, when reciprocally transplanted, a more southerly line with an 18°C optimum for flowering in the controlled environment studies, failed to flower in a nursery site at the 4°C warmer extreme of distribution of this species. Only the line with a 21–22°C optimum, which originated from this warmer site, flowered at this site. All lines flowered in a nursery at the cooler, more southerly site of origin. Thus, not only has this species of *Pimelea* developed a very sensitive thermoregulation of flowering, but, to survive at the warmer extreme of its range of natural distribution, its ‘thermoregulator’ tolerates higher temperatures characteristic of that site.

A further and reasonably demanding test of the adaptive advantage of mechanisms regulating seasonal flowering comes from studies with trans-global species. Darwin raised this question in relation to migration and isolation, which he suggested occurred following a period of glaciation. One such example involves bipolar species of the genus *Carex*. Seasonal regulation of flowering in such species is the same in the matching alpine environments of northerly and southerly alpine sites (Heide 2002). Furthermore, based on DNA fingerprinting studies (AFLP) there is no apparent genetic difference between northern and southern hemisphere representatives (Vollan *et al.* 2006). Thus, although geographically isolated, these *Carex* species have retained common mechanism(s) of flowering under matching environmental conditions in the two hemispheres.

In contrast, the cosmopolitan grass *Poa annua* L. illustrates the opposite situation, a micro-local distinction in the choice of

mechanisms for controlling flower initiation, Populations from two suburban parks in Canberra (35.3°S), separated by a distance of 7 km, exhibited very different flowering responses; one being a quantitative SD plant with no vernalisation response, the other flowering as a quantitative LD plant promoted by vernalisation (Heide 2001). Furthermore, while flowering responses were uniform within the latter population, the former segregated into early- and late-flowering types. This variation in earliness of flowering was shown to be genetically determined, and tests of selfed progenies indicated that individual plants collected from an area of 1 m<sup>2</sup> were an aggregate of several largely homozygous lines with divergent flowering responses. Likewise, two lowland *P. annua* populations from southern Norway were both shown to be quantitative LD plants with no response to vernalisation, while two alpine snow bed populations from southern Norway and two high-latitude, subarctic populations were perennial, quantitative SD plants with an obligatory SD or vernalisation requirement for flowering. Thus, virtually any kind of photoperiodic and vernalisation response could be found among various populations of *P. annua*.

The versatility in choice of mechanisms regulating flowering of *P. annua* reflects the contrasting flowering responses of its putative ancestors, *Poa infirma* Kunth and *Poa supina* Schrader, the former being a summer annual, quantitative SD plant with no response to vernalisation, whereas the latter is a perennial quantitative LD plant with an obligatory SD or low temperature vernalisation requirement (Heide 2001). Such findings demonstrate the large environmental adaptation potential of allotetraploid species such as *P. annua* in which two different genomes have been brought together (Thompson and Lumarat 1992). Clearly, such a system is of great evolutionary significance and may explain why *P. annua* has spread around the world as a successful cosmopolitan weed with a variety of new ecotypes, each apparently well adapted to its new environment. On the other hand, the putative ancestors have remained restricted to limited and well separated areas of Europe.

Overall, at any one site, plants may flower at the same time but may achieve this by quite different responses to the environment. Furthermore, as discussed above, for survival the critical 'floral' regulatory processes may include juvenility, perenniality and seasonal dormancy in addition to processes directly controlling flower initiation. Thus, it is unwise to focus on a limited number of processes and often on single genes as the basis of selection; what is essentially a selfish gene concept is unlikely to capture the variety of responses and gene complexes involved in adapting flowering to season and site. We return to this issue below as part of an examination of genetic aspects of evolutionary fitness.

### Genetic plasticity, selective advantage and strong heritability are central to environmental adaptation in the control of flowering

To argue evolutionary importance of a character requires evidence not only of broad latitudinal/geographical variation in flowering time but also of heritable responses. The foundation for much of our current knowledge of heritability of flowering time was provided by Laibach (1951) in his studies of flowering of the annual species *A. thaliana*. He established heritability of its flowering response to the seasonal environment. Subsequently,

the extensive natural variation in its flowering response to daylength and vernalisation has been documented by Karlsson *et al.* (1993) and screening of induced mutations and natural populations of *Arabidopsis* for vernalisation- and/or daylength-induced flowering has indicated roles for several candidate genes (see review in Koornneef *et al.* 2003).

Which genes are important is now becoming clear from molecular/genetic studies. For example, the CO and FT proteins play a central role in LD flowering of *Arabidopsis* (reviewed in Turck *et al.* 2008) and whether in SD or LD response types, proteins similar to CO are found in a wide range of higher plants (Griffiths *et al.* 2003) and in a moss (Shimizu *et al.* 2004). Furthermore, a CO homologue in the unicellular green alga *Chlamydomonas reinhardtii*, could be reproductively significant as, when expressed in *Arabidopsis*, it complements a *co* mutant (Serrano *et al.* 2009). In parallel, response to seasonal environment is seen not only across higher plants but also in some mosses and liverworts (Lang 1965; Thomas and Vince Prue 1997).

Quantitative trait loci (QTL) screens with hybrid populations have confirmed the importance for flowering of vernalisation response of the two genes *FLC* and *FRI* (Johanson *et al.* 2000) and of other genes including the photoreceptor *CRYPTOCHROME 2* (*CRY2*) (El-Assal *et al.* 2001). Subsequent analysis of allelic variants in 45 arbitrarily selected lines from natural populations of *Arabidopsis* showed that *FRI* accounted for up to a third of the heritable variation in vernalisation-induced flowering (Stinchcombe *et al.* 2004). In a larger grouping of 192 natural accessions where there was a wide range of expression of both *FRI* and *FLC*, Shindo *et al.* (2007) suggested a heritability of up to 70%. However, this may be a high estimate. While *FRI* could be identified as a gene of some importance in the variation of flowering time in European populations of *Arabidopsis*, there was no relationship between *FRI* and vernalisation in Asian lines (Shindo *et al.* 2007). Not only must other genes be important, but when Scarcelli *et al.* (2007) and Scarcelli and Kover (2009) used outbred populations developed by intermating 19 *Arabidopsis* accessions, they found that only 12% of the variation in flowering time was explained by *FRI*.

Interestingly, in the study of Scarcelli and Kover (2009), three generations of selection for early flowering in simulated spring conditions led to a reduced frequency of functional *FRI* alleles in these populations. Conversely, when vernalised in winter conditions the *FRI* limitation was removed and there was no large shift in the frequency of *FRI* alleles in the populations. These studies provide clear 'proof of concept', that *FRI* will contribute to fitness and, therefore, in natural populations it could have evolutionary importance. However, because *FRI* has pleiotropic effects, including changes in branching, the authors cautioned against concluding that selection optimises flowering time *per se*.

In wild populations, studies with the flowering time gene *FT* suggest its evolutionary importance for daylength responses of rice, an SD plant (Hagiwara *et al.* 2009). The genetic evidence for rice is compelling but little is known for other species although in wild poplar (*Populus* spp.), latitudinal variation in daylength-regulated autumn bud dormancy is matched by daylength regulation of *FT* expression (Bohlenius *et al.* 2006). Perhaps for poplar, *FT* expression is simply a good marker of dormancy. Alternatively, *FT* acts in a pleiotropic way on a range of



developmental process. This latter possibility appears more than likely as recent findings point to a pleiotropic effect of *FLC* acting on *FT*. Vernalising temperatures regulate flowering by suppressing *FLC* expression but also act in the same way during germination (Chiang *et al.* 2009). Thus, because *FLC* regulates *FT*, there is a broader role for *FT* in the regulation of plant development. The parallel between *FLC* regulation of *FT* (and other genes) both for flowering and germination (Chiang *et al.* 2009) not only highlights the importance of temperature in regulating *FLC* but also that the florigenic role for *FT* in flowering may involve chromatin remodelling as also for *FLC*.

Of further interest for field studies with *Arabidopsis*, temperature at germination may be an important determinant of flowering time (Wilczek *et al.* 2009). Thus, although *FLC* and the linked gene *FRI* may be important for flowering responses involving vernalisation, temperature sensing via *FLC* during germination may have been under most selective pressure in this annual species.

A second and very important issue for evolutionary studies with *Arabidopsis* is its ability to utilise alternative flowering pathways. Current genetic models list four pathways controlling its flowering; vernalisation, photoperiod, the gibberellin class of plant hormones and an autonomous path. Now, as discussed earlier, there is also evidence for a fifth path involving photosynthetic regulation of *FT* expression (King *et al.* 2008b).

Plasticity in the choice of floral pathways in *Arabidopsis* confers obvious evolutionary advantage but could also reduce selective pressure on any one pathway. Local genetic diversity within a species may in fact be quite extreme as in *P. annua* where different mechanisms for controlling flower initiation were evident in adjacent plants in a natural population (Heide 2001; see above). Conversely, selection pressure could be stronger where there is less plasticity in a species that flowers in response to one environmental input and by one genetic pathway.

Not only might gene/pathway flexibility complicate estimates of heritability, but it can mean that effective analysis of natural populations will require sampling of many plants in a population. A further qualification is that random genetic drift and not selection pressure may explain differences in the mechanism controlling flowering between adjacent plants or between geographical locations.

Random genetic drift may account for the identification of the *CRY2* gene as a QTL for flowering time in an *Arabidopsis* line from the Cape Verde Islands but not from other lines (El-Assal *et al.* 2001). To approach this issue, linkage disequilibrium tests for non-random association of polymorphisms in a population allow a formal assessment of this problem of random genetic drift; any divergence, if strong, may indicate selection pressure (Ehrenreich and Purugganan 2006). In the future, in association with intense micro-locational sampling, genome wide mapping (GWA) could provide an even more powerful way to look at the association between genome and phenotype (Norberg and Weigel 2008).

To avoid the challenge of population demography and non-homogeneity, in testing for selective pressure on the *FRI* gene, Scarcelli *et al.* (2007) and Scarcelli and Kover (2009) used an intermated, homogeneous *Arabidopsis* population containing various *FRI* and *FLC* alleles (see above). Similarly, genetic

homogeneity can be achieved by studying 'directed gene' responses in mutants and/or transgenic lines in a common background. For flowering, the first 'directed gene' effects have now been reported by Wilczek *et al.* (2009). Their field sowings of *Arabidopsis* lines across four sites in Europe over the year confirmed the natural control of flowering by genes considered as regulators of summer LD input (*CO*) and by winter vernalisation responses (*FRI* and *FLC*). However, unexpectedly, *FLC* repression of summer flowering could be overridden so substitution is possible by other unknown environmental inputs. Competitive advantage was not examined by Wilczek *et al.* (2009), so it is not possible to draw major conclusions about the evolutionary role of the two genes they used. The 'directed gene' approach also introduces *a priori* assumptions about which genes determine evolutionary significance but further studies are warranted.

## Overview

Darwin (1859) documented numerous characteristics of plants and animals that are beneficial for their survival. Here, we presented, in an evolutionary context, evidence supporting the use by plants of seasonal daylength, temperature and light intensity to time their flowering and seed set to avoid frost, heat or drought. Geographically, their choice of regulatory mechanisms has been selected to match their local environment.

A somewhat overlooked aspect of environmental regulation of flowering involves the dual requirements either for sequential exposure to different daylengths or to a combination of temperature and daylength and/or light intensity. Such dual responses greatly enhance the precision of seasonal timing of flowering and are evident in several species. While more difficult, simultaneous inheritance of two responses is not impossible especially if flowering is crucial for survival.

For the future, tests for selective advantage of any particular environmental input to flowering could involve reciprocal transplant experiments over a natural latitudinal cline (Mitchell-Olds *et al.* 2007). As an example, although the intensity of population sampling was limited, reciprocal transfers of *Pimelea* did establish differences in fitness that were related to their temperature responses for flowering and the temperature at their sites of origin (see discussion above and King *et al.* 1996). However, for other species there may be greater complexity both in environmental control of their flowering and in their natural environment. Thus, it may be difficult if not impossible to gain useful information from reciprocal transplantation studies.

Lacking our current genetic understanding, Darwin could not link inheritance and survival. Now modern molecular and genetic approaches allow tests of selective advantage of genes. One pair of floral regulatory genes, *FRI/FLC*, show a selective advantage for flowering in vernalising conditions but their low overall heritability (~12%) makes it likely that other flowering genes make substantial contributions to survival. A further issue is that studies of 'floral' genes have focussed on their effects on flowering time but various authors are beginning to consider their pleiotropic roles. Potentially how plants synchronise flowering to seasonal change could also involve regulation of germination, juvenility, dormancy or branching patterns.



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