*Journal of Ecology* 2002
**90**, 693–703

# Vernalization requirement of wild beet *Beta vulgaris* ssp. *maritima*: among population variation and its adaptive significance

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**Summary**

**1** Seven populations of *Beta vulgaris* ssp. *maritima* (wild beet) situated along a latitudinal cline were studied for their vernalization requirement and its consequences for fitness.

**2** Various cold regimes were applied in glasshouses and experimental gardens with plants of different ages. Three additional experimental sites (on the French Mediterranean, Atlantic and North Sea coasts) situated near three of the sampled populations, and thus including a reciprocal transplant design, were used to evaluate the influence of latitude under natural conditions. Survival and plant size were measured over 3 years.

**3** The vernalization requirement was greater in plants from more northern origins. The level of cold required to allow flowering overcompensated for the colder springs, so that northern plants in northern sites flowered less than southern plants in southern sites.

**4** Young seedlings were more difficult to vernalize than plants that had already developed vegetative rosettes.

**5** Differences in vernalization requirement seem to be an adaptive response to spring temperatures and season length in a particular latitude. A whole winter vernalization almost always led to flowering in the subsequent year whatever the latitude or geographical origin.

**6** Plants from the Atlantic and Channel coasts showed the highest lifetime reproductive success at all sites. Southern populations were better adapted to disturbed habitats as shown by their higher first-year reproductive success. The North Sea population had a lower reproductive success than the Atlantic populations, even in its native environment.

*Key-words*: latitudinal cline, lifetime reproductive success, reciprocal transplantation experiment, spring temperatures

*Journal of Ecology* (2002) **90**, 693–703

## Introduction

The distribution of within-species variation in life history traits has often been shown to be related to climatic variation, along geographical or altitudinal gradients (e.g. Smith 1927; Cooper 1952; Rao & Witcombe 1977; Reinartz 1984; Lacey 1988). The existence of such variation along environmental gradients is often cited as evidence of natural selection (e.g. Ollerton & Lack 1992; Fox & Kelly 1993), although, in most studies, it has been demonstrated by comparisons of genotypes from various origins under common glasshouse or common garden experiments (Venable 1984). These growing conditions are likely to be different to those where selection acts and such studies may therefore be of limited use in interpreting variation observed in the wild (Antonovics & Primack 1982). The traits of an individual plant phenotype are the result of both the adaptation of its genotype to the prevailing conditions and reaction of this genotype to the particular conditions. As the expression of genetic variability for life history traits, and therefore its estimation, can vary according to the environment (Mazer & Schick 1991), studies in a series of environments can help assess the relative importance of genetic and

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environmental factors in plant development. A single environment offers no opportunity for such separation (Lawrence 1984).

The timing of first reproduction in plants has been shown to be controlled by the interaction of environmental and internal factors (Murfet 1977; Bernier 1988; Bernier 1992; Simpson *et al*. 1999). In a seasonally varying environment, it can be regulated by temperature, e.g. through vernalization (Chouard 1960; Klinkhamer *et al*. 1987), day length (Vince-Prue 1975) or drought (Fox 1990). The internal (i.e. physiological) status of the plant, represented by levels of hormones or other substances, is determined by a combination of genetic and environmental factors. If genetic variation in the sensitivity to environmental factors exists, then genotype–environment interactions will occur. The physiological mechanisms, and the environmental stimuli they respond to, must therefore be taken into account when studying the adaptiveness of the variation.

Vernalization, i.e. the accumulation process during a period of cold by which the plant changes from the vegetative to the reproductive stage, is well documented. Genetically based within-population variation for the year of first reproduction, correlated with the vernalization requirement, has been reported in numerous wild plant species, e.g. *Thlaspi arvense* (McIntyre & Best 1978), *Arabidopsis thaliana* (Napp-Zinn 1987), *Daucus carota* (Lacey 1988) and *Beta vulgaris* ssp. *maritima* (Van Dijk *et al*. 1997). Similarly, 'winter' and 'spring' cultivars can be found in many crops, including wheat, barley and oil seed rape. Vernalization requirements are often under the control of a single or small number of genes (Napp-Zinn 1987) and, in A*rabidopsis thaliana*, considerable progress has been made in the identification of the genes concerned (Simpson *et al*. 1999). There is evidence for a common genetic basis for flowering induction, including the genes associated with vernalization, in all higher plant species (Levy & Dean 1998). In a recent review, Simpson & Dean (2002) discuss the molecular mechanism of vernalization, together with the other pathways controlling flowering time in *A. thaliana*.

First reproduction too early in the life of an individual could have a negative effect on its future reproduction, through either increased post-reproductive mortality or a detrimental effect on subsequent reproductive performance. In winter annuals this may just mean that flowering should occur in spring or summer rather than in the previous autumn. In perennials, on the other hand, flowering may even be postponed to later years, as a combined result of a vernalization requirement and a size threshold. In different environments, different sensitivities to environmental stimuli (low temperatures in our case) will optimize fitness.

*Beta vulgaris* subsp *maritima* (L) Arcangeli shows considerable variation in wild populations and genetic data are available for sugarbeet, its cultivated relative (see Letschert 1993 for a taxonomic review). It is a perennial salt-tolerant species, which in northern Europe is mainly found along the coast, but can grow further inland in the Mediterranean area. Adventitious forms can also be found on waste and fallow land and in cultivated fields (Hornsey & Arnold 1979; Boudry *et al*. 1993). Seeds germinate in autumn and in spring (Letschert 1993; H. van Dijk, personal observations) and rosettes bolt and flower in May to July. Plants are mostly self-incompatible and usually wind pollinated, although occasional selfing and insect pollination cannot be excluded. The seeds, which mature in July to October, remain fixed to the plant in 'seed balls' of two to six single-seeded fruit attached to each other by their flower bases, which become swollen during ripening. Plants die unless they develop new vegetative rosettes during the autumn.

Many studies have been conducted on the vernalization requirement of cultivated *Beta vulgaris* ssp. *vulgaris* due to the agronomic importance of this trait (Margara 1960; Lexander 1980; Smit 1983) and its presence or absence shown to be under the control of a single gene: the bolting gene *B* (Munerati 1931). Genotypes carrying the dominant *B* allele have no vernalization requirement and flower under long days (the threshold value being about 14 h, Van Dijk & Boudry 1992). Glasshouse experiments show that the distribution of the *B* allele in wild beet populations has a north–south cline along the Atlantic coastline of Europe (Van Dijk *et al*. 1997). All cultivated beets and wild beets from northern Europe are genotype *bb* and require a cold period to enable flowering induction under long days. The optimal temperature for vernalization of sugarbeet is about 8 °C; at 15 °C vernalization is still possible, but takes considerable time and at higher temperatures devernalization may happen (Smit 1983). Vernalization requirement can be quantified by the length of the period required at a given low temperature. This heritable, quantitative trait has been selected for in cultivated beets to prevent bolting (Desprez 1980; Le Cochec & Soreau 1989). Vernalization sensitivity has been reported to increase with age or size of the plant (Smit 1983), and with day length (Margara 1960), but few data are available concerning variation in wild populations.

We investigated the role of the *B* gene and, more particularly, the quantitative vernalization requirement of *bb* genotypes in the year of first flowering. To examine the variation across a range of environments, both among and within populations, we established glasshouse and multisite field experiments using seeds collected from wild populations at different latitudes (correlated with varying spring temperatures). Genetic differences between populations, i.e. phenotypic differences in a common environment may thus be interpreted as adaptations to latitudinal conditions. Year of first flowering, number of reproductive events, plant productivity (measured as size) and survival were recorded over 3 successive years in the multisite experiment. We used the latter data to examine the fitness consequences of a vernalization requirement.

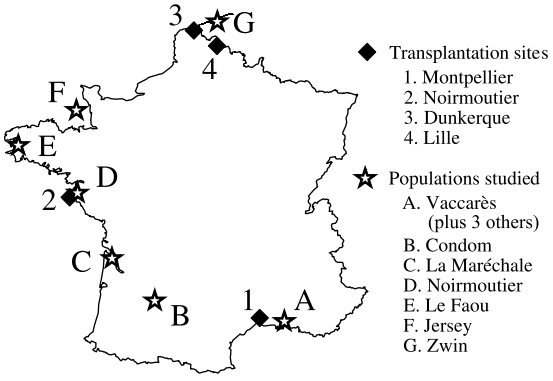


**Fig. 1** Location of the seven studied populations (A to G) and the four experimental sites (1–4) in France, the Channel Islands and Belgium.

## Materials and methods

### PLANT MATERIAL AND HABITAT CHARACTERISTICS

Seed-balls were sampled from seven wild beet populations (A–G, Fig. 1) chosen as representative of the variability observed along the north–south cline for the major gene *B* (Van Dijk et al. 1997). Due to the limited number of seeds per plant and the lower germination rate of the French Mediterranean coast populations in some of the experiments, we had to use seeds from four different populations in this area in order to obtain enough seeds. All were within 30 km of Vaccarès (Fig. 1) and are grouped for convenience ('population' A). The other (half-sib) families were sampled strictly within populations.

The seven accessions came from habitats, classified according to Hautekèete *et al*. (2002) as: A, roadside; B, field; C, seawall; D, harbour; E, rocks; F, cliff; G, estuary. Latitude and spring temperature data were obtained from the nearest meteorological stations (mean temperatures over the period 1961–90 provided by Météo France, Table 1). Mean spring temperatures are generally well correlated with latitude: only at Agen, near the inland population, and, to a lesser extent at Lille, the inland transplantation site, temperatures were slightly lower than expected for their latitude, with larger fluctuations.

### GLASSHOUSE EXPERIMENTS

The glasshouse experiments allowed us to make a comparison of the experimental populations under controlled conditions in order to classify flowering behaviour with regard to the first period of vernalization experienced. Did plants flower without any vernalization, or did they require light (spring) or longer (winter) vernalization. We also examined whether populations showed different flowering responses when exposed to a long cold period at the young seedling stage to represent autumn germination.

#### *The absolute vernalization requirement experiment*

The frequency of plants without any vernalization requirement was estimated in all populations except F. A total of 1378 seedballs, collected in August 1989 from 53 mother plants (eight or nine per population) were sown synchronously in autumn and the 788 germinated seedlings (mean number and standard deviation per family: 14.9 ± 4.2) were grown in 1-L pots, randomly distributed under homogeneous conditions in the University of Lille experimental glasshouses. Annual habit was detected by growing plants under 'standard glasshouse conditions': long days (16 h, additional light being provided by high pressure sodium lamps) at-non-vernalizing temperatures (20 °C). Only individuals carrying the *B* allele are then able to bolt and flower; '*bb*' genotypes, which require vernalization, remain vegetative (Margara 1960). Plants were classified as flowering or non-flowering 150 days after germination.

#### *The moderate vernalization experiment*

Non-flowering plants from the previous experiment were placed outside in May and received moderate vernalization during the spring. Plants were classified as flowering or non-flowering at the end of the summer.

**Table 1** Mean temperatures per month in the period January to June for eight meteorological stations near the sampled populations and transplantation sites. Mean maximum and mean minimum temperatures can be calculated by adding or subtracting the second number

| | Population or site, nearest meteorological station and the latitude of the latter | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Month | A/1 Sète 43°24′ | B Agen 44°11′ | C La Rochelle 46°09′ | D/2 Ile d'Yeu 46°42′ | E Lanvéoc 48°17′ | F Jersey 49°11′ | G/3 Dunkerque 51°03′ | 4 Lille 50°35′ |
| Jan | 7.4 ± 2.8 | 5.1 ± 3.4 | 5.9 ± 2.6 | 6.9 ± 2.2 | 6.7 ± 2.3 | 6.0 ± 2.3 | 4.3 ± 2.1 | 2.7 ± 2.4 |
| Feb | 8.4 ± 2.9 | 6.7 ± 4.1 | 6.9 ± 3.0 | 7.1 ± 2.3 | 6.7 ± 2.5 | 6.0 ± 2.3 | 4.4 ± 2.2 | 3.4 ± 2.9 |
| Mar | 10.3 ± 3.3 | 8.6 ± 5.0 | 8.7 ± 3.3 | 8.6 ± 2.7 | 7.9 ± 2.9 | 7.2 ± 2.8 | 6.4 ± 2.6 | 5.8 ± 3.6 |
| Apr | 12.8 ± 3.4 | 11.3 ± 5.1 | 11.1 ± 3.6 | 10.6 ± 3.1 | 9.5 ± 3.2 | 9.6 ± 3.0 | 8.6 ± 2.6 | 8.6 ± 4.3 |
| May | 16.4 ± 3.4 | 14.8 ± 5.4 | 14.3 ± 3.6 | 13.4 ± 3.1 | 12.1 ± 3.3 | 12.1 ± 3.3 | 12.0 ± 2.9 | 12.4 ± 4.6 |
| Jun | 20.2 ± 3.7 | 18.2 ± 5.6 | 17.5 ± 3.8 | 16.3 ± 3.4 | 14.8 ± 3.4 | 14.9 ± 3.3 | 14.8 ± 2.7 | 15.2 ± 4.7 |

*The seedling overwintering experiment*

To assess the ability of young autumn seedlings to be vernalized during their first winter, 1296 seedballs from the same six populations (a further six half-sib families per population) were sampled in August 1991 and sown in late autumn under the standard conditions. In December, 232 2-week-old seedlings (38.7 ± 11.9 per population) were transferred to a cold glasshouse with natural day length and temperatures maintained between 5 and 15 °C. The number of flowering plants was recorded during the following summer.

*The cold chamber and return experiments*

Two small-scale experiments were carried out with plants from all seven origins to quantify the vernalization requirement. Three-week-old seedlings ($n = 20$ per population per treatment) were given 20, 40 or 60 days of constant low temperatures between 5 and 7 °C in a cold growth chamber, with a low light intensity, after which flowering percentage was measured under standard glasshouse conditions 3 months later.

Two-month-old rosettes from the seven origins, grown under non-vernalizing conditions, were placed outside in pots at site 4 (see Fig. 1) on 1 September 1997. On the 1st of each following month a sample was brought back to the glasshouse, and 3 months later the number of flowering plants was recorded. All remaining plants were left outside from March 1998.

### MULTI-SITE FIELD EXPERIMENT

To assess the roles of the *B* gene and the quantitative vernalization requirement on the timing of first reproduction and the consequences for survival after first reproduction, a total of 3252 seedballs, from the same mother plants as used to determine absolute vernalization requirement, were sown under standard conditions. At the beginning of May 1991, 2000 3-week-old seedlings (37.7 ± 5.6 per family:) were transplanted into four experimental fields at different latitudes within France (1–4, Fig. 1) with plants from each family at each site. Seedlings were regularly separated by 50 cm in a random design in each experimental field. Weeding was performed several times per year to prevent competition from other plants and to eliminate young beet seedlings during the second and third years of the experiment. Three sites (Montpellier, Noirmoutier, Dunkerque) were located close to the coastal source populations and the fourth site (Lille) was inland at the University campus. Although Dunkerque is the most northerly site (Fig. 1), Lille, due to its inland position, is colder and therefore better positioned at the end of the cline than in the centre (see Results).

The experimental design includes six populations transplanted to four sites with reciprocal transplants between populations A, D and G (sites 1, 2 and 3, respectively). The plants were scored once every 2–3 months over 3 successive years and the following traits recorded: (i) life history traits (flowering and survival); and (ii) adult stage size characters (height of the central shoot and diameter of flowering plants at seed maturity). Overall size was calculated as the product of diameter and height of the central shoot, the resulting value being proportional to plant shoot 'surface' in three-dimensional space.

### DATA ANALYSIS

Variation of quantitative vernalization requirement among populations (considering only C–G) and environments or treatments was measured by comparing the percentage of flowering. The few plants without vernalization requirement in populations C and D were eliminated if possible, or else percentages were corrected. Each environment and treatment combination represents a certain cold dose, depending on the prevailing temperatures and other environmental conditions and also on the age of the plants. This cold dose, hereafter the 'vernalization index', was estimated by minimizing the combined sums of squares of the deviations from the linear regression of flowering percentage on vernalization index for each of the five populations using arcsine transformed percentages. Only the intermediate percentages including the last stable 0% and the first stable 100% were used. Vernalization index ranges from 0 (no cold) to 100 (the coldest treatment: complete overwintering of rosettes outdoors at site 4).

Differences in qualitative traits, flowering and survival, among sites and populations were examined by log-linear analysis using the Statgraphics' log-linear analysis procedure for categorical data (Sokal & Rohlf 1981). The analysis is based on fitting a log-linear model to the cell frequencies. Tests are based on maximum likelihood ratios. Differences among sites, populations and interactions between these main effects were tested separately in different models.

Differences in quantitative traits, such as plant size, among sites and populations, were analysed using the Statgraphics' multifactor analysis of variance procedure (using type III sums of squares because of the varying numbers of plants in the groups compared).

## Results

### ANALYSIS OF VERNALIZATION REQUIREMENT UNDER CONTROLLED CONDITIONS

*The absolute vernalization requirement experiment*

The ability to flower under non-vernalizing glasshouse conditions, a trait under the control of the *B* gene, varied significantly with plant origin (Table 2), along a

**Table 2** Vability for vernalization requirement among populations tested under non-vernalizing greenhouse conditions (separates *B* genotypes flowering from *bb* genotypes non-flowering), moderate vernalization (shows differences in requirement for flowering among *bb* genotypes) and overwintering of young seedlings (flowering plants include *B* genotypes and those *bb* genotypes where the vernalization requirement is fulfilled)

| | Population | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | | B | | C | | D | | E | | G | |
| Treatment | *n* | % | *n* | % | *n* | % | *n* | % | *n* | % | *n* | % |
| Non-vernalizing conditions | | | | | | | | | | | | |
| Total number of plants | 116 | | 137 | | 164 | | 125 | | 117 | | 129 | |
| Flowering plants and percentage | 72 | 62 | 125 | 91 | 14 | 3 | 4 | 3 | 0 | 0 | 0 | 0 |
| Moderate vernalization | | | | | | | | | | | | |
| Total number of plants | 34 | | 14 | | 100 | | 121 | | 116 | | 129 | |
| Flowering plants and percentage | 34 | 100 | 14 | 100 | 57 | 57 | 85 | 70 | 89 | 77 | 0 | 0 |
| Young seedlings exposed to long vernalization (overwintering) | | | | | | | | | | | | |
| Total number of plants | 22 | | 34 | | 36 | | 50 | | 55 | | 35 | |
| Flowering plants and percentage | 21 | 95 | 34 | 100 | 19 | 52 | 31 | 62 | 13 | 24 | 0 | 0 |

north–south cline, as previously described by Van Dijk *et al.* (1997). A high percentage of plants in southern populations (A and B) flower without vernalization, while no plants from northern populations E and G are capable of flowering under these conditions. A proportion of plants flower from populations A, B, C and D, confirming the presence of within-population polymorphism. Precise *B* allele frequencies would be difficult to estimate from these data due to partial penetrance of the annual habit in *Bb* genotypes (Owen 1954; Boudry *et al*. 1994; Abe *et al*. 1997).

Population F was not included in this experiment, but no Channel Island populations displayed flowering without vernalization in previous experiments with smaller plant numbers (Van Dijk *et al*. 1997).

### *The moderate vernalization experiment*

Some ($n = 514$) of the plants that had not flowered under non-vernalizing conditions were placed outside in May, when they were about 5 months old, and received moderate vernalization during the spring. Of these, 279 flowered during the summer of that same year (Table 2). The distribution differs significantly among populations and also shows a north–south cline.

### *The seedling overwintering experiment*

A north–south cline for vernalization requirement was again shown with 118 of the 232 young seedlings vernalized over winter flowering in the following summer (Table 2). Differences between populations included the effects of both absolute and quantitative vernalization requirements. The lower flowering percentages, compared with the effects of moderate vernalization on young rosette plants, imply that the effects on young plants, although present, may not be as strong.

### *The cold chamber experiment*

Three-week-old seedlings given 20, 40 or 60 days of constant low temperatures showed differences in vernalization requirement between populations (Table 3). The longest period was apparently sufficient for complete vernalization of the southern Atlantic plants, whereas none of the northernmost plants flowered.

### *The return experiment*

Sufficient cold exposure accumulated over 3 months (September to November) for almost all southern plants, whereas most northern plants needed a longer period (Table 4). The degree of vernalization achieved increased gradually during the rest of the winter, with the exception of January where, for unknown reasons, the plants were systematically less vernalized on the first of February than on the first of January A complete winter appeared to be sufficient for all plants, except for one of the 45 plants from population G.

## THE MULTISITE FIELD EXPERIMENT

Marked variability for the ability to flower in the first summer was observed among populations and

**Table 3** Flowering percentage of 20-day-old seedlings after 20, 40 or 60 days in a cold chamber. $n = 20$ in all cases

| | Number of days in the cold chamber | | |
|---|---|---|---|
| Population | 20 days | 40 days | 60 days |
| C | 30% | 85% | 100% |
| D | 0% | 50% | 75% |
| E | 10% | 55% | 65% |
| F | 0% | 5% | 30% |
| G | 0% | 0% | 0% |

**Table 4** The 'return experiment' in which 2-month-old plants were placed outside on 1 September. On the first of each following month the number of plants indicated was returned to the greenhouse, after which the percentage of plants flowering was measured

| | 1 Oct | | 1 Nov | | 1 Dec | | 1 Jan | | 1 Feb | | 1 Mar | | Left outside | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Population | *n* | % | *n* | % | *n* | % | *n* | % | *n* | % | *n* | % | *n* | % |
| C | 10 | 0 | 10 | 30 | 10 | 100 | 10 | 100 | 9 | 100 | 8 | 100 | 30 | 100 |
| D | 15 | 0 | 15 | 0 | 15 | 93 | 15 | 100 | 14 | 100 | 12 | 100 | 45 | 100 |
| E | 10 | 0 | 10 | 20 | 10 | 90 | 10 | 100 | 7 | 86 | 5 | 100 | 30 | 100 |
| F | 10 | 0 | 10 | 0 | 10 | 40 | 9 | 78 | 6 | 67 | 7 | 100 | 28 | 100 |
| G | 15 | 0 | 15 | 0 | 15 | 20 | 15 | 67 | 14 | 50 | 14 | 86 | 45 | 98 |

among sites (Fig. 2a). Differences among sites, among populations and site–population interactions were all highly significant ($P < 0.001$) for flowering in the first and second years. Individuals that flowered in their first year would have included *B* genotypes as well as those *bb* genotypes that were vernalized during the first spring. In southern populations A and B, where the *B* allele has a high frequency, almost all the plants flowered in the first summer and no great difference was observed among sites (Fig. 2a). In contrast, the other populations showed a large plasticity for first summer flowering between the experimental sites, which may be related to the threshold value of the quantitative vernalization requirement in *bb* genotypes. Thus, more *bb* genotypes will flower in their first summer when spring temperatures are lower, so that for all populations numbers were lowest at the southernmost site 1 (only one plant of each of the northern populations E and G, Fig. 2a). The highest percentage of first year flowering, over all populations, occurred in the coldest, most vernalizing site (4). In sites 3 and 4, only the most northern population (G) exhibited a threshold value high enough to prevent massive first year flowering.

All the plants that did not flower during the first summer flowered in their second summer or died before flowering at all, except a single plant from the G population in site 1, which flowered for the first time in its third year. First flowering in the second year therefore shows a pattern that is directly complementary to that in the first.

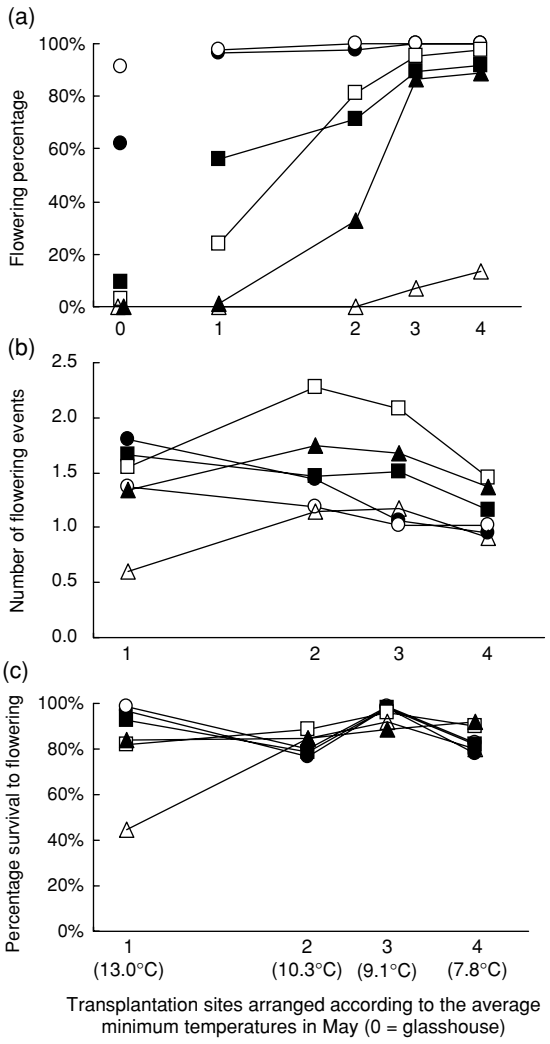


**Fig. 2** Variation among populations and among sites for: (a) the percentage of plants flowering in the first year (within the plants that flowered at least once); (b) the mean number of flowering events within 3 years; (c) percentage survival to flowering. Populations: A = ●, B = ○, C = ■, D = □, E = ▲, G = △.

## COMPARATIVE ANALYSIS OF COLD REQUIREMENT IN ALL EXPERIMENTS

We were able to compare the effects of cold at the four transplantation sites with those in the various glasshouse experiments by calculating the 'vernalization index' of each site or treatment (Table 5). Populations A and B, which have high frequencies for the *B* allele, were excluded, but a good fit to the linear regression model was obtained for the other populations ($R^2$ between 0.87 and 0.98; $P < 0.01$ in all cases). This means that all populations have about the same order of sensitivity to the various situations. Differences in slope are due to differences in within-population variability, steeper slopes corresponding to more homogeneity. The vernalization index at which 50% of the plants flowered is also given in Table 5, clearly confirming the north–south cline for the populations studied.

**Table 5** A synthesis of the vernalizing effects in all experiments. The presented arcsine transformed flowering percentages were used for the estimation of the vernalization index for each of the environments or treatments as well as the index value at which 50% of the plants of each population flower. The data used for the linear regression are shown in bold

| Experiment | Treatment or site | Flowering percentages (arcsine transformed) in populations | | | | | Vernalization index (arbitrary units) |
|---|---|---|---|---|---|---|---|
| | | C | D | E | F | G | |
| Greenhouse; Return | No cold; Return on 1 Oct | **0** | 0 | **0** | 0 | 0 | 0.0 |
| Return | Return on 1 Nov | **37** | 0 | **30** | 0 | 0 | 19.8 |
| Cold chamber | 20 days | **37** | **0** | **20** | 0 | 0 | 23.4 |
| Transplantation | Site 1 | **54** | **33** | **6** | | 0 | 28.0 |
| Seedling overwintering | | **49** | **56** | **33** | | 0 | 39.5 |
| Cold chamber | 40 days | **75** | **50** | **53** | **14** | 0 | 48.5 |
| Transplantation | Site 2 | **64** | **72** | **39** | | 0 | 48.9 |
| Moderate vernalization | | **51** | **62** | **68** | | 0 | 49.5 |
| Cold chamber | 60 days | **100** | **67** | **60** | **37** | **0** | 59.3 |
| Transplantation | Site 3 | **80** | **87** | **76** | | **17** | 64.7 |
| Transplantation | Site 4 | **82** | **91** | **78** | | **24** | 67.5 |
| Return | Return on 1 Dec | **100** | **83** | **80** | **44** | **30** | 68.9 |
| Return | Return on 1 Feb | 100 | **100** | **76** | **61** | **50** | 76.6 |
| Return | Return on 1 Jan | 100 | 100 | **100** | **69** | **61** | 84.5 |
| Return | Return on 1 Mar | 100 | 100 | 100 | **100** | **76** | 95.0 |
| Return | Left outside | 100 | 100 | 100 | 100 | **91** | 100.0 |
| Linear regression: | | | | | | | |
| $R^2$ | | 0.87 | 0.89 | 0.89 | 0.97 | 0.98 | |
| Slope | | 1.25 | 1.59 | 1.19 | 1.72 | 2.08 | |
| Vernalization index value with 50% flowering | | 34.7 | 43.6 | 47.4 | 69.7 | 80.1 | |

## FITNESS CONSEQUENCES OF VERNALIZATION AS MEASURED IN THE MULTISITE EXPERIMENT

### *Survival to first flowering and following first flowering*

Survival from the seedling stage to first flowering showed little variation between populations except at the southernmost site (1), where population G showed much greater mortality (Fig. 2c). This northernmost population G showed a delay in flowering to the second year in most sites (Fig. 2a). The total percentage of plants that flowered at all in population G was only 45% because of decreased survivorship (Fig. 2c). Population E showed a similar delay of nearly all flowering to the second year in site 1 without any decrease in survivorship. The apparent 'cost' effect in G is therefore population- or latitude-specific, rather than a general trade-off.

The percentage of plants flowering more than once shows a different distribution to the vernalization requirement: it is low in southern populations A and B (44% and 27%, respectively, over the four sites together), increases in populations C, D and E (58%, 78% and 66%), and decreases again in the northernmost population (G, 27%). Populations from the Atlantic coast appear therefore to have a higher post-reproductive survival than Mediterranean and North Sea populations. This is in accordance with the findings of Hautekèete *et al*. (2002), although the life spans they measured were generally longer because they kept their plants in the glasshouse, thus ruling out external causes of mortality.

### *Number of flowering events over the 3 years*

The combined effects of the variability of the year of first flowering and of flowering once or more than once leads to a large variability in the observed number of flowering events over the 3 years. This number can be considered as a quantitative trait, ranging from 0 to 3 for each plant. The low percentage of plants surviving to the end of year 3 (18%) reduces any right-censored bias generated by ending the experiment before all the plants were dead in all sites. The mean values for the number of flowering events were greatest in the middle latitude site (2) and for middle latitude populations (C, D and E, Fig. 2b). As would be expected, the percentage of plants flowering more than once shows a similar pattern. An analysis of variance shows that the site, population and site–population interaction all have highly significant effects on the frequency of flowering more than once ($P < 0.001$ in all cases).

Data from the reciprocal transplant component of the experiment show that the native population (A in 1, D in 2) had the highest mean number of flowering events over the 3 years, except at site 3 where the 'home' population (G) flowers significantly less often than some of the 'alien' populations. Tukey-Kramer mean comparisons showed that A was significantly higher than B, E and G, but not C and D at site 1, and D higher than all the other populations at site 2.

One qualitative trait, the year of first flowering (first or second, Fig. 2a), shows a north–south cline, while the other, flowering more than once, is higher on the Atlantic coast than on the Mediterranean and the North sea coasts, leading to two different shapes of

reaction norms for the number of flowering events within the 3 years. Populations A, B and C exhibit declining reaction norms for the four sites (Fig. 2b), ordered according to the percentage of first year flowering (Fig. 2a), while populations D, E and G show convex reaction norms, with maximum flowering events in site 2 on the Atlantic coast. Were annual reproductive output invariable between sites, we would conclude that populations A and D appear to be well adapted to their own 'native' sites (1 and 2), while the northern population G seems to have a lower reproductive success than some other populations in its 'native' site 3. A geographical cline is suggested by the fact that population C is intermediate between populations A and D for number of flowering events (Fig. 2b) and population E is intermediate between D and G.

### *Size of the flowering plants*

Plants were considerably bigger when grown in the northern sites 3 and 4 compared with sites 1 and 2, and plants from origins C, D, E and G are systematically bigger than plants from origins A and B (Table 6). Population and site effects are all highly significant ($P < 0.001$) for the variation observed in all years in both plant diameter and height of the central shoot. The population–site interactions are all significant at the 5% level. This implies genetic differences between the populations and variation in plasticity between sites for these characters.

We did not estimate seed-set or reproductive success. Evaluation of seed production in wild beet is very difficult due to the large size of the plants and because seeds may fall at maturity. Nevertheless, the size of the plants multiplied by the number flowering over 1 or 3 years can be used to compare relative reproductive success of the plants in our study (Fig. 3). Population A is the best in its native site (1) for first year reproductive success, whereas, over its lifetime, population D is the best in its native site (2), but at site 3, the native population G is relatively unsuccessful, outperforming only A and B when all years are considered.

## Discussion

### GEOGRAPHIC DISTRIBUTION OF THE GENETIC VARIABILITY FOR VERNALIZATION REQUIREMENT

The results show that there is a north–south cline for both the absolute (i.e. bolting gene *B*-related) and quantitative vernalization requirement through the studied area. The former is expressed under both glasshouse conditions and in multisite field trials. A colder spring climate leads to more bolting in plants of all origins, which is a logical consequence of vernalization. Thus there is considerable plasticity between sites with

**Table 6** Sizes of the flowering plants in the multi-site exeriment. For each combination of population and site the following are shown: the initial number of plants at age 0 and for each age the number of flowering plants, the mean diameter (d) and the mean height (h) of the central shoot. The mean values for all populations, for all sites and the overall means are weighted by plant number. The populations in their native sites are shown in bold

| | | Population | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | | | B | | | C | | | D | | | E | | | G | | | All populations | | |
| Site | Age | *n* | d | h | *n* | d | h | *n* | d | h | *n* | d | h | *n* | d | h | *n* | d | h | *n* | d | h |
| 1 | 0 | **64** | | | 86 | | | 81 | | | 96 | | | 96 | | | 87 | | | 510 | | |
| | 1 | **60** | **59** | **43** | 83 | 45 | 44 | 42 | 68 | 46 | 19 | 97 | 16 | 1 | 80 | 31 | 0 | | | 205 | 59 | 41 |
| | 2 | **47** | **73** | **37** | 33 | 60 | 38 | 66 | 98 | 59 | 78 | 100 | 44 | 81 | 97 | 35 | 39 | 100 | 33 | 344 | 91 | 42 |
| | 3 | **8** | **55** | **42** | 2 | 60 | 40 | 27 | 67 | 52 | 52 | 92 | 46 | 47 | 83 | 27 | 13 | 70 | 43 | 149 | 80 | 41 |
| 2 | 0 | 62 | | | 80 | | | 84 | | | **85** | | | 89 | | | 79 | | | 479 | | |
| | 1 | 47 | 85 | 53 | 64 | 71 | 52 | 47 | 154 | 69 | **62** | **165** | **40** | 25 | 169 | 9 | 0 | | | 245 | 123 | 48 |
| | 2 | 38 | 86 | 57 | 31 | 42 | 38 | 65 | 132 | 80 | **76** | **183** | **82** | 76 | 152 | 57 | 67 | 140 | 75 | 353 | 136 | 68 |
| | 3 | 4 | 95 | 60 | 0 | | | 11 | 132 | 55 | **56** | **152** | **57** | 54 | 118 | 40 | 24 | 94 | 44 | 149 | 127 | 49 |
| 3 | 0 | 61 | | | 87 | | | 88 | | | 93 | | | 91 | | | **77** | | | 497 | | |
| | 1 | 60 | 83 | 54 | 86 | 68 | 52 | 77 | 155 | 93 | 85 | 170 | 67 | 70 | 200 | 51 | **5** | **140** | **57** | 383 | 136 | 64 |
| | 2 | 5 | 74 | 52 | 3 | 80 | 53 | 53 | 127 | 79 | 82 | 130 | 68 | 70 | 105 | 54 | **71** | **124** | **72** | 284 | 120 | 67 |
| | 3 | 0 | | | 0 | | | 3 | 46 | 63 | 26 | 106 | 71 | 12 | 106 | 34 | **14** | **82** | **71** | 55 | 97 | 62 |
| 4 | 0 | 78 | | | 90 | | | 87 | | | 89 | | | 87 | | | 82 | | | 513 | | |
| | 1 | 61 | 66 | 65 | 75 | 59 | 66 | 65 | 130 | 100 | 78 | 149 | 84 | 71 | 160 | 68 | 9 | 124 | 16 | 359 | 114 | 75 |
| | 2 | 13 | 112 | 84 | 17 | 80 | 72 | 36 | 126 | 79 | 51 | 125 | 66 | 48 | 112 | 56 | 66 | 130 | 75 | 231 | 120 | 70 |
| | 3 | 0 | | | 0 | | | 0 | | | 0 | | | 0 | | | 0 | | | 0 | | |
| All sites | | | | | | | | | | | | | | | | | | | | | | |
| | 0 | 265 | | | 343 | | | 340 | | | 363 | | | 363 | | | 325 | | | 1999 | | |
| | 1 | 228 | 73 | 54 | 308 | 60 | 53 | 231 | 132 | 82 | 244 | 156 | 62 | 167 | 178 | 52 | 14 | 130 | 31 | 1192 | 113 | 60 |
| | 2 | 103 | 83 | 51 | 84 | 58 | 45 | 220 | 120 | 73 | 287 | 135 | 65 | 275 | 117 | 50 | 243 | 126 | 67 | 1212 | 117 | 61 |
| | 3 | 12 | 68 | 48 | 2 | 60 | 40 | 41 | 83 | 54 | 134 | 120 | 55 | 113 | 102 | 34 | 51 | 85 | 51 | 353 | 103 | 47 |
| All ages | | | 75 | 53 | | 60 | 52 | | 122 | 76 | | 140 | 62 | | 132 | 47 | | 119 | 63 | | 113 | 59 |

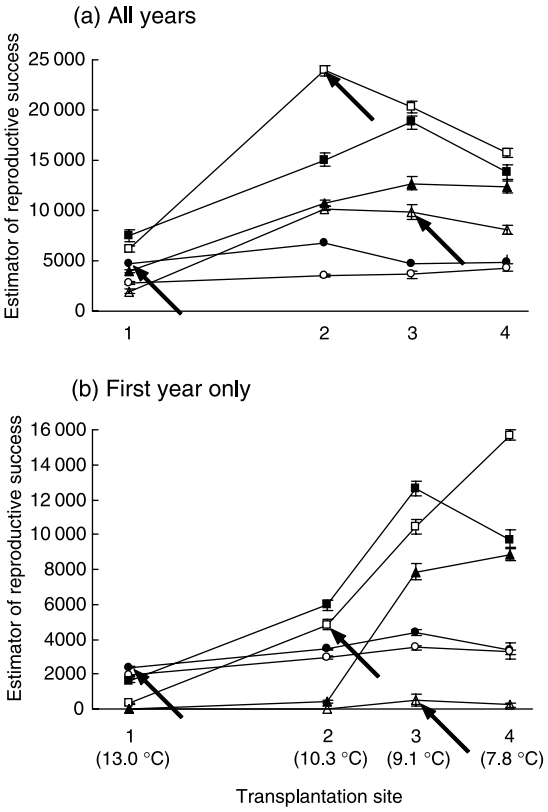


**Fig. 3** Reproductive success, estimated by the product of size and flowering proportion, over all years (a) and in the first year only (b) among populations and among sites. Populations in their own sites are indicated by arrows. 95% confidence intervals are included. Populations: A = ●, B = ○, C = ■, D = □, E = ▲, G = △.

different climates for the year of first flowering in genotypes with a moderate or strong vernalization requirement (our populations C–G).

The observed frequency of plants without any vernalization requirement (first glasshouse experiment) was found to be very similar to the estimation previously made on a smaller number of plants but from more populations in each of the geographical areas in the present experiment (Van Dijk *et al*. 1997). The populations studied therefore appear to be representative of the area from which they come. When we consider populations in their own native environment (A in 1, D in 2 and G in 3), far fewer plants flower in their first year as we move northwards, with the vernalization requirement appearing to increase faster than the 'vernalization index'. We might conclude therefore that early flowering is selected for in the southern part of our studied area, leading to the observed high frequency of the *B* allele and low quantitative vernalization requirement in *bb* genotypes. Similar clines for vernalization requirement have been shown for several other plant species (e.g. *Lolium* sp., Cooper 1963).

We were able to compare the effective vernalization potential of various treatments and transplantation sites because we used the same origins for all experiments (Table 5). The age of the plants, which was also taken into account in the experimental design, definitely plays a role apart from the cold *per se*. The cold chamber treatment, using 3-week-old seedlings, shows a vernalization index that is almost linear with the number of days exposure. The plants put outside in the return experiment, on the other hand, were already rosettes and were better vernalized during the 30 days of November than were seedlings exposed to more than 40 days at a constant optimal vernalizing temperature. The 3-week-old seedlings in the multisite experiment are in yet a different situation: in the spring, with long days but moderate temperatures, they are able to grow considerably, which seems to be favourable for vernalization (the favourable influence of growth rate and long days on vernalization is also reported for sugar beet by Smit 1983). The effect of small size in combination with short days is manifested in the young seedling overwintering experiment, where the vernalizing effect is less than in the moderate vernalization experiment carried out with large rosettes in the spring.

A complete overwintering of adult rosettes is sufficient to vernalize most plants of all origins at all sites (Table 4). Even site 1, located near the Mediterranean coast, regularly achieves the optimal vernalization temperature (5–10 °C). The adaptive significance of variation in quantitative vernalization requirement does not appear to have a relationship with overwintering in the latitudes we studied, but rather with spring temperatures.

## ADAPTIVE SIGNIFICANCE OF THE *B* GENE IN RELATION TO ITS GEOGRAPHICAL DISTRIBUTION

The *B* allele enables early flowering, which, following predictions based on mortality pressures (Murphy 1968), would be of fitness benefit in a disturbed environment. However, we did not observe a higher overall mortality rate in the southernmost site and cannot therefore explain its high incidence of the *B* allele, but our sites were protected against human (e.g. beach recreation) and other disturbances. A relationship between high mortality pressure and early flowering is demonstrated more clearly in the inland population, *B*, where wild beets can be found along roadsides, on waste or fallow land and as weeds in sunflower fields. They are exposed to mowing and weeding, which could lead to high mortality rates and therefore selection for a short life history with early flowering and low allocation to growth after first flowering. High frequency of the *B* allele would be expected under selection for early flowering because it removes the delay imposed by a vernalization requirement. The same pattern of evolution has been suggested for weed beet populations in some French sugar production areas (Van Dijk & Desplanque 1999). Similar types of demographic variation favouring high investment in early reproduction have been reported in *Poa annua* (Law *et al*. 1977; Till-Bottraud *et al*. 1990).

In contrast, in the northern part of the studied area, early flowering appears to be selected against, as demonstrated by the absence of the *B* allele, as well as the high vernalization requirement of *bb* plants. *B*-genotypes planted in the northern sites 3 and 4 flowered very early in their first year (i.e. at a smaller size) and exhibited higher mortality than *bb* genotypes. The resulting decreased overall reproduction may indicate that natural selection can eliminate *B*-genotypes, and therefore the allele, from northern areas.

### ADAPTIVE SIGNIFICANCE OF QUANTITATIVE DIFFERENCES IN VERNALIZATION REQUIREMENT IN RELATION TO GEOGRAPHICAL DISTRIBUTION

The optimal minimum age for first reproduction will, according to theory, depend on local mortality rates and on relative fecundity at each flowering event. As vernalization requirement appears to regulate the minimum age at which vegetative rosettes will first reproduce, then it would be expected to be under strong selection. Indeed, the *B* allele plays a special role in disturbed habitats where early reproduction is very important. The quantitative differences in vernalization requirement, on the other hand, are closely related to the trade-off between early but diminished reproduction and increased reproduction in following years as a result of reserve accumulation during the first year.

Vernalization requirement appears to be strongly genetically determined and highly variable within western Europe. Vernalization of a young rosette plant will depend on the quantitative 'dose' of cold received, which will vary with the local climate and its annual variation. It is also affected by when the seed germinates relative to the cold period, as this influences the time of exposure (germination can take place from early autumn to late spring, although not in winter, when temperatures are too low). The mechanism of vernalization is such that germination earlier in the spring gives a higher chance of flowering in the same year. Later germination implies milder temperatures and therefore a lower probability of flowering, which is advantageous, as the remaining growing season is shortened. It is less adaptive to bolt and flower early in life in the north than in the south, because of the generally shorter growing season. To achieve a geographical distribution with less bolting further north, the colder climate would need to be compensated for by an increasing vernalization requirement (see Van Dijk & Desplanque 1999 for a model of optimal vernalization requirement in relation to latitude and germination date). If the vernalization requirement is higher than can be achieved following spring germination, plants will overwinter and flower in their second year. In the southern areas in contrast, bolting is advantageous and may even be so when germination occurs late in spring when temperatures would not fulfil a vernalization requirement. In such a situation, the *B* allele will be selected for and regulation of the timing of reproduction will only be governed by the long day requirement.

Our finding that population A does better in site 1 than non-native populations, but only for first year reproductive success, suggests that disturbance may play an important role on the Mediterranean coast, although probably not as strong as in inland habitats (Van Dijk & Desplanque 1999). The adaptation of populations A and B to disturbed habitats may even be underestimated in the multisite experiment, because seedlings were germinated in April The low or absent vernalization requirement would allow even later germination to flower here, although not on the Atlantic coasts.

We do not have an explanation for the finding that population G seems suboptimally adapted to its native conditions. On the French, Belgian and Dutch North Sea coasts the species is rare and populations are rather small, whereas numerous large populations can found on Atlantic and Channel coasts (e.g. C, D, E and F). Possible explanations include: (i) our experimental site 3, although near the coast, differs in an essential way from the local natural habitat of wild beet; (ii) the evolutionary equilibrium has not been reached since the last ice age, in particular for the northernmost areas of the species distribution; and (iii) in the small and rather isolated North sea populations, like population G, plants suffer from inbreeding or lack of genetic variation, which makes them generally less fit.

## Acknowledgements

We would like to thank the Institut National de la Recherche Agronomique (Melgueil Research Station), Ph. Palvadeau (La Guérinière, Noirmoutier Island), Espace Naturel Régional Nord-Pas-de-Calais (Centre Régional de Ressources Génétiques) and Université des Sciences et Technologies de Lille for providing experimental fields, and O. Dehorter, R. Dron and B. Plancq for technical help. We are grateful to J. Cuguen, T. De Jong, G. Fox, P. Klinkhamer, J. Thompson, M. Valero and Ph. Vernet for helpful comments. Part of this work was supported by a grant from the CNRS and Region Nord Pas-de-Calais (P.B) and an MRT grant (n°90G0519).

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*Received 5 February 2002*
*revision accepted 26 April 2002*