

Long day plants and the response to global warming: rapid evolutionary change in day length sensitivity is possible in wild beet

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Abstract

Day length is a key factor in flowering induction in many plant species in a seasonal environment with flowering induction usually happening at shorter day lengths in lower latitudes. Now, the climate changes systematically at a considerable speed due to global warming. As a consequence, earlier flowering will be selected for in long day plants by favouring a lower threshold for day length sensitivity, on the condition of available genetic variability. Here, we show that there is considerable genetic variation for day length sensitivity in our study species, the seabest *Beta vulgaris* subsp. *maritima*. In the northernmost natural populations without vernalization requirement, in southwest France, the necessary day length for flowering induction could be reduced by artificial selection in <10 generations from >13 h to <11 h, the latter value corresponding to populations in the *Beta*-species complex from Northern Africa and the eastern part of the Mediterranean tested under the same conditions. A quantitative genetic analysis provided evidence of a gradual change without detectable major genes. Additional experiments were carried out to separate the response to photoperiod from age and energy effects. A certain effect of energy availability has been found, whereas age effects could be excluded. These results indicate a considerable potential for evolutionary change in adjusting flowering time in a changing climate.

Introduction

Temperatures on earth have been rising for several decades at a remarkable speed and the prediction is that this will not stop in the near future (Crowley, 2000; Intergovernmental Panel on Climate Change, 2002). Plant and animal species, or their local genotypes, must migrate to higher latitudes or change *in situ*. Recent species' range shifts are indeed observed (Walther *et al.*, 2005), but migration has to proceed at a speed that is beyond the capacity of many species (Malcolm *et al.*, 2002) and is increasingly difficult due to barriers and habitat fragmentation. The question of sufficient evolutionary potential is therefore gaining interest. A crucial aspect of climate change is its influence on the phenological processes and stage transitions. In plants, flowering induction is, next to germination, a critical moment

in the developmental cycle with obvious fitness consequences. Flowering induction stimuli are directly related with climate and consequently with latitude. Spring flowering plants that occur across a range of latitudes usually flower earlier in lower latitudes (White, 1995; Sparks & Menzel, 2002).

There are three well-known mechanisms that allow plants to adjust their flowering time to the prevailing seasonal climatic conditions. In the first place, flowering induction may take place after reaching a certain effective temperature sum, e.g. cumulative temperatures above 5 °C (Heikinheimo & Lappalainen, 1997). In warmer springs this is the case earlier and flowering consequently happens earlier. Global warming thus has an immediate effect which has been observed for many species since several decades, especially in higher latitudes (Post *et al.*, 2001; Bisgrove & Hadley, 2002; Fitter & Fitter, 2002; Walther *et al.*, 2002). Temperature sum implies a sort of phenotypic plasticity: a single genotype leads to different outcomes (phenotypes) in a range of circumstances. Natural selection favours the right plasticity (Scheiner, 1993), but it is unclear whether

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this mechanism still works if temperatures rise systematically and will thus lie more and more outside the original range.

Besides temperature sum, there are two other well-studied environmental cues with an influence on flowering phenology: vernalization and day length. Vernalization does not seem a very good mechanism to adapt flowering time to variable temperatures, because higher temperatures mean less vernalization and therefore later flowering, which means that the phenotypic plasticity goes in the wrong direction. This may explain why strong latitudinal clines exist for genetically determined vernalization requirement, often with no requirement at all in the most southern part of the species distribution, e.g. beet (Boudry *et al.*, 2001) and carrot (Lacey, 1988). A rise in temperature requires a strong decrease in vernalization requirement such that flowering happens earlier despite the less vernalizing temperatures (see Van Dijk & Desplanque, 1999 for a model).

Day length is a very reliable environmental signal with very little stochastic variation, and is used by many plant species as a cue for flowering induction (Thomas & Vince-Prue, 1997). Day length variation increases with latitude, which casts doubt on its importance in lower latitudes. The observation that flowering of many tropical plants is strongly influenced by day length (Thomas & Vince-Prue, 1984) suggests, however, that smaller variations may be compensated by a higher responsiveness. Day length varies across latitudes, but differences are small in the critical period of March and April on the northern hemisphere. Day length sensitive plants that flower earlier in lower latitudes must, therefore, have a lower threshold value for day length sensitivity, and responding to global warming requires evolutionary change in this character.

We studied day length requirement and the possibility of evolutionary change in this character in the seabed *Beta vulgaris* subsp. *maritima*, the wild ancestor of cultivated beet, mainly growing on the coasts of the Mediterranean and Western Europe (Letschert, 1993). In the southernmost population studied, the minimum day length is slightly <11 h with an amplitude of about 3.5 h. This species shows both vernalization and long day requirement, but in the southern part of the species distribution area vernalization requirement is suppressed by the partially dominant allele of the bolting gene B (Boudry *et al.*, 1994). The northernmost natural populations without vernalization requirement occur in southwest France. We first compared these populations with populations in the species complex from more southern regions: southern Europe, northern Africa and the eastern part of the Mediterranean. We then applied artificial selection for day length sensitivity starting with the populations from southwest France to get an idea of the evolutionary potential of this character (Conner, 2003). A final aim was to search for segregating major genes, if any, and to create the starting material for a

comparison with the genes involved in the photoperiod pathway known in other species, in particular in *Arabidopsis thaliana* (Mouradov *et al.*, 2002; Simpson & Dean, 2002; Boss *et al.*, 2004; Searle & Coupland, 2004).

Materials and methods

Study species

Beta vulgaris subsp. *maritima* is a perennial long day plant with a variable life span and a variable or absent vernalization requirement, in both cases among populations and among individuals within populations. The species is self-incompatible and wind-pollinated. It occurs along the coasts of West and South Europe, North Africa and West Asia and in the southern part of the distribution area occasionally inland like in southwest France. The other species and subspecies in the species complex are predominantly Mediterranean and are all interfertile with *B. v. maritima* (see Letschert, 1993 for a detailed description of the section *Beta*).

We sampled seeds in the populations near location 1 in Fig. 1 in 1989. Seeds from locations 10 and 15 were provided by the BGN gene bank in Wageningen, The Netherlands; seeds from all other locations by the BAZ gene bank in Braunschweig, Germany.

Artificial selection for day length sensitivity was carried out on populations from South West France where about 95% of the plants have no vernalization requirement (Van Dijk *et al.*, 1997). This is also the case for the more southern populations of *B. v. ssp. maritima* used in this study. The other subspecies and species in the species complex do not have any vernalization requirement at all.

Selection experiments and crosses

The starting material for artificial selection consisted of seeds individually sampled in 1989 from 43 plants in four populations and one bulk sample from a fifth population in the region near Condom between Toulouse and Bordeaux (southwest France). A preselection for early



Fig. 1 The geographical locations of the populations used. Further details are given in Table 1.

flowering was carried out under long days (16 h) by selecting the plants with the shortest time between germination and the first open flower. This was done to eliminate the few plants with vernalization requirement and to reduce the variation in lag time between flowering induction and the first open flower, which would be indistinguishable from variation in critical day length under our experimental conditions. The chosen day length of 16 h roughly corresponds to the maximum photoperiod in southwest France. We supposed that all plants that needed longer days in the glasshouse would never flower *in situ* without sufficient cold. They were, therefore, excluded with the argument of having a vernalization requirement. Inbreeding was avoided by making individual crosses between unrelated plants. The loss of other genetic variation was limited by selecting at least 40 plants from a total of several hundreds. The preselection was finished after three generations, leading to what we call the 'preselected material'. The artificial selection for early flowering under long days was continued, however, for other purposes (the 'long day selection line').

Selection for lower critical photoperiod started in 1991 with the preselected material, but now under increasing photoperiods starting with 10 h light and 14 h dark, leading to the 'short day selection line'. In each generation individual crosses were made between the 10 earliest flowering plants (five reciprocal crosses), avoiding crosses between full or half sibs. Of each parent 10 progeny were evaluated, leading to 100 plants per generation. The experiment was ended after nine generations when the selection response became very weak.

Plants from seeds collected in all wild populations (see Fig. 1) and all generations of the artificial short day selection experiment were sown together in November 2002 to avoid differences in environmental conditions, taking advantage of the fact that seeds of this species are very long-lived. The quantitative genetic analysis experiment started in November 2003.

Glasshouse conditions

Experiments were carried out in a glasshouse with a constant temperature of 20 °C. The photoperiod was adjusted by 400 W high pressure sodium lamps, PAR: 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ over the waveband 400–700 nm. The lamps were automatically switched off when daylight was sufficiently intense. Plants were grown in 1.7 L pots with commercial soil (N : P : K = 14 : 16 : 18 and oligo-elements). From day 60 on plants were supplied every 2 weeks with 1 g of fertilizer (N : P : K = 12 : 12 : 17).

Plants in the preselection phase received constant long days (16 h light/8 h dark). In the short day selection and evaluation experiments as well as in the quantitative analysis experiment plants were sown in early November and grown vegetatively during 80 days under short days (10 h light/14 h dark). Plants at day 80 typically had

10–14 leaves with a maximum length of 20–40 cm. After day 80, end January, day length was gradually increased with 15 min every 4 days, which led to photoperiods at least a bit longer than the natural day length outside.

Estimation of the day of flowering induction under increasing photoperiods

The moment of flowering induction under increasing photoperiods is directly related to the first signs of bolting (stem elongation from a rosette). To obtain a precise idea of the number of days between flowering induction and these first signs a separate experiment was carried out in which a number of plants were transferred from short days (10 h) to long days (16 h), considering the day of transfer as the day of induction. Visible bolting started about 10 days after the transfer. The progress of inflorescence height was measured every day and with these data an estimator could be developed based on one inflorescence height measure h_d of at least 1 cm at day d together with the height at the moment of first open flower h_f . In each case, a perfect linear relationship of $(h_d/h_f)^{0.4}$ with time was observed for all heights $<0.5 h_f$

$$(h_d/h_f)^{0.4} = kd,$$

where d is the number of days after of the start of bolting which occurs in its turn b days after the day of induction i (which is the day we wanted to estimate) and k is a parameter. This can be rewritten as

$$i = d - (1/k)(h_d/h_f)^{0.4} - b.$$

The relationship

$$i = d - 27.7(h_d/h_f)^{0.4} - 4.9$$

provided a very precise estimation of the day of induction i . The parameter 27.7 ($1/k$) could be derived from the data, giving the best fit for a large number of plants in the selection experiment with two inflorescence lengths available at a 5-day-interval. The i values thus obtained showed a frequency fluctuation with a period of exactly 4.0 days, which coincides with the interval of increase of the photoperiod thus confirming the reliability of the method. The parameter 4.9 (b) optimally synchronized this frequency fluctuation and the interval of photoperiod increase. Each i value was now substituted by the corresponding critical day length.

Checking for the role of age or size

The influence of age (and consequently size) on flowering induction was checked by transferring plants of ages between 0 and 80 days from short days (10 h) to long days (16 h). The number of days from transfer to flowering was recorded using 55 plants from the first (preselected material) and 42 plants from the last generation of the short day selection line homogeneously distributed over the ages.

Checking for the role of energy

Increasing day length in nature has two components: increasing photoperiod and increasing energy amount, which is the integral of light intensity over the light period. The artificial selection experiment for critical day length has *a priori* been carried out to measure sensitivity to photoperiod, but without excluding a response to increasing energy availability. We tested in a separate experiment for a correlated response of the influence of energy on flowering precocity. As reference material, we used a selection line for which the preselection for precocity under long days was continued (the 'long day selection line') compared with the plants from the last generation of the day length sensitivity experiment (the 'short day selection line'). We used an interrupted light regime with a photoperiod of 16 h but with only 11 h energy as visualized in the lower part of Fig. 3 and measured days from germination to flowering. We assumed, in accordance with the literature (Thomas & Vince-Prue, 1997), that photoperiod was exclusively determined by the longest dark period, which was 8 h in both light regimes. The interaction between selection line and light regime was tested by a two-way ANOVA.

Quantitative genetic analysis

Using the preselected material (parent high) and last generation of the short day selection line (parent low) as parental populations we made an F1, an F2 and backcrosses (F1 × parent low and F1 × parent high). In each case, this was done by means of 10 reciprocal crosses between 20 randomly chosen individuals from different families grown under long days (24 h) to have a good synchronization of flowering time. This analysis was done to get an idea whether only a few or rather several loci were involved in the selection process. In the former case, a more detailed genetic analysis of the different generations and families could provide more information, while in the latter case this is *a priori* unsuccessful. Although a best fitting number of loci will be generated by the following procedure, this is only a rough estimate due to the many assumptions it contains.

We used the quantitative genetics model and symbols of Falconer (1989). Assuming that more than one locus is involved, the number of variables is too high for a complete analysis which means that simplifications are necessary. We adopted the following hypotheses:

- (1) All loci are unlinked and diallelic and have an equal effect on the character, i.e. they have identical values of a and d *sensu* Falconer (1989) and identical allele frequencies q (low allele) and p (high allele) in the 'parent high' population.
- (2) The 'parent low' population is at its selection limit which means that all 'low alleles' have become fixed ($p = 0$). In the 'parent high' population p is relatively high but lower than one (because there is some genetic

variation). This also implies that the variance of the 'parent low' population is entirely due to the environment (V_E according to Falconer, 1989), whereas the variance of the 'parent high' population also has a genetic component ($V_E + V_G$) where V_G is caused by the segregating loci mentioned.

The analysis procedure was as follows:

- (1) for 1–3 loci all multilocus genotype frequencies were individually calculated. For higher numbers of loci the influence on variance was assumed to converge sufficiently to an overall genetic variance corresponding to the sum of the variances caused by each locus;
- (2) for each number of loci the values of p , a and d were chosen such that the variance of the 'parent high' population was equal to the observed value and the best fit was obtained with the means of F1, F2 and BCs. This was done by minimizing the relative sum of squares of the differences weighted by the number of individuals in the population;
- (3) the effect of the number of loci on the standard deviations was tested by a chi-square analysis comparing the numbers in the various classes of all crosses (F1, F2 and BCs) and the numbers generated by the model, combining classes where necessary to avoid expected numbers lower than two.

Results

Day length sensitivity in natural populations in the species complex

The southern populations, originating from regions with considerably higher spring temperatures, flower substantially earlier under glasshouse conditions with increasing day length than the plants from southwest France (population 1 in Table 1). We deduced for each population a critical day length which was always higher than the minimum day length in the latitude of origin, but in some cases the difference was very small, leading to a deduced flowering induction *in situ* in January or early February (Table 1). The plants from southwest France showed a substantially later deduced flowering induction, which was, however, biased by the presence of some vernalization requirement (see next section).

Artificial selection for reduced day length sensitivity

Before showing the results of the response to artificial selection, we first present the possible biasing factors that may play a role: (1) age, (2) vernalization requirement, (3) lag time between flowering induction and its realization.

We checked in a separate experiment that the size reached after 80 days of vegetative growth at short days was not a limiting factor. In the preselected material, the number of days between the moment of transfer from short days to long days and the moment of flowering

Table 1 Estimated critical day length and the environmental conditions in the evaluated populations of the species in the section *Beta*.

Location number	Taxon	District of origin (country)	Latitude of origin (north)	Minimum day length <i>in situ</i>	Temperature mean over February to April (°C)	Estimated mean critical day length \pm SD (<i>n</i>) in the glasshouse	Date in the district of origin corresponding to the estimated mean critical day length
1	mar	Nérac (F)	43°42'	9:31	8.9	15:23 \pm 1:24 (142)	17 May
		Idem after preselection				13:12 \pm 0:31 (27)	1 April
2	mar	Murcia (E)	38°	10:01	17.7	13:03 \pm 0:35 (11)	2 April
	mac					12:25 \pm 0:31 (3)	18 March
3	mac	Tavira (P)	37°06'	10:05	14.4	12:32 \pm 0:20 (20)	21 March
4	mar	Olhao (P)	37°	10:05	14.4	13:00 \pm 0:21 (9)	2 April
	mac					12:13 \pm 0:15 (15)	13 March
5	ada	Kos (GR)	36°54'	10:05	13.8	11:07 \pm 0:08 (7)	12 February
6	mac	Tunis (TN)	36°48'	10:06	14.0	11:51 \pm 0:14 (11)	4 March
7	mac	Puerto Real (E)	36°30'	10:08	14.7	12:19 \pm 0:25 (19)	16 March
8	ada	Rhodos (GR)	36°06'	10:09	14.3	11:04 \pm 0:16 (15)	9 February
9	mac	Limassol (CY)	34°36'	10:16	14.2	10:37 \pm 0:12 (15)	21 January
10	ada	Sharon Plain (ISR)	32°48'	10:24	15.3	10:55 \pm 0:11 (5)	30 January
	mac					10:37 \pm 0:09 (12)	15 January
11	pat	Madeira (P)	32°	10:27	16.5	13:35 \pm 0:41 (22)	24 April
12	mac4N	Tenerife (E)	28°30'	10:42	18.7	11:10 \pm 0:14 (18)	1 February

Location numbers refer to those in Fig. 1. Taxa: mar, *B. vulgaris* ssp. *maritima*; ada, *B.v.* ssp. *adanensis*; mac, *B. macrocarpa*; mac4N, tetraploid *B. macrocarpa*; pat, *B. patula*. Temperature means come from local websites.

diminished with age for ages between 0 and 60 at the moment of transfer but no further effect was observed for plants older than about 60 days. In the last generation of the artificial selection experiment, no age effect was observed for plants older than about 30 days at the moment of transfer (Fig. 2).

The elimination of plants with vernalization requirement as well as the reduction of variation in lag time by including the preselection phase appeared to be successful. After three generations of preselection we disposed of

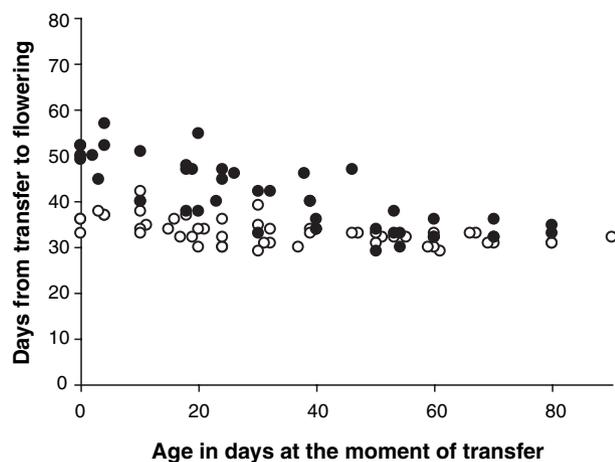


Fig. 2 The effect of size. Plants of various ages were transferred from short days (10 h) to long days (16 h) and the time between the moment of transfer and the first open flower was measured. Closed circles: the preselected material, open circles: the last generation of the short day selection line.

plants that all flowered between 29 and 35 days after a transfer from short days to long days (mean and standard deviation: 32.6 ± 1.8), whereas the lag time before the preselection was occasionally higher, up to 43 days, and in one case 48 days, with even a few plants that did not flower at all (mean and standard deviation for the flowering plants: 35.7 ± 3.0). The estimated critical photoperiod dropped from 15.2 directly measured in the natural populations to 13.2 h in the preselected material (see Table 1). The first figure was strongly influenced by a restricted number of plants which flowered at very long days (up to 24 h), which we, therefore, considered as having a vernalization requirement. The situation *in situ*, where vernalization effectively takes place, would probably be closer to the figure found for the preselected material. We only dispose of data about flowering date, which indeed confirm the considerably later flowering when compared with Mediterranean populations.

Starting with the preselected plant material, we showed that the critical day length was still very variable and could be changed by artificial selection in <10 generations from 13.2 h to <11 h (Fig. 3). The final value corresponds to the values of populations from warmer origins as shown in Table 1. For these populations, especially for *B. vulgaris* ssp. *adanensis* and *B. macrocarpa* the absence of vernalization is complete and cannot bias the results.

The overall h^2 under our conditions could be estimated as 0.56. A selection limit seems to be reached but may be a consequence of the experiment being limited to a day length of 10 h in the initial vegetative phase. At any rate shorter day lengths are ecologically of little importance

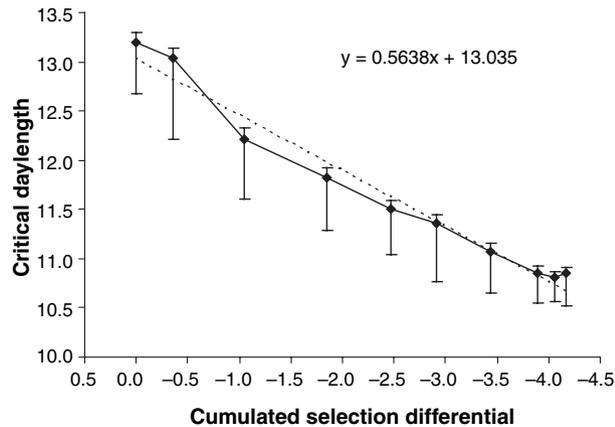


Fig. 3 The selection response in critical day length as a function of the cumulated selection differential. Bars indicate standard deviations (below) and standard errors (above).

because they only occur in higher latitudes during winter when growth is impossible due to low temperatures (Table 1).

The role of energy

The results in Fig. 4 clearly indicate that the plants from the 'long day selection line' flowered much later with less energy (71.0 days, $n = 43$ vs. 44.1 days, $n = 16$),

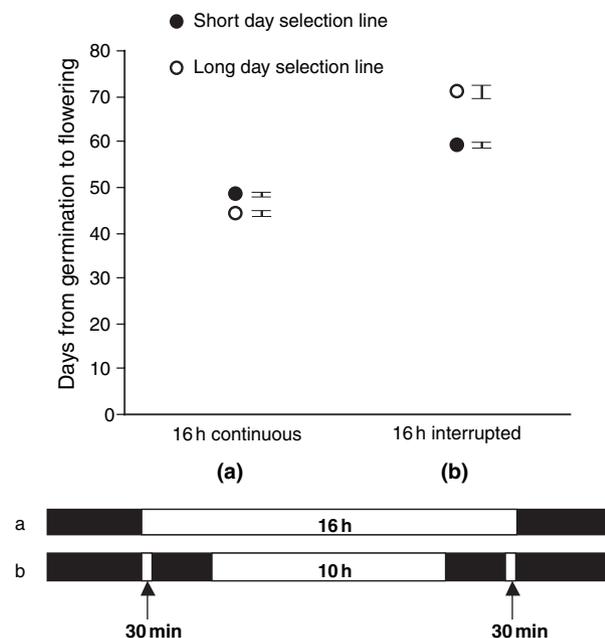


Fig. 4 The effect of two contrasting energy levels with the same photoperiod on the time from germination to the first open flower, comparing the last generations of the 'long day selection line' and the 'short day selection line'. Bars indicate standard errors on both sides of the mean.

whereas the plants from the 'short day selection line' showed a considerably smaller effect (59.0 days, $n = 38$ vs. 48.1 days, $n = 15$). In a two-way ANOVA all means were significantly different, and, crucial for the question we ask, this was also the case for the interaction between selection line and light regime ($P < 0.001$ in all cases). This means that selection has taken place for less sensitivity to lower energy levels during the artificial selection. Apparently both photoperiod and energy played a role in the realization of the lower critical day length.

Quantitative genetic analysis

The results of the parental populations, F1, F2 and backcrosses are visualized in Fig. 5. The means generated by the model we used gave a very good fit with the observed means of the various generations (Table 2) and were (due to the postulated additivity) insensitive to the number of loci. The number of loci was varied to obtain an optimal fit with the standard deviations of the F1, F2 and BCs. With one locus, bimodal distributions in the two backcrosses were generated by the model, giving a very bad fit with the observed distributions. At least 3 (backcross low) or 4 (backcross high) loci were necessary to reach a non-significant chi-square test. The best fit, corresponding to the lowest overall chi-square (combining backcrosses, F1 and F2), was obtained for 7 loci. The resulting standard deviations are presented in Table 2. The optimal values of Σa and Σd (Falconer, 1989) were 1.786 and -0.341 , giving a degree of dominance of -0.19 . The optimal frequency of the 'low' alleles in the 'parent high' population was 0.157 (these are the alleles supposed to be fixed in the 'parent low' population).

Discussion

Our findings demonstrate that considerable genetically based change of flowering date is possible in few generations in this outbreeding species, which apparently contains a considerable level of genetic variation in its natural populations. Several components are potentially responsible for such a change and each shows a significant level of genetic variation: (1) vernalization requirement; (2) the time between flowering induction and its realization; (3) photoperiod sensitivity; and (4) energy requirement. Evolutionary change in natural populations due to climate change can happen simultaneously through all these mechanisms.

We only indirectly discuss vernalization requirement here, since we decided to only consider in this study the effects of day length in plants without vernalization requirement. The problem that arises is the interaction between the two environmental cues in beet (studied in detail in sugarbeet, see Smit, 1983). For this reason, we cannot be sure that among the plants we eliminated during the preselection there were no individuals with,

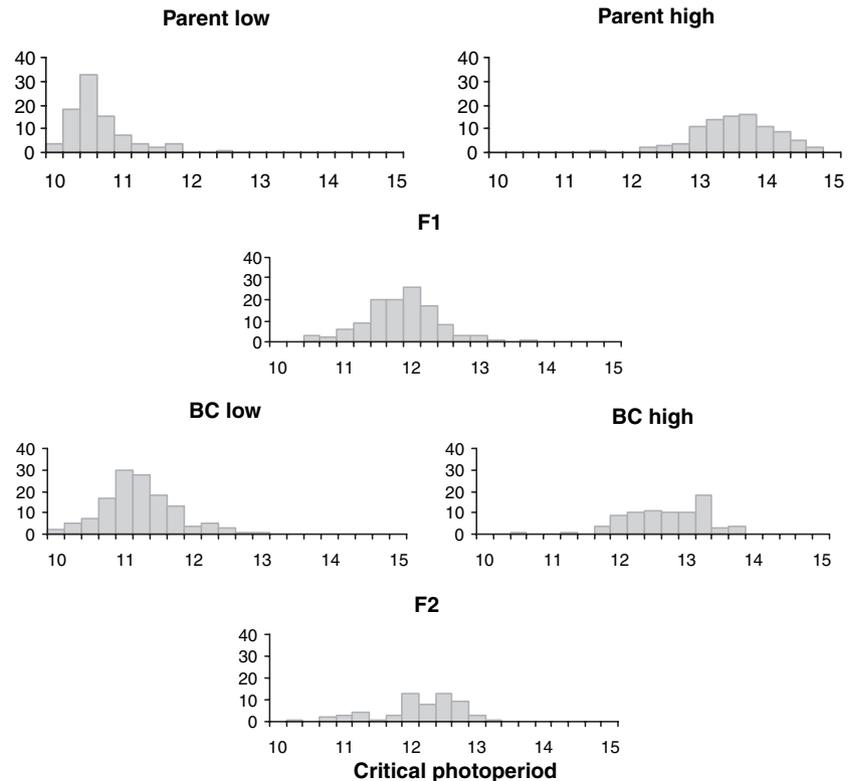


Fig. 5 The numbers of plants for each 15 min critical day length class in the various parental populations and crosses. Parent high = the parental population before selection (the preselected material); parent low = the parental population after selection (the last generation of the 'short day selection line'); BC = backcross.

Table 2 Observed means and standard deviations together with the values generated by the model for the optimal fitting case of seven loci with an equal effect.

Population	<i>n</i>	Mean		SD	
		Observed	Model generated	Observed	Model generated
Parent low	88	10.64	(10.64)	0.448	(0.488)
BC low	134	11.25	11.23	0.547	0.523
F1	119	11.86	11.85	0.558	0.490
F2	61	11.98	12.13	0.635	0.643
BC high	81	12.71	12.69	0.614	0.634
Parent high	93	13.57	13.55	0.597	(0.597)

The values in parentheses are the observed values as such used for the estimation of the others.

for instance, a weak vernalization requirement but a high critical day length. In the preselection phase, we therefore could already have selected for lowering day length requirement, thus underestimating the later selection response.

Genetic variation in the lag time between flowering induction and its realization allows an immediate response to a selection pressure on flowering time in natural conditions, but this variation was rather limited (no further change was observed in the third generation of the preselection) and gave the impression of being

caused by a few individuals with a 'developmental handicap', perhaps due to deleterious mutations, which were thus eliminated.

Experiments under completely controlled conditions in growth chambers at constant light intensity during 'day' only measure the effects of photoperiod. We have chosen for practical reasons to perform our experiments in a glasshouse, thus being limited to the winter months for getting short days and having variable light intensities, positively correlated with photoperiod. Actually, this reflects what happens under natural conditions, but the influence of light intensity on flowering induction is usually neglected in other studies. Our conclusion is that genetic variation for the sensitivity to energy availability is present although we showed this only indirectly by a limited experiment.

The quantitative genetic analysis suggested that the response to selection was a case of several loci with a small effect rather than few loci with a large effect. This does not completely exclude the possibility of detecting individual loci by means of a QTL analysis, because instead of all loci having an equal effect, the same effects on the variances can be explained by a larger number of loci with variable and therefore sometimes higher effects on the character. For the moment, no genes in the photoperiod pathway are known that show variable alleles or contrasting allele frequencies in natural populations of *A. thaliana* (but see El-Assal *et al.*, 2001, for a

difference between two laboratory strains of *A. thaliana* with different alleles of *CRY2*, a gene involved in the long day pathway). Nevertheless a search for a shift in the frequencies of the orthologues of genes known in other species may be worth the effort. Finally, what we are interested in is how (i.e. by which genes) natural selection acts in spatially heterogeneous and changing environments on the physiology of flowering induction of which the genetic basis is now already understood in much detail in several species. Our next step using a similar approach as in the present paper will be a study of the joint influences of day length and vernalization on flowering time to get an idea of what happens over a larger range of latitudes, including northwest Europe.

Few data are available thus far of this kind of potential evolutionary change in natural populations, which is certainly due to the fact that long-term experiments are needed for the physiological characters we studied. This may also be the reason why the interface of population genetics and whole plant physiology has remained an underdeveloped field. This is changing, however, now more and more is known about the genes responsible. In *Arabidopsis thaliana* and a few other species the major genes involved in flowering induction are known (Mouradov *et al.*, 2002; Simpson & Dean, 2002; Boss *et al.*, 2004; Putterill *et al.*, 2004). Recent studies of Stinchcombe *et al.* (2004) and Caicedo *et al.* (2004) describe a latitudinal cline in flowering time in *A. thaliana* where the genes *FRI* and *FLC* and their epistatic interaction play a role. Lempe *et al.* (2005) found considerable variation in response to vernalization, day length and ambient temperature among 155 wild populations. Although it concerns quite a different kind of species, the study on evolutionary change in photoperiod sensitivity as a reaction to global warming in the pitcher-plant mosquito *Wyeomyia smithii* by Bradshaw & Holzapfel (2001) and Bradshaw *et al.* (2003) should also be mentioned here. They compared populations sampled over the period from 1972 to 1996. We sampled our study populations in 1989 and have the advantage that the seeds are long-lived, which opens up the possibility of a comparison with future samples in a single experiment without the bias of maintaining the populations under artificial conditions.

Warming is not the only possible climate change in the near future. In our study region, temperatures could drop substantially and rapidly as a result of changes in the Atlantic circulation (Bryden *et al.*, 2005). Potential evolutionary change for day length requirement in the other direction (later flowering under increasing day length) would not be so easy to demonstrate. Later flowering, whatever the conditions, may always be caused by mutations with a negative effect on a plant's general metabolic efficiency. More complicated experiments that take fitness into account are then necessary. Nevertheless, we predict that genetic change is, at least initially,

equally well possible in both directions, because there seems to be no special reason for an asymmetric response to selection.

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