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Potential for evolutionary change in the seasonal timing of germination in sea beet (*Beta vulgaris* ssp. *maritima*) mediated by seed dormancy

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Abstract In sea beet (*Beta vulgaris* ssp. *maritima*), germination occurs in autumn or spring and is mediated by dormancy which can be released by cold or dry periods. Environmental change such as current climate change may require evolutionary response in seasonal timing. Here, we explore the potential for such evolutionary change. Seed dormancy was studied in a composite population based on seeds from all over the species range in France together with several generations of reciprocal crosses. We found high, repeatable variability for dormancy rate among individuals under greenhouse conditions and confirmed its relevance for germination phenology in the field. Our data fitted best with an exclusively maternal determination of the dormancy phenotype. Narrow-sense heritability, $h^2 \approx 0.5$ in the composite population and ≈ 0.4 in the original local populations, was such that rapid evolutionary change in the relative proportions of autumn and spring germination may be possible.

Keywords Global change · Dormancy release · Germination phenology · Heritability · Maternal effects · Repeatability

Introduction

The biotic and abiotic environment is continuously changing, constantly selecting for new and better adapted genotypes. Genetic diversity, the raw material for this adaptation, is thus of fundamental importance for the maintenance of species. Since this could reveal to be crucial in the current context of rapid climatic and anthropogenic change, the study of genetic diversity for adaptive traits has recently seen renewed interest (Jump et al. 2009). Among the many adaptive traits, life-history traits, i.e. traits associated with survival and reproduction, are susceptible to be highly affected by environmental changes. As such, their genetic variation in populations and species should be of extreme value.

In a plant's life, the (chronologically) first life-history trait is the timing of germination. Timing of germination is critical with obvious consequences for fitness and even for population persistence in certain locations (Donohue et al. 2005b). Not only are vulnerable seedlings at risk for mortality, but subsequent life-history traits also depend on the moment of germination as it determines whether—or at what size—a plant flowers and is capable of maturing seeds adequately by the end of the growing season (Donohue 2002; Forcella et al. 2000). The timing of germination can be controlled by seed dormancy, i.e. the reversible failure of a viable seed to germinate under conditions that normally favour the process (Bewley 1997; Bradford 2005; Finch-Savage and Leubner-Metzger 2006; Vleeshouwers et al. 1995). Germination of non-dormant seeds only depends on the suitability of the present environmental conditions, while dormant seeds await information from the environment for release from dormancy (Bradford 2005) in order to germinate at a later date with a higher fitness expectation. Such information is crucial for adaptation to a seasonal environment and is transmitted principally through temperature, moisture and light (Larsson 2002; Leinonen 1998). The direct effect of climate change on germination through a change in dormancy-releasing factors during autumn, i.e. through phenotypic plasticity,

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does not necessarily have positive fitness consequences. For instance, warmer, wetter autumns could decrease autumn germination even if fitness would be better promoted by an increase in autumn germination, since milder winters may be presaged. Evolutionary change in the sensitivity to factors that are involved in dormancy release is the only truly adaptive response.

To understand how natural selection could act on dormancy and, in particular, on sensitivity to the environmental cues involved, we need information on the variability of dormancy and its genetic control. For a number of species, it is known that seed dormancy has a more or less strong hereditary component, as reviewed by Baskin and Baskin (1998) and Meyer and Allen (1999). Most genetic studies conclude that seed dormancy is a quantitative trait controlled by multiple loci, in interaction with each other and with environmental factors (Bentsink et al. 2007; Foley and Fennimore 1998; Koornneef et al. 2002). These studies are complex due to the genetic structure of the seed: the embryo and endosperm are of zygotic origin (maternal and paternal genotypes) whereas the seed coat, or testa, is derived from maternal tissues (maternal genotype). The three structures, together with their interactions, determine germination and dormancy characteristics (Alonso-Blanco et al. 2003; Bentsink et al. 2007; Koornneef et al. 2002; Meyer and Pendleton 2000). In some species, seed dormancy is exclusively determined by the mother plant's phenotype, while in others the paternal genotype is also involved (see Baskin and Baskin 1998, for a literature survey). Knowledge on maternal determination is important for understanding whether natural selection acts on individual seeds or on maternal progenies as a whole. In the latter case, the production of different dormancy phenotypes by a single mother plant could be a strategy of spreading the risk, also known as bet-hedging (Cohen 1966; Simons and Johnston 2006). Bet-hedging requires that (1) a single plant produces all types of seeds and (2) variation in germination is not caused by genetic variance among seeds within progenies (Philippi 1993). Bet-hedging may maximize fitness over longer periods in the case of strong and unpredictable environmental variation (Simons 2009).

Our study species, the sea beet *Beta vulgaris* ssp. *maritima* (L.) Arcangeli, is the wild ancestor of the cultivated beet *B. v.* ssp. *vulgaris*. The dispersal unit is the fruit or a cluster of fruits, which means that there is an extra layer of maternal tissue involved, the pericarp, with important consequences for the control of germination in sugar beet (Hermann et al. 2007). In cultivated beets, rapid and synchronous germination has been selected for and has led to the total elimination of dormancy. As a consequence, there is no useful information available to extrapolate to the wild species. Although this suggests that this trait might also respond to selection in sea beet, the extrapolation to wild beet is difficult because the ancestors of cultivated beet probably originated from other climatic regions than France. Moreover, dormancy in sea beet is poorly understood and we do not have any idea of the amount of variation for this trait in natural populations. It is known that germination can take place either in autumn or in spring, depending on local conditions (Letschert 1993). This suggests that the seasonal timing of germination is subjected to natural selection and is susceptible to react in response to environmental change, e.g. due to a changing climate. The aim of this study was to evaluate how easily the seasonal timing of germination can be changed through natural selection. We (1) assessed the importance of seed dormancy in this timing and (2) explored its variability by estimating its heritability. To do so, we measured the dormancy rate of seeds obtained from plants of a composite, greenhouse population derived from plants from all over the species distribution in France. In this way the complications due to genetic disequilibrium among populations (those with a shorter life span may have dormancy rates different from those with a longer life span, Hautekèete et al. 2002) were avoided under the highly plausible hypothesis that linkage disequilibrium between the genes involved is low. A disadvantage of estimating heritability in a composite population instead of the local populations is that there is no information on the partitioning of variation within and among populations and therefore on the immediate potential response to selection without gene exchange between populations. For this reason we included data enlightening this partitioning. Evidence for gene flow among populations in the same region, which could reinforce within-population genetic variation and therefore local evolutionary change, is provided by Fénart et al. (2007) and Fievet et al. (2007).

In our experiments a number of reciprocal crosses were included, which were evaluated over several years in order to obtain information on the overall variability and its genetic and environmental components, together with the degree of maternal determination of the seed phenotype. To estimate dormancy rates, we applied three successive treatments (phases) that simulated possible climatic conditions in our study area: seed dissemination at the end of the summer followed by a wet autumn, a cold winter and a spring with dry periods, which was simulated by a phase of immediate germination in wet warm conditions, a phase of wet cold conditions followed by a test of germination (estimation of dormancy release) and a phase of dry warm conditions followed by a test of germination. Then, to better understand the role of natural selection in situ, we evaluated seed progenies simultaneously under standardized greenhouse conditions and in an experimental field in the species' natural habitat.

Materials and methods

Study species

Sea beets are found in a narrow zone along the European, North African and Middle East coasts, usually within 10–20 m of the high water mark. In the Mediterranean and Middle East regions, populations are also present in human-disturbed inland habitats (for more details, see Letschert 1993; Hautekèete et al. 2002; Van Dijk and Hautekèete 2007). Individuals are iteroparous with a variable life span (Hautekèete et al. 2002), self-incompatible (De Cauwer et al. 2010) and wind-pollinated. Seed maturation occurs from July to September depending on the region. Flowers are occasionally solitary, but usually occur in groups of two to eight flowers forming fruit clusters (seed balls) after ripening. As the number of seeds in a seed ball is variable among and within individuals and cannot be estimated without destruction, the per-seed germination rate cannot be estimated. However, dormancy was always measured using the proportion of total germination observed; consequently, the number of seeds per seed ball does not affect our estimation of germination proportions.

Plant material and crosses

In 1989, seeds were sampled from individual plants (see Van Dijk et al. 1997 for details) from 93 populations distributed throughout the species range in France, including some from neighbouring countries, e.g. Belgium, Great-Britain and The Netherlands (see Van Dijk et al. 1997 for the exact locations). Plants were grown from these seeds in a semi-natural greenhouse with natural day length and temperatures slightly higher than outdoor temperatures and were protected from frost during winter. Seeds were sown in September and plants were repotted each year in January in pots of the same size (2.4 L) using commercial soil (Neuhaus Huminsubstrat N3: 90% peat, 10% clay; pH 6; NPK 14:16:18, 1.3 kg/m^3; conductivity 35 mS/m). No fertilizer was used; each plant thereby received the same amount of nutrients each year through soil renewal.

Plant material and crosses, previously described by Van Dijk (2009), were also used here. In 1991, plants of all 93 populations (mean number of plants per population = 7.7) flowered together in the greenhouse and open pollination was allowed among plants arranged in a random design. When more than one plant per population was grown, plants were offspring from different originally sampled plants. Seed balls were harvested on one randomly chosen plant per original population and sown in autumn 1991, keeping only one seedling per plant. This composite population was grown up separately and after flowering the next year (open pollination in a random design as before), seeds were harvested and one seedling per plant was kept. This procedure guaranteed an effective composite population size of at least 93 different haploid genomes. Plants that flowered without vernalization were systematically eliminated so that all plants flowered for the first time after their first winter and thereafter once a year, in spring or early summer. We repeated this procedure each year until 1995, thus obtaining a fifth-generation composite population: the "1995 generation".

We then conducted several generations of reciprocal crosses starting from this material to assess the heritability and the parental determination of the trait. Crosses between closely related plants (full sibs, offspring and parents) were avoided. Eight controlled reciprocal crosses were done in 1998 using plants from the "1995" generation. The "1998" generation was formed with five offspring per parent plant (80 in total). This was repeated in 2000 and 2001 with five and four reciprocal crosses, respectively, using members of the "1998" generation. Finally, six reciprocal crosses were done in 2003 using plants of the "2000" and "2001" generations. The 46 selected parent plants involved in these crosses were used in only one of the years and were open-pollinated in the other years. A certain degree of positive assortative mating was carried out by including crosses where both parents had either a high or a low dormancy rate. Assortative mating does not lead to directional selection or cause any bias in the estimation of genetic parameters, but has a favourable effect on their precision (Falconer 1989). Five offspring were kept per parent plant (10 per reciprocal cross). All generations were grown in the semi-natural greenhouse as described above, but the total number of individuals gradually decreased over time due to some mortality.

Data analysis

Statistica 7.1 (StatSoft 2006) was used for statistical analyses except nested ANOVAs that were computed using $R_{2.6.1}$ (R Development Core Team 2008). We applied an arcsine-square root transformation on germination proportions for all statistical tests performed that require a normal distribution. A Kolmogorov–Smirnov test for goodness of fit confirmed that data met the normality requirement for ANOVA and homoscedasticity was achieved as demonstrated for the tested factors by a Bartlett's test of homogeneity of variances (Sokal and Rohlf 1995). Plants producing fewer than 10 seedlings in a given year were excluded from the data set for that year. Details on analyses done for each experiment are presented below in their corresponding sections.

Greenhouse germination test

This test was performed to measure the germination proportions in seed progenies under conditions chosen to

simulate winter conditions (cold treatment) and spring or summer conditions (drought treatment). For each parental generation, 1995, 1998, 2000 and 2001 the seed progeny of each plant was evaluated in the three following years (i.e. in 1996, 1997 and 1998 for the 1995 parental generation, *etc.*). The 2003 progenies were only evaluated in 2004 for technical reasons. Seed balls were always collected in August and sown the following month (September). All collected seeds balls were sown, but with a maximum of 100 (50 from 2003 on for capacity reasons) per progeny.

The germination test was divided into three successive phases. First (phase I: immediate germination), seed balls were put in optimal conditions for germination: in the greenhouse with watering, at a constant temperature of 20°C and a photoperiod of 16 h in trays with moist commercial soil as described above. After 4 weeks, the number of seedlings was recorded. Thereafter (phase II: germination after cold treatment), the trays were transferred to a dark cold room (5–7°C) keeping them moist. After these 4 weeks of cold treatment, germination was recorded after a second four-week-period of optimal conditions to estimate dormancy release. Finally (phase III: germination after drought treatment), trays underwent 4 weeks of drought (no watering) under otherwise optimal conditions. After these 4 weeks of drought treatment, germination was recorded after a third four-week-period of optimal conditions to estimate dormancy release. In all phases, seedlings were regularly eliminated. The following abbreviations are used for the three germination phases as proportions of their sum:

- Imm = Immediate Germination/Total Germination;
- Col = Germination after the Cold treatment/Total Germination;
- Dro = Germination after the Drought treatment/Total Germination.

Thus,

- Dormancy rate = 1-Imm = Col + Dro.

Mean germination rate per seed ball could be calculated from the sum over the three phases and the number of seed balls sown.

A one-way ANOVA was performed to assess the effects of the three phases of the greenhouse germination test (Imm, Col and Dro) in the overall dataset (all generations and all years of evaluation). A nested mixed-model ANOVA (generalized linear model procedure), was conducted to assess the effect of generation and accession within generation on germination rate. Generation was a fixed effect and accession was random. The ANOVA model was as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_{ij} + e_{ijk}$$

($Y_{ijk,}$ the germination rate of the *k*th individual of the *j*th accession nested in the *i*th generation; μ, the mean; α_i the fixed effect of the *i*th generation; β_{ij}, the random contribution of the *j*th accession of the *i*th generation and e_{ijk} is the error term). In both ANOVAs a post-hoc test (Tukey HSD) was performed to identify the different possible groups.

Correspondence between greenhouse and field conditions

Field experiments were conducted in 2003–2004 and 2004–2005 to compare the greenhouse and field germination patterns. The experimental field was located on the sea front (the natural sea beet habitat) at Wimereux in Northern France. We checked that no naturally occurring sea beet plants were present in our experimental plots. The soil was treated with an herbicide and manual weeding was performed in order to limit invasion by weeds. The experimental site was enclosed to exclude rabbits and was divided into 20 plots of 1 m^2 each.

In both years of the field experiment, eight seed progenies were chosen for their wide range of dormancy values (from 0.03 to 0.93) from the 2000/2001 generation complemented with two "wild" seed progenies per year (seeds produced by open pollination on plants from natural populations). In all cases, the seeds sown in September were collected in the previous month.

In both experimental years, seed balls from each chosen seed progeny were sown at the same time in the greenhouse and in the field site. In the latter, two separate 1 m^2 plots were used for each of the 10 seed progenies (about 16 randomly chosen seeds in each plot). On 4 September 2003 and 9 September 2004, 317 and 320 seed balls were thus sown, respectively. Each sowing hole was marked to ensure that only germination of the sown seed balls was recorded and not that of naturally occurring sea beets germinating from the seed bank. Water only came from natural rainfall. First rainfalls were recorded on 6 September 2003 and 11 September 2004.

In the greenhouse, the methods for germination trials were as described above. In the field site, germination was recorded twice a month, until 13 July 2004 or 19 July 2005. Germinations in the field were grouped into two periods: before and after winter (clearly separated by complete absence of germination in January and February) and are presented as proportions of total germination observed in the field. Correlations were calculated between the germination proportions in the greenhouse (Imm, Col and Dro) and those in the field (before and after winter).

Genetic determination

For all calculations, the arcsine-transformed data were further converted into standard deviates by calculating

means (μ) and standard deviations (σ) per year and per generation and then converting all individual data (x) into standard deviates $(x - \mu)/\sigma$. This enabled us to combine or compare different years and generations by eliminating the effects of year-to-year differences in environmental conditions.

The repeatability r (Falconer 1989) of the germination proportions of each treatment (Imm, Col and Dro) over the 3 years of evaluation on each progeny in each generation was calculated as the intraclass correlation of the transformed proportions (by applying a type I ANOVA). Average r values over all generations were obtained by combining all data for each germination proportion over the 3 years of evaluation as if all generations started in the same year.

For the evaluation of parental determination, the seeds sired by the known pollinator plant were compared with the seeds obtained from the same plant through open pollination in other years. Among the available 46 plants involved in crosses, only 36 could be used due to mortality or insufficient seed production. Under the hypothesis of complete maternal determination, no systematic differences in the germination proportions were expected between the progeny produced through controlled crosses and those produced through open pollination. Under the hypothesis of equal maternal and paternal determination of seed dormancy (50% maternal and 50% paternal), we first estimated the breeding value of each parent as twice the deviation of their open pollinated progeny from the generation mean (Falconer 1989) or the average breeding value over 2 years of open pollination. The expected value of the progeny produced through reciprocal crosses was then the mean of both parents' breeding values. Mixed determination is also possible in cases where the maternal plant has a greater influence than the paternal plant, in which case intermediate values would be expected. The expected values under all hypotheses from 100% maternal to 50% maternal determination with a 5% increment were compared with the observed values (all values as standard deviates). We used the variance of the differences between observed and expected values over all 36 plants as a criterion for the appropriateness of each hypothesis. Differences in variance were tested for significance (F-test, see Sokal and Rohlf 1995).

In each of the three parental generations (1995, 1998 and 2000/2001), controlled crosses were used to estimate the parent-offspring regression which directly corresponds to the narrow sense heritability (h^2). Mid-parent values in the three successive years were considered separately. Offspring data were averaged in the case of more than 1 year of evaluation, which resulted in three h^2 estimates per generation in most cases. Overall h^2 estimates per generation were obtained by combining all mid-parent/offspring pairs over the available years for that generation (19, 20 and 16 for the three generations, respectively; due to some mortality this is lower than the theoretical maximum of three times the number of crosses). Likewise, an overall h^2 value over the three generations was obtained (55 mid-parent/offspring pairs). Tests for h^2 values being different from zero are identical to those for regression coefficients (Sokal and Rohlf 1995).

To analyse the partitioning of the variance within and among populations we included data on 27 original populations, regularly distributed over the whole sampling range. Per population two individuals were grown, under the same experimental greenhouse conditions as described above, from seeds harvested on two different plants in the original populations. The germination data obtained were in the same way arcsine-transformed and converted into standard deviates. Variance among pairs of individuals from the same population was calculated as the mean of the 27 within-pair variances and was compared with the variance among individuals from the 27 different populations (relative decrease in variance $= \Delta V_P$). Within-population h^2 could now be estimated by ascribing the lower variance among plants of the same population to a greater genetic resemblance. This was done by converting $h^2_{comp} = V_A/V_P$ into $h^2_{pop} = (V_A - \Delta V_P)/(V_P - \Delta V_P)$. Standard errors were calculated by taking into account the errors of the decrease in variance, using the same conversion, and adding those for the h^2_{comp} estimations described above.

Results

Germination proportions observed in the greenhouse

Observed germination proportions were measured for all generations and years (Table 1). Mean total germination rate per seed ball was 1.19 (SD = 0.39). Whatever the generation and the year, more than half of germination occurred in the first four-week-period after sowing (Imm, from 0.52 to 0.75). The effect of cold (Col, from 0.04 to 0.27) was weaker than the subsequent effect of drought (Dro, from 0.12 to 0.37). A significant difference was observed between Imm, Col and Dro ($F_{2,2205} = 1171.49$, $P < 0.001$), with Imm > Dro > Col (HSD Tukey post-hoc test). Variation among individuals (within generations) and among generations were highly significant for all three treatments Imm, Col and Dro (Table 2). The post-hoc test identified different groups of generations, dependent on whether Imm, Col or Dro treatments were considered (Table 1). No one generation (or group of generations) was systematically different from the others.

Table 1 Germination proportions and germination rates in the greenhouse (means and standard deviations) under the different treatments in all generations and years

Generation	Year of evaluation	n_S	n_G	Germination rate	Germination proportions		
					Imm	Col	Dro
1995	1996	44	3,459	0.84 ± 0.40	0.56 ± 0.19	0.20 ± 0.12	0.24 ± 0.14
	1997	52	6,132	1.28 ± 0.34	0.54 ± 0.20	0.17 ± 0.13	0.29 ± 0.15
	1998	43	3,440	0.88 ± 0.45	0.65 ± 0.22	0.14 ± 0.12	0.21 ± 0.14
	All	139	13,031	1.01 ± 0.44	0.58^{ab} ± 0.21	0.17^{a} ± 0.13	0.25^{a} ± 0.15
1998	1999	52	2,642	1.41 ± 0.47	0.55 ± 0.24	0.16 ± 0.12	0.28 ± 0.20
	2000	57	4,690	1.08 ± 0.37	0.61 ± 0.19	0.10 ± 0.10	0.29 ± 0.18
	2001	36	3,510	1.30 ± 0.36	0.71 ± 0.22	0.04 ± 0.04	0.25 ± 0.21
	All	145	10,842	1.25 ± 0.43	0.61^{a} ± 0.22	0.11^{b} ± 0.11	0.28^{ab} ± 0.20
2000	2001	74	5,129	1.30 ± 0.38	0.53 ± 0.27	0.10 ± 0.07	0.37 ± 0.26
	2002	53	4,289	1.12 ± 0.32	0.52 ± 0.26	0.13 ± 0.09	0.35 ± 0.25
	2003	46	2,849	1.25 ± 0.32	0.71 ± 0.19	0.03 ± 0.05	0.25 ± 0.17
	All	173	12,267	1.23 ± 0.35	0.58^{b} ± 0.26	0.09^{b} ± 0.08	0.33^{b} ± 0.24
2001	2002	72	4,489	1.23 ± 0.45	0.58 ± 0.21	0.21 ± 0.13	0.21 ± 0.16
	2003	73	4,785	1.23 ± 0.26	0.60 ± 0.22	0.27 ± 0.17	0.12 ± 0.13
	2004	57	3,633	1.32 ± 0.31	0.79 ± 0.14	0.09 ± 0.07	0.12 ± 0.10
	All	202	12,907	1.26 ± 0.34	0.65^{a} ± 0.22	0.20^{c} ± 0.15	0.15^{c} ± 0.14
2003	2004	77	4,005	1.11 ± 0.33	0.75^{c} ± 0.22	0.11^{b} ± 0.09	0.14^{c} ± 0.16
All	All	736	53,052	1.19 ± 0.39	0.62 ± 0.23	0.14 ± 0.13	0.24 ± 0.20

n_S = number of evaluated seed progenies; n_G = number of germinated seeds observed; Imm = immediate germination; Col = germination after cold treatment; Dro = germination after cold and drought treatment

Different letters indicate significant differences between generations within treatments at $P < 0.05$

Correspondence between greenhouse and field conditions

The progress of germination in the field site was observed per progeny in both years (Fig. 1) with 277 germinations in 2003–2004 and 307 in 2004–2005. Most germination occurred during autumn shortly after sowing (varying among progenies from 38 to 100%) and this was particularly pronounced in progenies that had high immediate germination proportions in the greenhouse. In contrast, spring and summer germination in the field mainly occurred in progenies with a high dormancy rate in the greenhouse (e.g. progenies 10 and 20).

We tested for correlations between germination proportions in the greenhouse and in the field among progenies (Table 3). The significant correlations are clearly visible in Fig. 1: in both years, the proportions of delayed germination varied similarly in field and greenhouse. Consequently, we observed a highly significant negative correlation between germination after drought in the greenhouse and germination before winter in the field. In the second year, the proportions of immediate germination were also similar in the greenhouse and in the field: immediate germination in the greenhouse was significantly positively correlated with germination before winter in the field site. For after-winter germination proportions (the complement of before-winter germination proportions), correlations with immediate germination in the greenhouse were negative in both years.

Genetic determination

The repeatability r of the Imm, Col and Dro proportions was estimated over the three successive evaluation years of the generations 1995, 1998, 2000 and 2001 (Table 4). The overall value over generations was also calculated, assuming no systematic change in any crucial parameter over the generations. Repeatabilities of Imm and Dro varied little, whereas the value obtained for Col varied considerably (Table 4), with non-significant and significant values. Overall values were all highly significantly different from zero.

As for the parental determination, the observed progeny values were closer to the theoretical values expected under the 100% maternal hypothesis than to the expected values under any of the biparental hypotheses (Fig. 2). This analysis was only carried out for the Dro proportion (which showed the highest repeatability). The variances of the differences between the observed values and those expected under the two most extreme hypotheses were 0.93

Table 2 Type III nested ANOVA on arcsine square root transformed germination proportions for individual and generation effects

Source of variation	*df*	Imm		Col		Dro	
		Mean Square	*F*	Mean Square	*F*	Mean Square	*F*
Generations	4	2,713	7.62***	3,009	22.4***	6,233	20.71***
Individuals (generations)	340	356	2.84***	134	1.64***	301	3.63***
Error	391	125		82		83	

*** $P < 0.001$

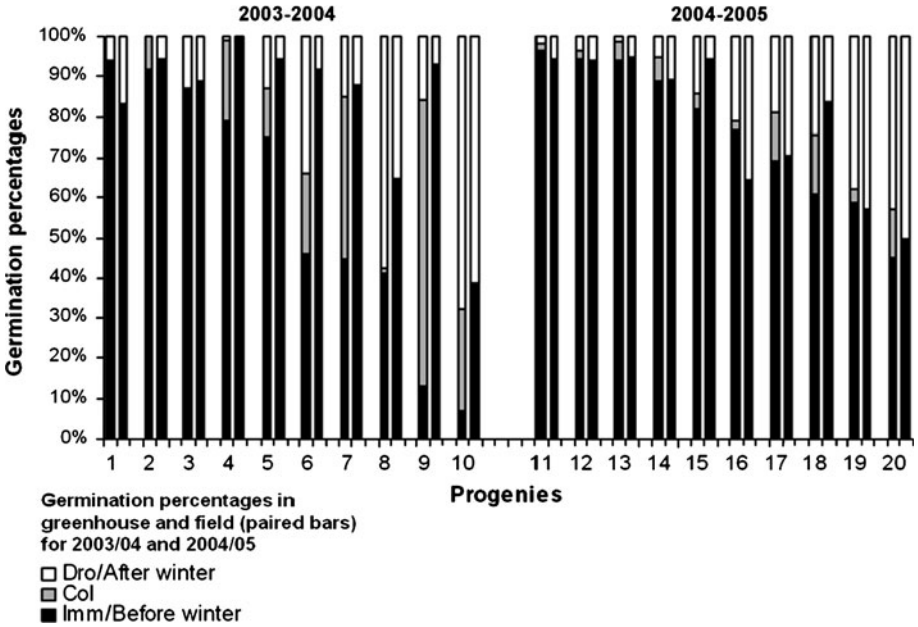

Fig. 1 Germination percentages in the greenhouse for Imm (immediate germination), Col (after a cold treatment), and Dro (after an additional drought treatment) and in the field before and after winter for the 20 progenies that were tested in both experiments in 2003–2004 and 2004–2005. Percentages are ordered by decreasing Imm values for each period. For each progeny (1–20), the *left bar* corresponds to the greenhouse experiment and the *right bar* to the field experiment

Table 3 Correlation coefficients between the germination proportions in the greenhouse and the proportions of germination before winter in the field in 2003–2004 (year 1) and 2004–2005 (year 2); $n = 10$ in both cases

Greenhouse	Field: germination before winter	
	Year 1	Year 2
Imm	0.57	0.86**
Col	0.11	−0.35
Dro	−0.88**	−0.91***

Correlations between germination proportions in the greenhouse and germinations after winter are not written explicitly since they are the exact complement to those with germinations before winter. Similarly, Imm + Col + Dro = 1 (see Material and methods), meaning that correlations with Imm + Col are the complement to those with Dro

*** $P < 0.001$, ** $P < 0.01$

(100% maternal) and 3.82 (50% maternal). These variances were significantly different from each other ($F_{35,35} = 4.10$; $P < 0.001$). When 100% maternal determination was

Table 4 Repeatability (*r* as calculated by intraclass correlation) of germination proportions over the 3 years of evaluation

Generation	Treatment	*n*	*r*
1995	Imm	38	0.52***
	Col		0.46***
	Dro		0.56***
1998	Imm	24	0.46***
	Col		−0.04
	Dro		0.44***
2000	Imm	42	0.65***
	Col		0.26**
	Dro		0.66***
2001	Imm	55	0.60***
	Col		0.52***
	Dro		0.66***
Overall	Imm	159	0.57***
	Col		0.35***
	Dro		0.60***

*** $P < 0.001$, ** $P < 0.01$

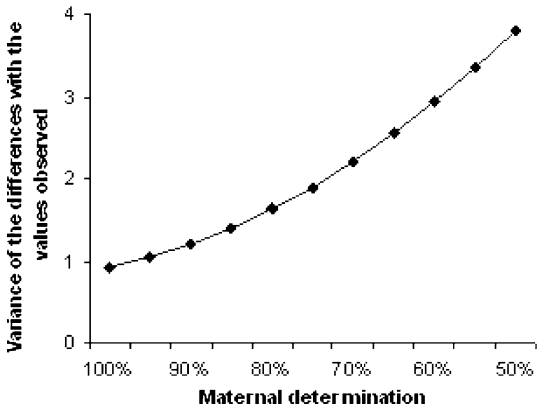

Fig. 2 Variance of the differences between the observed and expected values for the degree of maternal determination from 100 to 50%. Only the germination proportions after the drought treatment (Dro) were used

compared with decreasing degrees of determination, variances were significantly higher (at the 5% level) for all determination percentages lower than 80%.

Heritability values h^2 found for Imm and Dro were strongly significant and remarkably homogeneous among generations (Table 5). The Col proportion showed no significant heritability for any of individual generations, but the overall value was significant at the 5% level. The group of 27 populations used for the partitioning of the variance within and among populations showed the following germination proportions: 0.59 (SD = 0.26); 0.09 (SD = 0.09) and 0.33 (SD = 0.26) for Imm, Col and Dro, respectively, which was very similar to those of the composite population for both means and variances (Table 5). The variance among pairs of individuals from the same populations was lower than the variance among individuals from different populations. A reduction of 9.7% (SE = 24.3%) and 20.5% (SE = 18.1%) was found for Imm and Dro, respectively. Correcting the h^2 values found for these treatments (Table 5) by ascribing this reduction to a closer genetic resemblance of plants from the same population, and under the acceptance of 100% maternal determination, the within population h^2 values decreased to 0.42 (SE = 0.23) and 0.38 (SE = 0.20), respectively.

Table 5 Heritability values (h^2) as estimated by parent-offspring regression (means and standard errors)

Generation	Treatment	n	$h^2 \pm$ SE
1995	Imm	19	0.51 ± 0.08***
	Col		−0.09 ± 0.17
	Dro		0.52 ± 0.08***
1998	Imm	20	0.43 ± 0.09***
	Col		0.15 ± 0.11
	Dro		0.47 ± 0.09***
2000/2001	Imm	16	0.51 ± 0.09***
	Col		0.23 ± 0.13
	Dro		0.55 ± 0.05***
Overall	Imm	55	0.48 ± 0.05***
	Col		0.16 ± 0.08*
	Dro		0.51 ± 0.04***

n = the number of mid parent-offspring comparisons

*** $P < 0.001$, * $P < 0.05$

Discussion

Germination proportions observed in the greenhouse

Our experiments demonstrated that cold and drought had an important effect on sea beet dormancy release. About 40% of total germination resulted from dormant seeds, with dormancy mainly released by drought (after a cold period) and to a lesser extent by cold only. This suggests that both temperature and rainfall, which are crucial factors associated with seasonality, have an important effect on germination phenology in the field. A control experiment, where we extended the period for immediate germination to 1 year (unpublished results), confirmed that cold and/or drought are necessary releasing factors and that the ability to germinate is not just a matter of time. Among the factors that cause dormancy loss in many plant species, dry-wet cycles are rarely reported in the literature, compared to, for instance, cold stratification (Baskin and Baskin 1998). Peto (1964) showed that soaking and drying seeds of cultivated beet accelerated germination, possibly by removing inhibitors of germination or by loosening the seed caps on seed balls (Heydecker et al. 1971). Dormancy release through drought may therefore be important in our study species.

For efficiency reasons, we simulated just one seasonal scenario for all our greenhouse germination experiments. However, other scenarios are possible. Since sea beet seeds are naturally disseminated at the end of summer, the scenario we simulated for our experiments corresponded well to possible climatic conditions in our study area (wet autumn, cold winter and dry spring). While this is a plausible scenario, dry periods at moderate or higher temperatures may occur in autumn (i.e. drought before cold), and dry-wet cycles may be more realistic than a single drought period. Moreover, as we will discuss later, cold and drought are not necessarily entirely different in their effects as cold stratification alters the tegument integrity as well as drought. The implication of seed dormancy release by cold or by drought has therefore to be investigated more thoroughly to complete this first impression of the genetic variation in this trait in sea beet, for which we chose a simple and plausible scenario. We also neglected the role

of a long-lived seed bank, formed by seeds that remain dormant for more than 1 year, and this component was not included in the observed total germination. Seed banks may play an important role in the case of environmental instability and will be studied further with populations from ecologically different origins.

We found high variability in seed germination patterns among individuals. This variability is one of the prerequisites for natural selection to be able to adjust dormancy patterns to new environmental situations, such as new climatic conditions. The repeatability values were quite different from unity, indicating that there was considerable variation among years. Together with the variation among generations (Table 1), this shows that there is a non-negligible environmental component in the dormancy pattern. Germination is known to be highly plastic in response to environmental conditions experienced both during seed maturation and after seed release (Donohue et al. 2005a; Galloway 2002). However, conditions were standardized in our experiment. Moreover, the important correlation between the germination properties of siblings in greenhouse and field (Table 3) indicated limited plasticity during the germination phase. Taken together, these results suggest that the principal component of phenotypic plasticity in our experiments was caused by among-year variation in maternal conditions during seed ripening.

Correspondence between greenhouse and field conditions

Germination patterns in the field site were significantly correlated with those in the greenhouse. Indeed, seed progenies that germinated immediately in high proportions in the greenhouse also showed high proportions of germination before winter in the field site (Fig. 1, Table 3). Likewise, germination proportions observed after drought treatments were significantly correlated with germination proportions after winter in the field.

Although tendencies were similar, slight differences were noticed between the 2 years. In 2003–2004, autumn germination in the field was not significantly correlated with immediate germination (Imm) but was significantly positively correlated with Imm + Col (the exact complement to Dro). Apparently some of the dormant seeds that germinated only after the cold treatment in the greenhouse germinated already in autumn in the field. This might be due to short cold periods during that autumn. Germination after drought treatment in the greenhouse, Dro, was significantly positively correlated with spring and summer germination in the field (significant negative correlation with germinations before winter in the field, Table 3).

In 2004–2005, autumn germination in the field was significantly positively correlated with immediate germination in the greenhouse (Imm) and thus might correspond to non-dormant seeds exclusively. As well as in 2003–2004, germination after the drought treatment in the greenhouse, Dro, was significantly positively correlated with spring and summer germination in the field. It seems that dormancy was released during winter that year, mainly in relation to drought.

This very significant positive correlation between germinations due to the drought treatment in the greenhouse and after-winter germinations suggests that dormancy in sea beet is mainly released by drought often associated with frost and/or by short periods of drought that can occur during winter or spring and summer. The hypothesis that the *accumulation* of periods of cold or drought drives the germination phenology in the field is very probable and will be tested later.

The 2 years of observation with different climatic conditions allowed us to account for some natural environmental variability in time. However, we chose only one site for our field experiments, making it difficult to draw a general conclusion on the phenology of dormancy in the field. Moreover, we intend to study the geographical variation on germination phenology in sea beet to gain information on ecological factors selecting dormancy in this species.

Maternal determination of seed dormancy

Our data were best explained by the absence of any influence of the paternal genotype on the seed dormancy phenotype. This suggests that natural selection acts on maternal seed progenies as a whole and not on individual seeds, which is important under a hypothesis of bet-hedging and will be discussed below. However, we could not exclude the possibility of a minor influence (<20%) of the paternal genotype. Knowing the kind of parental determination is important for selection procedures and multiplication: the tendency of performing crosses using only seedlings from seeds that germinate immediately may inadvertently select for less dormancy. Dormancy would thus decrease over the generations. Fortunately, this type of bias in our experiments is unlikely because the within-maternal-family differences did not appear to have a genetic basis. Indeed, dormancy did not systematically decrease over the generations (Table 1).

Controlled crosses in perennial species designed to examine the effect of the paternal parent have been carried out in only few cases (Meyer and Pendleton 2000). Sib analyses are usually done on offspring borne of different pollinators for one seed parent in one crossing experiment. The method we used to measure parental determination is novel: in different years, progenies obtained by open pollination were compared with progenies from a known

pollinator plant. While our approach is only possible in iteroparous species studied over several years, it had the advantage that no additional experiments were needed beyond those already necessary for the repeatability and heritability estimations.

Heritability

Repeatability and heritability values averaged over all estimations were not significantly different from each other, with r values slightly higher than h^2 values (Tables 4 and 5), which is in accordance with theory (Falconer 1989). Dominance variance and/or "general environmental variance" (based on permanent environmental influences on individuals, see Falconer 1989) contributing to r but not to h^2, seemed to play a negligible role. The r and h^2 values for the proportions Imm and Dro were all highly significant, unlike those for the Col fraction with lower values that were not always significant. This means that the response to the cold treatment, apart from its weaker effect compared with the subsequent drought treatment, was less constant. Consequently, under slightly different conditions, it is possible that germination after the cold treatment could have occurred immediately or, alternatively, after the drought treatment. While we cannot completely exclude the possibility of a genetic disposition specifically favouring germination after a cold period, we found no strong evidence for it in this study.

The consequence of measuring heritabilities in a composite population instead of in separate natural populations is that the evolutionary potential of sea beet seed dormancy in the quantitative sense has to be interpreted as one in a metapopulation or region with substantial exchange between individual populations, which is suggested by previous molecular studies on sea beet populations (Fénart et al. 2007; Fievet et al. 2007). Actual within-population h^2 is probably lower due to local selection or random genetic drift that would not be fully compensated by gene flow. This was confirmed by the corrected h^2 values obtained by exploiting the partitioning of variance within and among populations. When estimating heritabilities for flowering date in sea beet, Van Dijk et al. (1997) found regional heritability to be 0.54 and the average within-population heritability to be 0.33. Although estimated on a different trait, these values -including an overall repeatability of 0.53 and obtained using the same plant material- were very close to those obtained for Imm and Dro in this study. The reduction in h^2 at the population level was of the same order for dormancy suggesting that the relative importance of local selection or random genetic drift *vs* gene flow is similar. These results suggest that rapid evolutionary change in the relative proportions of autumn and spring germination may be possible.

Bet-hedging

The within-progeny variation in dormancy suggests a strategy known as bet-hedging, i.e. spreading the mother plant's risk. Bet-hedging requires that a single plant produce all types of seeds without any influence of the genotypes of the seeds themselves (Philippi 1993). Our results appear to fit this requirement. Bet-hedging can be an optimal strategy in case of random variation in the best period to germinate. Most of the literature on bet-hedging of seed germination timing deals with year-to-year differences in seedling success, leading to higher long-term (geometrical mean) fitness when not all seeds germinate in the first year (Cohen 1966; Philippi 1993). The same reasoning may apply to seasonal differences (Simons 2009). If germination could only occur immediately, the entire progeny may be lost after a severe winter. On the contrary, if dormancy was complete and germination could only occur the following spring, this strategy cannot be evolutionarily stable when mild winters also occur, since autumn seedlings would then out-compete the smaller spring seedlings. However, the impact of the timing of germination is not independent of life span. In annual species, the importance of adopting the right bet-hedging strategy will be far more important than for a long-lived species where the risk is already spread over years. For this reason, a further study of seed dormancy in sea beet is necessary over its whole latitudinal distribution: life span is shorter in more southern regions, and even practically annual in inland human-disturbed habitats, while in other regions plants can reach as much as 10 years in age (Hautekèete et al. 2002).

Concluding remarks

Current climate change leads to a higher probability of mild winters and an increase in the length of the growing season across the latitudes of our study region (Bradshaw and Holzapfel 2007). As dormancy must be released as soon as the favourable season begins, evolutionary change then is the only sustainable response. Variability between individuals in combination with substantial heritability values for seed dormancy revealed in this study points to a strong potential for evolutionary change in germination phenology through optimization of autumn and spring germination proportions. The role of dispersal both needs to be further evaluated, since insufficient population connectivity through gene flow could limit the speed of evolution.

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References

Alonso-Blanco C, Bentsink L, Hanhart CJ, Vries HBE, Koornneef M (2003) Analysis of natural allelic variation at seed dormancy loci of *Arabidopsis thaliana*. Genetics 164:711–729

Baskin CC, Baskin JM (1998) Seeds: ecology, biogeography and evolution of dormancy and germination. Academic Press, London

Bentsink L, Soppe W, Koornneef M (2007) Genetic aspects of seed dormancy. In: Blackwell publishing Ltd Seed development, dormancy and germination: Oxford, UK, pp 113–132

Bewley JD (1997) Seed germination and dormancy. Plant Cell 9: 1055–1066

Bradford KJ (2005) Threshold models applied to seed germination ecology. New Phytol 165:338–341. doi:10.1111/j.1469-8137.2004.01302.x

Bradshaw WE, Holzapfel CM (2007) Genetic response to rapid climate change: it's seasonal timing that matters. Mol Ecol 17:157–166. doi:10.1111/j.1365-294X.2007.03509.x

Cohen D (1966) Optimizing reproduction in a randomly varying environment. J Theor Biol 12:119–129

De Cauwer I, Dufaÿ M, Cuguen J, Arnaud JF (2010). Effects of fine-scale genetic structure on male mating success in gynodioecious *Beta vulgaris* ssp. *maritima*. Mol Ecol 19:1540–1558

Donohue K (2002) Germination timing influences natural selection on life-history characters in *Arabidopsis thaliana*. Ecology 83:1006–1016. doi:10.1890/0012-9658(2002)083[1006:GTINSO]2.0.CO;2

Donohue K, Dorn L, Griffith C, Kim E, Aguilera A, Polisetty CR, Schmitt J (2005a) Environmental and genetic influences on the germination of *Arabidopsis thaliana* in the field. Evolution 59:740–757. doi:10.1554/04-419

Donohue K, Dorn L, Griffith C, Kim E, Aguilera A, Polisetty CR, Schmitt J (2005b) The evolutionary ecology of seed germination of *Arabidopsis thaliana*: variable natural selection on germination timing. Evolution 59:758–770. doi:10.1554/04-418

Falconer DS (1989) Introduction to quantitative genetics. Longman scientific and technical, 3rd edn. University of Edinburgh, UK

Fénart S, Auterlitz F, Cuguen J, Arnaud JF (2007) Long distance pollen-mediated gene flow at a landscape level: the weed beet as a case study. Mol Ecol 16:3801–3813. doi:10.1111/j.1365-294X.2007.03448.x

Fievet V, Touzet P, Arnaud JF, Cuguen J (2007) Spatial analysis of nuclear and cytoplasmic DNA diversity in wild sea beet (*Beta vulgaris* ssp. *maritima*) populations: do marine currents shape the genetic structure? Mol Ecol 16:1847–1864. doi:10.1111/j.1365-294X.2006.03208.x

Finch-Savage WE, Leubner-Metzger G (2006) Seed dormancy and the control of germination. New Phytol 171:501–523. doi:10.1111/j.1469-8137.2006.01787.x

Foley ME, Fennimore SA (1998) Genetic basis for seed dormancy. Seed Sci Res 8:173–182. doi:10.1017/S0960258500004086

Forcella F, Benech Arnold RL, Sanchez R, Ghersa CM (2000) Modeling seedling emergence. Field Crops Res 67:123–139. doi:10.1016/S0378-4290(00)00088-5

Galloway LF (2002) The effect of maternal phenology on offspring characters in the herbaceous plant *Campanula americana*. J Ecol 90:851–858. doi:10.1046/j.1365-2745.2002.00714.x

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. J Ecol 90:508–516. doi:10.1046/j.1365-2745.2002.00688.x

Hermann K, Meinhard J, Dobrev P, Linkies A, Pesek B, Heß B, Macháčková I, Fischer U, Leubner-Metzger G (2007) 1-Aminocyclopropane-1-carboxylic acid and abscisic acid during the germination of sugar beet (*Beta vulgaris* L.): a comparative study of fruits and seeds. J Exp Bot 58:3047–3060. doi:10.1093/jxb/erm162

Heydecker W, Chetram RS, Heydecker JC (1971) Water relations of beetroot seed germination II. Effects of the ovary cap and the endogenous inhibitors. Ann Bot 35:31–42. doi:10.2503/jjshs.50.355

Jump AS, Marchant R, Peñuelas J (2009) Environmental change and the option value of genetic diversity. Trends Plant Sci 14:51–58. doi:10.1016/j.tplants.2008.10.002

Koornneef M, Bentsink L, Hilhorst H (2002) Seed dormancy and germination. Curr Opin Plant Biol 5:33–36. doi:10.1016/S1369-5266(01)00219-9

Larsson EL (2002) Seed banks and seed dispersal in subarctic and arctic environments. Dissertation, University of Göteborg, Sweden

Leinonen K (1998) Picea abies seed ecology: effect of environmental factors on dormancy, vigor and germination. Dissertation, University of Helsinki, Finland

Letschert JPW (1993) *Beta* section *Beta*: biogeographical patterns of variation and taxonomy. Wagening Agric Univ Pap 93:1–155

Meyer SE, Allen PS (1999) Ecological genetics of seed germination regulation in *Bromus tectorum* L. 1. Phenotypic variance among and within populations. Oecologia 120:27–34. doi:10.1007/s004420050829

Meyer SE, Pendleton RL (2000) Genetic regulation of seed dormancy in *Purshia tridentata* (Rosaceae). Ann Bot 85:521–529. doi:10.1006/anbo.1999.1099

Peto FH (1964) Methods of loosening tight seed caps in monogerm seed to improve germination. J Am Soc Sugar Beet Techn 13:281–286

Philippi T (1993) Bet-hedging germination of desert annuals: variation among populations and maternal effect in *Lepidium lasiocarpum*. Am Nat 142:488–507. doi:10.1086/285551

R Development Core Team (2008) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

Simons AM (2009) Fluctuating natural selection accounts for the evolution of diversification bet hedging. Proc R Soc B 276: 1987–1992

Simons AM, Johnston MO (2006) Environmental and genetic sources of diversification in the timing of seed germination: implications for the evolution of bet hedging. Evolution 60:2280–2292. doi:10.1111/j.0014-3820.2006.tb01865.x

Sokal RR, Rohlf FJ (1995) Biometry. W. H. Freeman and Company 3rd, New York, USA

StatSoft Inc (2006) STATISTICA 7.1, Data analysis software system. Statsoft Inc, Tulsa

Van Dijk H (2009) Ageing effects in an iterparous plant species with a variable lifespan. Ann Bot 104(1):115–124. doi:10.1093/aob/mcp100

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. J Evol Biol 20:349–357. doi:10.1111/j.1420-9101.2006.01192.x

Van Dijk H, Boudry P, McCombie H, Vernet P (1997) Flowering time in wild beet (*Beta vulgaris* ssp. maritima) along a latitudinal cline. Acta Oecol 18:47–60. doi:10.1016/S1146-609X(97)80080-x

Vleeshouwers LM, Bouwmeester HJ, Karssen CM (1995) Redefining seed dormancy: an attempt to integrate physiology and ecology. J Ecol 83:1031–1037