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Ageing effects in an iteroparous plant species with a variable life span

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• *Background and Aims* Ageing effects may be due to dysfunction leading to decreasing reproduction and survival with age. In plants, however, other (physiological) causes, associated with size for example, may also play a role. Iteroparous plants with genetically variable life spans can be helpful in unravelling these two aspects of changes associated with growing older.
• *Methods* In a long-term experiment, *Beta vulgaris* ssp. *maritima* (sea beet) plants from the same set of populations but with different ages were compared for flowering date over several years. Flowering date, root growth and seed production were measured in a synthetic population and in progenies derived from reciprocal crosses over three consecutive years and analysed with respect to the number of years yet to live. Heritabilities of these three characters and of life span were estimated.
• *Key Results* Flowering occurred on average 1·3 d later each year over a plant's whole lifetime. In the year before dying, plants flowered on average 3·3 d later and both root investment and seed production decreased significantly compared with plants that remained alive for at least 1 further year. The negative relationship (trade-off) between reproduction and root investment in early life became positive near the end of life, and the positive relationship between flowering date and root growth became negative.
• *Conclusions* Effects of ageing – in the sense of a decline in reproduction and root storage – combined with later flowering were particularly pronounced in the year before death. The gradual change in flowering phenology, observed over the whole lifetime, could have a physiological basis unrelated to dysfunction.

Key words: Ageing, *Beta vulgaris* ssp. *maritima* (sea beet), flowering phenology, longevity, perennial, root investment, seed production, trade-offs, whole-plant senescence.

INTRODUCTION

Organisms change in several respects when they grow older. After maturity, i.e. the changeover from the vegetative to the reproductive state, two distinct components of ageing may exist. First, there is dysfunction with advancing age expressed through higher mortality rates, lower reproductive output or both. In evolutionary biology this is usually referred to as 'senescence' (e.g. Monaghan *et al.*, 2008) but since this word has a different meaning in the physiological literature where it is used at the tissue or cellular level (e.g. Jansson and Thomas, 2008, and references therein; Munné-Bosch, 2008) it will here be largely avoided. The second view of ageing is based on physiological changes unrelated to dysfunction. In species with a rigid developmental programme there is no strong evidence to distinguish between these two components of ageing. Other species, in particular plants, have the potential to continue to grow after maturity and thus gain performance with age. Negative effects of ageing are therefore not a straightforward matter in plants. Growth after maturity may lead to a positive rather than a negative relationship between reproduction or survival and age (Harper, 1977), leading to the idea of 'negative senescence' (Vaupel *et al.*, 2004), at least during a part of adult life. Unravelling the different aspects of ageing is experimentally complex, but species with genetic variability for life span may be helpful. The hypothesis adopted and tested here is that dysfunction is essentially associated with the last year(s) of life, whatever the life span, while other, e.g. size-related, phenomena may become manifest at any age.

Whole-plant ageing can be quite contrasted, ranging from dramatic senescence in semelparous plants to virtually absent in clonally reproducing iteroparous plants (Harper, 1977; Noodén, 1988; Watkinson, 1992; Roach, 1993; Pedersen, 1999; Thomas, 2002). In general, three classes of ageing are distinguished: rapid, gradual and negligible (Finch, 1990; Munné-Bosch, 2008), the first being associated with semelparity. The difference between the classes lies in the way in which rejuvenation is realized. Semelparity means that no effort is made to maintain the individual beyond the age of reproduction and that from that point on new tissues only appear in the form of seeds. Negligible ageing, defined as minimal or no ageing deterioration, requires efficient rejuvenation of the original individual's vegetative tissues, e.g. by clonal reproduction through the formation of physiologically autonomous ramets that may senesce individually without affecting the genet as a whole. Generally speaking, there is a trade-off between rejuvenation through seeds (reproduction) and investment in new vegetative tissues (survival). Investment in new vegetative tissues dominates in plants that have the potential of making new roots from shoots or new shoots from roots, which can be considered as a sort of clonal reproduction where the different parts of a genet remain more or less interconnected. This causes joint vulnerability to accidents and enemies, such as herbivores and pathogens, which may also

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lead to senescence effects at the whole-plant level (Roach, 1993; Gardner and Mangel, 1997; Silvertown *et al.*, 2001). Iteroparous plants that lack this possibility form 'integrated physiological units' and are the most comparable to animals for which the effects of ageing have been studied intensively (see Rose, 1991), in particular with respect to the decline in age-specific reproduction and survival. The iteroparous species studied here, *Beta vulgaris* ssp. *maritima*, commonly known as sea beet, never forms new roots from shoots or vice versa but forms a single physiological unit per genet. Sea beets are therefore expected to be sensitive to the negative effects of ageing with direct consequences for the plant performance and survival.

Ageing can be studied under natural or under protected conditions. In the wild, random, i.e. age-independent ('extrinsic'), mortality can be substantial (Roach, 2001), complicating the evaluation of ageing. Extrinsic mortality and mortality caused by ageing are not independent. For evolutionary reasons (Kirkwood and Austad, 2000), mortality through senescence is theoretically contributing more to total mortality in species or populations with higher extrinsic mortality in the wild, although this is not unequivocally true in practice (Silvertown *et al.*, 2001; Bronowski and Promislow, 2005; Williams *et al.*, 2006). Longevity in sea beet was studied under protected (glasshouse) conditions by Hautekèete *et al.* (2002*a*). They found that median life span was higher in populations with lower disturbance *in situ*, which confirms evolutionary theory. Although plants in a glasshouse are well-protected against most kinds of random mortality, there remains the possibility of attack by pathogens or parasites. Less well-functioning plants are more sensitive to such attacks than entirely sound plants. Thus, even under controlled conditions, extrinsic and intrinsic factors may interact in their contribution to mortality.

In iteroparous species, ageing effects are often studied at the cohort or age-class level (e.g. Roach, 2003; Roach and Gampe, 2004; Pico and Retana, 2008). Survival and reproduction vary among age classes, typically with a decline of one or both in the higher age classes. The sea beet is known for its variable life span, both within and among populations, with almost annual populations and populations where life span can exceed one decade (Hautekèete *et al.*, 2002*a*). In this type of species, the subdivision into age classes may not be very appropriate. A logical alternative approach is to study individual performance with respect to the number of years yet to live ('years to death') which synchronizes death instead of birth and seems well-adapted to the species studied here. The same approach was used by Reed *et al.* (2008) in a study on long-lived seabirds, adopting the abbreviation YBD (years before death).

Reproduction and survival in an iteroparous plant species can be measured by annual seed production and survival to the next flowering season. However, these traits are not directly comparable: seed production is an investment while survival is the consequence of an investment, which is here represented by root growth with the argument that the quantity of reserves is a criterion of survival to the next season. Since both seed production and root growth depend on available resources, a trade-off is to be expected between these resource allocations. In this study, flowering date was included, and is even the most intensively studied trait. It is easy to measure and is closely connected to investments in reproduction and survival. Many iteroparous plants flower in spring and finish flowering before or during summer at a more or less fixed moment, determined by day length, devernalization, drought, herbivory, etc. Later flowering in such situations means less time for seed production and more for investment in storage for survival and future reproduction. Flowering phenology is therefore a direct actor in the trade-off between the two biomass allocations (Lacey *et al.*, 2003; Bolmgren and Cowan, 2008). Senescence is considered to have a negative effect on both reproduction and survival and, for this reason, it is of particular interest to know how the relationship between both investments and flowering phenology behaves near the end of life.

Flowering date and survival data on sea beet were available over a period of 17 years for a large number of populations along a latitudinal gradient (see Van Dijk *et al.*, 1997) evaluated under common semi-natural glasshouse conditions. Given that life span can be longer than one decade (Hautekèete *et al.*, 2002*a*), observations of flowering date over long periods could be made on the same plants. Seed production and root growth were studied over a shorter period on fewer individuals. Instead of a detailed study of a series of populations, a single synthetic population was constructed, derived from populations from all over the study region (roughly covering France) obtained by open pollination in a series of successive generations. In this way the complications due to genetic disequilibrium among populations over the latitudinal gradient (populations with a longer life span are in general earlier flowering; see Van Dijk *et al.*, 1997; Hautekèete *et al.*, 2002*a*) were avoided. Seed production, root growth and flowering date were measured in the synthetic population with emphasis on the number of years yet to live as a criterion. Since flowering date was studied both directly in relation to age and with regard to years to death this trait was expected to give the most detailed information on both aspects of ageing in distinguishing between the end-of life phenomena and the general age effects. The link between flowering date and the investments in reproduction and storage reserves was demonstrated by calculating correlations between the three traits and supported by the calculation of heritabilities and genetic correlations.

MATERIALS AND METHODS

Plant material and crosses

Beta vulgaris ssp. *maritima* (sea beet) is a predominantly coastal, self-incompatible, wind-pollinated herb with a variable life span found in Europe, north Africa and west Asia (Letschert, 1993). Its seeds are grouped into seed balls and can remain viable for several decades. Flowering initiation requires long days (Van Dijk and Hautekèete, 2007) and, especially in northern areas, a variable vernalization intensity (Boudry *et al.*, 2002).

Seeds from 93 populations in France and some neighbouring countries were sampled from separate individuals in 1989 (for more details, see Van Dijk *et al.*, 1997). From 1990 until 1999, at least one seed from each population was

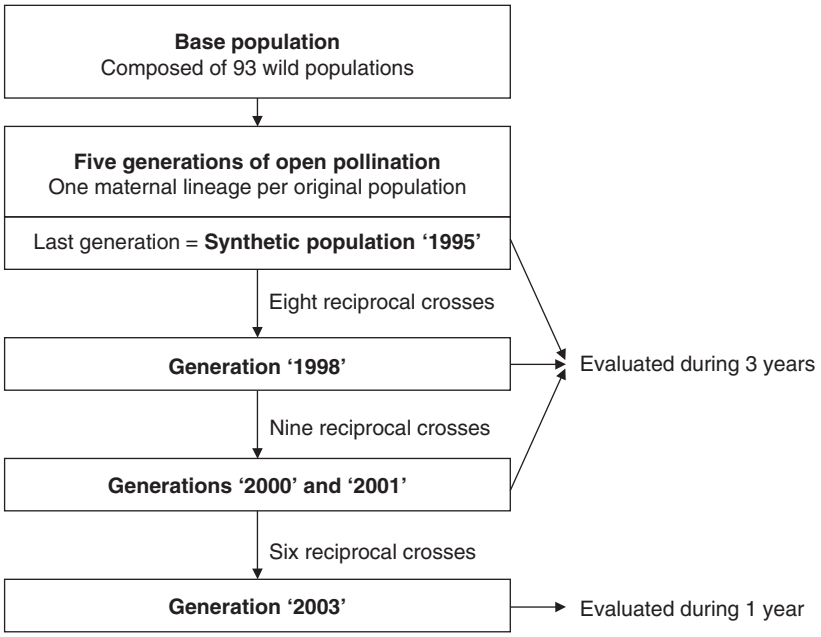

FIG. 1. Schematic presentation showing how the synthetic population and later generations were derived.

sown each year and the resulting plant(s) grown and kept in the glasshouse until death. Each year, insofar as possible, seeds were taken from different plants from the original population sample to represent the population. In total, ten cohorts were thus studied.

In 1991, plants of all 93 populations (mean number of plants per population = 7·7), arranged in a random design, flowered together in the glasshouse and open pollination was allowed. Seed balls were harvested on one randomly chosen plant per original population and sown in autumn 1991, keeping only one seedling per parent plant. The resulting mixed population was kept apart and after flowering the following year (with open pollination in a random design as before), seeds were harvested from each plant, and one seedling per parent plant was kept. This procedure was repeated each year until 1995, thus obtaining a single synthetic population called '1995' (see Fig. 1). Plants not requiring vernalization, frequent in inland and southern populations (Van Dijk *et al.*, 1997) and often showing a near-annual life cycle, were systematically eliminated; all plants thus flowered for the first time after their first winter and thereafter once a year in spring or early summer.

Eight controlled reciprocal crosses were made in 1998 between plants of the '1995' population. 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 (see Fig. 1). Inbreeding was limited by avoiding crosses between close relatives (first and second degree). Selection criteria mainly involved seed dormancy characters, which do not have any relationship with the traits studied here, and, in a few cases, root diameter for which positive assortative mating was carried out by choosing parents that had both a high or low value. No selection for flowering phenology was implemented, although crosses could only be made between plants that flowered simultaneously.

Culture conditions and measurements

Plants were sown in September (in June in 1990 and 1996) in a glasshouse compartment at 20 °C with a 16-h day length and remained there until December (September in 1990 and 1996). Thereafter, they were kept until death in a semi-natural glasshouse with natural day length and temperatures slightly warmer than external temperatures and protected against frost during winter. All plants were repotted in 2·4-L pots using standardized commercial soil each year in January. No fertilizer was added; each plant thus received the same amount of nutrients through annual soil renewal (Neuhaus Huminsubstrat N3: 90 % peat, 10 % clay; pH 6; NPK 14:16:18, 1·3 kg m^{-3}; conductivity 35 mS m^{-1}). Size differences were thus avoided as much as possible.

The day on which the first flower opened ('flowering date') was recorded each year for all plants described above. In addition, for the synthetic '1995' population and the later '1998', '2000' and '2001' generations, all plants were measured for seed production and root diameter for 3 years starting after their first flowering period (see Fig. 1). In the '2003' generation, these supplementary measurements were only made after the first flowering season in 2004. Survival was recorded for the three remaining years of the study, with a final evaluation in 2007.

Annual seed production was measured each year in August by separating seed balls – which were all ripe by that time – from other structures and determining the total weight per plant. Root diameter was measured each year in January when the plants were repotted. In the case of asymmetrical roots, two perpendicular diameters at the thickest point

were measured. The square diameter (or the product of perpendicular diameters) was used as root biomass criterion, since root length was probably limited by pot size. The difference in root biomass between two subsequent years was referred to as 'annual root growth'. Over the first period (from seedling to the winter after the first flowering period), final root biomass was used as the growth criterion.

Life span variability

All statistical analyses described here and in the following sections were carried out with Statistica 7·1 (Statsoft, France), unless otherwise indicated. Plants were considered dead when no more green parts were left (living plants always have inflorescences with leaves or one or more vegetative rosettes). Individual life span was determined for plants from all 93 populations and seven additional populations sampled later on, using all ten cohorts (total number of plants = 1680). Geographic regions were chosen *a priori* according to Van Dijk *et al.* (1997): A = French Mediterranean, B = inland populations in south-west France, C + D = French Atlantic coasts from the Spanish border up to and including south Brittany, E = west and north Brittany, Normandy and the Channel Islands, F = southern North Sea and around Dover Strait. A nested ANOVA (unequal sample sizes; Satterthwaite's approximation) was carried out to estimate what proportion of the total variance in life span was due to individuals within populations, populations within regions and between regions. A few plants were still alive after the winter of 2007–2008 and were considered as alive until the end of 2008. As they were almost all from region E, life span in this region was probably slightly underestimated, causing a bias. The '1995' synthetic population and '1998' generation were pooled; these were the only samples for which plants up to age 9 were available in 2007 (but with also a few plants still alive in early 2008).

Median life span per population is less sensitive to the presence of still-living plants and was estimated for all populations. As described earlier by Hautekèete *et al.* (2002*a*), survival percentage to a given age was arcsine transformed and median life span (age at which there is 50 % survival) was estimated by linear regression, excluding 0 and 100 %. Life span per region was estimated as the mean of the median population life spans. Due to within-region heterogeneity, pooling populations may lead to different, unrepresentative survival curves. Therefore, no quantitative analysis was applied to pooled data. Differences between regions were tested by a one-way ANOVA with a Tukey *post hoc* test.

Flowering date in relation to age

The same 1680 plants from ten cohorts presented above were used for testing the effect of age on flowering date. Although using individual trajectories over age (longitudinal data) is a powerful tool for such tests, systematic changes arising from environmental conditions, due to current climate change, could be erroneously interpreted as ageing effects. Flowering date is particularly sensitive to changes in vernalization intensity (where milder winters lead to less vernalization) and higher spring temperatures (leading to earlier flowering after induction). For this reason, plants were compared strictly within years. In each year from 1992 to 2007, plants from the same population but with an age difference of 1 year were compared for flowering date and this was done for all available populations. Comparisons were combined per age class over the whole study period, i.e. all comparisons of 1-year-old plants with 2-year-old plants were pooled, all comparisons of 2-year-old with 3-year-old plants, etc. To calculate the average difference in flowering time over all age classes, each individual comparison was used only once, averaged over the years that this comparison could be measured, thus avoiding a higher contribution by longer-living populations. In most cases, the plants' first flowering period occurred after an incomplete winter due to the transfer from the 20 °C compartment in December (all cohorts except 1990 and 1996). These data were not used because it is known that plants flower somewhat later when not sufficiently vernalized (Boudry *et al.*, 2002).

The shift in flowering time calculated over all age classes was tested with a null hypothesis of no differences, using Student's *t*-test. Heterogeneity among age classes was tested by a one-way ANOVA. For this test, all comparisons from 7-year-old with 8-year-old on, including plants alive at the end of the experiment, were pooled (7–8 +).

Root size before flowering may have a relationship with flowering date; the correlation between both traits was thus estimated using all available data, which were restricted to plants age 2 and older since root size was not measured before the first flowering period.

Effects near the end of life

The effects near the end of life were measured in the synthetic '1995' population and the later generations '1998', '2000', '2001' and '2003'. In each year and for each generation, the means (μ) and standard deviations (σ) for flowering date, seed production and root growth were calculated after which all individual data (x) were transformed into standard deviations $(x - \mu)/\sigma$. All generations were pooled to evaluate flowering date, seed production and root growth, thus exploiting the relative position that individuals had with regard to the mean of their population or generation, with the assumption that this position did not differ among years due to variable or non-linear reaction norms. The number of 'years to death' was defined as the number of future (potentially) reproductive periods (zero for plants in their last year of life). Differences in the measured traits as a function of the number 'years to death' were tested using Student's *t*-test or by ANOVA, in the latter case using Tukey's HSD (honestly significant differences). Plants with >2 'years to death' (i.e. ≥3) were pooled because the last measurements were made in 2004 and plants still alive in 2007 thus lived for at least three more years; they formed the '3 +' years-to-death class. Since measurements were repeated over the ages 1–3, it could be tested whether or not age had an effect on the difference between plants that were or were not in their last year of life ('persistence'). For this purpose, a two-way ANOVA was carried out for all three characters with persistence and age as factors.

Correlations between flowering date, annual seed production and annual root growth were compared for significant differences among years-to-death classes according to Sokal and Rohlf (1995, p. 581) also using the combined generations. For lack of a multiple-comparison test for calculating HSD, two-by-two tests were carried out for further analysis.

Heritabilities and genetic correlations

Heritabilities were estimated on transformed data (see previous section) for flowering date, annual seed production and annual root growth using midparent–offspring regression (Falconer, 1989), exploiting all useful midparent–offspring relationships ($n = 22$) in the successive generations. To improve interpretation of correlations, the corresponding genetic and environmental components and their standard errors were calculated according to Falconer (1989, pp. 316–317). Only data obtained for the first year of flowering were used for both parents and offspring. Although preferable, it was not possible to use the number of years to death as a criterion as in the previous sections, for lack of sufficient precision. All generations were combined to obtain a single estimation per character or correlation.

Life span heritability was estimated exploiting the same parents and their offspring but with the complication that a few plants involved were still alive at the moment of data analysis. As above, survival was artificially set to the end of 2008.

RESULTS

Life span variability

Total variance for life span ($s^2 = 7{\cdot}24$, 100 %) could be subdivided into individuals within populations ($s^2 = 3{\cdot}70$, 51·1 %), populations within regions ($s^2 = 0{\cdot}76$, 10·5 %) and between regions ($s^2 = 2{\cdot}78$, 38·4 %). The differences between populations within regions and the differences between regions were both highly significant ($F_{95,1580} = 4{\cdot}47$, $P < 0{\cdot}001$ and $F_{4,100} = 50{\cdot}92$, $P < 0{\cdot}001$, respectively). The variance among individuals in the synthetic population ('1995' synthetic population and '1998' generation pooled) was 6·45, indicating that the synthetic population covered a substantial proportion (89 %) of the total variance. When observations ceased (i.e. early 2008), 29 plants were still alive: 24 out of 495 from region E, one out of 852 from region D and four out of 120 in the two generations of the synthetic population. Two plants from the base population sown in 1990 were still alive in 2008.

Substantial differences in median life span were found between the geographical regions ($F_{4,95} = 56{\cdot}35$, $P < 0{\cdot}001$) as estimated by a one-way ANOVA with a Tukey *post hoc* test (Table 1). Survival curves per geographical region provide a more detailed picture (Fig. 2).

Since plants that do not require vernalization (almost all plants from region B and roughly half those from region A) were excluded, the synthetic population was principally derived from regions C, D, E and F and, to a lesser extent, from region A. The median life span of the synthetic population was, as expected, within the range of life spans determined for regions C to F (Table 1). The survival curve appeared to be less steep compared with the geographical regions (Fig. 2), which is a logic consequence of the combining of groups with different life spans. The variability of life span in the synthetic population had a significant genetic component: heritability ($h^2 \pm$ s.e.) was estimated to be $0{\cdot}48 \pm 0{\cdot}13$ ($P = 0{\cdot}003$).

TABLE 1. *Mean life span in the five geographical groups of populations and in the synthetic population ('1995' synthetic population and '1998' generation pooled)*

Populations and regions	n_P	n_I	L
A (Mediterranean)	9	102	$2{\cdot}9 \pm 1{\cdot}0^a$
B (inland)	10	96	$2{\cdot}2 \pm 0{\cdot}6^a$
C + D (Atlantic)	36	852	$4{\cdot}8 \pm 0{\cdot}9^b$
E (west Brittany, English Channel)	33	495	$7{\cdot}1 \pm 1{\cdot}3^c$
F (Dover Strait, North Sea)	12	135	$4{\cdot}8 \pm 1{\cdot}3^b$
S (synthetic)		120	5·7

n_P = number of natural populations; n_I = total number of individuals; L = life span (mean ± standard deviation among populations). Superscript letters refer to Tukey's *post hoc* test with different letters indicating significant differences at the 5 % level.

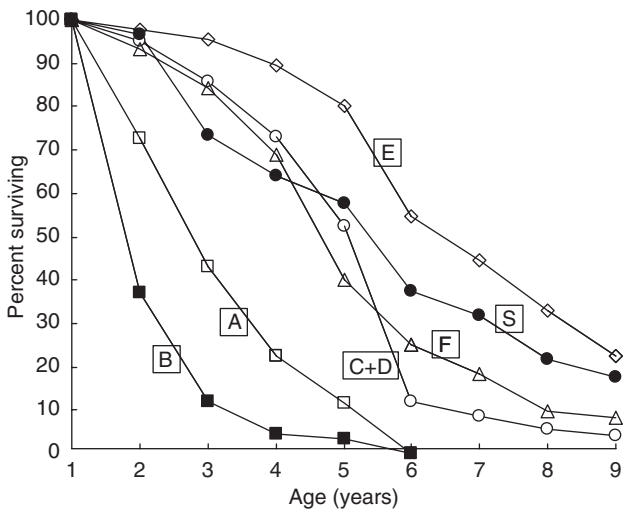

FIG. 2. Survivorship curves for the synthetic population S (pooled generations '1995' and '1998') and the combined populations from five regions: A (French Mediterranean); B (inland south-west France); C + D (French Atlantic); E (west and north Brittany, Normandy and the Channel Islands); F (southern North Sea and around Dover Strait).

Flowering date in relation to age

The comparison of plants from the same populations but with a 1-year age difference showed a highly significant, gradually later flowering of 1·31 d per year over all age classes ('All' in Fig. 3). This value was significantly different from zero ($n = 646$; $t = 3{\cdot}115$; $P = 0{\cdot}002$). Each age class showed the same trend (Fig. 3): there were no significant differences between them (one-way ANOVA, $F_{6,2071} = 0{\cdot}853$; $P = 0{\cdot}529$). It should be noted that variances were relatively high due to the comparison of different plants, often from different maternal origin, although from the same

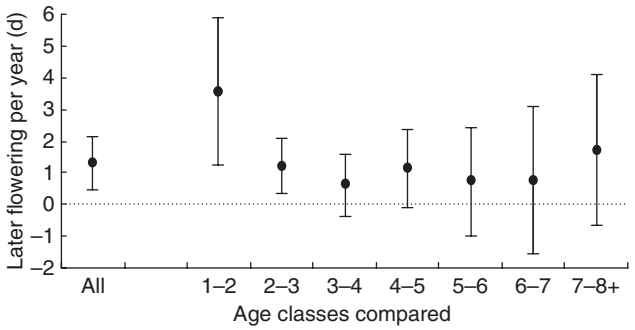

FIG. 3. Number of additional days for 1-year-older plants to flower (mean ± 95 % confidence interval). 1–2 = 1-year-old plants compared with 2-year-old plants, etc.; 7–8+ includes 8–9, 9–10, etc.

population. It was not possible to compare the same genotype at different ages in this outcrossing, non-clonal species. This approach would have required pure lines, or a comparison of ramets of the same genet.

Two checks for the validity of this result were made. First, the five available pairs of plants with a 9-year age difference (the maximum in this experiment) showed on the average 12·0 (s.e. = 2·8) d later flowering of the older ones compared with their younger counterparts from the same populations. This illustrates that iteroparous plants can indeed flower substantially later when much older.

The second check was made to exclude the possibility that the effects of later flowering were exclusively or principally brought about by plants in their last year before dying (see the next section). This type of phenology could have been more frequent in the older group, or in short-living ecotypes. Indeed, mortality was slightly lower in the first years of life, although in the whole data set the correlation between life span and the number of years to death was not significant at the 5 % level ($n = 912$; $r = -0{\cdot}058$; $P = 0{\cdot}082$). A subset of plants that flowered in at least five consecutive years was analysed for the first four years. With an overall value of 1·21 d later flowering per year ($n = 303$; $t = 2{\cdot}066$; $P = 0{\cdot}040$), the results were very similar to those for the whole set of plants, although the confidence intervals were higher due to the lower sample sizes.

A few plants stopped flowering all together after reaching a certain age. They were no longer included in the data set from that age on. This created a slight bias and the effects of ageing may have been slightly underestimated, but only with respect to flowering date as no plants stopped flowering during the first three years of evaluation of seed production and root growth.

The relationship between root size before flowering and the subsequent flowering date was evaluated by calculating the correlation between both traits which was found to be significantly positive ($n = 497$, $r = 0{\cdot}120$, $P = 0{\cdot}007$). Excluding plants in their last year of life reinforced the correlation ($n = 400$, $r = 0{\cdot}185$, $P < 0{\cdot}001$).

Effects near the end of life

Effects associated with the number of years yet to live ('years to death') were only examined in the synthetic population '1995' and later generations and not in the original

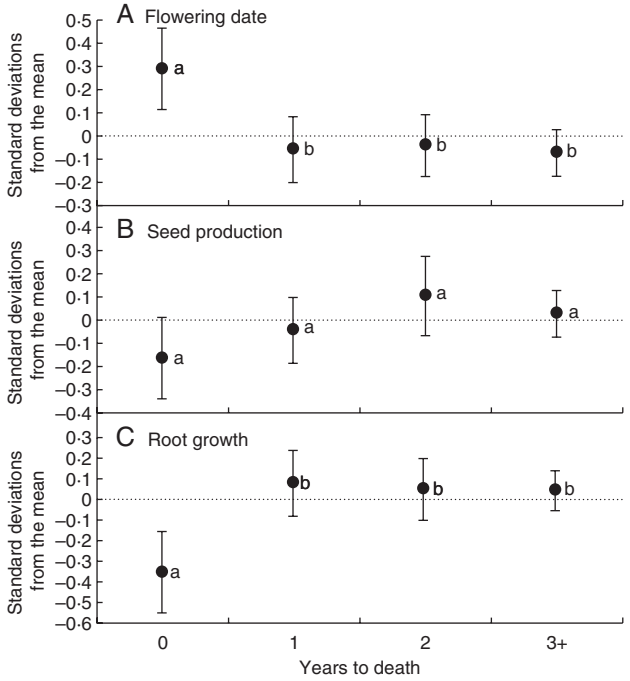

FIG. 4. Flowering date, annual seed production and annual root growth (data transformed into standard deviations, mean ± 95 % confidence interval) as a function of the number of years yet to live ('years to death'; 3 +, ≥3 years). Values with the same letter were not significantly different at the 5 % level.

populations. Plants in their last year of life systematically flowered later than plants that had at least 1 more year to live (0·287 vs. −0·062 standard deviations; $n = 908$, $t = 4{\cdot}09$, $P = 0{\cdot}0005$). This corresponds to a difference of about 3·3 d as the average standard deviation over years and generations was found to be 9·4 d. Figure 4A shows a more detailed picture of the plants that had 1, 2 and 3 or more ('3 +') years to live. Flowering date varied significantly among the different classes (ANOVA, $F_{3,904} = 5{\cdot}427$, $P = 0{\cdot}0008$) but multiple comparisons (Tukey's HSD test) indicated that there were no significant differences between the three categories with at least 1 more year to live and reproduce. The gradually later flowering with age inferred from Fig. 3 is independent of the later flowering in the last year of life reported here. Here, plants of the same age were compared, only differing in future lifetime.

Annual seed production was less influenced by the number of years to death. The difference between plants that died or not before the next flowering season (−0·165 vs. 0·030 standard deviations) was significant at the 5 % level ($n = 849$, $t = -2{\cdot}079$, $P = 0{\cdot}038$), but no significant heterogeneity among the four classes was revealed (Fig. 4B; one-way ANOVA, $F_{3,845} = 1{\cdot}979$, $P = 0{\cdot}116$).

Annual root growth was considerably lower in the last year of a plant's life. Here a highly significant difference of −0·353 vs. 0·056 standard deviations ($n = 868$, $t = -4{\cdot}197$, $P = 0{\cdot}0003$) was found. A more detailed analysis (Fig. 4C) showed a similar pattern as for flowering date: root growth varied significantly among all four classes (one-way ANOVA, $F_{3,864} = 5{\cdot}695$, $P = 0{\cdot}0006$) but no heterogeneity was revealed among the three classes of persisting plants.

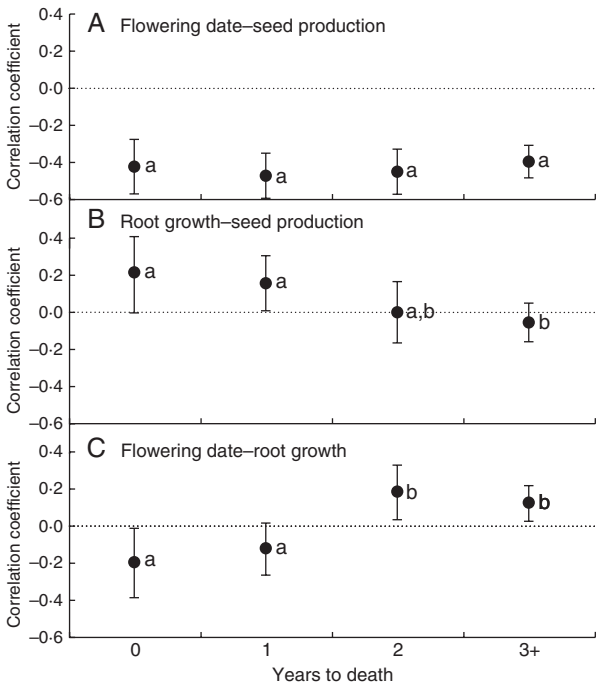

FIG. 5. Correlation coefficients between flowering date, annual seed production and annual root growth (± 95 % confidence intervals) as a function of the number of years yet to live ('years to death'; 3 +, ≥3 years). Values with the same letter were not significantly different at the 5 % level.

For all three variables, tests looking for differences between age classes 1, 2 or 3 were carried out. Age did not have a significant effect on the difference between plants in their last year of life vs. persisting plants (two-way ANOVAs: flowering date $P = 0{\cdot}987$; seed production $P = 0{\cdot}394$; root growth $P = 0{\cdot}723$), nor was there any significant interaction between persistence and age (flowering date $P = 0{\cdot}726$; seed production $P = 0{\cdot}067$; root growth $P = 0{\cdot}918$).

Correlations between annual seed production, annual root growth and flowering date were calculated over all measured plants of the synthetic population and later generations. The plants were subdivided into the same four classes of 'years to death' as used in the previous section. For the overall values, only the correlation between flowering date and seed production was strongly negative at a high significance level ($n = 845$, $r = -0{\cdot}428$, $P < 0{\cdot}001$). The detailed analysis showed no differences among the number of years to death: $\chi^2_3 = 3{\cdot}96$, $P = 0{\cdot}265$ (Fig. 5A).

The correlation between seed production and root growth was not significant when all plants were considered together ($n = 805$, $r = 0{\cdot}026$, $P = 0{\cdot}465$), but was heterogeneous among the years-to-death classes $\chi^2_3 = 8{\cdot}63$, $P = 0{\cdot}035$ (Fig. 5B).

Finally, the overall correlation between flowering date and root growth was not significant ($n = 864$, $r = 0{\cdot}004$, $P = 0{\cdot}901$), while further analysis showed a strongly significant heterogeneity $\chi^2_3 = 16{\cdot}81$, $P = 0{\cdot}0008$ (Fig. 5C).

Heritabilities and genetic correlations

Combining all available generations, 22 midparent–offspring pairs could be used for the regression analysis only using first-year data for both parents and offspring. All three characters showed significant h^2 values (Table 2). Phenotypic correlations r_P were limited to the first age class and roughly corresponded to the overall values in Fig. 5 but with a lower contribution of the soon dying plants as is to be expected in the first year. Only flowering date and seed production showed a significant genetic correlation; this trait combination also showed the only significant environmental correlation (Table 2).

DISCUSSION

Life span variability

The geographical differences in life span described by Hautekèete *et al.* (2002*a*) were confirmed in this study using the same populations but with data for a longer period. In the 2002 study, considerable variation between populations within regions (largely coinciding with those in Table 1) was reported with coefficients of variation (CV) between 15 % and 30 %. These values are generally comparable to the CV values that can be derived from Table 1. More generally, Ehrlén and Lehtilä (2002) showed that within-species variation was of the same magnitude as between-species variation in a literature survey based on 11 perennial plant species.

The variance in longevity within the original populations could be due, to an unknown relative extent, to genetic or to environmental components. An estimation of heritability could therefore not be made at the level of these populations. The variance among populations and regions, on the other hand, can be ascribed to genetic variation since environmental variation cancels out at those levels. The synthetic population covered part of the between-population variation and was, as expected, genetically variable for life span as illustrated by its highly significant heritability. The synthetic population and the later derived generations thus constitute a useful set of plant material to study to what extent changes in the measured life-history characteristics are specifically associated with the end of life, whatever the age.

Flowering date in relation to age

This study demonstrated that, even long before the end of life, there was a gradual later flowering of about 1·2–1·3 d year^{-1}. The mechanism behind this change in phenology remains unresolved: it may be due either to a slow, but steady type of deterioration (whole-plant-senescence) or to a consequence of a changing physiology with age, e.g. due to increasing plant size, in particular, roots. As reviewed by Munné-Bosch (2007), plant size can have a direct effect on the ageing process. Since size increases with age, it is difficult to distinguish between the two factors. However, data within years, and therefore within age classes, on the correlation between root size and the subsequent flowering date were available in this study. Although this correlation was significantly positive, the existence of a causal relationship, if any, between root size and flowering date cannot be determined. Even though the additive genetic component of the correlation (r_A) between flowering date and root growth was not significant at the 5 % level (Table 2), a genetic relationship

TABLE 2. *Heritabilities of flowering date, seed production and root growth obtained by midparent–offspring regression and the phenotypic (r_P), genetic (r_A) and environmental (r_E) correlations between all combinations of these traits*

Trait or correlation	$h^2 \pm$ s.e.	r_P	$r_A \pm$ s.e.	$r_E \pm$ s.e.
Flowering date	0·73 ± 0·12***			
Seed production	0·38 ± 0·13**			
Root growth	0·26 ± 0·10*			
Flowering date vs. seed production		−0·423***	−0·446 ± 0·139**	−0·460 ± 0·176**
Root growth vs. seed production		0·009	−0·084 ± 0·189	0·053 ± 0·123
Flowering date vs. root growth		0·152**	0·071 ± 0·177	0·272 ± 0·161

*** $P < 0·001$; ** $P < 0·01$; * $P < 0·05$.

between the two traits cannot be ruled out completely. A positive correlation between the two traits indicates that later flowering is associated with higher root growth and consequently with thicker roots in the following year. Since both traits have significant heritability and consequently significant repeatability (Falconer, 1989), plants with thicker roots would already only for this reason flower later the following year. Various relationships between plant size and flowering phenology have been reported (e.g. Ollerton and Lack, 1998; Torimaru and Tomaru, 2006), but with a tendency for larger plants to flower earlier. However, plant size in these studies was based on shoot rather than on root size. Since growth in this study was limited by soil renewal, the amount of storage tissue in roots was not *a priori* related to shoot size.

Whatever the underlying mechanism, flowering later and later each year may have consequences for assessing the response to selection for flowering date and for estimating realized heritability. A potentially elegant way to avoid year-to-year fluctuations due to the stochastic variation in temperatures during winter and spring is the comparison of parents and offspring in the same year, which is possible in iteroparous plants. However, the described ageing effect on flowering date would cause a bias since offspring are at least 1 year younger than the parents and will therefore systematically flower slightly earlier. Over several generations, this can cause a substantial overestimation of the cumulated selection response as well as the realized heritability of earlier flowering or an underestimation in the case of selection for later flowering.

Effects near the end of life

Flowering date was measured both as a function of age (especially on the data set limited to persisting plants) and in relation to the number of years to death. Both factors were significant and acted independently. This means that in a plant's lifetime, flowering tended to occur about 1·2–1·3 d later each year, with flowering occurring an additional approx. 3·3 d later in the plant's last year compared with persisting plants of the same age. Seed production and root growth, less easily measured on a large number of plants, were only studied in the various generations of the synthetic population during a limited number of years per generation. No direct conclusions can therefore be drawn on how investments in reproduction and survival changed with age over longer periods before the end of life (although the negative correlation between flowering date and seed production suggests that seed production could gradually decrease). The change in means and correlations near the end of life nevertheless provided useful information about the relationship between resource allocation and whole-plant-senescence.

Both seed production and root growth significantly decreased in the last year of life, but the decline in seed production was considerably weaker than the decline in root investment (Fig. 4B and C). One reason may be that, under protected conditions, the proximate cause of mortality is the lack of investment in maintenance, particularly in root storage reserves. Producing fewer seeds only is a consequence of general physiological deterioration and has no causal effect on survival (Hautekèete *et al.*, 2002*b*). Another possible reason is the *a priori* existing trade-off between both investments. Plants that invest relatively little in roots but much in seeds may be more vulnerable to senescence, and have a greater risk of dying, compared to plants with more investment in roots and less in seeds. A lower end-of-life seed production could thus be partially masked. A third possibility could be that seed production (end of summer) was measured earlier than root size (winter), which was therefore closer to the end of life. On the other hand, flowering date, occurring before seed production, was clearly influenced by end of life effects (Fig. 4A).

The optimal evolutionary strategy in iteroparous plants is to increase reproductive effort especially in the last year of life at the expense of all further, useless, investment in survival, as occurs – by definition – in semelparous species. This is apparently not what happened here. There may be no efficient physiological mechanism to 'warn' the plant of approaching death and set off this type of strategy.

The correlation between flowering date and seed production remained strongly negative, even when approaching the end of life (Fig. 5A). A negative relationship between the onset of flowering and reproductive output was also found by Lacey *et al.* (2003) for the short-lived perennial *Plantago lanceolata*, with virtually the same correlation coefficient. They showed that later flowering individuals had a shorter fruiting duration in such a way that the end of the seed maturation period happened at approximately the same date, which seems similar to what happens in sea beet. If the observed later flowering near the end of life means that part of the plants are not functioning as well due to senescence, seed production logically decreases for those plants, thus maintaining or reinforcing the negative correlation.

The other two correlations systematically changed when approaching the end of life (Fig. 5B, C). The theoretically expected negative correlation between survival and reproduction, based on the trade-off in combination with genetic

variation, assumes constancy in the amount of resources to allocate. This correlation can become positive if the total amount of available resources varies between individuals, which may be due to environmental variation in resource levels (Van Noordwijk and De Jong, 1986; Roff and Fairbairn, 2007), differences in size (Harper, 1977; Vaupel *et al.*, 2004) or, relevant to this study, variation in the degree of senescence between individuals with consequences for their physiological performance. The significant change in the correlation between survival and reproduction found here from slightly negative in '3 +' to clearly positive in '0' and '1' (Fig. 5B) suggests that in the last year of life there is a mixture of senescent plants with a negative impact on both types of resource allocation and plants dying from other weaknesses not associated with resource allocation (at least at the moment of the measurements).

The positive correlation between flowering date and root growth became negative in the last year of life. While healthy later-flowering plants leave more time for vegetative growth, later flowering due to senescence is associated with lower efficiency in general and is therefore expected to be correlated with diminished root growth in the same way as it was thought to be correlated with lower seed production.

Hautekèete *et al.* (2001) studied the correlation between seed production and investment in vegetative parts after the first flowering period among closely related semelparous and iteroparous taxa in the section *Beta*. A positive correlation was found for the semelparous taxa, where no investment was made in survival and the vegetative structures directly reflected the size of the plant as a whole including its reproductive structures. Small size differences sufficed for a significant positive correlation. They divided *B. v. maritima* into short-lived (regions A and B in Table 1) and long-lived (region D, selecting the rare plants without vernalization requirement). A negative correlation was found for the latter group, confirming the results presented here for the plants far from the end of life (3+ in Fig. 5B). The short-lived populations did not show significant positive nor negative correlations. According to the life span values for regions A and B in Table 1, considerable ageing effects can already be expected after the first flowering period. This result is thus in agreement with the correlations in Fig. 5B that show a higher contribution of the lower numbers of 'years to death'.

Conclusions

The gradient in life span in *B. v. maritima* from almost annual to more than one decade complicates the analysis of whole-plant ageing, but also provides the means of exploring the mechanisms behind life span variation in more detail. The overall picture emerging from this study is that whatever the life span, plants have similar 'last-year problems'. The gradually later flowering with age, independent from these end-of-life phenomena, confirms that, in plants, ageing effects different from general dysfunction have to be taken into account. The role of flowering date has often been studied in relationship to the reproduction–survival trade-off but has, thus far, been neglected in studies dealing with ageing. Here, it has turned out to be directly involved in ageing and to be an interesting methodological tool because of its simple and unambiguous measurement and its high heritability.

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