Journal of Experimental Botany, Vol. 60, No. 11, pp. 3143–3155, 2009
doi:10.1093/jxb/erp142 Advance Access publication 12 May, 2009

Journal of Experimental Botany
www.jxb.oxfordjournals.org

RESEARCH PAPER

Evolutionary change in flowering phenology in the iteroparous herb *Beta vulgaris* ssp. *maritima*: a search for the underlying mechanisms

Henk Van Dijk*

Laboratoire Génétique et Evolution des Populations Végétales, UMR 8016, CNRS, Université Lille 1, F-59655 Villeneuve d'Ascq, France

Received 17 February 2009; Revised 7 April 2009; Accepted 9 April 2009

Abstract

The potential for evolutionary change in flowering time has gained considerable attention in view of the current global climate change. To explore this potential and its underlying mechanisms in the iteroparous perennial *Beta vulgaris* ssp. *maritima* (sea beet), artificial selection for earlier and later flowering date was applied under semi-natural greenhouse conditions. Mean flowering date occurred more than 30 d earlier in 13 generations in the early selection line, but response was weaker in the late selection line. Taking advantage of the growing knowledge on the genetics and the physiology of flowering induction, particularly in *Arabidopsis thaliana*, the results obtained here were analysed in terms of the four different pathways of flowering induction known in this species. A first significant correlated response was stem elongation (bolting) in the vegetative stage, suggesting that plants were thus able to flower earlier as long as other requirements were satisfied. Vernalization had a clear influence on flowering date and its influence increased during the selection process, together with sensitivity to photoperiod. Vernalization and photoperiod could compensate for each other: each additional week of vernalization at 5 °C decreased the necessary daylength for flowering by about 15 min during the later selection stages, while in unselected plants, it was about 7 min. Devernalizing effects were observed at short days combined with higher temperatures. Special attention was given to the role of the *B* (bolting) gene that cancels the vernalization requirement. The results here obtained suggest that all four known pathways may simultaneously participate in evolutionary change.

Key words: Artificial selection, *Beta vulgaris* ssp. *maritima* (sea beet), correlated response, flowering time, heritability, perennial, photoperiod, stem elongation, vernalization.

Introduction

Growing knowledge on the genetics and the physiology of the onset of flowering (Bernier and Périlleux, 2005; Anthony, 2006; Bäurle and Dean, 2006; Roux *et al.*, 2006; Glover, 2007) opens up new possibilities in the field of evolutionary ecology, or in understanding how genes vary in heterogeneous and/or changing environments and how natural selection acts on this variation. The potential for evolutionary change in the timing of flowering has become more important in the context of current climate change (Franks *et al.*, 2007; Reusch and Wood, 2007). In this respect, flowering time variation over latitudinal gradients is particularly informative as it can help predict what could happen in a changing climate: the optimal future locally adapted phenotype at a given location may well be the present locally adapted phenotype at a distant latitude. In addition to migration, which does not always occur at a sufficient rate, evolutionary change is a solution for maintaining local adaptation. Genetic variation in flowering time at the within-population or the metapopulation level is a necessary condition for evolutionary change.

Latitudinal gradients in flowering phenology are well known (Cooper, 1963; Lacey, 1988; Van Dijk *et al.*, 1997; Stinchcombe *et al.*, 2004) and can have both environmental and genetic components (Riihimäki and Savolainen, 2004).

* E-mail: henk.van-dijk@univ-lille1.fr

Knowledge on the underlying mechanisms involved in flowering induction comes primarily from *Arabidopsis thaliana*. These mechanisms are related to daylength (Thomas *et al.*, 2006), vernalization (Gendall and Simpson, 2006), and internal physiological factors such as size or growth rate, which may, in turn, depend on environmental conditions such as ambient temperature (Blazquez *et al.*, 2003) and resource availability (Smit, 1983). *A. thaliana* accessions from different latitudes or climate zones vary in their sensitivity to environmental cues. This can, in principle, be interpreted as adaptations to different local situations resulting in different ecotypes (Shindo *et al.*, 2007). However, the term 'ecotype' is sometimes misused in the *Arabidopsis* literature where 'accessions' may be a more appropriate description of the material under study.

Some of the mechanisms underlying flowering have gradients that are related to latitude. The gradient in vernalization requirement is well known (Wesselingh *et al.*, 1994; Boudry *et al.*, 2002; Lempe *et al.*, 2005; Reeves *et al.*, 2007; Rhoné *et al.*, 2008). However, less is known about gradients in daylength requirement (Howe *et al.*, 1995; Van Dijk and Hautekèete, 2007) or both daylight and vernalization requirements in conjunction (Riihimäki and Savolainen, 2004; Heide and Sønsteby, 2007). At the gene level, gradients in allele frequencies have been described by Caicedo *et al.* (2004), Stinchcombe *et al.* (2004), and Balasubramanian *et al.* (2006) for *A. thaliana*. Evidence for fitness consequences of variation in flowering phenology in relation to latitude is provided by Montague *et al.* (2008) and Rhoné *et al.* (2008).

To verify that latitudinal variation is a plausible result of—and can be altered by—natural selection, the potential for evolutionary change has to be explicitly established. This can be done at the population, metapopulation or regional level. In the latter two cases, the relevance of evolutionary change also depends on the intensity of gene flow. A straightforward approach to assess the potential for evolutionary change is to apply artificial selection for flowering date and to see how genetic composition changes over generations (Sandring *et al.*, 2006; Burgess *et al.*, 2007). If there is sufficient genetic variation, i.e. a sufficient response to selection, the next step is to evaluate how the underlying mechanisms change genetically and, finally, which alleles increase in frequency. This method may be particularly successful in outcrossing species, where the relationship between genotype and environment is usually not as tight as in selfing species. Here, the first steps of this type of approach were applied to *Beta vulgaris* ssp. *maritima* (sea beet), an obligatory outcrossing species sampled along a large range of latitudes in view of its interest as the wild ancestor of cultivated beet. This species is an ideal study species because its populations range from virtual annuality (semelparity) to long-lived iteroparity (Hautekèete *et al.*, 2002) and because many experiments on flowering induction have been carried out on sugar beet, the economically most important cultivated form of *B. vulgaris*, in particular by Margara (1960) and Smit (1983). As the knowledge on flowering induction in iteroparous perennials primarily comes from woody species (Brunner and Nilsson, 2004; Wilkie *et al.*, 2008), sea beet bridges the gap between those species and intensively studied annuals such as *A. thaliana*, *Pisum sativum* (Hecht *et al.*, 2005), and cereals (Cockram *et al.*, 2007).

Sea beet shows important polymorphism in flowering induction coded by the *B*/*b* bolting gene that has, for the moment, no known orthologue with a similar function in *A. thaliana*. Homozygotes *bb* have a vernalization requirement, which is strict under natural conditions, and shows a latitudinal gradient with greater requirements in northern latitudes (Boudry *et al.*, 2002). On the contrary, genotypes *BB* and, under favourable conditions, also *Bb* are able to flower at constant, non-vernalizing temperatures (Boudry *et al.*, 1994). Populations almost fixed for the *B* allele are found particularly in ruderal inland areas in south-west France and have been intensively studied by Desplanque *et al.* (2002). These plants also have a short life span of about 1–2 years (Hautekèete *et al.*, 2002) and are thus ecologically comparable to *A. thaliana*. Both the absence of vernalization requirement and the short life span are most likely independent responses to the same selection pressure for rapid and abundant reproduction in disturbed environments. Indeed, Hautekèete *et al.* (2002) found that the rare plants without a vernalization requirement in less-disturbed populations do not live for significantly shorter periods of time than other members of those populations, suggesting that the there is no strict relationship between the two traits.

B. vulgaris is a long day plant. The critical photoperiod depends on the degree of vernalization and is therefore difficult to quantify in *bb* plants. Since Owen *et al.* (1940), authors speak of the 'photothermal' induction of flowering. On the other hand, in *BB* plants, daylength requirement can be analysed without this complication. Van Dijk and Hautekèete (2007) thus measured the critical photoperiod in several populations of species in the *Beta* section *Beta* species complex. Artificial selection showed that, starting with sea beet populations from south-west France, the critical photoperiod could be changed from more than13 h to less than 11 h in only seven generations. The shorter photoperiods are similar to those for natural populations in much lower latitudes. They failed to find evidence for segregating major genes.

B. vulgaris forms a rosette that bolts (i.e. produces one or more inflorescence stems with leaves) prior to flowering. Bolting is a necessary step in the realization of flowering, but may also happen in the vegetative state. Stem elongation then stops at a certain moment and is resumed after flowering induction. The role of gibberellins (GA) in bolting has been studied in detail by Margara (1967). In *B. vulgaris*, GA promotes stem elongation but cannot induce flowering by completely compensating for vernalization or long days, as it is possible in several other species including *A. thaliana* (see Mutasa-Göttgens and Hedden, 2009, for a review). According to Margara (1967) GA only promotes flowering in sugar beet when the photothermal induction is almost complete.

At a given stimulation due to environmental cues, for example, at saturated vernalization and standardized long

days, there may still be variation in flowering time. Such variation is usually ascribed to the 'autonomous pathway' (Marquardt *et al.*, 2006). This will be evaluated here in a short selection experiment with *BB* plants. In recent literature, the four above-mentioned factors, namely vernalization, photoperiod, gibberellins, and autonomous aspects, correspond to the four pathways that have been reported to contribute to flowering induction in *A. thaliana*. In the present artificial selection experiments, the aim was not only to measure the potential for evolutionary change in flowering time, but also to analyse such changes in terms of these four pathways and their interactions.

The joint evolution of vernalization and photoperiod requirement, together with the effects, if any, of the other pathways, was evaluated in *bb* plants using the successive generations obtained from a long-term selection experiment. This was done by variable vernalization durations followed by increasing photoperiods.

Materials and methods

Plant material

Beta vulgaris ssp. *maritima* is a perennial, self-incompatible, wind-pollinated species that grows along Mediterranean and NE Atlantic coasts and in some inland locations (Letschert, 1993). In 1989, seeds were sampled in 93 populations, mostly in France and some in neighbouring regions in other countries as described in Van Dijk *et al.* (1997). These seeds are long-lived and were still viable in 2004. Plants used for making the *BB* selection line came from four populations in south-west France. All other populations were used for constructing the *bb* selection line, with selection against plants that bolted without vernalization. A survey of the use of the plant material throughout this study is presented in Fig. 1.

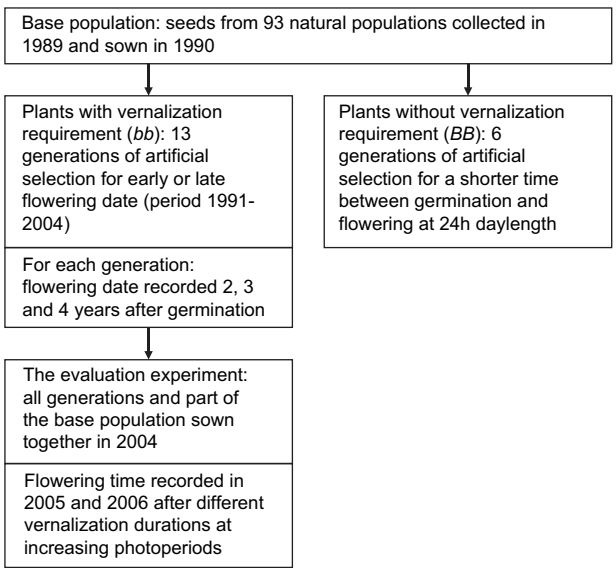

Fig. 1. A survey of the experiments and the plant material used.

Culture conditions

For the construction of the *BB* selection line, plants were grown in commercial soil (Neuhaus Huminsubstrat N3: 90% peat, 10% clay; pH 6; NPK 14:16:18, 1.3 kg m^{-3}; conductivity 35 mS m^{-1}) at 24 h daylight/artificial light (see Van Dijk and Hautekèete, 2007) and 20 °C using 0.7 l pots and adding 1 g of fertilizer (NPK: 12:12:17) every 2 weeks from day 40 onwards.

During the selection period of the *bb* line (1990–2004), plants were sown each year in the greenhouse under controlled conditions: 20 °C and 16/8 h light/dark. After 3 months, they were transferred to a compartment with natural daylength, no additional light, and temperatures a few °C above the outside temperatures, and only heated to prevent temperatures below 5 °C, hereafter called the 'semi-natural greenhouse', where they stayed for the rest of their lives. Each January, plants were groomed and repotted with new commercial soil in 2.4 l pots. They were supplied with sufficient water, but received no additional fertilizer.

During the two-year evaluation experiment to test the response to selection under various controlled conditions, plants were grown in 1.7 l pots and received 1 g of fertilizer every 2 weeks from day 60 onwards. Vernalization was effectuated in a cold cave (the 'vernalization cave') with a constant temperature of 5 °C and natural daylength. Photoperiod in the greenhouse was adjusted by 400 W high pressure sodium lamps, *PAR*: 200 µmol m^{-2} s^{-1} over the waveband 400–700 nm. The lamps were automatically switched off when daylight was sufficiently intense.

Selection procedure

Flowering time was defined as the moment of first anthesis, i.e. the opening of the first flower. In *BB* plants this was expressed as the number of days after germination, while in *bb* plants flowering date was recorded each year in the form of the 'Julian day': 01 January=1 etc.

A special selection line was set up for *BB* plants. Starting with 128 plants grown from seeds sampled in four populations from south-west France with almost 100% plants without vernalization requirement (Van Dijk *et al.*, 1997), artificial selection for early flowering was carried out at 20 °C and 24 h daylength. During six generations, the ten earliest flowering plants were selected and crossed, two by two (five reciprocal crosses). Inbreeding was avoided by excluding crosses between full sibs. Ten offspring per parent plant were sown each generation.

The *bb* selection line started with seeds from all other natural populations. They were sown in June 1990, thus obtaining a base population of 718 plants (mean number of plants per population, 7.7) which were sufficiently vernalized in the spring of 1991 to evaluate their flowering date correctly. The first selection for flowering date was done by selecting the 20 earliest and latest flowering individuals in 1991 and crossing them two by two (ten reciprocal crosses for each line, early and late). This was repeated in 1992 in the same way, mostly with different individuals, even

though the repeatability for flowering date is high (Van Dijk *et al.*, 1997). From 1993 to 2004, subsequent generations were made up each year by selecting the ten earliest and the ten latest flowering plants (five reciprocal crosses each; in 1993 exceptionally ten reciprocal crosses each) among all available well-vernalized plants. Crosses between full or half sibs and between parent and offspring were avoided. Plants without vernalization requirement (*BB* or *Bb*), as well as male sterile plants, were never chosen for performing crosses. Plants of the base population were kept until death, plants of later generations for four flowering seasons unless they died before then.

Seeds were collected after seed ripening in August and could not be sown before September and not transferred from the 20 °C/16 h conditions to the semi-natural greenhouse before the end of December, thus decreasing vernalization. Flowering systematically occurred about 1–2 weeks later than plants sown in June or plants from previous years (Boudry *et al.*, 2002). For this reason, plants sown the previous September were never chosen for crosses in their first year of flowering.

Because all available well-vernalized plants were used as the parental population for each new generation, there was no strict separation between generations. All plants that survived from previous generations were candidates, since using the largest possible collection of individuals probably leads to the greatest response to selection. Nevertheless, as a consequence of the response to selection, most plants chosen for the experiments belonged to the last available generation. A positive effect of the occasional use of older plants is that there was no risk of obtaining two separate selection lines, one from even years and one from odd years, which would otherwise have been the case if only plants in their second year of reproduction were used.

Evaluation of the response to selection for flowering date in the bb *selection line*

A general problem in measuring the change in flowering date over generations is the year-to-year variation due to variable spring temperatures and/or variation in vernalization intensities that cannot be avoided under the semi-natural greenhouse conditions. Three methods were used to estimate the cumulated response. The first and second methods were based on data obtained during the *bb* selection procedure; the third method on a group of plants used in the evaluation experiment described below.

Method 1. Always referring to the mean date of the base population, thus ignoring the year-to-year differences completely. This mean date (19 May) was calculated over the years with 2-, 3-, and 4-year-old plants because the same ages were available throughout the selection experiment (these are the ages of flowering after a complete winter during the study period). The reason for always taking the same ages is that plants gradually flower later when they grow older (Van Dijk, 2009).

Method 2. As each generation flowers each year for three years after a complete winter, it was possible to calculate the shift in flowering time due to stochastic between-year fluctuations and correct all results for those shifts. This is done by attributing a positive or negative value to each year. This value indicates how many days later or earlier the same plants flower, on average, in that year with respect to the previous year. By correcting for these values, all years could be set at the same level as the first year of the experiment. An additional correction was applied for the effect of ageing. Van Dijk (2009) showed that the plants here used systematically flowered about 1.31 d later per year.

Method 3. In the last year of the evaluation experiment (see next section), a limited number of plants of all generations were kept together under the same semi-natural greenhouse conditions as the plants of the *bb* selection line. This allowed a direct, unbiased evaluation of the changes over the generations under the same environmental conditions, but has two disadvantages: low numbers of plants per generation (not more than four in some cases) and an evaluation in only one year (2006) with its particular sequence of cold and warm periods over winter and spring.

The evaluation experiment of the bb *selection line*

Seed progenies (families) from virtually all generations of the *bb* selection line and seven natural populations (six *bb* populations and one *BB* population), chosen for their approximate latitudinal equidistance (Table 1), were

Table 1. The number of seed progenies (families) used in the evaluation experiment of the bb selection line

Natural populations are characterized by their genotype at the B locus and their latitude. Positive generation numbers represent selection for earlier flowering and negative numbers the selection for later flowering.

Population (*BB* or *bb*) or generation (early or late)	Latitude of origin (°N)	Generation number	Number of families used
BB	43.63		2
bb	45.15		2
	46.88		2
	47.60		2
	48.29		2
	49.17		2
	51.37		1
Early		+1	11
		+2	10
		+3	5
		+4	4
		+5	4
		+6	4
		+7	4
		+8	4
		+9	2
		+10	2
		+11	2
		+12	4
		+13	4
Late		−1	7
		−4	3

evaluated over two years for flowering phenology under various conditions. For the natural populations, the same, but still viable, seed samples were used which also contributed to the base population for the selection lines in 1990. Conditions were different in the two years of evaluation, and each family member was subjected to a different vernalization regime, as described hereafter in more detail.

Seeds of all families were sown together in early September 2004. For each generation or population, two or more families were chosen, for which germination was known to occur at a sufficient rate. A large number of families from the early stages of the selection procedure were included to determine with precision the starting point of the selection process, and fewer families from the later stages, which could be combined in case the selection limit had been reached (Table 1). In some cases, fewer than ten seedlings were obtained and the family in question was eliminated. Per family, ten randomly chosen seedlings were grown in the greenhouse for about 80 d at 20 °C and natural daylength, a sufficiently long period so that flowering was not either size- or age-limited (Van Dijk and Hautekèete, 2007). Starting on 24 November and then every 2 weeks over an 8-week period (8 December, 22 December, and 5 January), 20% of the plants (i.e. two per family or population) were placed in the vernalization cave. The remaining plants (20%) stayed in the greenhouse over the entire 8 week period. On 19 January, all plants in the cave were returned to the greenhouse. Five groups of plants that spent 8, 6, 4, 2, and 0 weeks at vernalizing temperatures were thus created (Table 2; first year). Daylength was then gradually increased through additional lighting. Starting with 10 h daylength on 19 January (natural daylength was then 9 h 23 min), 15 min was added every five days until 16 March and from then on every four days on until daylength reached 16 h on 6 May. Days in the greenhouse were always thus slightly longer than natural daylength during this period. After 6 May, the increase was 1 h every four days until daylength reached 24 h in early June.

Table 2. The different cold treatments per family member: the number of weeks in the cold cave or a complete winter in the semi-natural greenhouse

Plant no. in each family	Vernalization duration in the first year (weeks)	Vernalization duration in the second year (weeks)
1	0	0
2	0	9
3	2	3
4	2	6
5	4	Complete winter
6	4	Complete winter
7	6	3
8	6	6
9	8	0
10	8	9

At the end of the flowering period, in mid-July 2005, all plants were transferred to the semi-natural greenhouse and stayed there until the end of September. Eight plants per family were then transferred to a compartment with 20 °C and natural daylength; these plants were used later for the second year of the experiment. The remaining two plants per family stayed and overwintered in the semi-natural greenhouse (Table 2).

On 12 October 2005, the second year of the experiment started, now maintaining the temperature at 16 °C in the greenhouse instead of 20 °C. On 12 October, 2 November, and 23 November, two plants per family were placed in the vernalization cave, while the two remaining plants per family stayed in the greenhouse (Table 2; second year). On 14 December, all plants were returned from the cave to the greenhouse. Again, five groups of plants were created: those with 9, 6, 3, and 0 weeks at vernalizing temperatures and the group that stayed in the semi-natural greenhouse, each group counting two plants per family, except for some rare cases of mortality. To exclude uncontrolled effects of the first cold treatment on the second one, each family member was treated differently, as shown in Table 2.

Daylength in the second year started with 10 h on 14 December. Every 5 d, 15 min were added until 13 April (16 h) and then 1 h every 5 d until 24 h on 23 May.

Statistical analysis

All statistical operations were carried out using Statistica 7.1 (Statsoft France). Realized heritability (h^2) was calculated for the *BB* selection line as $\Sigma R/\Sigma S$ (Falconer, 1989), where R is the response to selection in each generation and S the selection differential, i.e. the difference between the means of the selected plants and the whole group they came from. In the *bb* selection line, generations could not strictly be placed on an ordinate scale and therefore no heritability analysis could be performed, since all available plants of former generations were potential parents. For the same reason, no linear regression analyses could be carried out to evaluate the change of stem elongation or flowering time as a function of generation number. Instead, heterogeneity among generations for these traits was tested by ANOVA.

The effect of vernalization duration in the first year on the earliness of flowering in the second year was tested by comparing, within each family, plant numbers 1 with 9 and 2 with 10 (Table 2). This was done by applying Wilcoxon's signed ranks test (two-tailed).

Results

The BB *selection line: days from germination to flowering*

Among the 128 plants used to start the *BB* selection line, three plants did not flower and were assumed to be *bb* genotypes. Mean flowering time for the other 125 plants was 51.6 (SD=9.1) d after germination (range, 35–93 d).

After six generations of artificial selection for early flowering, a rather homogeneous population was obtained with a mean flowering time of 29.7 (SD=1.2) d after germination (range, 26–31 d). The realized heritability was calculated as $\Sigma R/\Sigma S$=0.70. During the last two generations, virtually no further progress was made (both R and S very low) which was a sign that the limit of selective response was being approached. The earliest flowering plants under these conditions never formed a rosette: a clear internode of 1–2 cm was already present between the cotyledons and the first true leaves. Later flowering individuals in the earlier selection stages initially formed a rosette and bolted later on at various ages.

The response to selection for flowering date in the bb *selection line*

The initial response to artificial selection for early or late flowering among plants with a vernalization requirement (*bb* plants) has already been described for the base population (Van Dijk *et al.*, 1997). Here, further response to selection was shown. The cumulated response during the selection process is presented for all three calculation methods in Fig. 2. In the selection for earlier flowering, the last generation (generation 13) flowered 35.4, 39.2, or 30.6 d earlier according to Methods 1, 2, and 3, respectively, than the base population. Damping stochastic fluctuations by calculating the mean value over the last three generations gave very similar values: 33.0, 34.1, and 32.1 d, respectively. The necessity of the correction for age in method 2 was confirmed: without this correction, the total cumulated response would have been 54.9 d instead of 39.2, or 48.5 d instead of 34.1 d over the last three generations, leading to results completely different from the other two methods.

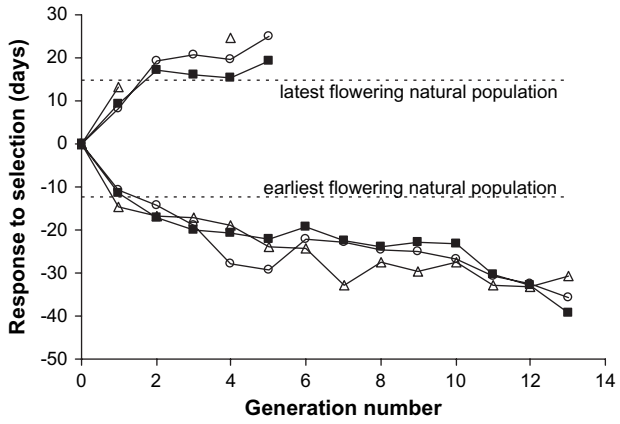

Fig. 2. The response to artificial selection for earlier or later flowering date among plants with a vernalization requirement (*bb* plants) according to three calculation methods. Circles: referring to the base population using plants of the same age; rectangles: applying corrections for stochastic between-year variations and ageing effects; triangles: using a limited number of plants of all generations evaluated together in the same experiment but in only one year. Dashed horizontal lines give the range limits of the natural populations used for constructing the base population.

Selection for later flowering after generation 5 is not shown in Fig. 2. Increasing numbers of plants did not flower at all: 21% in generation 5 and over 50% in generation 6. In addition, there was a strong negative effect on longevity (results not shown).

Since the experiment started with plants from different populations, which showed considerable among-population variation in flowering date (Van Dijk *et al.*, 1997), the initial, rapid response to selection is expected to be associated with the geographical origin of the selected plants. It is therefore informative to interpret the response to selection relative to the variation already existing in the base population in terms of extreme natural populations. The earliest natural population (49.43° N) flowered 12.6 d earlier and the latest *bb* population (51.14° N) 14.7 d later than the mean flowering time over all populations. This indicates that, during the selection experiment, a level of earliness in flowering was reached that was much earlier than the earliest natural population (Fig. 2). No considerable change in flowering time occurred after generation 2 in the late selection line, but the increasing number of non-flowering plants suggests that they surpassed a threshold beyond which the stimulus for flowering is no longer effective. This resulted in a strong bias towards lower apparent values for the response to selection when only flowering plants are considered.

Stem elongation in the vegetative stage of plants of the bb *selection line*

All plants in the evaluation experiment were measured for central shoot height 11 weeks after germination when all plants were still in their vegetative growth phase. Mean height was significantly different among generations as evaluated by ANOVA ($F_{15,749}$=30.95; P <0.001) and increased, although not quite regularly, during the selection process (Fig. 3A). The six natural '*bb*' populations that formed the reference generation 0 in this experiment were analysed in further detail. Mean population stem length varied from 1.8 cm to 18.5 cm which means that both the response in the selection line for later flowering and in the first generations of the selection line for earlier flowering can be explained by rapid selection acting on among-population variation. From generation 8 onwards in the early selection line, stems were considerably longer than those of the most extreme population.

A significant negative correlation was found between mean stem length and mean flowering date (the latter values were determined from the base population) among the natural populations involved (r=–0.938; n=6; P=0.006), suggesting that bolting in the vegetative stage is normally associated with early flowering in the next year. To test the generality of this finding that was based on the mean values of only six populations, old data from the base population (629 *bb* individuals) were reanalysed. Total stem length in the vegetative stage of 2-month-old *bb* plants had been

measured in August 1990 and flowering had been scored about every 10 d in the summer of 1991. Mean stem length for each of the flowering scoring dates (flowering date was taken as the mid-point of the scoring date interval) showed a clear negative relationship with flowering date (Fig. 3B). The correlation, based on individuals, was $r = -0.165$ ($n=629$; $P < 0.001$).

Flowering in the first year of the evaluation experiment of the bb *selection line*

In the first year of the evaluation experiment, the conditions to which plants were returned after their stay in the cold cave (20 °C and starting with a 10 h photoperiod) appeared to result in devernalization. Only two *bb* plants (all *BB* plants flowered as expected) started to develop flower buds in February, i.e. a few weeks after the return from the cold cave. Both had been exposed to the maximum vernalization of 8 weeks and were from later generations (10 and 12) of the early selection line. Flowering stopped soon after the opening of the first flowers and the remaining flower buds reverted to vegetative structures, a sign of devernalization (Margara, 1960). Nevertheless, useful information could be obtained later in the year, as, after the increase in photoperiod, some of the plants started flowering, but at photoperiods that were long in comparison with those necessary for well-vernalized plants. The percentage of flowering, over all vernalization treatments combined (assuming that these treatments were no longer playing a role—but see below) increased with generation number, but never reached 100% (Fig. 4A). The reconstructed photoperiod at which induction took place (32.6 d before flowering; see Van Dijk and Hautekèete, 2007) decreased with generation number (Fig. 4B). Accepting the hypothesis that the non-flowering plants have a hypothetical daylength requirement greater than 24 h, the trend in Fig. 4B underestimates the response to selection.

Although there was no statistically significant effect of the duration of vernalization on flowering rate and time, which was the argument for pooling all durations for the construction of Fig. 4, a closer inspection of the data used for Fig. 4A reveals that, in the first generations, there was a considerably lower flowering percentage in plants without vernalization (Table 3). The statistical justification of this conclusion is weak, however, because the separate pooling of generations 1–7 and 8–13 was made *a posteriori*, ignoring the individual generations. Further investigation with an experiment specifically designed to explore this relationship should be carried out to test its validity and magnitude. No significant effect of vernalization duration was found on the mean photoperiod at flowering induction when pooling generations 1–7 and 8–13 separately (Table 3).

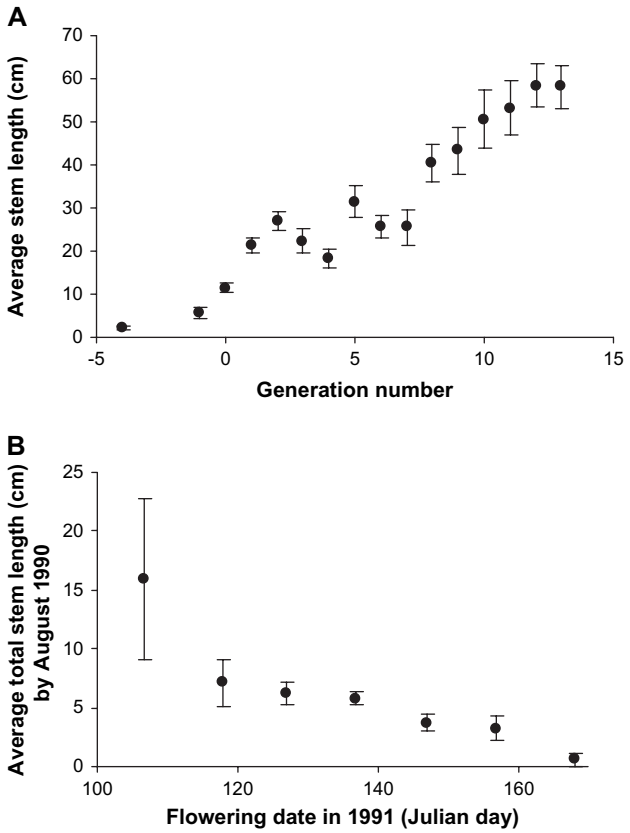

Fig. 3. (A) Plant height in the vegetative stage 11 weeks after germination (means ±SE per generation). Generation 0 refers to the mixture of six natural *bb* populations and negative numbers to selection for later flowering. (B) Total stem length (mean ±SE) of 2-month-old vegetative plants in August 1990 for each of the flowering date observations in 1991.

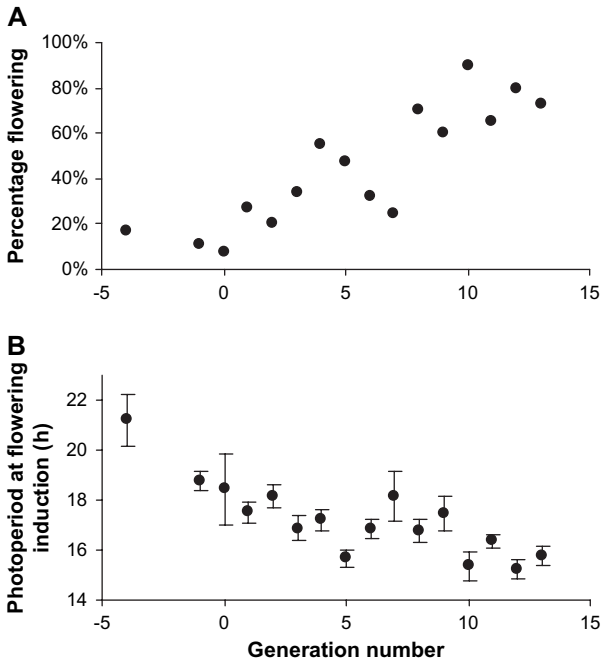

Fig. 4. Flowering in the first year of the evaluation experiment after devernalization: the percentage of plants that flowered (A) and the photoperiod (mean ±SE) at the moment of flowering induction for those plants (B). Generation number 0 refers to the mixture of six natural *bb* populations and negative numbers to selection for later flowering.

Table 3. Persistent effects of vernalization duration after possibly incomplete devernalization

Differences in numbers of flowering plants per treatment were tested by χ^2, differences in mean photoperiod at flowering induction per treatment were tested by ANOVA. Degrees of freedom can be derived from the data in this table.

Weeks of vernalization	Generations 1–7		Generations 8–13	
	Number of flowering plants	Photoperiod at flowering induction	Number of flowering plants	Photoperiod at flowering induction
0	12	17.7	23	16.8
2	24	17.6	25	16.6
4	31	17.8	28	15.7
6	31	17.6	30	15.8
8	33	16.9	26	16.0
Total flowering	131		132	
Total plants	420		180	
χ^2	11.40		1.11	
F		0.72		1.09
P	0.02	0.58	0.89	0.36

The joint effects of cold (vernalization) and daylength: flowering in the second year of the evaluation experiment

In the second year of the evaluation experiment, devernalization was prevented by keeping plants at 16 °C instead of 20 °C. All plants flowered in generations 1–13 of the early selection line, and only these generations were analysed further. Generation 0, and generations 1 and 4 from the late selection line had 81%, 61%, and 78% flowering, respectively. All plants in the semi-natural greenhouse and all *BB* plants flowered. The effect of the duration of the vernalizing period was reflected in a systematically earlier flowering after longer periods of cold (Fig. 5A). The finding that the 9 week treatment had a stronger effect than the 6 week treatment suggests that vernalization was not yet complete after 9 weeks.

No effect of the vernalization treatment in the first year of evaluation could be established over all generations 1–13 (P=0.79 for the comparison of plant numbers 1 and 9; P=0.63 for plant numbers 2 and 10, see Table 2). A special test was carried out for generations 1–7, for which the only significant effect after devernalization had been found (Table 3). Here P=0.40 was found for the comparison of plant numbers 1 and 9 and P=0.17 for plant numbers 2 and 10. In all cases these non-significant effects were in the 'wrong' direction: slightly later flowering for longer vernalized plants in the first year.

The generations with low numbers of families (3–13) showed considerable variation, probably due to stochasticity, with a particular effect in generation 6. Over the last generations (7–13), flowering time no longer changed significantly (see the regression lines in Fig. 5A) and showed an approximately linear relationship between daylength and the duration of vernalization: 15.2 min daylength per week of vernalization or 2.19 min less daylength required for each additional day of vernalization (Fig. 5B). The same procedure applied to the first generation in the selection process, which roughly corresponds to the earliest flowering

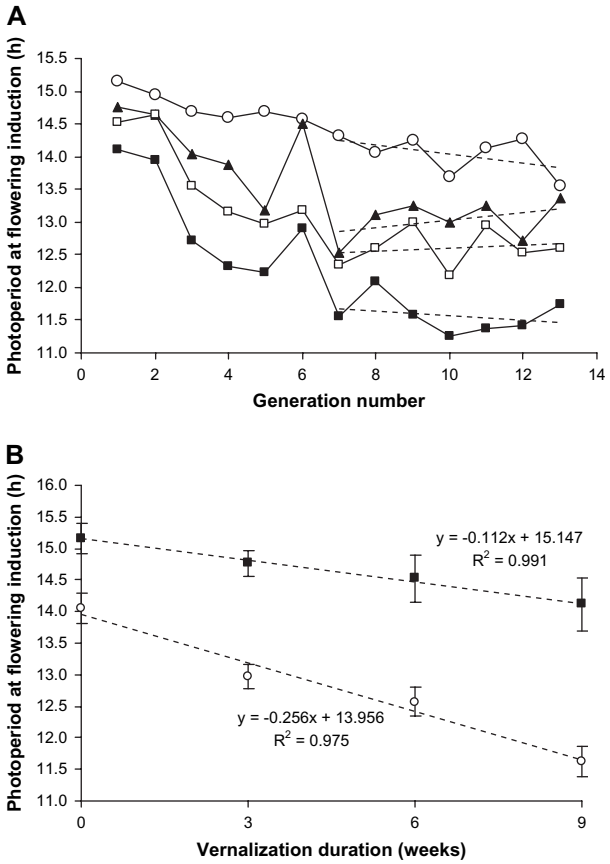

Fig. 5. Flowering in the second year of the evaluation experiment. (A) The mean photoperiod at the moment of flowering induction. Open circles: no vernalization; triangles: 3 weeks; open squares: 6 weeks: and filled squares: 9 weeks of vernalization prior to exposure to increasing daylength. Dashed lines correspond to the linear regression over generations 7–13. (B) Daylength at flowering induction of all plants with 0, 3, 6, or 9 weeks of vernalization (means and 95% confidence intervals) in the first generation (rectangles) and in the pooled generations 7–13 (open circles).

natural populations, showed that here each additional day of vernalization corresponded to 0.96 min less daylength (Fig. 5B).

Figure 5B also shows that, in the absence of vernalization (0 weeks), there is significant change due to the selection process: flowering occurred about 24 d earlier in the later generations of the early selection line compared with the first generation. This corresponds to a difference in photoperiod of 72 min. This can be ascribed to a lower photoperiod threshold and/or to the effects of other pathways. The second important finding is that sensitivity to vernalization (the slope of the regression line) was more than doubled.

The natural *BB* population included in the experiment did not show any change in flowering date with vernalization duration, although the numbers were very low. Remarkably, flowering date was very late compared with *bb* plants, which confirms earlier results (Van Dijk *et al.*, 1997).

As can be seen in Table 2, plants 1 and 8 had the same vernalization treatment in both years (0 weeks and 6 weeks, respectively) and could be directly compared for the photoperiod at flowering induction in both years. The correlation between years (i.e. the repeatability) for all plants in question (n=44) was measured as r=0.51, which is very close to the repeatability (r=0.53±0.02 SE) for flowering date between years found for the base population by Van Dijk *et al.* (1997). The difference between the two years in the photoperiod required was 3.6 h (SE=0.7) for the 0-week plants (n=11) and 4.0 h (SE=0.4) for the 6-week plants (n=33). In the case of the 0 week plants, the difference could be completely ascribed to the difference in temperature (16 °C versus 20 °C). The 6-week plants flowered earlier in the second year due to the vernalization, which may explain the additional 0.4 h.

Discussion

Consequences of the B/b *polymorphism*

The essential difference between *BB* and *bb* plants is not only that *BB* plants start reproducing one year earlier, but also that they are subjected to a different selection pressure. Especially in disturbed habitats, *BB* plants are selected for rapid flowering after germination (Van Dijk and Desplanque, 1999). The most extreme situation, where a weedy form of sea beet has evolved, is met in sugar beet cultures, where they cannot be eliminated by species-specific herbicides (Desplanque *et al.*, 2002). In these situations, rapid flowering has been selected for in order to reproduce before being eliminated by the farmer. In the *BB* populations from southern France included in this study, which are less associated with the agro-ecosystem, the time from germination to flowering appeared to be highly variable. The high h^2 could mean that the timing is not under very strong directional selection, but an alternative and more likely explanation is that the environment is heterogeneous with respect to the level of disturbance (Hautekèete *et al.*, 2002; H Van Dijk, personal observations). A low life expectancy, for example, due to ploughing or weeding, selects for small, but early flowering plants, while less disturbance favours a longer vegetative phase with larger plants that flower later.

At 24 h daylength and 20 °C, the most rapidly flowering plants did not form rosettes. The time it took from germination to flowering roughly corresponded to the time (about 4 weeks) that adult plants need to bolt and flower if they are transferred from short to long days in the greenhouse (Van Dijk and Hautekèete, 2007). This suggests that the induction of flowering takes place at the very moment of germination in these early *BB* plants. The period of about 4 weeks is just necessary to develop inflorescences and flowers and could only be further shortened by accelerating flower development at the expense of size.

The response to selection on flowering date in bb *plants: the role of vernalization and photoperiod*

The *bb* genotypes of sea beet are a perfect model to study how the optimal annual flowering date can be adjusted in iteroparous species. Both daylength sensitivity (Van Dijk and Hautekèete, 2007) and vernalization requirement (Boudry *et al.*, 2002) show considerable genetic variation along a latitudinal gradient. The present selection experiment for flowering date and the subsequent evaluation experiment confirms that vernalization and daylength requirement cannot be considered independently (cf. the above-mentioned 'photothermal induction' described by Owen *et al.*, 1940). In the first-year experiment, the vernalization effect apparently disappeared under the applied conditions. However, a large proportiont of the plants flowered at extremely long (mostly unnatural) daylengths, illustrating this compensatory mechanism. It appears that devernalization can be compensated by about 3.6 h daylength. In sugar beet, selected for bolting resistance, it has never been shown that extremely long photoperiods can completely compensate for vernalization. In the wild populations of this study, this compensation occurred only rarely (generation 0 in Fig. 2), but increased in the later generations of the early flowering selection line. The opposite situation, flowering under complete darkness at 21 °C after very long vernalization, was observed for a few sugar beet individuals by Fife and Price (1953).

In the second year of the experiment, the compensation between vernalization and daylength could more precisely be established since there was 100% flowering in all selection stages. In the later generations of the selection line, flowering was possible at photoperiods that were 2.23 min shorter for each extra day of vernalization. Interpreting this result in the light of knowledge gathered on *A. thaliana*, it can be assumed that the de-inhibition of the *FLC* gene through vernalization (*bvFLC* in *Beta*, Reeves *et al.*, 2007) and the effect of daylength (via the *Constans* gene: an orthologue is present in *Beta*; Chia *et al.*, 2008) both stimulate the genes involved in realizing flowering, called floral integrators, in a quantitative way and that the sum of their effects is determinant for surpassing the threshold.

The maximum vernalization duration of 9 weeks in the second experimental year was chosen to simulate the vernalization intensity of the field sites on the Atlantic French coasts (see Boudry *et al.*, 2002, for the correspondence between the effects of winters in the field and cold chamber regimes). The linear compensatory relationship with daylength requirement was unexpected and suggests that even longer durations will further decrease the critical daylength. Ecological relevance will set a limit: at higher latitudes; short photoperiods occur, but only in combination with temperatures that are too low for flowering to occur, at least under the present climatic conditions.

Vernalization and devernalization

The temperature after the cold period appeared to be critical for the persistence of the vernalization response. In the first year of the evaluation experiment, the post-vernalization period of 10 h daylength and 20 °C appeared to be strongly devernalizing, while at 16 °C, in the second year, the effect of the variable vernalization duration was clearly conserved. These results raise the question as to what extent there is a continuously changing effect of temperature from gradually weaker vernalization to gradually stronger devernalization and where the limit between the two responses lies. Little is known about devernalization based on the literature, although Chouard (1960) in his seminal paper on vernalization mentions the phenomenon in some detail. For sugar beet, Margara (1968) pointed out that the combination of higher temperatures (constantly higher than 20 °C) and short photoperiods leads to devernalization. Heide (1973) found devernalization at 18 °C under short days and at 24 °C under long days for red garden beet and stated that 'there is hardly any neutral temperature at which neither vernalization nor devernalization occurs'.

In mechanistic terms, these phenomena suggest that the epigenetic status of vernalization may easily be lost in iteroparous species, unlike in semelparous plants like *A. thaliana* where all meristems are irreversibly and more or less simultaneously destined to flower (Sablowski, 2007). Iteroparous plants develop a series of meristems with variable tendencies to develop into reproductive tissues (Tan and Swain, 2006; Rohde and Bhalerao, 2007), with vegetative meristems usually situated at the base of the plant. There appears to be a gradient within the plant that is under the influence of factors that stimulate flowering. In this system, it is not surprising that the balance may easily shift from reproductive to vegetative if stimulation for flowering decreases. Devernalization may thus take place by losing the epigenetic status brought about by vernalization. In semelparous species, this only happens in the transition to the next generation (Sheldon *et al.*, 2008). In iteroparous species, meristems destined to develop into reproductive tissues may become vegetative as a result of environmental stimuli, such as high temperatures. Here, it was found that this process can even be reversible after the formation of flowering buds that then develop into perched rosettes or leaves, a phenomenon also observed in other species (Tooke *et al.*, 2005).

Devernalization in the first year of evaluation may have been incomplete, as suggested by the significantly lower percentage of non-vernalized plants that flowered several weeks later at higher photoperiods (Table 3). This may indicate a vernalization effect that is independent of *FLC* as reported for *A. thaliana* by Alexandre and Hennig (2008). This vernalization unrelated to *FLC* may not be as easily reversible as *FLC*-associated vernalization. Its existence in *Beta* has to be confirmed by more appropriate experiments. Even if devernalization may have been incomplete for part of the plants during the following spring or early summer, there was no evidence of effects lasting to the next year, which means that plants have to be vernalized each year again.

The four pathways of flowering induction

An interesting phenomenon that emerged from the artificial selection experiment was the possibility to bolt even in the vegetative stage. Gibberellins clearly play a role in this phenomenon. Their role in the induction of flowering varies among species (Mutasa-Göttgens and Hedden, 2009). In some species, gibberellins can replace long days, for example in *A. thaliana*. The effect of GA in *B. vulgaris* was studied in detail by Margara (1960, 1967) who showed that it only acts on stem elongation without directly promoting flower formation. The GA level rises after flowering induction and results in bolting from a rosette. The present study suggests that a pre-existing, perhaps constitutive, GA level produces a more rapid onset of flowering. Apparently, bolting in the vegetative stage also occurs *in situ* as suggested by Fig. 3B. The earliest flowering plants (especially those from Brittany, see Van Dijk *et al.*, 1997) bolted significantly in their first, vegetative year.

All four pathways of flowering induction known in *A. thaliana* were found in this experiment. Genetic variability in the autonomous pathway was most clearly manifested in *BB* plants under long days, but its effect, in all cases, added up to the effects of the other mechanisms. Vernalization and photoperiod tended to be at the limit of selection after generation 7 in the plant material used; a trend for increased earliness after generation 10 may be brought about by progress in the autonomous or gibberellin pathways. Compensatory effects between the four pathways are clearest for vernalization intensity and photoperiod, but actually apply to all pathways. Apparently Justus von Liebig's 'law of the minimum' is not valid: there is no required strict minimum size, vernalization duration or photoperiod. This is in agreement with findings in other studies. For instance, Sung and Amasino (2005) stated that vernalization is a gradual process and has a progressive effect on the readiness to flower. In addition, Wesselingh *et al.* (1994) described the interaction between plant size and vernalization.

Annuals and perennials

A. thaliana appears to have mechanisms of flowering induction that have laxer requirements compared with those in sea beet, i.e. reproduction occurs under a larger range of

environmental conditions. This may be associated with its semelparous strategy. In iteroparous species, there is, in principle, a future reproductive season in which the individual can try again and for which investments have to be made, whereas in semelparous species it is ''now or never''. The genetic analysis of flowering induction in *A. thaliana* has provided deep insight as to its mechanism, but cannot fully explain what happens in other species. The attractiveness of sea beet as an alternative and complementary study species lies in the fact that the whole spectrum of flowering induction is represented: from tiny *BB* plants that flower after 4 week at 24 h daylength to plants that need an intense vernalization period and sufficient daylength and show gradual ageing effects over the years. Recently, more genetic details have become available for *B. vulgaris* (Reeves *et al.*, 2007; Chia *et al.*, 2008) but, apart from the *B* gene, there is, to date, no information available on the polymorphism of the genes involved in flowering time in natural populations.

Conclusion

The response to artificial selection shown here suggests that natural selection on flowering time has high potential in sea beet, at least at the metapopulation or regional scale. Current climate change with steadily rising temperatures leads to less vernalization and higher spring temperatures. Earlier flowering will be favoured and can occur either through phenotypic plasticity, or, in a more controlled way, through evolutionary change. All known flowering induction pathways potentially contribute to earlier flowering in sea beet. The sensitivity to photoperiod and to spring temperatures will change in the direction of lower critical values. The readiness to bolt will increase in association with rapid stem elongation in the vegetative stage. The role of vernalization is more complex. In the southern part of the species range, the *B* allele is expected to increase in frequency and to move northward (Hautekèete *et al.*, 2009). In *bb* plants, the quantitative vernalization requirement must decrease at such a speed that less vernalizing winters nevertheless lead to earlier flowering (see Van Dijk and Desplanque, 1999, for a model). The results obtained in this study suggest that all four pathways may simultaneously participate in evolutionary change.

Acknowledgements

I wish to thank Robert Dron, Eric Schmitt, and Angélique Bourceaux for meticulously taking care of the plants and Fabrice Roux for suggesting improvements on the manuscript.

References

Alexandre CM, Hennig L. 2008. *FLC* or not *FLC*: the other side of vernalization. *Journal of Experimental Botany* **59,** 1127–1135.

Anthony RG. 2006. Signal transduction regulating floral development. In: Jordan BR, ed. *The molecular biology and biotechnology of flowering*. Wallingford, UK: CABI, 50–78.

Balasubramanian S, Sureshkumar S, Agrawal M, Michael TP, Wesinger C, Maloof JN, Clark R, Warthmann N, Chory J, Weigel D. 2006. The PHYTOCHROME C photoreceptor gene mediates natural variation in flowering and growth responses of *Arabidopsis thaliana*. *Nature Genetics* **38,** 711–715.

Bäurle I, Dean C. 2006. The timing of developmental transitions in plants. *Cell* **125,** 655–664.

Bernier G, Périlleux C. 2005. A physiological overview of the genetics of flowering time control. *Plant Biotechnology Journal* **3,** 3–16.

Blázquez MA, Ahn JH, Weigel D. 2003. A thermosensory pathway controlling flowering time in *Arabidopsis thaliana*. *Nature Genetics* **33,** 168–171.

Boudry P, McCombie H, Van Dijk H. 2002. Vernalization requirement of wild beet *Beta vulgaris* ssp. *maritima*: among population variation and its adaptive significance. *Journal of Ecology* **90,** 693–703.

Boudry P, Wieber R, Saumitou-Laprade P, Pillen K, Van Dijk H, Jung C. 1994. Identification of RFLP markers closely linked to the bolting gene B and their significance for the study of the annual habit in beets (*Beta vulgaris* L.). *Theoretical and Applied Genetics* **88,** 852–858.

Brunner AM, Nilsson O. 2004. Revisiting tree maturation and floral initiation in the poplar functional genomics era. *New Phytologist* **164,** 43–51.

Burgess KS, Etterson JR, Galloway LF. 2007. Artificial selection shifts flowering phenology and other correlated traits in an autotetraploid herb. *Heredity* **99,** 641–648.

Caicedo A, Stinchcombe JR, Olsen KM, Schmitt J, Purugganan D. 2004. Epistatic interaction between *Arabidopsis FRI* and *FLC* flowering time genes generates a latitudinal cline in a life history trait. *Proceedings of the National Academy of Sciences, USA* **101,** 15670–15675.

Chia TYP, Müller A, Jung C, Mutasa-Göttgens ES. 2008. Sugar beet contains a large *CONSTANS-LIKE* gene family including a *CO* homologue that is independent of the early-bolting (*B*) gene locus. *Journal of Experimental Botany* **59,** 2735–2748.

Chouard P. 1960. Vernalization and its relations to dormancy. *Annual Review of Plant Physiology* **11,** 191–238.

Cockram J, Jones H, Leigh FJ, O'Sullivan D, Powell W, Laurie DA, Greenland AJ. 2007. Control of flowering time in temperate cereals: genes, domestication, and sustainable productivity. *Journal of Experimental Botany* **58,** 1231–1244.

Cooper JP. 1963. Species and population differences in climatic response. In: Evans LT, ed. *Environmental control of plant growth*. NY: Academic Press, 381–403.

Desplanque B, Hautekèete N, Van Dijk H. 2002. Transgenic weed beets: possible, probable, avoidable? *Journal of Applied Ecology* **39,** 561–571.

Falconer DS. 1989. *Introduction to quantitative genetics*. Harlow, UK: Longman.

Fife JM, Price C. 1953. Bolting and flowering of sugar beets in continuous darkness. *Plant Physiology* **28,** 475–480.

Franks SJ, Sim S, Weis AE. 2007. Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proceedings of the National Academy of Sciences, USA* **104,** 1278–1282.

Gendall AR, Simpson GG. 2006. Vernalization. In: Jordan BR, ed. *The molecular biology and biotechnology of flowering*. Wallingford, UK: CABI, 26–49.

Glover B. 2007. *Understanding flowers and flowering*. UK: Oxford University Press.

Hautekèete N-C, Piquot Y, Van Dijk H. 2002. Life span in *Beta vulgaris* ssp. *maritima*: the effects of age at first reproduction and disturbance. *Journal of Ecology* **90,** 508–516.

Hautekèete N-C, Van Dijk H, Piquot Y, Teriokhin A. 2009. Evolutionary optimization of life-history traits in the sea beet *Beta vulgaris* subsp. *maritima*: comparing model to data. *Acta Oecologica* **35,** 104–116.

Hecht V, Foucher F, Ferrándiz C, *et al*. 2005. Conservation of Arabidopsis flowering genes in model legumes. *Plant Physiology* **137,** 1420–1434.

Heide OM. 1973. Environmental control of bolting and flowering in red garden beets. *Meldinger fra Norges Landbrukshøgskole* **52,** 1–17.

Heide OM, Sønsteby A. 2007. Interactions of temperature and photoperiod in the control of flowering of latitudinal and altitudinal populations of wild strawberry (*Fragaria vesca*). *Physiologia Plantarum* **130,** 280–289.

Howe GT, Hackett GR, Klevorn RE. 1995. Photoperiodic responses of a northern and southern ecotype of black cottonwood. *Physiologia Plantarum* **93,** 695–708.

Lacey EP. 1988. Latitudinal variation in reproductive timing of a short-lived monocarp. *Daucus carota (Apiaceae). Ecology* **69,** 220–232.

Lempe J, Balasubramanian S, Sureshkumar S, Singh A, Schmid M, Weigel D. 2005. Diversity of flowering responses in wild *Arabidopsis thaliana* strains. *PLoS Genetics* **1,** 109–118.

Letschert JPW. 1993. *Beta* section *Beta*: biogeographical patterns of variation and taxonomy. *Wageningen Agricultural University Papers* **93-1,** 1–155.

Margara J. 1960. Recherches sur le déterminisme de l'élongation et de la floraison dans le genre *Beta*. *Annales Amélioration des Plantes* **10,** 362–471.

Margara J. 1967. Recherches sur les gibbérellines et le développement floral chez la betterave. *Comptes-rendus de l'Institut International de Recherches Betteravières* **2,** 242–254.

Margara J. 1968. Physiologie de l'induction florale chez la betterave. *Comptes-rendus de l'Institut International de Recherches Betteravières* **3,** 17–26.

Marquardt S, Boss PK, Hadfield J, Dean C. 2006. Additional targets of the *Arabidopsis* autonomous pathway members, FCA and FY. *Journal of Experimental Botany* **57,** 3379–3396.

Montague JL, Barrett SCH, Eckert CG. 2008. Re-establishment of clinal variation in flowering time among introduced populations of purple loosestrife (*Lythium salicaria*, Lythraceae). *Journal of Evolutionary Biology* **21,** 234–245.

Mutasa-Göttgens E, Hedden P. 2009. Gibberellin as a factor in floral regulatory networks. *Journal of Experimental Botany* **60,** 1979–1989.

Owen FV, Carsner E, Stout M. 1940. Photothermal induction of flowering in sugar beets. *Journal of Agricultural Research* **61,** 101–124.

Reeves PA, He Y, Schmitz RJ, Amasino RM, Panella LW, Richards CM. 2007. Evolutionary conservation of the *FLOWERING LOCUS C*-mediated vernalization response: evidence from sugar beet (*Beta vulgaris*). *Genetics* **176,** 295–307.

Reusch TBH, Wood TE. 2007. Molecular ecology of global change. *Molecular Ecology* **16,** 3973–3992.

Rohde A, Bhalerao RP. 2007. Plant dormancy in the perennial context. *Trends in Plant Science* **12,** 217–223.

Rhoné B, Remoué C, Galic N, Goldringer I, Bonnin I. 2008. Insight into the genetic basis of climatic adaptation in experimentally evolving wheat populations. *Molecular Ecology* **17,** 930–943.

Riihimäki M, Savolainen O. 2004. Environmental and genetic effects on flowering differences between northern and southern populations of Arabidopsis lyrata (Brassicaceae). *American Journal of Botany* **91,** 1036–1045.

Roux F, Touzet P, Cuguen J, Le Corre V. 2006. How to be early flowering: an evolutionary perspective. *Trends in Plant Science* **11,** 375–381.

Sablowski R. 2007. Flowering and determinacy in *Arabidopsis*. *Journal of Experimental Botany* **58,** 899–907.

Sandring S, Riihimäki MA, Savolainen O, Ågren J. 2006. Selection on flowering time and floral display in an alpine and a lowland population of *Arabidopsis lyrata*. *Journal of Evolutionary Biology* **20,** 558–567.

Sheldon CC, Hills MJ, Lister C, Dean C, Dennis ES, Peacock WJ. 2008. Resetting of *FLOWERING LOCUS C* expression after epigenetic repression by vernalization. *Proceedings of the National Academy of Sciences, USA* **105,** 2214–2219.

Shindo C, Bernasconi G, Hardtke CS. 2007. Natural genetic variation in Arabidopsis: tools, traits and prospects for evolutionary ecology. *Annals of Botany* **99,** 1043–1054.

Smit AL. 1983. Influence of external factors on growth and development of sugar-beet (*Beta vulgaris* L.). *Agricultural Research Reports* 914. Pudoc, Wageningen, The Netherlands.

Stinchcombe JR, Weinig C, Ungerer M, Olsen KM, Mays C, Halldorsdottir S, Purugganan MD, Schmitt J. 2004. A latitudinal cline in flowering time in *Arabidopsis thaliana* modulated by the flowering time gene *FRIGIDA*. *Proceedings of the National Academy of Sciences, USA* **101,** 4712–4717.

Sung S, Amasino RM. 2005. Remembering winter: toward a molecular understanding of vernalization. *Annual Review of Plant Biology* **56,** 491–508.

Tan F-C, Swain SM. 2006. Genetics of flower initiation and development in annual and perennial species. *Physiologia Plantarum* **128,** 8–17.

Thomas B, Carré I, Jackson S. 2006. Photoperiodism and flowering. In: Jordan BR, ed. *The molecular biology and biotechnology of flowering*. Wallingford, UK: CABI, 3–25.

Tooke F, Ordridge M, Chiurugwi T, Battey N. 2005. Mechanisms and function of flower and inflorescence reversion. *Journal of Experimental Botany* **56,** 2587–2599.

Van Dijk H. 2009. Ageing effects in an iteroparous plant species with a variable lifespan. *Annals of Botany* DOI: 10.1093/aob/mcp100.

Van Dijk H, Boudry P, McCombie H, Vernet Ph. 1997. Flowering time in wild beet (*Beta vulgaris* ssp. *maritima*) along a latitudinal cline. *Acta Oecologica* **18,** 47–60.

Van Dijk H, Desplanque B. 1999. European *Beta*: crops and their wild and weedy relatives. In: Van Raamsdonk LWD, Den Nijs JCM, eds. *Plant evolution in man-made habitats*. Amsterdam: Hugo de Vries Laboratory, 257–270.

Van Dijk H, Hautekèete N. 2007. Long day plants and the response to global warming: rapid evolutionary change in day length sensitivity is possible in wild beet. *Journal of Evolutionary Biology* **20,** 349–357.

Wesselingh RA, Klinkhamer PGL, de Jong TJ, Schlatmann EGM. 1994. A latitudinal cline in vernalization requirement in *Cirsium vulgare*. *Ecography* **17,** 272–277.

Wilkie JD, Sedgley M, Olesen T. 2008. Regulation of floral initiation in horticultural trees. *Journal of Experimental Botany* **59,** 3215–3228.